This information is not intended as a complete guide and is not a substitute for textbooks or formal guidelines. It covers the basic information relevant for a busy routine microbiology laboratory involved with infectious disease diagnosis, from specimen collection to the final report. Priority is given to common causative agents detected using basic equipment. Common agents detectable only using complex molecular methods and rare causes of infection are low priority in developing countries with limited health budget. It is assumed that automated organism identification such as Vitek, MALDI-TOF, sequencing and PCR testing are not available.

As health resources improve, and newer commercially available rapid PCR testing becomes more affordable, additional causative agents (common and unusual) will receive attention. This guide is aimed at countries with limited laboratory resources in microbiology, and should demonstrate that early clinically useful information can be found despite the non-availability of expensive technology. Some basic familiarity with laboratory terminology simple bench tests and workflow is assumed.

It is hoped that this guide will encourage health administrators to consider using relatively cheap basic microbiology investigations as routine in their pathology program, the results of which can be utilised BEFORE prescribing broad spectrum antibiotics. In many countries, overuse of these agents is rapidly causing a predominance of antibiotic resistant bacteria in community and hospital settings.
**Part 1. Stool microbiology and gastroenteritis**

**Patient and Specimen collection.**

Obtain some details from patient such as duration of symptoms, have close contacts also been affected ?, recent travel ?, exposure to seafood or undercooked meat ?, etc.

Samples should be sent in clean (not necessarily sterile) containers and transported to the lab without lengthy delay, urine collection pots are ideal. For bacterial pathogens, as delay increases, pathogen viability DECREASES leading to false negative results. Some guidelines suggest no more than 2 hour delay! In most rural areas, this is impractical. Culture within 24 hours of collection will probably detect most enteropathogens. Transport medium (Cary-Blair) should be used if lengthy delay is likely.

If parasites are sought, 2-3 samples should be collected on different days (as excretion of some parasites can be intermittent). Samples do not need to be sent in transport medium and can be stored in a fridge (if available). Samples should arrive at the lab on the day of collection where possible.

**Stool culture should NOT be ordered on patients admitted to hospital for > 3 days, and whose diagnosis on admission was NOT diarrhoea. If the test is available, these patients should be investigated for Clostridium difficile only.**

**Specimen processing**

Wear protective gloves if available. Check request form details and name on container match, preferably with 2 identifiers (name/ DOB).

**Microscopy**

Prepare a wet film on a whole slide using saline using a thick-thin technique. The causative agent of some diarrhoeal diseases can be diagnosed immediately by microscopy, but do not forget the possibility of MIXED causes.

Look for white cells, and parasites such as Giardia, cysts of Cyclospora, Isospora and eggs of worms known to be endemic in the location. Remember that patients might have been exposed to parasites during travel to other regions or other countries. Be familiar with ova of Ascaris, Strongyloides, trichostrongylus (round worms), Trichuris (whip-worm), Ancylostoma (hookworm), Enterobius (pin-worm), Schistosoma spp. and Taenia spp., (tapeworms).

Also look for Strongyloides larvae, trophozoites of Entamoeba histolytica with ingested red cells and trophozoites of Dientamoeba fragilis. Note that finding CYSTS of amoebae is not diagnostic; some cysts are normal flora and are commonly found. Special stains are needed to help speciate cysts, such as iron haematoxylin. A modified ZN stain will show oocysts of cryptosporidium and Cyclospora more clearly.
The use of concentration techniques will improve diagnosis of parasites. Recognition of *Microsporidium spp.* requires special stains and considerable experience. Not all possible parasites have been mentioned. Seek knowledge of common local parasites.

**Culture set up**

Keep all culture media in original packaging to avoid dehydration of the agar. Use MacConkey and selective agars such as XLD, Campylobacter agar. (In countries where Shigellae are common, the mac plate covers for strains which might grow poorly on XLD). Include a TCBS plate for *Vibrio spp.* if ingestion of shellfish or contact with salt water has occurred.

Learn an effective plating out (streaking out) technique and stick to it. As long as single colonies are obtained, the precise technique does not matter.

