HEALTH SERVICES LABORATORIES

Clinical Parasitology User Manual

Version 1
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HSL (Analytics) LLP, Department of Clinical Parasitology

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CONTENTS

1 Introduction – About the Department of Clinical Parasitology

1.1 SERVICES PROVIDED

The Health Services Laboratories, Department of Clinical Parasitology serves as a National Parasitology Reference Laboratory. It services requests from all General practitioners, PHE and Medical laboratories in the NHS and private sector. The Department has an international reputation and provides a parasitology service to Clinicians and Laboratories worldwide.

The Department offers a wide range of investigations including diagnosis and identification of parasites in clinical material, diagnosis of human parasitic disease by immunological methods in addition to culture of parasitic organisms and detection of parasitic genomic material from clinical material.

A twenty-four hour service for microscopic diagnosis of malaria, trypanosomiasis and amoebiasis (via hot stools) is available.

An advisory service on investigation of patients for parasitic disease, the appropriateness of tests, their timing and interpretation together with advice on treatment is also available.
Individual tuition for technical, scientific and medical staff in faecal and blood parasitology can be provided by special arrangement.

The Department of Clinical Parasitology processes over 33,000 requests per annum.

1.2 REMIT OF THE DEPARTMENT OF CLINICAL PARASITOLOGY

1) To provide a comprehensive diagnostic, identification and advisory service on human parasites and the diseases they cause.

2) To develop, evaluate and advise on new parasite diagnostic techniques.

3) To produce epidemiological data for the PHE.

4) To liaise with other diagnostic and research parasitology laboratories in the UK and overseas, so that best practice is shared globally.

2 How to use this manual

The manual is intended to assist you in making the best use of the services offered by the Department of Clinical Parasitology. The manual is divided into six sections. If you have difficulty finding information that you think should be here, please let us know, so that we can improve the manual. This can be done via email to spencer.polley@hslpathology.com

The page numbers on which specific items appear are listed in the Table of Contents at the front of the manual.

Section 1 (Introduction – About the Department of Clinical Parasitology) provides an overview of the Department of Clinical Parasitology.

Section 2 (How to use this manual) explains how to use this manual

Section 3 (The Department of Clinical Parasitology – Staff and Organisation) provides information about the Department of Clinical Parasitology, its staff and organisation and contact details.

Section 4 (How to use the Diagnostic and Advisory Service) provides suggestions on how to use the Diagnostic and Advisory Service.

Section 5 (Repertoire of Services offered by the Department of Clinical Parasitology) provides a repertoire of services available from the Department of Clinical Parasitology.

Section 6 (Results and advisory service) describes how results are normally sent to you and how they can be obtained if they are required urgently.

3 The Department of Clinical Parasitology – Staff and Organisation

3.1 THE DEPARTMENT OF CLINICAL PARASITOLOGY STRUCTURAL OVERVIEW.

The Department of Clinical Parasitology is a service within Health Services Laboratories (http://hslpathology.com/services-divisions/).

The Department of Clinical Parasitology serves as a National Parasitology Reference Laboratory.

3.2 STAFFING:

3.2.1 Clinical staff:

Professor P L Chiodini - Consultant Parasitologist and Clinical Lead for Parasitology
Dr Gauri Godbole - Consultant Microbiologist and Parasitologist
Specialist Registrar (on rotation) in Parasitology

3.2.2 Laboratory staff:

Dr S D Polley - Scientific Lead
Ms P Lowe - Serology Section Head (BMS 8a)
Ms J Watson - Microscopy & PCR Section Head (BMS 8a)

3.3 ENQUIRIES

For enquiries requesting information and/or advice regarding any item identified in the list of services offered by the Department of Clinical Parasitology, requests for information and/or advice regarding suitability of specimens, safe arrival of specimens, availability of tests or their results, etc please contact:

Scientific Lead
Or appropriate section: Microscopy / PCR
Serology

For equires relating to the clinical interpretation of test results, patient treatment options and suitability of tests in light of patient symptoms/history please contact:

Consultant Parasitologist
Specialist Registrar

Information on how to contact the department is shown in section **3.4**.

3.4 CONTACTING THE DEPARTMENT

To contact the above staff or relevant section (with Microscopy covering Molecular testing):

Phone 02073079400 (switchboard)
When connected ask for one of the following:

1) Parasitology microscopy,
2) Parasitology serology
3) Dr Spencer Polley (Scientific Lead)
4) Parasitology medical staff.

For Out of Hours Urgent Malaria/ Hot stool/ African Trypanosomiasis Diagnosis ONLY please call On-call BMS as follows

Phone +44 (0)845 155 5000 or +44 (0)20 34567890
Ask for On Call Parasitologist

For urgent out of hours advice on clinical matters please phone switchboard (0203 456 7890) and ask to be transferred to the duty tropical medicine SPR.

Please note: The department will only release test results to recognised health care providers. It is unable to release results to member of the general public, patients or their friends and family.

We are always happy to recieve feeback on the quality or scope of service offered. If you would like to offer any such feedback please send it to spencer.polley@hslpathology.com

3.5 HOURS OF BUSINESS

Information and advice is available from staff in the Department of Clinical Parasitology within normal working hours (0900 - 1700 Monday to Friday).

3.6 EMERGENCY ON-CALL SERVICE

A 24 hour, 7 day service is provided for urgent diagnosis of malaria, trypanosomiasis and amoebiasis (via hot stools).

