Aetna Better Health®
2000 Market Street, Suite 850
Philadelphia, PA 19103

AETNA BETTER HEALTH®

Clinical Policy Bulletin: Polymerase Chain Reaction Testing: Selected Indications

Revised February 2015

Number: 0650

Policy

I. Aetna considers polymerase chain reaction (PCR) testing medically necessary for the following indications (not an all-inclusive list):

- Actinomyces, for identification of actinomyces species in tissue specimens
- Adenovirus, to diagnose adenovirus myocarditis, and to diagnose adenovirus infection in immunocompromised hosts, including transplant recipients
- Avian influenza A virus, for diagnosis of avian influenza A (H5N1) in persons with both: (i) symptoms consistent with Avian influenza A virus (see background); and (ii) a history of travel to or contact with persons or birds from a country with documented H5N1 avian influenza infections within 10 days of symptom onset. Ongoing listings of countries affected by avian influenza are available from the World Organization for Animal Health, available at http://www.oie.int/eng/en_index.htm
- Babesiosis (*Babesia spp*.), for diagnosis in malaria-endemic areas, where the morphologic characteristics observed on microscopic examination of blood smears do not allow an unambiguous differentiation between Babesia and Plasmodium
- Bartonella species (*B. henselae*, *B. quintana, B. clarridgeiae, B. elizabethae*), to confirm diagnosis in acutely or severely ill members with systemic symptoms of Cat-Scratch Disease, particularly person with hepatosplenomegaly or persons with large painful adenopathy and immunocompromised hosts; and to distinguish *B. henselae* from *B. quintana* infection in HIV-infected members and other immunocompromised members with signs and symptoms of bacillary angiomatosis or peliosis hepatitis (Also see CPB 0215 - Lyme Disease and other Tick-Borne Diseases)
- Beta-tyrosinase, to detect hematogenous spread of melanoma cells in members with melanoma
- BK polyomavirus in transplant recipients receiving immunosuppressive therapies and persons with immunosuppressive diseases (e.g., immune complex glomerulonephritis associated with HIV/AIDS)
- *Bordetella pertussis* and *B. parapertussis* , for diagnosis of whooping cough in individuals with coughing
- Breast and ovarian cancer (BRCA1, BRCA2), for persons who meet criteria for BRCA testing (see CPB 0227 - BRCA Testing, Prophylactic Mastectomy, and Prophylactic Oophorectomy)

- *Brucella spp*., for members with signs and symptoms of Brucellosis, and history of direct contact with infected animals and their carcasses or secretions or by ingesting unpasteurized milk or milk products
- *Burkholderia* infections (including *B. cepacia, B. gladioli*), diagnosis
- Chancroid (*Haemophilus ducreyi*), for diagnosis of persons with genital ulcer disease
- Chikungunya virus infection, diagnosis
- *Chlamydophila* (*Chlamydia*) *pneumoniae*, in members with signs or symptoms suggestive of Chlamydial pneumonia
- *Chlamydia trachomatis,* for screening* and diagnosis (See CPB 0433 - Chlamydia Trachomatis - Screening and Diagnosis)
- Chromosome 18q assay for persons with colorectal cancer
- *Clostridium difficile*, diagnosis (e.g., persons with diarrhea, not for asymptomatic or “test of cure”)
- Colorado tick fever virus, for diagnosis of Colorado tick fever within the first 14 days, before serologic tests become positive
- Colorectal cancer, hereditary non-polyposis colorectal cancer (MLH1, MSH2, MSH6 and microsatellite instability) and familial adenomatous polyposis (FAP, APC), for persons who meet criteria in CPB 0140 - Genetic Testing
- *Coxiella burnetii,* for confirmation of acute Q fever
- Cytomegalovirus (CMV), for persons with signs or symptoms suggestive of CMV infection
- Dengue, for laboratory confirmation of clinical diagnosis
- Ebola, diagnosis
- Ehrlichiosis (*Ehrlichia spp*.), for diagnosis in acute phase in persons from endemic areas with signs or symptoms suggesting this diagnosis (including persons residing in areas where human ehrlichiosis is endemic who either have very high fever (higher than 38 degrees C) or develop fever in the absence of erythema migrans after an Ixodes tick bite or have residual symptoms after treatment for Lyme disease)
- *Entamoeba histolytica*, to distinguish *E. histolytica* from *E dispar* and *E moshkovskii*
- Enterovirus infections (Group A and B coxsackieviruses, polioviruses, and echoviruses), for detecting viral RNA in cerebrospinal fluid (CSF), for immunocompromised persons suspected of having persistent central nervous system (CNS) infection (aseptic meningitis)
- Epidemic typhus (*Rickettsia prowazekii*), diagnosis
- *Epstein Barr Virus (EBV):* for detection of EBV in post-transplant lymphoproliferative disorder; or for testing for EBV in persons with lymphoma; or for those who are immunocompromised for other reasons.
- Factor II (prothrombin) G20210A mutation, for persons who meet criteria in CPB 0140 - Genetic Testing
- Factor V Leiden mutation, for persons who meet criteria in CPB 0140 - Genetic Testing
- Fragile X syndrome, for persons who meet medical necessity criteria for Fragile X genetic testing (see CPB 0140 - Genetic Testing), FMR1 gene analysis by PCR is considered medically necessary to confirm diagnosis of fragile X syndrome and to rule out FRAXE and FRAXF.
- *Francisella tularensis*, for presumptive diagnosis of tularemia
- Genetic testing in Canavan disease, Niemann Pick disease, cystic fibrosis, Gaucher disease, Tay Sachs, connexin 26, Rett syndrome, fetal sickle cell anemia, Huntington's disease, and Angelman and Prader Willi syndromes, for persons who meet criteria in CPB 0140 - Genetic Testing
- Gonorrhea (*Neisseria gonorrhoeae),* for screening* and diagnosis
- Group B streptococcal (GBS) infection screening, for the following: 1) intrapartum testing of women with unknown GBS colonization status and no intrapartum risk factors (temperature of greater than o equal to 100.4º F [greater than or equal to 38.0ºC] or rupture of amniotic membranes greater than or equal to18 hours) at the time of testing and who are delivering at term; and 2) antepartum testing with broth enrichment for pregnant women at 35 to 37 weeks gestation.

- *Haemophilus influenzae*, for capsular typing of invasive disease
- Hantavirus, diagnosis
- Hemochromatosis mutation in persons who meet criteria for genetic testing for hereditary hemochromatosis in CPB 0140 - Genetic Testing
- Hemorrhagic fevers and related syndromes caused by viruses of the family *Bunyaviridae* (Rift Valley fever, Crimean-Congo hemorrhagic fever, hemorrhagic fever with renal syndromes), for diagnosis in acute phase in persons with clinical presentation suggestive of these conditions
- Hepatitis B virus, for selection of candidates to receive antiviral therapy and to monitor the response to therapy
- Hepatitis C virus genotyping, for determining the risks/benefits and duration of treatment
- Hepatitis C virus, for diagnosis of infection, and for monitoring members receiving anti-viral therapy
- Hepatitis D virus, for confirmation of active infection in persons with anti-HDV antibodies
- Hepatitis E virus, for definitive diagnosis in persons with anti-HEV antibodies
- Herpes simplex virus (HSV), for diagnosis of HSV infection
- Human granulocytic anaplasmosis (*Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophilum*))
- Human herpesvirus type 6 (HHV-6), for diagnosis of infection in immunocompromised persons such as persons with AIDS patients or transplant recipients, and to diagnose HHV-6 infection in members with mononucleosis-like syndrome in members without heterophile antibodies or antibodies specific to Epstein-Barr virus (EBV)
- Human immunodeficiency virus (HIV), to diagnose HIV infection in infants and young children less than 18 months of age
- Human leukocyte antigen (HLA) typing: for assessing histocompatability in tissue grafts and organ transplants; for diagnosis of ankylosing spondylitis or Reiters syndrome (HLA B27); for persons suspected of having celiac disease who meet criteria in CPB 0561 - Celiac Disease Laboratory Testing
- Human metapneumovirus, diagnosis
- Human papilloma virus (HPV), for indications listed in CPB 0443 - Cervical Cancer Screening and Diagnosis
- Human T Lymphotropic Virus type 1 and type 2 (HTLV-I and HTLV-II), to confirm the presence of HTLV-I and HTLV-II in the cerebrospinal fluid of persons with signs or symptoms of HTLV-I/HTLV-II myeloradiculopathy who have traveled to areas where HTLV-I/HTLV-II infection is endemic (Japan, the Caribbean, and parts of South America)
- Influenza virus (including influenza A and B), for hospitalized persons with suspected influenza and for other persons with suspected influenza for whom a diagnosis of influenza will inform decisions regarding clinical care, infection control, or management of close contacts
- Janus Kinase 2 gene mutation, for initial diagnostic assessment of BCR-ABL negative adults presenting with symptoms of myeloproliferative disorders, and for diagnostic assessment of polycythemia vera in adults
- JC polyomavirus, in transplant recipients receiving immunosuppressive therapies, in persons with immunosuppressive diseases (e.g., AIDS), and for diagnosing progressive multifocal leukoencephalopathy in persons with multiple sclerosis or Crohn's disease receiving natalizumab (Tysabri)
- Leishmaniasis, diagnosis
- Leukemias, acute myelogenous (FLT3 mutation), acute myelocytic (AM1/ETO t(8;21) translocation), acute myelomonocytic (CBFB/MYH11 inv(16) inversion), acute promyelocytic (PML/RARA t(15;17) translocation), acute lymphocytic (bcr-abl gene rearrangement), chronic myelogenous (bcr-abl gene rearrangement), and chronic lymphocytic (IgVh mutation analysis)
- Lymphogranuloma venereum (*Chlamydia trachomatis*)

- Lymphomas, B cell (bcl-2 gene translocation t(14;18)), mantle cell (bcl-1 gene translocation t(11;14)(q13;q32)) and T cell (gene rearrangements)
- Lymphomas, B cell or T cell, to determine clonality
- Malaria, for confirming the species of malarial parasite after the diagnosis has been established by either smear microscopy or a rapid diagnostic test
- Measles virus (Morbilliviruses), for diagnosis of measles (rubeola)
- Methicillin resistant *Staphylococcus aureus* (MRSA), to distinguish MRSA from non-resistant forms o *S. aureus* (not for detection of the mere presence of *S. aureus*)
- Microsporidia, diagnosis
- Mucosa-associated lymphoid tissue (MALT) lymphomas and marginal zone lymphomas, for evaluating persons who have non-diagnostic atypical lymphoid infiltrates that are positive for *H. pylor* infection, to assess likelihood of response to antibiotic therapy
- Mumps, in persons with acute parotitis of 2 or more weeks' duration
- *Mycobacterium tuberculosis*, for rapid diagnostic testing of acid fast stain positive respiratory tract specimens
- *Mycoplasma hominis*, diagnosis
- *Mycoplasma pneumoniae*, for diagnosis
- *Neisseria meningitidis*, to establish diagnosis where antibiotics have been started before cultures have been obtained
- Oncotype Dx test for breast cancer, when criteria in CPB 0352 - Tumor Markers are met
- Parvovirus, for detecting chronic infection in immunocompromised persons (e.g., pregnant women with known exposure to parvovirus B19 infection and serologic testing is negative)
- Psittacosis, for diagnosis of *Chlamydophila (Chlamydia) psittaci* infection and distinguishing C. psittaci from other *Chlamydophila* infections
- Respiratory syncytial virus (RSV), for confirming the result of rapid antigen detection assay.
- RET proto-oncogene mutations, for diagnosis of multiple endocrine neoplasia type 2 (MEN2) and familial medullary thyroid carcinoma (FMTC) in persons who meet criteria in CPB 0319 - RET Proto-Oncogene Testing
- Rocky Mountain Spotted Fever (*Rickettsia rickettsii*), for diagnosis in acute phase (first 2 weeks of infection) in persons from endemic areas with signs or symptoms suggestive of this diagnosis
- Rubella, diagnosis
- Severe acute respiratory syndrome (SARS), for detection of SARS coronavirus RNA in persons with signs or symptoms of SARS who have traveled to endemic areas or have been exposed to persons with SARS
- Shiga toxin (from *E. coli* and Shigella), detection
- Streptococcal pharyngitis (group A Streptococcus), diagnosis
- Syphilis (*Treponema pallidum*), for diagnosis of oral or other lesions where contamination with commensal treponemes is likely
- Thiopurine methyltransferase (TPMT) genotyping (see CPB 0249 - Inflammatory Bowel Disease: Serologic Markers and Pharmacogenomic and Metabolitic Assessment of Thiopurine Therapy)
- *Toxoplasma gondii*, for detection of *T. gondii* infection in immunocompromised persons with signs and symptoms of toxoplasmosis, and for detection of congenital Toxoplasma *gondii* infection (including testing of amniotic fluid for toxoplasma infection)
- Trichomoniasis *(Trichomonas vaginalis)*, diagnosis of of trichomonas in symptomatic men and women, and screening of women at high risk of infection (i.e., women who have new or multiple partners, have a history of STDs, exchange sex for payment, or use injection drugs)
- Ureaplasma urealyticum, diagnosis
- Varicella-Zoster infections, for diagnosis and also to distinguish wild-type virus from vaccination in previously immunized persons with signs or symptoms of Varicella zoster infection

- West Nile Virus, to confirm diagnosis in acute-phase serum, CSF or tissue specimens, and to screen bone marrow transplant donors in endemic areas.
- Whipple's disease (*T. whippeli*), biopsy tissue from small bowel, abdominal or peripheral lymph nodes, or other organs of persons with signs and symptoms, to establish the diagnosis, and of CSF tissue in persons with long-standing systemic involvement or with suspected CNS manifestations.
- Yellow fever, diagnosis

*Note: Screening tests are covered only for members with preventive services benefits. Please check benefit plan descriptions for details.

II. Aetna considers the following quantitative PCR tests medically necessary:

- Adenovirus viral load, to monitor response to antiviral therapy in infected immunocompromised hosts, including transplant recipients
- BK polyomavirus viral load, for diagnosis and monitoring response to therapy in infected kidney transplant recipients
- Cytomegalovirus (CMV) viral load, to monitor response to therapy
- EBV viral load, to monitor for EBV viral replication in solid organ transplant recipients by measuring blood quantitative EBV PCR at intervals appropriate to the specific organ transplantation
- Hepatitis B viral load, to monitor response to therapy
- Hepatitis C viral load, to monitor response to therapy
- Human herpesvirus type 6, to monitor response to therapy in immunocompromised hosts, including transplant recipients
- HIV RNA viral load testing, to monitor disease progression and response to therapy
- Acute myeloid leukemia: AM1/ETO t(8, 21) translocation, to monitor disease progression and response to therapy
- Chronic myelogenous leukemia and acute lymphocytic leukemia: bcr/abl gene rearrangement, to monitor disease progression and response to therapy
- Acute myelomonocytic leukemia: CBFB/MYH11 inv(16), to monitor disease progression and response to therapy
- Acute promyelocytic leukemia: PML/RARA t(15;17), to monitor disease progression and response to therapy
- Mantle cell lymphoma: bcl-1/JH t(11;14) gene rearrangement, to monitor disease progression and response to therapy
- B cell lymphomas: bcl-2 gene translocation, to monitor disease progression and response to therapy
- T cell lymphomas: gene rearrangements, to monitor disease progression and response to therapy.

- Varicella-zoster infection diagnosis in previously immunized persons, to distinguish wild-type virus from vaccination

III. Aetna considers PCR testing for the following indications experimental and investigational because of insufficient evidence in the peer-reviewed literature:

- Acinetobacter baumannii
- Aspergillosis
- Astrovirus
- Autoimmune lymphoproliferative syndrome
- Bacterial vaginosis (*Gardnerella vaginalis, Atopobium vaginae, Mobiluncus mulieris, M. curtisii,* and *megasphaera type 1* and *type 2*)
- Bacterial vaginosis associated bacteria 2 (BVAB2)
- *Bacteroides spp*. (*B. fragilis, B. ureolyticus*)
- Blastomycosis

- BRAF mutation analysis for hairy cell leukemia
- Caliciviruses (noroviruses and sapoviruses)
- Campylobacteriosis (*Campylobacter* infection)
- Candidiasis (*Candida albicans*)
- Castleman's disease
- Cervical intraepithelial neoplasia (CIN) metastasis
- *Chlamydia pneumoniae*, for assessment of atherosclerotic cardiovascular disease, asthma, Alzheimer disease, multiple sclerosis, or Kawasaki disease
- Coagulase-negative staphylococcus (including *Staphylococcus saprophyticus*, and *Staphylococcus lugdunensis*)
- Coccidiodomycosis (*Coccidioides* species)
- Colorectal cancer screening (PreGen Plus) (see CPB 0516 - Colorectal Cancer Screening)
- Coronavirus (other than SARS-coronavirus)
- Creutzfeldt-Jakob disease
- Cryptococcus (*Cryptococcus neoformans*)
- Cryptosporidiosis (cryptosporidium infection)
- Cyclosporiasis (Cyclospora infection)
- Cytochrome P450 genotyping (see CPB 0715 - Pharmacogenetic and Pharmacodynamic Testing)
- Donovanosis, or granuloma inguinale (*Klebsiella granulomatis*)
- Eastern equine encephalitis
- *Enterobacter aerogenes*
- *Enterobacter cloacae*
- *Enterococcus faecalis*
- *Enterococcus faecium*
- *Escherichia coli* (except for detection of Shiga toxin)
- Giardia lamblia
- Hepatitis A virus
- Hepatitis G virus (HGV)/GB virus type C
- Histoplasma capsulatum histoplasmosis
- Human bocavirus
- Human herpesvirus type 7 (HHV-7)
- Human herpesvirus type 8 (HHV-8)
- Kawasaki disease
- LaCrosse encephalitis
- Lactobacillus vaginitis
- *Legionella pneumophila*
- Leptospirosis (*Leptospira* organisms)
- Listeria
- Lyme disease (*Borrelia burgdorferi*)
- Malaise and fatigue (including chronic fatigue syndrome)
- Melanoma (p16, Melaris) (see CPB 0140 - Genetic Testing) and melanoma micrometastases
- MTHFR mutation in persons with hyper-homocysteinemia
- *Molluscum contagiosum*
- *Moraxella catarrhalis*
- *Myocbacterium* species (other than *M. tuberculosis*), including *Mycobacterium-avium intracellulare (MIA)*
- *Mycoplasma fermentans*
- *Mycoplasma genitalium*
- *Mycoplasma penetrans*
- Nanobacteria

- Non-albicans Candida
- Onychomycosis
- Parainfluenza virus
- Peptic ulcer disease (*Helicobacter pylori*) (other than in persons with MALT lymphomas and margina zone lymphomas)
- Peripheral neuropathy
- Pneumococcal infections (*S. pneumoniae*)
- Pneumocystis pneumonia (*Pneumocystis jiroveci* (formerly *P. carinii*))
- *Prevotella spp.*
- Prostate cancer micrometastasis
- *Proteus mirabilis*
- Pseudomonas (*P. aeruginosa*)
- Pleuropulmonary coccidioidomycosis
- Rhinovirus
- Rotavirus
- Routine screening of trichomonas in asymptomatic women
- *Saccharomyces cerevisiae*
- Salmonella
- Screening newborns for congenital cytomegalovirus infection
- Serratia spp. (including *S. marcescens*)
- Sporotrichosis (*Sporothrix schenckii*)
- St. Louis encephalitis
- Staphylococcus epidermidis
- *Staphylococcus aureus*
- Stenotrophomonas maltophilia
- Trichosporonosis (Trichosporon spp.)
- Vancomycin-resistant enterococcus
- Vibrio cholerae
- Vibrio vulnificus
- Western equine encephalitis
- Wound infection
- Xenotropic murine leukemia
- Yersinia enterocolitica

IV. Aetna considers the following quantitative PCR tests experimental and investigational because their role in patient management has not been established:

- Bartonella (*B. henselae, B. quintana*)
- Candidiasis (*Candida albicans*)
- Chlamydia species (*Chlamydia (Chlamydophila) pneumoniae, C. trachomatis*)
- *Gardnerella vaginalis*
- Hantavirus
- Hepatitis G virus (HGV)/GB virus type C
- Herpes simplex virus (HSV)
- Influenza
- *Legionella pneumophila*
- Lyme disease (*Borrelia burgdorferi*)
- *Mycobacterium* species (including *Mycobacterium avium-intracellulare* and *Mycobacterium tuberculosis*)
- Mycoplasma

- *Neisseria gonorrhoeae*
- Parvovirus (including chronic fatigue immune dysfunction syndrome secondary to parvovirus B19 infection)

Background

The development of the polymerase chain reaction (PCR) has greatly simplified DNA analysis and shortened laboratory time (ACOG, 2002). Polymerase chain reaction allows the exponential amplification of the targeted gene or DNA sequence. Only minute quantities of DNA, typically 0.1 to 1.0 mg, are necessary for PCR. DNA can be amplified from a single cell. One important prerequisite of PCR is that the sequence of the gene, or at least the borders of the region of DNA to be amplified, must be known.

The PCR procedure has 3 steps: (i) DNA is denatured by heating to render it single stranded, (ii) the PCR primers, which are short pieces of DNA (oligonucleotides) 20 to 30 base pairs in length exactly complementary to the ends of each piece of the double-stranded DNA to be amplified, anneal to their complementary regions of the DNA, and (iii) synthesis of the complementary strand of DNA occurs in the presence of the enzyme Taq polymerase and nucleotides triphosphates (dATP, dCTP, dGTP, and dTTP). The reaction cycle of denaturation, annealing, and extension is repeated 25 to 30 times to produce millions of copies of DNA. Typically, fragments several kilobases (kb) in size can be amplified, but sequences up to 10 kb have been successfully amplified. The exact cycling parameters and conditions for PCR must be determined empirically for each set of primers.

Polymerase chain reaction is very sensitive; therefore, extreme care must be taken to avoid amplification of contaminant DNA from aerosolized secretions or sloughed skin cells. These precautions are particularly important when DNA from a single cell is being amplified.

PCR amplification techniques raise considerable concerns regarding contamination from one specimen to another creating the potential for false-positive results. The clinical interpretation of PCR results may also be challenging. Amplification of organisms representing latent infection or colonization can not be distinguished from active, clinically significant infections. Additionally, PCR may amplify fragments of nucleic acids, representing dead microorganisms, thus further clouding the clinical interpretation. Finally, specificities, sensitivities, and positive and negative predictive values of PCR have not been reported in large groups of patients for many of the microorganisms.

Polymerase chain reaction may be useful when culture is difficult due to the low numbers of the organisms, for fastidious or lengthy culture requirements, or when there is difficulty in collecting an appropriate sample. Quantification of viral load via PCR may be useful when the viral load can be used as a prognostic indicator, or when necessary follow the patient's response to therapy.

Several manufacturers have produced PCR Assays designed to detect multiple pathogens. The INFINITI® Bacterial Vaginosis QUAD Assay is designed to detect the following pathogens: Bacteroides fragilis, Gardnerella vaginalis, Mobiluncus mulieris, Mobiluncus curtisii, Atopobium vaginae, and Prevotella bivia (AutoGenomics, Bacterial Vaginosis, 2010). The INFINITI® Candida Vaginitis QUAD Assay is designed to detect 5 fungal species: C. albicans, C. glabrata, C. krusei, C. parapsilosis, and C. tropicalis (AutoGenomics Candida Vaginitis 2010). Quest Diagnostics has developed the Quest SureSwab, which includes tests for C. trachomatis, N. gonorrhoeae, and T. vaginalis as well as tests for bacterial vaginosis and Candida spp.

Vaginal Discharge

The 3 diseases most frequently associated with vaginal discharge are trichomoniasis (caused by *T. vaginalis*), bacterial vaginosis (caused by a replacement of the normal vaginal flora by an overgrowth of anaerobic microorganisms, mycoplasmas, and *Gardnerella vaginalis*), and candidiasis (usually caused by *Candida albicans*).

C. trachomatis or *N. gonorrhoeae* can sometimes cause vaginal discharge, but can be distinguished in that the vaginal discharge is accompanied by a mucopurulent cervicitis.

Current Centers for Disease Control and Prevention Guidelines on management of diseases characterized by vaginal discharge (CDC, 2002) do not indicate any role for PCR tests in the assessment of vaginal discharge unless the sexually transmitted diseases *C. trachomatis* or *N. gonorrhoeae* are suspected based on history of sexual activity and presence of mucopurulent cervicitis. Otherwise, the cause of vaginal infection can be diagnosed by pH and microscopic examination of the discharge.

Bacterial Vaginosis

Bacterial vaginosis (BV) is a condition in which the natural balance of organisms found in the vagina is changed from a predominance of Lactobacillus to an over-growth of other bacteria including *Gardnerella vaginalis*, *Mobiluncus* and other anaerobes. Atopobium vaginae is a recently recognized bacterium that has been found in bacterial vaginosis (Ferris et al, 2004). Its clinical significance is unknown. Bacterial vaginosis can be diagnosed by the use of clinical or Gram-stain criteria (CDC, 2002; AGM-MSSVD, 2002). The most widely accepted method for diagnosis of bacterial vaginosis is the presence of 3 of the following four Amsel criteria: (i) a homogenous vaginal discharge; (ii) a vaginal pH greater than 4.5; (iii) the presence of clue cells; and (iv) a fishy odor after addition of potassium hydroxide to the vaginal secretions (the amine test). Diagnosis on the basis of Amsel criteria has a reported sensitivity of 81 % and a specificity of 94 % (WHO, 1999). Gram stain of a vaginal smear has a sensitivity of 89 % and a specificity of 93 %. According to the CDC, culture of *G. vaginalis* is not recommended as a diagnostic tool because it is not specific. A DNA probe based test for high concentrations of G. vaginalis (Affirm™ VP III, manufactured by Becton Dickinson, Sparks, MD) does have clinical utility (CDC, 2002). This test has sensitivity for *G. vaginalis* of 94 % and a specificity of 81 % (WHO, 1999). Other commercially available tests that may be useful for the diagnosis of BV include a card test for the detection of elevated pH and trimethylamine (FemExam® test card, manufactured by Cooper Surgical, Shelton, CT) and prolineaminopeptidase (Pip Activity TestCard™, manufactured by Litmus Concepts, Inc., Santa Clara, CA). The FemExam is a rapid test that measures vaginal pH and volatile amines, corresponding to 2 of the 4 Amsel criteria. The proline aminopeptidase test is an indirect test for a chemical produced by the organisms associated with bacterial vaginosis. Prolineaminopeptidase assay has a reported sensitivity of 93 % and a specificity of 93 % for BV (WHO, 1999). Current guidelines do not recommend PCR testing for bacterial vaginosis (CDC, 2010; BASHH, 2007; AAP, 2009; NYSDOH, 2009). Guidelines on BV from the CDC (Workowski et al, 2010) stated that "PCR also has been used in research settings for the detection of a variety of organisms associated with BV, but evaluation of its clinical utility is uncertain." Sobel (2011) noted that PCR-based tests are being investigated for molecular diagnosis of BV, mostly based upon molecular quantification of *Gardnerella vaginalis* and *Atopobium vaginae*. "These tests are expensive and of questionable advantage."

Trichomoniasis

Diagnosis of vaginal trichomoniasis (*T. vaginalis*) is usually performed by microscopy of vaginal secretions, but this method has a sensitivity of only about 60 % to 70 % (CDC, 2002). According to the CDC, culture is the most sensitive commercially available method of diagnosis. The AGM-MSSVD (2002) states that up to 95 % of female cases can be diagnosed by culture; 60 to 80 % of male cases can be diagnosed by urethral culture or culture of first-void urine, and that sampling both simultaneously will significantly increase the diagnostic rate. According to the American Academy of Pediatrics (AAP, 2006), culture of the organism and antibody tests using an enzyme immunoassay and immunofluorescence techniques for demonstration of the organism are more sensitive than wet mount preparations but generally are not required for diagnosis. A rapid antigen detection assay for Trichomonas and Candidiasis has a sensitivity of 86 % and a specificity of 99 % for *T. vaginalis* (WHO, 1999). A commercially available, rapid, automated hybridization assay is available that uses DNA probes to directly detect Candida, Trichomonas and Gardnerella in vaginal swab samples (WHO, 1999). This assay has a sensitivity of 88 to 91 % and a specificity of 100 % (WHO, 1999) for *T. vaginalis*. PCR assay for trichomonas is somewhat more sensitive (93 %) than antigen detection or hybridization assay for *T. vaginalis* but less sensitive than culture or microscopy

(WHO, 1999). In addition, the PCR assay is less specific (96 %) than microscopy, culture, antigen or hybridization assay, with the latter methods having sensitivities of 99 to 100 % (WHO, 1999).

Guidelines from the CDC (Workowski et al, 2010) stated that an FDA-cleared PCR assay for detection of gonorrhea and chlamydial infection (Roche Amplicor) has been modified for *T. vaginalis* detection in vaginal or endocervical swabs and in urine from women and men; sensitivity ranges from 88 % to 97 % and specificity from 98 % to 99 %. T. vaginalis RNA can also be detected by transcription-mediated amplification (TMA) (Gen-Probe APTIMA). The CDC guidelines stated that, in men, wet preparation is not a sensitive test. Culture testing of urethral swab, urine, or semen is one diagnostic option; however, PCR or TMA have superior sensitivity for *T. vaginalis* diagnosis in men.

Lee et al (2012) evaluated the usefulness of PCR for diagnosis of T. vaginalis infection among male patients with chronic recurrent prostatitis and urethritis. Between June 2001 and December 2003, a total of 33 patients visited the Department of Urology, Hanyang University Guri Hospital and were examined for T. vaginalis infection by PCR and culture in TYM medium. For the PCR, these researchers used primers based on a repetitive sequence cloned from T. vaginalis (TV-E650). Voided bladder urine (VB1 and VB3) was sampled from 33 men with symptoms of lower urinary tract infection (urethral charge, residual urine sensation, and frequency). Culture failed to detect any T. vaginalis infection whereas PCR identified 7 cases of trichomoniasis (21.2 %); 5 of the 7 cases had been diagnosed with prostatitis and 2 with urethritis. PCR for the 5 prostatitis cases yielded a positive 330 bp band from bothVB1 and VB3, whereas positive results were only obtained from VB1 for the 2 urethritis patients. The authors concluded that they showed that the PCR method could detect T. vaginalis when there was only 1 T. vaginalis cell per PCR mixture. They stated that these findings strongly support the usefulness of PCR on urine samples for detecting T. vaginalis in chronic prostatitis and urethritis patients.

Muzny and Schwebke (2013) noted that T. vaginalis is the most common curable sexually transmitted infection worldwide. T vaginalis infections in women can range from asymptomatic to acute inflammatory vaginitis. In men, this infection is typically asymptomatic but is increasingly being recognized as a cause of non-gonococcal urethritis. Diagnosis of T vaginalis has traditionally been made by direct microscopic examination of a wet mount of vaginal fluid or through the use of culture. The recent commercial availability of nucleic acid amplification tests for the detection of T vaginalis has seen these replace culture as the gold standard for diagnosis. Nitroimidazoles (i.e., metronidazole and tinidazole) are the mainstay of therapy. In the case of treatment failure due to drug resistance or in the case of a severe nitroimidazole allergy, alternative intra-vaginal therapies exist, although their effectiveness has not been evaluated systematically. Novel systemic agents other than nitroimidazoles for the treatment of T vaginalis are needed, and efforts to promote and support anti-microbial drug development in this setting are necessary.

Furthermore, an UpToDate review on “Trichomoniasis” (Sobel, 2014) states that “Men -- The most reliable methods for diagnosis of trichomonas urethritis in the male are by culture or a nucleic acid amplification test (i.e., PCR or transcription-mediated amplification [TMA]) of first fraction urine or a urethral swab specimen, but these tests are not widely available. Saline microscopy of a urethral swab specimen has low sensitivity and is not recommended”.

Candidiasis

A diagnosis of *Candida vaginitis* is suggested clinically by pruritus and erythema in the vulvovaginal area; a white discharge may be present (CDC, 2002). The diagnosis can be made in a woman who has signs and symptoms of vaginitis when either a) a wet preparation (saline, 10 % KOH) or Gram stain of vaginal discharge demonstrates yeasts or pseudohyphae, or b) a culture or other test yields a positive result for a yeast species (CDC, 2002; AGM-MSSVD, 2002). *Candida vaginitis* is associated with a normal vaginal pH (less than 4.5). According to the CDC (2002), identifying Candida by culture in the absence of symptoms is not an indication for treatment, because approximately 10 % to 20 % of women harbor Candida species and other yeasts in the vagina. Culture may be indicated in women with recurrent vulvovaginal candidiasis (defined as more than four episodes of vulvovaginal

Candidiasis per year) to confirm the clinical diagnosis and to identify unusual species, including non-albicans species, including *C. glabrata*. A rapid antigen detection assay for Trichomonas and Candidiasis has a sensitivity of 61 to 81 % and a specificity of 97 % for *C. albicans* (WHO, 1999). A commercially available, rapid, automated hybridization assay is available that uses DNA probes to directly detect Candida, Trichomonas and Gardnerella in vaginal swab samples (WHO, 1999). This assay has a reported sensitivity 80 % and specificity of 98 % for Candida vaginalis. Current guidelines do not include a recommendation for PCR testing for candidiasis (ACOG; 2006; BASHH, 2006; CDC, 2006; AAP, 2009; Workowski et al, 2010).

According to guidelines from the CDC (2006), *C. glabrata* may be suspected in persons with recurrent vulvovagina candidiasis. The CDC explains that *C. glabrata* and other nonalbicans Candida species are observed in 10 to 20 % of patients with recurrent vulvovaginal candidiasis, and that conventional anti-myoctic therapies are not as effective against these species as against *C. albicans*. The CDC guidelines state that *C. glabrata* does not form pseudohyphae or hyphae and is not easily recognized on microscopy. The CDC states that the clinical diagnosis of *C. glabrata* can be confirmed by vaginal cultures, and that such cultures should be obtained from patients with RVVC to confirm the clinical diagnosis and to identify unusual species.

Candida albicans, *C. dubliniensis*, and *C. stellatoidea* can be identified morphologically via germ-tube formation (hyphae are produced from yeast cells after 2 to 3 hrs of incubation) on microscopy, or with biochemical assays.

CHROMagar Candida is a specialized media for Candida isolation, which allows for the presumptive identification of several Candida species by using color reactions in specialized media that demonstrate different colony colors depending on the species of Candida. CHROMagear distinguishes *C. albicans*, *C. tropicalis*, and *C. krusei* based on the species' distinctive pigments.

Also, fluorescent in-situ hybridization (FISH) has been used to distinguish *C. albicans* from non-albicans species. The peptide nucleic acid FISH (PNA-FISH) is used to distinguish *C. albicans* from non-albicans Candida species. The *C. albicans* PNA-FISH test can be used to identify *C. albicans* in 24 to 48 hrs when the probe is added to smears that are made directly from the blood culture bottle and followed by hybridization. A newer version of this test now allows for the simultaneous identification of either *C. albicans* or C*. glabrata*.

Culture of Candida species allows for susceptibility testing. According to current guidlines, susceptibility testing may be considered in situations where there is a failure to respond to initial antifungal therapy.

Updated guidelines on the management of candidiasis from the Infectious Diseases Society of America (Pappas e al, 2009) concluded: "Real-time PCR is a non-validated but intriguing methodology that holds promise as an early diagnostic aid for candidemia. These encouraging data offer new perspectives for early diagnosis of Candida infections, but continued evolution of these assays will be required before they can be used routinely." These recommendations are in line with previous guidelines from other authorities on the management of candida infectious that indicated no role for PCR testing (CDC, 2006; White et al, 2006; Pickering et al, 2006). According to guidelines from the British Association for Sexual Health and HIV (White et al, 2006), PCR testing for Candida species "is currently of use only as a research tool." More recently, Sobel (2011) stated that "PCR tests are now available commercially, but offer no proven benefit over culture in symptomatic women."

Chlamydia Trachomatis

Chlamydia trachomatis is an important cause of urethritis and cervicitis (WHO, 1999; AGM-MSSVD, 2002). Laboratory detection of *C. trachomatis* is necessary because as many as 70 % to 80 % of women and up to 50 % of men who are infected do not experience any symptoms. The U.S. Preventive Services Task Force (USPSTF) strongly recommends that clinicians routinely screen all sexually active women 25 years and younger, and other asymptomatic women at increased risk of infection, for chlamydial infection (USPSTF, 2002). The conventional method for the laboratory diagnosis of *C. trachomatis* has been inoculation of a cell culture with a genital specimen. According to the WHO (1999), however, this method is expensive, labor-intensive, time-consuming,

and requires considerable expertise. For these reasons, culture tests are now used less frequently and antigen and nucleic acid detection techniques have become common methods for detection of *C. trachomatis* infection.

