Urine bench

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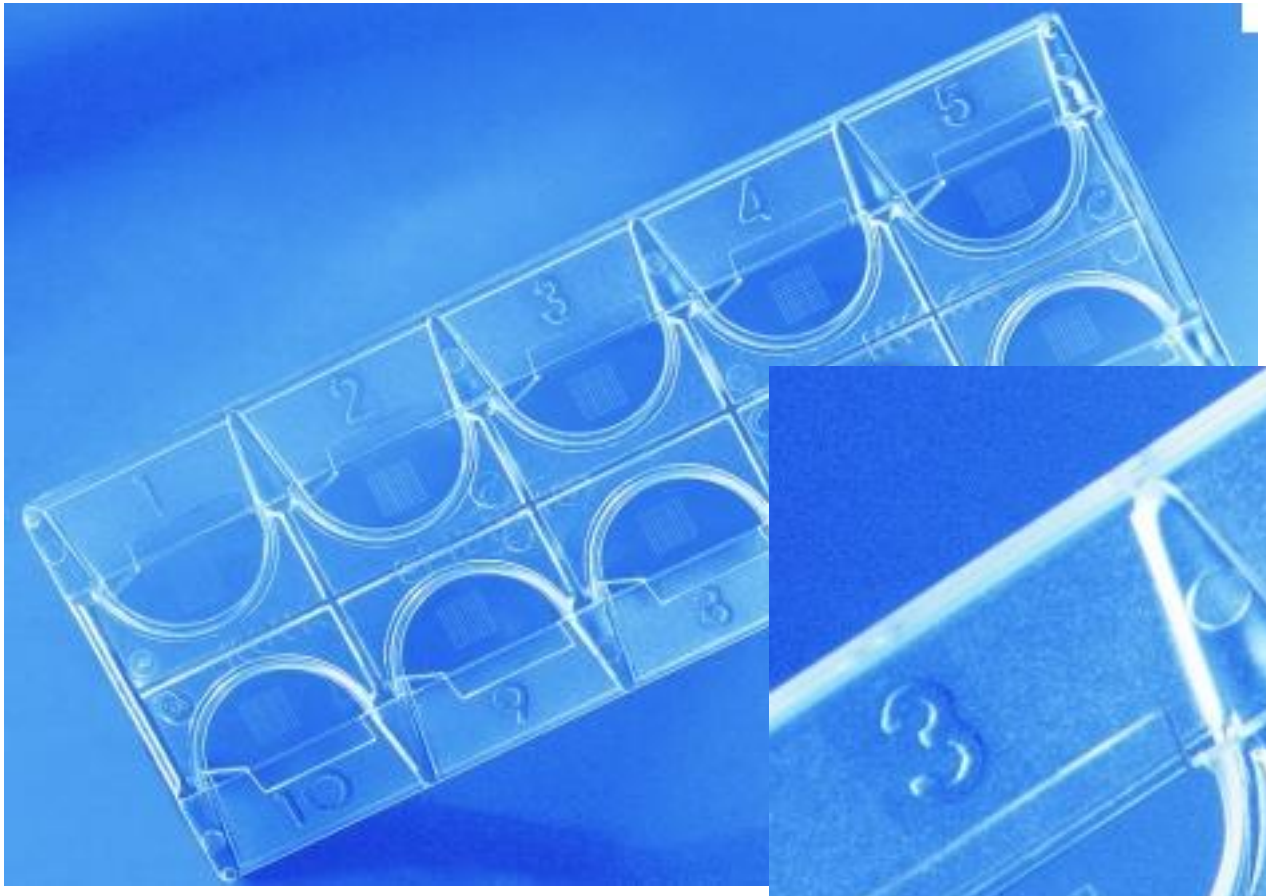
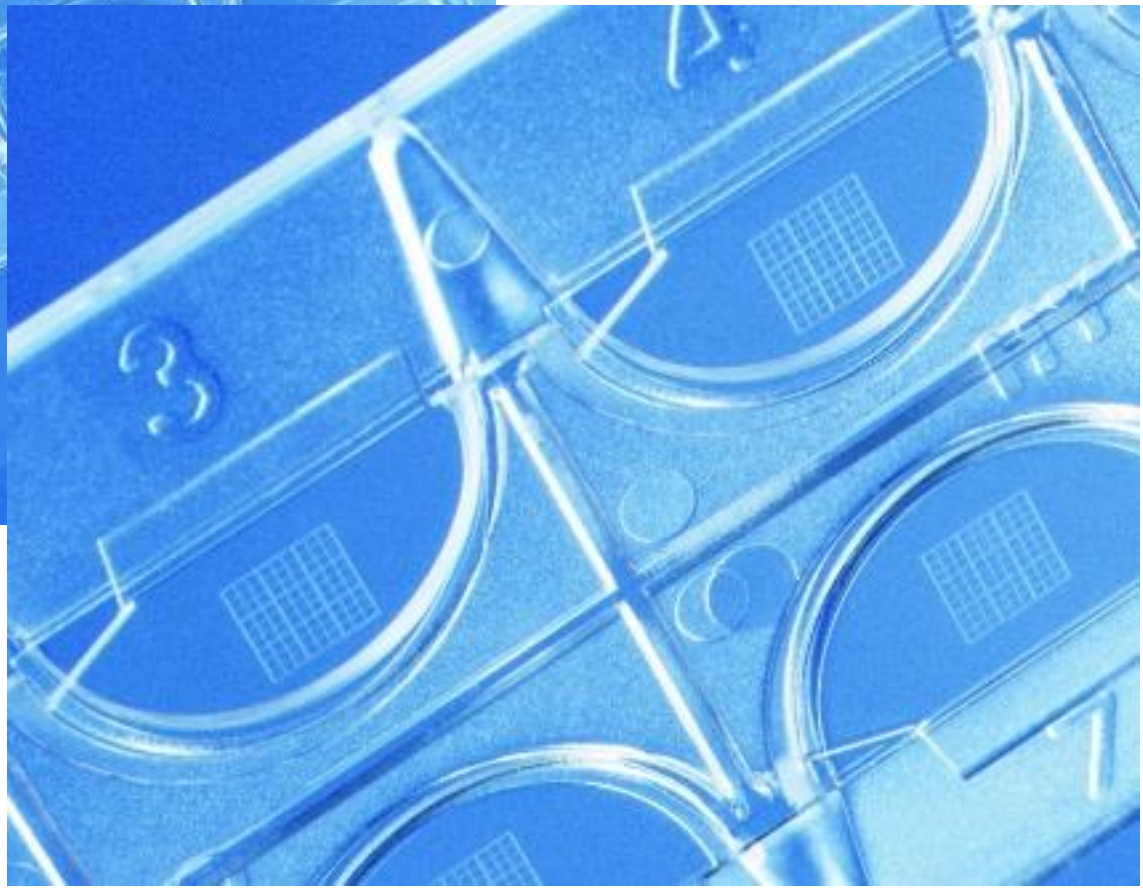
Sept 2013