3.7 URGENT REQUESTS DURING NORMAL HOURS

Please telephone to say that an urgent sample is en route as follows:

Phone 02073079400 (switchboard)
When connected ask for one of the following:

1) Parasitology microscopy,
2) Parasitology serology

A responsible person (and deputy), capable of accepting and transmitting the result(s), in the submitting organisation must be identified at this time. The results of urgent tests will be telephoned by a senior

member of staff to the identified person (or deputy) in the submitting organisation as soon as the result is verified

3.8 FABRIC AND FACILITIES

The department is situatated in two purpose built laboratories. Sample reception and urgent Malaria/ Trypanosomiais/ Amoebiasis is performed in the Mortimer Market Building. All other diagnostic services are carried out in the Halo Building at 1 Marbledon Place.

Routine access to the laboratories is restricted to the laboratory staff, with controlled entry for visitors.

4 How to use the Diagnostic and Advisory Service

4.1 TRANSPORT AND COLLECTION OF SPECIMENS

Specimens are received (by vacuum tube, post, DX, hospital van, taxi, or by courier) at the department. A regular van delivery / pickup of specimens between local centres is maintained by the UCLH Transport department.

If specimens are to be brought to the laboratory personally by medical or nursing staff they must be carried in an approved container for transport.

4.2 SAFETY

Current guidelines must be followed to avoid needle stick injuries or accidental exposure to blood and blood-contaminated body fluids of those persons taking, transporting and processing the samples.

Any accident should be reported at once to your immediate superior as urgent action may be required; please refer to your local Safety Policy/Infection Control guidelines.

Neither the request form nor the outside of the container should be contaminated with the sample.

Ensure that the container is correctly sealed. All specimens from human sources must be regarded as potentially infectious

4.3 PACKAGING OF SPECIMENS

Label all samples clearly with hospital number, name, and date of collection.

Location, consultant code/name, doctor’s name, bleep/extension and test or tests required in addition to the patient details above should be put on the request form.

The test requestor must be an authorised person, not a member of the public.

The Department of Clinical Parasitology is unable to receive samples sent by members of the public that are not accompanied by a request for from an approved laboratory, medical practitioner or health care provider.

The recipient of the results must be a recognised laboratory, medical practitioner or health care provider.

Specimens MUST be packaged according to Packing Instructions P650 and UN3373 requirements. See "Transport of infectious substances - best practice guidance for microbiology laboratories" available on the Department of Health website (www.dh.gov.uk)

The outside must be marked conspicuously with the following:

'BIOLOGICAL SUBSTANCE, CATEGORY B'

It is essential that such substances are properly packed and labelled and appropriate instruction and protection provided to the carrier(s).

The sender is responsible for ensuring the health and safety of any courier or taxi service that is used to transport samples to the Parasitology laboratory.

4.4 HAZARDOUS SPECIMENS

Any specimens from known or suspected cases of hepatitis, tuberculosis, Viral Hemorraghic Fever (VHF – see 4.7.10) or HIV/AIDS must be clearly identified as a 'RISK OF INFECTION'.

Spillage of body fluids / leaking containers. - This may necessitate the rejection of the specimen. If this occurs, a member of the Department of Clinical Parasitology staff will inform a responsible person in the submitting organisation by telephone and advise that a request for a repeat sample be made.

4.5 REQUEST FORMS

Where possible, use a Parasitology request form personalised to your location. A personalised request form will have the code assigned to your laboratory or practice, this ensures speedy processing of the specimen and ensures the report is returned to the requesting address. If a laboratory would like a copy of the new parasitology request form please email the Scientific Lead providing the laboratory address, responsible person (where appropriate) to whom results are to be sent, telephone and fax number:

Spencer.Polley@hslpathology.com

Request forms can be dispatched to you by prior arrangement. Use a separate form for each specimen type. Personalised request forms will ensure your tests are booked into the correctly and you receive the results in a timely manner. Complete all sections of the form using a ball-point pen or ink. Mark clearly the name of the responsible person (and deputy where appropriate) to whom results are to be sent.

Please give complete patient identification and relevant clinical details, including risk category and travel history. This information is needed to help determine which special precautions are required and which tests are to be done. If you use your own form please include your address and a contact telephone number we can use in case of a clinically urgent result.

Processing times for different specimens vary according to clinical priority, as does the frequency of individual tests.

CLINICALLY IMPORTANT REQUESTS WILL BE GIVEN PRIORITY AND THE RESULTS TELEPHONED TO YOU BY A SENIOR MEMBER OF STAFF AT THE EARLIEST OPPORTUNITY.

4.6 TYPES OF SPECIMENS

Confirmation of Parasitic infection can often be obtained directly following the analysis of a clinical specimen for the presence of the parasite. Indirect methods can also be used to test for evidence of a parasitic infection. Negative results do not necessarily exclude a diagnosis.

Faeces, blood and sera constitute the majority of samples received for analysis. Other samples include adhesive tape smears, urine, semen, skin snips, biopsies, liver aspirates, CSF, ocular fluid, corneal scrapes and whole organisms such as arthropods and worms for identification.

Please send separated serum rather than whole blood for routine serology requests (to prevent lysis of sample if delayed in post).

If you are uncertain of the type(s) of specimen(s) you should submit for analysis, telephone prior to sending the sample, in order that you can discuss the appropriateness of the specimen with a senior member of staff from the Department of Clinical Parasitology.