The leukocyte esterase assay is a rapid urine dipstick test for the presence of an enzyme found in the urine when leukocytes are present due to inflammation. The LE test can diagnose urethritis but can not identify the specific cause of the infection. The sensitivity and specificity of LE for the detection of chlamydial and gonococcal infection are 54 % to 97 % and 36 % to 95 %, respectively (WHO, 1999). Microscopy detection of *C. trachomatis* has a reported sensitivity and specificity of 74 % to 90 % and a specificity of 9 8% to 99 %. According to the AAP's Committee on Infectious Diseases, nucleic acid amplification methods, such as PCR, transcription-mediated amplification (TMA), and strand displacement amplification (SDA) are more sensitive than cell culture and more specific and sensitive than DNA probe, direct fluorescent antibody (DFA) tests, or enzyme immunoassays (EIAs), although specificity is variable compared with culture (AAP, 2006). According to the USPSTF (2002), the sensitivities and specificities of nucleic acid amplification tests are all high, ranging from 82 to 100 %. The sensitivity of antigen detection tests (EIA, DFA) is slightly lower (70 to 80 %) but specificity remains high (96 to 100 %). The AAP states that tests for detection of chlamydial antigen or nucleic acid are useful for evaluating urethral specimens from males, cervical specimens from females, and conjunctival specimens from infants. The PCR and LCR tests are useful for evaluating urine specimens from either sex. The Scottish Intercollegiate Guidelines Network (2000) states that nucleic acid amplification tests (LCR or PCR) are the recommended laboratory test for *Chlamydia trachomatis.* The British Association of Sexual Health and HIV (BASHH, 2006) makes similar recommendations about PCR testing for *Chlamydia trachomatis.*

Lymphogranuloma Venereum

Lymphogranuloma venereum (LGV) is caused by *C. trachomatis* serovars L1, L2, or L3. The disease occurs rarel in the United States. The most common clinical manifestation of LGV among heterosexuals is tender inguinal and/or femoral lymphadenopathy that is most commonly unilateral (CDC, 2002). Women and homosexually active men may have proctocolitis or inflammatory involvement of peri-rectal or peri-anal lymphatic tissues resulting in fistulas and strictures. According to the CDC (2002), the diagnosis of LGV is usually made by complement fixation and by exclusion of other causes of inguinal lymphadenopathy of genital ulcers. The diagnostic utility of serologic methods other than complement fixation is unknown (CDC, 2002). The British Association of Sexual Health and HIV (BASHH, 2006) states that detection of nucleic acid (DNA) by amplification techniques such as the ligase chain reaction (LCR) or PCR are becoming established for routine testing of urethral, cervical, or urine specimens but have rarely been used in the context of LGV, until recent outbreaks in Western Europe. BASHH (2006) notes that these methods are highly sensitive and specific, and have now widely become available commercially. Positive samples should be confirmed by real-time PCR for LGV specific DNA.

Gonorrhea

Infection of the genital tract with *Neisseria gonorrhoeae* can cause urethritis, cervicitis, proctitis, or bartholinitis (WHO, 1999; AGM-MSSVD, 2002). Complications of untreated disease include epididymitis, prostatitis, and infertility in men and pelvic inflammatory disease and infertility in women. Because most cases in females are asymptomatic, detection of infection using laboratory tests is needed to prevent sequelae and transmission to sexual partners and, for pregnant women, to neonates (WHO, 1999).

According to the AAP (2006), microscopic examination of Gram-stained smears of exudate from the eyes, the endocervix of postpubertal females, the vagina of prepubertal girls, male urethra, skin lesions, synovial fluid, and, when clinically warranted, cerebrospinal fluid (CSF) is useful in the initial evaluation. According to the WHO, the sensitivity and specificity of Gram-stain for detection in urethral samples in symptomatic men is 90 to 95 % and the specificity is 98 to 100 % (WHO, 1999). However, Gram stain is not as useful for endocervical smears (sensitivity of 50 to 70 %) because the presence of other Gram-negative diplococci makes interpretation difficult.

Neisseria gonorrhoeae can be cultured from normally sterile sites, such as blood, CSF, or synovial fluid, using specialized culture media. Selective media that inhibit normal flora and nonpathogenic Neisseria organisms are used for culture from non-sterile sites, such as the cervix, vagina, rectum, urethra, and pharynx. According to the WHO (1999), the sensitivity of culture for *N. gonorrhoeae* ranges from 81 to 100 % with a specificity of 100 %. An advantage of culture is that isolates are available for further testing. Disadvantages of culture are the need for stringent handling and up to 3 days for results.

The AAP (2006) notes that nucleic acid amplification methods by PCR, mediated amplification (TMA), and strand-displacement assays are highly sensitive and specific when used on urethral (males) and endocervical swab assays. They also can be used with good sensitivity and specificity on first-void urine specimens, which has led to increased compliance with testing and follow-up in hard-to-access populations, such as adolescents. These techniques also permit dual testing of urine for *C. trachomatis* and *N. gonorrhoeae*. According to the WHO, the sensitivity and specificity of DNA hybridization assays for gonorrhea are 86 to 100 % and 99 %, respectively. The sensitivity of PCR testing ranges from 89 to 97 % and the specificity ranges from 94 to 100 %. The sensitivity of LCR ranges from 95 to 100 % and the specificity ranges from 98 to 100 %.

According to guidelines from the CDC (2002), PCR tests for *Neisseria gonorrhoeae* are recommended for testing urethral swabs from males and endocervical swabs when conditions during holding and transport of inoculated culture media are not adequate to maintain the viability of organisms. The CDC guidelines note that commercial PCR assays have cross-reacted with non-gonococcal Neisseria; such cross-reactivity has not been reported for commercial LCR and un-amplified probe assays.

Polymerase chain reaction testing of the urine may be indicated for urinary screening in women when pelvic examination is not indicated, and in men (CDC, 2002). The CDC guidelines note that the sensitivity of urine testing with PCR may be lower than with urethral (males) or endocervical swabs. Polymerase chain reaction tests may be indicated in screening vaginal swabs of prepubescent children for possible sexual abuse if culture is not available (CDC, 2002). According to CDC guidelines, additional review is needed before a recommendation can be made for use of PCR tests in vaginal swabs of post-menarcheal adolescents and adults.

According to guidelines from the CDC, PCR tests for gonorrhea are not recommended for vaginal, rectal, conjunctival or pharyngeal swabs, or for detecting disseminated gonococcal infection (CDC, 2002; see also AAP, 2006; AAP, 2009).

Neisseria Meningitidis

Neisseria meningitidis is a gram-negative diplococcus with at least 13 serogroups based on capsule type. Strains belonging to groups A, B, C, Y, and W-135 are implicated most commonly in invasive disease worldwide. Invasive infection usually results in meningococcemia, meningitis, or both. According to current guidelines (AAP, 2009; CDC, 2009), cultures of blood and CSF are indicated for patients with suspected invasive meningococcal disease. Bacterial antigen detection in CSF supports the diagnosis of a probable case if the clinical illness is consistent with meningococcal disease. Polymerase chain reaction testing is available in some research and public health laboratories. A serogroup-specific PCR test to detect N meningitidis from clinical specimens is used routinely in the United Kingdom, where up to 56 % of cases are confirmed by PCR testing alone. This test particularly is usefu in patients who receive antimicrobial therapy before cultures are obtained.

Chancroid

Chancroid is a genital ulcer disease caused by the bacterium *Haemophilus ducreyi*. In the United States, chancroid usually occurs in discrete outbreaks, although the disease is endemic in some areas. The accuracy of clinical diagnosis varies due to the atypical presentation of the ulcer. According to the AAP (2006), the diagnosis of chancroid usually is made on the basis of clinical findings and the exclusion of other infections associated with genital ulcer disease, such as syphilis or HSV, or adenopathies, such as lymphogranuloma venereum. Direct

examination of clinical material by Gram stain may strongly suggest the diagnosis if large numbers of gram-negative coccobacilli, sometimes in "school of fish" patterns, are seen. Confirmation by recovery of *H. ducreyi* from a genital ulcer or lymph node aspirate is the more available alternative diagnostic test. The AAP (2009) notes that fluorescent monoclonal antibody stains and PCR assays can provide a specific diagnosis but are not available in most laboratories.

According to the Association for Genitourinary Medicine (AGUM) of the Medical Society for the Study of Venereal Disease (MSSVD) (2002), in addition to culture or direct examination of gram stain, *H. ducreyi* may be identified by detection of nucleic acid (DNA) by amplification techniques such as PCR techniques, using nested techniques.

According to the CDC, a culture for *H. ducreyi* should be performed in patients with genital ulcer disease from regions where *H. ducreyi* is prevalent (CDC, 2002). According to the WHO (1999), the sensitivity and specificity of culture is 56 to 90 % and 100 %, respectively; the WHO notes that the sensitivity of culture can only be estimated because there is no gold standard on which to base the diagnosis of chancroid. The resolved sensitivity of PCR using *H. ducreyi* compared to culture is 77 to 98 %, and the specificity is 98 to 100 % (WHO, 1999). Conversely, culture may be only 75 % sensitive relative to PCR. Yet, PCR may be negative in a number of culture-proven chancroid cases, owing to the presence of Taq polymerase inhibitors in the DNA preparations extracted from genital ulcer specimens.

To circumvent the many problems of positive diagnosis of chancroid, the CDC proposes that a "probable diagnosis", for both clinical and surveillance purposes, be made if the patient has one or more painful genital ulcers, and (a) no evidence of *T. pallidum* infection by dark field examination of ulcer exudates or by a serologic test for syphilis, and (b) the clinical presentation, appearance of the genital ulcers and regional lymphadenopathy, i present, is typical for chancroid and a test for HSV is negative (CDC, 2002).

Donovanosis

Donovanosis, or granuloma inguinale, is one cause of genital ulcer disease (WHO, 1999; AGM-MSSVD, 2002). The disease occurs rarely in the United States, although it is endemic in certain tropical and developing areas, including India; Papua, New Guinea; central Australia; and southern Africa. The disease is caused by *Klebsiella granulomatis* (previously known as *Calymmatobacterium granulomatis*), which can be seen in infected tissue as intracellular bacterial inclusions known as Donovan bodies but can not be cultured on artificial media. Diagnosis o donovanosis is made by direct visualization under microscope of Donovan bodies. Alternatively, a piece of clean granulation tissue is removed from the leading edge of the genital ulcer with a scalpel, smeared on a slide, and stained. According to the WHO (1999), the sensitivity and specificity of tissue microscopy or swab is 60 to 80 % and 100 %, respectively. The AAP (2009) notes that diagnosis by PCR assay and serologic testing is available on a research basis. The sensitivities and specificities of antibody detection and PCR have not been determined (WHO, 1999).

Babesiosis

Babesiosis is a tick-borne disease caused by hemoprotozoan parasites of the genus Babesia. While more than 100 species have been reported, only a few have been identified as causing human infections. *Babesia microti* and *Babesia divergens* have been identified in most human cases, but variants (considered different species) have been recently identified. Little is known about the occurrence of Babesia species in malaria-endemic areas where Babesia can easily be misdiagnosed as Plasmodium.

Worldwide, but little is known about the prevalence of Babesia in malaria-endemic countries, where misidentification as Plasmodium probably occurs. In the United States, *B. microti* is the agent most frequently identified (Northeast and Midwest).

Infectious Disease Society of America Lyme disease guidelines (Wormser, 2000) stated that the diagnosis of Babesiosis should be suspected in patients from areas where babesiosis is endemic who develop fever (especially

if fever is very high (greater than 37 degrees)) in the absence of erythema migrans after an *Ixodes* tick bite. Infection may also be suspected in patients who have residual symptoms after treatment for early Lyme disease.

According to the CDC Guidelines on Identification and Diagnosis of Parasites of Public Health Concern (2001), PCR testing has a limited role in the diagnosis of Babesia infection. Diagnosis of Babesia infection should be made by microscopy detection of parasites in patients' blood smears. However, indirect fluorescent antibody (IFA) tests are useful for detecting infected individuals with very low levels of parasitemia (such as asymptomatic blood donors in transfusion-associated cases), for diagnosis after infection is cleared by therapy, and for discrimination between *Plasmodium falciparum* and Babesia infection in patients whose blood smear examinations are inconclusive and whose travel histories cannot exclude either parasite.

According to the CDC, molecular techniques, such as PCR, are necessary only in limited situations, specifically:

> Where the morphologic characteristics observed on microscopic examination of blood smears do not allow an unambiguous differentiation between Babesia and Plasmodium.
> In research investigations of new Babesia variants (or species) observed in recent human infections in the U.S. and in Europe.

Malaise and Fatigue (including Chronic Fatigue Syndrome)

"Shotgun" testing for a variety of infectious etiologies in patients with symptoms of fatigue is not medically necessary or appropriate. Testing for specific individual infectious etiologies is only appropriate when the patient exhibits signs or symptoms suggestive of active infection with that virus. (See also Aetna CPB 0369 - Chronic Fatigue Syndrome.)

According to the CDC (2000), in clinical practice, no tests can be recommended for the specific purpose of diagnosing chronic fatigue syndrome. Tests should be directed toward confirming or excluding other possible clinical conditions.

In a statement on the "Theoretical and experimental tests" for chronic fatigue syndrome, the CDC (2000) states: "No diagnostic tests for infectious agents, such as Epstein-Barr virus, enteroviruses, retroviruses, human herpesvirus 6, Candida albicans, and Mycoplasma incognita, are diagnostic for CFS and as such should not be used (except to identify an illness that would exclude a CFS diagnosis, such as mononucleosis)."

A Clinical Practice Guideline from the Collège des médecins du Québec (1998) reached the same conclusion about the value of diagnostic testing for infectious agents in patients with chronic fatigue syndrome.

A review of the literature on the diagnosis of viruses in patients suspected of having chronic fatigue syndrome from Chronic Fatigue and Immune Dysfunction Syndrome (CFIDS) Association of America (2001) states that viral tests are only appropriate when a specific active viral infection is suspected based on clinical signs: "Because research has documented no clear association between a virus and CFIDS, testing patients for viral infection has limited us unless clinical signs indicate that an active viral infection may be present and requiring treatment. The results are also difficult to interpret, because the immune system in CFIDS may be up-regulated and latent viruses may not be fully suppressed. Tests to determine elevated antibody titers for EBV and other viruses are not considered diagnostic for CFIDS by most physicians, and are usually performed only when a specific viral infection is suspected as a cause of the patient's symptoms."

A review of diagnosis and management of chronic fatigue syndrome (2002) published in the journal *American Family Physician* explains that there is no clear evidence that chronic fatigue syndrome is caused by an infection: "Although a number of other viral pathogens (such as the Coxsackie virus, human herpes virus 6, cytomegalovirus measles, and the human T-cell lymphotropic virus [HTLV-II]) have also been implicated as etiologic agents for CFS, there is no consistent or conclusive data to suggest any causal relationships. It is now believed that CFS is

not specific to one pathogenic agent but could be a state of chronic immune activation, possibly of polyclonal activity of B-lymphocytes, initiated by a virus."

Regarding laboratory tests, the authors stated that "[l]aboratory tests should be limited to complete blood cell counts and tests specific for the patient's symptoms. For example, serologic and neurologic analyses for Lyme disease or multiple sclerosis need only be conducted if the patient presents with appropriate symptoms."

Thus, panels of PCR tests to detect various infections are not indicated in patients with symptoms suggestive of chronic fatigue syndrome. It is only appropriate for individual tests to be selected to detect particular infectious agents if the patient's clinical presentation suggests active infection with that infectious agent.

Genital Ulcer Diseases

According to guidelines from the CDC (2002), a diagnosis of genital ulcer disease based only on the patient's medical history and physical examination often is inaccurate. Therefore, evaluation of all patients who have genita ulcers should include a serologic test for syphilis and a diagnostic evaluation for genital herpes; in settings where chancroid is prevalent, a test for *Haemophilus ducreyi* should also be performed.

According to the CDC, specific tests for evaluation of genital ulcers include:

Serology, and either darkfield examination or direct immunofluorescence test for *T. pallidum*;
Culture or antigen test for herpes simplex virus (HSV); and
Culture for *H. ducreyi*.

According to CDC guidelines, no FDA-approved PCR test for these organisms is available in the United States, bu such testing can be performed by commercial laboratories that have developed their own PCR tests.

Herpes Simplex Virus

Herpes simplex virus (HSV) is one of the major causes of genital ulcer disease. Primary infection is followed by latency and variable periods of reactivation. Although clinical diagnosis may be accurate if based on the presence of typical vesicles, up to 2/3 of individuals acquire HSV asymptomatically, and most infected persons shed virus during latent periods. Laboratory diagnosis is necessary to detect HSV in asymptomatically infected people to prevent transmission to sexual partners and to children born to infected mothers.

The Association for Genitourinary Medicine and the Medical Society for the Study of Venereal Diseases (2002) stated that the clinical utility of HSV serologic tests has not been fully assessed, and that virus detection remains the method of choice. According to the CDC's current guidelines for diagnosing HSV infection, type-specific serology for HSV type 2 may be helpful in identifying persons with genital herpes (CDC, 2002). Biopsy of ulcers may be helpful in identifying the cause of unusual ulcers or ulcers that do not respond to initial therapy.

The British Association for Sexual Health and HIV (BASHH, 2007) recommends real-time PCR as the preferred diagnostic method for genital herpes. BASHH guidelines stated that HSV DNA detection by PCR increases HSV detection rates by 11 % to 71 % compared with virus culture. The guidelines state that PCR-based methods allow less stringent conditions for sample storage and transport than virus culture and new real-time PCR assays are rapid and highly specific.

According to guidelines from the AAP Committee on Infectious Diseases (AAP, 2009), a PCR assay often can detect HSV DNA in CSF from patients with CNS infection during the neonatal period (neonatal HSV CNS disease) and with herpes simplex encephalitis in older children and adults and is the diagnostic method of choice for CNS HSV involvement. The AAP (2009) also stated that blood PCR may be of benefit in the diagnosis of neonatal HSV disease, but its use should not supplant the standard work-up of such patients (which includes surface cultures and CSF PCR).

Current guidelines indicate no role for quantification of HSV viral load in the diagnosis or management of herpes simplex virus infection (AAP, 2009; CDC, 2002). The AAP (2009) stated that there is no role for serial blood PCR in monitoring response to therapy.

Human Immunodeficiency Virus (HIV)

Most individuals can be diagnosed as infected with human immunodeficiency virus (HIV) based on the detection of HIV specific IgG antibodies (WHO, 1999). For detection of early infection before seroconversion occurs, or to detect HIV infection in neonates, assays that detect HIV p24 protein or HIV DNA or RNA are used. Quantitative HIV RNA assays are not necessary for diagnosis of infection but are useful for monitoring treatment.

The laboratory diagnosis of HIV infection during infancy depends on detection of virus or virus nucleic acid. The transplacental transfer of antibody complicates the serologic diagnosis of infant infection. According to the AAP (2006),HIV nucleic acid detection by PCR of DNA extracted from peripheral blood mononuclear cells is the preferred test for diagnosis of HIV infection in infants.

Plasma HIV RNA PCR may be used to diagnose HIV infection if the result is positive. However, this test result may be negative in HIV-infected persons. The test is licensed by the Food and Drug Administration only in quantitative format and, according to the AAP (2006), currently is used for quantifying the amount of virus present as a measurement of disease progression, not for diagnosis of HIV infection in infants.

According to the AAP (2006), enzyme immunoassays (EIAs) are used most widely as the initial test for serum HIV antibody. These tests are highly sensitive and specific. Repeated EIA testing of initially reactive specimens is required to reduce the small likelihood of laboratory error. Western blot or immunofluorescent antibody tests should be used for confirmation, which will overcome the problem of a false-positive EIA result.

Lyme Disease

According to available evidence-based guidelines, PCR has not been validated for either the diagnosis of Lyme disease or monitoring response to therapy.

American College of Physicians-American Society of Internal Medicine guidelines (1997) stated that PCR of serum or cerebrospinal fluid "need[s] further validation" and that "[p]ublished experience with these techniques [PCR] is insufficient to allow development of guidelines for their use."

The CDC (2001) stated that "PCR has not been standardized for routine diagnosis of Lyme Disease."

The National Institute of Arthritis and Infectious Disease (2001) has explained the reasons why PCR has limited utility in the diagnosis of Lyme disease: "To be sure, the polymerase chain reaction (PCR) is an extremely sensitive laboratory test that is capable of detecting very few molecules of bacterial DNA. However, the numbers of Borrelia likely to be present -- if at all -- in patients suspected of having Lyme disease are too small to generate sufficient amounts of bacterial DNA to be detected by this procedure."

The AAP's Committee on Infectious Diseases (2003) stated: "New, more sensitive and more specific diagnostic tests such as the polymerase chain reaction assay, which may be able to identify the presence of even small quantities of spirochetal DNA, are in development. However, physicians should be cautious when interpreting the results of these investigational tests until their clinical usefulness has been proven."

The American Lyme Disease Foundation (2002) stated: "The polymerase chain reaction (PCR) test is a very sensitive assay that detects the DNA of *B. burgdorferi*. However, certain limitations prevent the PCR from being widely used. First, *B. burgdorferi* bacteria do not persist in easily obtainable fluids such as blood, synovial (joint) fluid or spinal fluid, but typically bind to joint and nerve tissues. A PCR done on spinal fluid may be positive in early neurologic disease (e.g., Lyme meningitis) but is usually negative in a patient with long-term central nervous

system damage. Second, a PCR can be easily contaminated, producing false positive results. For this reason, a positive PCR in a patient whose standard blood tests (ELISA and Western blot) are negative must be viewed with skepticism."

Puotinen et al (2002) concluded that "[t]he Lyme multiplex polymerase chain reaction (PCR) has not been standardized; therefore, it is not employed currently in routine testing." Edlow (2001) explained that PCR remains a research technique, in part because labs performing PCR tests must be meticulous in technique to minimize the likelihood of false-positive results". In addition, Edlow explained, "no large clinical series have been reported that assess the performance of the test in the non-research setting."

The AAP (2006) stated that PCR tests for spirochete DNA have no role in diagnosis of Lyme disease. The AAP (2009) noted that PCR testing "has been used in to detect *B. burgdorferi* DNA in joint fluid", but make no specific recommendation for its use.

Thus, the clinical utility of PCR in the diagnosis or monitoring of Lyme disease has not been established. In addition, current guidelines do not indicate any role for PCR quantification of spirochete load in the diagnosis or management of patients with Lyme disease.

Syphilis

Syphilis, a chronic infection with clinical manifestations occurring in distinct states, is caused by the spirochete *Treponema pallidum*. Diagnostic studies for syphilis include a targeted clinical history and physical examination, serologic tests, investigations of sexual contacts and, if appropriate, darkfield microscopic examination of fluids from lesions, CSF tests, and radiologic examination (CDC, 2002; Beer and Berkow, 1999).

According to the AAP, the non-treponemal antibody tests (VDRL, RPR, and ART) are useful for screening; the treponemal tests (FTA-ABS and MHA-TP) are used to establish a presumptive diagnosis (AAP, 2006). Quantitative non-treponemal antibody tests are used to assess the adequacy of therapy and to detect re-infection and relapse. Most current guidelines indicate no specific role for PCR testing in the screening or diagnosis of syphilis (AAP, 2006; CDC, 2002; AAP, 2009). The Association for Genitourinary Medicine and the Medical Society for the Study of Venereal Diseases (2002) suggested that either the direct fluorescent antibody test or the PCR tes may be useful for used for oral or other lesions where contamination with commensal treponemes is likely.

Two classes of serologic tests for syphilis (STS) aid in diagnosing syphilis and other related treponemal diseases:

> Screening, non-treponemal tests using lipoid antigens detect syphilitic reagin and include the Venereal Disease Research Laboratory (VDRL) and the rapid plasma reagin (RPR) tests.
> Specific treponemal tests detect anti-treponemal antibodies and include fluorescent treponemal antibody absorption (FTA-ABS) test, microhemagglutination assay for antibodies to T. pallidum (MHA-TP), and Treponema pallidum hemagglutination assay (TPHA).

The VDRL test is a flocculation test for syphilis in which reagin antibody in the patient's serum reacts visibly with cardiolipin, the antigen. Reactive and weakly reactive VDRL tests are considered positive for *T. pallidum*, and should be confirmed by one of the more specific treponemal tests, and the reactive tests should be quantitated by serial dilution.

Before treatment (except in infections of less than 1 yr), CSF examination is recommended to exclude neurosyphilis. The cell count and differential and total protein are usually measured, and VDRL or other nonspecific (reagin) serologic tests performed. Treponemal tests of CSF are not helpful.

Human Papillomavirus (HPV)

Human papillomaviruses (HPVs) produce epithelial tumors (warts) of the skin and mucous membranes (AAP, 2006). Cutaneous non-genital warts include common skin warts, plantar warts, flat warts, thread-like (filiform)

warts, and epidermodysplasia verruciformis. Those affecting the mucous membranes include anogenital, oral, nasal, and conjunctival warts, as well as respiratory papillomatosis.

More than 100 types of HPV exist, more than 40 of which can infect the genital area (Workowski et al, 2010). Mos HPV infections are asymptomatic, unrecognized, or subclinical. Oncogenic, or high-risk HPV types (e.g., HPV types 16 and 18), are the cause of cervical cancers. These HPV types are also associated with other anogenital cancers in men and women, including penile, vulvar, vaginal, and anal cancer, as well as subset of oropharyngeal cancers. Non-oncogenic, or low-risk HPV types (e.g., HPV types 6 and 11), are the cause of genital warts and recurrent respiratory papillomatosis.

Most cutaneous and anogenital warts are diagnosed by clinical inspection. Detection of cervical HPV infection may be enhanced by use of colposcopy with application of acetic acid (vinegar), which causes the lesion to turn white. This characteristic, however, is not specific for HPV infection, and false-positive test results are common. When the diagnosis is questionable, histologic examination of a biopsy specimen can be diagnostic.

Human papillomavirus can not be cultured. According to the American College of Obstetricians and Gynecologists (ACOG, 2002), the Hybrid Capture II is FDA-approved for HPV DNA. This test uses nucleic acid amplification and hybridization to assess exfoliated cervical cells for the presence of one or more of 13 high- and intermediate-risk HPV types. Although this test appears to be very sensitive, rare cross-reactivity with low-risk HPV types and HPV types of undetermined significance has been reported.

According to the CDC (Workowski et al, 2010), HPV tests are available for women aged greater than 30 years undergoing cervical cancer screening. These tests should not be used for men, for women less than 20 years of age, or as a general test for sexually transmitted diseases. These HPV tests detect viral nucleic acid (i.e., DNA or RNA) or capsid protein. Four tests have been approved by the FDA for use in the United States: the HC II High-Risk HPV test (Qiagen), HC II Low-Risk HPV test (Qiagen), Cervista HPV 16/18 test, and Cervista HPV High-Risk test (Hologics). Since the updated CDC guidelines were published, an additional test, the Roche cobas 4800 HPV test was approved by the FDA; this PCR test identifies genotypes 16 and 18 plus 12 other high-risk genotypes.

Given the prevalence of HPV infection, the clinical benefit of testing for the presence of HPV, other than as an adjunct to cancer screening, is of unknown clinical benefit. According to the CDC, "[n]o data support the use of type-specific HPV nucleic acid tests in the routine diagnosis or management of visible genital warts." The Advisory Committee on Immunization Practices does not recommend HPV testing to select persons for HPV vaccination. The AAP (2009) explained that testing for HPV types is used in combination with Pap test to determine whether patients need to be sent for colposcopy; otherwise, screening for clinically inapparent HPV infection or evaluating anogenital warts using HPV DNA or RNA tests is not recommended.

The National Cancer Institute's (2002) interim guidelines for managing abnormal cervical cytology and the American Society of Colposcopy and Cervical Pathology recommend human papilloma virus (HPV) DNA testing fo women with ASCUS (atypical cervical squamous cells of undetermined significance) using the a hybridization assay. The hybridization assay can distinguish low-risk HPV (not usually found in pre-cancerous lesions) from high-risk HPV (found in pre-cancerous and cancerous lesions) in ASCUS lesions. According to the CDC, typing HPV DNA is not useful in types of cervical abnormalities other than ASCUS lesions. See Aetna CPB 359 -- HPV Testing in Cervical Cancer Screening. The CDC (2002) noted that screening for subclinical genital HPV infection using DNA or RNA tests is not recommended.

Ehrlichiosis

Ehrlichiosis is a febrile illness resembling Rocky Mountain spotted fever caused by rickettsial-like bacteria of the genus Ehrlichia and transmitted to humans by ticks (Merck Manual). Most cases have been identified in the southeastern and south-central parts of the United States. Two species of Ehrlichia are human pathogens in the

United States: *E. chaffeensis* causes human monocytic ehrlichiosis and *E. phagocytophilia* or a related organism causes human granulocytic ehrlichiosis. *E. canis* is now regarded as human monocytic ehrlichiosis.

Regardless of the species causing the infection, the symptoms and signs are similar. Although some infections are asymptomatic, most cause an abrupt onset of illness with fever, chills, headache, and malaise, usually beginning about 12 days after the tick bite. Some patients develop a maculopapular or petechial rash involving the trunk and extremities, although rash is rare with *E. canis*. Abdominal pain, vomiting and diarrhea, disseminated intravascula coagulation, seizures, and coma may occur. Hematologic and hepatic abnormalities include leukopenia, thrombocytopenia, and abnormal liver function tests, especially elevated levels of transaminases.

According to guidelines from the Infectious Disease Society of America, ehrlichiosis may be suspected in patients with Lyme disease symptoms who have a very high fever (greater than 38 degrees C) (Wormser, 2000), or patients who develop fever in the absence of erythema migrans after an *Ixodes* tick bite in areas where these infections are endemic.

The CDC defines a confirmed case of ehrlichiosis as a 4-fold or greater change in antibody titer by IFA between acute and convalescent serum samples (ideally collected 3 to 6 weeks apart), PCR amplification of ehrlichial DNA from a clinical sample, or detection of intraleukocytoplasmic Ehrlichia microcolonies (morulae) and a single IFA tite of more than 64. A probable case is defined as a single IFA titer of more than 64 or the presence of morulae withi infected leukocytes. Examination of peripheral blood smears to detect morulae in peripheral blood monocytes or granulocytes is insensitive, but this test is warranted for patients for whom a high index of suspicion exists. The PCR test is useful in detecting erlichiosis in the acute phase before immunohistochemical tests are positive (AAP, 2006). The use of the PCR test to amplify nucleic acid from acute phase peripheral blood of patients with ehrlichiosis seems sensitive, specific, and promising for early diagnosis but currently is unstandardized and is available only in research laboratories and at the CDC (AAP, 2006; CDC, 2000; AAP, 2009).

Doxycycline is the drug of choice for treatment of human ehrlichiosis and is also effective against Lyme disease. Ehrlichiosis may be severe or fatal in untreated patients, and initiation of therapy early in the course of the disease helps minimize complications of the illness.

Polymerase chain reaction testing is also recommended for diagnosis of human granulocytic anaplasmosis (*Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophilum*)) (AAP, 2009).

Malaria

Malaria is infection with any of 4 different species of Plasmodia, causing periodic paroxysms of chills, fever and sweating, anemia, and splenomegaly. Malaria is endemic in Africa, much of South and Southeast Asia, Central America, and northern South America. Malaria once was endemic in the United States but has been virtually eliminated from North America. The 4 important Plasmodium species are *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*.

P. falciparum infection is a medical emergency. Recurrent attacks of chills and fever without apparent cause should always suggest malaria, particularly if the patient has been in an endemic area within 3 to 5 yrs, has an enlarged spleen, or has been recently transfused. Finding Plasmodium in a blood smear is diagnostic. The infecting species must be identified, since this influences therapy and prognosis.

According to the CDC (2010), parasite nucleic acids are detected using PCR. The CDC states that, although this technique may be slightly more sensitive than smear microscopy, it is of limited utility for the diagnosis of acutely ill patients in the standard healthcare setting. Polymerase chain reaction results are often not available quickly enough to be of value in establishing the diagnosis of malaria infection. It is most useful for confirming the species of malarial parasite after the diagnosis has been established by either smear microscopy or rapid diagnostic test.

Genital Mycoplasma Infections: Ureaplasma urealyticum and Mycoplasma hominis

Because detection of mycoplasma or ureaplasma is currently impractical, guidelines from the AAP and CDC recommend performing diagnostic tests for mycoplasmas and ureaplasmas when a patient presents with a clinical condition known to be caused by or associated with these organisms and when more common etiologies are excluded (AAP, 2006; CDC, 2002). The correct microbiological diagnosis takes on greater importance in patients who are immunosuppressed and at greater risk for disseminated infection with a poor outcome.

The standard method of diagnosing genital mycoplasma infections is by cell culture. Specialized culture media and growth conditions are necessary. Both *M. hominis* and *U. urealyticum* can be detected in culture within 2 to 5 days. According to the CDC, serologic studies are not useful for evaluating genital mycoplasma infections (CDC, 2002). Molecular techniques such as PCR are not needed when culture is available for *M. hominis* and *Ureaplasma* species. The AAP (2009) noted that sensitive PCR methods for *U. urealyticum* have been developed but are not available routinely.

Mycoplasma genitalium has been identified as a potential cause of nongonococcal urethritis and a possible cause of genital tract inflammation in women. Current guidelines indicate that the place of diagnostic testing for *Mycoplasma genitalium* has not been established (BASHH, 2007).

Evidence is emerging on other mycoplasma organisms, including *Mycoplasma fermentans* (see below) and *Mycoplasma penetrans*, and their possible roles in certain pathologic conditions in humans. Although PCR testing can be used to detect these organisms, relatively little is known about their pathogenic importance.

Helicobacter Pylori

Current guidelines do not provide any indication for PCR testing in the diagnosis of *Helicobacter pylori*. To establish the presence of *H. pylori*, tests for antibody to *H. pylori* in blood are greater than 90 % specific and sensitive. Other tests include a urea breath test or rapid urease test, and histology of antral biopsies obtained at endoscopy.

Helicobacter pylori infection can be diagnosed by culture of gastric biopsy tissue (AAP 2006). Organisms usually can be visualized on histologic sections using special stains. Because of production of urease by the organisms, urease testing of a gastric specimen can give a rapid and specific microbiologic diagnosis. Each of these tests requires endoscopy and biopsy. Non-invasive, commercially available tests include the breath test, which detects labeled carbon dioxide in expired air after oral administration of isotopically labeled urea, and serology for the presence of immunoglobulin G to *H. pylori*. According to the AAP (2006), each of the diagnostic tests has a sensitivity and specificity of 95 % or more. A stool antigen test is also available commercially.

Mucosa-Associated Lymphoid Tissue (MALT) Lymphomas and Marginal Zone Lymphomas

Mucosa-Associated Lymphoid Tissue lymphomas have recently been reclassified as extra-nodal marginal-zone lymphomas of MALT-type. The most common and well-studied MALT lymphoma is gastric MALToma. This neoplasm is intimately associated with *H. pylori*, with the organism being present in more than 90 % of pathologic specimens of MALTomas. This etiologic factor serves as the basis for treatment. Treatment of gastrointestinal MALT lymphomas is with antibiotics designed to eradicate *H. pylori*.

Guidelines from the National Comprehensive Cancer Network (NCCN, 2003) on non-Hodgkin's lymphoma indicated that PCR testing is useful in evaluating individuals with MALT lymphomas and marginal zone lymphomas who have non-diagnostic atypical lymphoid infiltrates that are positive for *H. pylori* infection. According to the NCCN guidelines, detection by PCR of a t(11;18) gene rearrangement, a specific translocation of genes 11 and 18 in these persons predicts no response to antibiotic therapy for *H. pylori* infection, and alternative treatment should be considered

Trichomoniasis

Trichomoniasis is the term for infection of the vagina or male genital tract with *Trichomonas vaginalis* (Beers and Berkow, 1999). *T. vaginalis* is a flagellated protozoan found in the GU tract of both men and women. The organism is more common in women, affecting about 20 % during the reproductive years and causing vaginitis, urethritis, and possibly cystitis. *T. vaginalis* is more difficult to detect in men; probably causes prostatitis and cystitis; and may account for 5 to 10 % of all cases of male urethritis in some areas. Asymptomatic infected men often infect their sex partners. The infection may co-exist with gonorrhea and other sexually transmitted diseases.

The diagnosis of *T. vaginalis* in women is discussed above in the section above on vaginal discharge. In women, an immediate diagnosis of trichomoniasis can usually be made by examining vaginal secretions under microscopy (wet mount). The lashing movements of the flagella and striking motility of the oval-shaped organisms are readily observed. Cultures and antibody tests using an enzyme immunoassay and immunofluorescence techniques for demonstration of the organism are more sensitive and specific than direct examination, but according to the AAP, are generally not required for the diagnosis. Trichomoniasis is also commonly diagnosed on a Papanicolaou smear.

T. vaginalis may sometimes cause non-gonococcal urethritis in men. According to the CDC (2002), diagnostic procedures for *T. vaginalis* reserved for situations in which these infection is suspected (e.g., contact with trichomoniasis and genital lesions suggestive of genital herpes) or when non-gonococcal urethritis is not responsive to therapy. In men, an immediate diagnosis of *T. vaginalis* can be made by examining a wet mount of urethral secretions and by culture. Examining the centrifuged sediment of urine and prostatic secretions may also be helpful.

The CDC guidelines indicated no role for PCR testing in the diagnosis of *T. vaginalis* (CDC, 2002; CDC, 2006). The AAP (2006) noted that an FDA-licensed PCR test for *T. vaginalis* is not available in the United States but may be available as a research diagnostic test or from commercial laboratories. Other guidelines indicated no role for PCR testing for this indication (ACOG, 2006; BASHH, 2007; AAP, 2009).

Rocky Mountain Spotted Fever

Rocky Mountain spotted fever (RMSF) is an acute febrile disease caused by *Rickettsia rickettsii* and transmitted by ixodid ticks, producing high fever, cough, and rash (Beers and Berkow, 1999). Rocky Mountain spotted fever is limited to the Western Hemisphere. Initially recognized in the Rocky Mountain states, it occurs in practically all states (except Maine, Hawaii, and Alaska) in the United States, especially the Atlantic states. In humans, infection occurs mainly from May to September, when adult ticks are active and persons are most likely to be in tick-infeste areas. In southern states, cases occur throughout the year. The incidence is high in children less than 15 years of age and in others who frequent tick-infested areas for work or recreation.

Serologic tests, isolation and identification of *Rickettsia rickettsii* from blood or tissues, and identification of the agent in skin or other tissues by immunofluorescence help confirm the diagnosis, particularly in RMSF. To be useful, serologic tests require 3 serum samples, taken during the 1st, 2nd, and 4th to 6th weeks of illness. Polymerase chain reaction is useful in early identification of specific rickettsial nucleic acids.