Overview

- Specimen collection- separate presentation
- Urinalysis: protein, blood, white cells, nitrite
- Microscopy-
  - crystals and casts- separate presentations
  - quantitative cell counts
- Culture
- Susceptibility
- Reporting
- Examples

Kova counting chamber slide





Microscopy

- Cells
  - WC < 10, 10-50, 50-100, > 100/ uL
  - RC < 10, 10-100, >100/uL
    - Glomerular bleeding – dysmorphic RBC
  - Squamous epithelial cells < 50, > 50
  - Other cells- malignant cells, ‘DECOY cells”
- Casts
- Crystals

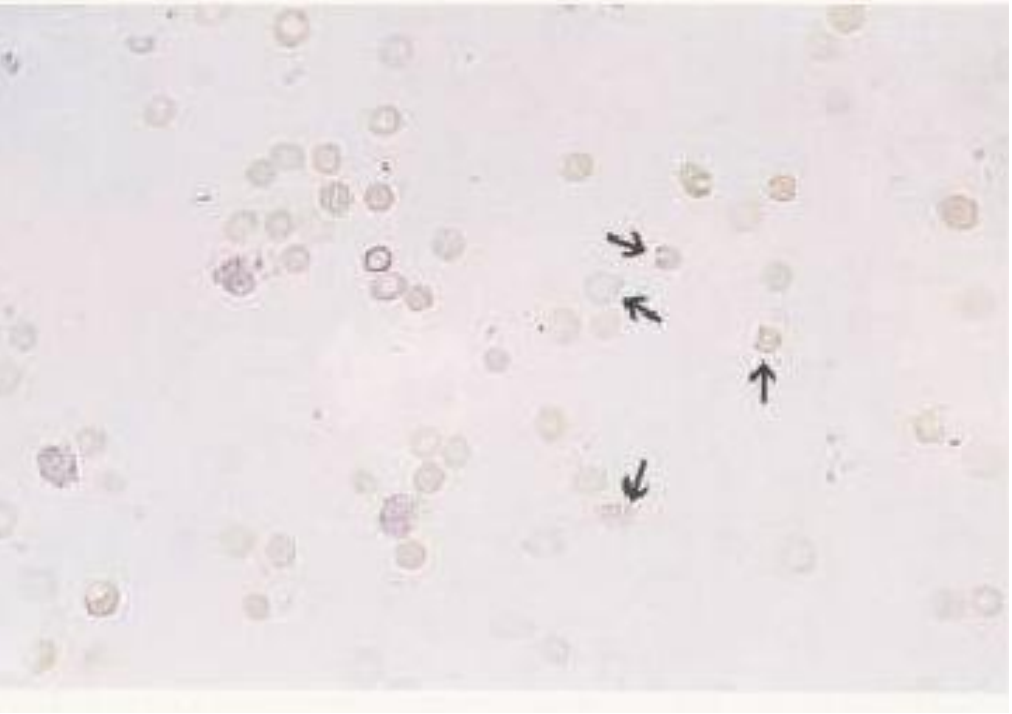
Microscopy- notes

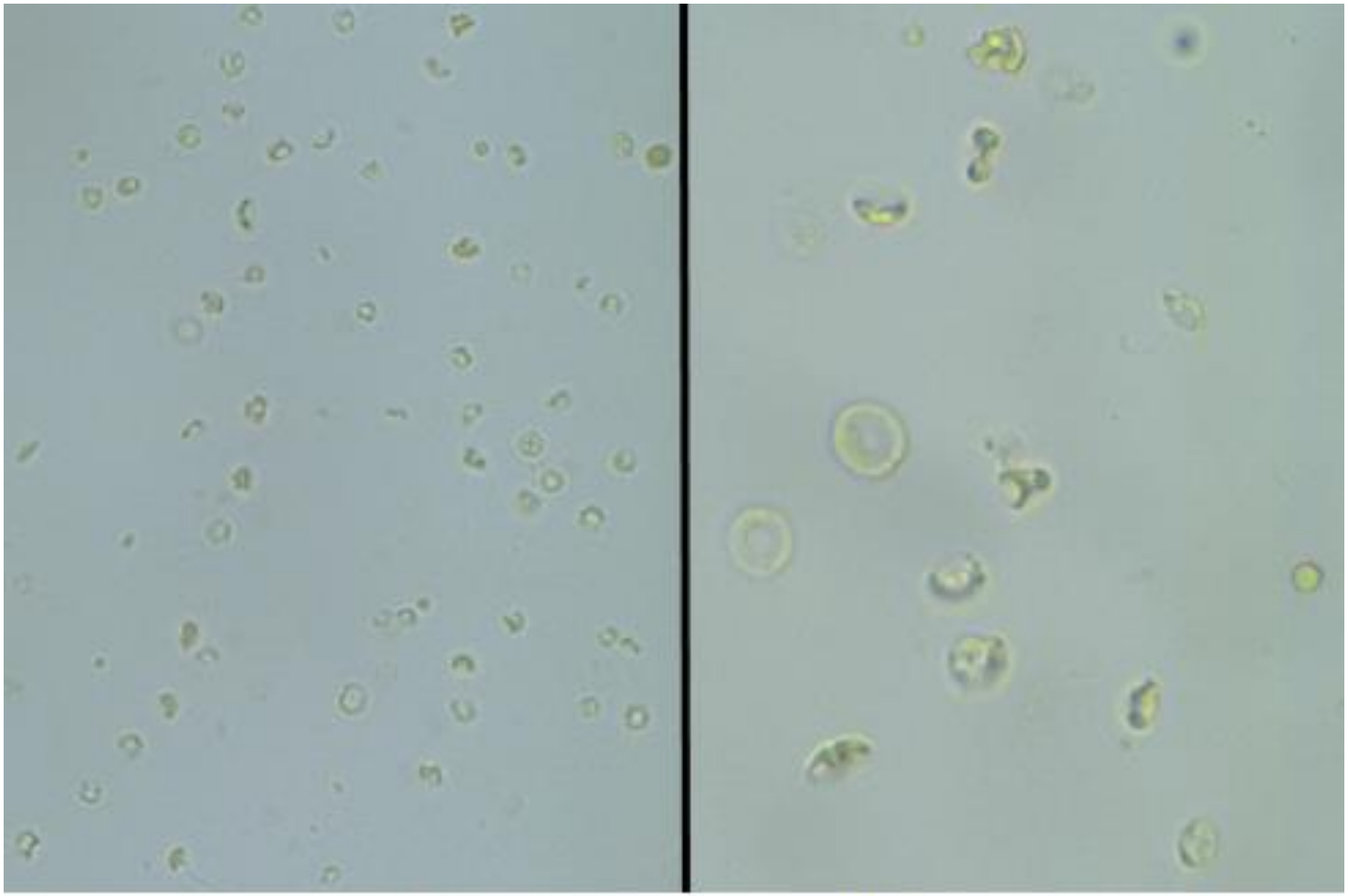
- white cell excretion:normal pattern of excretion that has been quantitated at around 5-8 WC x 10^6/L. ie, = < 10/uL
- Neutropenic patients – no urine white cells

Urine Microscopy Findings

- White cells are almost always present in symptomatic urinary tract infections (but may be absent in infections with *Proteus* spp. if there has been a delay in transport)
- The presence of white cells is non-specific – there is a long list of causes
- In dysuria, the presence of red cells is specific for urinary tract infection
- The presence of bacteria is also specific (seen only if $>10^8$ cfu/l bacteria are present)
- Both the presence of red cells and finding of bacteria on microscopy are insensitive

