Laboratory Diagnosis of Infection
The Role of Microbiology Laboratory

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Learning objectives

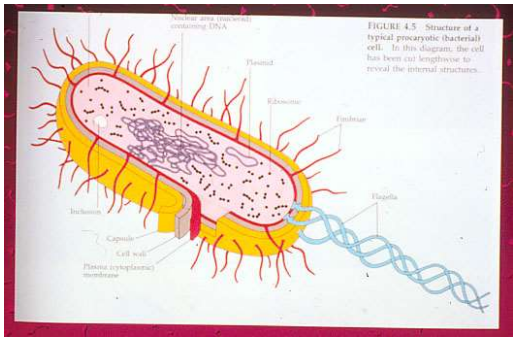
- List the reasons why microbiological laboratory diagnosis is important for good clinical care?
- Describe the factors that need to be considered in collection and transport of specimens for microbiological laboratory diagnosis?
- Describe the precautions to be followed when collecting blood for bacteriological culture?
- Describe the precautions to be followed when collecting urine for diagnosis of urinary tract infection?
- Describe the available options for diagnosis of viral infections?
- Describe how serological results are interpreted to arrive at a microbiological diagnosis?

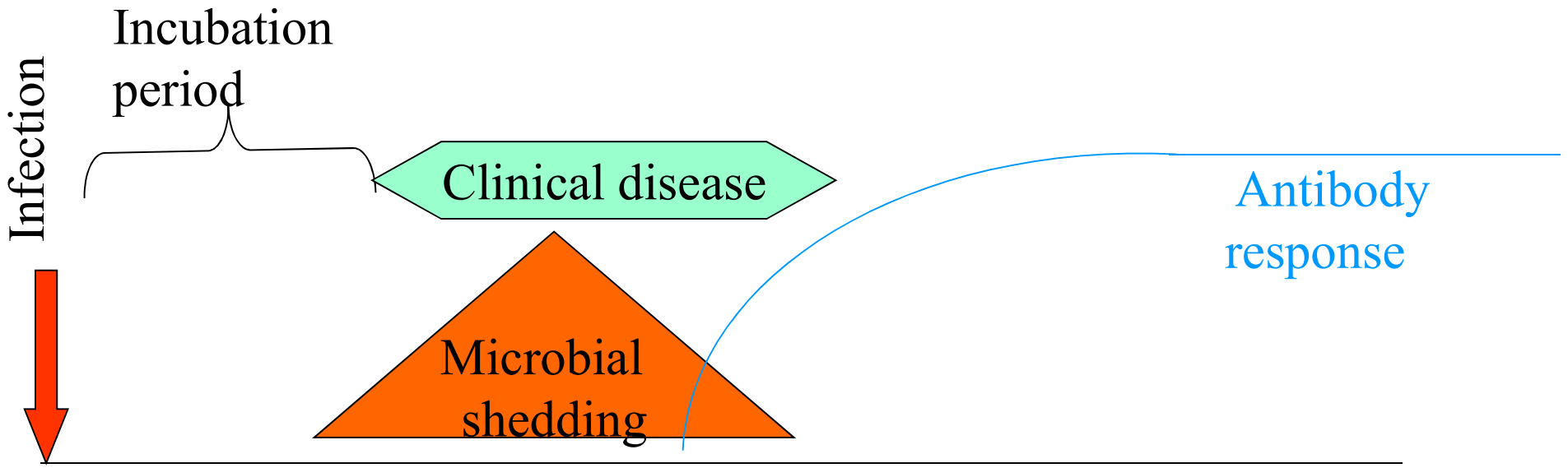
Microbiological diagnosis: Why?

- Anatomical (clinical) diagnosis vs. microbiological diagnosis.
  - *A disease may be caused different pathogens*
  - e.g. pyogenic meningitis in a child is most often caused by *Streptococcus pneumoniae, Haemophilus influenzae, Neisseriae meningitidis*
- Antibiotic sensitivity test: *Accurately target antimicrobial therapy – specific vs. broad spectrum or combination therapy*
- Monitor effectiveness of therapy
- Infection control
- Public health and epidemiology

Microbial diagnosis: How?

- Detect the microbe
- Detect antibody response - serology





Detect the microbe: How

- see - microscopy, *minutes*

- grow (culture) – *days*

- Microbial antigen detection – *hours*
- Microbial nucleic acid detection (e.g. Polymerase chain reaction) (PCR) - *hours / days*

Collection and transport of clinical specimens for microbiological examination

- What specimen? representation of the infectious disease process
- Well collected specimen
- Appropriate transport medium or procedure
- Appropriate storage, packing and transport to the laboratory.
- Specimen request form: Relevant clinical details
  - *Date of onset of illness*
  - *Suspected clinical diagnosis*
  - *Prior antibiotic therapy*
  - *Known biological hazards (e.g. HIV positive) clearly stated on the request form and biohazard labels be put on the specimen bag.*

Queen Mary Hospital Department of Microbiology
Bacteriology & Virology Investigation Form

2nd Label

Date of collection: _____ Time: _____ AM/PM
(Day/Month/Year)

Biohazard: unknown/known (specify) _____ Date of admission:

H.K.I.D. No. _____ Hosp Name & No. _____
AE
HN _____

Surname _____

Sex/Age: _____

Firstname _____

Ward/Bed: _____

Physician in charge/unit _____ OUTPATIENT ☐

Date received:

Lab no.:

Diagnosis/Clinical history
(Specimen without relevent details may be rejected)

NATURE OF SPECIMEN

(For Virus Culture: Must be in Virus transport medium)

1. ASPIRATE
   - BAL [120]
   - BA [27]
   - ETA [24]
   - NPA [14]
   - Gastric [102]
   - Tracheal [21]
   - Other site
2. BILE (please specify)
   Site: GB/PTBD/CBD /T-tube
3. BLOOD: (culture) [30]
   (clotted) [31]
   EDTA [106]
   Heparin [121]
4. C.S.F [32]
   V.P. Shunt [109]
5. FLUID:
   - Pleural [38]
   - Peritoneal [42]
   - Peritoneal dial [122]
   - Joint [40]
   - Pericardial [39]

   Other :
6. IV CATHETER
   - Broviac/Hickman [91]
   - Other site: (please specify)
7. PUS [66]
8. SPUTUM: [20]
9. SWAB:
   - Endocervical [88]
   - Ear Rt. [55] Lt. [56]
   - Eye Rt. [57] Lt. [58]
   - High/vaginal [85]
   - Nasal [17]
   - Nasopharyngeal [18]
   - Oral [16]
   - Perineal [80]
   - Peritoneal [128]
   - Placental [130]
   - Rectal [97]
   - Throat [19]
   - Umbilical [79]
   - Urethral [86]
   - Ulcer [131]
   - Superficial wound swab [63]
   - Other site swab ☐ (please specify)
10. Saliva [133]
11. Skin Scraping [78]
12. Skin vesicle fluid [134]
13. STOOL [96]
14. STERILITY TEST:
    - Milk [98]
    - Spore Strip [99]
15. TISSUE
    Site [69]
16. URINE
    - Bag [4]
    - CSU (indwelling) [3]
    - CSU (once) [142]
    - Early morning (AFB) [10]
    - Midstream [1]
    - Suprapubic [5]
    - Nephrostomy urine [12]
17. OTHERS (please specify)

Sputum for AFB : Day 1 / Day 2

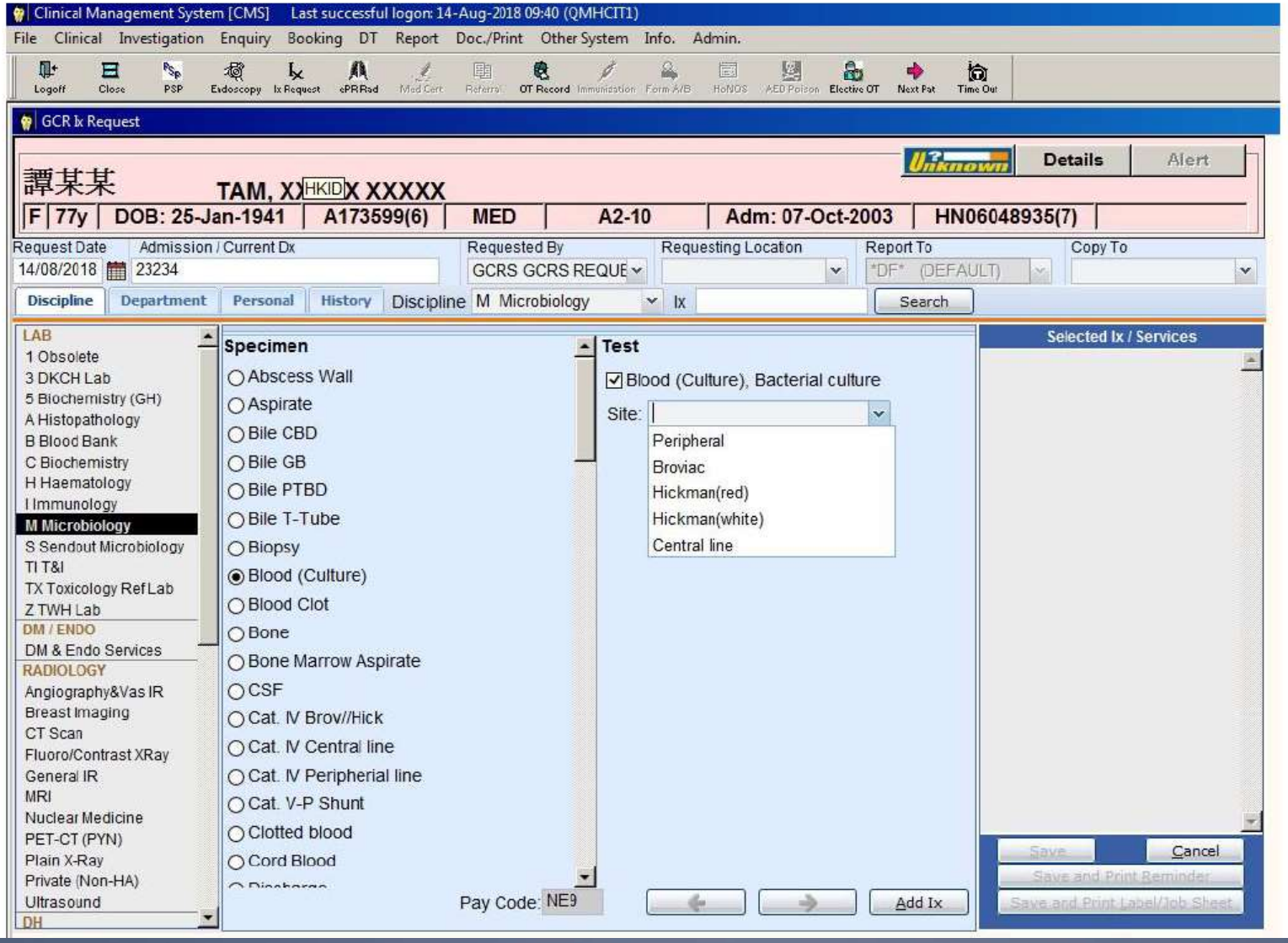
Chest x-ray abnormal .....