According to the AAP (2006), the diagnosis of RMSF can be established by one of the multiple rickettsial group-specific serologic tests. A 4-fold or greater change in titer between acute- and convalescent-phase serum specimens is diagnostic when determined by IFA, enzyme immunoassay (EIA), complement fixation (CF), latex agglutination (LA), indirect hemagglutination (IHA), or microagglutination (MA) tests. The IFA is the most widely available confirmatory test. Antibodies are detected by IFA 7 to 10 days after onset of illness. According to the AAP, the non-specific and insensitive Weil-Felix serologic test (Proteus vulgaris OX-19 and OX-2 agglutinins) is no recommended (AAP, 2006).

The AAP guidelines state that culture of *R. rickettsii* usually is not attempted because of the danger of transmission to laboratory personnel (AAP, 2009). *Rickettsia rickettsii* have been identified by immunofluorescent staining or

PCR testing of tissue specimens. The AAP guidelines stated that PCR for detection of *R. rickettsii* in blood and biopsy specimens during the acute phase of the illness confirms the diagnosis and is available from CDC reference laboratories.

Colorado Tick Fever

Colorado tick fever is an acute viral infection transmitted from the bite of an infected wood tick. The disease is found almost exclusively in the western United States and Canada, mostly in high mountain areas such as Colorado and Idaho. Colorado tick fever can be confirmed by measurement of virus-specific antibody in serum or CSF. The assay, with complement fixation or immunofluorescent techniques, must be performed in a laboratory with experience in performing this test. Serologic tests are often not positive for 10 to 14 days after symptom onset. In comparison, diagnostic PCR may be positive from the first day of symptoms (AAP, 2009).

Mosquito-Borne Arboviruses

Mosquito-borne arboviruses are viral diseases that are spread to humans through the bite of infected mosquitos. These viruses do not normally infect humans but if they do, they usually cause a mild infection such as a fever or a rash. Others however are epidemic and can cause serious infections such as meningitis and encephalitis. St. Louis encephalitis is found throughout much of the United States, as well as parts of Canada, the Caribbean, and South America. Eastern equine encephalitis virus is a rare illness in humans, and only a few cases are reported in the United States each year. Most cases occur in the Atlantic and Gulf Coast states. Western equine encephalitis virus is a mosquito-borne virus closely related to eastern and Venezuelan equine encephalitis viruses, and is found mainly in the plains regions of the western and central United States. Most cases of La Crosse encephalitis virus disease occur in the upper Midwestern and mid-Atlantic and southeastern states.

According to the CDC (2007), a presumptive diagnosis of an arboviral disease is often based on the patient's clinical features, places and dates of travel (if the patient is from a non-endemic country or area), activities, and epidemiologic history of the location where infection occurred. Laboratory diagnosis of arboviral infections is generally accomplished by testing of serum or CSF to detect virus-specific IgM and neutralizing antibodies. According to the CDC, in fatal cases, nucleic acid amplification [PCR], histopathology with immunohistochemistry, and virus culture of biopsy or autopsy tissues can also be useful. Only a few state laboratories or other specialize laboratories, including those at CDC, are capable of doing this specialized testing.

Chlamydophila Pneumoniae

Chlamydophila (formerly Chlamydia) pneumoniae is a species of Chlamydia that is antigenically, genetically, and morphologically distinct from Chlamydia species. *C. pneumoniae* has been found in 5 to 10 % of older adults with community-acquired pneumonia and often produces disease severe enough to require hospitalization. This organism has also been implicated in 5 to 10 % of cases of nosocomial pneumonia, but relatively little is known about its epidemiology. Transmission of *C. pneumoniae* is presumably by respiratory aerosol transmission between humans.

Clinical features of *C. pneumoniae* resemble those of mycoplasmal pneumonia, including pharyngitis, bronchitis, and pneumonitis, primarily in older children and young adults. Most patients have cough, fever, and sputum production but are not seriously ill.

In addition to acute respiratory tract disease, some investigators have associated *C. pneumonia*e with atherosclerotic cardiovascular disease. This association is based on the increased frequency of serum antibodies in patients compared with controls, the detection of antigen or DNA in atheromatous plaques, the production of arterial lesions in experimentally infected animals, and small human trials demonstrating that treatment of high-risk patients with macrolides decreases the risk of subsequent cardiovascular events. According to the AAP, large, prospective, randomized trials are underway to further explore this association and to determine whether treatment is beneficial (AAP, 2003). Other investigators have associated *C. pneumoniae* with asthma, Alzheimer disease,

multiple sclerosis, and Kawasaki disease, but the AAP has concluded that the evidence supporting any of these associations is limited.

C. pneumoniae may be detected by cultivating it in embryonated egg cultures (as with other chlamydiae), by using direct stains with immunofluorescence or a polymerase chain reaction, or by using serial serologic tests to show seroconversion. However, these tests are usually unavailable in most clinical laboratories. The diagnosis is suspected in a patient who has typical symptoms, has no established alternative diagnosis, and does not respond to beta-lactam antibiotics. *C. pneumoniae* may be treated with erythromycin or tetracycline.

Polymerase chain reaction is not available routinely but may be used to established a probable diagnosis of psittacosis and distinguish *Chlamydophila psittaci* from other chlamydial infections (AAP, 2009; NASPHV, 2010).

Cytomegalovirus

Cytomegalovirus (CMV) causes various infections, occurring congenitally, post-natally, or at any age, ranging from inconsequential silent infection to disease manifested by fever, hepatitis, pneumonitis, and, in newborns, severe brain damage, stillbirth, or perinatal death.

Especially in the immunocompromised host, CMV may be isolated from urine, other body fluids, or tissues. However, CMV can be excreted for months or years after infection without causing active disease, and a positive CMV culture must be interpreted with regard to the particular host and disease manifestation. Examination of cells shed in urine for intranuclear inclusions is an insensitive test. Biopsy showing CMV-induced pathology is often important in demonstrating invasive disease.

Recovery of virus from a target organ provides unequivocal evidence that the disease is caused by CMV infection. However, according to the AAP (2006), a presumptive diagnosis can be made on the basis of a 4-fold antibody tite rise in paired serum samples or by demonstration of virus excretion.

Complement fixation is the least sensitive serologic method for diagnosis of CMV infection and should not be used to establish previous infection or passively acquired maternal antibody. Various immunofluorescence assays, indirect hemagglutination, latex agglutination, and enzyme immunoassays are preferred for this purpose.

Techniques for detection of viral DNA in tissues and some fluids, especially CSF, by PCR or hybridization are available from reference laboratories. Detection of pp65 antigen in white blood cells is used to detect infection in immunocompromised hosts.

Cytomegalovirus viral load tests using quantitative PCR are used to monitor disease progression.

Recently updated guidelines from the AAP Committee on Infectious Diseases (2009) commented on the use of PCR testing to detected intrauterine CMV infection: "Amniocentesis has been used in several small series of patients to establish the diagnosis of intrauterine infection. Proof of congenital infection requires isolation of CMV from urine, stool, respiratory tract secretions, or CSF obtained within 2 to 4 weeks of birth. Recent findings suggest that congenital CMV infection also might be diagnosed by using polymerase chain reaction assay to detec CMV DNA in newborn dried blood spots, although additional work is needed to define the sensitivity and feasibility of this approach. Differentiation between intrauterine and perinatal infection is difficult later in infancy unless clinical manifestations of the former, such as chorioretinitis or intracranial calcifications, are present. A strongly positive CMV-specific IgM is suggestive during early infancy, but IgM antibody assays vary in accuracy for identification of primary infection".

Pneumocystis Pneumonia

Pneumocystis jiroveci (formerly *P. carinii*), now considered a fungus rather than a protozoan, causes disease only when defenses are compromised, most commonly when there are defects in cell-mediated immunity as in hematologic malignancies, lymphoproliferative diseases, cancer chemotherapy, and AIDS (Beers and Berkow,

1999). About 30 % of patients with HIV infection have *P. jiroveci* pneumonia as the initial AIDS-defining diagnosis, and geater than 80 % of AIDS patients have this infection at some time if prophylaxis is not given. Patients with HIV infection become vulnerable to *P. jiroveci* pneumonia when the CD4 count is less than 200/µL. Most patients have fever, dyspnea, and a dry, non-productive cough that may evolve subacutely over several weeks or acutely over several days.

A definitive diagnosis of PCP is made by demonstration of organisms in lung tissue or respiratory tract secretions (AAP, 2006). The most sensitive and specific diagnostic procedures have been open lung biopsy and transbronchial biopsy. However, bronchoscopy with bronchoalveolar lavage, induction of sputum in older children and adolescents, and intubation with deep endotracheal aspiration are less invasive and often diagnostic and have been sufficiently sensitive in patients with HIV infection who have an increased number of organisms compared with non-HIV-infected patients with PCP. According to the AAP (2009), "polymerase chain reaction assays for detecting *P. jiroveci* infection are experimental and are not approved by the U.S. Food and Drug Administration for diagnosis." The AAP notes that serologic tests are not useful. Guidelines from Cincinnati Children's Hospital Medical Center (2001) stated that PCR testing for *P. jiroveci* infection is investigational. Other guidelines indicate no role for PCR testing for this indication (NYSDOH, 2006).

Haemophilus influenzae

Haemophilus influenzae type b (Hib) causes pneumonia, occult febrile bacteremia, meningitis, epiglottitis, septic arthritis, cellulitis, otitis media, and purulent pericarditis. The mode of transmission is person to person by inhalation of respiratory tract droplets or by direct contact with respiratory tract secretions. According to current guidelines for diagnosis (AAP, 2009), CSF, blood, synovial fluid, pleural fluid, and middle-ear aspirates should be cultured on a specialized medium such as chocolate agar. Gram stain of an infected body fluid specimen can facilitate presumptive diagnosis. Latex particle agglutination for detection of type b capsular antigen in CSF can b helpful, but a negative test result does not exclude the diagnosis, and false-positive results have been recorded. All *H. influenzae* isolates associated with an invasive infection should be serotyped.

Human Herpesvirus Type 6

Human herpesvirus 6 (HHV-6) is a T-cell lymphotrophic virus with high affinity for CD4 lymphocytes. HHV-6 has 2 variants, A and B. Variant B causes the childhood illness roseola infantum, while variant A has been isolated mainly from immunocompromised hosts. The disease manifestations of variant A still are undefined, but both variants may turn out to be pathogenic in the settings of transplantation and AIDS.

Primary HHV-6 infection usually occurs in infants and is the most common cause of febrile-induced seizures in children aged 6 to 24 months. Acute infection in immunocompetent adults is rare but may present as a mononucleosis-like illness with fever, lymphadenopathy, hepatitis or encephalitis, and negative test results for CMV or Epstein-Barr virus (EBV).

Infection remains latent in lymphocytes and monocytes and can persist in some tissues at low levels. In the immunocompetent host, this persistent infection generally is of no consequence. In the immunosuppressed host, HHV-6 may be associated with opportunistic disease. Whether this represents reactivation of latent infection or superinfection is unclear.

In transplant patients, HHV-6 infection has been linked with infection/reactivation and increased severity of CMV disease. In HIV patients, HHV-6 infection may up-regulate HIV replication and hasten the progression towards AIDS. HHV-6 also has been implicated in the pathogenesis of white matter demyelination in AIDS dementia complex. Causality has yet to be demonstrated clearly.

HHV-6 infection often is asymptomatic. Symptomatic disease occurs predominately after primary infection in infants and after either primary or reactivation disease in immunocompromised adults.

An Expert Working Group convened by the Health Canada Laboratory Centre for Disease Control (LCDC, 2000) concluded that the most appropriate clinical scenarios in which HHV-6 laboratory diagnosis may be indicated appear to be: a) primary infection in febrile children less than 3 years of age; b) primary infection or viral reactivation in immunocompromised individuals such as AIDS patients or transplant patients; and c) mononucleosis-like syndrome in patients without heterophile antibodies or antibodies specific to EBV.

The diagnosis of primary HHV-6 infection currently necessitates use of research techniques to isolate the virus from a peripheral blood specimen (AAP, 2006). A 4-fold increase in serum antibody alone does not necessarily indicate new infection, as an increase in titer also may occur with reactivation and in association with other infections. However, sero-conversion from negative to positive in paired sera is good evidence of recent primary infection. The LCDC Expert Working Group (2000) concluded that serologic and PCR tests have been developed to diagnose an active or recent HHV-6 infection, "further evaluation in the clinical context (specificity, sensitivity, predictive values) needs to be done to improve confidence in and reliability of HHV-6 laboratory testing." Guidelines from the AAP (2009) stated that PCR tests for HHV-6 are available in reference laboratories. However, chromosomal integration of HHV-6 DNA always will result in a positive PCR test result with a high viral load, potentially confounding the interpretation of a positive test result. Chromosomal integration has been reported in 0.8 % of adult blood donors. The diagnosis of active HHV-6 infection should not be made without first excluding chromosomal integration by measuring DNA load in serum or CSF and comparing it with the DNA load in whole blood.

According to the AAP (2009), treatment for HHV-6 infection is supportive. Therapy usually is unnecessary with primary infection of immunocompetent patients.

Therefore, HHV-6 testing in immunocompetent individuals is not necessary. For immunocompromised patients with serious HHV-6 disease, some experts recommend a course of ganciclovir. Therefore, HHV-6 testing may be necessary in immunocompromised patients such as AIDS patients and transplant recipients. In addition, HHV-6 testing may be necessary to rule out other potential diagnoses in patients presenting with a mononucleosis-like illness with fever, lymphadenopathy, hepatitis or encephalitis, and negative test results for CMV or EBV.

Human Herpesvirus Type 7

According to the AAP (2006), recognition of the varied clinical manifestations of human herpesvirus 7 (HHV-7) infection is evolving. Many, if not most, primary infections with HHV-7 may be asymptomatic or mild; some may present as typical roseola and may account for second or recurrent cases of roseola. Febrile illnesses associated with seizures also have been reported. Some investigators suggest that the association of HHV-7 with these clinical manifestations results from the ability of HHV-7 to reactivate HHV-6 from latency.

An Expert Working Group convened by the Health Canada Laboratory Centre for Disease Control (LCDC, 2000) concluded that "presumably" the most appropriate clinical scenarios in which HHV-7 laboratory diagnosis may be indicated appear to be similar to those for HHV-6 - children with febrile illness and immunocompromised individuals. However, the LCDC Expert Working Group stated that HHV-7 "has not been linked to any specific clinical scenarios."

The AAP (2009) concluded that "[d]iagnostic tests for HHV-7 are also limited to research laboratories, and reliable differentiation between primary infection and reactivated is problematic." The LCDC Working Group stated that HHV-7 serologic assays must be carefully selected to avoid cross-reaction with antibodies to HHV-6. The LCDC Working Group commented that, while PCR-based assays can differentiate between HHV-6 and HHV-7, "further studies are required to determine the most appropriate samples and the most appropriate PCR method format (i.e. qualitative or quantitative) for detecting an active HHV-7 infection." Diagnosis of HHV-7 will not alter the patient's management because no effective treatment is known for HHV-7; treatment of HHV-7 is supportive (AAP, 2009).

Human Herpesvirus Type 8

Human herpesvirus 8 (HHV-8) is the most recently discovered member of the herpesvirus family. In adults, HHV-8 is etiologically associated with Kaposi's sarcoma. Evidence of HHV-8 infection in children is rare, and no clinical associations are known.

Diagnostic tests for detection of HHV-8 infections are limited to research laboratories, and reliable differentiation of primary versus latent infection is problematic. According to the AAP (2009), both serologic and nucleic acid amplification tests for HHV-8 are available, but no HHV-8 screening method has been approved by the FDA. The AAP stated that screening for HHV-8 may be advisable for blood transfusion and organ transplantation procedures once a suitable method is available, but existing diagnostic tests are of limited clinical utility.

No effective treatment is known for HHV-8. Thus, diagnosis of HHV-8 will not alter the patient's management.

Trichosporonosis

Trichosporon species are fungal soil inhabitants and common colonizers of human skin and GI tracts. *Trichosporon beigelii* causes the superficial dermatomycosis known as white piedra, a distal infection of the hair shaft. *T beigelii* can also cause onychomycosis, otomycosis, or superficial skin infections. This organism is also associated with summer-type hypersensitivity pneumonitis, a type of hypersensitivity pneumonitis commonly found in Japan.

Trichosporon has been implicated in severe, disseminated infections (trichosporonosis) associated with several immunocompromised states, particularly hematologic malignancies.

No role has been established for PCR for diagnosing trichosporonosis. According to the AAP (2009), trichosporon infection is diagnosed by blood culture and histopathological examination of tissue. The diagnosis of trichosporonosis is usually confirmed by a positive blood culture result obtained in the evaluation of a febrile (usually neutropenic) patient (Hale, 2002). The urine may be the first body fluid to grow Trichosporon in culture in the setting of disseminated disease, and it should not be presumed to be a contaminant or colonizer in the high-risk host (i.e., in the setting of neutropenic fever).

Saccharomyces Cerevisiae

Saccharomyces cerevisiae is a type of acospore-forming food yeast, also known as brewer's/baker's yeast. Yeasts are fungi that have a unicellular growth form and yield mucoid, bacteria-like colonies on laboratory media. Food yeasts, primarily *S. cerevisiae*, may cause allergic symptoms in occasional atopic persons but are rarely implicated with certainty (Middleton, 1998). Cases of vaginitis caused by *Saccharomyces cerevisiae* have been reported, an may be associated with baking (Mandell, 2000). Guidelines on treatment of vaginitis, however, do not include a recommendation for PCR testing for *S. cerevisiae* in patients suspected of having none.

Two serum antibodies, anti-neutrophilic cytoplasmic antibodies (ANCA) and anti-*Saccharomyces cerevisiae* (ASCA) have been investigated as a technique to improve the efficiency and accuracy of diagnosing inflammatory bowel disease in order to potentially decrease the extent of the diagnostic work up or to avoid invasive diagnostic imaging. See CPB 0249 - Inflammatory Bowel Disease: Serologic Markers and Pharmacogenomic and Metabolic Assessment of Thiopurine Therapy.

Parvovirus

Infection with parvovirus B19 is recognized most often as erythema infectiosum (EI), which is characterized by mild systemic symptoms, fever in 15 % to 30 % of patients, and, frequently, a distinctive rash (Cunningham and Rennels, 2002). Before onset of these manifestations, a brief, mild, non-specific illness consisting of fever, malaise, myalgias, and headache, followed approximately 7 to 10 days later by the characteristic exanthema, may occur in some patients. The facial rash is intensely red with a "slapped cheek" appearance and often accompanied by circumoral pallor.

Infection with the causative agent of EI, human parvovirus B19, also can cause asymptomatic infection, a mild respiratory tract illness with no rash, a rash atypical for EI that may be rubelliform or petechial, arthritis in adults (in the absence of manifestations of EI), chronic bone marrow failure in immunodeficient patients, and transient aplastic crisis lasting 7 to 10 days in patients with hemolytic anemias (e.g., sickle cell disease, and autoimmune hemolytic anemia) and other conditions associated with low hemoglobin levels, including hemorrhage, severe anemia, and thalassemia (Cunningham and Rennels, 2002). Chronic parvovirus B19 infection has been detected in some human immunodeficiency virus (HIV)-infected patients with severe anemia. In addition, parvovirus B19 infection has been associated with thrombocytopenia and neutropenia. Patients with aplastic crisis may have a prodromal illness with fever, malaise, and myalgia, but rash usually is absent. The red blood cell aplasia is related to lytic infection in erythrocyte precursors.

Parvovirus B19 infection occurring during pregnancy can cause fetal hydrops and death but is not a proven cause of congenital anomalies. The risk of fetal death is probably between 2 % and 6 %, with the greatest risk when infection occurs during the first half of pregnancy.

Parvovirus B19 is distributed worldwide and is a common cause of infection in humans, who are the only known hosts. Modes of transmission include contact with respiratory tract secretions, percutaneous exposure to blood or blood products, and vertical transmission between a mother and her fetus. Parvovirus B19 infections are ubiquitous, and cases of EI can occur sporadically or as part of community outbreaks, which often occur in elementary or junior high schools during the late winter and early spring.

According to the AAP (2006), the most feasible methods of diagnosis are direct detection of parvovirus B19 antigen or DNA in clinical specimens and serologic tests. In the immunocompetent host, detection of serum parvovirus B19-specific immunoglobulin (Ig) M antibody is preferred, and detection indicates infection probably occurred within the previous 2 to 4 months. By using a radioimmunoassay or enzyme immunoassay, antibody may be detected in 90 % or more of patients at the time of the EI rash and by the 3rd day of illness in patients with transient aplastic crisis. Serum IgG antibody indicates previous infection and immunity. These assays are available through commercial laboratories and through some state health and research laboratories. However, their sensitivity and specificity may vary, particularly for IgM antibody. The optimal method for detecting chronic infection in the immunocompromised patient is demonstration of virus by nucleic acid hybridization or PCR assay, because parvovirus B19 antibody is variably present in persistent infection. Since parvovirus B19 DNA can be detected by PCR in serum after the acute viremic phase for up to 9 months in some patients, PCR detection of parvovirus B19 DNA does not necessarily indicate acute infection. Less sensitive nucleic acid hybridization assay usually are positive for only 2 to 4 days after onset of illness. For HIV-infected patients with severe anemia associated with chronic infection, dot blot hybridization of serum may be a more appropriate assay. Parvovirus B19 has not been grown in standard cell culture, but the virus has been cultivated in experimental cell culture.

For most patients, only supportive care is indicated. Patients with aplastic crises may require transfusion. For the treatment of chronic infection in immunodeficient patients, intravenous immunoglobulin therapy has been used. Some cases of B19 infected hydrops fetalis have been treated successfully with intra-uterine blood transfusions.

Cryptococcus

Primary *Cryptococcus neoformans* infection is acquired by inhalation of aerosolized fungal elements and often is unapparent or mild (AAP, 2006). Pulmonary disease, when symptomatic, is characterized by cough, hemoptysis, chest pain, and constitutional symptoms. Hematogenous dissemination to the central nervous system, bones and joints, skin, and mucous membranes can occur, but dissemination is rare in persons without defects in cell-mediated immunity (e.g., transplantation, malignant neoplasm, collagen-vascular disease, long-term corticosteroid administration, or sarcoidosis). Cryptococcal meningitis, the most common and serious form of cryptococcal disease, often follows an indolent course. Cryptococcal fungemia, without apparent organ involvement, occurs in

patients with human immunodeficiency virus (HIV). Cryptococcosis is one of the acquired immunodeficiency syndrome (AIDS)-defining diseases.

Encapsulated yeast cells can be visualized by India ink or other stains of CSF. Definitive diagnosis requires isolation of the organism from body fluid or tissue. The lysis-centrifugation method is the most sensitive technique for recovery of C. neoformans from blood cultures. According to the AAP (2006), the latex agglutination and enzyme immunoassay tests for detection of cryptococcal capsular polysaccharide antigen in serum or CSF are excellent rapid diagnostic tests. Antigen detection in CSF or serum is positive in 90 % of patients with cryptococca meningitis. The AAP guidelines (2006) stated that cryptococcal antibody testing is useful, but skin testing is of no value. The AAP guidelines (2009) stated that PCR tests for cryptococcus are investigational.

Adenovirus

Adenoviruses are DNA viruses. The most common site of adenovirus infection is the upper respiratory tract. Manifestations include symptoms of the common cold, pharyngitis, pharyngoconjunctival fever, tonsillitis, otitis media, and keratoconjunctivitis, often associated with fever. Life-threatening disseminated infection, severe pneumonia, meningitis, and encephalitis occasionally occur, especially among young infants and immunocompromised hosts. Adenoviruses are infrequent causes of acute hemorrhagic conjunctivitis, a pertussis-like syndrome, croup, bronchiolitis, hemorrhagic cystitis, and genitourinary tract disease. A few adenovirus serotypes can cause gastroenteritis.

Infection in infants and children may occur at any age. Adenoviruses causing respiratory tract infection usually are transmitted by respiratory tract secretions through person-to-person contact, fomites, and aerosols. Because adenoviruses are stable in the environment, fomites may be important in their transmission. Other routes of transmission have not been defined clearly and may vary with age, type of infection, and environmental or other factors.

According to AAP guidelines, although PCR testing has been used to detect adenovirus DNA, detection of adenovirus infection by culture or antigen is the preferred diagnostic method. Adenoviruses associated with respiratory tract disease can be isolated from pharyngeal secretions, eye swabs, and feces by inoculation of specimens into a variety of cell cultures. Adenovirus antigens can be detected in body fluids of infected persons by immunoassay techniques, which are especially useful for diagnosis of diarrheal disease, because enteric adenovirus types 40 and 41 usually can not be isolated in standard cell cultures. Enteric adenoviruses also can be identified by electron microscopy of stool specimens. Multiple methods to detect group-reactive hexon antigens in body secretions and tissue have been developed. Also, detection of viral DNA can be accomplished with genomic probes, synthetic oligonucleotide probes, or gene amplification by polymerase chain reaction. Serodiagnosis is based on detecting a 4-fold or greater rise in antibodies to a common adenovirus antigen (e.g., hexon). According to the AAP (2006), serodiagnosis is used primarily for epidemiologic studies.

Polymerase chain reaction has been used to diagnose adenovirus myocarditis (Martin et al, 1994; Towbin et al, 1994; Shirali et al, 2001). Routine viral cultures and histopathology are rarely positive in cases of presumed viral myocarditis (AAP, 2006).

Re-activation of adenovirus infection occurs in greater than 80 % of autologous and allogeneic hematopoietic stem cell transplant recipients but causes severe disease in fewer than 2 % (Anaissie, 2008). There are four clinically significant adenoviral syndromes: pneumonitis, nephritis, diarrhea and hemorrhagic colitis, and hemorrhagic cystitis. Disseminated disease with multi-organ failure can also occur. Quantitative PCR assays have been developed to detect viremia in hematopoietic stem cell transplant recipients. In several studies, rising blood viral loads were associated with invasive adenovirus disease. Adenovirus viral loads also can be utilized to help monitor responses to therapy (Flomenberg and Munoz, 2008).

Treatment of adenovirus infection in immunocompetent persons is supportive.

Whooping Cough (Pertussis)

Pertussis begins with mild upper respiratory tract symptoms similar to the common cold (catarrhal stage) and progresses to cough and then usually to paroxysms of cough (paroxysmal stage) characterized by inspiratory whoop and commonly followed by vomiting. Pertussis is caused by a fastidious, gram-negative, pleomorphic bacillus, *Bordetella pertussis*. *Bordatella parapertussis* is another cause of prolonged cough illness.

Culture still is considered the "gold standard" for laboratory diagnosis of pertussis. Polymerase chain reaction is being used increasingly for detection of *B. pertussis* because of its improved sensitivity and more rapid result (AAP, 2009). However, the PCR test lacks sensitivity in previously immunized people, and unacceptably high rates of false-positive results are reported from some laboratories. The AAP (2009) stated that no FDA-licensed PCR test is available, and there are no widely accepted standardized protocols, reagents, or reporting formats. The sensitivity of direct fluorescent antibody is low and is not recommended for laboratory confirmation of pertussis.

Enteroviruses

Enteroviruses are a subgroup of the Picornaviridae family. They are subclassified into polioviruses, Group A and coxsackieviruses, and echoviruses (Dua and Berkowicz, 2003). Enteroviruses cause a wide range of infections. Poliovirus infections can be subclinical or can cause mild illness, aseptic meningitis, or poliomyelitis. Coxsackie virus, an RNA virus, is one of several non-polio enteroviruses that are responsible for significant and frequent illnesses in infants and children and result in protean clinical manifestations. Coxsackievirus infections are the most common cause of viral heart disease. Group A viruses cause flaccid paralysis, while group B viruses cause spastic paralysis. Other diseases associated with coxsackievirus infections are hand-foot-and-mouth (HFM) disease and hemorrhagic conjunctivitis, caused by group A, while group B coxsackievirus is associated with herpangina, pleurodynia, myocarditis, pericarditis, and meningoencephalitis. Aseptic meningitis and colds are associated with both group A and group B. Echovirus infections range from the common cold and fever to aseptic meningitis and acute hemorrhagic conjunctivitis (AHC). The enteroviruses are spread from person to person via the fecal-oral route (Marx, 2002).

All enteroviruses enter the body through the oropharynx and multiply in the tissues around the oropharynx (Marx, 2002). Most enteroviral infections are unapparent. The most common clinical manifestation is that of a nonspecific febrile illness. Young children may be admitted to hospitals with enteroviral fevers that simulate bacterial sepsis. Coxsackie B virus and some of the echoviruses may cause severe perinatal infection associated with fever, meningitis, myocarditis, and hepatitis. Immunocompromised patients with humoral deficiencies can have persistent central nervous system infections lasting for several months or more.

Polymerase chain reaction assays for detection of enterovirus RNA is more rapid and more sensitive than cell culture and can detect all enteroviruses, including enteroviruses that are difficult to culture (AAP, 2009). Enteroviruses can be detected by PCR assay and culture from stool, rectal swab, throat specimens, urine, and blood during acute illness and from CSF when meningitis is present. Polymerase chain reaction testing is most useful for detecting enterovirus RNA in the CSF (AAP, 2009; Dua and Berkowicz, 2003).

No specific treatments for the enteroviruses exist (Marx, 2002; AAP, 2009). Thus, care is supportive and the results of diagnostic testing will not direct clinical management in most cases. An antiviral agent, pleconaril, is undergoing clinical evaluation. For chronic enteroviral meningoencephalitis in an immunodeficient patient, intravenous immunoglobulin (IVIG) containing high antibody titer to the infecting virus has been used for treatment of persistent enterovirus infection (AAP, 2009).

Human T Cell Leukemia Virus

Human T-Cell Lymphotropic Virus Type I (HTLV-I) is a retrovirus that is endemic in Japan, the Caribbean, and parts of South America, and is associated with development of malignant neoplasms and neurologic disorders among adults (Armstrong, 2000). HTLV-I can be transmitted by sexual intercourse, inoculation of infected blood or blood products and peri-natal exposure.

Only a small proportion of those infected with HTLV-I develop adult T-cell leukemia or HTLV-I-associated myelopathy: the lifetime risk of these diseases in HTLV-I-infected Japanese is estimated at 2 to 4 % and 0.25 %, respectively. The usual age at onset of is the fifth decade of life and more women than men are affected.

The myeloradiculopathy produced by HTLV-I mainly affects the pyramidal tracts and, to a lesser extent, the sensory system. HTLV-I-associated myelopathy is clinically characterized by a chronic syndrome with a combination of upper- and lower-motor neuron signs. Patients often complain of difficulty walking, dragging pains and stiffness of the legs, together with numbness and paresthesia, urinary retention and/or incontinence and impotence. About 1/3 of patients have weakness in the upper limbs, but the cranial nerves are only very rarely involved. Examination reveals a symmetric spastic paraparesis with mild sensory abnormalities indicative of posterior column involvement (diminished vibration and proprioception). Most patients progress gradually over months or years.

There may be confusion of HTLV-I-associated myelopathy with multiple sclerosis. There is, however, a lack of optic neuritis or ocular movement problems in the former and the latter tends to run a relapsing-remitting course. The WHO has published diagnostic guidelines for HTLV-I myelopathy.

The diagnostic hallmark of HTLV-I infection is the presence of 'flower lymphocytes' (T-helper cells with multi-lobulated nuclei that are similar to the cells of ATL) in the blood. These cells only comprise about 1 % of the circulating white cells, however, and the diagnosis of HTLV-I infection requires the demonstration of specific antibodies in the serum.

In HTLV-I CNS disease, the CSF examination may be normal or show a slightly elevated protein concentration an a mild lymphocytosis. Flower lymphocytes are found in a minority of cases. A definitive diagnosis of HTLV-I-associated myelopathy requires detection of HTLV-I DNA in the CSF by polymerase chain reaction or evidence of intra-thecal synthesis of HTLV-I antibody.

No therapy has been proven to be of benefit in HTLV-I-associated myelopathy. At present the management of HTLV-I-associated myelopathy is similar to that of myelopathies of any cause, with supportive therapy of spasticity and urinary sphincter disturbance. Occasional patients have improved while receiving oral corticosteroids or systemic a-interferon, and plasmapheresis has also been claimed to lead to a temporary benefit.

Human T-cell lymphotropic virus type II (HTLV-II), also a retrovirus, has been detected among American and European injection drug users and some indigenous Native American groups. Limited data are available regarding the association of clinical disease with HTLV-II infection. In contrast to the clear association of adult T-cell leukemia with HTLV-I infection, no convincing link of HTLV-II to malignancy has been observed (Feigin, 1998). Although HTLV-II has been isolated in some patients with myeloneuropathies resembling HTLV-1 myelopathy, there is also no clear link between HTLV-II and myeloradiculopathies. Feigin (1998) concluded that "[t]he natural history and clinical manifestations of HTLV-II need further delineation in the context of ongoing prospective natural history studies."

Hepatitis G Virus

Although hepatitis G virus (HGV) can cause chronic infection and viremia, it is a rare cause of hepatic inflammation, and most infected persons are asymptomatic (AAP, 2003). Histologic evidence of HGV infection is rare, and serum aminotransferase concentrations usually are normal. Although high levels of HGV RNA are found in blood, the liver is not a significant site of replication. Currently, no conclusive evidence indicates that HGV

causes fulminant or chronic disease, and co-infection does not seem to worsen the course or severity of concurrent infection with hepatitis B virus (HBV) or hepatitis C virus (HCV).

The HGV has been reported in adults and children throughout the world and is found in about 1.5 % of blood donors in the United States. Infection has been reported in 10 % to 20 % of adults with chronic HBV or HCV infection, indicating that co-infection is a common occurrence. The primary route of spread is thought to be through transfusions, but HGV also can be transmitted by organ transplantation. Other important risk factors for infection include injection drug use, hemodialysis, and homosexual and bisexual relationships, indicating that sexual transmission also may occur.

Although PCR testing can detect HGV testing, such testing would not influence management because the disease is mild, and there is no known method to treat or prevent it. Currently, HGV infection can be diagnosed only by identifying viral genomes by using polymerase chain reaction assay, which is not widely available (AAP, 2003). No serologic test is available.

According to the AAP (2003), no treatment is indicated for this virus that causes mild, if any, disease. No method to prevent infection with HGV is known.

Hepatitis C Virus

The signs and symptoms of hepatitis C virus (HCV) infection usually are indistinguishable from those of hepatitis A or B. Persistent infection with HCV occurs in 75 % to 85 % of infected persons. Chronic hepatitis develops in approximately 60 % to 70 % of chronically infected patients, and cirrhosis develops in 10 % to 20 %; primary hepato-cellular carcinoma can occur in these patients. Infection with HCV is the leading reason for liver transplantation in the United States.

The prevalence of HCV infection in the general population of the United States is estimated at 1.8 %. Sero-prevalence rates vary among individuals according to their associated risk factors. Infection is spread primarily by parenteral exposure to blood and blood products from HCV-infected persons.

The highest sero-prevalence rates of infection (60 % to 90 %) occur in persons with large or repeated direct percutaneous exposure to blood or blood products, such as injection drug users and persons with hemophilia who were treated with clotting factor concentrates produced before 1987. Rates are moderately high among those with frequent but smaller direct percutaneous exposures, such as patients receiving hemodialysis (10 % to 20 %). Lower rates are found among persons with unapparent percutaneous or mucosal exposures, such as persons with high-risk sexual behaviors (1 % to 10 %), or among persons with sporadic percutaneous exposures, such as health care personnel (1 %).

In persons with no risk factors, sero-prevalence rates are less than 0.5 %. For most infected children and adolescents, no specific source of infection can be identified.

According to the AAP (2009), there are 2 major types of tests available for the laboratory diagnosis of HCV infections: (i) antibody assays for anti-HCV, and (ii) assays to detect HCV nucleic acid (RNA). Diagnosis by antibody assays involves an initial screening enzyme immunoassay (EIA); repeated positive results are confirmed by a recombinant immunoblot assay (RIBA), analogous to testing for HIV infection. The current EIA and RIBA assays are at least 97 % sensitive and more than 99 % specific. False-negative results early in the course of acut infection result from the prolonged interval between exposure or onset of illness and sero-conversion that may occur. Within 15 weeks after exposure and within 5 to 6 weeks after the onset of hepatitis, 80 % of patients will have positive test results for serum HCV antibody.

According to the AAP (2009), highly sensitive FDA-licensed PCR assays for detection of HCV RNA are available from several commercial laboratories. Hepatitis C virus RNA can be detected in serum or plasma within 1 to 2 weeks after exposure to the virus and weeks before onset of liver enzyme abnormalities or appearance of anti-

HCV. Polymerase chain reaction assays for HCV infection are used commonly in clinical practice in the early diagnosis of infection, for identifying infection in infants early in life (i.e., peri-natal transmission) when maternal serum antibody interferes with the ability to detect antibody produced by the infant, and for monitoring patients receiving anti-viral therapy (AAP, 2009; CDC, 1998). However, false-positive and false-negative results can occur from improper handling, storage, and contamination of the test samples. Viral RNA may be detected intermittently and, thus, a single negative PCR assay result is not conclusive. The AAP (2009) guidelines noted that quantitative assays for measuring the concentration of HCV RNA also are available but are less sensitive than qualitative assays. These quantitative assays have primarily been used a prognostic indicator for patients undergoing or about to undergo antiviral therapy. A Consensus Conference convened by the Health Canada Laboratory Centre for Disease Control (1999) concluded that pretreatment quantitative HCV RNA assays provide important information with respect to the risks and benefits of treatment and duration of therapy and should be made available. In addition, the LCDC Consensus Conference concluded that pretreatment genotyping provides important information with respect to the risks/benefits and duration of treatment. Interferon given alone or in combination with ribavirin is FDA-approved for treatment of chronic HCV infection in adults.

Hepatitis B Virus

Hepatitis B virus (HBV) causes a wide spectrum of manifestations, ranging from asymptomatic sero-conversion, subacute illness with nonspecific symptoms (e.g., anorexia, nausea, or malaise) or extra-hepatic symptoms, and clinical hepatitis with jaundice, to fulminant fatal hepatitis (AAP, 2009). Chronic HBV infection with persistence of hepatitis B surface antigen (HBsAg) occurs in as many as 90 % of infants infected by peri-natal transmission, in an average of 30 % of children 1 to 5 years of age infected after birth, and in 2 % to 6 % of older children, adolescents, and adults with HBV infection. Chronically infected persons are at increased risk for developing chronic liver disease (e.g., cirrhosis, chronic active hepatitis, or chronic persistent hepatitis) or primary hepatocellular carcinoma in later life.