For successful growth of *Campylobacter spp.*, incubation in a jar or lunchbox using commercially available sachets (which produce the required microaerophillic atmosphere) is needed, and the incubation temperature should be 42-43°C. Incubation at 35-37°C is less reliable; the lower temperature will not minimise rapid growing normal bowel flora, and slower growing Campylobacter will be overgrown. Many species of normal bowel flora do not grow at 42-43°C, but the major *Campylobacter spp.* prefer this temperature.

Use of selective agar for Yersinia is less important in tropical areas (it seems to be more important in cool climates). In addition, routine culture for Edwardsiella, Plesiomonas, Aeromonas and Hafnia is not recommended, unless reliable tests and experience are available.

Attempting culture for pathogenic *Escherichia coli* is not indicated, even if antisera for Enteropathogenic E. coli (EPEC) antisera are available. Gut infection with EPEC is now known to be complex and beyond simple antisera testing. False positive and false negative results will be obtained and the real cause of the diarrhoea will be overlooked. Complex molecular testing which detects certain virulence factors is the only method of proving a pathogenic role for any *E. coli* isolated.

Use of enrichment media, such as selenite broth, improves detection of Salmonella spp. but should be used with caution as positive results only in the enrichment broth might be due to carriage following previous infections, and therefore produce misleading results. A comment should be issued on reports where Salmonellae is found only in an enrichment culture to explain this.

Superinfections (or atypical heavy growth) of *Pseudomonas aeruginosa* or *Candida spp.* are difficult to interpret and probably best ignored unless special requests have been made.

**Culture reading**

The target bacteria are: *Salmonella typhi* /paratyphi, non-typhoid Salmonellae, Shigellae, Campylobacters, *Vibrio cholera*, other Vibrios
After 18-24 hrs incubation, examine XLD plate. Salmonella appear as red colonies usually with a black centre. Absence of H2S production suggests slow growing serovars or H2S-negative serovar, such as *S.paratyphi A*.

Shigellae appear as small pink clear colonies; sometimes a whitish tinge is seen. Normal bowel enteric flora produce yellow-orange colonies.

Look for clear colourless colonies on mac agar; these are non-lactose fermenters and could be Shigella or Salmonella. Note that Proteus, Pseudomonas and some other non-pathogens will look very similar on mac agar.

Subculture suspect colonies to a new blood agar and/or MacConkey plate for further identification the following day. If enough colonies are accessible, perform oxidase test and set up urea slope. A positive oxidase eliminates Salmonella and Shigella immediately, and positive urea also excludes these pathogens. Note that direct cultures are often mixed; non-pathogens mask the pathogens.

Identify suspect Salmonella/Shigella using a series of simple preliminary substrate tests and when these results suggest a pathogen, check using commercial kits such as API, Crystal or Remel. Confirmation using a limited range of specific Salmonella and Shigella antisera is desirable.

Examine Campylobacter plates after 2 days incubation (in a microaerophilic atmosphere). On charcoal agars, look for small off-white colonies sometimes starting to spread, and sometimes with a metallic sheen. Remove any suspect colonies for Gram stain. Counter stain using dilute carbol fuchsin (1 in 10), if available. Look for typical small curved (and double-curved) Gram negative rods. Oxidase testing can be unreliable as many strains give slow borderline reactions. On lysed blood selective agars, colonies are small greyish/off white. Consult media supplier information sheets or internal QC data.

Traditionally, preliminary steps use Kohn’s tubes, Gillies tubes or SIM medium. If these can be prepared cheaply, and used reliably, laboratory resources are saved; however, if laboratory funding allows, leave this delaying step out and this should reduce the time to obtaining final results.

If possible, become familiar with the colonial appearances of enteric pathogens and normal flora on the media being used with the help of control strains from a recognised culture collection such as ATCC/NTCC. At a minimum, use wild strains to develop plate reading skills.

**Susceptibility testing and reporting**

Using a standardised method such as CLSI or EUCAST, set up the test strictly following the method guidelines. The laboratory should perform regular quality control of susceptibility testing which is important for reliability of results.