If CXR normal, test must be endorsed by ward specialist in-charge

_____
(Specialist name / staff no.)

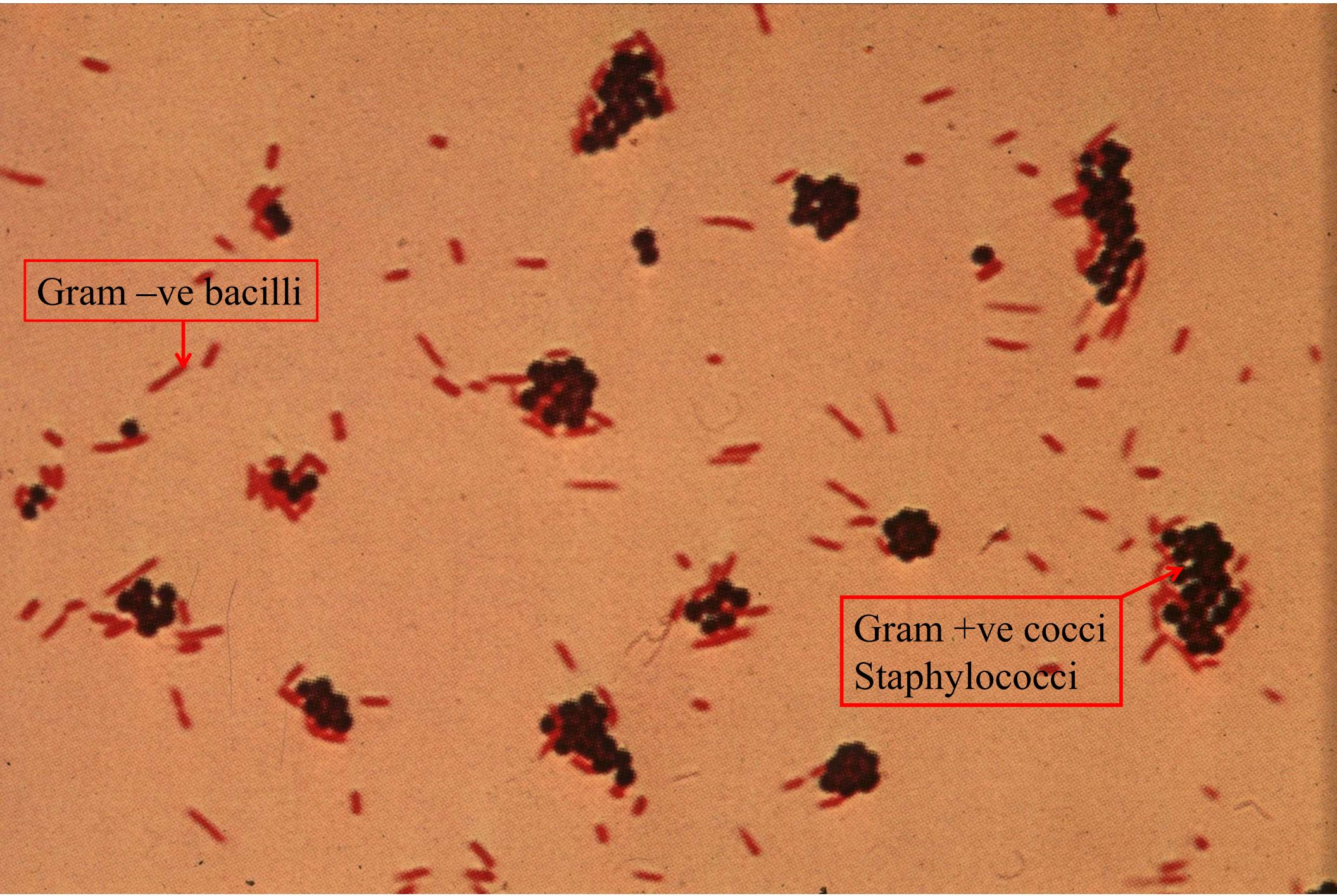
Bacteriology investigation

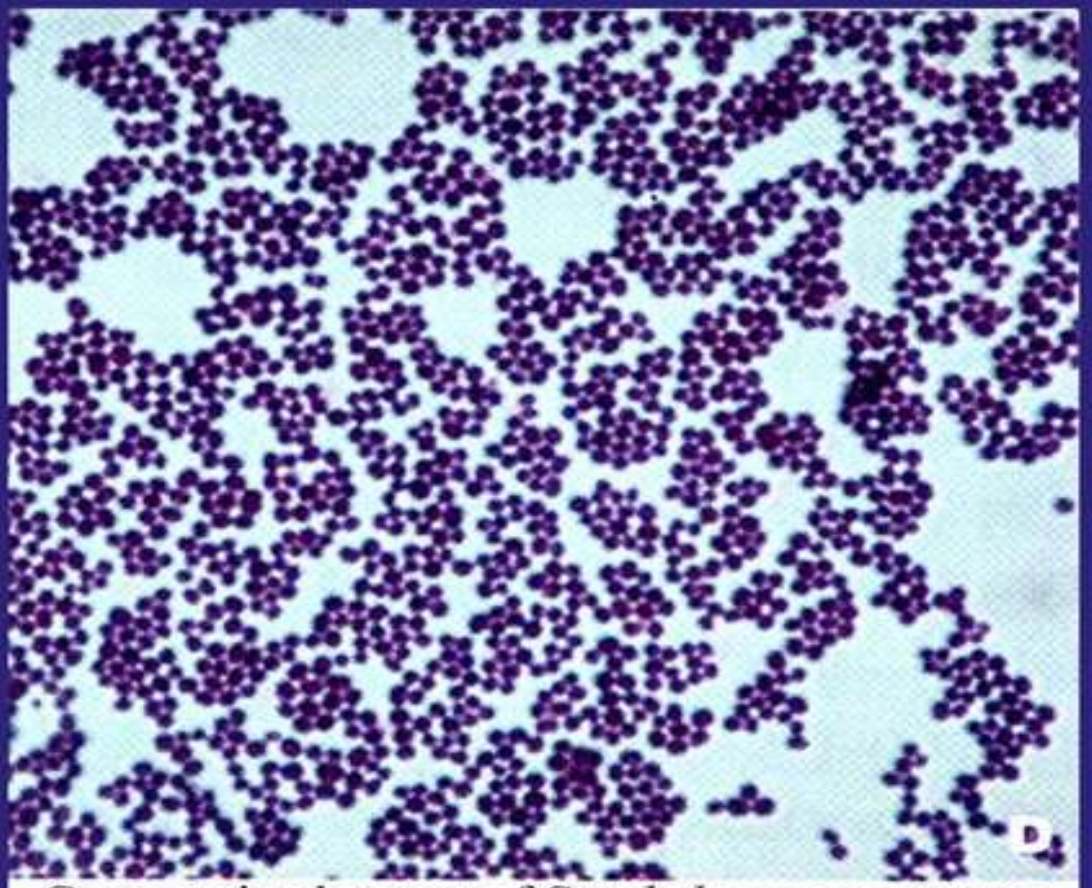
Ward ordering on CMS (Clinical Management System)




BD
40 mL
Queen Mary Hospital
Bacteriology & Virology Laboratory
Bacteriology Investigation
(one separate form for one box)
OTHERS (please specify)
Sputum for AFB : Day 1 / Day 2
(Specialist name / staff no.)
Urine routine
Urine Cx/Se
FOR ANTIBIOTIC ASSAY
Serum level: Creatinine
Dosage & Frequency:
Amikacin
Gentamicin
Netilmicin
Aerobic culture for
AFB
Bacteria
Fungus
Anaerobic culture
Smear for
AFB
Bacteria
Fungus
Serology for
Anti-nuclear Ab
ASOT
Aspergillosis Ag
Brucellosis Ab
Cryptococcal Ag
Cytotoxosis Ab
Diphtheria Ab
Melioidosis
Peritoneal
Placental
Sputum
Throat
Urethrogal
Urethral
Ulcer
Peritoneal dial
Joint
Other


SIZE
Large
Medium
Small
LATEX EXAMINATION GLOVES
POWDERED
FOR URINE


Gram –ve bacilli
Gram +ve cocci
Staphylococci



Gram stained smear of *Staphylococcus aureus* on blood agar plate

Staphylococcus aureus

Antibiotic sensitivity test

NAME : XXXXX XXXX XXX
SEX/AGE : F/65
WARD/BED : K8N/24
HOSP.NO : 9456877
REQ. DATE : 1/4/2014

LAB.NO :94156788
SPECIMEN : Blood

INVEST : C&ST

Culture : Pure growth of *E. coli*

Ampicillin	R
Cephalothin	S
Gentamicin	S
Co-trimoxazole	R

Bacterium is Resistant
Cannot use for treatment

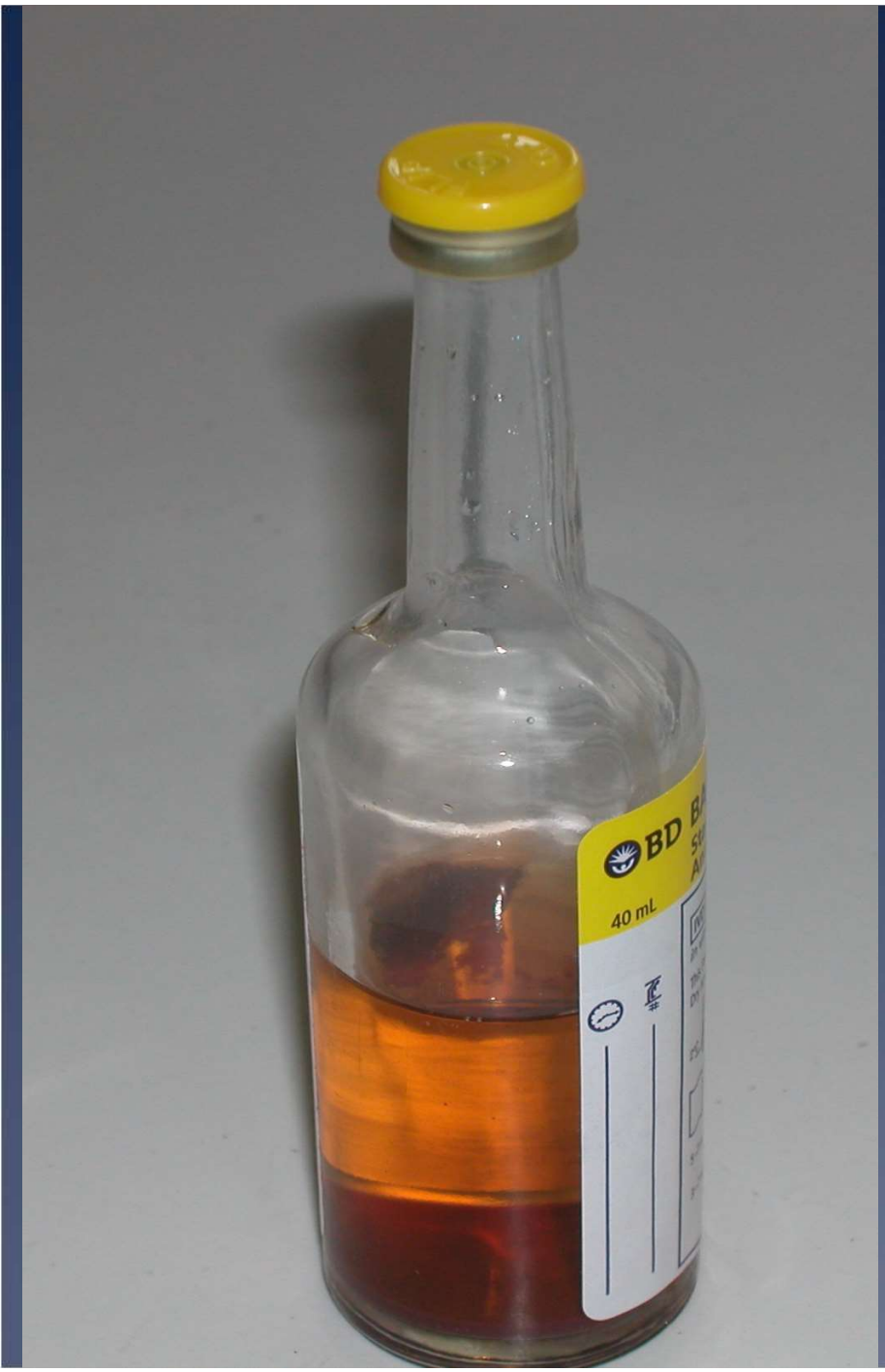
Bacterium is Sensitive
to antibiotic.
Can use for treatment

