Use of the diagnostic bacteriology laboratory: a practical review for the clinician

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Effective utilisation and understanding of the clinical bacteriology laboratory can greatly aid in the diagnosis of infectious diseases. Although described more than a century ago, the Gram stain remains the most frequently used rapid diagnostic test, and in conjunction with various biochemical tests is the cornerstone of the clinical laboratory. First described by Danish pathologist Christian Gram in 1884 and later slightly modified, the Gram stain easily divides bacteria into two groups, Gram positive and Gram negative, on the basis of their cell wall and cell membrane permeability to organic solvents (box 1).

Box 1: Gram stain technique
(1) Air dry specimen and fix with methanol or heat.
(2) Add crystal violet stain.
(3) Rinse with water to wash unbound dye, add mordant (for example, iodine: potassium iodide).
(4) After waiting 30–60 seconds, rinse with water.
(5) Add decolorising solvent (ethanol or acetone) to remove unbound dye.
(6) Counterstain with safranin.

Gram positive bacteria stain blue (retained crystal violet).
Gram negative bacteria stain red (decolorised and then counterstained).

The Gram stain classifies bacteria (fig 1) phenotypically based on differences in cell wall thickness with differing glycosaminopeptide and lipoprotein compositions: Gram positive bacteria have a peptidoglycan layer 10–15 times thicker than Gram negative bacteria. The cell wall, synonymous with the peptidoglycan layer, is a rigid framework of cross linked peptidoglycan forming the outermost component of the cell. The more complex Gram negative bacteria also have an outer membrane beyond the peptidoglycan layer that consists of lipopolysaccharide (endotoxin), lipoprotein, and phospholipids. In some Gram negative species there also exists a periplasmic space between the outer membrane and the inner cytoplasmic membrane with β-lactamases that degrade β-lactam antibiotics.

The present hypothesis for the mechanism of the Gram stain states the cell wall acts as a physical permeability barrier restricting diffusion of the stain complex, and any microorganism with a cell wall able to retard the efflux of the crystal violet-iodine complex
should be Gram positive. The mechanism further implies that solvent decolorisation causes significant damage to the cell surfaces of Gram negative bacteria, and only limited damage to Gram positive bacteria. This suggests Gram negative bacteria are more “leaky”, causing these thin walled lipid-rich cells to lose their crystal violet stain and appear red from the counterstain. Gram positive cells, thick walled and lipid-poor, appear blue from retaining the original crystal violet.

**Gram stain utility**
Gram stain interpretation gives immediate information regarding the presence or absence of bacterial disease and can guide initial antibiotic treatment. Additionally, epithelial and inflammatory cells are stained in a Gram stain, thus providing information about the host immune response and quality of the specimen. A well prepared sample can showcase the organism’s colour, size, shape, and arrangement, allowing cellular morphology to further separate bacteria into four major groups. Cocci are spherical or oval, bacilli are rod-like or cylindrical, vibrios are comma-like or curved, and spirochetes are flexible (spirilla if rigid) and helical. Additionally, coccobacilli are unusually short bacilli, and fusiform bacilli are bacilli with tapered ends.

**Limitations**
Several substances have been shown to convert Gram staining results. Ultraviolet light, antibiotics, prolonged heat fixation, crushing of Gram staining results. Ultraviolet light, antibiotics, prolonged heat fixation, crushing of Gram-stained cells can cause the loss of Gram positivity, with cells 48 hours old sometimes more Gram positive than younger cells. Several bacteria are unable to be Gram stained for a variety of reasons. Mycobacteria and nocardia have a high concentration of lipids called mycolic acids in their cell walls and are “acid-fast” because they resist decolorisation with an organic solvent. The spirochetes (treponema, borrelia, leptospira, spirillum) are too thin and are best seen with darkfield microscopy. Legionella, rickettsia, coxiella, ehrlichs, and chlamydiae are primarily intracellular and although possess outer and inner membranes similar to Gram negative bacteria, lack a peptidoglycan layer to take up a Gram stain adequately. Mycoplasma and ureaplasma do not have a cell wall to absorb the stain.

**Specific bacteria**

**GRAM POSITIVE COCCI**
The two principal medically important genera are staphylococcus and streptococcus, arranged in irregular grapelike clusters and chains, respectively (fig 2). The orientation and degree of attachment at the time of cell division determines the type of arrangement: staphylo cocci divide in three planes while streptococci divide in only one plane. Enterococci are closely related to the streptococci yet are now known to be phylogenetically distinct and therefore comprise their own genus. The enzyme catalase, which degrades hydrogen peroxide to oxygen and water, differentiates catalase positive staphylococci from catalase negative streptococci and enterococci.