4.7 POSTAL ADDRESS

Send your specimens, together with an official request form or signed letter containing as much clinical information as is deemed necessary and requesting the service(s) required to:

The Department of Clinical Parasitology
The Hospital for Tropical Diseases
3rd Floor Mortimer Market Centre
Mortimer Market
London WC1E 6JB

Dx Number: DX 6640701
Exchange: TOTTENHAM CT RD 91 WC

We would request your form has and address, contact phone number, sample time and date added to the patient identifiable information and travel history.

Bespoke request forms can be obtained from spencer.polley@hslpathology.com, these will ensure the correct booking of your request and resulting in a timely manner.

Please Note: Specimens sent for diagnosis or further investigation to a clinical laboratory must comply with the conditions set down in the Post Office Regulations governing the transport of pathological specimens. For insurance purposes, the value of a routine specimen is not likely to exceed £1 sterling.

Specimens which are known or suspected to contain Hazard Group 4 pathogens should not be sent by post (see www.hse.gov.uk/pubns/misc208.pdf for a list of group 4 pathogens)

5 Repertoire of Services offered by the Department of Clinical Parasitology

The Department of Clinical Parasitology offers the following services:

Diagnosis and identification of parasites in clinical material
Examples of this service would include:
Identification or confirmation of identity of ova, cysts, larvae and worms in faeces, tissues, urine and other fluids. The department aims to provide a 24 hour turnaround time within the working week for the above-mentioned specimens. If histology is required the sample will be dealt with in conjunction with a histopathologist.
Identification of malaria parasites in thick and thin blood films. The department aims to provide a 2 hour turnaround time within the working week, for these specimens. Communication with the laboratory before the specimen is dispatched is recommended for urgent samples.

Diagnosis of human parasitic diseases by immunological methods.

Culture of Leishmania from clinical material by prior arrangement.
Culture of *Leishmania* from clinical material can take up to THREE WEEKS. Prior arrangement is advised to obtain the most efficient service.

PCR assays
PCR assays for *Leishmania*, *Microsporidia,* the triple assay for *E. histolytica*, *Giardia* and *Cryptosporidia*, free living amoebae and detection of subpatent (repeatedly slide negative) malarial infections are available on request.

Advisory service
We provide an advisory service on the investigation of patients for parasitic disease, the appropriateness of tests, their timing and interpretation together with advice on treatment.
Information regarding this service can normally be provided by telephone, fax or email.

6 SPECIMENS REQUIRED FOR THE DIAGNOSIS OF INDIVIDUAL PARASITIC DISEASES

6.1 AMOEBIASIS (ENTAMOEBA HISTOLYTICA)

6.1.1 Detection of Entamoeba histolytica / Entamoeba dispar cysts by microscopy:

Intestinal - stool samples for examination can be sent by conventional means. Examination for **trophozoites** requires that the stool is examined within **15 to 20 minutes of voiding**. Please phone the the laboratory must be advised in advance of submission. Microscopy of rectal scrapings must be arranged with the laboratory in advance.

6.1.2 Detection of Cryptosporidium species, Giardia intestinalis and Entamoeba histolytica by PCR:

This test offers several advantages over standard microscopy based diagnostics. The assay is significantly more sensitive (greater than ten fold improvement in the limit of detection for some species) than light microscopy. In addition the assay is semi quantitative and can therefore reveal detailed information on the response of a patient's parasite load to subsequent drug therapy. For Entamoeba histolytica the assay also has the advantage of being specific for this pathogen, and does not pick up morphologically related but non pathogenic cysts such as Entamoeba dispar. Finally, the assay can be run on a much wider range of samples, such as biopsies and liver aspirates since it does not rely on the presence of morphologically intact parasites, although the assay is not currently validated for anything other than stool samples.

Stool samples or liver aspirates for the molecular test must **NOT** be in any fixative as this may cause false negatives.

6.1.3 Amoebic serology

For such test a **minimum** of 0.5ml of **serum** is required.

The IFAT (screening titre 1/80) is an essential test in cases of suspected amoebic liver abscess (ALA). Such cases produce high titres of about 1/160-1/320, and the test is positive in over 95% of cases of ALA by the end of the first 14 days. However, it appears to give false positives in some cases of non-amoebic liver disease. Consequently it is necessary to confirm a positive result by the Cellulose Acetate Precipitin test (CAP).

The IFAT also gives very good results in cases of amoeboma. In amoebic colitis the test is positive, often at low titre, in about 75% of cases. In cyst passers it is often negative and in other cases it may be positive because of past infection. The test is therefore not suitable for the investigation of vague abdominal symptoms or as a routine check.

However, in addition to hot stool microscopy, PCR on stool nucleic acids, rectal scrapes or biopsies, a negative result should be obtained before the use of steroids or surgery for presumed ulcerative colitis or Crohn's Disease.

A Cellulose Acetate Precipitin test (CAP) will be performed if the IFAT is positive. This test is less sensitive than the IFAT. A positive is confirmatory evidence of an active or recently treated infection. A negative CAP in the presence of a positive IFAT may suggest early infection, a treated case, past infection or occasionally, a false positive IFAT. After treatment the CAP is the first to become negative, sometimes as soon as one month but occasionally after one year.

6.2 BABESIOSIS

A tick borne parasitic infection caused by *Babesia microti* and *Babesia divergens*

Diagnosis is via microscopy examination of thick and thin blood films. Please send a **minimum** of 2ml of **EDTA anti-coagulated blood**

Serological testing by IFAT is available for *Babesia microti* only upon discussion with the laboratory. For serological testing please send 0.5ml of **serum**.