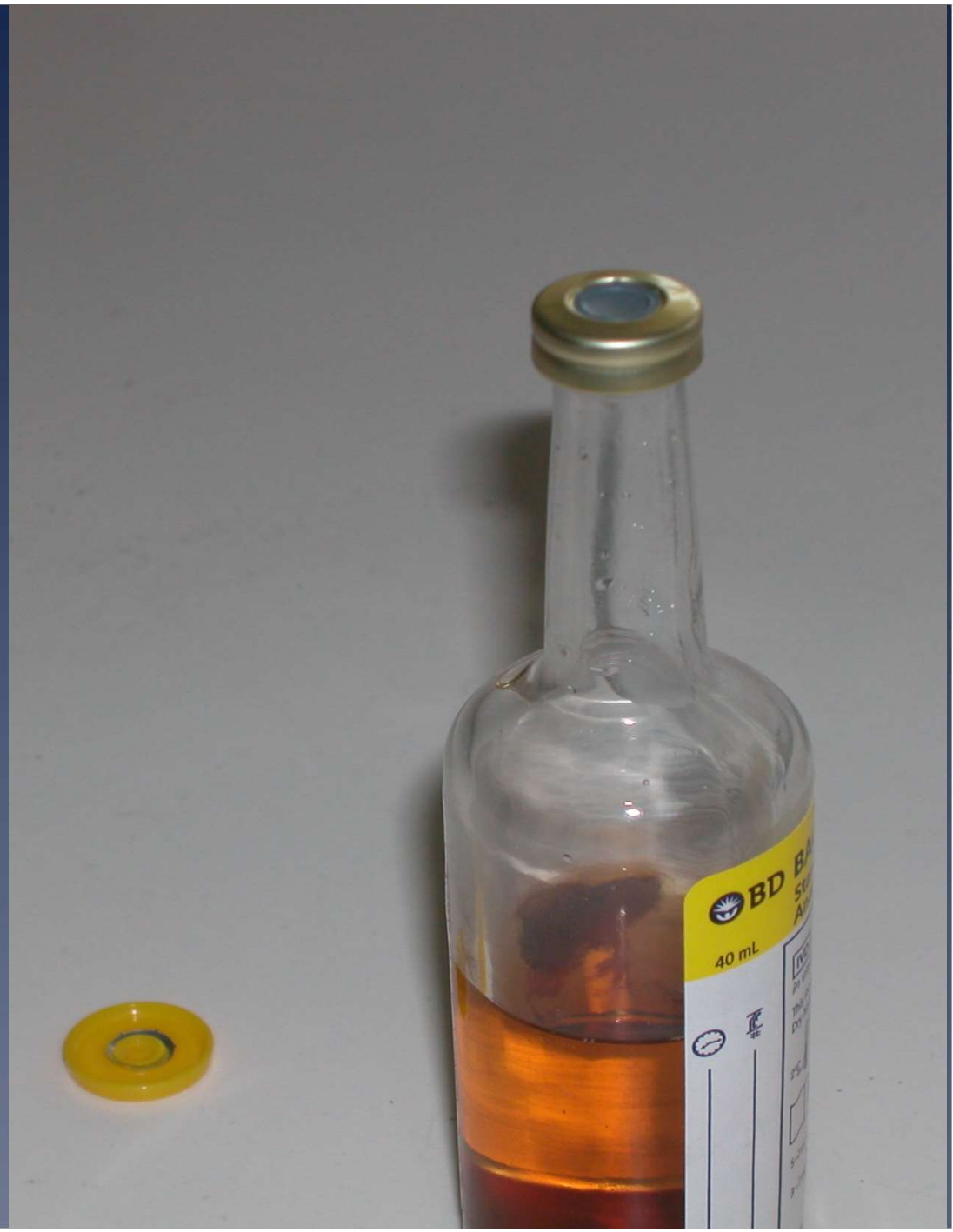
Blood Culture

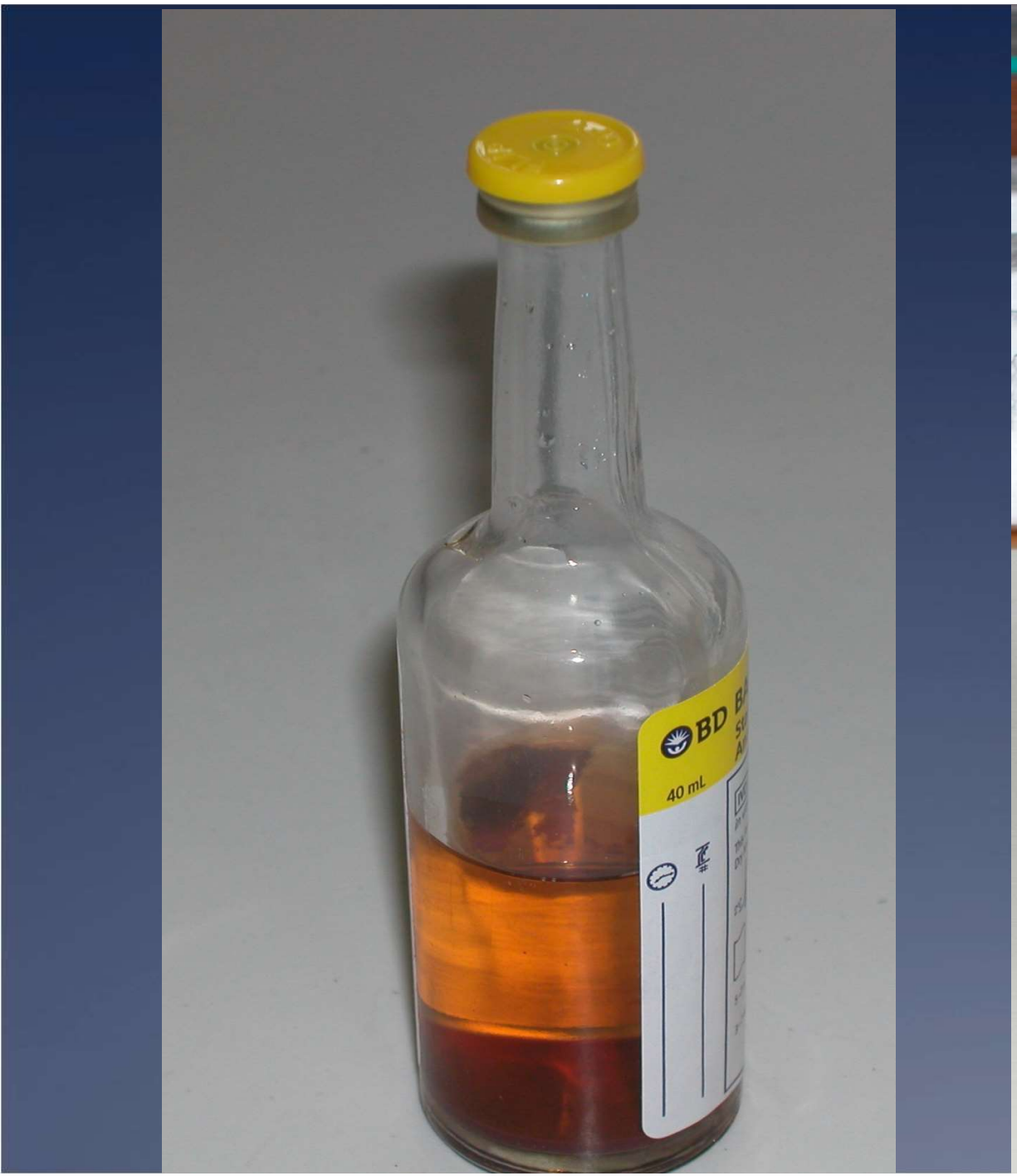
Common Indications: endocarditis, suspected sepsis

- Unexplained fever
- Sudden relative increase in pulse rate, temperature;
- change in consciousness, confusion
- onset of chills, prostration and hypotension;
- prolonged, mild, intermittent fever without/with a heart murmur.


BACTEC™
Standard
Anaerobic/F
BD BACTEC™
PLUS +
Aerobic/F
25 mL
1-3 mL


BD
40 mL


BD
40 mL


BD
40 mL


BD
40 mL
D510005(5) M 82y
CHU, CHI CHANG
HN03007043(Y) D3 3 MED
24/01/2003 15:36 EP1
HN03007043(Y) D3 3 MED
24/01/2003 15:36 EP1
5 mL

Blood Culture

- At least 2 blood cultures should be taken; in febrile patients with a central venous catheter, one set should be taken through the catheter and another set by peripheral venous puncture
- Aerobic / Anaerobic micro-organisms;
- Aseptic technique;
- Adequate volume (~20 ml)
- Sample collection before antibiotic administration
- Take specimens to the laboratory promptly, or place them in an incubator at 37 C.

Blood Culture

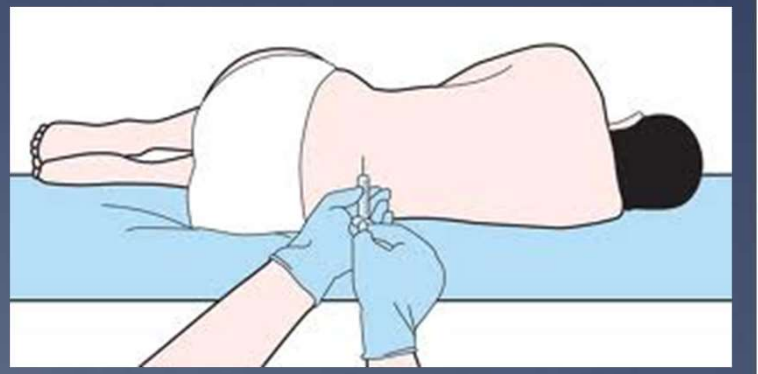
- Broth (BACTEC) with resins to adsorb (inactivate) antibiotics.

Cerebrospinal Fluid (CSF); Sterile Body Fluid

Cerebro-spinal fluid (CSF)

The representative specimen for meningitis.

Lumbar puncture should be undertaken whenever meningitis is suspected after a intracranial space occupying lesion is excluded.