**STAPHYLOCOCCI**
Staphylococci are a major component of the normal human flora and the presence of coagulase, which accelerates the formation of a fibrin clot from fibrinogen, differentiates the species. Although there are 29 species of coagulase negative staphylococci, most clinical isolates are either *Staphylococcus epidermidis* or *Staphylococcus saprophyticus*. *Staphylococcus epidermidis* is part of the normal skin flora. Although often occurring as a contaminant in blood culture specimens, *S epidermidis* may cause infection in neonates, the immunocompromised, and in patients with an indwelling central line, shunt placement, or prosthetic implant. *Staphylococcus saprophyticus* occurs chiefly in the periurethral and urethral flora where it shows a tropism for urinary tract epithelium and causes urinary tract infections in sexually active adolescent girls, second only to *Escherichia coli* in this age group.

*Staphylococcus aureus* is an important pathogen, causing skin infections, osteomyelitis, pneumonia, and septicemia. It is distinguished on the positive results of coagulase, mannitol fermentation, and deoxyribonuclease tests. Selective media, such as mannitol salt agar, may be used for isolating *S aureus* when screening for carriage in infection control investigations. In the last decade the prevalence of resistance to penicillin G among isolates of *S aureus* and *S epidermidis* has consistently
Capsular polysaccharides can be identified using an antigen latex particle agglutination test for the cerebrospinal fluid, serum, or urine. Group B streptococci as well as *Listeria monocytogenes*, both major neonatal pathogens, are treated with a penicillin while gentamicin is added in the nursery for Gram negative coverage, namely *E. coli*.

Group C streptococci species (chiefly *Streptococcus equisimilis*) are β-haemolytic and have been identified as a cause of pharyngitis but are not associated with non-suppurative complications like rheumatic fever due to decreased virulence of the group specific carbohydrate compared to the M protein. Group G streptococci also produce a wide zone of β-haemolysis and occasionally cause cellulitis and bone and joint infections, often requiring the addition of an aminoglycoside with a penicillin for therapy.

Viridans streptococci derive their name from the Latin word *viridis*, a reference to the green colour seen in the α-haemolysis, however some species are β or γ-haemolytic. Viridans streptococci, the preferred term since “Streptococcus viridans” implies a single species and not a group of species, lack classic virulence factors possessed by other streptococci and therefore have a low pathogenic potential in the normal host. *Viridans* streptococci are ubiquitous inhabitants of the mouth and produce an extracellular dextran which may have a role in mediating bacterial adherence to heart valves in endocarditis. Viridans streptococci account for 40.3% of bacterial endocarditis cases while other bacteria account for a minority of cases: *S. aureus* (23.8%), *S. epidermidis* (4.7%), and enterococci (4.0%).

Streptococci that grow in the intestine are now designated enterococci. Before recent reclassification raised enterococci to genus level, group D streptococci were divided into enterococcal species (chiefly *Enterococcus faecalis*, *Enterococcus faecium*) and non-enterococcal species (*Streptococcus bovis*) based on the differential ability of the enterococci to grow in hypertonic 6.5% saline solution. Most enterococci produce γ or α-haemolysis on blood agar and all are able to grow on MacConkey medium that contains bile salts. Most human clinical isolates are either *E. faecalis* (74–90%) or *E. faecium* (5%–16%) and biochemical tests can further differentiate these two, important in planning therapy since *E. faecium* is more antibiotic resistant than *E. faecalis*. Enterococci are resistant to multiple drugs, including uniform resistance to cephalosporins, and empirical treatment requires a penicillin plus an aminoglycoside for synergy.

*Streptococcus pneumoniae* shows a characteristic diplococci on Gram staining and is consistently α-haemolytic and optochin sensitive. *S. pneumoniae* possess a polysaccharide capsule which interferes with phagocytosis; this capsule can be made to swell for rapid identification (quellung reaction) and differentiation into one of more than 90 serotypes. Penicillin and cephalosporin resistant *S. pneumoniae* are emerging as a result of alterations in penicillin binding proteins. Because resistance is not a result of the production of β-lactamases,
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Figure 4 Differentiating aerobic Gram-positive cocci.

Antibiotics with β-lactamase inhibitors such as clavulonic acid are not helpful.

In recent years the proportion of penicillin non-susceptible pneumococcal invasive isolates has varied from 0%–41%

Of these isolates, 5–21% exhibit penicillin resistance. The incidence of cefotaxime and ceftriaxone non-susceptible pneumococcal isolates has increased to 20% in some areas. The Streptococcus pneumoniae Therapeutic Working Group recently advocated using higher dose amoxicillin in less invasive infections, such as acute otitis media, to overcome penicillin binding protein resistance in high risk patients.