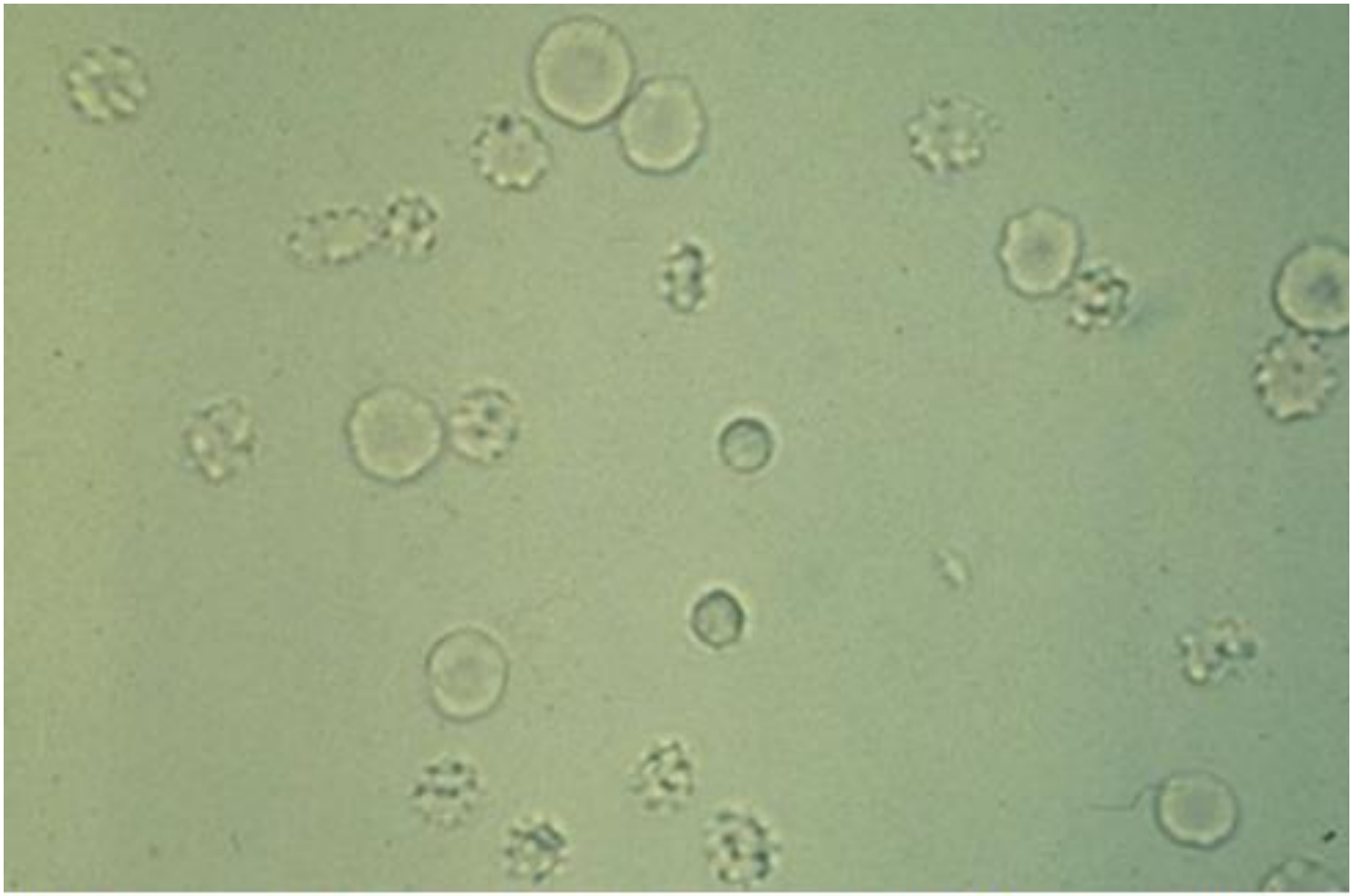
Microscopy: dysmorphic vs normal RBC





Note the irregular outlines of many of these RBC's, compared to two relatively normal RBC's at the center left of the right panel. These abnormal RBC's are dysmorphic RBC's.

http://library.med.utah.edu/WebPath/TUTORIAL/URINE/URIN082.html



Red blood cells in urine appear as refractile disks. With hypertonicity of the urine, the RBC's begin to have a crenated appearance.

http://library.med.utah.edu/WebPath/TUTORIAL/URINE/URIN082.html

Sterile pyuria

Dob 07-Apr-1970 Wd F1 (JHH) Dr J O'SULLIVAN c05:00 17-May-99

MICROBIOLOGY-Urine mid stream Result status - VALIDATED BY M.O.
JH99M30757

MICROSCOPY

White cells	: >100	x10^6/L
Red cells	: <10	x10^6/L
Squamous cells	: <10	x10^6/L
Organisms	: Nil seen	
Casts	: Nil seen	
Other	:	

Comment :

CULTURE : No growth (<10^5/L)
:

1.
2.
3.

Sterile pyuria

- excess white cells indicate inflammation which may be infective or noninfective
- infective causes might include uti due to a fastidious organism eg *Haemophilus*, a slow growing fastidious organism eg M. *tuberculosis*, previous antibiotic treatment rendering the urine sterile
- non-infective causes might include vasculitis (are there casts), chemical cystitis or nephritis (eg interstitial nephritis from drugs), inflammation following surgery (usually blood cells present)
- another possibility can be tubular epithelial cells being shed into urine; they are indistinguishable from normal white cells under phase contrast microscopy. That occurs in interstitial nephritis.