Common indications :

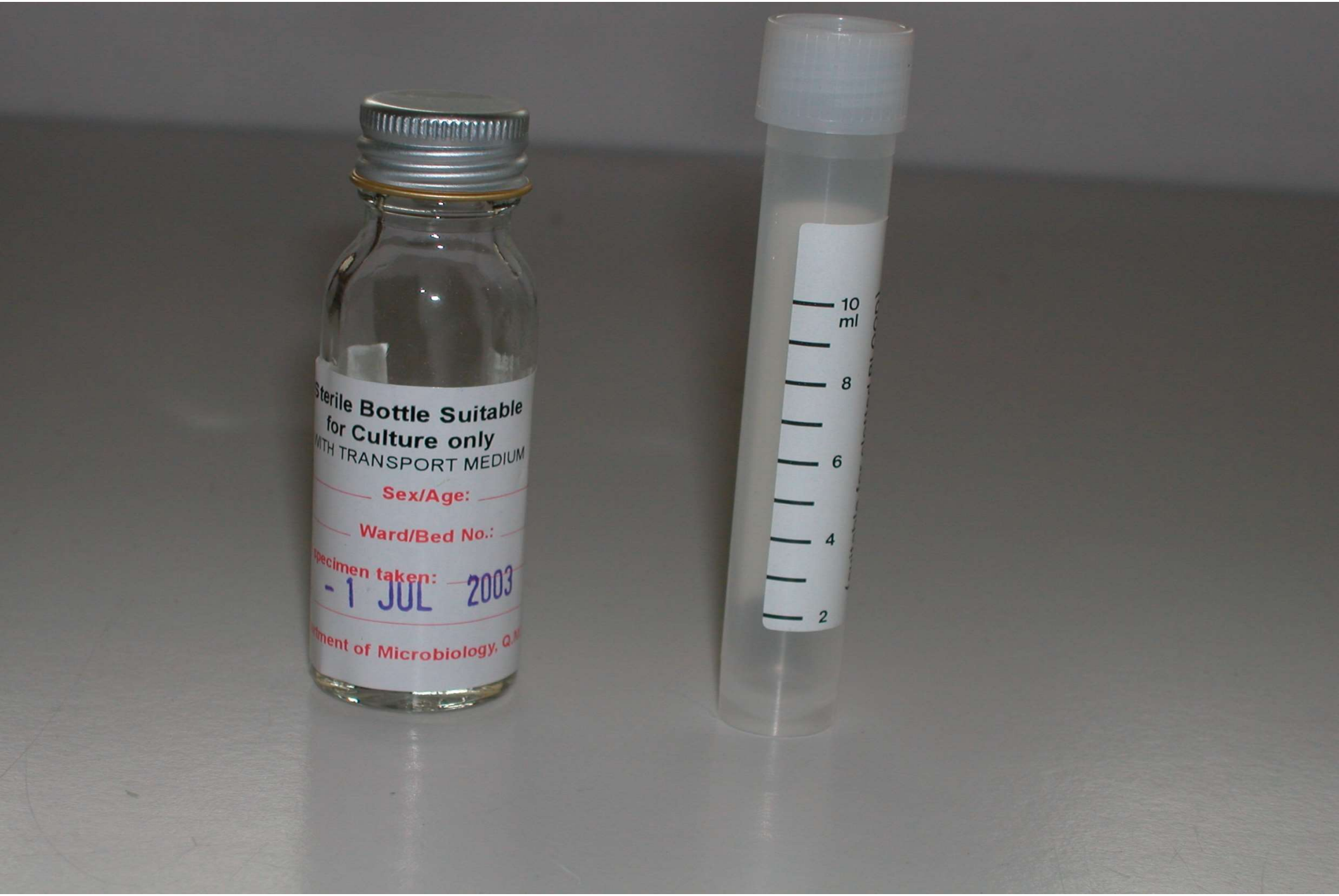
- Fever, headache and meningeal irritation
- unexplained febrile illness in an irritable infant who is feeding poorly.

CSF

- Strict aseptic technique
- Use of new (Non-reused) sterile glass bottles (labeled: microbiology only) to prevent falsely positive gram smear results.
- Aliquots for sample bottles
- Immediate transport to the laboratory.

Sterile Body Fluid

- Including pleural, peritoneal (dialysis), pericardial and synovial (joint) fluid.
- Strict aseptic technique : per-cutaneous needle aspiration.
- Use of bottles containing citrate or heparin may be necessary if the specimen is prone to clotting (e.g bone marrow).


Sterile Bottle Suitable
for Culture only
WITH TRANSPORT MEDIUM
Sex/Age:
Ward/Bed No.:
Specimen taken:
- 1 JUL 2003
10
ml
8
6
4
2

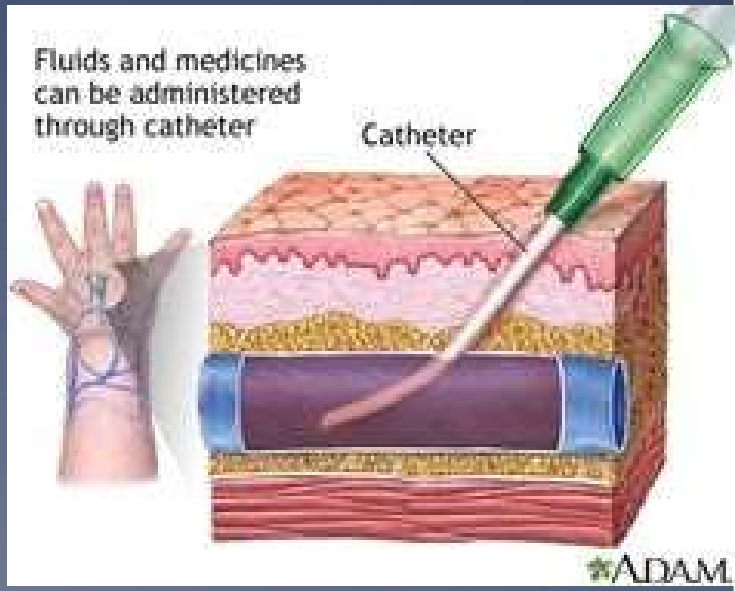
Wound Swabs

Swabs

- Wound swabs, ear and eye swabs, drainage fluid, abscess (pus), sterile aspirate, tissue
- Tissue biopsy specimens should be put into sterile, wide mouth, screw-cap containers e.g. universal bottles.

Intravascular catheter

- The exit site of the catheter is disinfected; after the area is dry, the catheter is removed and the distal 5 cm segment is aseptically cut off and directly dropped into a dry screw-cap container.
- The catheter segment is rolled over the blood agar plate 4 times.
- The presence of $\geq$ 15 colony forming units of a single organism after 48 hours of incubation is associated with an increased risk of catheter-related infections.



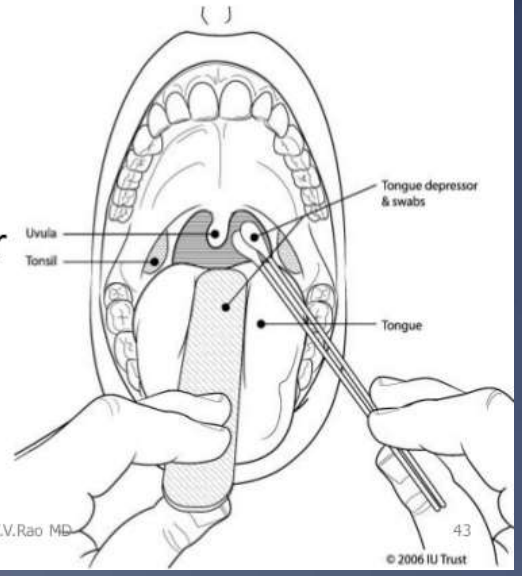
Faeces (Stool/Rectal swab)

Upper Respiratory Tract

Throat Swab

Specimen collection in Throat Infections

- A plain cotton wool swab should be used to collect as much exudates as possible from tonsils, posterior pharyngeal wall and other area that is inflamed or bears exudates



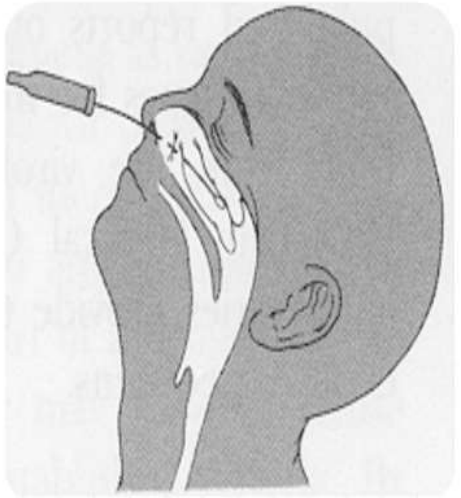
Nasopharyngeal Swab for

Bordetella pertussis (Whooping cough)

Virus diagnosis

Nasopharyngeal swab

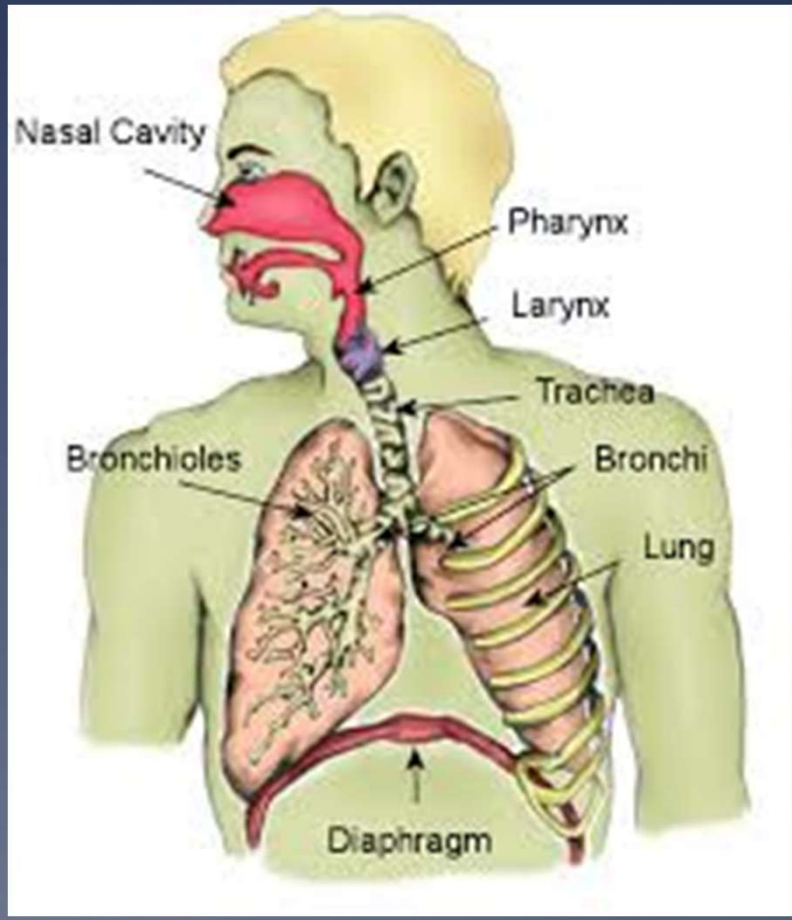
- A deep nasal swab generally yields the same information as throat swab.
- Nasal swabs are taken to detect healthy carriers than diagnose deep infection
- Deep nasal are taken to diagnose S.pyogenes and Diphtheria bacillus.