**For Salmonellae and Shigellae**, test ampicillin, co-trimoxazole, ciprofloxacin, azithromycin and ceftriaxone.
Note: Antibiotic therapy is NOT indicated for uncomplicated Salmonella infections. The antibiogram should only be reported for the following patient groups: neonates – 3 months old, 3-12 months (IF toxic or febrile), >65 yrs

And severe illness/bacteraemia/haematology patients/ immunocompromised

Report antibiogram for all S. typhi and S. paratyphi. (azithromycin, ciprofloxacin and ceftriaxone).

For Shigellae, report full antibiogram. Resistance to some antibiotics is now common in some areas.

For Campylobacter spp. routine susceptibility testing is not recommended. Add comment to positive reports suggesting treatment ONLY in severe cases, the very young and the elderly, with erythromycin stearate, azithromycin or ciprofloxacin.

For Vibrios, test and report doxycycline, co-trimoxazole, ceftriaxone, ciprofloxacin and azithromycin.

For V. cholerae, report ciprofloxacin and azithromycin.

Note – for dosages, route and duration of therapy, see appropriate guidelines.

Incidence of Campylobacter in tropical countries.

Campylobacter is clearly the most common cause of gastroenteritis in sub-central and southern parts of Australia and many European countries, but in warm to hot climates, data shows that cases of Salmonellae outnumber Campylobacter.

Future testing strategies

Routine testing for viral agents of gastroenteritis is beyond the health budget of most developing countries. In time, convenient kits using of solid phase EIA tests for rotavirus, norovirus and adenovirus might be introduced. Similar kits are readily available for Giardia, E. histolytica and Cryptosporidium.

In addition, multiplex PCR testing for the most common bacteria, viruses and parasites might be a more reliable and cost-effective alternative when costs allow. Commercially available PCR testing for C. difficile is becoming more affordable.

XLD xylene lysine deoxycholate
TCBS thiosulphate citrate bile-salts sucrose
ATCC American typed culture collection
PCR Polymerase chain reaction
EIA Enzyme immune assay
Part 2. **Urine microscopy and culture.**

**Specimen collection.**

A high proportion of urine specimens are heavily contaminated at the time of collection with a mixture of normal skin bacteria or faecal bacteria or both. Finding of the causative organism of UTI is then difficult or impossible. Considerable effort must be continually put into education of health care/nursing staff or anyone involved with specimen collection. Patients (both sexes) must be given relevant verbal instructions aimed at minimising contamination. A clean catch (midstream) sample is the usual collection method, catheter specimens can be collected and suprapubic aspiration is performed to avoid contamination when clear-cut results are needed urgently.

Sterile urine containers are ideal or cheaper “clean room” containers are satisfactory. Patients-supplied containers are usually unsuitable. About 20ml of urine is collected and the lid tightly closed.

Prompt transport to the laboratory is often difficult, but important, as small numbers of contaminating bacteria multiply in urine. This gives a false high bacterial colony count and therefore misleading information. Urines should be stored in a fridge, if possible, before delivery to the lab. Storage up to 24 hrs is reasonable at fridge temperature, NOT room temperature. Use of a preservative solution is another option.

Urines should be processed promptly on arrival to minimise inaccurate results. The traditional habit of centrifuging urine for “urinalysis” is unnecessary and can cause inconsistent results unless carefully planned with some standardisation.

**Microscopy**

Fill a counting chamber with well mixed uncentrifuged urine, allow to settle, then examine under low power then under higher power. Disposable plastic slides which accommodate up to 10 urine specimens are now cost effective (such as “Kovaslides”). Record the count for white cells, red cells and epithelial cells. Also look for significant casts and only significant crystals. Note the approx. quantity of organisms present (zero – 3+). Look out for large moving structures which could be Trichomonas or a hatched miracidium from ova of *Schistosoma haemotobium*.

Gram stain can be performed if urgent information is needed. Specimen collection requires special attention. Using oil immersion (x1000) 5 of the same organism per hpf field, in a FRESH, CAREFULLY COLLECTED sample, correlates well with UTI. Gram stains should not be attempted routinely.