6.3 CYCLOSPORIASIS

Stool samples may be sent for microscopy. Up to three samples may be necessary due to the intermittent excretion of this parasite.

Malabsorption is a relatively common finding in patients with the recently recognised parasite *Cyclospora cayetanensis*.

6.4 CYSTICERCOSIS (LARVAL TAENIA SOLIUM INFECTION)

Cysticercosis, caused by the presence of the larval stage (cysticercus) of *Taenia solium* in various organs, especially the CNS, is diagnosed by a variety of methods including imaging and serology. A serological service (EITB Immunoblotting) is provided. A **minimum** of 0.5ml of **serum** is required, CSF testing is also available, please provide as much CSF as you are able to spare. This detects the presence of human antibodies to the parasite.

The cysts may occur in almost any situation but are most likely to draw attention to their presence in the brain or eye. Any patient with 'epilepsy' who has resided overseas should be investigated.

Intestinal infections with *Taenia solium or saginata* will usually give negative results by serology

Microscopy of stools for ova is recommended in these cases but cannot differentiate to species level.

If sending segments for identification please send without fixative (see below under identification of worms). **HIGH RISK** stickers must be used if *Taenia solium* is suspected

For individuals with a high suspicion/ confirmed infection with *Taenia solium*, detection of circulating Cysticerosis antigens is also available. This test detects the presence of antigens secreted by live Cystcerci within the patient. A **minimum** of 0.5ml of **serum** is required, CSF testing is also available, please provide as much CSF as you are able to spare (minimum volume for the test is 0.2ml).

6.5 ENTEROBIASIS

An **adhesive tape smear** (Sellotape, Scotch tape (i.e. clear transparent adhesive tape)) taken first thing in the morning from the perianal skin and attached sticky side down to a microscope slide, is the appropriate specimen for detecting *Enterobius vermicularis* ova. While adult worms may be present in stool samples, a negative stool result for worms and ova does not exclude the diagnosis because the ova are laid on the perianal skin.

6.6 FASCIOLIASIS

Fascioliasis is caused by *Fasciola hepatica* (the liver fluke of sheep and cattle). Eggs in faeces are often scanty and may not be found in up to 30% of cases. Serology can be helpful and an IFAT test for antibody is available. A **minimum** of 0.5ml of **serum** is required.

Outbreaks of infection with *Fasciola hepatica* (typically following consumption of infected watercress) are uncommon but have occurred in the UK. Occasional sporadic cases are encountered, usually from abroad.

Patients with upper abdominal pain, thought to be hepatic, eosinophilia and fever, should be investigated. Serology is the best method of diagnosis in the early stage of the infection.

The IFAT (screening titre 1/32) has given reliable results. It is species specific. In proven *Fasciola hepatica* infections the titre is in the order of 1/128.

6.7 FILARIASIS

The syndromes produced by the various species of filarial worms are usually associated with eosinophilia. A patient with an eosinophilia who has lived in, or visited, a filaria endemic area might reasonably be tested for filariasis.

The major human filariases are *Wuchereria bancrofti, Onchocerca volvulus, Brugia malayi,* and *Loa loa.*

With the exception of *Onchocerca volvulus*, a definitive diagnosis of filariasis is usually made by the demonstration of microfilariae in the peripheral blood. *Onchocerca volvulus* is diagnosed by demonstration of microfilariae in **skin snips.**

Twenty millilitres of **anti-coagulated blood (citrate tube)** are required so that the microfilariae can be detected by filtration. Day blood (for *Loa loa*) should be taken between 12noon and 2pm local time and night blood (for *Wuchereria bancrofti*) at 12 midnight. Samples should be kept at room temperature until processed.

6.7.1 Table showing correct blood collection times for diagnosis of human filariasis

	PERIODICITY	**COLLECTION TIME(hr/local)**
Wuchereria bancrofti	Nocturnal (except in Pacific Islands)	2400-0200
Brugia malayi	Nocturnal	2400-0200
Loa loa	Diurnal	1200-1400
Mansonella perstans	No periodicity	Anytime
Mansonella ozzardi	No periodicity	Anytime

A filaria ELISA, using *Brugia pahangi* as antigen is used as a “generic” screening test. A **minimum** of 0.5ml of **serum** is required. A negative result does not exclude the diagnosis and this is especially so with onchocerciasis.

The filaria ELISA is a non-specific screening test that is positive in many types of filariasis and in strongyloidiasis. It is most useful in the diagnosis of TPE (Tropical Pulmonary Eosinophilia) where high antifilarial antibody levels are required to make the diagnosis. Positive results are reported at Levels 1 to 9. Levels 1 and 2 are regarded as weak positives; Levels 5 and over are strong positives.

Reactive symptomatic cases with moderate eosinophilia tend to give high level positives. Non-reactive cases, which may be asymptomatic though microfilariae are present, give low levels of positivity and may be negative. Known causes of false positive results are Hookworm (about 50% of cases) and occasionally *Ascaris* infection. We are unable to speciate Filaria infections using our ELISA test. This may be done if microfilaria are seen in a blood film or by staining the microfilaria obtained by filtration.

6.8 FREE LIVING AMOEBA

A PCR is available upon discussion with the Consultant Parasitologist or SpR for the diagnosis of *Naegleria fowleri, Balamuthia mandrillaris* and Acanthamoeba species.