Culture: Bacteriological loops

Lower Limits of detection:

1 µL loop	$1x10^6$cfu/L
7.5 µL loop	$1.33x10^5$ cfu/L
10 µL loop	$1x10^5$cfu/L

Culture counts

- No growth with 1 uL loop
  - Report issued as No growth (< 10^6/L)
- No growth 10 uL loop
  - Report issued as No growth (< 10^5/L)

Quantitation of Bacterial Counts in Urine

In asymptomatic women

- $>10^8$ cfu/l of a pathogen on one occasion = 80% probability of representing bacteriuria rather than contamination
- $>10^8$ cfu/l of the same pathogen of two occasions = 95% probability of representing bacteriuria rather than contamination
- $<10^8$ cfu/l of a pathogen on one occasion = <5% probability of representing bacteriuria rather than contamination

In symptomatic women

- the cutoff of $>10^8$ cfu/l is insensitive (misses about one-third of true bacteriuria)
- $>10^5$ cfu/L pure or predominant pathogen may be significant

In symptomatic men

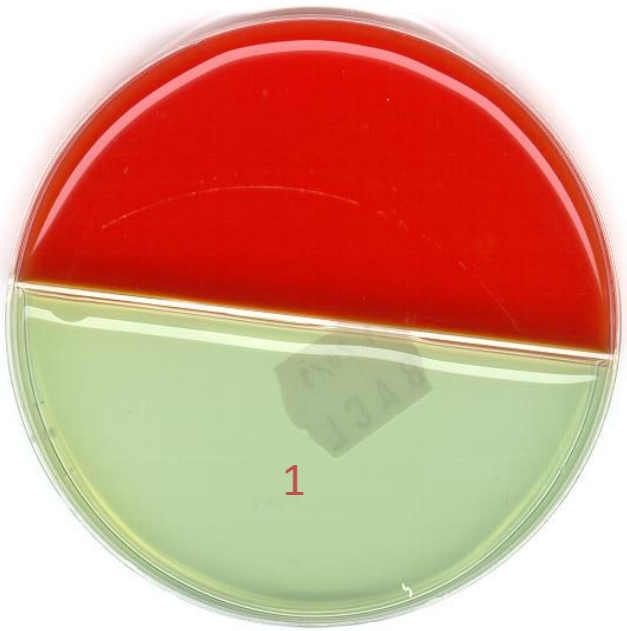
- $>10^6$ cfu/L pure or predominant pathogen is the appropriate cut-off

MSU Culture: Inoculation of Media

- Mix urine
- Label the reverse side of the culture media
- Introduce a sterile **1µL loop** vertically into the urine
- Remove the loop with a vertical action
- Inoculate the culture medium
- Using the same loop and inoculate all other media with the same procedure.

HAPS

Culture: Media



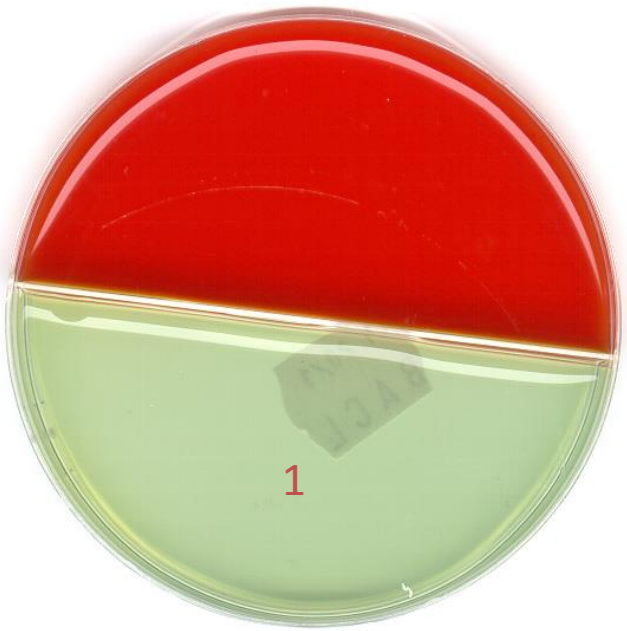
Some of the common media used:

- MacConkey agar
- CLED
- combination of blood agar and MacConkey or CLED

1= CLED medium

CLED agar (cysteine lactose electrolyte deficient medium) : non-inhibitory growth medium Being electrolyte deficient, it prevents the swarming of *Proteus* species. Cysteine promotes the formation of cysteine-dependent dwarf colonies. Lactose fermenters produce yellow colonies on CLED agar; non-lactose fermenters appear blue.

Culture: Media



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Culture: Media

on media

blood agar

or CLED

CLED agar (cysteine lactose electrolyte deficient medium) : non-inhibitory growth medium
Being electrolyte deficient, it prevents the swarming of *Proteus* species. Cysteine promotes the formation of cysteine-dependent dwarf colonies. Lactose fermenters produce yellow colonies on CLED agar; non-lactose fermenters appear blue.