**Culture set up**

Culture the urine on chromogenic agar, or if this is unavailable, a bi-plate of blood agar and MacConkey agar. Failing this, MacConkey agar alone will support the growth of the majority of common uropathogens.
The volume cultured is important. Use plastic disposable loops or ready-prepared metal loops (lab prepared loops are likely to be inaccurate). Use of 1 ul loops are satisfactory for most investigations of UTI. (Some labs use 10 ul, or a combination of both volumes depending on certain UTI risk factors for organisms which can cause UTI when present in counts lower than $10^5$ per ml ($10^8$ per litre). Remove the urine from the container with the loop upright and avoid patches of mucous or other debris. Streak-out the inoculum to obtain single colonies after incubation. Incubate in air at 35-37°C.

**Setting up direct susceptibility test**

Refer to the microscopy: for urines with few organisms present, using a sterile plastic transfer pipette, place 5 drops of urine into 4 ml of sterile saline or distilled water; for urines with abundant organisms, place only 2 drops. Using a swabstick, inoculate a susceptibility plate in 3 different directions, allow to dry for 10-20 minutes, then place first-line urinary antibiotic discs on the plate.

This practice is controversial as it deviates from the strict guidelines of the official method, however, studies have shown that within certain boundaries, the results correlate well with tests set up the following day using the exact inoculum size. The advantage of the direct test is that the result is available the following morning which helps with early patient management.

**Culture reading**

Estimate the approx. colony total count. This will be a pure growth or a mixed growth. Look for a predominant species in mixed cultures. Mixed cultures (without a predominant type) are reported as such and no further action is needed.

On urine chrome agars, colonies of *E. coli* are usually purple/mauve/pink (depending of the brand of agar used) while Klebsiella, Citrobacter, Enterobacter and Serratia produce green colonies. Proteus, Providence, Morganella and Pseudomonas appear as off-white or beige coloured. Enterococci are green-blue, staphylococci and group B Strep are off-white-faint blue. Yeasts are off-white. Use media makers guidelines.

Perform a rapid spot indole test to confirm suspect *E. coli*. In laboratories where collecting complete data on urines is not a priority, green colonies can be reported simply as Kleb/Citro/Enterobacter/Serratia“group”. Indole test separates Proteus mirabilis/Morganella from *Proteus vulgaris* group, and oxidase is used to presumptively identify *Pseudomonas*. Other oxidase positive (non-*Ps. aeruginosa*) should be further identified or reported as miscellaneous GNR if no further identification is possible.

On MacConkey agar, colonies of *E. coli* are nearly always pink (except for some non-biochemically active strains). The other enteric organisms mentioned above are also usually lactose positive and are pink-red. Basic identification will require setting up a series of substrate tests. A short series of commercially available tests should be cost-effective. The degree of accuracy for routine identification of uropathogens should be decided in consultation with clinicians whilst considering laboratory resources. Half CNA- blood agar/ MAC plates work well (colistin and nalidixic acid –
CNA, is good for finding Gram-positive uropathogens such as Staphylococci, Streptococci, Corynebacteria, Enterococcus and others. Use bench tests and Gram stains to aid identification.

Pure/predominant growths of yeast should be reported and any pure growths of *Staph. aureus*.

Results from the direct susceptibility plate can be used on the final report if the growth is satisfactory (not too heavy; not too light) and pure, or clearly the predominant likely causative organism. Measure the zones sizes and refer to the laboratory SOP for guidance.

Report the usual first line antibiotics; ampicillin, augmentin, cephalexin, trimethoprim, nitrofurantoin. Report gentamicin and norfloxacin if resistance occurs to the first-line antibiotics.

**Interpretation of results**

Deciding on the likely presence of a UTI needs 3 values to be considered with clinical details. The aim is to differentiate between infection, colonisation and contamination.

1. **White cells**, usually > 100/mm³, may be 50-100/mm³
2. Presence of $10^5+$ /ml (= $10^8+$ /litre) of the causative organism
3. A pure or predominant growth

Bacterial counts of $10^4$- $10^5$/ml, with > 10 white cells/cu.mm might be significant in pyelonephritis and in women with uncomplicated UTI. Any growth is significant in suprapubic collections, ileal conduit and cystoscopy specimens, but these specimens sometimes get contaminated during collection.