The concern for resistance has also brought about the practice of simultaneously using vancomycin and a third generation cephalosporin, ceftriaxone or cefotaxime, as empiric coverage in patients beyond 3 months of age. Vancomycin addresses the possibility of cephalosporin resistance while a well absorbed cephalosporin compensates for the poor cerebrospinal fluid penetrability of vancomycin.

GRAM POSITIVE BACILLI

Bacillus, clostridium, listeria, and corynebacterium are the four medically important genera of Gram positive rods (fig 4), with anaerobic growth differentiating the spore forming clostridium and bacillus while mobility differentiates the two non-spore forming Gram positive bacilli. Most Bacillus spp are non-pathogenic, but Bacillus anthracis is the cause of the disease anthrax while Bacillus cereus is a cause of food poisoning. Clostridium spp include the causative agents of gas gangrene, food poisoning, tetanus, botulism, and antibiotic associated colitis.

Listeria monocytogenes can be diagnosed by Gram stain alone with the appearance of Gram positive rods in small, grey colonies with a narrow zone of β-haemolysis resembling diphtheroids; it may be assumed to be a contaminant. Listeria monocytogenes is a common cause of infection in neonates and the immunocompromised and infection in pregnancy accounts for 27% of all cases of listeriosis, usually occurring in the third trimester due to a decline in cell mediated immunity seen at 26–30 weeks’ gestation. Because L monocytogenes are uniformly not susceptible to cephalosporins, the practice of beginning ampicillin and cefotaxime as empiric neonatal sepsis coverage is questionable. Additionally, routine use of these antibiotics may contribute to cephalosporin resistance among strains of Enterobacter cloacae, Klebsiella spp, and Serratia spp in the nursery.

Corynebacterium diphtheriae, the cause of diphtheria, are non-motile Gram positive rods with metachromatic granules, often arranged as “Chinese lettering” on Gram stain. A throat swab should be cultured on Löffler’s medium.

Figure 3 Differentiating aerobic Gram negative cocci.

There are three medically important Gram negative cocci (fig 3): Neisseria meningitidis, a major cause of meningitis and sepsis, N gonorrhoeae, the cause of gonorrhoea and pelvic inflammatory disease, and Moraxella catarrhalis (formerly Branhamella catarrhalis), which can cause respiratory infections including otitis media, sinusitis, and pneumonia. These cocci, all diplococci, possess the enzyme cytochrome c and consequently are oxidase positive.

Because the trace metals and fatty acids found in blood agar inhibit both neisseria species they are cultured on “chocolate” agar, a blood agar heated to 80°C to inactivate the inhibitors. Non-selective chocolate agar is used for usually sterile sites such as cerebrospinal fluid, blood, or synovial fluid. Thayer-Martin or Martin-Lewis selective media is used for sites where contamination of other bacterial flora is suspected, such as urethral cultures.

The two major Neisseria spp can be differentiated from each other by carbohydrate utilisation tests.

Moraxella catarrhalis is one of the three major agents in acute otitis media and sinusitis besides S pneumoniae and non-typhable Haemophilus influenzae. Nearly 100% of strains of M catarrhalis produce β-lactamases. Although amoxicillin remains an effective empiric therapy for acute otitis media, suspicion or isolation of M catarrhalis warrants the addition of a β-lactamase inhibitor (that is, clavulonic acid).

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to inhibit normal flora and enhance the metachromasia and a tellurite plate to highlight the reduction of tellurium salt in the organism. In patients with the clinical picture of tonsillopharyngeal diphtheria characterised by a thick, grey, adherent membrane over the tonsils and throat the Gram stain can make the diagnosis as the methylene blue stain reveals the typical metachromatic granules.

**GRAM NEGATIVE BACILLI**

**Enteric tract**

The family enterobacteriaceae, often called “enterics” due to their normal habitat in the colon of humans and animals, are differentiated by a range of biochemical tests but all ferment glucose (fermentation of other sugars varies), reduce nitrates to nitrites, and are oxidase negative (figs 5–7). Suspected enteric bacteria are inoculated on a blood agar plate as well as a selective medium such as MacConkey’s agar or eosin-methylene blue agar to suppress unwanted Gram positive organisms by bile salts and bacteriostatic dyes. Lactose fermenters form coloured colonies while triple sugar iron agar, composed of ferrous sulfate and three sugars (glucose, fructose, and sucrose), determine fermentation as well as hydrogen sulphide production. Urea agar is used to determine urease production, which hydrolys urea to ammonia and carbon dioxide and turns the pH alkaline.