Hepatitis B virus is transmitted through blood or body fluids, such as wound exudates, semen, cervical secretions, and saliva of people who are HBsAg-positive.

Commercial serologic antigen tests are available to detect hepatitis B surface antigen (HbsAg) and hepatitis B e antigen (HbeAg). Assays also are available for detection of antibody to HBsAg (anti-HBs), total antibody to hepatitis B core antigen (anti-HBc), IgM anti-HBc, and antibody to HBeAg. In addition, hybridization assays and gene amplification techniques (e.g., PCR, branched DNA methods) are available to detect and quantitate HBV DNA. Tests for HBeAg and HBV DNA are useful in the selection of candidates to receive antiviral therapy and to monitor the response to therapy. Quantitative PCR viral load tests are used to monitor response to therapy.

Hepatitis A Virus

Hepatitis A characteristically is an acute, self-limited illness associated with fever, malaise, jaundice, anorexia, and nausea. The most common mode of transmission is person to person, resulting from fecal contamination and oral ingestion. Current guidelines (AAP, 2009) recommend for the diagnosis of hepatitis A virus serologic tests for HAV -specific total antibody. The presence of serum IgM anti-HAV indicates current or recent infection. IgG anti-HAV is detectable shortly after the appearance of IgM. A positive total anti-HAV test result and a negative IgM anti-HAV test result indicate past infection and immunity.

Varicella Zoster Virus

Primary varicella zoster virus (VZV) infection results in chickenpox, manifested by a generalized, pruritic, vesicular rash and mild fever and systemic symptoms (AAP, 2009). Most cases of varicella in the United States occur in children younger than 10 years of age. Immunity generally is lifelong. Immunocompromised persons with primary (varicella) or recurrent (zoster) infection are at increased risk of severe disease.

The virus establishes latency in the dorsal root ganglia during primary infection. Re-activation results in herpes zoster ("shingles"), which are grouped vesicular lesions appearing in a dermatomal distribution, sometimes accompanied by pain localized to the area. Zoster occasionally can become disseminated in immunocompromised patients, with lesions appearing outside the primary dermatomes and with visceral complications.

Varicella virus can be isolated from scrapings of vesicle base during the first 3 to 4 days of the eruption but rarely from other sites, including respiratory tract secretions. A significant increase in serum varicella IgG antibody by any standard serologic assay can retrospectively confirm a diagnosis. According to the AAP (2009), these antibody tests are reliable for determining immune status in healthy hosts after natural infection but are not necessarily reliable in immunocompromised. Many commercially available tests are not sufficiently sensitive to demonstrate a vaccine-induced antibody response.

According to the AAP (2009), rapid diagnostic tests (PCR, direct fluorescent antibody) are the methods of choice o diagnosing varicella virus infection. Varicella virus infection can be diagnosed using PCR testing of body fluid or tissue. The advantages of PCR testing over other methods is that it is very sensitive and can distinguish wild-type strains from vaccine virus.

Varicella and zoster may be treated with intravenous or oral acyclovir, valacyclovir, famciclovir, and foscarnet. The decision to use therapy and the duration and route of therapy should be determined by specific host factors, extent of infection, and initial response to therapy. Oral acyclovir is not recommended for routine use in otherwise healthy children with varicella, because it results in only a modest reduction in symptoms. Oral acyclovir should be considered for otherwise healthy persons at increased risk of moderate-to-severe varicella. Intravenous therapy is recommended for immunocompromised patients.

In 2012 the American Academy of Pediatrics Red Book recommendations stated that vesicular fluid or a scab can be used to identify VZV using a PCR test and to distinguish between wild-type and vaccine-strain VZV, which may especially be desirable and informative in immunized children who develop herpes zoster. The Red Book recommendations specify PCR assay currently is the diagnostic method of choice. Viral culture and DFA assay are less sensitive than PCR assay and do not distinguish vaccine strain from wild-type viruses (Red Book Online, 2012).

Influenza Virus

Influenza is characterized by the sudden onset of fever, frequently with chills or rigors, headache, malaise, diffuse myalgia, and a nonproductive cough (AAP, 2009). Subsequently, the respiratory tract signs of sore throat, nasal congestion, rhinitis, and cough become more prominent.

Influenza is spread from person to person by inhalation of small particle aerosols, by direct contact, by large droplet infection, or by contact with articles recently contaminated by nasopharyngeal secretions. In temperate climates, epidemics usually occur during the winter months and, within a community, peak within 2 weeks of onset and last 4 to 8 weeks or longer.

When viral cultures are performed, specimens should be obtained during the first 72 hours of illness because the quantity of virus shed subsequently decreases rapidly. Rapid diagnostic tests for identification of influenza A and antigens in nasopharyngeal specimens are available commercially, although their sensitivity and specificity have been variable. Serologic diagnosis can be established retrospectively by a significant change in antibody titer between acute and convalescent serum samples, as determined by complement fixation, hemagglutination inhibition, neutralization, or enzyme immunoassay tests. The AAP guidelines (2009) noted that reverse transcriptase-PCR (RT-PCR) testing of respiratory tract specimens may be available at some institutions, and offers potential for high sensitivity and specificity.

Amantadine and rimantadine are approved for treatment of influenza A; treatment with either drug diminishes the severity of influenza A infection when administered within 48 hours of onset of illness. Neither amantadine nor rimantadine is effective against influenza B infections. Two neuraminidase inhibitors, zanamivir and oseltamir, have been approved for treatment of influenza A and B.

According to the CDC (2003), testing for highly pathogenic Avian influenza A (H5N1) virus can be performed by PCR. Testing of hospitalized patients for influenza A (H5N1) infection is indicated when both of the following exist: (i) radiographically confirmed pneumonia, acute respiratory distress syndrome (ARDS), or other severe respiratory illness for which an alternative diagnosis has not been established; and (ii) a history of travel within 10 days of symptom onset to a country with documented H5N1 avian influenza infections in poultry or humans. Ongoing listings of countries affected by avian influenza are available from the World Organization for Animal Health at http://www.oie.int/eng/en_index.htm.

Testing for influenza A (H5N1) also should be considered on a case-by-case basis in consultation with state and local health departments for hospitalized or ambulatory patients with all of the following: (i) documented temperature of greater than 100.4º F (greater than 38 ºC); (ii) cough, sore throat, or shortness of breath; and (iii) history of contact with poultry or domestic birds (e.g., visited a poultry farm, a household raising poultry, or a bird market) or a known or suspected patient with influenza A (H5N1) in an H5N1-affected country within 10 days of symptom onset (CDC, 2003).

According to guidelines from the CDC (2009), a confirmed case of H1N1 influenza A (swine flu) virus infection is defined as a person with an acute respiratory illness with laboratory confirmed H1N1 influenza A virus infection at CDC by either real-time PCR or by viral culture. According to the CDC (2009), a suspected case of H1N1 influenza A virus infection is defined as: (i) a person with acute respiratory illness who was a close contact to a confirmed case of H1N1 influenza A virus infection during the case's infectious period; or (ii) a person with an acute respiratory illness who traveled to or resides in an area where there are confirmed cases of H1N1 influenza A virus infection. The CDC defines "close contact" as being within about 6 feet of an ill person who is a confirmed or suspected case of H1N1 influenza A virus infection during the case's infectious period. The infectious period for a confirmed case of H1N1 influenza A virus infection is defined as 1 day prior to the case's illness onset to 7 days after onset. "Acute respiratory illness" is defined as recent onset of at least 2 of the following: rhinorrhea or nasal congestion, sore throat, cough (with or without fever or feverishness).

According to CDC guidelines for the 2009 to 2010 influenza season, clinicians should consider 2009 H1N1 influenza A (swine flu) virus infection in the differential diagnosis of patients with febrile respiratory disease and who (i) live in areas in the U.S. with confirmed human cases of H1N1 influenza A virus infection; or (ii) who travele recently to Mexico or were in contact with persons who had febrile respiratory illness and were in the areas of the U.S. with confirmed H1N1 influenza cases or Mexico in the 7 days preceding their illness onset. Ongoing information about areas of the world affected by H1N1 influenza Avirus is available at the WHO's Influenza A (H1N1) website: http://www.who.int/csr/disease/swineflu/en/index.html.

Parainfluenza Virus

Parainfluenza viruses are the major cause of laryngotracheobronchitis (croup), but they also commonly cause upper respiratory tract infection, pneumonia, and/or bronchiolitis. Virus may be isolated from nasopharyngeal secretions by culture inoculation or by staining for viral antigen (shell viral assay) (AAP, 2009). Confirmation is made by rapid antigen detection, usually immunofluorescent. Rapid antigen identification techniques, including immunofluorescent assays, enzyme immunoassays, and fluoroimmunoassays, can be used to detect the virus in nasopharyngeal secretions, but the sensitivities of the tests vary. In addition, sensitive and specific PCR tests are available; however, the role of PCR testing in the management of persons with parainfluenza virus infection has not been established. Treatment of parainfluenza virus infection is supportive, and no specific antiviral therapy is available.

Bartonella

The predominant sign of *Bartonella henselae* (cat-scratch disease, CSD) is regional lymphadenopathy in an immunocompetent person. Fever and mild systemic symptoms occur in one-third of patients (AAP, 2009; CPS, 2002).

Bartonella henselae is the causative organism for most cases of CSD. Other less common causes of CSD are *B. clarridgeiae* and *B. elizabethae*. *B. henselae* are fastidious, slow-growing, gram-negative bacilli that also have been identified as the causative agent of bacillary angiomatosis and peliosis hepatitis, 2 infections that have been reported primarily in patients infected with the human immunodeficiency virus.

Infection with *B. henselae* results in disease syndromes of variable severity, ranging from lymphadenopathy only (CSD) to systemic disease. The severity and presentation of disease are related to immune status. In general, immunocompetent patients who are otherwise healthy tend to present with classic CSD when infected with *B. henselae*. Patients who are immunocompromised by having AIDS, chronic alcoholism, immunosuppression, or other serious health problems tend to have systemic disease. However, there have been rare reports of systemic disease, including bacillary angiomatosis, in immunocompetent persons.

Cat-scratch disease is believed to be a relatively common infection, although the true incidence is unknown. Most cases occur in patients younger than 20 years of age. Cats are the common reservoir for human disease, and bacteremia in cats associated with patients with CSD is common. More than 90 % of patients have a history of recent contact with cats, often kittens, which usually are healthy. No evidence of person-to-person transmission exists.

The IFA test for detection of serum antibody to antigens of Bartonella species is sensitive and specific and useful for the diagnosis of CSD (AAP, 2009; CPS, 2002). Enzyme immunoassays for detection of antibody to *B. henselae* have been developed; however, they have not been demonstrated to be more sensitive or specific than the IFA test. If involved tissue is available, the putative agent of the disease may be visualized by the Warthin-Starry silver impregnation stain; however, this test is not specific for *B. henselae*. Pathologic and microbiologic examinations also are useful to exclude other diseases. Histologic findings in lymph node sections are characteristic but not pathognomonic for CSD. A cat-scratch antigen skin test, which was used formerly to confirm the clinical diagnosis, was prepared from aspirated pus from suppurative lymph nodes of patients with apparent CSD. The AAP (2006) stated that this test should not be used.

Polymerase chain reaction assays are available in some commercial laboratories, and from reference laboratories and the CDC (AAP, 2009). According to the Canadian Paediatric Society (2002), PCR assays can differentiate between *B. henselae* and *B. Quintana*, the case of trench fever and of bacillary angiomatosis and bacillary peilosis hepatitis in HIV-infected patients.

Management is primarily symptomatic since the disease usually is self-limited, resolving spontaneously in 2 to 4 months. Painful suppurative nodes can be treated with needle aspiration for relief of symptoms; surgical excision generally is unnecessary.

Antibiotic therapy may be considered for acutely or severely ill patients with systemic symptoms, particularly persons with hepatosplenomegaly or persons with large painful adenopathy and immunocompromised hosts. No well-controlled randomized clinical trials have been performed that clearly demonstrate a clinically significant benefit of antimicrobial therapy for CSD. Reports suggest that several oral antibiotics (rifampin, trimethoprim-sulfamethoxazole, azithromycin, and ciprofloxacin) and parenteral gentamicin may be effective in CSD. Doxycycline, erythromycin, and azithromycin are effective for treatment of signs and symptoms associated with bacillary angiomatosis if administered for prolonged periods to immunocompromised persons.

Bartonellosis

Bartonellosis, or Carrion's disease, is a biphasic disease caused by *Bartonella bacilliformis* and transmitted by sandflies. The disease is characterized by an initial life-threatening febrile phase known as Oroya fever followed by an eruptive phase known as verruga peruana. *Bartonella bacilliformis*, is a small, gram-negative, intra-cellular bacteria. Bartonellosis is endemic to certain areas of the Andean regions of Peru, Columbia, and Ecuador. The diagnosis of Oroya fever is made by blood culture or by identifying *B. bacilliformis* organisms on Giemsa-stained blood smears. The diagnosis of Bartonellosis in patients with verruga peruana is generally based on the characteristic clinical features with or without a skin biopsy specimen that shows compatible findings on a Giemsa-stained sample viewed under light microscopy. A more definitive diagnosis can be made by visualizing inclusions with light microscopy or by visualizing individual microorganisms with electron microscopy. Antibody tests for *B. bacilliformis* infection have been developed. Although these tests have been useful in epidemiologic studies, their sensitivity and specificity for clinical practice have not been determined. There is a lack of evidence on the performance characteristics and clinical utility of PCR tests for *B. bacilliformis*. Anti-microbial therapy is essential in patients suspected of having Oroya fever. Rifampin has been recommended as the preferred therapy for patients with verruga peruana.

Bacteroides and Prevotella Infections

Bacteroides species (including *B. fragilis*, *B. ureolyticus*) and *Prevotella* species are anaerobic bacteria that are predominant components of the bacterial flora of mucous membranes and are therefore a common cause of endogenous infections. Bacteroides and Prevotella infections can develop in all body sites, including the central nervous system, the head, the neck, the chest, the abdomen, the pelvis, the skin, and the soft tissues. Anaerobic culture media are necessary for recovery of *Bacteroides* and *Prevotella* species (AAP, 2009). Because infections usually are polymicrobial, aerobic cultures also should be obtained. Nucleic acid probes and PCR methods, available in research laboratories, are being developed for rapid identification.

Proteus Mirabilis

Proteus species are part of the Enterobacteriaceae family of gram-negative bacilli. *Proteus* organisms are implicated as serious causes of infections in humans. *Proteus* species are most commonly found in the human intestinal tract as part of normal human intestinal flora, *Proteus mirabilis* causes 90 % of *Proteus* infections. *Proteus* organisms are easily recovered through routine laboratory cultures. Cultures may be indicated when patients do not respond to empiric therapy or when they have recurrent symptoms. A role for PCR testing in the management of *Proteus mirabilis* has not been established.

Brucella

Brucella species are small, non-motile, gram-negative coccobacilli. The species that infect humans are *Brucella abortus, B. melitensis, Brucella suis,* and, rarely, *Brucella canis* (AAP, 2006).

Brucellosis in children frequently is a mild self-limited disease compared with the more chronic disease observed among adults. However, in areas where Brucella melitensis is the endemic species, disease can be severe. Onset of illness can be acute or insidious. Manifestations are nonspecific and include fever, night sweats, weakness, malaise, anorexia, weight loss, arthralgia, myalgia, abdominal pain, and headache. Physical findings include lymphadenopathy, hepatosplenomegaly, and, occasionally, arthritis. Serious complications include meningitis, endocarditis, and osteomyelitis.

Brucellosis is a zoonotic disease of wild and domestic animals. Humans are accidental hosts, contracting the disease by direct contact with infected animals and their carcasses or secretions or by ingesting unpasteurized milk or milk products. Persons in occupations such as farming, ranching, and veterinary medicine, as well as abattoir workers, meat inspectors, and laboratory personnel, are at increased risk. Approximately 100 cases of brucellosis occur annually in the United States.

A definitive diagnosis is established by recovery of Brucella organisms from blood, bone marrow, or other tissues. A presumptive diagnosis can be made by serologic testing. The serum agglutination test (SAT), which is the most commonly used test, will detect antibodies against *B. abortus, B. suis,* and *B. melitensis*, but not *B. canis*. Detection of antibodies against *B. canis* requires use of *B. can*is-specific antigen. Enzyme immunoassay is a sensitive method for determining IgG, IgA, and IgM anti- Brucella antibodies, but until better standardization is established, EIA should be used for suspected cases with negative SAT titers or for evaluation of patients with suspected relapse or re-infection. The PCR test has been developed but is not available in most clinical laboratories (AAP, 2009).

Prolonged therapy is imperative for achieving a cure. Oral doxycycline or tetracycline given orally should be administered for 4 to 6 weeks. Oral trimethoprim-sulfamethoxazole is appropriate therapy for younger patients. For life-threatening complications of brucellosis, such as meningitis or endocarditis, the duration of therapy often is extended for several months.

Epstein Barr Virus (EBV)

Current guidelines do not indicate a role for PCR testing in the routine diagnosis of EBV infection. According to available guidelines, PCR testing for EBV may be considered in immunocompromised persons. Polymerase chain reaction testing for EBV may also be indicated in persons with lymphoma when CNS involvement is suspected in the presence of focal neurologic deficits, seizures, or changes in mental status and when CT scan or magnetic resonance imaging (MRI) reveals a mass lesion.

Epstein-Barr virus, a DNA virus, is a B-lymphotropic herpesvirus and is the most common cause of infectious mononucleosis. Replication of EBV in B lymphocytes and the resulting lymphoproliferation usually is inhibited by natural killer and T-cell responses, but in patients who have congenital or acquired cellular immune deficiencies, fatal disseminated infection or B-cell lymphomas can occur.

Epstein-Barr virus causes several other distinct disorders, including the X-linked lymphoproliferative syndrome (also known as Duncan syndrome), post-transplantation lymphoproliferative disorders, Burkitt lymphoma, nasopharyngeal carcinoma, and undifferentiated B-cell lymphomas of the CNS. The X-linked lymphoproliferative syndrome occurs in persons with an inherited, maternally derived, recessive genetic defect characterized by several phenotypic expressions, including occurrence of infectious mononucleosis early in life among boys, nodular B-cell lymphomas often with CNS involvement, and profound hypogammaglobulinemia.

Epstein-Barr virus-associated lymphoproliferative disorders result in a number of complex syndromes associated with immunosuppression, including HIV infection, and occur in approximately 2 % of graft recipients. The highest incidence occurs after heart transplantation.

Other EBV syndromes are of greater importance outside the United States, including Burkitt lymphoma (a B-cell tumor), found primarily in Central Africa, and nasopharyngeal carcinoma, found in Southeast Asia.

According to the AAP (2006), the chronic fatigue syndrome is not related specifically to EBV infection. A small group of patients with recurring or persistent symptoms have abnormal serologic test results for EBV, as well as fo other viruses.

Isolation of EBV from oropharyngeal secretions is possible, but techniques for performing this procedure usually are not available in routine diagnostic laboratories, and viral isolation does not necessarily indicate acute infection (AAP, 2006). Hence, diagnosis depends on serologic testing. Non-specific tests for heterophil antibody, including the Paul-Bunnell test and slide agglutination reaction, are most commonly available. The results of these tests are often negative in infants and children younger than 4 years of age with EBV infection, but they identify approximately 90 % of cases (proven by EBV-specific serology) in older children and adults. An absolute increase

in atypical lymphocytes in the 2nd week of illness with infectious mononucleosis is a characteristic but not specific finding.

Multiple specific serologic antibody tests for EBV are available in diagnostic virology laboratories. The most commonly performed test is for antibody against the viral capsid antigen (VCA). Since immunoglobulin (Ig) G antibody against VCA occurs in high titers early after onset of infection, testing of acute and convalescent serum samples for anti-VCA may not be useful for establishing the presence of infection. Testing for IgM anti-VCA antibody and for antibodies against early antigen is useful for identifying recent infections. Since serum antibody against EBV nuclear antigen (EBNA) is not present until several weeks to months after onset of the infection, a positive anti-EBNA antibody test excludes acute infection.

Serologic tests for EBV are particularly useful for evaluating patients who have heterophil-negative infectious mononucleosis. Testing for other viral agents, especially CMV, is indicated for these patients. In research studies culture of saliva or peripheral blood mononuclear cells for EBV, in-situ DNA hybridization, or PCR can determine the presence of EBV or EBV DNA and may implicate EBV with a syndrome, such as lymphoproliferation (AAP, 2006).

The AAP guidelines (2009) stated that detection by DNA PCR assay of serum, plasma, and tissue and RNA PCR assay of lymphoid cells or tissue are available commercially and may be useful in evaluation of immunocompromised patients and in complex clinical problems.

According to available guidelines, PCR testing of CSF for EBV is necessary in immunosuppressed persons and persons with lymphoma when CNS involvement is suspected. CNS involvement is suspected in the presence of focal neurologic deficits, seizures, or changes in mental status and when CT scan or MRI reveals a mass lesion (New York State Department of Health, 2003). Persons with lymphoma detected outside the CNS should be vigorously assessed for possible intra-cranial involvement. Lumbar puncture for EBV PCR and cytology (assuming no evidence of mass effect on neuroimaging studies), and functional neuroimaging (SPECT scan) are non-invasiv methods by which to diagnose lymphoma. A brain biopsy may be necessary to confirm diagnosis of lymphoma.

According to available guidelines, all patients who have had solid organ transplants should be monitored for evidence of EBV viral replication by measuring whole blood quantitative EBV PCR at regular intervals for 6 months after transplantation (Cincinnati Children's Hospital Medical Center, 2003).

Supportive therapy should include rest in the acute stages of illness (AAP, 2006). Corticosteroid use is considered only for cases with complications such as marked tonsillar inflammation with impending airway obstruction, massive splenomegaly, myocarditis, hemolytic anemia, and hemophagocytic syndrome. Although acyclovir has in-vitro anti-viral activity against EBV, the clinical benefits of treatment have not been demonstrated, with the possible exception of HIV-infected patients with hairy leukoplakia.

A 2012 revision of the Cincinnati Children’s Hospital Medical Center guidelines on monitoring of EBV following transplantation recommend that all patients be monitored for evidence of increased EBV-induced B-cell proliferation or EBV reactivation (by measuring blood quantitative EBV PCR at regular intervals after transplantation. The suggested frequency for renal transplantation is at baseline and with presentation of symptoms. The suggested frequency for liver transplantation is every 2 weeks for 3 months, then monthly for 9 months, then yearly and with presentation of symptoms. The suggested frequency for heart transplantation is at baseline, then every 3 months and with presentation of symptoms or rising PCR. The suggested frequency for small intestine transplantation is every 2 weeks for 3 months followed by monthly for 9 months, then yearly and with presentation of symptoms (Cincinati Children's Hospital Medical Center, 2012).

Aspergillosis

Aspergillosis comprises a variety of manifestations of infection. Allergic bronchopulmonary aspergillosis manifests as episodic wheezing, expectoration of brown mucus plugs, low-grade fever, eosinophilia, and transient pulmonary

infiltrates (AAP, 2009). This form of aspergillosis occurs most frequently in immunocompetent children with chronic asthma or cystic fibrosis. Allergic sinusitis is a far less common allergic response to colonization by Aspergillus species than allergic bronchopulmonary syndrome. It occurs in children with nasal polyps or previous episodes of sinusitis or who have undergone sinus surgery and is characterized by symptoms of chronic sinusitis with dark plugs of nasal discharge.

Aspergillomas and otomycosis are 2 syndromes of non-allergic colonization by Aspergillus species in immunocompetent children (AAP, 2009). Aspergillomas grow in pre-existing cavities or bronchogenic cysts withou invading pulmonary tissue; almost all patients have underlying lung disease, typically cystic fibrosis. Patients with otomycosis have underlying chronic otitis media with colonization of the external auditory canal by a fungal mat that produces a dark discharge.

Invasive aspergillosis occurs almost exclusively in immunocompromised patients with neutropenia or an underlying disease (e.g., chronic granulomatous disease) or medication use (e.g., corticosteroids) that causes neutrophil dysfunction or after cytotoxic chemotherapy or immunosuppressive therapy (e.g., organ transplantation) (AAP, 2009). Invasive infection usually involves pulmonary, sinus, cerebral, or cutaneous sites, and the hallmark is angioinvasion with resulting thrombosis, dissemination to other organs, and, occasionally, erosion of the blood vessel wall and catastrophic hemorrhage. Rarely, endocarditis, osteomyelitis, meningitis, infection of the eye or orbit, and esophagitis occur.

Dichotomously branched and septate hyphae, identified by microscopic examination of wet preparations, tissue specimens or bronchoalveolar lavage, are suggestive of the diagnosis. Isolation of an Aspergillus species in culture is required for definitive diagnosis. The organism usually is not recoverable from blood but is isolated readily from lung, sinus, and skin biopsy specimens cultured on special media.

Biopsy of a lesion usually is required to confirm the diagnosis. According to the AAP (2009), a serologic assay for detection of galactomannan, a molecule found in the cell wall of Aspergillus species, is available commercially but has not been evaluated widely in infants and children. A positive test result in adults supports a diagnosis of invasive aspergillosis, and monitoring of serum antigen concentrations may be useful to assess response to therapy.

In allergic aspergillosis, diagnosis is suggested by a typical clinical syndrome and elevated concentrations of total and Aspergillus-specific serum immunoglobulin E, eosinophilia, and a positive skin test to Aspergillus antigens. In persons with cystic fibrosis, the diagnosis is more difficult because wheezing, eosinophilia, and a positive skin test unassociated with allergic bronchopulmonary aspergillosis often are present.

According to the Infectious Diseases Society of America (2000), although PCR assays for Aspergillus RNA and DNA have been developed, these PCR assays must be tested with body fluids in prospective trials of invasive aspergillosis, and reproducibility must be verified before a role for these tests are established.

Rotavirus

Guidelines indicate no specific role for PCR testing in the diagnosis of rotavirus infection. Rotaviruses (Rv) are RNA viruses. Infection can result in diarrhea, usually preceded or accompanied by emesis and fever (AAP, 2009). In severe cases, dehydration, electrolyte abnormalities, and acidosis may occur. In immunocompromised children including those with HIV infection, persistent infection can develop.

The AAP (2009) notes that EIA and latex agglutination assays for group A Rv antigen detection in stool are available commercially. However, EIAs are more sensitive for the detection of antigen late in the course of illness. Both assays have high specificity, but false-positive and non-specific reactions can occur in neonates and in persons with underlying intestinal disease. These non-specific reactions can be distinguished from true positive ones by the performance of confirmatory assays. Virus also can be identified in stool by electron microscopy and by specific nucleic acid amplification techniques.

The ISDA (2001) noted, however, that routine fecal testing to diagnose rotavirus infection is not necessary. "Rotavirus infection, a leading cause of diarrhea in young children (especially in winter months in temperate climates) can be diagnosed with commercial assays, and Norwalk-like virus infections can be diagnosed with research assays, but these tests are usually not necessary for managing an individual case."

No specific antiviral therapy is available (AAP, 2009). Oral or parenteral fluids are given to prevent and correct dehydration.

Rhinovirus

Rhinoviruses are the most frequent causes of the common cold or rhinosinusitis. Transmission occurs predominantly by person-to-person contact with self-inoculation by contaminated secretions on hands. Inoculation of nasopharyngeal secretions in appropriate cell cultures for viral isolation has been the primary means to diagnose infection but is insensitive for many strains (AAP, 2009). Although PCR assays have been developed, they are not commercially available. Treatment of rhinovirus infection is symptomatic, and diagnostic testing does not alter management such that clinical outcomes are improved.

Nanobacteria

Nanobacteria are tiny bacteria (less than 0.5 mum) recently discovered in human and bovine blood samples and in commercial batches of cell culture quality serum (Pitcher and Fry, 2000). The extraordinary property possessed by nanobacteria is their ability to excrete calcium phosphate in the form of a crystalline apatite shell. This ability to deposit minerals in media and blood has aroused considerable speculation about their possible role in the formation of kidney stones, and also to other pathologies involving abnormal mineral deposition. The existence of nanobacteria and their potential role in the pathogenesis of disease remains controversial. Pitcher and Fry (2000) cited evidence that the nanobacteria may be a PCR artifact. Because the existence of nanobacteria and their role in disease have not been established, PCR testing for nanobacteria is considered experimental and investigationa

Human Bocavirus

Human bocavirus (HBoV) has been identified in 5 % to 10 % of all children with acute respiratory tract infections. Cough, rhinorrhea, and fever are the most prominent symptoms. Transmission is presumed to be from respiratory tract secretions. HBoV circulates worldwide and throughout the year. No commercial test is available to diagnose HBoV infection (AAP, 2009). HBoV PCR and detection of HBoV-specific antibody are used by research laboratories to detect the presence of virus and infection, respectively. However, there is no evidence that these tests alter management, and no specific therapy is available.

Whipple's Disease

Whipple's disease (WD) is a syndrome with a wide variety of clinical manifestations (Pitcher and Fry, 2000). Blood cultures of affected patients are negative. The diagnosis of WD is usually made by upper endoscopy showing whitish or yellowish plaques distributed on a friable mucosa (Mandell, 2000). The diagnosis usually can be established on histopathological examination of tissue obtained from biopsy of the small bowel if characteristic PAS-positive material is present in the lesions. However, electron microscopy or PCR to detect Whipple bacilli (*Tropheryma whippeli*, formerly *T. whippelii*) is now recommended in every newly identified patient, and is mandatory in doubtful cases. Thus, a PCR should now be routinely performed on small bowel tissue and additionally (in patients with long-standing systemic involvement or with suspected CNS manifestation) on CSF. Biopsies from abdominal or peripheral lymph nodes or from other organs may yield diagnostic material as well.

A number of antibiotic regimens have been used for the treatment of Whipple's disease with variable success. In addition to specific antibiotic therapy, various symptomatic treatments and supportive measures may be required, depending upon the clinical manifestations of the disease.

Mycoplasma Pneumonia

According to available guidelines, the diagnosis of Mycoplasma pneumonia may be established by a rise of specific antibody titer. Although this occurs in most instances, it requires paired samples separated by 1 week or more, and is therefore not useful in the initial diagnosis.

Other methods of diagnosis include cold hemagglutination serology, which is present in about 50 % of cases but may produce false-positives in measles, infectious mononucleosis, adenovirus pneumonias, and certain tropical diseases and collagen vascular disease.

The diagnosis may also be established by isolation of *Mycoplasma pneumoniae*.

The AAP (2009) reported, that, where available, PCR has replaced other tests, because PCR enables more rapid diagnosis in acutely ill patients. However, no PCR kits are available commercially in the United States, and tests prepared at different institutions use different primer sequences and target different genes, which precludes generalizations about sensitivity and specificity.

Mycoplasma Fermentans

M. fermentans originally was isolated from the genital tract of men and women 45 years ago, but it has not been established as a cause of genitourinary disease (Feigin 1998). This organism has been isolated from the blood of leukemia patients, from joint fluid of patients with arthritis, and from the blood and urine of patients with AIDS. In recent studies, *M. fermentans* has been identified by PCR in peripheral blood mononuclear cells and lymph nodes of HIV-infected patients. The organism also has been recovered from the blood of homosexual men without HIV infection.

M. fermentans has been identified in synovial fluid samples from patients with inflammatory arthritis diseases, including rheumatoid arthritis. The detection of *M. fermentans* in joints of rheumatic patients incriminates this microorganism as a cause of arthritis in humans. Although *M. fermentans* is a human pathogen suspected to be involved in the induction of arthritis since 1970, its pathogenesis mechanisms are poorly understood (Rivera et al, 2002). It is not known how *M. fermentans* reaches the joints and induces arthritis.

M. fermentans has been frequently found in normal healthy persons. This organism has been detected in the saliva samples from 44 % of healthy people (Chingbingyoung et a., 1996), and in 55 % of healthy people (Shibata et al, 1999).

This organism has also been detected in the throat, urine, or peripheral blood mononuclear cells of 33 % of HIV-seronegative patients attending a venereal diseases clinic (Katseni et al, 1993), in 11 % of the peripheral blood mononuclear cells from HIV-seronegative subjects (Kovacic et al, 1996), and in the synovial fluid of 14 % of patients with rheumatoid and other inflammatory arthritides (Schaeverbeke et al, 1996), although the organism wa present in 40 % of the biopsy specimens of the RA patients' synovial lining cells.

Cultivation of these organisms directly from patient material on cell-free medium has proved to be difficult, and prior animal and tissue culture passage is required. This difficulty in direct culturing has fueled a controversy over whether these organisms are pathogens or contaminants in these patients. A further controversy exists regarding the causative or disease-enhancing role of these organisms in AIDS versus their role as only one of the many opportunistic infectious agents in this condition.

In summary, although *M. fermentans* has been found in rheumatoid arthritis, AIDS, and other conditions, *M. fermentans* is also frequently found in normal healthy persons. *M. fermentans* role in the pathogenesis of disease has not been established. Although PCR assays have been developed to detect *M. fermentans*, there are no prospective studies demonstrating that diagnosis and treatment of *M. fermentans* infections improves patient

outcomes in rheumatoid arthritis, AIDS, chronic fatigue syndrome, or other conditions. Therefore, PCR testing for *M. fermentans* is considered experimental and investigational.

Human Immunodeficiency Virus (HIV)

According to the AAP (2006), EIAs are used most widely as the initial test for serum HIV antibody in adults, adolescents and children older than 18 months of age. These tests are highly sensitive and specific. Repeated EIA testing of initially reactive specimens is required to reduce the small likelihood of laboratory error. Western blo or immunofluorescent antibody tests should be used for confirmation, which will overcome the problem of a false-positive EIA result. A positive HIV antibody test result in a child 18 months of age or older usually indicates infection. In adults, adolescents, and children infected by other than peri-natal exposure, plasma viral RNA nucleic acid tests should not be used in lieu of licensed HIV screening tests (e.g., repeatedly reactive enzyme immunoassay). In addition, a negative (i.e., undetectable) plasma HIV-1 RNA test result does not rule out the diagnosis of HIV infection.

Human immunodeficiency virus nucleic acid detection by PCR of DNA extracted from peripheral blood mononuclear cells is the preferred test for diagnosis of HIV infection in infants up to 18 months of age. Although HIV culture can be used for this purpose, it is more complex and expensive to perform and is less well standardized than nucleic acid detection tests. The use of p24 antigen testing to exclude infection in children aged less than 18 months is not recommended because of its lack of sensitivity. Although plasma HIV RNA PCR may be used to diagnose HIV infection if the result is positive, this test result may be negative in HIV-infected persons. The test is licensed by the FDA only in quantitative format and currently is used for quantifying the amount of virus present as a measurement of disease progression, not for diagnosis of HIV infection in infants.

Metastatic Melanoma

The use of reversed transcriptase and PCR to detect circulating melanoma cells was described in 1991 as the first example of detecting hematogenous spread of melanoma cells from a solid tumor in peripheral blood.

Because of the higher rate of treatment failure in the subset of clinical stage I melanoma patients with occult nodal disease, clinical trials are evaluating new techniques to detect submicroscopic sentinel lymph node metastasis, in order to identify those patients who may benefit from regional lymphadenectomy with or without adjuvant therapy (NCI, 2002). One of the objectives of the phase III "Sunbelt Melanoma Trial" is to determine the effects of lymphadenectomy with or without adjuvant high-dose interferon alfa-2b versus observation on disease-free and overall survival in patients with submicroscopic sentinel lymph node metastasis detected only by PCR (i.e., sentine lymph node negative by histology and immunohistochemistry) (Urist, 2001). No survival data have been reported from this study. An ongoing diagnostic study is testing the combination of reverse transcription and PCR (RT-PCR) in the detection of melanoma tumor antigen transcripts in lymph nodes and peripheral blood samples (Gajewski, 2000). Currently, the use of PCR testing to detect melanoma micrometastases in the serum is considered experimental and investigational.

Prostate Cancer Micrometastases

Reverse transcription of messenger RNA followed by the PCR (RT-PCR) can identify circulating prostate cancer cells that express PSA or prostate-specific membrane antigen (PSMA) (Sieden et al, 1994). Studies in clinically localized patients have shown very variable results, which are at least partly due to significant technical differences in how separate laboratories have performed the assay. Hematogenous PSA-expressing cells have been identified by RT-PCR in 0 to 81 % of patients prior to radical prostatectomy. In patients documented to have metastatic disease, RT-PCR detected circulating cancer cells in 31 to 100 % of patients (de la Taille et al, 1999). In a pooled analysis of available data of RT-PCR in peripheral blood, 174 of 757 (23 %) patients with pathologic T1 or T2 disease and 151 of 401 (38 %) patients with pT3 disease were found to be RT-PCR positive for PSA or PSMA (Bast et al, 2000). All but 2 of the studies evaluated showed no additional advantage to the use of RT-PCR

over conventional staging modalities. According to Bast et al (2000), "in its current form, RT-PCR for PSA or PSMA remains an investigational staging tool."

Cervical Intraepithelial Neoplasia (CIN) Metastases

Current evidence-based guidelines from the National Comprehensive Cancer Network (NCCN) (2003) and information from the National Cancer Institute (NCI) (2002) mentioned no role for PCR testing in the detection of metastases from cervical cancer.

Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) is a clonal disease of multi-potent hematopoietic cells associated with specific cytogenetic changes involving a translocation t(9;22) (q34:q11), more commonly known as Philadelphia Chromosome (Ph1). Ph1-negative CML is a poorly defined entity that is less clearly distinguished from other myeloproliferative syndromes. Patients with Ph1-negative CML generally have a poorer response to treatment an shorter survival than Ph1-positive patients. However, Ph1-negative patients who have bcr-abl gene rearrangemen detectable by Southern blot analysis have prognoses equivalent to Ph1-positive patients. A small subset of patients has bcr-abl detectable only by RT-PCR, which is the most sensitive technique currently available. Patients with RT-PCR evidence of the bcr-abl fusion gene appear clinically and prognostically identical to patients with a classic Ph1; however, patients who are bcr-abl-negative by RT-PCR have a clinical course more consistent with CML, a distinct clinical entity related to myelodysplastic syndrome. Fluorescent in-situ hybridization of the bcr-abl translocation can be performed on the bone marrow aspirate or on the peripheral blood of patients with CML.

Fragile X Syndrome

Fragile X syndrome (a.k.a. Martin-Bell Syndrome, FRAX) is an X-linked syndrome of mental retardation associated in a proportion of cases with dysmorphic features including large everted ears, coarse facies, elongated face and macro-orchidism. Behavioral disturbances including hyperactivity or autistic-like behavior may be present. Females can also be affected. Approximately 1/3 of female carriers of full mutations will have mild to moderate mental retardation. Most recent publications on the prevalence of FRAX estimate that the disease affects approximately 1 in 5,000 males.

The gene involved (termed FMR-1 for Fragile X mental retardation) is located in chromosomal band Xq27.3 and encodes an RNA-binding protein. FRAX is caused in the vast majority of cases by expansions at a (CGG)n repeat sequence in the promoter region of the FMR-1 gene. Expansion of the array above about 200 repeats, accompanied by methylation of the adjacent CpG island, extinguishes expression of the gene. It appears that FRAX is due to absence of functional FMR-1 gene product, as deletions and point mutations in the FMR-1 coding sequence have also been reported to cause the syndrome.

According to available guidelines (McIntosh et al, 2000), Fragile site mental retardation 1 (FMR1) gene analysis by PCR/Southern Blot is medically necessary to confirm the diagnosis of fragile X syndrome (McIntosh et al, 2000). If the diagnosis of fragile X syndrome was made by cytogenetic testing, FMR1 gene analysis by PCR is recommended to confirm the diagnosis and to rule out the presence of other fragile sites in the same region of the X chromosome (i.e., FRAXE and FRAXF).

Streptococcal Infections (Including Screening for Hemolytic Streptococcus in Pregnancy)

The AAP (2009) stated that presumptive diagnosis of group B Streptococcal infection can be made by identifying gram-positive cocci in body fluids that typically are sterile (such as cerebrospinal, pleural, or joint fluid). Cultures o blood, other typically sterile body fluids, or a suppurative focus are necessary to establish the diagnosis. Serotype identification is available in reference laboratories. The AAP (2009) stated that commercially available real-time PCR tests for group B streptococci in vaginal swab specimens have high sensitivity and specificity, but data are limited regarding their usefulness for women with unknown colonization status at admission for delivery.

Group B beta-hemolytic streptococcus colonizes 20 % of pregnant women. Intrapartum fetal colonization leads to invasive disease in 1 to 2 infants of every 1,000 births in the U.S., and has a mortality of approximately 6 %. Several protocols using intra-partum chemoprophylaxis have been devised to improve management of the disease, but confusion continues about details and implementation. The 2002 CDC's recommendations on the prevention of perinatal Group B streptococcal (GBS) disease recommended that cultured-based screening for vaginal and rectal GBS colonization of all pregnant women at 35 to 37 weeks' gestation. These guidelines further stated that, although a PCR test is under development, "further studies are needed to determine whether this type of test can be adapted for use outside the research setting."

Updated guidelines from the CDC (2010) stated that, despite the availability of PCR and other nucleic acid amplification tests (NAAT) for GBS, "utility of such assays in the intrapartum setting remains limited." The guidelines explain that, although a highly sensitive and specific test with rapid turnaround time could be used to assess intra-partum GBS colonization and therefore obviate the need for antenatal screening, "data on currently available assays do not support their use in replacement of antenatal culture or risk-based assessment of women with unknown GBS status on admission for labor." The CDC guidelines explain that the additional time required fo enrichment of samples (which is necessary to increase the sensitivity of NAATs to acceptable levels) makes it not feasible for intrapartum testing, and the sensitivity of assays in the absence of enrichment is not adequate in comparison to culture. In addition, the CDC cited concerns regarding real-world turnaround time, test complexity, availability of testing at all times, staffing requirements, and costs. The CDC guidelines stated that, in settings that can perform NAAT, such tests might prove useful for the limited circumstance of a woman at term with unknown colonization status and no other risk factors. The CDC guidelines explained that even optimal NAAT would have drawbacks in the intra-partum setting, including a delay in administration of antibiotics while waiting for the result, and no anti-microbial susceptibility testing for penicillin-allergic women.

Current guidelines from the American College of Obstetricians and Gynecologists (2002) on prevention of early-onset group B streptococcal disease in newborns have concluded: "Current rapid tests for the detection of GBS colonization at the time of labor or rupture of membranes do not have sufficient sensitivity and specificity to eliminate the need for culture-based prenatal screening."

An assessment of the evidence for PCR testing and other rapid diagnostic tests for GBS colonization reached similar conclusions about the poor quality of available evidence (Honest et al, 2006). The investigators found that the quality of available studies of PCR and other rapid diagnostic testing for GBS was generally poor, and that "[b] efore implementation in practice, a robust technology assessment of their accuracy, acceptability, and cost-effectiveness is required."

The AAP (2012) stated that group A streptococcal pharyngitis can be confirmed by culture on sheep blood agar, and latex agglutination, fluorescent antibody, coagglutination, or precipitation techniques performed on colonies growing on the agar plate can differentiate group A from other ß-hemolytic streptococci. The AAP (2012) noted that several rapid diagnostic tests for group A streptococcal pharyngitis are available using techniques such as optical immunoassay and chemiluminescent DNA probes. The specificities of these tests generally are high, but the reported sensitivities vary considerably. Because of the very high specificity of these rapid tests, a positive test result generally does not require throat culture confirmation. However, because of their variable sensitivity, when patient suspected on clinical grounds of having group A streptococcal pharyngitis has a negative rapid streptococcal test, a throat culture should be obtained to ensure that the patient does not have group A streptococcal infection. Current published evidence-based guidelines from the Infectious Diseases Society of America make similar recommendations for rapid diagnostic tests and throat culture for diagnosis of group A streptococcal pharyngitis (Shulman, et al., 2012). There is no recommendation for the use of PCR testing in the diagnosis of group A streptococcal pharyngitis.

Guidelines from the Institute for Clinical Systems Improvement (Snellman, et al., 2013) state that a study indicates the utility of a real-time polymerase chain reaction assay as a replacement for both rapid antigen testing and

culture. According to the guidelines, this recommendation is based upon low quality evidence, citing a 2003 study by Uhl, et al.. The guidelines note that a polymerase chain reaction (PCR) method requires a minimum of 30 to 60 minutes to perform the test, and in order to be used efficiently, it would require batch testing. The ICSI guidelines state that, when PCR testing is used, a backup plated culture is not necessary.

In order to evaluate the performance of a PCR test for group A streptococcus, Anderson, et al. (2013) collected a total of 796 pharyngeal swabs at three separate clinical centers. Each specimen was analyzed using the illumigene group A strep DNA amplification assay (Meridian Bioscience Inc., Cincinnati, OH). To confirm GAS identification, the results were compared to those from direct and extracted culture methods using Gram staining and a GAS-specific latex agglutination test. Discrepant results were resolved using an alternative PCR test. The prevalence of culture-detected GAS in this study was 12.8% (102/796 specimens). The illumigene assay detected GAS in 74/74 direct culture-positive specimens (100% sensitivity) and 100/102 extracted culture-positive specimens (98.0% sensitivity). GAS was detected by the illumigene assay in an additional 42 specimens that were direct culture negative (94.2% specificity) and 16 specimens that were extracted culture negative (97.7% specificity). Compared to culture, the positive predictive value of the illumigene group A strep DNA amplification assay was 63/8% (54-72) and the negative predictive value was 100% (99-100). Analysis of discrepant results using an alternative PCR assay detected GAS nucleic acid in 13/16 (81.3%) false-positive specimens and 1/2 false-negative specimens. Using the alternative PCR test as the gold standard for discrepant results, the authors reported a final sensitivity of 99.0% and a specificity of 99.6% for the detection of GAS in pharyngeal swabs using the illumigene assay. Limitations of this study included the use of another PCR test as the gold standard for evaluating discrepant results; it is now known whether the alternative PCR assay shares the same limitations as the illumigene DNA amplification assay. It is not known whether the additional specimens detected by DNA amplification were more likely than culture to represent a group A strep carrier state in a patient with a concurrent viral pharyngitis. In addition, unlike culture and similar to rapid detection asssays, DNA amplification assays cannot distinguish viable from nonviable organisms. In addition, culture is still needed to detect other causes of pharyngitis.

Unlike most rapid detection tests for group A streptococcal pharyngitis, group A strep DNA amplification assays ar not point of care tests and results are not available during the initial office visit. Although DNA amplification assays take 30 minutes to one hour, the total amount of time for results would depend upon the time to transport the specimen to a reference laboratory and the timing of the batch runs during the day.

Cultures or rapid diagnostic tests may be useful in other group A streptococcus infections, such as pyoderma. According to the AAP (2012), cultures of impetiginous lesions are not indicated routinely, because lesions often yield both streptococci and staphylococci, and determination of the primary pathogen may not be possible. In suspected invasive group A streptococcus infections, cultures of blood and focal sites of possible infection are indicated. In necrotizing fasciitis, magnetic resonance imaging can be helpful for confirming the anatomic diagnosis.

According to the AAP (2009), microscopic examination of fluids that ordinarily are sterile can yield presumptive evidence of infections by enterococci and non-group A or group B streptococcus. Diagnosis is established by culture and serogrouping of the isolate, using group specific antisera. Identification of the Enterococcus species may be useful to predict anti-microbial susceptibility. In some circumstances, biochemical testing may be necessary to accurately identify the organism. Anti-microbial susceptibility testing of enterococci isolated from sterile sites is important to determine ampicillin and vancomycin hydrochloride susceptibility as well as gentamicin sulfate susceptibility to assess potential of gentamicin for synergy with ampicillin.

Updated guidelines on management of perinatal group B streptococcal infection from the CDC (Verani et al, 2010) state that, despite the availability of nucleic acid amplification tests (NAAT) such as PCR for group B streptococcal infection, "the utility of such assays in the intrapartum setting remains limited." The guidelines stated: "Although a highly sensitive and specific test with rapid turnaround time could be used to assess intrapartum GBS [group B streptococcal] colonization and therefore obviate the need for antenatal screening, data on currently available

assays do not support their use in replacement of antenatal culture or risk-based assessment of women with unknown GBS status on admission for labor."

The CDC guidelines expressed concern about the amount of time for enrichment of samples that is necessary for PCR testing (Verani et al, 2010). The guidelines explained that the additional time required for enrichment of samples makes it not feasible for intra-partum testing, and the sensitivity of assays in the absence of enrichment is not adequate in comparison to culture. The guidelines also noted concerns regarding real-world turnaround time, test complexity, availability of testing at all times, staffing requirements, and costs.

The guidelines stated: "In settings that can perform NAAT, such tests might prove useful for the limited circumstance of a woman at term with unknown colonization status and no other risk factors" (Verani et al, 2010). The guidelines noted, however, that even optimal nucleic acid amplification tests would have drawbacks in the intr -partum setting, including a delay in administration of antibiotics while waiting for the result, and no anti-microbial susceptibility testing for penicillin-allergic women.

The CDC guidelines commented that other rapid tests in addition to PCR and other NAAT have been developed to detect group B streptococcal infection rapidly from non-enriched samples, including optical immunoassays and enzyme immunoassays. The guidelines concluded, however, that none of these rapid tests is sufficiently sensitive when used on a direct specimen to detect group B streptococcal colonization reliably in the intra-partum setting (Verani et al, 2010).

Guidelines from the American Academy of Pediatrics (AAP, 2011) indicate that PCR testing of broth enrichment as an option for antepartum screening for GBS. ACOG guidelines (2011) also mention this option. The AAP guidelines indicated however, that this use of PCR is a “C” recommendation, meaning that there is “insufficient evidence for efficacy, or efficacy does not outweigh possible adverse consequences.”

Toxoplasma Gondii

According to the CDC, *Toxoplasma gondii* infection is generally asymptomatic, although 20 % of infected persons may develop cervical lymphadenopathy and/or a flu-like illness. However, immunocompromised patients may develop central nervous system (CNS) disease, myocarditis, or pneumonitis. Toxoplasmosis is caused by accidental ingestion of contaminated substances (e.g., soil contaminated with cat feces, on fruits and vegetables, raw or partly cooked meat [especially pork, lamb, or venison]).

Diagnosis may be made by isolating parasites from blood or other body fluids, and observing them in patient specimens via microscopy or histology. Detection of organisms is rare, serology (reference laboratory needed) can be a useful adjunct in diagnosing toxoplasmosis. However, IgM antibodies may persist for 6 to 18 months and thus may not necessarily indicate recent infection.

For congenital infection, diagnosis may be made by isolation of *T. gondii* from placenta, umbilical cord, or infant blood. PCR of white blood cells, CSF or amniotic fluid, or IgM and IgA serology, performed by a reference laboratory.

Asymptomatic healthy, but infected, persons do not require treatment. Spiramycin or pyrimethamine plus sulfadiazine may be used for immunocompromised persons, in specific cases. Pyrimethamine plus sulfadiazine (with or without steroids) may be given for ocular disease when indicated. Folinic acid is given with pyrimethamine plus sulfadiazine to counteract bone marrow suppression.

Coronaviruses

Severe acute respiratory syndrome (SARS) is a viral respiratory illness caused by a coronavirus, called SARS-associated coronavirus (SARS-CoV). SARS was first reported in Asia in February 2003. Over the next few months, the illness spread to more than 2 dozen countries in North America, South America, Europe, and Asia

before the SARS global outbreak of 2003 was contained. According to guidelines from the Infectious Disease Society of America (Mandell et a., 2003), diagnostic criteria for SARS include clinical and epidemiologic features and may include diagnostic studies for SARS-CoV. Recommended virologic studies for laboratory confirmation are: (i) culture for SARS-CoV, (ii) detection of antibody during the acute phase of illness or any time after onset, or (iii) detection of SARS-CoV ribonucleic acid (RNA) by RT-PCR confirmed by second PCR assay by using a second aliquot of the specimen or a different set of primers.

Coronaviruses are also a cause of the "common cold" and other upper respiratory illnesses (AAP, 2009). Although PCR tests for coronaviruses have been developed, such testing is not necessary as infection is self-limited and treatment is symptomatic.

Mycobacterium Species

According to the AAP (2009), one PCR tests for rapid diagnosis of *Mycobacterium tuberculosis* is licensed by the FDA only for acid-fast stain positive respiratory tract specimens, and another for any respiratory tract specimen. The AAP noted that the PCR assay has decreased sensitivity for gastric aspirate, CSF, and tissue specimens, with false-negative and false-positive results reported.

Current guidelines do not indicate any role for PCR testing for diagnosis of *Mycobacterium avium intracellulare* or other Mycobacteria species (NYS DOH, 2006). According to the AAP (2009), definitive diagnosis of non-tuberculous Mycobacteria disease requires isolation of the organism.

Actinomycosis

Actinomycosis is a subacute or chronic infection due to *Actinomyces israelii* or one of several other *Actinomyces* species. The body's reaction tends to be granulomatous or suppurative. Hematogenous spread can occur to distant sites but lymphatic spread is uncommon. The 3 major anatomic types of actinomycosis are cervicofacial, thoracic, and abdominal (AAP, 2006). *Actinomyces* species are slow-growing, microaerophilic or facultative anaerobic gram-positive, filamentous, branching bacilli that can be part of the normal oral, gastrointestinal, or vaginal flora. Diagnosis is suspected clinically and confirmed by x-rays and identification of *Actinomyces* spp. in sputum, pus, or biopsy specimen. In pus or tissue, the microorganism appears as the distinctive sulfur granules or as tangled masses of branched and unbranched wavy bacterial filaments, pus cells, and debris, surrounded by an outer zone of radiating, club-shaped, hyaline, and refractive filaments that take hematoxylin-eosin stain in tissue but are positive on Gram stain. Current serologic tests have no role in diagnosis. Nucleic acid probes and PCR methods are being developed for more rapid and more accurate identification. Actinomyces species can be identified in tissue specimens using the 16s rRNA sequencing and PCR assay (AAP, 2009). Initial therapy should include intravenous penicillin G or ampicillin for 4 to 6 weeks followed by high-doses of oral penicillin, amoxicillin, erythromycin, clindamycin, doxycycline, or tetracycline for a total of 6 to 12 months.

Molluscum Contagiosum

Molluscum contagiosum is a benign, usually asymptomatic viral infection of the skin with no systemic manifestations (AAP, 2009). It usually is characterized by discrete, flesh-colored to translucent papules. Lesions commonly occur on the trunk, face, and extremities but rarely are generalized. People with eczema, immunocompromising conditions, and HIV infection tend to have more widespread and prolonged eruptions. The diagnosis usually can be made clinically from the characteristic appearance of the lesions (AAP, 2009). Wright or Giemsa staining of cells expressed from the central core of a lesion reveals characteristic intracytoplasmic inclusions. There is a lack of reliable evidence for PCR testing in the diagnosis and management of molluscum contagiosum. Lesions usually regress spontaneously, but curettage of the central core of each lesion may result i more rapid resolution.

Moraxella Catarrhalis

Moraxella catarrhalis is a gram-negative aerobic diplococcus that is part of the normal flora of the upper respiratory tract. Common infections include acute otitis media and sinusitis. Bronchopulmonary infection occurs predominantly among patients with chronic lung disease or impaired host defenses. Rare manifestations are bacteremia and conjunctivitis or meningitis in neonates. The organism can be isolated on blood or chocolate agar culture media after incubation in air or with increased carbon dioxide (AAP, 2009). Culture of middle ear or sinus aspirates is indicated for patients with unusually severe infection, patients with infection that fails to respond to treatment, and immunocompromised children. The role of PCR testing for *M. catarrhalis* has not been established

Methicillin-Resistant Staphylococcus Aureus (MRSA)

Methicillin-resistant *Staphylococcus aureus* (MRSA) must be differentiated from other strains of *S. aureus* because special infection control precautions are recommended for patients with MRSA, but are not necessary for patients with low-level oxacillin-resistant (so-called "borderline-resistant") *S. aureus* that do not contain the mecA gene characteristic of MRSA.

Methicillin resistance is mediated by the mecA gene, which encodes an abnormal low-affinity binding protein, PBP-2a, that permits the organism to grow and divide in the presence of methicillin and other beta-lactam antibiotics, thus rendering the antibiotics ineffective.

According to available guidelines, the most accurate methods of detecting MRSA are PCR methods for detection of the mecA gene and latex agglutination tests for PBP-2a.

Staphylococcus Saprophyticus

Staphylococcus saprophyticus is a coaguloase-negative species of Staphylococcus that is associated with urinary tract infection in adolescent girls and young adult women, often after sexual intercourse. Persons with urinary trac infections may be treated empirically based upon evidence of infection from dipstick analysis of urine and urine microscopy. Urine culture may be indicated in high-risk persons or in persons who have failed to respond to empiric treatment. The role of PCR testing in *S. saprophyticus* has not been established.

Legionella Pneumophila

According to available guidelines, the diagnosis of *Legionella pneumophila* is confirmed by urinary antigen detection, change in antibody titers (with a 4-fold increase at 2 to 5 weeks) and immunofluorescent staining of the organism in the pleural fluid, sputum or bronchial washings. Available guidelines provide no specific indication for PCR testing in the diagnosis of *L. pneumophila*. To date, clinical experience has not shown PCR to be more sensitive than culture, and therefore the CDC does not recommend the routine use of PCR for the detection of *Legionella* in clinical samples. Guidelines from the Infectious Diseases Society of America and the American Thoracic Society do not include recommendations for PCR testing for *Legionella* (Mandell et al, 2007).

Klebsiella Pneumoniae

Klebsiella pneumoniae is a member of the Klebsiella genus of Enterobacteriaceae and belongs to the normal flora of human mouth and intestine. Infections with *K. pneumoniae* are usually hospital-acquired and occur primarily in patients with diminished resistance. The diagnosis of *K. pneumoniae* infection is confirmed by culture of blood, sputum, urine, or aspirated body fluid, including pleural effusion, pericardial effusion, synovial fluid, CSF, and abscess material. In the setting of bacterial pneumonia, sputum Gram stain may provide a presumptive identification for an etiologic agent. Serology results are not useful for detection of infection with Klebsiella organisms. There is a lack of reliable evidence of the clinical performance and utility of PCR testing for *K. pneumoniae*.

Escherichia Coli

Escherichia coli is a gram negative bacterium that is commonly found in the intestine. Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rarer cases, virulent strains are also responsible for hemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicemia and gram-negative pneumonia. Microscopy shows gram negative rods. *E. coli* can be cultured using specialized media. Rapid methods for detecting *E. coli* in stool include ELISA tests, colony immunoblots, and direct immunofluorescence microscopy. The role of PCR testing in *E. coli* infection has not been established.

BK and JC Polyomaviruses

BK virus (BKV) and JC virus (JCV) are double-stranded DNA, human polyomaviruses (Quest Diagnostics, 2005). More than 70 % of the adult population has antibodies to BKV and JCV, with primary infections typically occurring in childhood. In immunocompetent individuals, primary BKV infections usually cause a mild respiratory illness and, rarely, cystitis, whereas primary JCV infections are typically asymptomatic. After initial infection, polyomaviruses establish latency in various tissues. The primary sites of latency are uroepithelial cells for BK viru and B-lymphocytes and renal tissue for JCV. Re-activation of latent as well as primary BKV and JCV infections may occur in immunocompromised individuals. BKV infections can lead to interstitial nephritis, hemorrhagic cystitis, and kidney allograft rejection. BKV nephropathy is associated with BK viremia of greater than 5,000 copies/mL (plasma) and BK viruria greater than 107 copies/mL and is seen in approximately 8 % of kidney transplant recipients. Though latency is typically associated with the absence of viremia, low levels (less than 2,000 copies/mL plasma) are seen in some asymptomatic individuals. JCV is responsible for progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disease of the central nervous system seen in up to 70 % of AIDS patients. Additionally, BKV and JCV viruria are found in approximately 40 % of bone marrow transplant patients.

Polymerase chain reaction testing detects the presence of the virus, not antibodies to the virus; thus, the detection of viral DNA may be indicative of an active infection (Quest Diagnostics, 2005; Randhawa et al, 2005). The identification of viral DNA may warrant the institution of antiviral therapies and/or a decrease of immunosuppressive therapies. Determination of viral DNA presence or concentration is also useful in establishing the cause of allograft rejection.

JC virus has been identified as the cause of PML in persons receiving natalizumab (Tysabri), which is indicated for certain persons with Crohn's disease and multiple sclerosis. Natalizumab increases the risk of PML, which is a rare and frequently fatal demyelinating disease of the CNS. Use of natalizumab requires enrollment of the prescriber, patient, and infusion center and pharmacy in a risk-minimization program, called the TOUCH Prescribing Program, in an attempt to identify cases of PML as early as possible. Prior to initiating the therapy a MRI scan must be obtained for each patient with multiple sclerosis to help differentiate potential, future symptoms of multiple sclerosis from PML. A baseline brain MRI may also be helpful in patients with Crohn's disease, although baseline lesions are uncommon. The FDA-approved labeling recommends that clinicians monitor patients for any new sign or symptom that may be suggestive of PML. Natalizumab should immediately be withheld at the first sign or symptom suggestive of PML. For diagnosis of PML, the labeling recommends an evaluation that includes a gadolinium-enhanced MRI scan of the brain and, when indicated, cerebrospinal fluid analysis for JC viral DNA.

Human Metapneumovirus (hMPV)

The human metapneumovirus (hMPV) is a newly reported respiratory virus belonging to the Paramyxoviridae family that appears to be one of the leading causes of bronchiolitis in infants and also causes some cases of pneumonia and croup (AAP, 2009). Otherwise healthy young children infected with hMPV usually have mild or moderate symptoms, but some young children have severe disease requiring hospitalization. Risk factors for severe hMPV infection include immunodeficiency disease or therapy causing immunosuppression at any age. Serologic studies suggest that all children are infected at least once by 5 years of age. Recurrent infection

appears to occur throughout life and, in healthy people, usually is mild or asymptomatic. According to the AAP Committee on Infectious Diseases (AAP, 2009), rapid diagnostic immunofluorescent assays based on hMPV antigen detection by monoclonal antibodies are available commercially. The assays for hMPV developed and used by research laboratories include PCR and viral isolation from nasopharyngeal secretions using cell culture. However, only about 50 % of nasopharyngeal cultures that have positive results for hMPV by PCR yield cultivable virus by current techniques. There is no specific treatment for hMPV. Treatment is supportive and includes hydration, careful clinical assessment of respiratory status, including measurement of oxygen saturation, use of supplemental oxygen, and if necessary, mechanical ventilation (AAP, 2009).

Caliciviruses

Calcifiviruses are RNA viruses. The 2 recognized genera that cause disease in humans are noroviruses (formerly Norwalk-like viruses) and sapoviruses. Symptoms of infection include diarrhea and vomiting, commonly accompanied by fever, headache, malaise, myalgia, and abdominal pain. Symptoms last from 1 day to 2 weeks. According to the AAP (2009), commercial assays for diagnosis are not available in the United States. Tests available in some research and reference laboratories include electron microscopy, PCR, and serologic testing. There is no specific treatment for calicivirus infection; supportive therapy includes oral rehydration solution to replace fluids and electrolytes. No specific control measures are available. Given the self-limited nature of infection and the lack of specific treatment, there is no indication for testing other than by public health laboratories in investigating outbreaks.

Microsporidia

Microsporidia are obligate intracellular, spore-forming protozoa. Patients with intestinal infection have watery, non bloody diarrhea, generally without fever, although asymptomatic infection may be more common than originally suspected (AAP, 2006). Intestinal infection is most common in immunocompromised people, especially people who are infected with HIV, and often results in chronic diarrhea. Chronic infection in immunocompetent people is rare. According to the AAP (2009), infection with gastrointestinal Microsporidia species can be documented by identification of organisms in biopsy specimens from the small intestine. Microsporidia species spores also can be detected in stool specimens or duodenal aspirates. Polymerase chain reaction assay also can be used for diagnosis (AAP, 2009).

Chromic Lymphocytic Leukemia: IgVH Mutation Analysis

Chronic lymphocytic leukemia (CLL) patients can be divided into 2 basic groups on the basis of the mutational status of the immunoglobulin heavy-chain, variable-region (IgVH) gene in leukemic cells (Chin et a., 2006). Patients with IgVH mutations have longer survival than those without IgVH mutations. Thus, mutation analysis may be useful for assessing prognosis of patients with CLL and planning management strategies.

Chronic Myelogenous Leukemia and Acute Lymphocytic Leukemia: bcr/abl Gene Rearrangement

The bcr/abl fusion gene, formed by rearrangement of the breakpoint cluster region (bcr) on chromosome 22 with the c-abl proto-oncogene on chromosome 9, is present in 95 % of patients with chronic myelogenous leukemia (Chin et al, 2006). It is also identified in 30 % of patients with acute lymphocytic leukemia, in which it is associated with poor prognosis. The t(9;22)(q34;q11) translocation associated with bcr/abl leads to a cytogenetic aberration known as Philadelphia chromosome, although this rearrangement may also be detected in the absence of cytogenetically defined Philadelphia chromosome. The bcr/abl rearrangement causes production of an abnormal tyrosine kinase molecule with increased activity, postulated to be responsible for the development of leukemia. Increasing levels of bcr/abl are associated with clinical progression. Identification of the bcr/abl rearrangement is important for the diagnosis of CML, whereas in ALL, the presence of bcr/abl is associated with poor prognosis and maywarrant more aggressive therapy. In both diseases, increasing levels of bcr/abl may be associated with clinical progression.

Acute Myeloid Leukemia: AM1/ETO t(8, 21) Translocation

The translocation t(8;21)(q22;q22) is one of the most common structural chromosomal aberrations in patients with AML, occurring in about 15 % of adult AML cases and 40 % of AML cases with differentiation (AML-M2) (Chin et al, 2006). AML with t(8;21) has a mean age onset of about 30 years and is most common in children and younger adults; it is relatively rare in elderly persons. The presence of t(8;21) is associated with the highest complete remission rate (90 %) and the highest probability (50 % to 70 %) of remaining in complete remission at 5 years. However, the disease may become resistant to therapy upon relapse.

The t(8;21)(q22;q22) translocation results in constant expression of AML1/ETO fusion mRNA, which can be detected by reverse transcription-polymerase chain reaction (RT-PCR). The quantitative t(8;21) assay can be used not only to diagnose AML, but also to serially monitor patients, evaluate the effectiveness of treatment, and predict early relapse.

Acute Myelomonocytic Leukemia: CBFB/MYH11 inv(16)

The pericentric inversion of chromosome 16(p13;q22), and less frequently the t(16;16) (p13;q22) translocation, accounts for 16 % of the chromosomal aberrations associated with acute myelomonocytic leukemia (Chin et al, 2006). This inversion results in fusion of the core binding factor ß (CBFß) gene on 16q22 with the smooth muscle myosin heavy chain gene (MYH11) on 16p13, leading to the formation of a chimeric CBFß/MYH11 fusion protein. Clinically, the inv(16) or t(16;16) is associated with AML with abnormal eosinophils (French-American-British classification M4E0 subtype), with abnormal eosinophils being part of the malignant clone. Patients with inv(16) or t(16;16) generally have relatively good response and long-term disease-free survival rates.

Acute Promyelocytic Leukemia: PML/RARA t(15;17)

Acute promyelocytic leukemia (APL or AML-M3) is a subtype of acute myeloblastic leukemia characterized by distinct clinical and histopathologic features as well as a unique cytogenetic abnormality, t(15;17)(q22;q12-21) (Chin et al, 2006). Historically one of the most lethal forms of acute myeloid leukemia, APL leads to disseminated intravascular coagulation and death when not diagnosed and treated. Treatment with all-trans-retinoic acid substantially improves survival in patients who have failed anthracycline chemotherapy or for wom anthracycline is contraindicated. Sequential treatment with all-trans-retinoic acid and chemotherapy results in remission in the majority of cases. If chemotherapy is given initially, retinoic acid should be given upon confirmation of the molecular diagnosis of APL.

The PML/RAR-alpha/ t(15;17) translocation results in fusion of the retinoic acid receptor alpha (RARA) gene on chromosome 17 with the PML gene on chromosome 15. Detection of the PML/RARA fusion transcript by RT-PCR is more sensitive than conventional cytogenetic detection of the t(15;17) translocation and best predicts therapeuti response to all-trans-retinoic acid.

More than 99 % of APL patients harbor this translocation. A positive RT-PCR test is diagnostic for APL; thus the test can also be used for initial diagnosis as well as detection of minimal residual disease or recurrence.

Acute Myelogenous Leukemia: FLT3 Mutation

Mutations in FLT3 are common in acute myelogenous leukemia (AML) and have been associated with poorer survival in children and in younger adults with normal cytogenetics receiving intensive chemotherapy (Chin et al, 2006). FLT3 mutation analysis is used to predict survival in AML patients.

B-Cell Lymphomas: bcl-2 Gene Translocation

The bcl-2 gene translocation, t(14;18), is the rearrangement of the bcl-2 proto-oncogene on chromosome 18 with the immunoglobulin heavy chain region on chromosome 14 (Chin et al, 2006). The majority of translocations occu

in the major breakpoint cluster region (mbr) of the bcl-2 gene and result in over-expression of the bcl-2 protein. Over-expression, in turn, results in resistance to apoptosis (natural cell death), which leads to abnormally high levels of B-cell lymphocytes in the lymph nodes, spleen and peripheral blood.

The bcl-2 translocation is a characteristic of B-cell lymphomas. It is observed in 70 to 90 % of follicular non-Hodgkin B-cell lymphomas, 20 to 30 % of large diffuse B-cell lymphomas, and 50 % of undifferentiated B-cell lymphomas, but not in other lymphomas. Thus, the bcl-2 translocation is useful in the differential diagnosis of B-cell neoplasms. In addition, presence of the bcl-2 translocation is an indicator of poor prognosis in large cell diffuse lymphoma. Testing during and after treatment may assist in monitoring therapeutic response and detection of minimal residual disease or recurrent lymphoma.

Mantle Cell Lymphoma: bcl-1 Translocation

Mantle cell lymphoma is an aggressive non-follicular small B-cell lymphoma that is associated with significantly shorter survival despite a low-grade histology. Because of the aggressive nature of mantle cell lymphoma, accurate diagnosis is important for prognosis and management.

The t(11;14)(q13;q32) translocation causes deregulation of the bcl-1 gene and over-expression of cyclin D1, which may in turn lead to lymphoma genesis (Chin et al, 2006). The bcl-1 translocation is specific for mantle cell lymphoma and occurs in the majority of cases. The bcl-1 translocation is specific for mantle cell lymphoma. Detection of the bcl-1/JH gene rearrangement [t(11;14)(q13;q32)] can be helpful in the differential diagnosis of mantle cell lymphoma and in following up patients during and after treatment. However, because this translocation occurs in only about 2/3 of cases, a negative result does not preclude the diagnosis of mantle cell lymphoma.

T-Cell Lymphomas: Gene Rearrangements

T-cell receptor gene rearrangement helps distinguish between benign lymphadenopathy and malignant lymphoma This test is specifically used to detect clonal gene rearrangements in the T-cell receptor beta-chain constant region. The presence of a monoclonal gene rearrangement usualy, but not always, reflects the presence of a T-lymphocytic neoplasm while polyclonal gene rearrangement patterns are found in benign reactive conditions.

The evaluation of lymph nodes, bone marrows, and other tissues for the presence of lymphoma usually involves a multiparameter approach. The obligatory first step when evaluating a tissue for suspected lymphoma is to examine the tissue microscopically for morphology. In many cases, morphologic examination is sufficient to establish a diagnosis of malignant lymphoma. There is, however, a significant proportion of cases in which additional studies are needed in order to establish a definitive diagnosis. Those additional studies include immunoperoxidase staining of the tissue sections, flow cytometric evaluation of fresh cells from the specimen, and molecular analysis. Molecular analysis includes such modalities as cytogenetics (including FISH) and PCR. All of these special studies are intended to provide some evidence that can help to distinguish between benign lymphadenopathy and malignant lymphoma. In addition, the special tests can sometimes help to establish both the lineage and the presence of prognostically significant subtypes of malignant lymphoma.

Chromosome 18q for Colorectal Carcinoma

Colorectal cancer patients with tumors with chromosome 18 deletions are more likely to have disease recurrence and have a shorter disease-free survival period when compared to patients with 2 copies of this chromosome (Chi et al, 2006). The chromosome 18q assays is used in the diagnosis of colorectal cancer, and in predicting recurrence of disease.

Clostridium Difficile

Clostridium difficile the leading cause of antibiotic-associated diarrhea and pseudomembranous colitis, which have significant morbidity and mortality. Accurate and timely diagnosis is critical. Repeat enzyme immunoassay testing

or PCR testing for *C. difficile* toxin has been recommended because of less than 100 % sensitivity. Aichinger and colleagues (2008) reported that the diagnostic gains of repeat testing for *C. difficile* by enzyme immunoassay and PCR (i.e., initial negative result followed by positive result) within a 7-day period were 1.9 % and 1.7%, respectively. The authors concluded that there is little value of repeat testing for *C. difficile* by enzyme immunoassay or PCR.

According to the AAP Committee on Infectious Diseases (2009), "[e]ndoscopic findings of pseudomembranes and hyperemic, friable rectal mucosa suggest pseudomembranous colitis. To diagnose *C difficile* disease, stool should be tested for presence of *C difficile* toxins. Commercially available enzyme immunoassays detect toxins A and B, or an enzyme immunoassay for toxin A may be used in conjunction with cell culture cytotoxicity assay, the 'gold standard' for toxin B detection.".

According to United Kingdom National Health Services' guidelines on management of *C difficile* (2009), "although PCR has been described, its diagnostic role remains to be determined". The guidelines also note that a positive PCR test does not necessarily mean that toxin has been produced.

New guidelines from the Infectious Diseases Society of America (Cohen et al, 2010) indicate PCR testing as a preferred method of diagnosing *C. difficile*. Although more than 90 % of U.S. laboratories use commercial enzyme immunoassays to diagnose *C. difficile* infection, these tests are considered suboptimal because of their relatively low sensitivity (63 % to 94 %). The ISDA guidelines stated that they should be supplemented with either toxin testing or molecular assays (e.g., PCR).

The Committee on Infectious Diseases of the AAP's position statement on "Clostridium difficile infection in infants and children" (Schutze and Willoughby, 2013) stated that (i) testing for C difficile colonization or toxin should only be performed in children with diarrhea who meet the clinical and age-related conditions, (ii) test of cure is not recommended, and 9iii) testing for recurrence less than 4 weeks after initial testing is only useful when the results of repeat testing are negative.