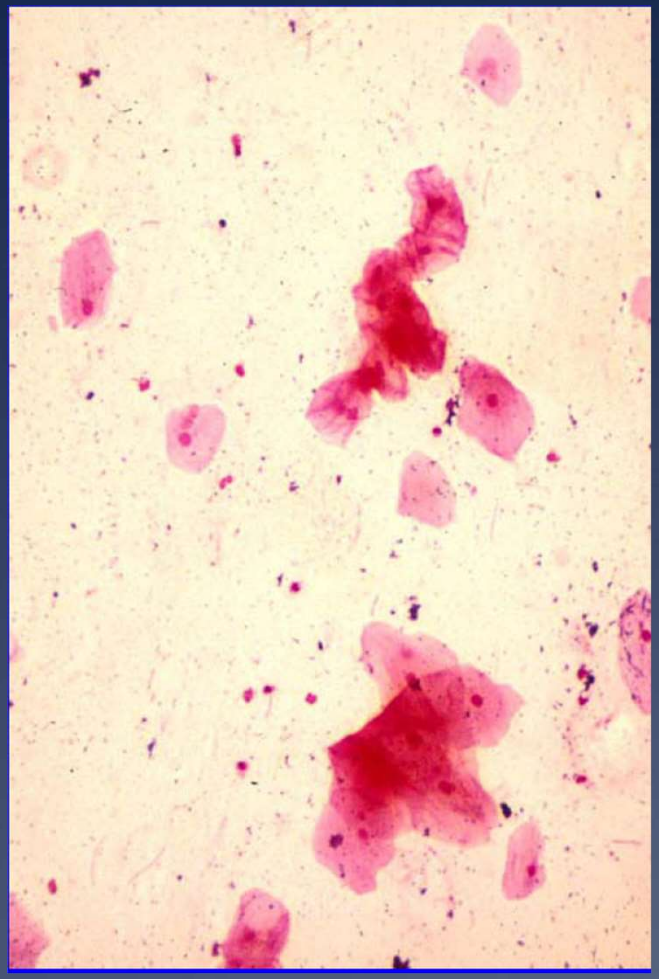


Lower Respiratory Tract Infections

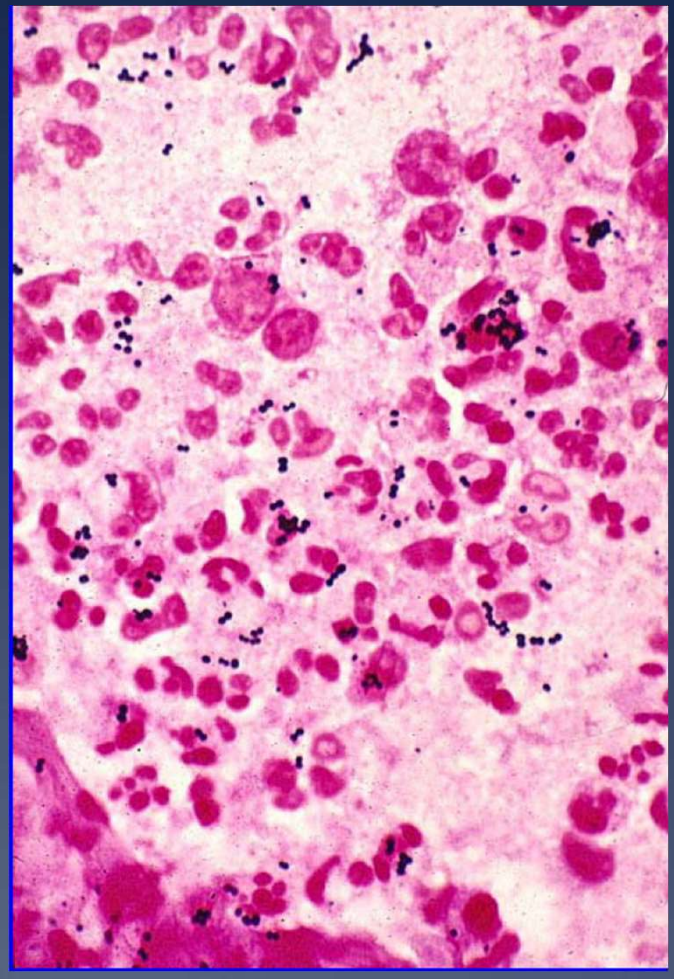
Expectorated sputum :

- Cough from lower respiratory tract. Not saliva.
- A high squamous epithelial cell content (from the buccal mucosa) together with a low white cell count on gram staining suggest oral-pharyngeal contamination).





Squamous epithelial cells from the buccal mucosa



Mainly neutrophils (pus cells) indicative of specimen from the site of infection.

http://www.fletcherallen.org/upload/photos/6088Sputum_Gram_Stain.pdf

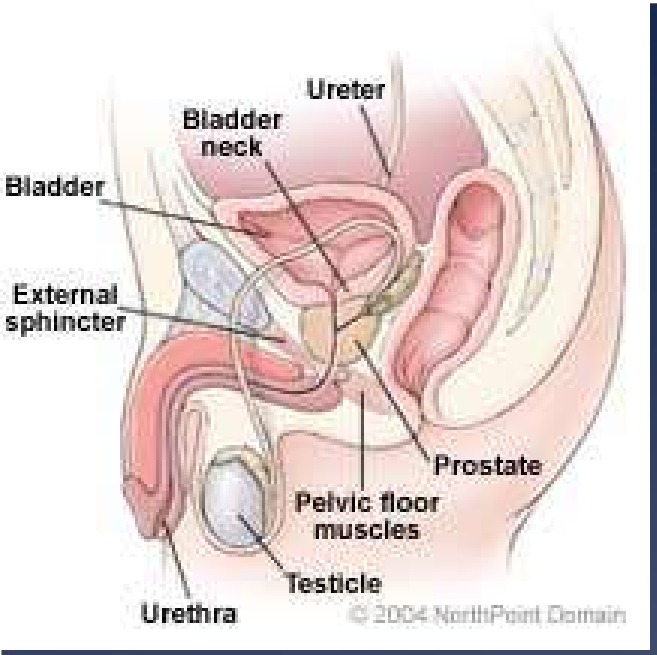
Urine


FOR URINE CULTURE ONL
Name:
Ward/BED No
Time/Date
MSU
CSU
Please send specimen to Laboratory within 1/2 hr
Or Store at 4—10°C overnight
Microbiology, Q.M.H. Tel.: 28553216


FOR URINE CULTURE
Name:
Ward/BED No
Time/Date
Please send specimen to Laboratory
Or Store at 4—10°C overnight
Microbiology, Q.M.H. Tel.:

Urine

- Proper instruction regarding the collection of clean-voided (catch), mid-stream urine (MSU) is critical to ensure accuracy of results.
- All urine specimens must be transported to the laboratory for processing within an hour of collection unless refrigerated at 4^{o}C.
- Quantitative culture

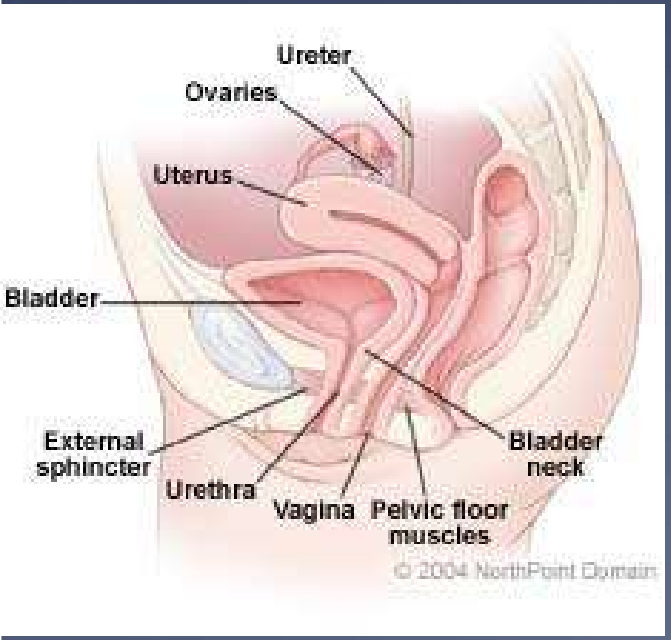


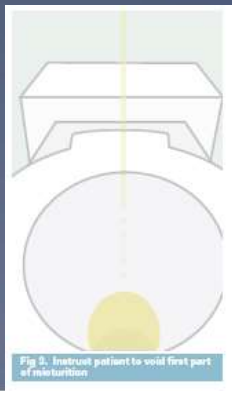
Mid-stream Urine

• Uncircumcised men → instructed to retract the foreskin before micturition. Women should be instructed to part the labia.

• Clean with sterile water.

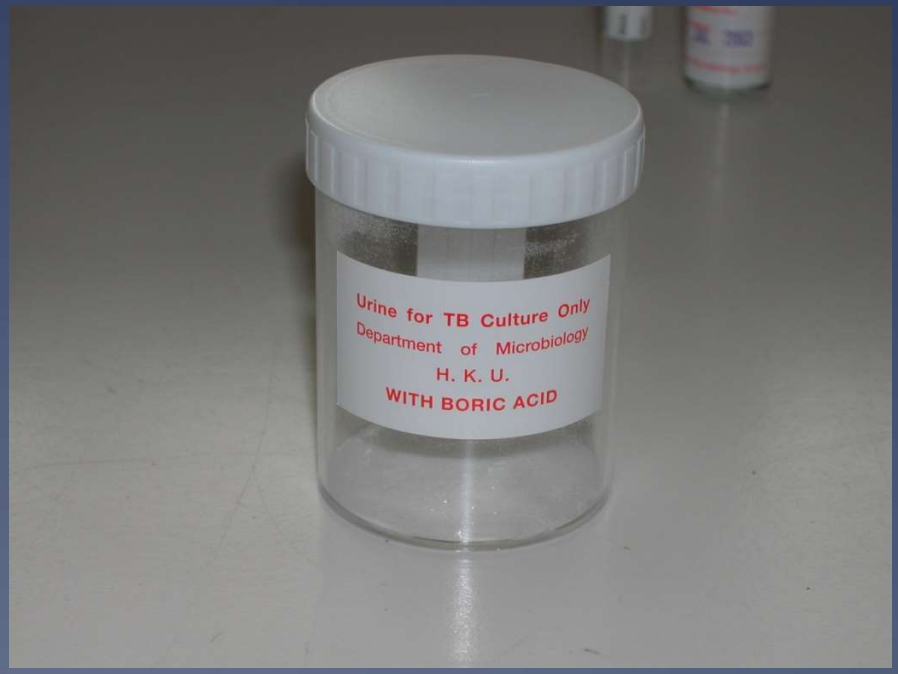
• Strong urine flow is important in clearing bacteria from the urethral meatus. Obtaining the sample when bladder is full will result in the least contaminated sample.



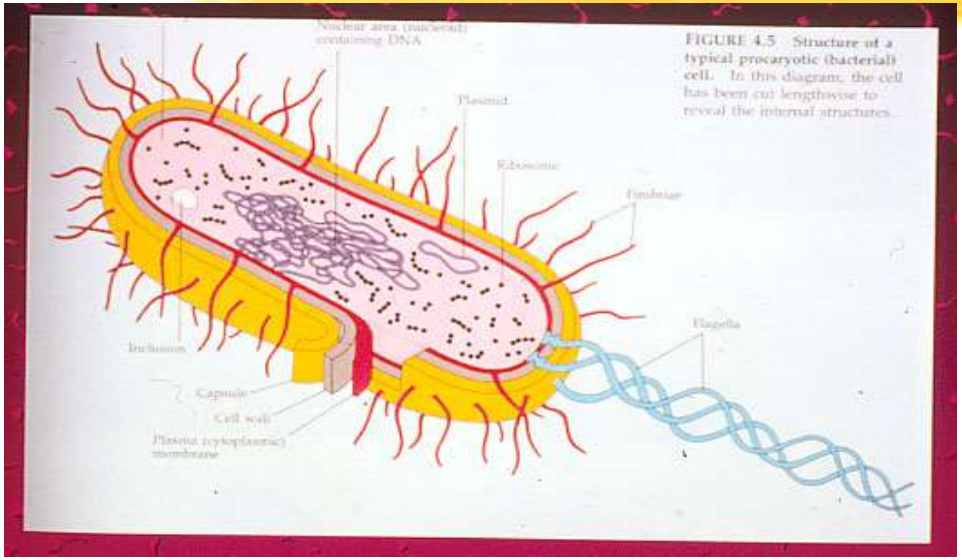


Urine for *Mycobacterium tuberculosis* (TB) culture

- Three consecutive early morning urine should be collected in patients with suspected renal tuberculosis