Absence of white cells does not favour infection, but can indicate bacteriuria or colonisation. Mixed growths usually indicate contamination and low bacterial counts are usually associated with contamination, but not always. Presence of white cells is not specific for UTI; contaminating white cells due to vaginitis might be present.

When patients symptoms are considered, it can be difficult to decide how to report some results. Borderline results might include a request for another specimen, if clinically indicated.

It should be accepted that some agents of UTI will not be detected using simple culture methods and poorly collected specimens. Also, some bacteria are suppressed by urine transport preservatives. Fastidious and slow growing organisms will be missed (Haemophilus spp, anaerobes, Actinobaculum, AFB, etc). Each lab must decide how much money and effort can be spared to detect unusual cases of UTI. Even the best equipped labs have to address this problem.
Part 3. Respiratory specimens

Throat swabs

In many areas, throat swabs are not routinely sent for culture. About 90% of sore throat are viral, and the small proportion due to bacterial causes might be considered low priority. However, detection of beta-haemolytic Strep. group A (Strep. pyogenes) is not difficult and more attention to this could contribute to reducing the incidence of complications of group A Strep infection; rheumatic fever and glomerulonephritis.

A throat swab is collected in the usual manner and sent preferably in a suitable swab-transport medium or a dry swab will be satisfactory. A single plate is set up and streaked out. The best medium is blood agar (BA) with colistin and nalidixic acid (CNA-blood agar). Plain BA allows overgrowth of Gram negative normal throat flora and missing the group A Strep. Place a bacitracin disc near the end of the first set of streak lines. This helps recognition of group A Strep. Incubation at 35-37°C in air is satisfactory, but incubation in anaerobic conditions should improve yield of group A Strep.

Gram stains for group A Strep from throat swabs are NOT helpful; group A Strep is indistinguishable from other normal URT Streptococci, which will nearly always be present, but a Gram stain could show large numbers of yeast cells suggesting oral thrush. A stain using only dilute carbol fuchsin can be useful for detecting Borrelia vincenti, still common in some countries. Look for polymorphs, fusiform rods and weakly stained spirochaetal organisms. Culture of B. vincenti is not attempted.

After incubation of 18-24 hrs, look for small colonies with beta haemolysis. Group A BHS are inhibited by bacitracin. Look for a zone of inhibition (normal flora will grow inside the zone). Group C BHS will look the same as group A but will be resistant to bacitracin. Remove some colonies for simple strep latex grouping test, or subculture to a new plain BA plate for the following day, if few colonies are available for work up. If tiny beta-haemolytic colonies have grown after 48 hrs, suspect Arcanobacterium haemolyticum. Gram stain a few colonies and look for medium to small Gram positive rods. It needs longer incubation to recognise beta-haemolysis (48 hrs). Treatment is the same as for group A Strep.

Susceptibility testing for erythromycin resistance, which is common in some locations, is easily set up. Susceptibility to penicillin can reasonably be assumed. Treatment is penicillin or erythromycin if penicillin allergy exists.

Culture for other bacteria such as Corynebacterium diphtheriae requires selective media and a reliable method for testing isolates for toxin production. Unless these are available, and the experience needed, culture is not recommended. Culture of a heavy growth of Staph aureus in conditions such as peritonsillar abscess should be reported. Attempting cultures for Fusobacterium spp. is not recommended.
Lower respiratory tract

Specimen suitability

Diagnosis of pneumonia from samples of expectorated sputum provide the likely causative organism in only a small proportion of investigations. Collection of better quality material such as bronchoalveolar lavage or bronchial biopsy is not often possible. As patients are not given instructions about sample collection, a high proportion of specimens consist of only saliva and throat mucus. This should be rejected as unsuitable. Deep cough purulent samples, collected in early morning are required. The patient should rinse the mouth with clean water twice before deep cough collection.

Safety for lab personnel

Attempting culture of sputum samples in areas where TB is common is hazardous unless the lab is equipped with a well-maintained biosafety hood and properly trained staff. Simple fume hoods are NOT adequate. If the correct safety equipment is not available, routine sputum cultures should not be attempted.