Chromogenic agar

Urine specimens

chromID™ CPS® / Columbia CNA 5% sheep blood

Ref. 43473: kit of 20 plates

Enumeration of organisms in urine specimens and the direct identification of *Escherichia coli*, Enterococcus, KESC* and *Proteeae*.

Selective isolation of fastidious bacteria. Determination of hemolysis.

**Klebsellia, Enterobacter, Serratia, Citrobacter*

What is the likely mechanism of betalactam resistance?

Dob 22-Dec-1936 Wd H3N-JHH Dr K MURREE-ALLEN c11:00 03-Sep-9

MICROBIOLOGY-Urine mid stream Result status - VALIDATED BY M.O.
JH99M55804

MICROSCOPY

White cells	: >100	x10^6/L
Red cells	: >100	x10^6/L
Squamous cells	: <10	x10^6/L
Organisms	: Rods - Scanty	
Casts	: Nil seen	
Other	: Nil seen	

Comment :

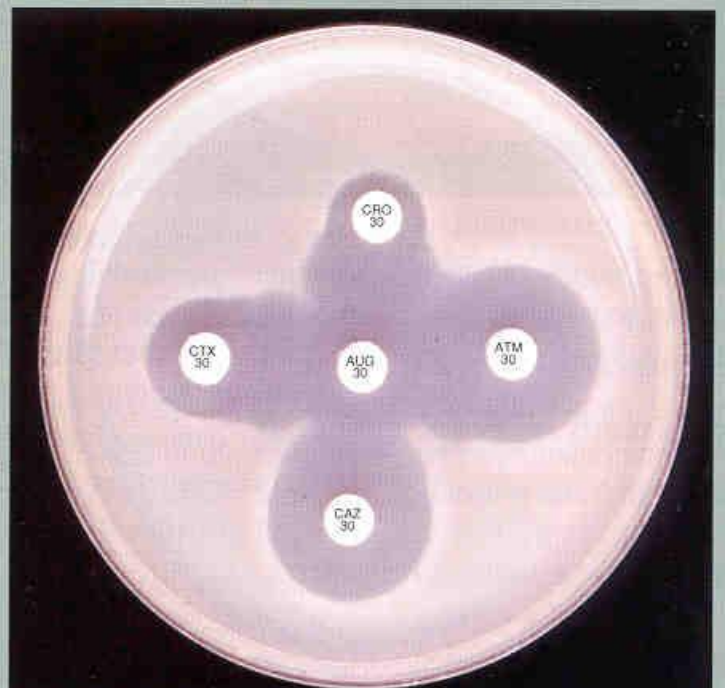
CULTURE :

:

1. Escherichia coli >10^8/L predom
2.
3.

R- Amp, Aug, Ceftriaxone, Gent, Aztreonam
S- Nor, Pip+tazobactam, Meropenem

Figure 2. Keyhole test (Jarlier [18]) demonstrating ESBL production by an extension of the zone of growth inhibition around aztreonam (ATM), ceftazidime (CAZ), cefotaxime (CTX) and ceftriaxone (CRO) toward the central amoxycillin clavulanate (AUG) disk.



Under CLSI below (2013), cefotaxime disc zone cutoff detects ESBL without need for keyhole test

Table 2A. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm)			MIC Interpretive Criteria (µg/mL)		
			S	I	R	S	I	R
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)								
B	Cefepime	30 µg	≥18	15–17	≤14	≤8	16	≥32
B B	Cefotaxime or ceftriaxone	30 µg 30 µg	≥26 ≥23	23–25 20–22	≤22 ≤19	≤1 ≤1	2 2	≥4 ≥4

What relevance is this organism?

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Dob  07-Aug-1929  Wd  J3M~JHH        Dr  Bernard JONES       c15:00 12-Feb-99