The sample should be CSF or brain tissue and received in the laboratory as soon as possible.

Molecular detection of Free Living Amoeba is not currently validated as a clinical test or covered under our accreditation by UKAS. Please phone the Consultant Parasitologist or SpR to discuss its relevance to patient management.

Culture is available for *Naegleria fowleri* or *Balamuthia mandrillaris* on discussion with the Consultant Parasitologist or SpR. The sample should be CSF or brain tissue and received in the laboratory as soon as possible.

Diagnosis of Amoebic Keratitis can be done by culture for *Acanthamoeba* species. Samples should be referred directly to:

Diagnostic Parasitology Lab
The London School of Hygiene and Tropical Medicine.
Keppel Street, London WC1E 7HT
DX address: HPA Malaria Reference Lab
DX 6641200
Tottenham Crt RD92WC
Tel: +44 (0)207 927 2427
Fax: +44(0)207 637 0248

This is best diagnosed by sending **corneal scrapings** suspended in a small volume (0.2ml) sterile saline or sterile distilled water

We can also perform culture from **contact lenses or fluids**; isolation from these specimens, whilst suggestive, does not necessarily implicate the amoeba as causing the patient's symptoms."

Request forms can be found at: http://www.parasite-referencelab.co.uk/

6.9 GIARDIASIS (SEE ALSO INTESTINAL PROTOZOA)

Giardia trophozoites are only detectable when stools are examined within **4 hours of voiding**. *Giardia* cysts are frequently excreted intermittently so that a minimum of **six stools** may be required for microscopic exclusion. *Giardia* can be demonstrated in **duodenal / jejunal juices** if examined within 4 hours.

The molecular test for *Cryptosporidium* species, *Giardia intestinalis* and *Entamoeba histolytica* offers several advantages over standard microscopy based diagnostics. The assay is significantly more sensitive (greater than ten fold improvement in the limit of detection for some species) than light microscopy. In addition the assay is semi quantitative and can therefore reveal detailed information on the response of a patient's parasite load to subsequent drug therapy.

Stool samples for the molecular test must **NOT** be in any fixative as this may cause false negatives.

Giardia serology is no longer available, please send an unfixed stool sample for microscopy and PCR.

6.10 HYDATID DISEASE

Hydatid ELISA is performed at HTD. The diagnosis should be considered in individuals who have visited an endemic area and have a space-occupying lesion in any organ, especially the liver. The diagnosis of hydatid disease depends upon a compatible clinical picture, serology and imaging.

Serological cross-reactions, giving rise to false positives, can occur with sera from patients with other parasitic infections, notably larval cestodes and filarial worms, and with some neoplasms. False negatives may occur and are more common in the case of non-hepatic hydatid cysts.

A **minimum** of 0.5ml of **serum** is required, CSF testing is available, please provide as much CSF as you are able to spare.

Aspiration of a cyst should be considered only after taking expert advice and, if felt to be indicated, should be conducted in a centre experienced in the management of hydatid disease. If viability is required the aspirate should be kept at room temperature and reach us with 24 hours.

For watchful waiting of confirmed cases of Hydatid disease or to determine patient response to surgical intervention and/or drug treatment an IgG2 subclass ELISA is available. Please contact medical staff for advice upon this service.

If you have any queries concerning the diagnosis in a suspected case of hydatid disease, please contact us for advice.

6.11 IDENTIFICATION OF WORMS

Tapeworm segments for identification (**see also 6.4 Cysticercosis**) – please send in saline, **DO NOT** send in Formalin or other fixative as this prevents identification beyond genus

HIGH RISK stickers must be used if *Taenia solium* is suspected.

Other worms, part or whole, please send as they are or in saline, DO NOT send in formalin or other fixative.

6.12 INTESTINAL HELMINTHIASIS

(Excluding *Enterobius* infections). **Stool samples** should be forwarded with the minimum delay. (A minimum of two separate samples should be examined before a diagnosis is excluded.)

Serology is **NOT** available for Ascaris, Anisakis, Capillaria, Clonorchis, or Hookworm infections.

6.13 INTESTINAL PROTOZOA (SEE ALSO AMOEBIASIS, GIARDIASIS AND MICROSPORIDIA).

Stool samples for the demonstration of trophozoites, cysts and oocysts should be forwarded with the minimum of delay.

6.14 LEISHMANIASIS

6.14.1 Microscopy based Diagnosis of Cutaneous and Mucocutaneous Leishmaniasis.

For Diagnosis and Species Determination of Cutaneous and Mucocutaneous infections, please send:

Punch Biopsy; Take from the edge of the lesion. Place in viral transport media containing antibiotics (but not antifungals) or sterile saline if this is unavailable and send to Parasitology at HTD.

If histology is required take second biopsy, or cut original biopsy in half vertically through the epidermis and tissue. Put half in viral transport media (as above) for Parasitology at HTD and half in formal saline for histology.

Slit skin smears; Take from the edge of the lesion, onto a slide. Air dry and then fix with methanol.

PCR can be used to detect (with very high sensitivity) and speciate *Leishmania* from cutaneous cases. Contact microscopy section of laboratory for advice.

6.14.2 Microscopy based Diagnosis of Visceral Leishmaniasis.

For Diagnosis and Species Determination of **Visceral** infections, please send:

Bone marrow or Splenic aspirate; Please send 2 methanol fixed slides and a **small** amount (less than 1ml) of sample in a sterile EDTA tube (e.g. Vacutainer purple top).