Pneumococcal Disease

Avni and colleagues (2010) stated that the use of molecular-based methods for the diagnosis of bacterial infection in blood is appealing, but they have not yet passed the threshold for clinical practice. These researchers performed a systematic review of prospective and case-control studies assessing the diagnostic utility of PCR directly with blood samples for the diagnosis of invasive pneumococcal disease (IPD). A broad search was conducted to identify published and unpublished studies. Two reviewers independently extracted the data. Summary estimates for sensitivity and specificity with 95 % confidence intervals (CIs) were calculated by using the hierarchical summary receiver operating characteristic method. The effects of sample processing, PCR type, the gene-specific primer, study design, the participants' age, and the source of infection on the diagnostic odds ratios were assessed through meta-regression. A total of 29 studies published between 1993 and 2009 were included. By using pneumococcal bacteremia for case definition and healthy people or patients with bacteremia caused by other bacteria as controls (22 studies), the summary estimates for sensitivity and specificity were 57.1 % (95 % CI: 45.7 to 67.8 %) and 98.6 % (95 % CI: 96.4 to 99.5 %), respectively. When the controls were patients suspected of having IPD without pneumococcal bacteremia (26 studies), the respective values were 66.4 % (95 % CI: 55.9 to 75.6 %) and 87.8 % (95 % CI: 79.5 to 93.1 %). With lower degrees of proof for IPD (any culture or serology result and the clinical impression), the sensitivity of PCR decreased and the specificity increased. All analyses were highly heterogeneous. The use of nested PCR and being a child were associated with low specificity, while the use of a cohort study design was associated with a low sensitivity. The lack of an appropriate reference standard might have caused under-estimation of the performance of the PCR. The authors concluded that currently available methods for PCR with blood samples for the diagnosis of IPD lack the sensitivity and specificity necessary for clinical practice.

Hantavirus

Diagnosis of Sin Nombre virus (SNV) RNA has been detected uniformly by RT-PCR assay of peripheral blood mononuclear cells and other clinical specimens from the first few days of hospitalization up to 10 to 21 days after symptom onset, and the duration of viremia is unknown (AAP, 2009). According to the CDC (2011), RT-PCR can be used to detect hantaviral RNA in fresh frozen lung tissue, blood clots, or nucleated blood cells. However, "RT-PCR is very prone to cross-contamination and should be considered an experimental technique." Differences in viruses in the United States complicate the use and sensitivity of RT-PCR for the routine diagnosis of hantaviral infections (CDC, 2011).

Q Fever (C. Burnetti)

According to current guidelines (AAP, 2009; CDC, 2011), confirmation of acute Q fever requires one of the following: (i) a fourfold change in immunoglobulin (Ig) G-specific antibody titer between acute and convalescent specimens taken 3 to 6 weeks apart by immunofluorescent antibody assay (IFA) or enzyme linked immunosorbent assay; (ii) detection of C burnetii DNA in a clinical sample using PCR assay; (iii) culture of C burnetii from a clinical specimen; or (iv) positive immunohistochemical staining of C burnetii in a tissue sample. Confirmation of chronic Q fever is based on a single IgG titer of 800 or more by IFA.

Tularemia

Growth of *F. tularensis* in culture is the definitive means of confirming the diagnosis of tularemia (CDC, 2011). Some clinical laboratories can identify presumptively *F. tularensis* in ulcer exudate or aspirate material by direct fluorescent antibody or PCR assays (AAP, 2009; CDC, 2011). Suspect growth on culture may be identified presumptively by direct fluorescent antibody or PCR. The diagnosis of tularemia can also be established serologically by demonstrating a 4-fold change in specific antibody titers between acute and convalescent sera.

Cyclosporiasis (Cyclospora infection)

Cyclospora infection is diagnosed by examination of stool specimens. Microscopic methods for diagnosis involvin ultraviolet light and acid fast staining are used (CDC, 2009; CDC, 2010). "Investigational" molecular diagnostic assays (e.g., PCR) are available at the CDC and some other reference laboratories (AAP, 2009). Trimethoprim/sulfamethoxazole is the usual treatment for Cyclospora infection. No highly effective alternative antibiotic regimen has been identified yet for patients who do not respond to the standard treatment or have a sulfa allergy (CDC, 2010).

Astrovirus

Commercial tests for diagnosis of astrovirus are not available in the United States, although enzyme immunoassays are available in many other countries (AAP, 2009). The following tests are available in some research and reference laboratories: electron microscopy for detection of viral particles in stool, enzyme immunoassay for detection of viral antigen in stool or antibody in serum, latex agglutination in stool, and RT-PCR assay for detection of viral RNA in stool. Of these tests, RT-PCR assay is the most sensitive. Rehydration with oral or intravenous fluid and electrolyte solutions is recommended for treatment of astrovirus infection (AAP, 2009). No specific control measures are available.

Measles

Measles virus infection can be diagnosed by a positive serologic test result for measles immunoglobulin (Ig) M antibody, a significant increase in measles IgG antibody concentration in paired acute and convalescent serum specimens by any standard serologic assay, or isolation of measles virus or identification of measles RNA (by RT-PCR assay) from clinical specimens, such as urine, blood, throat, or nasopharyngeal secretions (AAP, 2009; CDC 2009). The simplest method of establishing the diagnosis of measles is testing for IgM antibody on a single serum specimen obtained during the first encounter with a person suspected of having disease.

Leptospirosis

Clinical features and routine laboratory findings of leptospirosis are not specific, and a high index of suspicion mus be maintained for the diagnosis. The organism can be cultured, but the diagnosis is more frequently made by serologic testing. Polymerase chain reaction assays for detection of *Leptospira* organisms are being explored for the diagnosis of leptospirosis, and are available only in research laboratories (AAP, 2009; Everett, 2011).

Leishmaniasis

The diagnosis of cutaneous leishmaniasis relies on the demonstration of Leishmania in tissue biopsy, scraping or impression preparations by microscopy and/or culture in a specialized medium (CDC, 2010). Species identificatio is recommended because management may vary depending on the infecting species. Recently, assays based on the use of PCR, including multiplex assays that can distinguish among several species simultaneously, have become more widely available. For visceral leishmaniasis, definitive diagnosis requires the demonstration of the parasite by smear or culture in tissue, usually bone marrow or spleen, and thus entails an invasive procedure (CDC, 2010). Parasites can be detected in tissue samples by light microscopy of stained slides, culture in a specialized medium, or by specific PCR assays. Serological tests can be used to demonstrate anti-leishmanial antibodies. These assays have high sensitivity for visceral leishmaniasis in patients without HIV infection, but may show positive results due to subclinical infection or cross-reactions, and are therefore less specific than tissue sampling.

Sporotrichosis

Sporotrichosis can be confirmed by obtaining a swab or a biopsy of a freshly opened skin nodule and submits it for fungal culture (Kauffman, 2010). Serologic testing and PCR assay show promise for accurate and specific diagnosis but are available only in research laboratories (AAP, 2009).

Cryptosporidiosis (Cryptosporidium infection)

Diagnosis of cryptosporidiosis is made by examination of stool samples. Most often, stool specimens are examined microscopically using different techniques (e.g., acid-fast staining, direct fluorescent antibody, and/or enzyme immunoassays for detection of *Cryptosporidium* sp. antigens) (CDC, 2010). Molecular methods (e.g., PCR) are increasingly used in reference diagnostic labs to identify *Cryptosporidium* spp. at the species level. Although PCR assays can be used to identify species and genotyp, immunocompetent people need no specific therapy for Cryptosporidiosis (AAP, 2009). Thus, the results of such PCR testing would not alter clinical management.

Hemorrhagic Fevers and Related Syndromes Caused by Viruses of the Family Bunyaviridae

Polymerase chain reaction assay performed in containment laboratories can be a useful complement to serodiagnostic assays on samples obtained during the acute phase of Crimean-Congo hemorrhagic fever, Rift Valley fever, or hemorrhagic fever with renal syndrome (AAN, 2009).

Campylobacteriosis (Campylobacter infection)

Campylobacter infection is diagnosed when a culture of a stool specimen yields the organism (CDC, 2009; CDC, 2010). Visualization of motile and curved, spiral or S-shaped rods by stool phase-contrast or darkfield microscopy can provide rapid presumptive evidence for Campylobacter enteritis. Campylobacter species can be detected directly in stool specimens by commercially available enzyme immunoassay or in research laboratories by PCR assay (AAP, 2009).

Burkholderia Infections

Culture is the appropriate method to diagnose *B. cepacia* complex infection. In cystic fibrosis lung infection, culture of sputum on selective agar is recommended to decrease the potential for over-growth by mucoid *P. aeruginosa*. *B. cepacia* and *B. gladioli* can be identified by PCR assay, but this assay is not available routinely (AAP, 2009).

Epidemic typhus

Rickettsia prowazekii can be isolated from acute blood specimens by animal passage or through tissue culture but can be hazardous. Definitive diagnosis requires immunohistochemical visualization of rickettsiae in tissues, isolation of the organism, detection of the DNA of rickettsiae by PCR assay, or antibody detection in paired serum specimens obtained during the acute and convalescent phases of disease (AAP, 2009).

B-Cell/T-Cell Clonality for Lymphomas

According to Kim et al (2013) a clonality test for immunoglobulin (IG) and T-cell receptor (TCR) is a useful adjunctive method for the diagnosis of lympho-proliferative diseases (LPDs). Recently, the BIOMED-2 multiplex PCR assay has been established as a standard method for assessing the clonality of LPDs. Clonality in LPDs wa tested in Korean patients using the BIOMED-2 multiplex PCR and compared the results with those obtained in European, Taiwanese, and Thai participants. The usefulness of the test as an ancillary method for diagnosing LPDs was also evaluated. A total of 219 specimens embedded in paraffin, including 78 B-cell lymphomas, 80 T-cell lymphomas and 61 cases of reactive lymphadenitis, were used for the clonality test. Mature B-cell malignancies showed 95.7 % clonality for IG, 2.9 % co-existing clonality, and 4.3 % polyclonality. Mature T-cell malignancies exhibited 83.8 % clonality for TCR, 8.1 % co-existing clonality, and 16.2 % polyclonality. Reactive lymphadenitis showed 93.4 % polyclonality for IG and TCR. The majority of these results were similar to those obtained in Europeans. However, the clonality for IGK of B-cell malignancies and TCRG of T-cell malignancies was lower in Koreans than Europeans. The authors concluded that the BIOMED-2 multiplex PCR assay was a useful adjunctive method for diagnosing LPDs.

Shin et al (2013) stated that the evaluation of bone marrow (BM) involvement is important for diagnosis and staging in patients with lymphoid neoplasia. These researchers evaluated IG and/or TCR gene re-arrangements in the BM for diagnosis and staging in patients with lymphoid neoplasia, using the standardized BIOMED-2 multiplex PCR clonality assays. The results were compared with microscopic findings such as histology and CD10, CD20, CD79a, CD3 and CD5 immunohistochemistry. A total of 151 samples were enrolled; 119 B-cell neoplasia, 29 T-cell neoplasia, and 3 Hodgkin's lymphoma. The molecular clonality assay and microscopic diagnosis were concordant in 66.9 % (n = 101) and discordant in 33.1 % (n = 50); Ig/TCR gene clonality assay detected 43 cases of BM involvement which was not presented in the morphology. Two cases among them turned into microscopic BM involvement during a close follow-up. Clonal TCR gene re-arrangements were detected in 12.6 % of B-cell neoplasia and Ig gene re-arrangement were found in 3.4 % of T-cell neoplasia. The authors concluded that this molecular clonality assay is valuable particularly in diagnosing BM involvement of lymphoid neoplasia if it is morphologically uncertain. But it should be carefully interpreted because molecular clonality may be present in the reactive lympho-proliferation. Therefore, comprehensive analysis with morphologic analysis should be important to reach a final diagnosis.

Shaw et al (2014) stated that re-biopsy rates as high as 12 % have been reported in previous studies of primary central nervous system lymphoma (PCNSL). This can lead to secondary operations, increasing risks of morbidity to the patient and costs for the NHS. Polymerase chain reaction testing for clonality in hematological malignancies has been applied to cases of lymphoma outside the CNS, but is less commonly used in the diagnosis of CNS lymphomas. Clonality in B- and T-cell populations may indicate the presence of malignancy. The present study aimed to identify factors to reduce the re-biopsy rate in PCNSL. A cohort of 102 suspected cerebral lymphoma cases biopsied at Frenchay Hospital, Bristol over a 10-year period (2000 to 2010) was examined. Clinical data, including age, sex, location, pre-biopsy steroid use, the need for re-biopsy and histological diagnosis, were collected. Re-biopsied cases were retrospectively reviewed and they subsequently underwent PCR testing for

clonality. Overall, 96/102 (94 %) cases achieved a histological diagnosis after 1 or more biopsies; 81/96 (84 %) of these were lymphomas involving the brain and 15/96 (16 %) were spinal lymphomas. The majority of these were B -cell lymphomas (95/96 (99 %)), with 1 case of peripheral T-cell lymphoma (1/96 (1 %)). Due to insufficient histological evidence of PCNSL after the 1st biopsy, 9/102 (9 %) of cases had required re-biopsy. In 7/9 (78 %) of these cases, these investigators undertook PCR testing for clonality on tissue from the 1st biopsy; 3/7 (43 %) cases were monoclonal for B or T populations, raising the possibility of PCNSL. Based on these results, the authors recommended that all CNS lympho-proliferative lesions be assessed by hematopathologists, with the inclusion of PCR testing particularly in equivocal cases. This would reduce the number of patients going for re-biopsy and reduce the patient morbidity and costs for the NHS.

Ribera et al (2014) noted that in up to 5 to 15 % of studies of LPDs, flow cytometry (FCM) or immunomorphologic methods cannot discriminate malignant from reactive processes. These researchers determined the usefulness of PCR for solving these diagnostic uncertainties. Immunoglobulin heavy-chain gene (IGH) and TCRγ genes were analyzed by PCR in 106 samples with inconclusive FCM results. A clonal result was registered in 36/106 studies, with a LPD being confirmed in 27 (75 %) of these cases. Specifically, 9/9 IGH clonal and 16/25 TCRγ clonal results were finally diagnosed with LPD. Additionally, 2 clonal TCRγ samples with suspicion of undefined LPD were finally diagnosed with T LPD. Although polyclonal results were obtained in 47 of the cases studied (38 IGH and 9 TCRγ), hematologic neoplasms were diagnosed in 4/38 IGH polyclonal and in 1/9 TCRγ polyclonal studies. There were also 14 PCR polyclonal results (4 IGH, 10 TCRγ), albeit non-conclusive. Of these, 2/4 were eventually diagnosed with B-cell lymphoma and 3/10 with T-cell LPD. In 8 IGH samples, the results of PCR techniques were non-informative but in 3/8 cases a B-lymphoma was finally confirmed. The authors concluded that PCR is a useful technique to identify LPD when FCM is inconclusive. A PCR clonal B result is indicative of malignancy but IGH polyclonal and non-conclusive results do not exclude lymphoid neoplasms. Interpretation of T-cell clonality should be based on all the available clinical and analytical data.

Furthermore, the NCCN' clinical practice guideline on "Non-Hodgkin's lymphomas" (Version 2.2014) recommends determination of T-cell clonality in T-cell lymphomas, including mycosis fungoides/Sezary syndrome. The guideline also recommends determining B-cell clonality for the B-cell lymphomas.

Pleuropulmonary Coccidioidomycosis

Thompson et al (2013) stated that in patients with positive serum serology for coccidioidomycosis, the differential diagnosis of concurrent pleural effusions can be challenging. These researchers sought to clarify the performance characteristics of biochemical, serologic, and nucleic-acid-based testing in an attempt to avoid invasive procedures. The utility of adenosine deaminase (ADA), coccidioidal serology, and PCR in the evaluation of pleuropulmonary coccidioidomycosis has not been previously reported. A total of 40 consecutive patients evaluated for pleuropulmonary coccidioidomycosis were included. Demographic data, pleural fluid values, culture results, and clinical diagnoses were obtained from patient chart review. Testing of ADA was performed by ARUP Laboratories, coccidioidal serologic testing was performed by the University of California-Davis coccidioidomycosis serology laboratory, and PCR testing was performed by the Translational Genomics Research Institute using a previously published methodology. Fifteen patients were diagnosed with pleuropulmonary coccidioidomycosis by European Organization for the Research and Treatment of Cancer/Mycoses Study Group criteria. Pleural fluid ADA concentrations were less than 40 IU/L in all patients (range of less than 1.0 to 28.6 IU/L; median of 4.7). The sensitivity and specificity of coccidioidal serologic testing was 100 % in this study. The specificity of PCR testing was high (100 %), although the overall sensitivity remained low, and was comparable to the experience of others i the clinical use of PCR for coccidioidal diagnostics. The authors concluded that contrary to prior speculation, ADA levels in pleuropulmonary coccidioidomycosis were not elevated in this study. The sensitivity and specificity of coccidioidal serologic testing in non-serum samples remained high, but the clinical usefulness of PCR testing in pleural fluid was disappointing and was comparable to pleural fluid culture.

Wound Infection

Gentili et al (2012) stated that the impact of poly-microbial bacterial infection on chronic wounds has been studied extensively, but standard bacteriological analysis is not always sensitive enough. Molecular approaches represent a promising alternative to the standard bacteriological analysis. This work aimed to assess the usefulness of a panbacterial quantitative real-time PCR reaction to quantitate the total bacterial load in chronic wounds treated wit Cutimed™ Sorbact™, a novel therapeutic approach based on hydrophobic binding of bacteria to a membrane. The results obtained by panbacterial real-time PCR on conserved sequences of the bacterial 16S gene showed that the bacterial burden significantly decreased in 10 out of 15 healing chronic wounds, and did not change in 5 out of 5 non-healing chronic wounds. On the contrary, classical culture for S. aureus and P. aeruginosa, and real-time PCR for bacteroides and fusobacterium did not show any correlation with the clinical outcome. This study also showed that quantification of chronic wounds by panbacterial real-time PCR is to be performed on biopsies and not on swabs. The authors concluded that these results showed that panbacterial real-time PCR is a promising and quick method of determining the total bacterial load in chronic wounds, and suggested that it might be an important biomarker for the prognosis of chronic wounds under treatment.

Also, and UpToDate review on “Clinical assessment of wounds” (Armstrong and Meyr, 2014) does not mention the use of PCR testing as a management tool.

Miscellaneous Indications

Boppana et al (2010) stated that reliable methods to screen newborns for congenital cytomegalovirus (CMV) infection are needed for identification of infants at increased risk of hearing loss. Since dried blood spots (DBS) are routinely collected for metabolic screening from all newborns in the United States, there has been interest in using DBS polymerase chain reaction (PCR)-based methods for newborn CMV screening. These researchers determined the diagnostic accuracy of DBS real-time PCR assays for newborn CMV screening. Between March 2007 and May 2008, infants born at 7 U.S. medical centers had saliva specimens tested by rapid culture for early antigen fluorescent foci. Results of saliva rapid culture were compared with a single-primer (March 2007 to December 2007) and a 2-primer DBS real-time PCR (January 2008 to May 2008). Infants whose specimens screened positive on rapid culture or PCR had congenital infection confirmed by the reference standard method with rapid culture testing on saliva or urine. Main outcome measures were sensitivity, specificity, and positive and negative likelihood ratios (LRs) of single-primer and 2-primer DBS real-time PCR assays for identifying infants with confirmed congenital CMV infection. Congenital CMV infection was confirmed in 92 of 20,448 (0.45 %; 95 % CI: 0.36 % to 0.55 %) infants. Ninety-one of 92 infants had positive results on saliva rapid culture. Of the 11,422 infants screened using the single-primer DBS PCR, 17 of 60 (28 %) infants had positive results with this assay, whereas, among the 9,026 infants screened using the 2-primer DBS PCR, 11 of 32 (34 %) screened positive. The single-primer DBS PCR identified congenital CMV infection with a sensitivity of 28.3 % (95 % CI: 17.4 % to 41.4 %), specificity of 99.9 % (95 % CI: 99.9 % to 100 %), positive LR of 803.7 (95 % CI: 278.7 to 2,317.9), and negative LR of 0.7 (95 % CI: 0.6 to 0.8). The positive- and negative-predictive values of the single-primer DBS PCR were 80.9 % (95 % CI: 58.1 % to 94.5 %) and 99.6 % (95 % CI: 99.5 % to 99.7 %), respectively. The 2-primer DBS PCR assay identified infants with congenital CMV infection with a sensitivity of 34.4 % (95 % CI: 18.6 % to 53.2 %), specificity of 99.9 % (95 % CI: 99.9 % to 100.0 %), positive LR of 3,088.9 (95 % CI: 410.8 to 23,226.7), and negative LR of 0.7 (95 % CI: 0.5 to 0.8). The positive- and negative-predictive values of the 2-primer DBS PCR were 91.7 % (95 % CI: 61.5 % to 99.8 %) and 99.8 % (95 % CI: 99.6 % to 99.9 %), respectively. The authors concluded that among newborns, CMV testing with DBS real-time PCR compared with saliva rapid culture had low sensitivity (since they missed approximately 2/3 of the infections), limiting its value as a screening test.

An UpToDate review on "Diagnosis of invasive aspergillosis" (Marr, 2012) states that "Investigational DNA detection assays (e.g., by polymerase chain reaction [PCR]) have shown mixed results, with some studies suggesting superior performance compared to antigen based assays, and others reporting the opposite. Results of multiple assays that use different technologies and microbial targets have been reported. A systemic review an meta-analysis suggested that sensitivity and specificity of PCR to detect invasive aspergillosis was 88 and 75

percent. However, this review emphasized that results cannot be generalized with non-homogeneity of methods and patients evaluated".

An UpToDate review on "Clinical manifestations and diagnosis of blastomycosis" (Bradsher, 2012) states that "Molecular identification of B. dermatitidis has been accomplished by a variety of techniques, including PCR assays for ribosomal genes, the ITS-region, repetitive sequences, and species- or genus-specific genes. PCR techniques, although promising, are labor-intensive, are not routinely available, and have not been examined in large prospective studies".

Wheat and Kauffman (2012) stated that the role of PCR for diagnosis of histoplasmosis is uncertain. In one study, 2 of 6 culture-positive broncho-alveolar lavage (BAL) fluid specimens and 9 of 9 other respiratory or tissue specimens were positive using a PCR assay developed in a commercial laboratory, but comparison to microscopy was not reported, leaving open the question of whether PCR improves the sensitivity for diagnosis. In another report, PCR was positive only if organisms were seen by microscopy. Other studies noted a sensitivity of 8 % of urine and 22 % of BAL specimens that were positive for Histoplasma antigen, whereas results in cerebrospinal fluid (CSF) and serum were uniformly negative.

An UpToDate review on "Polymerase chain reaction" (Klanderman, 2012) does not mention the use of PCR for listeria, salmonella, and vibrio species; and an UpToDate review on “"Infection with less common Campylobacter species and related bacteria" (Skirrow, 2012) does not mention the use of PCR.

An UpToDate review on "Epidemiology and prevention and control of vancomycin-resistant enterococci" (Anderson, 2012) states that "PCR techniques for identification of vanA and vanB genes from stool/rectal samples have been developed but their impact on surveillance for VRE has yet to be determined". Furthermore, the AAP Committee on Infectious Diseases has no recommendation for use of PCR testing for enterococci (2009). There is some mention by the CDC about PCR being an option for detecting clonal spread in a hospital. However, that would be for hospitalized patients on a hospital claim; and there is no recommendation for PCR for standard clinical management of outpatients. http://www.cdc.gov/HAI/settings/lab/VREClinical-Laboratory.html

Xenotropic murine leukemia virus-related virus (XMRV) is a gamma-retrovirus that was first described in 2006. Initial reports linked the virus to prostate cancer, and later to chronic fatigue syndrome (CFS), but these were followed by a large number of studies in which no association was found. It has not been established that XMRV can infect humans, nor has it been demonstrated that XMRV is associated with or causes human disease. Numerous researchers have suggested that XMRV detection may result from contamination of clinical specimens and laboratory reagents with mouse retroviruses or related nucleic acids. http://en.wikipedia.org/wiki/Xenotropic_murine_leukemia_virus-related_virus.

Trevino et al (2012) noted that although most HTLV infections in Spain have been found in native intravenous drug users carrying HTLV-2, the large immigration flows from Latin America and Sub-Saharan Africa in recent years may have changed the prevalence and distribution of HTLV-1 and HTLV-2 infections, and hypothetically open the opportunity for introducing HTLV-3 or HTLV-4 in Spain. These investigators assessed the current sero-prevalence of HTLV infection in Spain; a national multi-center, cross-sectional, study was conducted in June 2009. A total of 6,460 consecutive outpatients attending 16 hospitals were examined. Overall, 12 % were immigrants, and their main origin was Latin America (4.9 %), Africa (3.6 %) and other European countries (2.8 %). Nine individuals were sero-reactive for HTLV antibodies (overall prevalence, 0.14 %). Evidence of HTLV-1 infection was confirmed by Western blot in 4 subjects (prevalence 0.06 %) while HTLV-2 infection was found in 5 (prevalence 0.08 %). Infection with HTLV types 1, 2, 3 and 4 was discarded by Western blot and specific PCR assays in another 2 specimens initially reactive in the enzyme immunoassay. All but 1 HTLV-1 cases were Latin-Americans while all persons with HTLV-2 infection were native Spaniards. The authors conclude that the overall prevalence of HTLV infections in Spain remains low, with no evidence of HTLV-3 or HTLV-4 infections so far.

Pinto et al (2012) noted that the sero-prevalence and geographic distribution of HTLV-1/2 among blood donors are extremely important to transfusion services. These investigators evaluated the sero-prevalence of HTLV-1/2 infection among first-time blood donor candidates in Ribeirao Preto city and region. From January 2000 to December 2010, 1,038,489 blood donations were obtained and 301,470 were first-time blood donations. All samples were screened with serological tests for HTLV-1/2 using enzyme immunoassay (EIA). In addition, the frequency of co-infection with hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), Chagas disease (CD) and syphilis was also determined. In-house PCR was used as confirmatory test for HTLV-1/2. A total of 296 (0.1 %) first-time donors were serologically reactive for HTLV-1/2. Confirmatory PCR of 63 samples showed that 28 were HTLV-1 positive, 13 HTLV-2 positive, 19 negative and 3 indeterminate. Regarding HTLV co-infection rates, the most prevalent was with HBV (51.3 %) and HCV (35.9 %), but co-infection with HIV, CD and syphilis was also detected. The authors stated that the real number of HTLV-infected individual and co-infection rate in the population is under-estimated and epidemiological studies like theirs are very informative.

An UpToDate review on “Human T-lymphotropic virus type I: Disease associations, diagnosis, and treatment” (Scadden et al, 2013) states that “Polymerase chain reaction (PCR)-based testing to detect proviral DNA in peripheral blood mononuclear cells is an alternative diagnostic test. This test will also differentiate HTLV-I from HTLV-II infection. Advantages of this type of analysis are its ability to provide quantitation of proviral load in the blood [62], and its applicability in detecting proviral DNA in tumor cells or other tissue samples”.

Pakneshan et al (2013) states that BRAF represents one of the most frequently mutated protein kinase genes in human tumors. The mutation is commonly tested in pathology practice. BRAF mutation is seen in melanoma, papillary thyroid carcinoma (including papillary thyroid carcinoma arising from ovarian teratoma), ovarian serous tumors, colorectal carcinoma, gliomas, hepato-biliary carcinomas and hairy-cell leukemia (HCL). In these cancers various genetic aberrations of the BRAF proto-oncogene, such as different point mutations and chromosomal rearrangements, have been reported. The most common mutation, BRAF V600E, can be detected by DNA sequencing and immunohistochemistry on formalin fixed, paraffin embedded tumor tissue. Detection of BRAF V600E mutation has the potential for clinical use as a diagnostic and prognostic marker. In addition, a great deal of research effort has been spent in strategies inhibiting its activity. Indeed, recent clinical trials involving BRAF selective inhibitors exhibited promising response rates in metastatic melanoma patients. Clinical trials are underway for other cancers. However, cutaneous side effects of treatment have been reported and therapeutic response to cancer is short-lived due to the emergence of several resistance mechanisms. In this review, the authors gave an update on the clinical pathological relevance of BRAF mutation in cancer. It is hoped that the review will enhance the direction of future research and assist in more effective use of the knowledge of BRAF mutation in clinical practice.

An UpToDate review on “Clinical features and diagnosis of hairy cell leukemia” (Tallman and Aster, 2013) states that “The pathogenesis of HCL is largely unknown. However, studies indicate that most cases are associated with a V600E activating mutation in the serine/threonine kinase BRAF (an isoform of RAF), implicating BRAF signaling in HCL. Response to BRAF inhibitor therapy has been described in a patient with refractory HCL, in line with the idea that oncogenic BRAF signaling enhances HCL proliferation and survival …. While numerous genetic abnormalities have been described, none has been incorporated into the diagnostic criteria for HCL yet. As described above, initial studies suggest that the vast majority of cases demonstrate BRAF mutations. Further study is needed to define the sensitivity and specificity of these mutations for HCL before testing for BRAF mutations becomes a routine part of diagnosis …. It also appears that HCL-v lacks BRAF mutations, though furthe study is needed to confirm this finding”.

An UpToDate review on "Treatment of enterococcal infections" (Murray, 2013) does not mention the use of PCR.

Wei et al (2013) noted that Hantaan viruses cause 2 severe diseases lacking efficient treatment, yet no effective prophylactic vaccines are available. Continued exploration of alternative anti-viral agents to treat hantavirus-

related syndromes remains compulsory. The fluorescence-based quantitative real-time PCR (qPCR) has become the touchstone for target gene quantification. In the present study, standard curves for Hantaan virus (HTNV), mouse, and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were generated by serial 10-fold dilutions of the constructed recombinant plasmid pGEM-T/HTNV, pGEM-T/mouse-GAPDH, and pGEM-T/human-GAPDH, respectively. Comparisons between the indirect immunofluorescence assay and qPCR assay in the detection of HTNV-infected Vero E6 cells showed improved detection limit and sensitivity of latter method. To characterize the inhibitory effect of several conventional antivirals (arbidol and ribavirin) and unconventional anti-virals (indomethacin and curcumin) on HTNV, the levels of viral RNAs were measured for 4 days post-treatment of HTNV-infected Vero E6 cells and 18 days post-inoculation of HTNV-infected suckling mice. The authors noted tha these findings validated that HTNV was sensitive to ribavirin and arbidol treatment, while indomethacin and curcumin may also be therapeutically effective in treating HTNV infection. They concluded that the establishment and application of qPCR may be a useful tool for the evaluation of potential anti-virals for Hantaan virus infection in -vitro and in-vivo.

Mohamed et al (2013) described the design and evaluation of a rapid and robust quantitative real-time PCR (QRT-PCR) assay able to detect a wide range of hantaviruses. Primers with the potential to detect different hantaviruses were designed from conserved regions of different hantavirus L segments, as identified from multiple sequence alignments. By using SYBR-green-based QRT-PCR 100-1000 target molecules of in-vitro produced RNA and less than 100 copies of hantavirus RNA from different hantavirus clades and regions of the world were detected. When using the assay on clinical samples from patients with acute HFRS, Puumala hantavirus (PUUV) RNA was confirmed in all previously positive samples. Notably, the broad reacting L-segment QRT-PCR also detected viral RNA in HFRS patient samples, previously negative by a QRT-PCR targeting the S segment of PUUV. The authors concluded that this novel assay provides a powerful tool for diagnosis of hantaviruses from different clades and regions and may also be useful in surveys with the purpose of finding new hantaviruses in rodent or insectivore species.

UpToDate reviews on “Clinical features and diagnosis of chronic fatigue syndrome” (Gluckman, 2013), “Clinical manifestations and pathogenesis of human parvovirus B19 infection” (Jordan, 2013), and “Epidemiology and diagnosis of hantavirus infections” (Hjelle, 2013) do not mention the use of quantitative PCR.

An UpToDate review on "Polymerase chain reaction" (Raby, 2013) does not mention the use of PCR for giardia lamblia, vibrio cholera and yersinia enterocolitica.

An UpToDate review on “Epidemiology, clinical manifestations, and diagnosis of giardiasis” (Leder and Weller, 2013) states that “Research tools include serology, culture and polymerase chain reaction (PCR) techniques. Serologic tests are not of value in the diagnosis of acute giardiasis. IgG and IgM antibodies persist after infection so may be useful in epidemiologic studies. Studies utilizing quantitative PCR are facilitating increased understanding of the relationship between infection and clinical disease. In one study using PCR to detect Giardia in stool samples, parasite concentrations as low as 10 parasites/100 microL could be detected. This study also showed 100 percent correlation between PCR, microscopy and ELISA in patients with symptoms suggestive of giardiasis. PCR could also be a valuable tool for screening of water supplies”.

An UpToDate review on “Infection with less common Campylobacter species and related bacteria" (Allos, 2013) states that “Studies using PCR assays identified the organism more frequently in the stool samples of children recently diagnosed with Crohn’s than in samples from controls. Campylobacter concisus is a part of oral flora; it may contribute to the development of periodontal disease. Additionally, the organism has been identified in a brai abscess in a patient with chronic sinusitis”. There is no mentioning of vibrio cholerae and yersinia enterocolitica.

The American College of Medical Genetics and Genomics’ practice guideline on “MTHFR polymorphism testing” (Hickey et al, 2013) states that “MTHFR polymorphism testing is frequently ordered by physicians as part of the clinical evaluation for thrombophilia. It was previously hypothesized that reduced enzyme activity of MTHFR led to mild hyperhomocysteinemia which led to an increased risk for venous thromboembolism, coronary heart

disease, and recurrent pregnancy loss. Recent meta-analyses have disproven an association between hyperhomocysteinemia and risk for coronary heart disease and between MTHFR polymorphism status and risk for venous thromboembolism. There is growing evidence that MTHFR polymorphism testing has minimal clinical utility and, therefore should not be ordered as a part of a routine evaluation for thrombophilia".

Wikipedia states that "In a recent study, samples of lesions on the skin, eyes, and lung from 182 patients with presumed herpes simplex or herpes zoster were tested with quantitative PCR or with viral culture. In this comparison, viral culture detected VZV with only a 14.3 % sensitivity, although the test was highly specific (specificity = 100 %). By comparison, quantitative PCR resulted in 100 % sensitivity and specificity. Overall testin for herpes simplex and herpes zoster using PCR showed a 60.4 % improvement over viral culture. http://en.wikipedia.org/wiki/Herpes_zoster.

Furthermore, the CDC's Manual for the Surveillance of Vaccine-Preventable Diseases notes that real-time PCR [also known as quantitative PCR] has been designed that distinguishes vaccine strain from wild-type [varicella]; results rapidly available (within 3 hours). (Last updated April 1, 2014). http://www.cdc.gov/vaccines/pubs/surv-manual/chpt17-varicella.html.

Chikungunya Virus

The Centers for Disease Control and Prevention (CDC) states that preliminary diagnosis of chikungunya virus infection is based on the patient's clinical features, places and dates of travel, and activities (Staples, et al., 2014). Laboratory diagnosis is generally accomplished by testing serum to detect virus, viral nucleic acid, or virus-specific IgM and neutralizing antibodies.

During the first week after onset of symptoms, Chikungunya virus infection can often be diagnosed by using viral culture or RT-PCR on serum (Staples, et al., 2014). Chikungunyavirus-specific IgM and neutralizing antibodies normally develop toward the end of the first week of illness. Therefore, to definitively rule out the diagnosis, convalescent-phase samples should be obtained from patients whose acute-phase samples test negative.

Testing for Chikungunyavirus IgM and IgG is commercially available (Staples, et al., 2014). However, confirmatory neutralizing antibody testing is only available through CDC and a few state health laboratories.