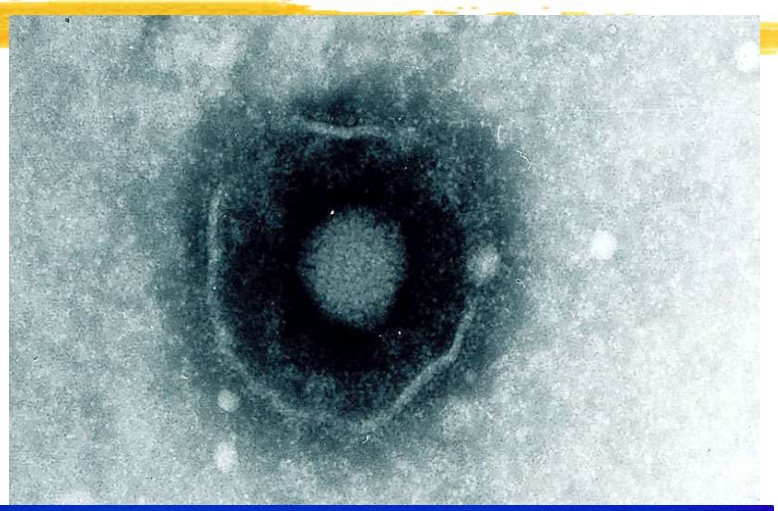


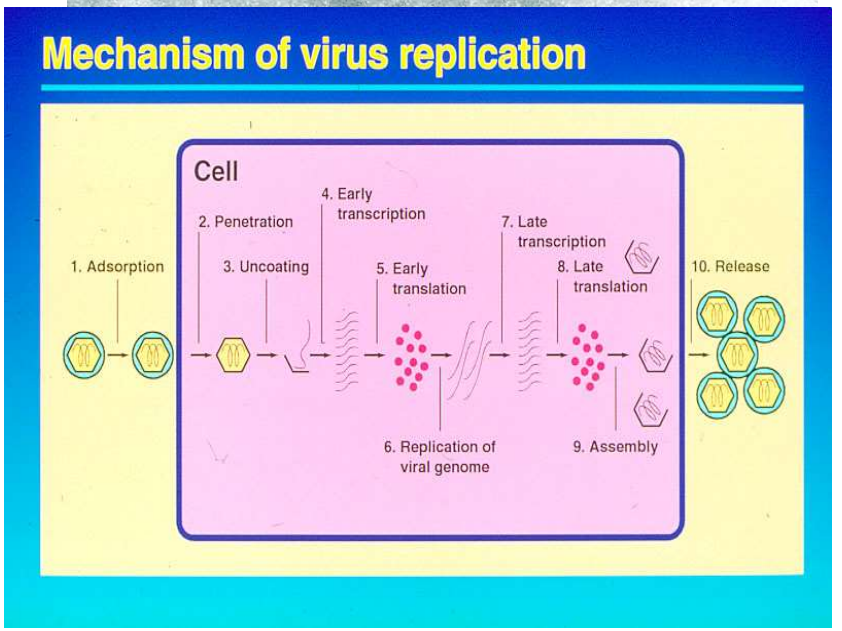
Bacteria



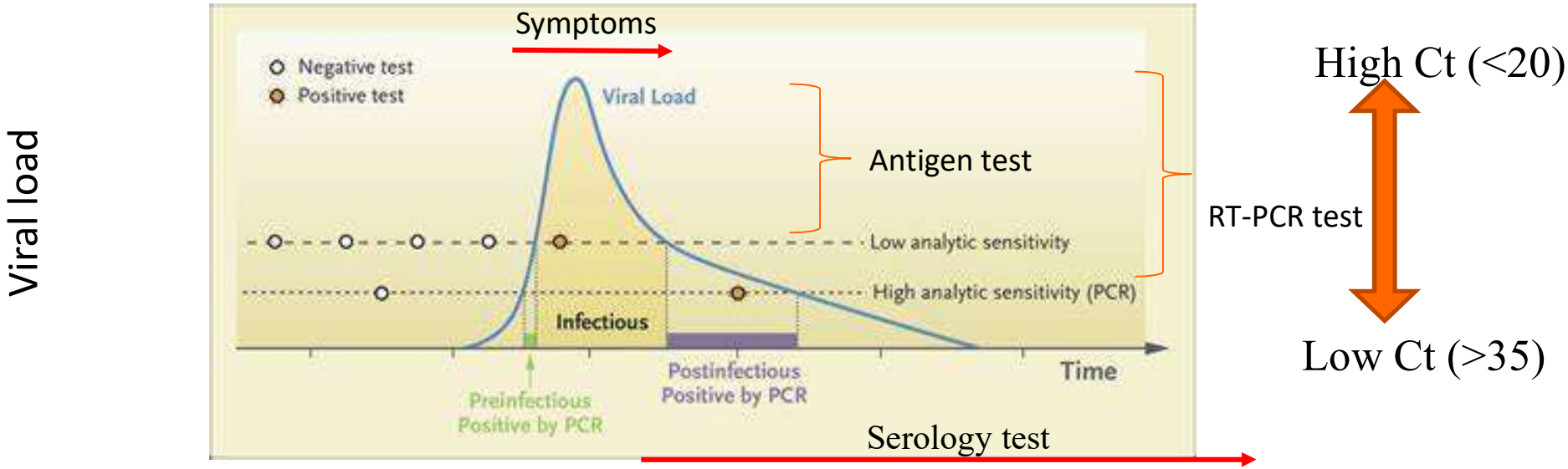
	No of genes
⌘ Human	>20,000
⌘ Bacterium	~ 4000
⌘ Herpesvirus	~ 100
⌘ Hepatitis B v	<10

Virus





Infectiousness and testing strategy in COVID-19



Modified from Mina et al NEJM Oct 2020

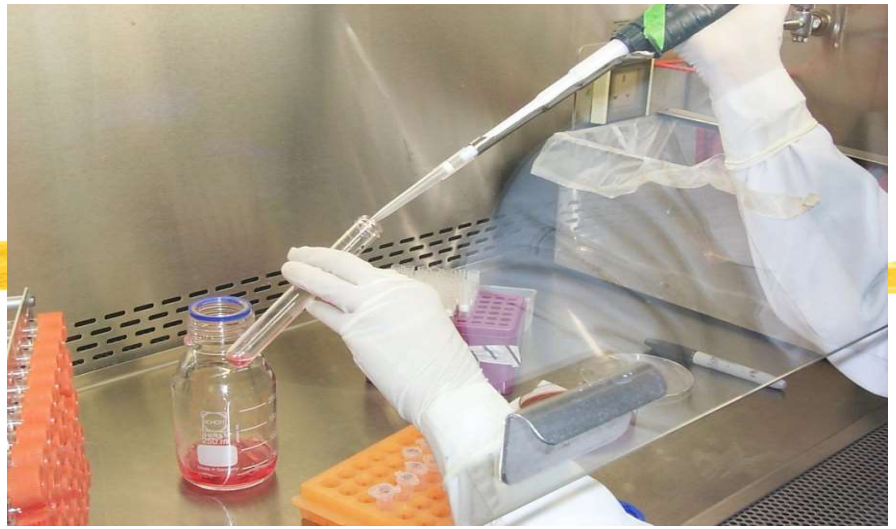
RT-PCR positive does not necessarily mean the person is infectious

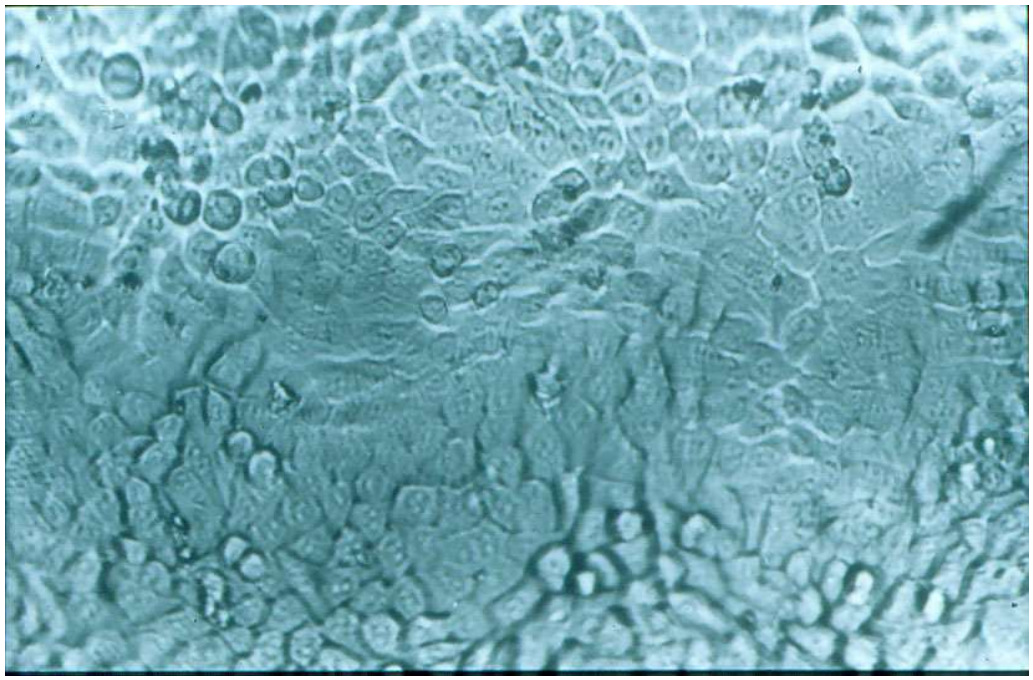
Use the test appropriate for the question you are trying to answer

Viral diagnosis: Detect virus

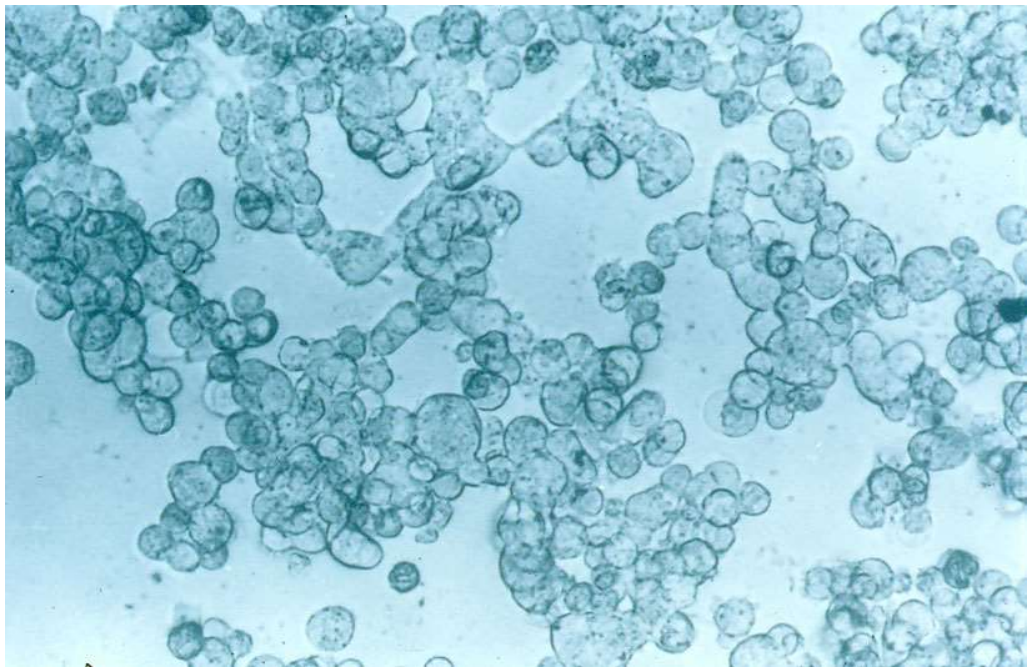
- grow (virus culture in cell lines) - *days / weeks*
- viral antigen detection - *hours*
- viral nucleic acid detection (e.g. Polymerase chain reaction) (PCR) - *hours / days*