An attempt should always be made to find *Leishmania* from aspirated material (bone marrow or spleen) by microscopy or PCR - contact laboratory for advice.

For Diagnosis and Species Determination of **all of the above conditions.**

Histology Sections; Please send at least 6 normal thickness sections in a small screw capped or Eppendorf (snap lip) tube.

Please do not send samples over the weekend.

Please send a travel history with all specimens.

6.14.3 Serology for Visceral and Mucocutaneous Leishmania

For Serology a **minimum** or 0.5ml of **serum** is required.

Note: negative serology does NOT exclude the diagnosis of visceral leishmaniasis, particularly in sera from HIV positive patients.

Serology is **NOT** helpful in the diagnosis of cutaneous infections.

In mucocutaneous leishmaniasis serology is usually seropositive except in early cases.

A Direct Agglutination Test (DAT) for Leishmaniasis using formalinised promastigotes of *Leishmania donovani* stained with Coomassie blue is the standard serology test and a rapid test (rK39) antibody detection assay is also provided. The DAT is considered positive when the titre exceeds 1600 and in visceral leishmaniasis titres may rise to 51,000 or above. The rK39 test is reported as positive or negative, with no titre available.

6.15 MICROSPORIDIA

The molecular test for microsporidial species offers several advantages over standard microscopy based diagnostics. The assay is significantly more sensitive (greater than one hundred fold improvement in the limit of detection) than light microscopy. In addition the assay is semi quantitative and can therefore reveal detailed information on the response of a patient's parasitic load to subsequent drug therapy. Finally, the assay can differentiate between morphologically identical microsporidia, a feat only possible previously with electron microscopy. Therefore phenomena such as resistance of *Enterocytozoon bieneusi* to albendazole therapy can be considered in a real time process.

Intestinal and tissue microsporidia are found almost exclusively in immunocompromised patients. **Stool, tissue and urine samples** may be sent for examination as appropriate.

PCR is the preferred diagnostic tool

Requests for microsporidiosis should be clearly marked

No serology is available at HTD for these parasites

Stool samples for the molecular test must **NOT** be in any fixative as this may cause false negatives.

Microscopy can still be performed on corneal scrapes.

6.16 SUSPECTED MALARIA AS A MEDICAL EMERGENCY.

The infection is best diagnosed by submitting a **minimum of 2ml of EDTA anti-coagulated blood** with the minimum of delay, so that thick and thin films can be made in the laboratory. A less satisfactory alternative is to submit stained thin blood films plus unstained thick films for examination. Delay in receipt of EDTA sample can adversely affect the integrity of the sample and consequently make accurate diagnosis difficult.

Antigen detection by immunochromatography is available on request.

Malaria serology is **NOT** suitable for diagnosing current infection.

6.17 DIAGNOSIS OF SUSPECTED SUBPATENT (SLIDE NEGATIVE) ON GOING MALARIA INFECTIONS.

For suspected malaria infections that are repeatedly negative by slide microscopy, highly sensitive diagnosis may be made by the use of species specific PCR. Please submit a minimum of 0.5ml of EDTA anti-coagulated blood for nucleic acid extraction and amplification.

6.18 MALARIA (PAST INFECTION)

Serology for malaria may be requested for the following reasons:

1) If for some reason it is important to attempt a retrospective diagnosis.

2) For the investigation of splenomegaly or nephrotic syndrome in a patient who might have been exposed to malaria.

It is **NOT** recommended for the investigation of acute fever, as urgent blood film examination is the method of choice. An ELISA assay is performed using *Plasmodium falciparum* and *vivax* antigens. Positive results will be reported as an optical density and a cut-off point will be stated. A **minimum** of 0.5ml of **serum** or EDTA **plasma** is required.

Sera/plasma from suspected Tropical Splenomegaly Syndrome patients will be tested by IFAT if the ELISA is positive.

The malaria ELISA used at HTD cannot be used to speciate malaria infections.

6.19 SCHISTOSOMIASIS

6.19.1 Diagnosis of Schisotosmiasis by microscopy

Definitive diagnosis is by demonstration of the characteristic ova in clinical material. For *S. haematobium,* a terminal urine sample (the last 10 to 20ml of urine passed) is required. Faecal samples are the best specimens for the detection of *S. mansoni* (and *S. japonicum*) but as *S. mansoni* and *S. haematobium* overlap in geographical distribution and can affect both genitourinary and alimentary systems a **terminal urine sample and a minimum of three faecal samples** should be sent from all patients being investigated for schistosomiasis when serology is positive. Biopsy material (unfixed) from rectum, sigmoid or bladder is valuable for the detection of ova by crush preparation and permits assessment of their viability. If biopsies are taken, fixed material should also be sent for histology. Rectal / sigmoid scrapings are also useful samples for the diagnosis of schistosomiasis. Such samples must be sent to the laboratory by prior arrangement only.

6.19.2 Schistosomiais; diagnosis via serology:

A **minimum** of 0.5ml of **serum** is required.

The test should be requested on patients known to have been exposed to fresh water in endemic areas. It starts to become positive approximately six weeks after exposure.

Deposition of ova commences at about this time but their first appearance (e.g. in urine) may be delayed for several months. Confirmation of the diagnosis by finding ova should be sought where possible.

The ELISA is reported to detect about 96% of *Schistosoma mansoni* and 92% of *Schistosoma haematobium* infections. The test does not distinguish active from treated infections. The actual time taken to become seronegative post treatment varies, but in some patients the test may remain positive for over two years after treatment.