CPT Codes / HCPCS Codes / ICD-9 Codes *PCR*

***testing for genetic or inherited disorder*: CPT**

codes covered if selection criteria are met:

81206 - 81208	BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis
81220 - 81224	CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis
81225	CYP2C19 (cytochrome P450, family 2, subfamily C, polypeptide 19) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3, *4, *8, *17)
81226	CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3, *4, *5, *6, *9, *10, *17, *19, *29, *35, *41, *1XN, *2XN, *4XN)
81227	CYP2C9 (cytochrome P450, family 2, subfamily C, polypeptide 9) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3, *5, *6)

81240	F2 (prothrombin, coagulation factor II) (eg, hereditary hypercoagulability) gene analysis, 20210G>A variant
81242	FANCC (Fanconi anemia, complementation group C) (eg, Fanconi anemia, type C) gene analysis, common variant (eg, IVS4+4A>T)
81243 - 81244	FMR1 (Fragile X mental retardation 1) (eg, fragile X mental retardation) gene analysis
81251	GBA (glucosidase, beta, acid) (eg, Gaucher disease) gene analysis, common variants (eg, N370S, 84GG, L444P, IVS2+1G>A)
81252 - 81253	GJB2 (gap junction protein, beta 2, 26kDa, connexin 26) (eg, nonsyndromic hearing loss) gene analysis; full gene sequence or known familiar variants
81255	HEXA (hexosaminidase A [alpha polypeptide]) (eg, Tay-Sachs disease) gene analysis, common variants (eg, 1278insTATC, 1421+1G>C, G269S)
81256	HFE (hemochromatosis) (eg, hereditary hemochromatosis) gene analysis, common variants (eg, C282Y, H63D)
81257	HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis, for common deletions or variant (eg, Southeast Asian, Thai, Filipino, Mediterranean, alpha3.7, alpha4.2, alpha20.5, and Constant Spring)
81261	IGH@ (Immunoglobulin heavy chain locus) (eg, leukemias and lymphomas, B-cell), gene rearrangement analysis to detect abnormal clonal population(s); amplified methodology (eg, polymerase chain reaction)
81264	IGK@ (Immunoglobulin kappa light chain locus) (eg, leukemia and lymphoma, B-cell), gene rearrangement analysis, evaluation to detect abnormal clonal population(s)
81270	JAK2 (Janus kinase 2) (eg, myeloproliferative disorder) gene analysis, p.Val617Phe (V617F) variant
81292 - 81294	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis
81295 - 81297	MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis
81298 - 81300	MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis
81315 - 81316	PML/RARalpha, (t(15;17)), (promyelocytic leukemia/retinoic acid receptor alpha) (eg, promyelocytic leukemia) translocation analysis
81317 - 81319	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis
81330 - 81331	SMPD1(sphingomyelin phosphodiesterase 1, acid lysosomal) (eg, Niemann-Pick disease, Type A) gene analysis, common variants (eg, R496L, L302P, fsP330)

81340 - 81342	TRB@ (T cell antigen receptor, beta) (eg, leukemia and lymphoma), gene rearrangement analysis to detect abnormal clonal population(s)
81370 - 81377	HLA Class I and II typing, low resolution (eg, antigen equivalents)
81378 - 81383	HLA Class I typing, high resolution (ie, alleles or allele groups)
81400 - 81408	Molecular pathology
86711	Antibody; JC (John Cunningham) virus
86828 - 86835	Antibody to human leukocyte antigens (HLA), solid phase assays (eg, microspheres or beads, ELISA, flow cytometry)

CPT codes not covered for indications listed in the CPB:

81210	BRAF (v-raf murine sarcoma viral oncogene homolog B1) (eg, colon cancer), gene analysis, V600E variant
81291	MTHFR (5,10-methylenetetrahydrofolate reductase) (eg, hereditary hypercoagulability) gene analysis, common variants (eg, 677T, 1298C)

HCPCS codes covered if selection criteria are met:

S3854	Gene expression profiling panel for use in the management of breast cancer treatment [see CPB 352 for criteria]

Other HCPCS codes related to the CPB:

J2323	Injection, natalizumab, 1 mg

ICD-9 codes covered if selection criteria are met:

153.0 - 154.1	Malignant neoplasm of colon, rectosigmoid junction, and rectum
172.0 - 172.9	Malignant melanoma of skin
200.20 - 200.28	Burkitt's tumor or lymphoma
200.30 - 200.38	Marginal zone lymphoma [Diffuse large B cell lymphoma (DLBCL)]
200.40 - 200.48	Mantle cell lymphoma
200.60 - 200.68	Anaplastic large cell lymphoma
200.70 - 200.78	Large cell lymphoma
201.00 - 201.98	Hodgkinâ€™s disease
202.00 - 202.08	Nodular lymphoma [Follicular]
202.10 - 202.18	Mycosis fungoides
202.70 - 202.78	Peripheral T-cell lymphoma
202.80 - 202.88	Other lymphomas [Small cell lymphocytic]

204.00 - 204.02	Acute lymphoid leukemia
204.10 - 204.12	Chronic lymphoid leukemia
205.00 - 205.02	Acute myeloid leukemia
205.10 - 205.12	Chronic myeloid leukemia
211.3	Benign neoplasm of colon
272.7	Lipodystrophy
275.0	Disorders of iron metabolism
277.00 - 277.09	Cystic fibrosis
282.60 - 282.69	Sickle cell disease
289.81	Primary hypercoagulable state
330.1	Cerebral lipidoses
330.8	Other cerebral degenerations in childhood
333.4	Cerebral ataxia in diseases classified elsewhere
579.0	Celiac disease
720.0	Ankylosing spondylitis
759.81	Prader-Willi syndrome
759.83	Fragile X syndrome
V59.0 - V59.9	Donors
V77.6	Special screening for cystic fibrosis
V78.2	Special screening for sickle-cell disease or trait

ICD-9 codes not covered for indications listed in the CPB:

185	Malignant neoplasm of prostate
202.40 - 202.48	Leukemic reticuloendotheliosis [hairy-cell leukemia]
207.80 - 207.82	Other specified leukemia [Xenotrophic murine leukemia]
233.1	Carcinoma in situ of cervix uteri
270.4	Disturbances of sulphur-bearing amino-acid metabolism
356.0 - 356.9	Hereditary and idiopathic peripheral neuropathy
622.10 - 622.12	Dysplasia of cervix (uteri)
785.6	Enlargement of lymph nodes [Castlemans disease]

V10.41	Personal history of malignant neoplasm of cervix uteri
V10.46	Personal history of malignant neoplasm of prostate
V76.3	Special screening for malignant neoplasm of skin
V76.44	Special screening for malignant neoplasm of prostate
V76.50 - V76.52	Special screening for malignant neoplasm of intestine

Other ICD-9 codes related to the CPB:

046.3	Progressive multifocal leukoencephalopathy
340	Multiple sclerosis
555.0 - 555.9	Crohn's disease

PCR testing for microbial identification - amplified probe:

CPT codes covered if selection criteria are met:

81302 - 81304	MECP2 (methyl CpG binding protein 2) (eg, Rett syndrome) gene analysis
87150	Culture, typing; identification by nucleic acid (DNA or RNA) probe, amplified probe technique, per culture or isolate, each organism probed
87471	Infectious agent detection by nucleic acid (DNA or RNA); Bartonella henselae and Bartonella quintana, amplified probe technique
87486	chlamydia pneumoniae, amplified probe technique
87491	chlamydia trachomatis, amplified probe technique
87493	clostridium difficile, toxin gene(s), amplified probe technique [not covered for asymptomatic persons or for "test of cure"]
87496	cytomegalovirus, amplified probe technique
87498	enterovirus, amplified probe technique
87501	Infectious agent detection by nucleic acid (DNA OR RNA); influenza virus, reverse transcription and amplified probe technique, each type or subtype
87502	Infectious agent detection by nucleic acid (DNA or RNA); influenza virus, for multiple types or subtypes, reverse transcription and amplified probe technique, first 2 types or subtypes
87503	Infectious agent detection by nucleic acid (DNA or RNA); influenza virus, for multiple types or sub-types, multiplex reverse transcription and amplified probe technique, each additional influenza virus type or sub-type beyond 2
87516	hepatitis B virus, amplified probe technique
87521	hepatitis C virus, amplified probe technique

87529	herpes simplex virus, amplified probe technique
87532	herpes virus-6, amplified probe technique
87535	HIV-1, amplified probe technique
87538	HIV-2, amplified probe technique
87556	mycobacterium tuberculosis, amplified probe technique
87581	mycoplasma pneumoniae, amplified probe technique
87591	neisseria gonorrhoeae, amplified probe technique
87621	papillomavirus, human, amplified probe technique
87631 - 87633	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), multiplex reverse transcription and amplified probe technique, multiple types or subtypes, 3-5 targets, 6-11 targets or 12-25 targets
87641	staphylococcus aureus, amplified probe technique
87651	streptococcus, group A, amplified probe technique
87653	streptococcus, group B, amplified probe technique
87661	trichomonas vaginalis, amplified probe technique
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87801	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; amplified probe(s) technique
87910 - 87912	Infectious agent genotype analysis by nucleic acid (DNA or RNA); cytomegalovirus or Hepatitis B virus

CPT codes not covered for indications listed in the CPB:

87476	Infectious agent detection by nucleic acid (DNA or RNA); Borrelia burgdorferi, amplified probe technique
87481	candida species, amplified probe technique
87511	gardnerella vaginitis, amplified probe technique
87526	hepatitis G virus, amplified probe technique
87541	legionella pneumophilia, amplified probe technique
87551	mycobacteria species, amplified probe technique
87561	mycobacteria avium-intracellulare, amplified probe technique

ICD-9 codes covered if selection criteria are met:

004.0 - 004.9	Shigellosis
006.0 - 006.9	Amebiasis
008.45	Clostridium difficile
010.0 - 018.9	Tuberculosis
021.0 - 021.9	Tularemia
023.0 - 023.9	Brucellosis
025	Melioidosis [when caused by Burkholderia infection]
033.0 - 033.9	Whooping cough
034.0	Streptococcal sore throat [Streptococcus, group A]
036.0	Meningococcal meningitis [Neisseria meningitis]
038.11	Methicillin susceptible staphylococcus aureus septicemia [to distinguish MRSA from non-resistant forms of S. aureus (not for detection of the mere presence of S. aureus)]
038.12	Methicillin resistant Staphylococcus aureus septicemia
039.0 - 039.9	Actinomycotic infections
040.2	Whipple's disease
041.02	Bacterial infection, streptococcus group B
041.11	Methicillin susceptible staphylococcus aureus [to distinguish MRSA from non-resistant forms of S. aureus (not for detection of the mere presence of S. aureus)]
041.12	Methicillin resistant Staphylococcus aureus
041.41 - 041.43	Shiga toxin-producing E. coli
041.5	Hemophilus influenza [H. influenza]
041.81	Mycoplasma [not covered for mycoplasma fermentans, genitalium, or penetrans]
041.86	Helicobacter pylori (H pylori)
042	Human immunodeficiency virus disease
045.0 - 045.9	Acute poliomyelitis
046.3	Progressive multifocal leukoencephalopathy
047.0 - 047.9	Meningitis due to enterovirus
048	Other enterovirus diseases of central nervous system
052.0 - 052.9	Chickenpox
053.0 - 053.9	Herpes zoster

054.0 - 054.9	Herpes simplex
055.0 - 055.9	Measles
056.00 - 056.9	Rubella
058.11	Roseola infantum due to human herpesvirus 6
058.21	Human herpesvirus 6 encephalitis
058.81	Human herpesvirus 6 infection
060.0 - 060.9	Yellow fever
061	Dengue
065.0	Crimean hemorrhagic fever (CHF Congo fever]
065.4	Dengue hemorrhagic fever
065.8	Other specified arthropod-borne hemorrhagic fever [Ebola virus]
066.1	Tick-borne fever [Colorado fever]
066.3	Other mosquito-borne fever
066.40 - 066.49	West Nile fever
070.20 - 070.33	Viral hepatitis B
070.41 - 070.44, 070.51 - 070.54, 070.70 - 070.71	Viral hepatitis C, hepatitis delta, and hepatitis E
072.0 - 072.9	Mumps
073.0 - 073.9	Ornithosis [Psittacosis]
076.0 - 076.9	Trachoma
077.0	Inclusion conjunctivitis
077.98	Unspecified diseases of conjunctiva due to chlamydiae
078.3	Cat-scratch disease
078.5	Cytomegaloviral disease
078.88	Other specified diseases due to chlamydiae
079.0	Adenovirus infection
079.1	ECHO virus
079.2	Coxackievirus
079.50 - 079.59	Retrovirus

079.6	Respiratory syncytial virus (RSV)
079.81	Hantavirus
079.82	SARS-associated coronavirus
079.83	Parvovirus B19
079.88	Other specified chlamydial infection
079.98	Unspecified chlamydial infection
080 - 081.0	Louse-borne [Epidemic] Typhus
082.0	Spotted fevers
082.40 - 082.49	Ehrlichiosis
083.0	Q Fever
084.0 - 084.9	Malaria
085.0 - 085.9	Leishmaniasus
088.0	Bartonellosis [not covered for Bartonella baciliformis]
088.82	Babesiosis
090.0 - 097.9	Syphilis
098 - 098.8	Gonococcal infections
099.0	Chancroid
099.1	Lymphogranuloma venereum
099.3	Reiter's disease
130.0 - 130.9	Toxoplasmosis
131.00 - 131.9	Trichomoniasis
238.4	Polycythemia vera
238.77	Post-transplant lymphoproliferative disorder (PTLD)
466.11	Acute bronchiolitis due to RSV
480.1	Pneumonia due to RSV
482.42	Methicillin resistant pneumonia due to Staphylococcus aureus
483.1	Pneumonia due to chlamydia
487.0	Influenza with pneumonia
487.1	Influenza with other respiratory manifestations

487.8	Influenza with other manifestations
488.01 - 488.09	Influenza due to identified avian influenza virus
488.11 - 488.19	Influenza due to identified novel H1N1 influenza virus
647.50 - 647.54	Rubella infection complicating pregnancy, childbirth, or the puerperium
771.0	Congenital Rubella
786.2	Cough
787.91	Diarrhea [for Clostridium difficile diagnosis]
795.00 - 795.05	Abnormal papanicolaou smear of cervix and cervical HPV
795.71	Nonspecific serologic evidence of HIV
V01.4	Contact with or exposure to rubella
V01.71	Contact with or exposure to varicella
V01.79	Contact with or exposure to other viral diseases
V01.82	Contact with or exposure to SARS-associated coronavirus
V02.61	Hepatitis B carrier
V02.62	Hepatitis C carrier
V02.7	Gonorrhea
V02.8	Other venereal diseases
V08	Asymptomatic HIV infection status
V09.0	Infection with microorganisms resistant to penicillins
V42.0	Kidney replaced by transplant
V69.2	High-risk sexual behavior
V72.32	Encounter for papanicolaou cervical smear to confirm findings of recent normal smear following initial abnormal smear
V73.0	Special screening examination for poliomyelitis
V73.3	Special screening examination for rubella
V73.4	Special screening examination for rubella
V73.88	Other specified chlamydial diseases
V73.89	Special screening examination for other specified viral diseases
V73.98	Special screening examination for unspecified chlamydial disease

V74.1	Special screening examination for pulmonary tuberculosis
V74.5	Special screening examination for venereal disease [not covered for routine screening of trichomonas in asymptomatic men and women]

ICD-9 codes not covered for indications listed in the CPB (not all-inclusive):

003.0 - 003.9	Other salmonella infections
007.1	Giardiasis [Giardia lamblia]
007.4	Cryptosporidiosis
007.5	Cyclosporiasis
008.2	Aerobacter aerogenes [Enterobacter aerogenes]
008.43	Campylobacter
008.44	Intestinal infections due to yersinia enterocolitica
008.66	Astrovirus
027.0	Listeriosis
038.2	Pneumococcal septicemia
038.42	Escherichia coli [E. coli] septicemia
038.43	Pseudomonas septicemia
038.44	Serratia septicemia
041.00 - 041.01 041.03 - 041.09	Bacterial infection Streptococcus [other than group B]
041.10, 041.19	Unspecified and other Staphylococcus [Coagulase-negative staphylococcus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Staphylococcus lugdunensis]
041.2	Pneumococcus
041.49	Other and unspecified escherichia coli [E. coli] [non-Shiga toxin producing E. coli]
041.6	Proteus (mirabilis) (morganii)
041.7	Pseudomonas
041.82	Bacteroides fragilis
041.85	Other gram-negative organisms [Acinetobacter baumannii, Enterobacter cloacae, Stenotrophomonas maltophilia, Vibrio vulnificus, Vibrio cholerae]
046.11 - 046.19	Jakob-Creutzfeldt disease
057.8	Other specified viral exanthemata

058.10	Roseola infantum, unspecified
058.12	Roseola infantum due to human herpesvirus 7
058.29	Other human herpesvirus encephalitis
058.82	Human herpesvirus 7 infection
058.89	Other human herpesvirus infection
070.49, 070.59	Other specified viral hepatitis [GB virus type C]
078.0	Molluscum contagiosum
079.4	Human papillomavirus
079.89	Other specified viral infection [Coronavirus other than SARS-coronavirus] [Parainfluenza virus]
088.81	Lyme disease
099.2	Granuloma inguinale
100.0 - 100.9	Leptospirosis
110.1	Onychomycosis
111.2	Tinea blanca
112.0 - 112.9	Candidiasis
114.0 - 114.9	Coccidioidomycosis
115.00 - 115.09	Infection by Histoplasma capsulatum
116.0 - 116.2	Blastomycotic infections
117.1	Sporotrichosis
117.3	Aspergillosis
117.5	Cryptococcosis
136.3	Pneumocystosis
176.0 - 176.9	Kaposi's sarcoma
202.40 - 202.48	Leukemic reticuloendotheliosis [hairy-cell leukemia]
233.1	Carcinoma in situ of cervix uteri
279.41	Autoimmune lymphoproliferative syndrome
331.0	Alzheimer's disease
414.00 - 414.07	Coronary atherosclerosis

429.2	Cardiovascular disease, unspecified
440.0 - 440.9	Atherosclerosis
446.1	Acute febrile mucocutaneous lymph node syndrome [MCLS] [Kawasaki disease]
481	Pneumococcal pneumonia [Streptococcus pneumoniae pneumonia]
482.0	Pneumonia due to Klebsiella pneumoniae
482.1	Pneumonia due to Pseudomonas
482.83 - 482.49	Pneumonia due to Streptococcus pneumoniae
493.00 - 493.92	Asthma
531.00 - 534.91	Gastric, duodenal, peptic or gastrojejunal ulcer
616.10	Vaginitis and vulvovaginitis, unspecified [bacterial vaginosis associated bacteria 2 (BVAB2), megasphaera type 2]
616.11	Vaginitis and vulvovaginitis in diseases classified elsewhere [bacterial vaginosis associated bacteria 2 (BVAB2), megasphaera type 2]
622.10 - 622.12	Dysplasia of cervix (uteri)
647.40 - 647.44	Malaria in pregnancy
771.1	Malaria in pregnancy
780.71 - 780.79	Malaise and fatigue
795.05	Cervical high risk human papillomavirus (HPV) DNA test positive
V09.80 - V09.81	Infection with microorganisms resistant to other specified drugs [vancomycin-resistant enterococcus]
V10.41	Personal history of malignant neoplasm of cervix uteri
V12.71	Personal history of peptic ulcer disease
V22.0 - V22.2	Supervision of normal pregnancy [not covered for routine screening of trichomonas in asymptomatic men and women]
V25.40 - V25.49	Surveillance of previously prescribed contraceptive methods [not covered for routine screening of trichomonas in asymptomatic men and women]
V28.6	Antenatal screening for Streptococcus B
V70.0	General medical examinations [not covered for routine screening of trichomonas in asymptomatic men and women]
V72.31	Routine gynecological examinations [not covered for routine screening of trichomonas in asymptomatic women]
V73.81	Special screening for human papillomavirus (HPV)

V74.8	Special screening examination for other specific bacterial and spirochetal diseases [not covered for routine screening of trichomonas in asymptomatic men and women]
V74.9	Special screening examination for other specific bacterial and spirochetal diseases [not covered for routine screening of trichomonas in asymptomatic men and women]
V75.1	Special screening for malaria
V81.2	Special screening for other and unspecified cardiovascular conditions
V81.6	Special screening for other and unspecified genitourinary conditions [not covered for routine screening of trichomonas in asymptomatic men and women]

PCR testing for microbial identification - quantification:

CPT codes covered if selection criteria are met:

87497	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus, quantification
87517	hepatitis B virus, quantification
87522	hepatitis C virus, quantification
87533	Herpes virus-6 quantification
87536	HIV-1, quantification
87539	HIV-2, quantification
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism

CPT codes not covered for indications listed in the CPB:

87472	Infectious agent detection by nucleic acid (DNA or RNA); Bartonella (B. henselae, B Quintana), quantification
87477	Borrelia burgdorferi, quantification
87482	Candida species, quantification
87487	Chlamydia pneumoniae, quantification
87512	Gardnerella vaginitis, quantification
87527	hepatitis G, quantification
87530	Herpes simplex virus, quantification
87533	Herpes virus-6, quantification
87542	Legionella pneumophilia, quantification
87562	Mycobacteria avium-intracellulare, quantification
87582	Mycoplasma pneumoniae, quantification

87592	Neisseria gonorrhoeae, quantification
87652	Streptococcus, group A, quantification

ICD-9 codes covered if selection criteria are met:

042	Human immunodeficiency virus disease
052.0 - 052.9	Chickenpox [for diagnosis and also to distinguish wild-type virus from vaccination in previously immunized persons with signs or symptoms of varicella-zoster infection]
058.11	Roseola infantum due to human herpesvirus 6
058.21	Human herpesvirus 6 encephalitis
058.81	Human herpesvirus 6 infection
070.20 - 070.33	Viral hepatitis B
070.41, 070.44, 070.51, 070.54, 070.70, 070.71	Viral hepatitis C
078.5	Cytomegaloviral disease
079.0	Adenovirus infection [to diagnose and monitor infection in hematopoietic stem cell transplant recipients]
771.1	Congenital cytomegalovirus infection
996.81	Complications of transplanted kidney
V08	Asymptomatic HIV infection status
V42.0	Kidney replaced by transplant
V42.82	Organ or tissue replaced by transplant, peripheral stem cells

ICD-9 codes not covered for indications listed in the CPB:

031.0 - 031.9	Diseases due to other mycobacterium
041.81	Mycoplasma
041.89	Other specified bacteria [gardnerella vaginalis]
053.0 - 053.9	Herpes zoster
054.0 - 054.9	Herpes simplex
058.11	Roseola infantum due to human herpesvirus 6
058.12	Roseola infantum due to human herpesvirus 7
058.21	Human herpesvirus 6 encephalitis
058.29	Other human herpesvirus encephalitis

058.81	Human herpesvirus 6 infection
058.82	Human herpesvirus 7 infection
058.89	Other human herpesvirus infection
070.49, 070.59	Other specified viral hepatitis [GB virus type C]
079.81	Hantavirus infection
079.83	Parvovirus B19
079.88	Other specified chlamydial infection
079.98	Unspecified chlamydial infection
088.0	Bartonellosis
088.81	Lyme disease
098.0 - 098.8	Gonococcal infections
112.0 - 112.9	Candidiasis
482.84	Legionnaires' disease
483.1	Pneumonia due to chlamydia
487.0 - 487.8	Influenza
488.01 - 488.09	Influenza due to certain identified influenza viruses
616.10	Vaginitis and vulvovaginitis, unspecified [gardnerella vaginalis]
616.11	Vaginitis and vulvovaginitis in diseases classified elsewhere [gardnerella vaginalis]
780.71	Chronic fatigue syndrome [secondary to parvovirus B19 infection]

The above policy is based on the following references:

1. Centers for Disease Control and Prevention (CDC). Sexually Transmitted Diseases Treatment Guidelines -- - 2002. MMWR. 2002;51(RR 6):1-80. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5106a1.htm. Accessed July 1, 2002.
2. Centers for Disease Control and Prevention (CDC), National Center for Infectious Diseases, Division of Parasitic Diseases. Babesiosis. Parasites and Health. DPDx. Identification and Diagnosis of Parasites of Public Health Concern. Atlanta, GA: CDC; January 31, 2002. Available at: http://www.dpd.cdc.gov/dpdx/HTML/Babesiosis.htm. Accessed July 1, 2002.
3. Centers for Disease Control and Prevention (CDC), National Center for Infectious Diseases. Diagnosis of CFS. CFS Information. Chronic Fatigue Syndrome. Atlanta, GA: CDC; February 4, 2002. Available at: http://www.cdc.gov/ncidod/diseases/cfs/diagnosis.htm#Theoretical. Accessed July 1, 2002.
4. Chronic Fatigue and Immune Dysfunction Syndrome (CFIDS) Association of America. Diagnosis: Viruses. About CFIDS. Charlotte, NC: CFIDS Association of America, Inc.; 2001. Available at: http://www.cfids.org/about-cfids/viruses.asp. Accessed July 1, 2002.

5. Craig T, Kakumanu S. Chronic fatigue syndrome: Evaluation and treatment. Am Fam Physician. 2002;65:1083-1090,1095. Available at: http://www.aafp.org/afp/20020315/1083.html. Accessed July 1, 2002.
6. U.S. Department of Defense (DoD), Veterans Health Administration (VHA). Management of medically unexplained symptoms: Chronic Pain and Fatigue Working Group. VHA/DoD clinical practice guideline for the management of medically unexplained symptoms: Chronic pain and fatigue. Washington, DC: VHA, DoD; July 2001.
7. American College of Physicians - American Society of Internal Medicine. Guidelines for laboratory evaluation in the diagnosis of Lyme disease. Clinical Guideline, Part 1. Ann Intern Med. 1997;127:1106-1108. Available at: http://www.acponline.org/journals/annals/15dec97/pplyme1.htm. Accessed July 1, 2002.
8. Tugwell P, Dennis DT, Weinstein A, et al. Laboratory evaluation in the diagnosis of Lyme disease. Clinical Guideline, Part 2. Ann Intern Med. 1997;127:1109-1123. Available at: http://www.acponline.org/journals/annals/15dec97/pplyme2.htm. Accessed July 1, 2002.
9. Centers for Disease Control and Prevention (CDC), National Center for Infectious Diseases, Division of Vector-Borne Infectious Diseases. Lyme disease. Diagnosis. CDC Lyme Disease Home Page. Ft. Collins, CO: CDC; September 19, 2001. Available at: http://www.cdc.gov/ncidod/dvbid/lyme/diagnosis.htm. Accessed July 1, 2002.
10. National Institutes of Health (NIH), National Institute of Arthritis and Infectious Diseases, Division of Microbiology and Infectious Diseases. Diagnosis of Lyme disease. Lyme Disease Research. Bethesda, MD NIH; January 5, 2001. Available at: http://www.niaid.nih.gov/dmid/lyme/diagnosis.htm. Accessed July 1, 2002.
11. Wormser GP, Nadelman RB, Dattwyler RJ, et al. Practice Guidelines for the Treatment of Lyme Disease. Guidelines from the Infectious Diseases Society of America. Clin Infectious Diseases. 2000;31:1-14. Available at: http://www.journals.uchicago.edu/CID/journal/issues/v31nS1/000342/000342.html. Accessed July 1, 2002.
12. American Lyme Disease Foundation, Inc. (ALDF). Testing (medical). Frequently Asked Questions. Somers, NY: ALDF; March 15, 2002. Available at: http://www.aldf.com/FAQ.asp#PARA18. Accessed July 1, 2002.
13. Puotinen JL, Cheng EY, Peltier W, et al. Lyme disease. eMedicine J. 2002;3(1). Available at: http://www.emedicine.com/NEURO/topic521.htm. Accessed July 1, 2002.
14. Edlow JA. Lyme disease. eMedicine J. 2001;2(10). Available at: http://www.emedicine.com/derm/topic536.htm. Accessed July 1, 2002.
15. Waites KB. Ureaplasma infection. eMedicine J. 2002;3(1). Available at: http://www.emedicine.com/med/topic2340.htm. Accessed July 1, 2002.
16. Elrod W, Plewa MC. Urethritis, male. eMedicine J. 2001;2(9). Available at: http://www.emedicine.com/emerg/topic623.htm. Accessed July 1, 2002.
17. Hale BR. Trichosporon infections. eMedicine J. 2002;3(4). Available at: http://www.emedicine.com/MED/topic2310.htm. Accessed July 1, 2002.
18. Shanley JD. Papillomavirus. eMedicine J. 2002;3(3). Available at: http://www.emedicine.com/med/topic1729.htm. Accessed July 1, 2002.
19. Centers for Disease Control and Prevention (CDC), Viral and Rickettsial Zoonoses Branch. Rocky Mountain spotted fever. Laboratory detection. Atlanta, GA: CDC; August 7, 2000. Available at: http://www.cdc.gov/ncidod/dvrd/rmsf/Laboratory.htm. Accessed July 9, 2002.
20. Centers for Disease Control and Prevention (CDC), Viral and Rickettsial Zoonoses Branch. Human Ehrlichiosis in the United States. Laboratory detection. Atlanta, GA: CDC; April 5, 2000. Available at: http://www.cdc.gov/ncidod/dvrd/ehrlichia/Laboratory/Laboratory.htm. Accessed July 9, 2002.
21. Varde S, Beckley J, Schwartz I. Prevalence of tick-borne pathogens in Ixodes scapularis in a rural New Jersey county. Emerg Infect Dis. 1998;4(1):97-99.
22. Health Canada, Laboratory Centre for Disease Control (LCDC) Expert Working Group on Canadian Guidelines for Sexually Transmitted Disease. Canadian STD Guidelines. 1998 ed. Ottawa, ON: LCDC; 1998.

23. Centers for Disease Control and Prevention (CDC), National Center for Infectious Diseases, Division of Vector-Borne Infectious Diseases. Lyme disease. CDC Wonder. Atlanta, GA: CDC: January 1, 1991.
24. Alberta Medical Association, Alberta Clinical Practice Guidelines Working Group for Microbiology. Laboratory testing for serological testing for suspected viral hepatitis. Alberta Clinical Practice Guideline Program. Edmonton, AB; Alberta Clinical Practice Guidelines Program; March 1997 (reviewed September 2000).
25. Ontario Medical Laboratories Association. Guidelines for testing for viral hepatitis. Guideline No. CLP012. North York, ON: Ontario Association of Medical Laboratories; March 2000.
26. Collège des médecins du Québec. Chronic fatigue syndrome. Clinical Practice Guidelines. Montreal, QC: Collège des médecins du Québec; August 1998.
27. Health Canada, Laboratory Centre for Disease Control (LCDC). Expert working group on HHV-6 and 7 laboratory diagnosis and testing. Canada Communicable Disease Report. 2000;26S4.
28. No authors listed. Babesiosis - Connecticut. MMWR Morb Mortal Wkly Rep. 1989;38(38):649-650.
29. Shivers SC, Wang X, Li W, et al. Molecular staging of malignant melanoma: Correlation with clinical outcome. N Engl J Med. 1998;280:1410-1415.
30. Smith B, Selby P, Southgate J, et al. Detection of melanoma cells in peripheral blood by means of reverse transcriptase and polymerase chain reaction. Lancet. 1991;338:1227-1229.
31. Centers for Disease Control and Prevention (CDC), National Center for Infectious Diseases, Respiratory and Enteric Viruses Branch. Adenoviruses. Disease Information. Atlanta, GA: CDC; August 20, 2001. Available at: http://www.cdc.gov/ncidod/dvrd/revb/respiratory/eadfeat.htm. Accessed July 9, 2002.
32. Lindblom A, Liljegren A. Regular review: Tumour markers in malignancies. Br Med J. 2000;320:424-427.
33. Scottish Intercollegiate Guidelines Network (SIGN). Management of genital Chlamydia trachomatis infection. A national clinical guideline. SIGN Pub. No. 42. Edinburgh, Scotland: SIGN; March 2000.
34. Wright TC Jr, Cox JT, Massad LS, et al. 2001 Consensus Guidelines for the management of women with cervical cytological abnormalities. JAMA. 2002;287:2120-2129.
35. Sabella C, Goldfarb J. Parvovirus B19 infections. Am Fam Physician. 1999;60:1455-1460.
36. No authors listed. Human exposure to brucella abortus strain RB51 - Kansas, 1997. MMWR Morb Mortal Wkly Rep. 1998;47(9):172-175.
37. Centers for Disease Control and Prevention. Appendix A. Methods for diagnosing avian chlamydiosis. MMWR. 1998;47(RR10):10-12.
38. Sherman M. Management of viral hepatitis: Clinical and public health perspectives - a consensus statement CASL Hepatitis Consensus Group. Canadian Association for Study of the Liver. Can J Gastroenterol. 1997;11(5):407-416.
39. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of *Chlamydia trachomatis* genital tract infection. London, UK: AGUM, MSSVD; 2002.
40. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of bacterial vaginosis. London, UK: AGUM, MSSVD 2002.
41. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of genital herpes. London, UK: AGUM, MSSVD; 2002.
42. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline on the management of gonorrhoea in adults. London, UK: AGUM, MSSVD; 2002.
43. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline on the management of trichomonas vaginalis. London, UK: AGUM, MSSVD; 2002.

44. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline on the management of vulvovaginal candidiasis. London, UK: AGUM, MSSVD; 2002.
45. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of anogenital warts. London, UK: AGUM, MSSVD; 2002.
46. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of balanitis. London, UK: AGUM, MSSVD; 2002.
47. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of chancroid. London, UK: AGUM, MSSVD; 2002.
48. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of donovanosis (granuloma inguinale). London, UK: MGUM, MSSVD; 2002.
49. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of early syphilis. London, UK: AGUM, MSSVD; 2002.
50. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of epididymo-orchitis. London, UK: AGUM, MSSVD; 2002.
51. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of late syphilis. London, UK: AGUM, MSSVD; 2002.
52. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of lymphogranuloma venereum. London, UK: AGUM, MSSVD; 2002.
53. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of molluscum contagiosum. London, UK: AGUM, MSSVD; 2002.
54. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of non-gonococcal urethritis. London, UK: AGUM, MSSVD; 2002.
55. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of pelvic infection and perihepatitis. London, UK: AGUM, MSSVD; 2002.
56. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of sexually acquired reactive arthritis. London, UK: AGUM, MSSVD; 2002.
57. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Diseases (MSSVD). 2002 national guideline for the management of the viral hepatitides A, B, and C. London, UK: AGUM, MSSVD; 2002.
58. Association for Genitourinary Medicine (AGUM), Medical Society for the Study of Venereal Disease (MSSVD). 2002 national guideline for the management of prostatitis. London, UK: AGUM, MSSVD; 2002.
59. Health Canada, Health Protection Branch, Laboratory Centre for Disease Control. Hepatitis C - prevention and control: A public health consensus. Ottawa, Canada, October 14 - 16, 1998. Can Commun Dis Rep. 1999;25 Suppl 2:I-iii, 1-25.
60. Canadian Paediatric Society (CPS), Infectious Diseases and Immunization Committee. Cat scratch disease Diagnosis and management. Paediatr Child Health. 1997;2(4):275-278 (reaffirmed April 2002).
61. Society of Obstetricians and Gynaecologists of Canada (SOGC), Policy and Practice Guideline Committee. Human papillomavirus infections: Guidelines for investigation and management. SOGC Clinical Practice Guidelines. Policy Statement No. 69. J Soc Obstet Gynaecol Can. 1997;19:1435-1437.

62. Stevens DA, Kan VL, Judson MA, et al. Practice guidelines for diseases caused by Aspergillus. Guidelines from the Infectious Diseases Society of America. Clin Infect Diseases. 2000;30:696-709.
63. Saag MS, Graybill RJ, Larsen RA, et al. Practice guidelines for the management of cryptococcal disease. Guidelines from the Infectious Diseases Society of America. Clin Infect Diseases. 2000;30:710-718.
64. Infectious Diseases Society of America (IDSA). Practice guidelines for the management of patients with sporotrichosis. Alexandria, VA: ISDA; April 2000.
65. Infectious Diseases Society of America (ISDA). Practice guidelines for the treatment of candidiasis. Alexandria, VA: ISDA; April 2000.
66. Infectious Diseases Society of America (ISDA). Practice guidelines for the treatment of coccidiodomycosis. Alexandria, VA: ISDA; April 2000.
67. Infectious Diseases Society of America (ISDA). Practice guidelines for the treatment of Lyme disease. Alexandria, VA: ISDA; July 2000.
68. Infectious Diseases Society of America (ISDA). Practice guidelines for the treatment of tuberculosis. Alexandria, VA: ISDA; September 2000.
69. U.S. Preventive Services Task Force. Screening for bacterial vaginosis in pregnancy: recommendations and rationale. Recommendations of the United States Preventive Services Task Force. 3rd ed. Rockville, MD: Agency for Healthcare Research and Quality; 2001.
70. U.S. Preventive Services Task Force. Screening for chlamydial infection: recommendations and rationale. Recommendations of the United States Preventive Services Task Force. Rockville, MD: Agency for Healthcare Research and Quality; 2001.
71. National Institutes of Health (NIH) Consensus Development Panel on Management of Hepatitis C. Management of hepatitis C. Bethesda, MD: NIH; March 1997.
72. No authors listed. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. MMWR Recomm Rep. 1998;47(RR-19);1-39.
73. No authors listed. Recommendations to prevent hepatitis B virus transmission-United States-Update. MMWR Morb Mortal Wkly Rep. 1999;48(2):33-34.
74. No authors listed. Recommendations for the prevention and management of Chlamydia trachomatis infections, 1993. MMWR Recomm Rep. 1993;42(RR-12):1-39.
75. Williams HA, Roberts J, Kachur SP, et al. Malaria surveillance - United States, 1995. MMWR CDC Surveill Summ. 1999;48(1):1-23.
76. No authors listed. Recommendations for the use of Lyme disease vaccine. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 1999;48(RR-7):1-17, 21-25.
77. No authors listed. Prevention of varicella. Updated Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 1999;48(RR-6):1-5.
78. No authors listed. Guidelines for the prevention and control of congenital syphilis. MMWR Morb Mortal Wkly Rep. 1988;37(Suppl 1):1-13.
79. No authors listed. 1999 USPHS/IDSA guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus. MMWR Recomm Rep. 1999;48(RR-10);1-59, 61-66.
80. Holmes GP, Chapman LE, Stewart JA, et al. Guidelines for the prevention and treatment of B-virus infections in exposed persons. Clin Infect Dis. 1995;20:421-439.
81. Harper SA, Fukuda K, Uyeki TM, et al. Prevention and control of influenza: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2004;53(RR-6):1-40..
82. No authors listed. Management of hepatitis C. NIH Consens Statement. 1997;15(3):1-41.
83. American College of Obstetricians and Gynecologists (ACOG). Diagnosis and treatment of cervical carcinomas. ACOG Practice Bulletin No. 35. Clinical Management Guidelines for Obstetrician-Gynecologists. Washington, DC: ACOG; May 2002.
84. World Health Organization, Regional Office for the Western Pacific (WPRO). Laboratory Tests for the Detection of Reproductive Tract Infections. Mannilla, Phillipines: WPRO; 1999.