Cell culture



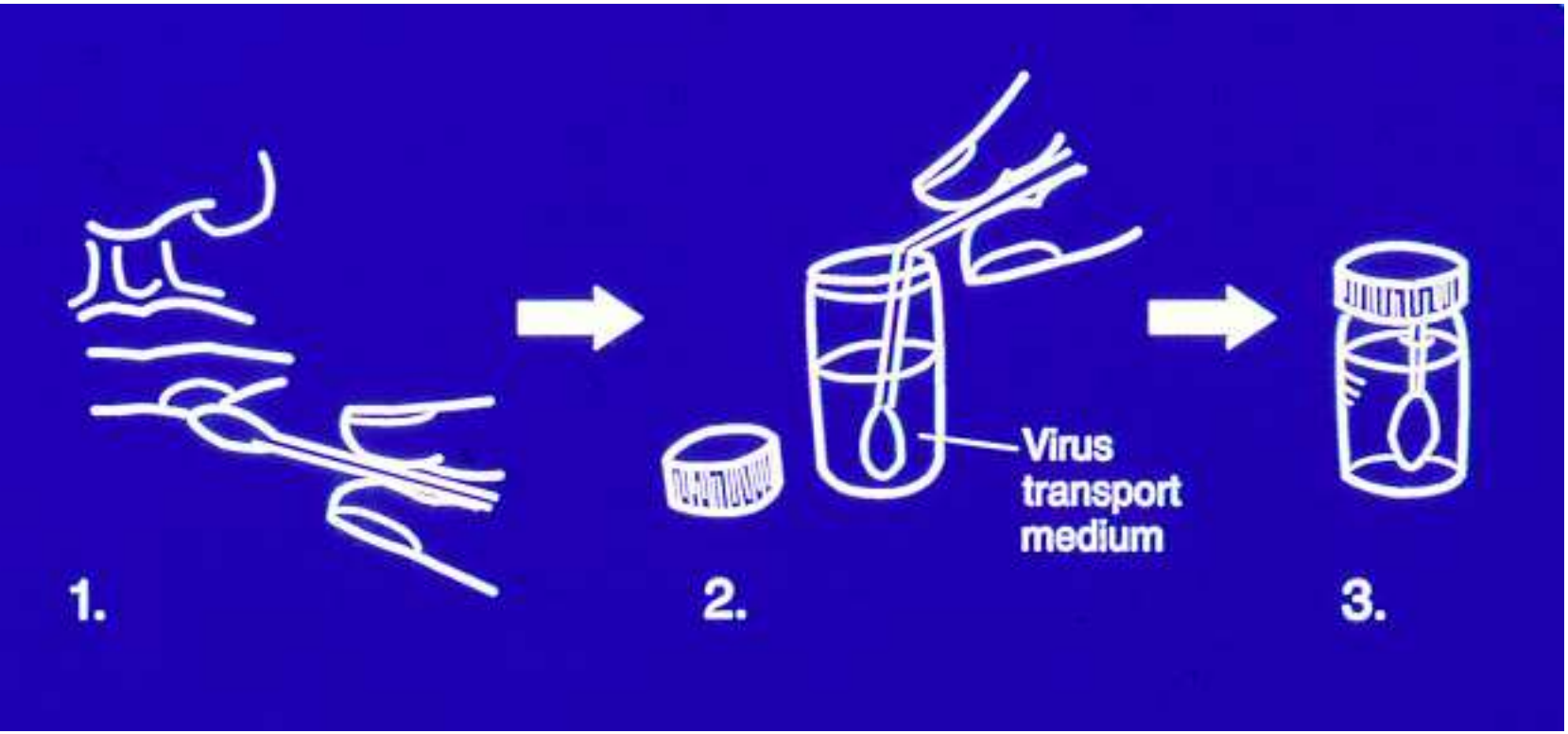


Normal cells



Virus cytopathic effect

Virus transport medium



Virus transport medium CANNOT be used for culture of bacteria

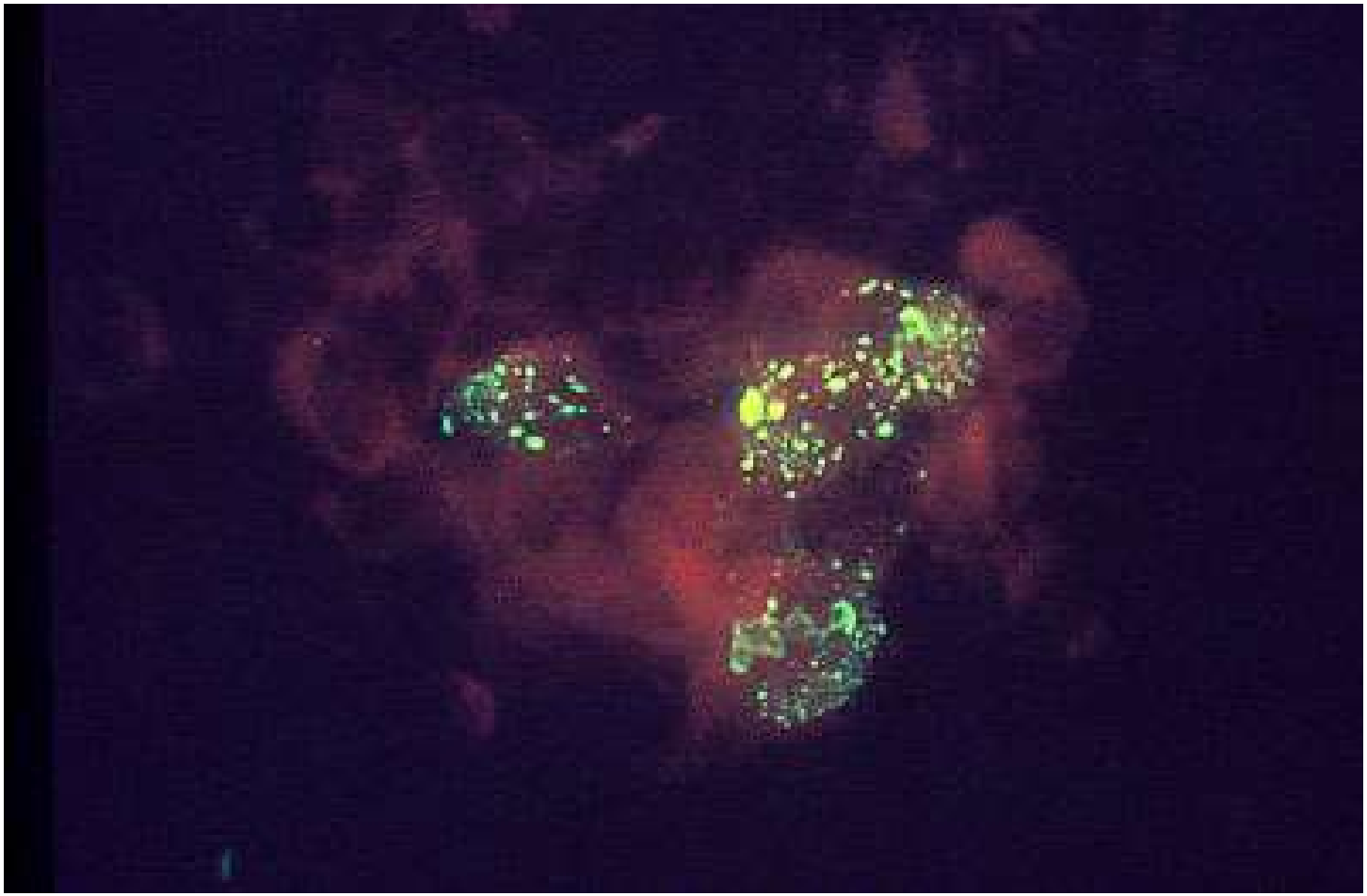
Buffer – pH
pH indicator

Protein – maintain virus stability
Antibiotics – prevent bacterial overgrowth

Viral diagnosis: Detect virus

- grow (virus culture in cell lines) - *days / weeks*
- viral antigen detection - *hours*
- viral nucleic acid detection (e.g. Polymerase chain reaction) (PCR) - *hours / days*

Viral antigen detection by immunofluorescence

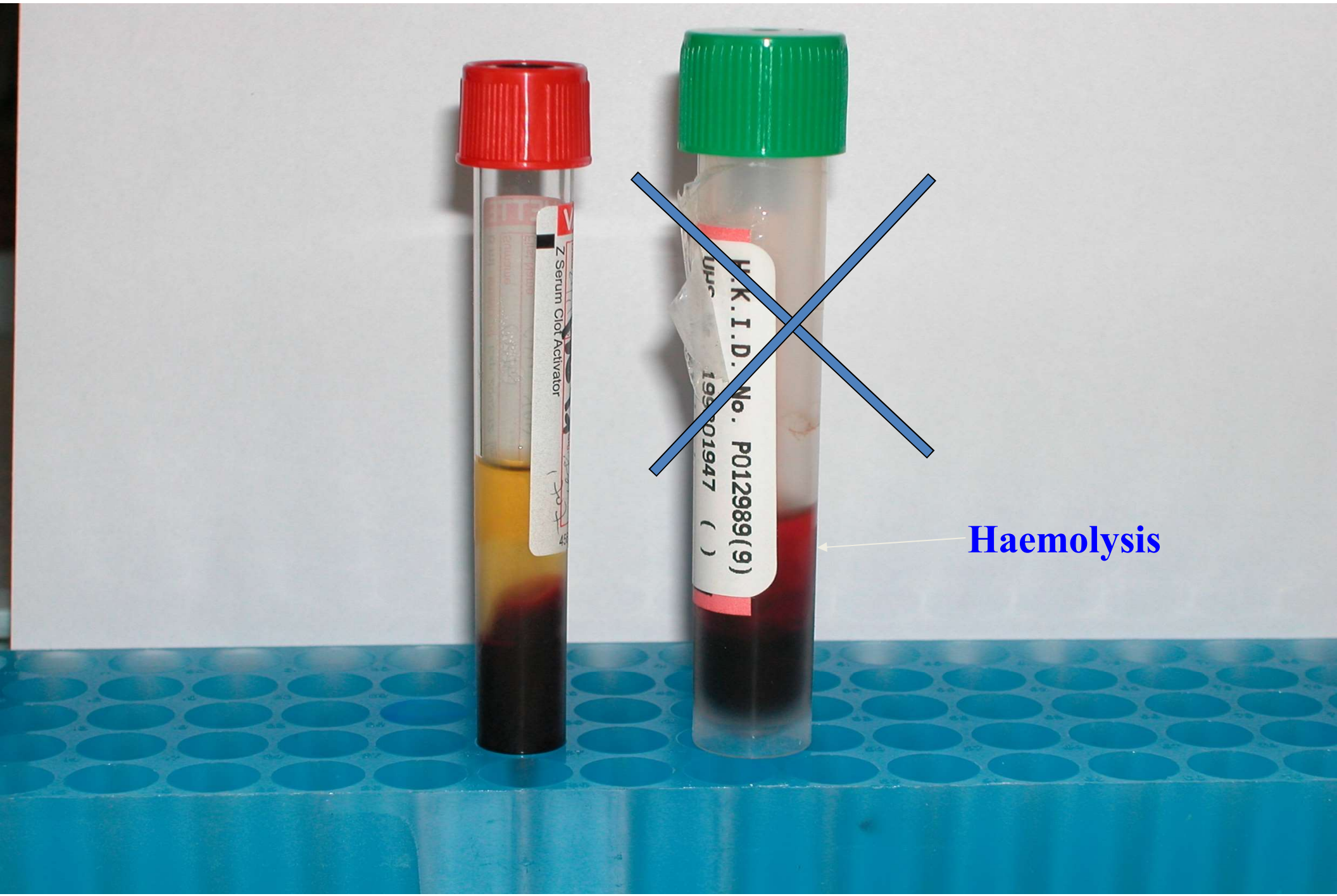


Viral diagnosis: Detect virus

- grow (virus culture in cell lines) - *days / weeks*
- viral antigen detection - *hours*
- viral nucleic acid detection (e.g. Polymerase chain reaction) (PCR) - *hours / days*