MICROBIOLOGY-Urine mid stream            Result status - VALIDATED BY M.O.
MICROSCOPY                                                   JH99M9275
White cells    : <10      x10^6/L
Red cells      : >100     x10^6/L
Squamous cells: <10       x10^6/L
Organisms      : Nil seen
Casts          : Nil seen
Other          :
Comment     :
CULTURE :
         :
1. Enterococcus faecium 10^6-10^7/L
2
```

Enterococcus species, no white cells

- Elderly male
- Very likely to have been collected without good reason (eg symptoms)
- Represents either chronic bacteruria or more likely contamination of the specimen from perineal flora

What about now! ?

Dob 07-Aug-1929 Wd J3M~JHH Dr Bernard JONES c15:00 12-Feb-99

MICROBIOLOGY-Urine mid stream Result status - VALIDATED BY M.O.
MICROSCOPY JH99M9275

White cells	: <10	x10^6/L
Red cells	: >100	x10^6/L
Squamous cells	: <10	x10^6/L
Organisms	: Nil seen	
Casts	: Nil seen	
Other	:	

Comment :

CULTURE :
:

1. Enterococcus faecium 10^6-10^7/L
2

Comment : Ampicillin MIC >256 mg/L (by E-Test) Resistant
Vancomycin MIC = 128 mg/L (by E-Test) Resistant

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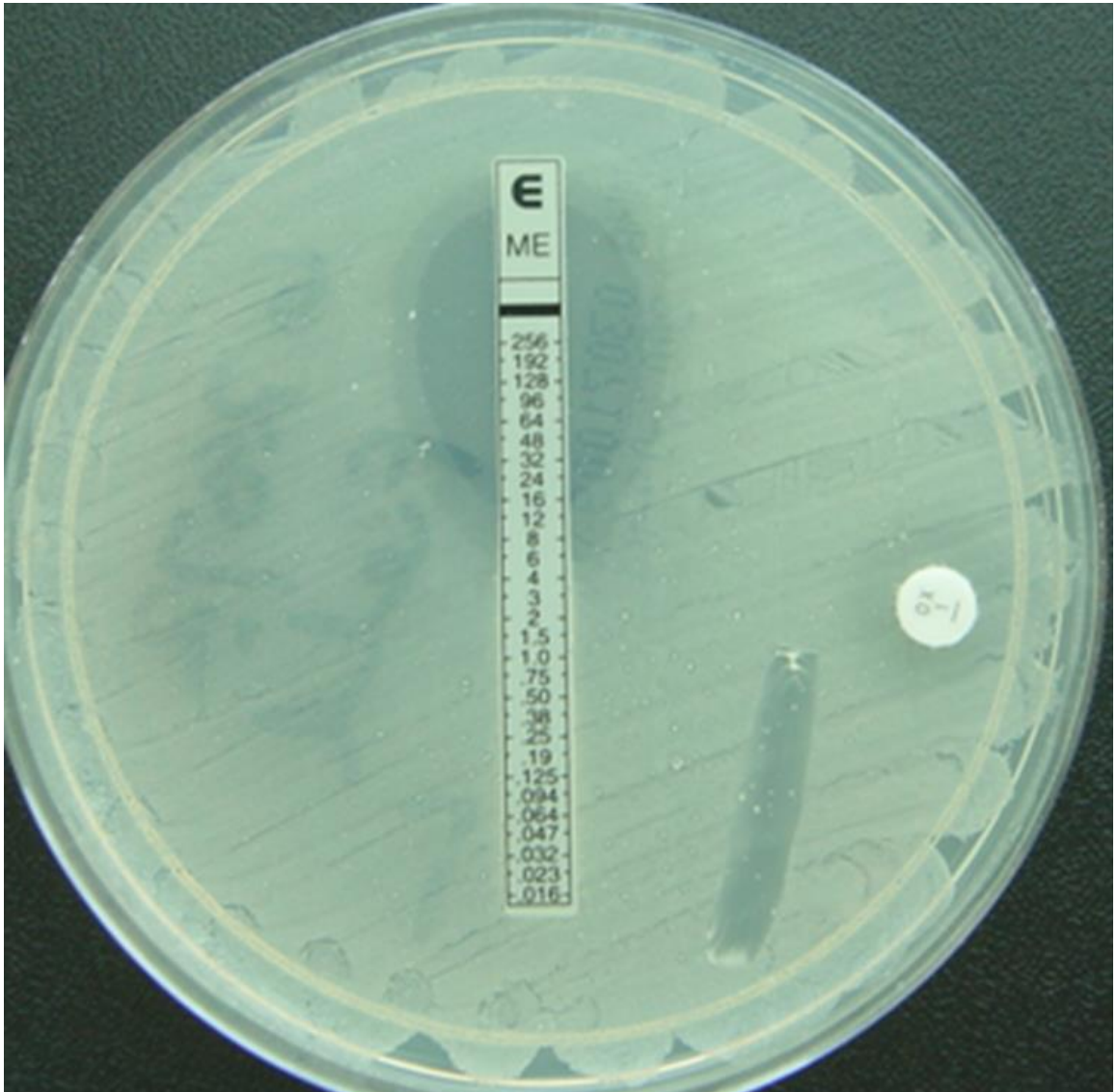
Comment :   Ampicillin MIC >256 mg/L (by E-Test) Resistant
            Vancomycin MIC = 128 mg/L (by E-Test) Resistant
```

- The ampicillin resistance is expected – *E. faecium* expresses a penicillin “binding” protein that does not bind ampicillin or other betalactams – it is a penicillin non-binding protein!
- The vancomycin test indicates that the isolate is a vancomycin-resistant Enterococcus (MIC >=32 g/L is the cutoff for resistance. This does not need treatment but in Australia , this patient will be isolated to reduce transmission to other patients

E-test example – Methicillin strip MIC=16



Asymptomatic bacteriuria

- Occurs in:
  - 5% healthy adult women, including those in early pregnancy (increases to 10% in late pregnancy)
  - 25% of elderly women
  - 25% of diabetic women
  - 15% – 20% of elderly men
- Only group with asymptomatic bacteriuria that has been proven to require treatment is pregnant women.

This patient is febrile post-op from abdominal surgery and has a catheter in situ. ? interpretation of her urine result Is therapy for uti indicated?