Positive results are reported at Levels 1 to 6. Levels 1 and 2 are regarded as weak positives; Levels 5 and over are strong positives.

It is known that patients may become seropositive through contact with cercaria from animal species of schistosome and probably when harbouring unisexual infection with human species. The schistosomal egg antigen used in the ELISA may cross-react with the sera of trichinosis cases or with those of hepatitis cases in some instances.

Currently it is not possible to speciate using our serology ELISA test.

6.20 STRONGYLOIDIASIS

Often associated with mild abdominal symptoms, strongyloidiasis is also an occasional cause of Loeffler's syndrome and, in fulminating cases, may cause secondary bacterial septicaemia or meningitis. Strongyloidiasis may be diagnosed by the directed observation of parasites in cultured and neat clinical material and serological anlaysis.

Direct observation of *Strongyloides* larvae is achieved by **faecal microscopy** and stool culture. The larvae may not be present in every specimen. *Strongyloides* larvae (and adults) can also be demonstrated in **duodenal / jejunal aspirates**. Duodenal string testing is more sensitive.

Faecal specimens should NOT be refrigerated before sending if Strongyloides culture is required.

Serology for strongyloidiasis (ELISA) is available. The test is indicated for the investigation of eosinophilia or if there is a good clinical history to suggest strongyloidiasis.

A **minimum** of 0.5ml of **serum** is required. There is known to be cross reaction between filaria and strongyloides in ELISA tests.

6.21 TOXOCARIASIS

Serology is the method of choice for the diagnosis of toxocariasis. The ELISA is usually performed on serum, but can be undertaken on aqueous humour, vitreous humour or CSF under the guidance of the Consultant Parasitologist. Please contact the serology section if you intend to send any non-serum samples.

A **minimum** of 0.5ml of **serum** is required.

The Toxocara IgG antibody ELISA test against larval excretory/secretory antigen is the most appropriate method for diagnosis. Sensitivity is 91% and specificity is 86% (with cross reactivity possible with strongyloidiasis, trichinosis, amoebiasis and fascioliasis). Results are expressed as an optical density value.

Positive ELISA tests will be confirmed using a Western blot.

A Negative ELISA on serum does **NOT** exclude ocular toxocariasis. Ocular sampling may be necessary to exclude ocular toxocariasis. Please contact the Consultant Parasitologist for queries about ocular toxocariasis.

Although serology is the method of choice for the diagnosis of toxacariasis, stool samples may be examined for putative intestinal infections.

6.22 TOXOPLASMOSIS

Please refer samples to the Toxoplasma Reference Laboratory (TRL) at Singleton Hospital, Swansea. (General enquiries: 01792 285058)

6.23 TRICHINOSIS

Serology is usually deployed for the diagnosis of this condition.

A **minimum** of 0.5ml of **serum** is required.

Except in the rare event of an outbreak in the UK, serology is usually requested for symptoms suggestive of the stage of muscle encystment: myalgia, eosinophilia, and, in the early stages, fever. The IFAT (screening titre 1/32) has proved reliable and specific with positive titres of about 1/128.

Crush preparations of fixed muscle biopsy specimens may reveal larvae. Biopsies should also be fixed and sent for histology.

6.24 TRYPANOSOMIASIS (OVERVIEW)

African trypanosomiasis is caused by *Trypanosome brucei rhodesiense* or *gambiense*. This disease is restricted to Africa. Diagnosis is made by examining stained blood films or by antibody detection. Microscopy and serology using CSF may occasionally be required in cases with neurological involvement.

American trypanosomiasis (Chagas disease) is caused by *Trypanosoma cruzi*. Once confined entirely to the Americas it has now spread to other continents.

6.25 AFRICAN TRYPANOSOMIASIS

Diagnosis is made by **microscopy** examination of stained blood films (and CSF where neurological involvement is suspected) and by **serological** analysis.

For microscopy please send a **minimum** of 2ml of **EDTA anti-coagulated blood** and/or as much CSF as you can spare.

Trypanosomes quickly disintegrate upon removal from the body, therefore, it is vital that specimens for microscopy are examined rapidly. EDTA whole blood must be examined within 24 hrs and CSF within 20 minutes of taking the sample.

For serological testing please send a **minimum** of 0.5ml of **serum**.

For serological testing of CSF, please provide as much CSF as you are able to spare.

Sera are screened by IFAT for *Trypanosoma brucei.* The usual titre for screening is 1/20.

Please give the relevant travel history so that the appropriate species can be tested for.

6.26 AMERICAN TRYPANOSOMIASIS (CHAGAS DISEASE)

Screening and serodiagnosis of *T. cruzi* is performed by ELISA, with IFAT performed on ELISA positive samples.

A **minimum** of 0.5ml of **serum** is required for serological analysis.

Microscopy on blood films can be performed for diagnosis of *T. cruzi* following consultation with the Clinical Parasitologist. Bloods should be taken for examination within two months of the acute phase of infection or reactivation in cases of immunosuppression.

6.27 VISCERAL LARVA MIGRANS

Serology offers almost the only prospect of specific diagnosis. Requests should be made for tests for filariasis, strongyloidiasis and toxocariasis. A **minimum** of 0.5ml of **serum** is required.