85. Centers for Disease Control and Prevention (CDC), Division of Bacterial and Mycotic Diseases. Aspergillosis. Disease Information. Atlanta, GA: CDC; March 6, 2001. Available at: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/aspergillosis_t.htm. Accessed July 9, 2002.
86. Seiden MV, Kantoff PW, Krithivas K, et al. Detection of circulating tumor cells in men with localized prostate cancer. J Clin Oncol. 1994;12(12):2634-2639.
87. de la Taille A, Olsson CA, Katz AE. Molecular staging of prostate cancer: Dream or reality? Oncology (Huntingt). 1999;13:187-198, 204-205 passim.
88. Oh WK, Hurwitz M, D'Amico AV, et al. Neoplasms of the prostate. In: Holland and Frei Cancer Medicine. 5t ed. RC Bast, DW Kufe, RE Pollock, et al., eds. Hamilton, ON: BC Decker; 2001.
89. Collins JF, Donta ST, Engel CC, et al. The antibiotic treatment trial of Gulf War Veterans' Illnesses: Issues, design, screening, and baseline characteristics. Control Clin Trials. 2002;23(3):333-353.
90. Chingbingyong MI, Hughes CV. Detection of Mycoplasma fermentans in human saliva with a polymerase chain reaction-based assay. Arch Oral Biol. 1996;41(3):311-314.
91. Katseni VL, Gilroy CB, Ryait BK, et al. Mycoplasma fermentans in individuals seropositive and seronegative for HIV-1. Lancet. 1993;341:271-273.
92. Kovacic R, Launey V, Tuppin P, et al. Search for the presence of six Mycoplasma species in peripheral blood mononuclear cells of subjects seropositive and seronegative for human immunodeficiency virus. J Clin Microbiol. 1996;34:1808-1810.
93. Taylor-Robinson D, Schaeverbeke T. Mycoplasmas in rheumatoid arthritis and other human arthritides. J Clin Pathol. 1996;49(10):781-782.
94. Schaeverbeke T, Gilroy CB, Bébéar C, et al. Mycoplasma fermentans, but not M. penetrans, detected by PCR assays in synovium from patients with rheumatoid arthritis and other rheumatic disorders. J Clin Pathol. 1996;49:824-828.
95. Gilroy CB, Keat A, Taylor-Robinson D. The prevalence of Mycoplasma fermentans in patients with inflammatory arthritides. Rheumatology (Oxford). 2001;40(12):1355-1358.
96. Ainsworth JG, Clarke J, Lipman M, et al. Detection of Mycoplasma fermentans in broncho-alveolar lavage fluid specimens from AIDS patients with lower respiratory tract infection. HIV Med. 2000;1(4):219-223.
97. Ainsworth JG, Easterbrook PJ, Clarke J, et al. An association of disseminated Mycoplasma fermentans in HIV-1 positive patients with non-Hodgkin's lymphoma. Int J STD AIDS. 2001;12(8):499-504.
98. Lo SC, Levin L, Ribas J, et al. Lack of serological evidence for Mycoplasma fermentans infection in army Gulf War veterans: A large scale case-control study. Epidemiol Infect. 2000;125(3):609-616.
99. Horowitz S, Evinson B, Borer A, et al. Mycoplasma fermentans in rheumatoid arthritis and other inflammatory arthritides. J Rheumatol. 2000;27(12):2747-2753.
100. Ainsworth JG, Clarke J, Goldin R, et al. Disseminated Mycoplasma fermentans in AIDS patients: Several case reports. Int J STD AIDS. 2000;11(11):751-755.
101. Henry CH, Hughes CV, Gerard HC, et al. Reactive arthritis: Preliminary microbiologic analysis of the human temporomandibular joint. J Oral Maxillofac Surg. 2000;58(10):1137-1144.
102. Ainsworth JG, Hourshid S, Webster AD, et al. Detection of Mycoplasma fermentans in healthy students and patients with congenital immunodeficiency. J Infect. 2000;40(2):138-140.
103. Buskila D. Fibromyalgia, chronic fatigue syndrome, and myofascial pain syndrome. Curr Opin Rheumatol. 2000;12(2):113-123.
104. Nasralla M, Haier J, Nicolson GL. Multiple mycoplasmal infections detected in blood of patients with chronic fatigue syndrome and/or fibromyalgia syndrome. Eur J Clin Microbiol Infect Dis. 1999;18(12):859-865.
105. Johnson S, Sidebottom D, Bruckner F, Collins D. Identification of Mycoplasma fermentans in synovial fluid samples from arthritis patients with inflammatory disease. J Clin Microbiol. 2000;38(1):90-93.
106. Shibata K, Kaga M, Kudo M, et al. Detection of Mycoplasma fermentans in saliva sampled from infants, preschool and school children, adolescents and adults by a polymerase chain reaction-based assay. Microbiol Immunol. 1999;43(6):521-525.
107. Gray GC, Kaiser KS, Hawksworth AW, et al. No serologic evidence of an association found between Gulf War service and Mycoplasma fermentans infection. Am J Trop Med Hyg. 1999;60(5):752-757.

108. Vojdani A, Choppa PC, Tagle C, et al. Detection of Mycoplasma genus and Mycoplasma fermentans by PCR in patients with Chronic Fatigue Syndrome. FEMS Immunol Med Microbiol. 1998;22(4):355-365.
109. Horowitz S, Horowitz J, Hou L, et al. Antibodies to mycoplasma fermentans in HIV-positive heterosexual patients: Seroprevalence and association with AIDS. J Infect. 1998;36(1):79-84.
110. Guerrant RL, Van Gilder T, Steiner TS, et al. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis. 2001;32(3):331-351.
111. British Columbia Medical Association (BCMA), Guidelines and Protocols Advisory Committee. Clinical approach to adult patients with dyspepsia. G&P2001-046. Victoria, BC: BCMA; revised 2001.
112. British Columbia Medical Association (BCMA), Guidelines and Protocols Advisory Committee. Clinical approach to adult patients with gastroesophageal reflux disease. Victoria, BC: BCMA; revised 2001.
113. British Columbia Medical Association (BCMA), Guidelines and Protocols Advisory Committee. Investigation of suspected infectious diarrhea. Victoria, BC: BCMA; 1997 (reviewed 2000).
114. British Columbia Medical Association (BCMA), Protocol Steering Committee. Protocol for viral hepatitis testing. Victoria, BC: BCMA; 1998.
115. British Columbia Medical Association (BCMA), Guidelines and Protocols Advisory Committee. Protocol for detection and treatment of Helicobacter pylori infection in adults. Victoria, BC: BCMA; 2000.
116. Veldhuyzen van Zanten SJO, Flook N, Chiba N, et al. An evidence-based approach to the management of uninvestigated dyspepsia in the era of Helicobacter pylori. Canadian Dyspepsia Working Group. CMAJ. 2000;162(12 Supp): S1-S23.
117. American Academy of Pediatrics (AAP), Committee on Infectious Diseases. 2003 Red Book: Report of the Committee on Infectious Diseases. 26th ed. LK Pickering, G Peter, CJ Baker, et al., eds. Elk Grove Village, IL: AAP; 2003.
118. Beers MH, Berkow R. The Merck Manual of Diagnosis and Therapy. 17th ed. Centennial Edition. Whitehouse Station, NJ: Merck & Co.; 1999.
119. Cunningham D, Rennels MB. Parvovirus B19 infection. eMedicine J. 2002;3(2). Available at: http://www.emedicine.com/ped/topic192.htm. Accessed September 11, 2002.
120. University of Michigan Medical Center Peptic Ulcer Guideline Team. Peptic ulcer disease. Guidelines for Clinical Care. Ann Arbor, MI: University of Michigan Health System; 1999.
121. Urist MM, University of Alabama at Birmingham Comprehensive Cancer Center. Phase III study of adjuvant interferon alfa-2b in patients with invasive cutaneous melanoma with early lymph node metastasis detected by intraoperative lymphatic mapping and sentinel lymph node biopsy. UAB-9735, clinical trial, active, 08/24/2001 (summary last modified October 2001).
122. Gajewski TF, University of Chicago Cancer Research Center. Diagnostic study of reverse transcriptase-polymerase chain reaction to detect melanoma markers in lymph nodes or peripheral blood of patients with melanoma. UCCRC-9308, clinical trial, active, 06/01/1998 (summary last modified June 2000).
123. National Cancer Institute (NCI). Melanoma (PDQ): Treatment. Health Professionals. Bethesda, MD: NCI; July 2002.
124. National Cancer Institute (NCI). Cervical Cancer (PDQ): Treatment. Health Professionals. Bethesda, MD: NCI; August 2002.
125. National Cancer Institute (NCI). Colon Cancer (PDQ): Treatment. Health Professionals. Bethesda, MD: NCI September 2002.
126. National Comprehensive Cancer Network (NCCN). Practice Guidelines in Oncology. Rockledge, PA: NCCN; 2003.
127. Benson AB, Desch CE, Flynn PJ, et al. 2000 Update of American Society of Clinical Oncology Colorectal Cancer Surveillance Guidelines. Alexandria, VA: ASCO; 2000.
128. Glantz JC, Kedley KE. Concepts and controversies in the management of group B streptococcus during pregnancy. Birth. 1998;25(1):45-53.
129. Medical Services Advisory Committee (MSAC). Hepatitis C viral load testing. Canberra, Australia: MSAC; 2000.

130. Birkebaek NH. Bordetella pertussis in the aetiology of chronic cough in adults. Diagnostic methods and clinic. Dan Med Bull. 2001;48(2):77-80.
131. von Konig CH, Halperin S, Riffelmann M, Guiso N. Pertussis of adults and infants. Lancet Infect Dis. 2002;2 (12):744-750.
132. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A,. Prevention of perinatal group B streptococcal disease, MMWR Morbid Mortal Wkly Rep. 2002;51(RR11):1-22. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5111a1.htm. Accessed April 20, 2004.
133. National Comprehensive Cancer Network (NCCN). Practice Guidelines in Non-Hodgkin's Lymphoma. Rockledge, PA: NCCN; 2003.
134. National Cancer Institute (NCI). Chronic myelogenous leukemia (PDQ®): Treatment. Health Professionals. Bethesda, MD: NCI; 2004. Available at: http://www.cancer.gov/cancerinfo/pdq/treatment/CML/healthprofessional/. Accessed September 14, 2004.
135. Fry NK, Tzivra O, Li YT, et al. Laboratory diagnosis of pertussis infections: The role of PCR and serology. J Med Microbiol. 2004;53(Pt 6):519-525.
136. Centers for Disease Control and Prevention (CDC). Public health guidance for community-level preparedness and response to severe acute respiratory syndrome (SARS). Version 2. Supplement F: Laboratory Guidance. Atlanta, GA: CDC; January 8, 2004.
137. McIntosh N, Gane LW, McConkie-Rosell A, Bennett RL. Genetic counseling for fragile X syndrome: Recommendations of the National Society of Genetic Counselors. J Genet Counsel. 2000;9(4):303-325.
138. New York State Department of Health. Neurologic complications in HIV-infected children and adolescents. New York, NY: New York State Department of Health; March 2003.
139. Outbreaks of avian influenza A (H5N1) in Asia and interim recommendations for evaluation and reporting of suspected cases--United States, 2004. MMWR Morb Mortal Wkly Rep. 2004;53(5):97-100.
140. Cincinnati Children's Hospital Medical Center. Evidence based clinical practice guideline for management o post transplant lymphoproliferative disease (PTLD) following solid organ transplant. Cincinnati, OH: Cincinnati Children's Hospital Medical Center; February 4, 2003.
141. Cincinnati Children's Hospital Medical Center. Evidence based clinical practice guideline for pneumocystis carinii pneumonia prophylaxis following solid organ or blood and marrow transplants. Cincinnati, OH: Cincinnati Children's Hospital Medical Center; January 12, 2001.
142. U.S. Department of Health and Human Services, Henry J. Kaiser Family Foundation. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Bethesda, MD: Department of Health and Human Services, Henry J. Kaiser Family Foundation; March 23, 2004.
143. Mandell LA, Bartlett JG, Dowell SF, et al. Update of practice guidelines for the management of community-acquired pneumonia in immunocompetent adults. Clin Infect Dis. 2003;37(11):1405-1433.
144. Scottish Intercollegiate Guidelines Network (SIGN). Dyspepsia. A national clinical guideline. SIGN Publication No. 68. Edinburgh, Scotland: SIGN; March 2003.
145. Institute for Clinical Systems Improvement (ICSI). Dyspepsia and GERD. ICSI Healthcare Guidelines. Bloomington, MN: ICSI; July 2004.
146. Medical Services Advisory Committee (MSAC). Polymerase chain reaction in the diagnosis and monitoring of patients with PML-RARa and PLZF-RARa gene rearrangements in acute promyelocytic leukaemia. Assessment Report. MSAC Reference 9a (ii). Canberra, Australia; MSAC; March 2003.
147. Medical Services Advisory Committee (MSAC). Polymerase chain reaction in the diagnosis and monitoring of patients with AML1-ETO and CPFß-MYH11 gene rearrangement in acute myeloid leukemia. Assessmen Report. MSAC Reference 9a (iii). Canberra, Australia; MSAC; August 2003.
148. Medical Services Advisory Committee (MSAC). Polymerase chain reaction in the diagnosis and monitoring of patients with BCR-ABL gene rearrangement in acute lymphoblastic leukaemia. Assessment Report. MSAC Reference 9a (iv). Canberra, Australia: MSAC; March 2004.
149. Medical Services Advisory Committee (MSAC). Antenatal screening for heritable thrombophilia. Assessment Report. MSAC Reference 9b. Canberra, Australia: MSAC; August 2002.

150. Quest Diagnostics. BK and JC virus DNA, real-time PCR. Test Summary. Lyndhurst, NJ: Quest Diagnostics 2005.
151. Randhawa P, Shapiro R, Vatas A. Quantitation of DNA of polyomaviruses BK and JC in human kidneys. J Infect Dis. 2005;192:504-509.
152. Schrag S, Gorwitz R, Fultz-Butts K, Shuchat A, et al. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. MMWR Recomm Rep. 2002;51(RR-11):1-22.
153. Centers for Disease Control and Prevention. Screening tests to detect Chlamydia trachomatis and Neisseri gonorrhoeae infections - 2002. MMWR Recomm Rep. 2002;51(RR-15):1-40.
154. Dua P, Berkowicz LB. Enteroviruses. eMedicine Infectious Diseases Topic 681. Omaha, NE: eMedicine.com; updated November 24, 2003.
155. Quest Diagnostics. Interpretive Guide. Lyndhurst, NJ: Quest Diagnostics; 2005. Available at: http://www.questdiagnostics.com/hcp/intguide/hcp_ig_main.html. Accessed November 14, 2005.
156. Miller WH J., Kakizuka A, Frankel SR, et al. Reverse transcription polymerase chain reaction for the re-arranged retinoic acid receptor a clarifies diagnosis and detects minimal residual disease in acute promyelocytic leukemia. Proc Natl Acad Sci USA. 1992;89:2694-2698.
157. Aisenberg AC, Wilkes BM, Jacobson JO. The bcl-2 gene is rearranged in many diffuse B-cell lymphomas. Blood. 1988;71:969-972.
158. Crescenzi M, Seto M, Herzig GP, et al. Thermostable DNA polymerase chain amplification of t(14;18) chromosome breakpoints and detection of minimal residual disease. Proc Natl Acad Sci USA. 1988;85:4869 -4873.
159. Korsmeyer SJ. bcl-2: An antidote to programmed cell death. Cancer Surv. 1992;15:105-118.
160. Offit K, Lo Coco F, Louie DC, et al. Rearrangement of the bcl-6 gene as a prognostic marker in diffuse large -cell lymphoma. N Engl J Med. 1994;331:74-80.
161. Pezzella F, Ralfkiaer E, Gatter KC, Mason DY. The 14;18 translocation in European cases of follicular lymphoma: comparison of Southern blotting and the polymerase chain reaction. Br J Haematol. 1990;76:58-64.
162. Segal GH, Jorgensen T, Scott M, Braylan RC. Optimal primer selection for clonality assessment by polymerase chain reaction analysis: II. Follicular lymphomas. Hum Pathol. 1994;25:1276-1282.
163. Stetler-Stevenson M, Raffeld M, Cohen P, Cossman J. Detection of occult follicular lymphoma by specific DNA amplification. Blood. 1988;72:1822-1825.
164. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. Science. 1985;228:1440.
165. Weiss LM, Warnke RA, Sklar J, et al. Molecular analysis of the t(14;18) translocation in malignant lymphomas. N Engl J Med. 1987;317:1185.
166. Martin AB, Webber S, Fricker FJ, et al. Acute myocarditis. Rapid diagnosis by PCR in children. Circulation. 1994;90(1):330-339.
167. Towbin JA, Griffin LD, Martin AB, et al. Intrauterine adenoviral myocarditis presenting as nonimmune hydrops fetalis: diagnosis by polymerase chain reaction. Pediatr Infect Dis J. 1994;13(2):144-150.
168. Shirali GS, Ni J, Chinnock RE, et al. Association of viral genome with graft loss in children after cardiac transplantation. N Engl J Med. 2001;344(20):1498-1503.
169. National Horizon Scanning Centre (NHSC). IDI-MRSA detection test for methicillin-resistant Staphylococcu aureus (MRSA) colonisation - horizon scanning review. Birmingham, UK: NHSC; 2004.
170. National Horizon Scanning Centre (NHSC). The LightCycler Staphylococcus and MRSA detection kits for methicillin-resistant Staphylococcus aureus (MRSA) - horizon scanning review. Birmingham, UK: NHSC; 2004.
171. American Academy of Pediatrics (AAP), Committee on Infectious Diseases. 2006 Red Book: Report of the Committee on Infectious Diseases. 27th ed. LK Pickering, CJ Baker, SS Long, JA McMillan, eds. Elk Grove Village, IL: AAP; 2006.

172. Szczepura A, Westmoreland D, Vinogradova Y, et al. Evaluation of molecular techniques in prediction and diagnosis of cytomegalovirus disease in immunocompromised patients. Health Technol Assess. 2006;10 (10):1-176.
173. American College of Obstetricians and Gynecologists (ACOG), Committee on Obstetrics Practice. Prevention of early-onset group B streptococcal disease in newborns. ACOG Committee Opinion No. 279. Washington, DC: ACOG; December 2002.
174. Mandell LA, Bartlett JG, Dowell SF, et al. Update of practice guidelines for the management of community-acquired pneumonia in immunocompetent adults. Clin Infect Dis. 2003;37(11):1405-1433.
175. Chin KM, Wessler B, Chew P, Lau J. Genetic tests for cancer. Technology Assessment. Prepared for the Agency for Healthcare Research and Quality (AHRQ) by the Tufts-New England Medical Center Evidence-Based Practice Center. Rockville, MD: AHRQ; January 9, 2006.
176. Honest H, Sharma S, Khan KS. Rapid tests for group B Streptococcus colonization in laboring women: A systematic review. Pediatrics. 2006;117(4):1055-1066.
177. American College of Obstetricians and Gynecologists (ACOG). Vaginitis. ACOG Practice Bulletin No. 72. Washington, DC: American College of Obstetricians and Gynecologists (ACOG); May 2006.
178. Centers for Disease Control and Prevention, Workowski KA, Berman SM. Diseases characterized by vaginal discharge. Sexually transmitted diseases treatment guidelines 2006. MMWR Morb Mortal Wkly Rep 2006;55(RR-11):49-56.
179. British Association for Sexual Health and HIV (BASHH). Sexually transmitted infections: UK national screening and testing guidelines. London, UK: British Association for Sexual Health and HIV (BASHH); 2006.
180. Bailey JW, Williams J, Bain BJ, et al.; General Haematology Task Force. Guideline for laboratory diagnosis of malaria. London, UK: British Committee for Standards in Haematology; 2007.
181. Clinical Effectiveness Group, British Association for Sexual Health and HIV (BASHH). National guideline for the management of lymphogranuloma venereum (LVG). London, UK: British Association for Sexual Health and HIV (BASHH); 2006.
182. Clinical Effectiveness Group, British Association for Sexual Health and HIV (BASHH). National guideline for the management of bacterial vaginosis. London, UK: British Association for Sexual Health and HIV (BASHH); 2006.
183. Clinical Effectiveness Group, British Association for Sexual Health and HIV (BASHH). United Kingdom national guideline on the management of trichomonas vaginalis. London, UK: British Association for Sexual Health and HIV (BASHH); 2007.
184. Clinical Effectiveness Group, British Association for Sexual Health and HIV (BASHH). 2007 national guideline on the management of non-gonococcal urethritis. London, UK: British Association for Sexual Health and HIV (BASHH); 2007.
185. New York State Department of Health. Lymphogranuloma venereum (LVG). New York, NY: New York State Department of Health; August 2007.
186. New York State Department of Health. Mycobacterial infections. New York, NY: New York State Departmen of Health; September 2006.
187. New York State Department of Health. Infectious complications associated with HIV infection: Parasitic infections. New York, NY: New York State Department of Health; November 2006.
188. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis. 2007;44 Suppl 2:S27-S72.
189. Ferris MJ, Masztal A, Aldridge KE, et al. Association of *Atopobium vaginae*, a recently described metronidazole resistant anaerobe, with bacterial vaginosis. BMC Infect Dis. 2004;4:5.
190. Flomenberg P, Munoz FM. Diagnosis and treatment of adenovirus infection in children. UpToDate [online serial]. Waltham, MA: UpToDate; 2008.
191. Flomenberg P, Kojaoghlanian T. Diagnosis and treatment of adenovirus infection in adults. UpToDate [online serial]. Waltham, MA: UpToDate; 2008.

192. Anaissie E. Overview of infections following hematopoietic cell transplantation. UpToDate [online serial]. Waltham, MA: UpToDate; 2008.
193. Centers for Disease Control and Prevention (CDC). Interim guidance on antiviral recommendations for patients with confirmed or suspected swine influenza A (H1N1) virus infection and close contacts. Swine Flu Guidance for Health Professionals. Atlanta, GA: CDC; updated April 25, 2009. Available at: http://www.cdc.gov/swineflu/recommendations.htm. Accessed April 27, 2009.
194. World Health Organization (WHO). Influenza A (H1N1). Epidemic and Pandemic Alert and Response (EPR). Geneva, Switzerland; WHO: 2009. Available at: http://www.who.int/csr/disease/swineflu/en/index.html. Accessed April 27, 2009.
195. White DJ, Vanthuyne A. Vulvovaginal candidiasis (VVC). In: Sexually transmitted infections: UK national screening and testing guidelines. J Ross, C Ison, C
196. Carder, et al., eds. London, UK: British Association for Sexual Health and HIV (BASHH); August 2006.
197. Sobel JD. Candida vulvovaginitis. UpToDate [online serial]. Waltham, MA: UpToDate; January 2011.
198. Centers for Disease Control and Prevention, Workowski KA, Berman SM. Diseases characterized by vaginal discharge. Sexually transmitted diseases treatment guidelines 2006. MMWR Morb Mortal Wkly Rep 2006;55(RR-11):49-56.
199. Hidalgo JA, Vazquez JA. Candidiasis. eMedicine Infectious Diseases. Omaha, NE: eMedicine.com; updated July 14, 2008.
200. Pappas PG, Kauffman CA, Andes D, et al.; Infectious Diseases Society of America. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(5):503-535.
201. Aichinger E, Schleck CD, Harmsen WS, et al. Nonutility of repeat laboratory testing for detection of Clostridium difficile by use of PCR or enzyme immunoassay. J Clin Microbiol. 2008;46(11):3795-3797.
202. American Academy of Pediatrics, Committee on Infectious Diseases. Red Book: Report of the Committee on Infectious Diseases. Elk Grove Village, IL: AAP; 2009.
203. National Health Service (NHS), Department of Health and Health Protection Agency. Clostridium difficile infection: How to deal with the problem. Publication Policy and Guidance. 287860. London, UK: NHS; 2009. Available at: http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_093220 Accessed August 14, 2009.
204. Centers for Disease Control and Prevention (CDC), National Center for Zoonotic, Vector-borne and Enteric Diseases (NCZVED), Division of Vector-Borne Infectious Diseases (DVBID). Arboviral Diagnostic Testing. Arboviral Encephalitides. Atlanta, GA: CDC; December 7, 2007.
205. Campbell PJ, Scott LM, Buck G, et al; United Kingdom Myeloproliferative Disorders Study Group; Medical Research Council Adult Leukaemia Working Party; Australasian Leukaemia and Lymphoma Group. Definition of subtypes of essential thrombocythemia and relation to polycythaemia vera based on JAK2V617F mutation status: A prospective study. Lancet. 2005;366(9501):1945-1953.
206. Humar A, Kumar D, Mazzulli T, et al. A surveillance study of adenovirus infection in adult solid organ transplant recipients. Am J Transplant. 2005;5(10):2555-2559.
207. Campbell PJ. Green AR. The myeloproliferative disorders. N Engl J Med. 2006;355(23):2452-2462.
208. James C, Delhommeau F, Marzac C, et al, Detection of JAK2V617F as first intention diagnostic test for erythrocytosis. Leukemia 2006;20(2):350-353.
209. Michiels JJ, Bernema Z, Van Bockstaele D, et al. Current diagnostic criteria for the chronic myeloproliferative disorders (MPD) essential thrombocythemia (ET), polycythemia vera (PV) and chronic idiopathic myelofibrosis (CIMF). Pathol Biol. 2007;55(2):92-104.
210. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007;44 Suppl 2:S27-S72.
211. Richert B, Lateur N, Theunis A, Andre J. New tools in nail disorders. Semin Cutan Med Surg. 2009;28(1):44-48.

212. Avni T, Mansur N, Leibovici L, Paul M. PCR using blood for diagnosis of invasive pneumococcal disease: Systematic review and meta-analysis. J Clin Microbiol. 2010;48(2):489-496.
213. Centers for Disease Control and Prevention (CDC). Prevention of perinatal group B streptococcal disease — Revised guidelines from CDC, 2010. MMWR Recomm Rep. 2010;59(RR10):1-32.
214. Boppana SB, Ross SA, Novak Z, et al.; National Institute on Deafness and Other Communication Disorders CMV and Hearing Multicenter Screening (CHIMES) Study. Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection. JAMA. 2010;303(14):1375-1382.
215. Cohen SH, Gerding DN, Johnson S, et al.; Society for Healthcare Epidemiology of America; Infectious Diseases Society of America. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). Infect Control Hosp Epidemiol. 2010;31(5):431-455.
216. Kaufman CA, Bustamante B, Chapman SW, Pappas PG. Clinical practice guidelines for the management o sporotrichosis: 2007 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2007;45:1255-1265.
217. Kauffman CA. Clinical features and diagnosis of sporotrichosis. UpToDate [online serial]. Waltham, MA: UpToDate; updated January 2011.
218. Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC). Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. MMWR Recomm Rep. 2010;59(RR-10):1-36.
219. Workowski KA, Berman S; Centers for Disease Control and Prevention (CDC). Sexually transmitted diseases treatment guidelines, 2010. MMWR Recomm Rep. 2010;59(RR-12):1-110.
220. New York State Department of Health (NYSDOH). Bacterial vaginosis (BV). New York, NY: NYSDOH; August 2009.
221. Sobel JD. Bacterial vaginosis. UpToDate [online serial]. Waltham, MA: UpToDate; updated February 2011.
222. National Association of State Public Health Veterinarians (NASPHV). Compendium of measures to control *Chlamydophila psittaci* infection among humans (psittacosis) and pet birds (avian chlamydiosis), 2010. NASPHV Compendia. St. Paul, MN: NASPHV; 2010.
223. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Vector-Borne Diseases (DVBD). Tularemia. For Clinicians. Atlanta, GA: CDC; updated January 2011.
224. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Vector-Borne Diseases (DVBD). Q Fever. Symptoms, Diagnosis, and Management. Atlanta, GA: CDC; updated April 2011.
225. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of High-Consequence Pathogens and Pathology (DHCPP), Viral Special Pathogens Branch (VSPB). Hantavirus. HPS Clinical/Technical Information. Atlanta, GA: CDC; updated May 2011.
226. Centers for Disease Control and Prevention, Global Health - Division of Parasitic Diseases and Malaria. Parasites - Cyclosporiasis (Cyclospora infection). Resources for Health Professionals. Atlanta, GA: CDC; updated November 2010.
227. Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases. Measles (Rubeola). Lab Tools. Atlanta, GA: CDC; updated August 2009.
228. Centers for Disease Control and Prevention, Global Health - Division of Parasitic Diseases and Malaria. Parasites - Leishmaniasis. Resources for Health Professionals. Atlanta, GA: CDC; updated November 2010.
229. Everett ED. Microbiology, epidemiology, clinical manifestations, and diagnosis of leptospirosis. UpToDate [online serial]. Waltham, MA: UpToDate; updated March 2011.

230. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED). Parasites - Cryptosporidum (also known as "Crypto"). Resources for Health Professionals. Atlanta, GA: CDC; update November 2010.
231. Allos BM. Clinical features and treatment of Campylobacter infection. UpToDate [online serial]. Waltham, MA: UpToDate; updated January 2011.
232. Centers for Disease Control and Prevention, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, Division of Foodborne, Bacterial, and Mycotic Diseases. Campylobacter. Atlanta, GA: CDC; updated July 2010.
233. Centers for Disease Control and Prevention. CDC Health Information for International Travel 2010. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2009.
234. Boppana SB, Ross SA, Novak Z, et al; National Institute on Deafness and Other Communication Disorders CMV and Hearing Multicenter Screening (CHIMES) Study. Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection. JAMA. 2010;303(14):1375-1382.
235. Marr KA. Diagnosis of invasive aspergillosis. Last reviewed May 2012. UpToDate Inc. Waltham, MA.
236. Bradsher RW. Clinical manifestations and diagnosis of blastomycosis. Last reviewed May 2012. UpToDate Inc. Waltham, MA.
237. Aster JC, Brown, JR, Fredman AS. Castleman's disease. Last reviewed May 2012. UpToDate Inc. Waltham, MA.
238. Brown HG, Lee JM. Creutzfeldt-Jakob disease. Last reviewed May 2012. UpToDate Inc. Waltham, MA.
239. Wheat J, Kauffman CA. Diagnosis and treatment of pulmonary histoplasmosis. Last reviewed May 2012. UpToDate Inc. Waltham, MA.
240. Klanderman BJ. Polymerase chain reaction. Last reviewed May 2012. UpToDate Inc. Waltham, MA.
241. Skirrow MB. Infection with less common Campylobacter species and related bacteria. Last reviewed May 2012. UpToDate Inc. Waltham, MA.
242. Bisno A, Lichtenberger P. Evaluation of acute pharyngitis in adults. Last reviewed May 2012. UpToDate Inc. Waltham, MA.
243. Fleisher GR. Evaluation of sore throat in children. Last reviewed May 2012. UpToDate Inc. Waltham, MA.
244. Anderson DJ. Epidemiology and prevention and control of vancomycin-resistant enterococci. Last reviewed May 2012. UpToDate Inc. Waltham, MA.
245. Cincinnati Children's Hospital Medical Center. Management of EBV-Associated Post-Transplant Lymphoproliferative Disease (PTLD) in Solid Organ Transplant. Cincinnati, OH: Cincinnati Children's Hospital Medical Center; Revised January, 2012.
246. AutoGenomics. INFINITI® Bacterial Vaginosis QUAD. Spot On. Vista, CA: AutoGenomics, Inc.; 2010. Available at: http://www.autogenomics.com/infectious_Bacterial_Vaginosis_Quad.php. Accessed: August 21, 2012.
247. AutoGenomics. INFINITI® Candida Vaginitis QUAD. Spot On. Vista, CA: AutoGenomics, Inc.; 2010. Available at: http://www.autogenomics.com/infectious_Candida_Vaginitis_Quad.php. Accessed: August 21, 2012.
248. Quest Diagnostics. SureSwab® - Bacterial Vaginosis and Vaginitis Test. Madison, NJ: Quest Diagnostics; 2012. Available at: http://http//www.questdiagnostics.com/home/physicians/testing-services/by-test-name/sureswab.html. Accessed: August 22, 2012.
249. Red Book Online. Summaries of infectious diseases varicella-zoster infections: Clinical manifestations. Elk Grove Village, IL: American Academy of Pediatrics; 2012. Available at http://aapredbook.aappublications.org/content/1/SEC131/SEC289.body?sid=48de265b-7eaf-4cf5-8e14-3394b3adf337. Accessed: August 22, 2012.
250. Snellman L, Adams W, Anderson G,et al. Diagnosis and treatment of respiratory illness in children and adults. Bloomington, MN: Institute for Clinical Systems Improvement (ICSI); January 2013.

251. Shulman ST, Bisno AL, Clegg HW, et al. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. Clin Infect Dis. 2012;55(10):e86-e102.
252. Anderson NW, Buchan BW, Mayne D, et al. Multicenter clinical evaluation of the illumigene group A Streptococcus DNA amplification assay for detection of group A Streptococcus from pharyngeal swabs. J Clin Microbiol. 2013;51(5):1474-1477.
253. Trevino A, Aguilera A, Caballero E, et al; HTLV Spanish Study Group. Trends in the prevalence and distribution of HTLV-1 and HTLV-2 infections in Spain. Virol J. 2012;9:71.
254. Pinto MT, Rodrigues ES, Malta TM, et al. HTLV-1/2 seroprevalence and coinfection rate in Brazilian first-time blood donors: An 11-year follow-up. Rev Inst Med Trop Sao Paulo. 2012;54(3):123-129.
255. Scadden DT, Freedman AR, Robertson P. Human T-lymphotropic virus type I: Disease associations, diagnosis, and treatment. Last reviewed May 2012. UpToDate Inc. Waltham, MA.
256. Pakneshan S, Salajegheh A, Smith RA, Lam AK. Clinicopathological relevance of BRAF mutations in human cancer. Pathology. 2013;45(4):346-356.
257. Tallman MS, Aster JC. Clinical features and diagnosis of hairy cell leukemia. Last reviewed May 2013. UpToDate Inc. Waltham, MA.
258. Murray BE. Treatment of enterococcal infections. Last reviewed May 2013. UpToDate Inc. Waltham, MA.
259. Wei F, Li JL, Ling JX, et al. Establishment of SYBR green-based qPCR assay for rapid evaluation and quantification for anti-Hantaan virus compounds in vitro and in suckling mice. Virus Genes. 2013;46(1):54-62.
260. Mohamed N, Nilsson E, Johansson P, et al. Development and evaluation of a broad reacting SYBR-green based quantitative real-time PCR for the detection of different hantaviruses. J Clin Virol. 2013;56(4):280-285.
261. Gluckman SJ. Clinical features and diagnosis of chronic fatigue syndrome. Last reviewed May 2013. UpToDate Inc., Waltham, MA.
262. Jordan JA. Clinical manifestations and pathogenesis of human parvovirus B19 infection. Last reviewed May 2013. UpToDate Inc., Waltham, MA.
263. Hjelle B. Epidemiology and diagnosis of hantavirus infections. Last reviewed May 2013. UpToDate Inc., Waltham, MA.
264. Anderson NW, Buchan BW, Mayne D, et al. Multicenter cinical evaluation of the illumigene Group A Streptococcus DNA amplification assay for detection of Group A Streptococcus from pharyngeal swabs. J Clin Microbiol. 2013;51(5):1474-1477.
265. Raby BA. Polymerase chain reaction. Last reviewed June 2013. UpToDate Inc., Waltham, MA.
266. Leder K, Weller PF. Epidemiology, clinical manifestations, and diagnosis of giardiasis. Last reviewed June 2013. UpToDate Inc., Waltham, MA.
267. Allos BM. Infection with less common Campylobacter species and related bacteria. Last reviewed June 2013. UpToDate Inc., Waltham, MA.
268. Hickey SE, Curry CJ, Toriello HV. ACMG Practice Guideline: Lack of evidence for MTHFR polymorphism testing. Genet Med. 2013:15(2):153–156. Available at: http://www.acmg.net/docs/MTHFR_gim2012165a_Feb2013.pdf. Accessed August 5, 2013.
269. Gentili V, Gianesini S, Balboni PG, et al. Panbacterial real-time PCR to evaluate bacterial burden in chronic wounds treated with Cutimed™ Sorbact™. Eur J Clin Microbiol Infect Dis. 2012;31(7):1523-1529.
270. Lee JJ, Moon HS, Lee TY, et al. PCR for diagnosis of male Trichomonas vaginalis infection with chronic prostatitis and urethritis. Korean J Parasitol. 2012;50(2):157-159.
271. Schutze GE, Willoughby RE; Committee on Infectious Diseases; American Academy of Pediatrics. Clostridium difficile infection in infants and children. Pediatrics. 2013;131(1):196-200.
272. Muzny CA, Schwebke JR. The clinical spectrum of Trichomonas vaginalis infection and challenges to management. Sex Transm Infect. 2013;89(6):423-425.

273. Kim Y, Choi YD, Choi C, et al. Diagnostic utility of a clonality test for lymphoproliferative diseases in Koreans using the BIOMED-2 PCR Assay. Korean J Pathol. 2013;47(5):458-465.

274. Thompson GR1, Sharma S, Bays DJ, et al. Coccidioidomycosis: Adenosine deaminase levels, serologic parameters, culture results, and polymerase chain reaction testing in pleural fluid. Chest. 2013;143(3):776-781.
275. Shaw A, Iyer V, Rooney N, et al. Diagnosis of primary cerebral lymphomas: Possible value of PCR testing in equivocal cases requiring rebiopsy. Br J Neurosurg. 2014;28(2):214-219.
276. Shin S, Kim AH, Park J, et al. Analysis of immunoglobulin and T cell receptor gene rearrangement in the bone marrow of lymphoid neoplasia using BIOMED-2 multiplex polymerase chain reaction. Int J Med Sci. 2013;10(11):1510-1517.
277. Ribera J, Zamora L, Junca J, et al. Usefulness of IGH/TCR PCR studies in lymphoproliferative disorders with inconclusive clonality by flow cytometry. Cytometry B Clin Cytom. 2014;86(1):25-31.
278. Sobel JD. Trichomoniasis. UpToDate [serial online]. Waltham, MA: UpToDate; reviewed May 2014.
279. Armstrong DG, Meyr AJ. Clinical assessment of wounds. UpToDate [serial online]. Waltham, MA: UpToDate; reviewed May 2014.
280. National Comprehensive Cancer Network (NCCN). Non-Hodgkin's lymphomas. NCCN Clinical Practice Guidelines in Oncology, v.2.2014. Fort Washington, PA: NCCN; 2014.

281. Staples JE, Hills SL, Power AM. Chikungunya. In: Infectious Disease Related to Travel. Ch. 3. CDC Health Information for International Travel 2014 (Yellow Book). GW Brunette, PE Kozarsky, eds. New York, NY: Oxford University Press; 2014.

Copyright Aetna Inc. All rights reserved. Clinical Policy Bulletins are developed by Aetna to assist in administering plan benefits and constitute neithe offers of coverage nor medical advice. This Clinical Policy Bulletin contains only a partial, general description of plan or program benefits and does not constitute a contract. Aetna does not provide health care services and, therefore, cannot guarantee any results or outcomes. Participating providers are independent contractors in private practice and are neither employees nor agents of Aetna or its affiliates. Treating providers are solely responsible for medical advice and treatment of members. This Clinical Policy Bulletin may be updated and therefore is subject to change.

CPT only copyright 2008 American Medical Association. All Rights Reserved.

Copyright 2001-2015 Aetna Inc. Web Privacy Statement | Legal Statement | Privacy Notices | Member Disclosure