Culture set up

Working in a biosafety hood, add an equal volume of digesting agent such as “Sputolysin” to the sample, mix well, leave for about 10 min. Transfer material using a swab stick. (If Sputolysin is unavailable, proceed by selecting a purulent portion of the sample with a swab stick). Inoculate a blood agar and a chocolate plate and streak out to obtain single colonies. Place an optochin disc at the end of the first set of streak lines to help detect pneumococcus. Place a bacitracin disc on the same part of the choc plate to help detect Haemophilus influenzae (or if possible, use choc agar with bacitracin in the medium). Incubate at 35-37°C in a CO₂ incubator if possible, or in a CO₂ jar. Incubate for 48 hours total; most pathogens will be found after 18-24 hrs. Further incubation is needed for Nocardia (typically 2-5 days) and Cryptococcus. Incubation in air produces a slightly lower yield of H. influenzae and interferes with the function of the optochin disc.

Prepare a Gram stain using a thick/thin technique; prepare another slide for Zn stain. Note that AFB can remain viable in stained slides, therefore heat fix Zn slide for 1 hr using a heating block at about 70-80°C, or thoroughly fix in a flame.

Microscopy

Perform Gram stain. Report the numbers of polymorphs and epithelial cells. If > 20 epithelial cells and no (or only a few) polymorphs are found, the specimen is unsuitable for culture. Add comment to report to this effect and suggest repeat carefully collected sample, if clinically indicated.

In most cases of bacterial pneumonia, the causative organism is present in large numbers; 10⁸/ml or more. (This does not apply to TB, fungi or Legionella). Therefore, look for a predominant organism morphotype. Estimate the rough quantity present. Record as 1-3+. If there is no
predominant morphotype, diagnosis of a bacterial cause of pneumonia from THIS sample is very unlikely.

Zn stain: Examine slide for 2-3 minutes before reporting as negative. Screen using x40 or x56 objective lens, then use oil immersion (x100) to view in more detail, and to exclude artefacts. Double check all slide labelling to avoid cross-over of samples. Note - cultures should only be attempted for AFB using proper safety equipment by properly trained staff. Refer sample to a specialist lab if possible.

**Culture reading**

For safety reasons, plates should be examined in a biosafety hood. There is a high risk of TB aerosols in countries where TB is common.

Glance at the Gram stain result; this should predict the culture result. Typically, large numbers of mixed and variable normal URT flora will be grown, so the best approach is to consider only a small number of possible pathogens and if all these are absent, do not attempt identifications of what has grown, unless there is a predominant growth with supporting Gram evidence of infection..

Organisms to look for:

1. **Strep pneumoniae.** Look for a zone of inhibition around the optochin disc and for typical dimple-shaped colonies. Note that some strains of *Strep. pneumoniae* do not have this appearance, and look like viridians Streps and some strains produce large mucoid colonies. Also, if the BA plate was incubated in air, inhibition around the optochin disc might be *Strep. pneumoniae* OR it could be the non-pathogen *Strep.pseudopneumoniae*. Check colony morphology: dimpled colonies favour *Strep.pneumoniae*, non-dimpled could be either!

2. **Haemophilus influenzae.** Small translucent colonies on the choc plate with corresponding pinpoint colonies on the BA plate. Gram suspect colonies and look for small pleomorphic Gram negative coccobacilli. Differentiation from the non-pathogen *H. parainfluenzae* might require using growth factor tests (X and V factors), but with experience colony differences can be observed for most strains.

3. **Moraxella catarrhalis.** Look for medium to large sized off-white colonies on BA plate which break up when touched with a loop. Gram suspect colonies, look for Gram negative cocci with a few coccobacillary forms. Oxidase test will be positive. Testing the isolate with a butyrate disc helps confirm this organism.

4. **Enteric Gram negative rods.** Klebsiella, *E. coli*, Enterobacter, Serratia are the most common. Report (as interim report) 2-3+ growths, with Gram evidence of infection, as “Enteric GNR” and attempt identification later. Scant growths of enteric GNRs are usually URT contaminants, particularly when mixed types are grown.

5. **Staph. aureus.** Check identification by coagulase (slide and tube) or use a staph latex kit. This is a common URF coloniser therefore scant growths can usually be ignored. If 2-3+ growth is found,
with Gram evidence of infection (2-3+ polymorphs and 2-3+ GPC) work these up with susceptibility tests.

