**DIAGNOSTICS 3  
THE DIAGNOSIS OF INFECTION AND THE USE OF THE   
BACTERIOLOGY LABORATORY**

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**Learning Objectives:**

1. Explain the concept of best-guess microbiological diagnosis and the contribution of the laboratory to it.
2. Describe the investigations the microbiology laboratory does and their limitations
3. Describe the limitations of microbiology laboratory investigations.
4. Be aware of the turn-around times of different investigations, particularly the delays inherent in making cultural diagnoses.
5. To know how to interpret laboratory results of the commonly used tests.

The initial diagnosis of infection is based on the principles of making an informed best-guess clinical diagnosis.

Present illness: date of onset, symptoms, signs (esp. rashes)

Past history: previous infections particularly with resistant organism e.g. MRSA, hospitalizations, travel, antimicrobial use.

**Types of investigation made by the microbiology lab:**

* **Most microbiology samples are cultured on agar plates, which takes time:**

For organisms to multiply sufficiently: usually 24-48 hours

(Some need longer incubation: e.g. TB, brucella, actinomycetes)

To culture again for antibiotic sensitivities: another 24 hours

* **Microscopy**

direct under the microscope – urine

various stains (Gram, Ziehl-Nielsen (ZN), *etc*.) – pus, tissue fluids

fluorescence, with conjugated antibodies to specific antigens

* **Direct antigen detection** (particle agglutination tests, ELISA)
* **Molecular probes and amplification** (PCR, *etc*.)
* **Serology**: looking for antibodies as evidence of infection/immunity

**Optimal time of Collection of Specimen**

* In the acute phase of illness and before staring antimicrobials
* Collection from proper site, avoiding contamination by normal flora
* Prompt transport to lab since micro-organisms multiply in transit
* Adequate quantity and appropriate number of specimens
* Acute sera and Convalescent sera (paired), for rising antibody titres

**EXAMPLES:**

**Microbiological examination of Urine**

* Bedside: Naked eye - clear, cloudy, haemorrhagic.   
  note: although these are NOT microbiological investigations dipstick tests for nitrites, leucocytes, blood, protein, bilirubin, ketones may provide indication of there being an infection. Nitrites strongly suggest bacteriuria as many species of gram-negative bacteria convert nitrates to nitrites.
* Microscopy: WBC (pyuria suggests infection), RBC (may also indicate tumour/microemboli/trauma), epithelial cells (suggest the specimen has been contaminated during collection), crystals, casts.
* Culture on MacConkey agar (urine should be sterile so any microbial growth is potentially significant in an appropriately taken sample)
* Quantitative colony count for “significant” bacteruria (>105 bacteria/mL)
* Antibiotic sensitivity testing of bacteria that grow

**Microbiological examination of faeces**

* Naked eye, consistency, blood stained, colour, presence of worms
* Microscopy: ova, cysts, parasites
* Culture on inhibitory media – e.g. deoxycholate-citrate agar (DCA), selenite (Faeces contains 1012-14 bacteria per gram, so selective media are used to suppress background ‘flora’ organisms)
* Certain organism such as *Vibrio cholerae* are not looked for routinely therefore it is important adequate clinical information is provided on the request to allow the appropriate laboratory investigations to be carried out.
* Toxin detection (*Clostridium difficile*)
* Special stains, *e.g.* for cryptosporidia

**Examples - microscopy**

* Gram stain of CSF, joint fluid, purulent exudates.
* ZN/auramine stain of *e.g*. sputum, for TB
* FTA (fluorescent treponemal antibody) for antibodies to *T. pallidum*

**Examples – direct antigen detection**

* Meningococcal antigen in CSF
* *C. difficile* toxin in faeces
* Legionella and Pneumococcal antigen in urine

**Examples – PCR**

* Chlamydia in genital specimens
* Rapid PCR for MRSA

**Investigations on Billy** (case study)

**Diarrhoea**

* **Microscopy of stool for parasites**, especially giardia, amoeba (which cause diarrhoea but not rashes) and for higher parasites (may cause rashes but rarely diarrhoea). Stool culture for the common bacterial pathogens – salmonella, campylobacter, shigella. Stool result often negative.
* **Rash/skin lumps**. Often viral aetiology. Also, infected insect bites, syphilis, gonococcal infection, typhoid, endocarditis, systemic parasites.   
  ? Take skin biopsy – prolonged cultures for TB, fungi.