Serum

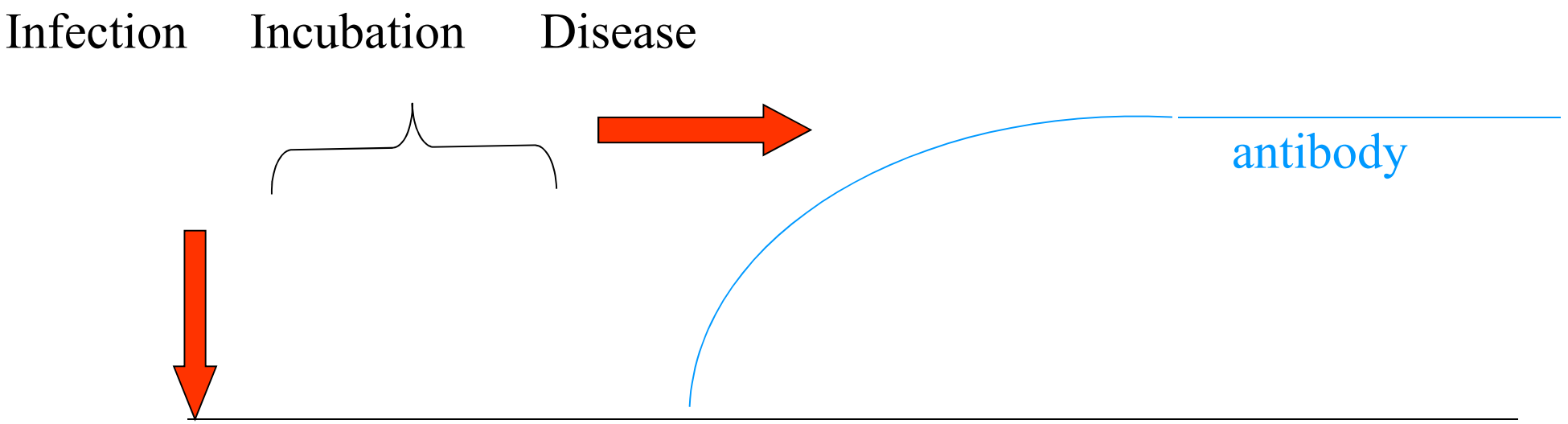
Z Serum Clot Activator
H.K.I.D. No. P012989(9)
199801947


Z Serum Clot Activator
H.K.I.D. No. P012989(9)
Haemolysis

Blood

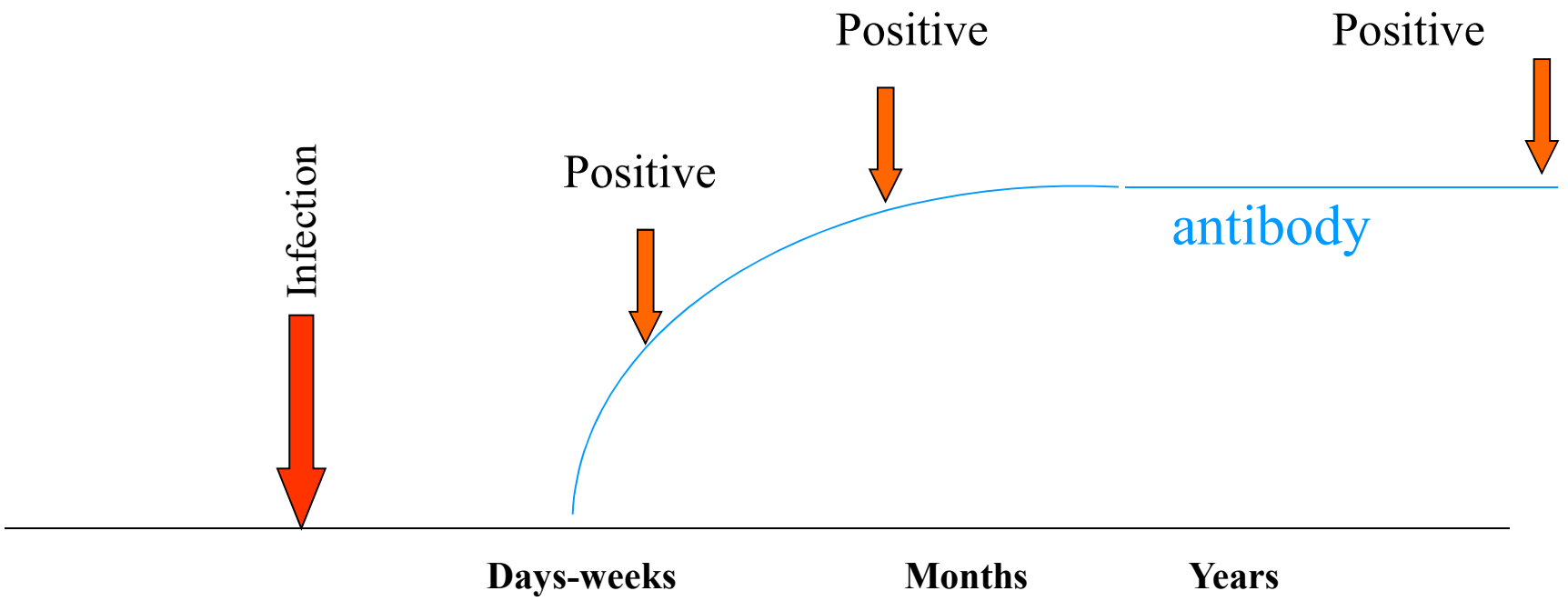
- Serum
- Plasma

Detect antibody response



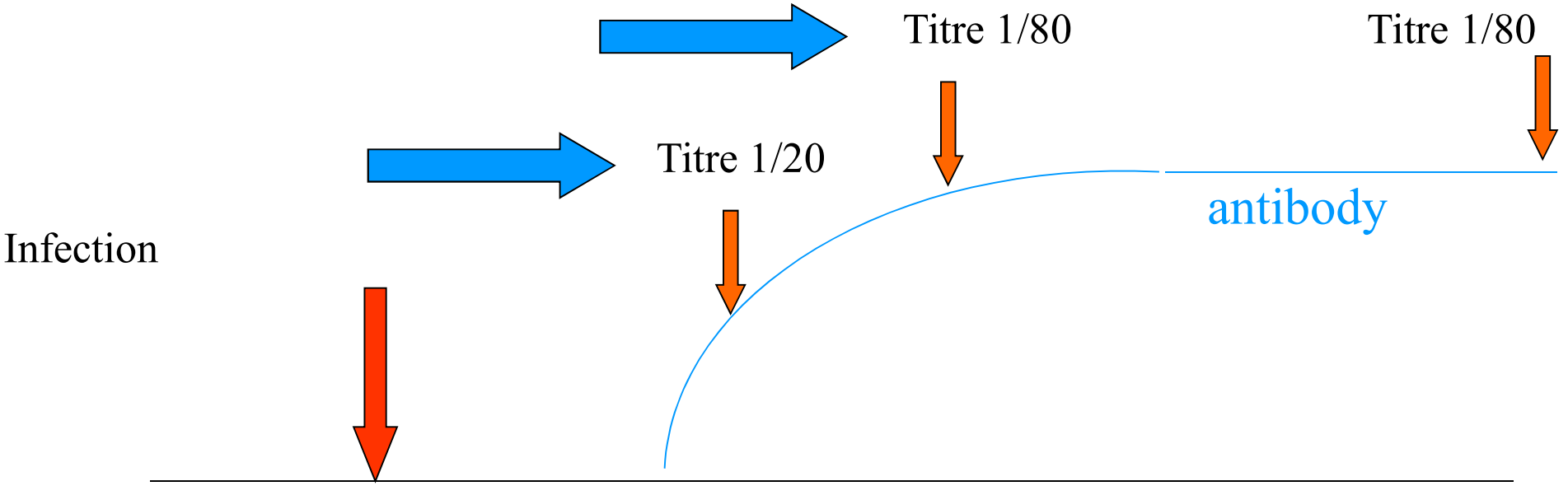
Detect antibody response

- A single antibody test? What does it mean?
- Infection at some time during lifetime.



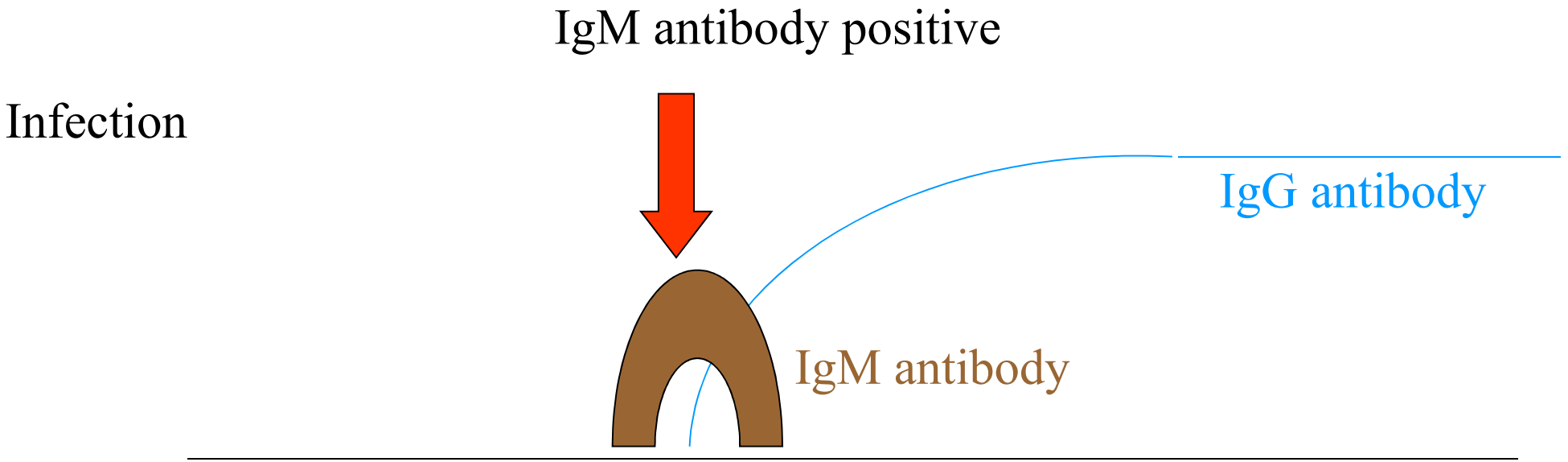
Detect antibody response

- Paired sera 10-14 days apart, tested for antibody titre (quantitation)
- Rising antibody titre: ≥4 fold increase



Detect antibody response

- Antibody classes: IgM antibody / IgG antibody
- Recent infection within last 1-3 months



Thank you

Any questions