6. **Pseudomonas aeruginosa.** Check oxidase is positive. Report 2-3+ growths when there is supporting Gram evidence. Scant growths are not likely to be significant (except, perhaps, in cystic fibrosis or bronchiectasis when the patient might be on long-term antibiotics). Beware of mucoid strains which have no greenish pigment and look nothing like normal P. aeruginosa. Deciding the likely relevance of this organism can be difficult as it may be a pathogen, a coloniser or a contaminant. Discussion with caring team should be helpful.

7. **Burkholderia pseudomallei.** The agent of Melioidosis, related to Pseudomonas and underdiagnosed in many tropical countries. Smallish Gram negative rod, oxidase positive. Finding this organism in sputum cultures usually requires using the selective medium Ashdown agar. Colonies appear dry-looking, pink-purple and wrinkled. On MacConkey agar colonies are pink with similar appearance, but this agar is much less reliable than using the selective medium. Do not get this organism confused with *Pseudomonas stutzeri*, which looks similar! Identify any suspect colonies using a set of substrates or kits such as API 20E or 20NE. *B. pseudomallei* is a hazardous organism to laboratory staff; handle all plates with care, preferably in a biosafety cabinet.

8. **Less common organisms.** These include some species of Corynebacterium (*C. striatum, C. pseudodiptheriticum*), group A BHS, group C BHS, *Pasteurella spp* and *Neisseria meningitidis*. There should be 2-3+ predominant growth, with the corresponding organism seen in the Gram stain to be of likely significance. Gram stain suspect colonies of the above and proceed using catalase/oxidase tests and Strep grouping kit as appropriate. Speciation for Corynebacterium is usually successful using the API Coryne strip or the Crystal Gram positive kit.

9. **Nocardia.** The Gram stain should show branching Gram positive rods, some with a beaded appearance. Note that Streptomyces looks similar and is much more likely to be transient colonisation than Nocardia. If growth of dry chalky colonies occurs (which can be vivid white, off-white, pale yellow or orange, with variable textures, perform a modified ZN stain using 5% sulphuric acid for the decolouriser. If all the cells stain blue (or green if malachite green is the counterstain), this suggests Streptomyces spp. Nocardia stain acid fast with a variable proportion of the cells retaining the carbol-fuchsin, sometimes a little as 10%.

10. **Fungi.** All procedures involved with making wet mounts for fungal morphology or other procedures must be done in the safety hood by experienced staff. Some fungi are potential hazardous species and a serious risk to lab personnel, such as Histoplasma, Coccidioides and Blastomyces (mostly in N and S. America). *Aspergillus fumigatus* is commonly grown from sputum cultures. Deciding its clinical relevance may be difficult. Scant growths in repeat, carefully collected samples might be significant, single-occasion isolates probably represent contamination or colonisation. For *Scedosporium spp.*, repeat isolation might help decide its relevance. Seek assistance from staff trained in mycology identification. Cryptococcus neoformans/gattii are sometimes grown, mainly in immunosuppressed patients such as HIV. This must not be confused with various Candida spp.
which are very commonly grown from sputum cultures. Colonies of Cryptococcus tend to be large, soon become mucoid, and fawn/pale brown coloured.

11. Other findings. Culture for other agents such as anaerobes, Actinomyces and Legionella spp requires special techniques and is beyond the scope of this document.

Evidence of the parasite Strongyloides in the cultures is sometimes observed. Rhabtidiform larve produce peculiar-looking irregular tracks through the bacterial growth. This should not be confused with entry to the culture plate of a tiny ant or a maggot track, after a fly has deposited eggs down the side of the plate before plate incubation. The maggot will hatch during the incubation time and then burrow its way through the agar and bacterial growth.

The list of potential organisms (bacteria, viruses, fungi and parasites) causing pneumonia is extensive! Detection of many of these agents requires specialist molecular-based techniques and experienced staff. See text books or on-line guides for more information.

Note that a large proportion (about 50%) of pneumonia patients do NOT have the causative organism identified.