Dob 28-Feb-1938 Wd G1~JHH Dr P ANSELINE c14:00 16-Mar-99

MICROBIOLOGY-Urine catheter specimen Result status - VALIDATED BY M.O.
MICROSCOPY JH99M16957

White cells	: <10	x10^6/L
Red cells	: >100	x10^6/L
Squamous cells	: <10	x10^6/L
Organisms	: Rods - Moderate	
Casts	: Nil seen	
Other	: Nil seen	

Comment :
CULTURE :
:
1. Escherichia coli >10^8/L
2.
3.

Question responses

1. *What is the likely interpretation of her urine result?*

If the operation has taken place recently and the catheter has only been in for a short time, then this isolate could well be significant even in the absence of white cells. However still prudent to check abdominal wound, chest, iv drips etc. As well fever day 2 or 3 very common post operatively without sinister cause. Most patients who develop symptoms from catheter-related uti get them soon after bacterial invasion of the catheter system.

2. Is therapy for uti indicated?

Probably not. If the catheter can be removed then recommend this ASAP

In summary

Patients with Indwelling Urinary Catheters

- Colonisation is common (invariable if the catheter remains in long enough)
- Treatment with antibiotics is only warranted if symptoms are present
- presence of protein or nitrite on urinalysis or wbte/red cells on microscopy is a non-specific finding in catheterised patients
- presence of cultured bacteria is NOT predictive of the cause of fever in elderly catheterised patients who do not have urinary infection symptoms

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Dob  12-Sep-1936  Wd  RENALC~JHH     Dr  S CARNEY          c??:?? 24-Jun-99

MICROBIOLOGY-Urine mid stream            Result status - VALIDATED BY M.O.
MICROSCOPY                                                JH99M39276
White cells    : >100      x10^6/L
Red cells      : 10-100    x10^6/L
Squamous cells: >10        x10^6/L
Organisms      : Rods - Profuse
Casts          : Nil seen
Other          : Nil seen
Comment     :The presence of squamous cells indicates contamination of this
           CULTURE : Mixed growth of organisms >10^8/L
        :
1.
```

Patient with chronic renal failure

1. What is the significance of > 10 epithelial cells?
2. Given that white cells are present, what is your interpretation of this result? Is any treatment indicated?

Responses

1. *What is the significance of > 10 epithelial cells?*

These are squamous epithelial cells from skin around the perineum. Their presence in the sample indicates that contamination / poor collection may have occurred. This can then be responsible for mixed contaminated growth.

2. *Given that white cells are present, what is your interpretation of this result? Is any treatment indicated?*

The patient has chronic renal failure and presumably passes a low volume of urine per day. They may have an abnormal urinary tract with a high residual volume after passage. Both of these predispose to bacteruria and infection. The patient may have true mixed bacteruria with white cells resulting from subclinical cystitis. Alternatively the bacteria may relate to perineal contamination (squamous epithelial cells > 10) with white cells from some chronic non-infective process in the urinary tract eg analgesic nephropathy. Antibiotics would NOT be indicated without other symptoms or signs.

Major organisms associated with UTI

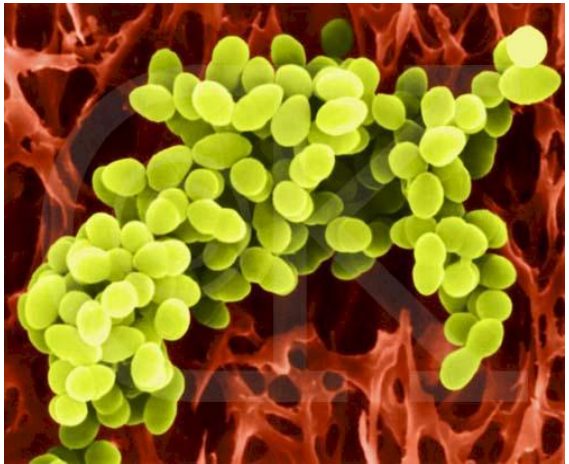
Organism	Outpatient UTI's	Hospital-Acquired UTI's
Escherichia coli	80%	40%
Staphylococcus saprophyticus	7%	-
Proteus spp.	6%	11%
Other Gram-negative rods	4%	25%
Other Gram-positive cocci	3%	19%
Candida spp.	-	5%

Significance of certain organisms isolated from urine

- Coagulase negative staphylococci
  - *S. saphrophyticus* – common cause of UTI in women of child-bearing age; penicillin susceptible, novobiocin resistant
  - Other strains of CoNS – most usually represent contamination and do not require treatment; exception – urological patients who have been recently instrumented

Significance of certain organisms isolated from urine

- *Staphylococcus aureus :*
  - *Contamination from vaginal flora in woman*
  - *Catheter-associated UTI*
  - *Systemic sepsis with secondary renal abscesses (often small) that leads to positive urine culture*



Other Gram positives that cause UTI

- *Streptococcus agalactiae* (Group B Strep)
- *Streptococcus pyogenes* (Group A Strep; in children)
- *Streptococcus pneumoniae*
- *Enterococcus* species (should be pure growth with white cells; not mixed)
- Urease positive *Corynebacterium* species – associated with renal tract struvite stones / calculi