Chapter 25

Microscopy

SMEAR PREPARATIONS

Acid-Fast Bacilli: 5% phenol in ethanol fixed Kinyoun and Truant's fluorochrome, modified Ziehl-Neelsen stain

Anaerobes: heat fixed Kopeloff

Bacteria: heat fixed Gram, alcohol/acetone fixed direct immunofluorescence

Bacterial Flagella: basic fuchsin-tannic acid flagellar stain

***Chlamydia*:** alcohol/acetone fixed direct immunofluorescence, heat fixed Gimenez, glutaraldehyde fixed electron microscopy

***Corynebacterium*:** Loeffler's methylene blue stain, Albert's stain, Neisser's stain

***Cryptococcus*:** nigrosine negative stain wet preparation, India ink wet preparation, 10% formalin fixed mucicarmine stain

***Cryptosporidium*:** Sheather's wet preparation, modified Ziehl-Neelsen stain, immunofluorescence

Faeces: saline wet preparation, iodine stain wet preparation, MIF stain wet preparation, Schaudinn's fixed trichrome stain; look for leucocytes, erythrocytes and parasites (particularly trophozoites) in mucus strands if present (particularly with formed specimens)

Fungi: KOH-Parker Quink wet preparation, lactophenol cotton blue wet preparation, calcofluor white wet preparation, alcohol/acetone fixed direct immunofluorescence, 10% formalin fixed periodic acid-Schiff, Gridley and Grocott's methenamine silver stain

General Morphology: saline wet preparation

***Giardia*:** zinc sulphate flotation or formalin-ether wet preparation

Histopathology: 10% formalin fixed haematoxylin and eosin stain

***Histoplasma*:** Giemsa stain

***Legionella pneumophila*:** 10% formalin fixed Dieterle silver, immunofluorescence (direct and indirect)

Leucocytes: methylene blue wet preparation, Romanowsky stain

***Mycobacterium*:** acid-fast stain

***Mycoplasma*:** Dienes stain wet preparation

***Nocardia*:** 10% formalin fixed Gram-Weigert, acid-fast stain

Parasites: iodine and formalin-ether wet preparations, unfixed dried Wright stain, alcohol/ether fixed Giemsa and direct immunofluorescence, Schaudin's fixed trichrome

***Pneumocystis jiroveci*:** unfixed dried toluidine blue O

***Prototheca*:** Grocott's silver stain, PAS

***Rickettsia*:** heat fixed Gimenez

***Schistosoma*:** 10% formalin fixed modified Ziehl-Neelsen stain

***Toxoplasma qondii*:** immunoperoxidase stain

***Treponema*:** darkfield wet preparation

Viruses: electron microscopy and immune electron microscopy of unfixed negative stained wet preparations and glutaraldehyde fixed dried preparations, alcohol/ether fixed Papanicolaou and indirect immunofluorescence

GRAM STAIN

Amies transport medium and culture collection devices have been associated with false positive Gram stains.

The importance of the Gram stain in determining the quality of a sputum specimen (absence of squamous epithelial cells, absence of mixed normal flora, presence of histiocytes) and in identifying likely pathogens present should not be underestimated. The isolation of a light growth of an organism which has not been seen in a Gram stain is unlikely to be of significance unless there is a virtual absence of other organisms and the patient has been

on antibiotics. *Haemophilus* requires at least a minute of counterstaining with safranin and is in any case frequently difficult to see in Gram stains of sputum. Unless the patient is hospitalised, bed-ridden, alcoholic or immunocompromised, and/or the Gram stain shows clear evidence of a lower respiratory tract specimen in which the organism is present together with a significant number of neutrophils, coliforms and non-mucoid strains of *Pseudomonas* can safely be ignored.

A Gram stain of faeces is useful in diagnosing enterocolitis. In both *Staphylococcus aureus* and *Campylobacter jejuni* infections, specimens will typically contain large numbers of leucocytes and erythrocytes. In the case of a *Staphylococcus aureus* infection, the Gram stain will show large numbers of Gram positive cocci, usually recognisably staphylococci; mannitol salt agar may be used as an isolation medium. In *Campylobacter jejuni* infections, the 'squiggly' Gram negative bacilli may be seen in a Gram stain; microaerophilic culture at 42°C is necessary.

In plague infections, the presence of bipolar staining Gram negative rods in lymph node aspirate can be diagnostic. The search for anaerobic bacteria begins with the direct examination by Gram stained smear. This technique
provides immediate information regarding the types of organisms present and may be sufficient to permit a presumptive diagnosis and choice of therapy.

Pseudomonas and Enterobacteriaceae may be differentiated in Gram stained direct smears.

In CSF, a Gram stain detects between 10^5 and ? 10^7 cfu/mL of both Gram positive and Gram negative organisms in < 5 minutes. There is a 75-80% correlation with culture.

A Gram stain detects urinary tract infections in 2 minutes, with a sensitivity of 97% at 10^5 cfu/mL (decreased sensitivity at < 10^5 cfu/mL).

The Gram stain serves as a quality control. Organisms corresponding to all the types seen in the Gram should be cultured.

Methanol fixation is superior in every instance to heat fixation and should especially be used for specimens, such as CSF and synovial fluid, containing large amounts of protein.