6.28 RETENTION OF SAMPLES

Please note that we do not keep all samples once tested so if extra tests are required please phone the laboratory at the earliest opportunity to request the additions, please see table below for approximate sample retention times:

6.28.1 Table of standard retention times for samples

Serum and CSF supernatant for serology tests	2 months unless specifically requested to be saved (or found to be positive)
Citrated blood for filarial microscopy	Discarded after filtration
EDTA Blood for microscopy	7 days
Body fluids inc semen sputum Aspirates BAL duodenal and jejunal cyst fluids stool Urine Perianal swab	7 days after final report produced by Parasitology Laboratory 7 days after final report produced by Parasitology Laboratory
String test Skin scrapes Skin snips Swabs Rectal scrapes Rectal snips Sellotape slide	Discarded after processing and testing
Biopsies Bone marrow Slit skin smears	6 months (unless all sample used in testing)
Ectoparasites	1 year

Adult worms	1 year
Tapeworm Segments	48 hours after final report produced by Parasitology Laboratory
Ticks	Not kept (sent to ref lab for further ID)

6.29 REPORTING TIMES FOR LABORATORY INVESTIGATIONS

The reporting time is defined as the period from the receipt and booking in of a specimen to the time the report is **issued** to the individual requesting the test.

Clinically important requests will be given priority and the results telephoned to you at the earliest opportunity.

A table listing the range of tests for parasitic diseases that are undertaken in the Department of Clinical Parasitology is available as a separate turn around times document. Some tests are restricted to UCLH requests only.

Routine serological analyses are undertaken in batches.

The following serology tests are referred to the Mahidol University, Thailand.

Angiostrongylus
Paragonimus

We would hope for a 28 day turnaround from sending of a sample to another reference laboratory to receiving a result and reporting it on our computer system.

7 Results and advisory service

7.1 NORMAL REPORTING PRACTICE

Reports are currently issued in either paper format (via postal system) or secure email.

Where possible the Department would prefer to issue reports by the secure email system to ensure the speed and security of data delivery (including compliance with GDPR).

Secure emails as sent as soon as the results are ready, and ensure the fastest routine reporting system available.

If you would like to set up secure email reporting you will need an email address that is regularly monitored. Please email Spencer.Polley@hslpathology.com

7.2 TELEPHONED RESULTS:

Results are telephoned under the following circumstances:

1) If it is thought that a result might lead to an immediate change in patient management (including positive Malaria films, Positive Leishmania results, Positive African Trypanosomiasis results (HAT).
2) If further information is required to decide whether the submitted sample should be processed further.

3) If a telephoned result has been requested.

4) All On Call results.

5) Results will usually be telephoned by the Specialist Registrar or by the individual who has performed the test, but if clinical advice is likely to be needed the call may be made by the Consultant Parasitologist or Deputy. If a telephone number, telephone extension or bleep number has been indicated on the report, the call will be made to that number.

7.3 OBTAINING RESULTS BY TELEPHONE

Although written and Email reports are issued as soon as they are available, the laboratory is happy to make results available by telephone when these would be clinical assistance. Users are asked to use this service only when necessary as it does delay the routine work of the laboratory. The use of Email reports offers a significant increase in the speed of returing results to our users and has been found to largely negate the requirement for telephoning the department.

7.4 MICROSCOPY TURNAROUND TIMES.

For microscopy for the majority of tests, we aim to provide a 24-hour turnaround time, within the working week, although some tests have an official Turn Around Time which is longer than this period. All microscopy tests can be performed and reported by telephone within 24 hours of receiving an urgent specimen if prior notice is given.

7.5 SEROLOGY TURNAROUND TIMES.

It is most economical to carry out serological tests in batches and, in general, serological tests are not necessarily performed as soon as a specimen is received. Most tests are batched weekly, thus when tests are carried out at the Department of Clinical Parasitology, written reports may not be available for eight days after the specimen has reached the laboratory. See the table published on the same web page as this manual for laboratory turnaround times.

When several tests are to be carried out on the same specimen then reports are issued as results become available. Reports are not necessarily delayed to allow all tests to be reported at once.

If urgent results are required or if you want to know when a particular result will be available when please contact the relevant department via Switchboard.

7.6 STORAGE OF RESULTS

All records are currently maintained in the department for a minimum period of ten years.

7.7 INFORMATION GOVERNANCE POLICY:

Wherever possible personal information should not be transferred by FAX. If, for reasons of urgency, it is necessary to use FAX, rather than mail or courier, the FAX should be sent to a designated Safe Haven. Therefore if we receive a request to fax results we require a safe haven fax number, and we will only fax results if a paper copy is required urgently. The use of Email reporting negates the requirement for faxing results and should therefore be the prefered method of resulting from a GDPR perspective.

7.8 OBTAINING CLINICAL ADVICE AND INFORMATION

If you need of advice on the clinical interpretation of results, Professor P.L. Chiodini or the Specialist Registrar can be reached via Switchboard as show in **Contacting the Department** (**3.4)**. If the advice relates to a particular result it is helpful if the clinical details and laboratory reference number are available.
For urgent out of hours advice on clinical matters please phone switchboard (0203 456 7890) and ask to be transferred to the duty tropical medicine SPR.

For advice on the types of samples and containers appropriate for different tests please contact the relevant section as shown in in **Contacting the Department** (**3.4)**.

If you are unsure which of the above numbers is appropriate please telephone either section and the Department of Clinical Parasitology staff will put you in touch with the appropriate section/ people.

Please note: Laboratory staff can only give out results to a recognised laboratory or medical practitioner and NOT members of the general public.