**Enterobacteriaceae**

- Gram negative oxidase negative, nitrates reduced to nitrites, glucose fermented
- Citrobacter
- Edwardsiella
- Enterobacter
- Escherichia
- Hafnia
- Klebsiella
- Morganella
- Proteus
- Providencia
- Salmonella
- Serratia
- Shigella
- Yersinia

**Non-enterobacteriaceae**

- Fermentative
- Proteus
- Pasteurella
- Pseudomonas
- Vibrio
- Acinetobacter
- Alcaligenes
- Burkholderia
- Flavobacterium
- Pseudomonas
- Stenotrophomonas

**Lactose fermenter**

- Positive:
  - Escherichia coli
  - Enterobacter aerogenes, cloacae
  - Citrobacter (50%)
  - Klebsiella pneumoniae
  - Vibrio vulnificus

- Negative:
  - Edwardsiella tarda
  - Morganella morganii
  - Pasteurella multocida
  - Proteus mirabilis
  - Providencia
  - Pseudomonas
  - Salmonella typhi
  - Shigella dysenteriae
  - Vibrio cholerae, para-haemolyticus
  - Yersinia pestis, enterocolitica, pseudotuberculosis

**Urease**

- Positive:
  - Citrobacter
  - Klebsiella
  - Morganella
  - Proteus
  - Providencia
  - Y enterocolitica
  - Y pseudotuberculosis

- Negative:
  - Alcaligenes
  - Eikenella corrodens
  - Pasturella
  - Salomonella
  - Shigella
  - Y pestis

**H₂S production**

- Positive:
  - Citrobacter freundii
  - Edwardsiella tarda
  - P vulgaris, mirabilis
  - Salmonella

- Negative:
  - Citrobacter diversus
  - Escherichia coli
  - Klebsiella
  - Morganella
  - Providencia
  - Serratia marcescens
  - Shigella
  - Y pseudotuberculosis, enterocolitica

**Oxidase**

- Positive:
  - Aeromonas hydrophila
  - Alcaligenes
  - Burkholderia cepacia
  - Campylobacter jejuni, C fetus
  - Flavobacterium
  - Helicobacter pylori
  - Kingella kingae
  - Pasteurella multocida
  - Pseudomonas shigelloides
  - Pseudomonas aeruginosa
  - V cholerae, V parahaemolyticus

- Negative:
  - Acinetobacter
  - Klebsiella pneumoniae
  - Stenotrophomonas maltophilia

**Motile**

- Yes:
  - Enterobacter
  - Flavobacterium
  - Proteus
  - Pseudomonas
  - Salmonella
  - Serratia
  - Y pestis

- No:
  - Escherichia
  - Klebsiella
  - Shigella
  - Y pseudotuberculosis, enterocolitica

*Figure 5*  Differentiating aerobes Gram negative bacilli.
A methylene blue stain of a fecal sample will determine whether polymorphonuclear cells (PMNs) are present. The presence of PMNs indicates the involvement of an invasive organism, such as shigella, salmonella, campylobacter, rather than a toxin-producing organism such as V. cholerae, E. coli, or Clostridium perfringens. *Escherichia coli* and salmonella produce disease both within and outside the enteric tract; in contrast, shigella, vibrio, campylobacter, and helicobacter produce disease primarily within the enteric tract.

*Escherichia coli* is the most abundant facultative anaerobe in the colon and faeces, although vastly outnumbered by the obligate anaerobe *Bacteroides fragilis*, and the five major subdivisions each cause different clinical pictures. *Escherichia coli* O157:H7, famous in the public media for outbreaks of food poisoning, is so named by its antigens. The “O” or somatic antigen, is the outer polysaccharide portion of the lipopolysaccharide; the “H” antigen is the flagellar antigen. In haemolytic uraemic syndrome *E. coli* O157:H7 produces a shiga-like verotoxin, named because it is toxic to Vero (African green monkey) cell culture. *Escherichia coli* O157:H7 is easily separated as it does not ferment sorbitol and forms pale colonies on sorbitol MacConkey agar.

*Salmonella spp* include the causes of typhoid and paratyphoid fevers, gastroenteritis, sepsis, and osteomyelitis, especially in patients with sickle cell disease. Unlike salmonella, shigella does not produce hydrogen sulphide gas (neither ferment lactose) and is immobile. Shigella produces bloody diarrhoea by invasion of the mucosa of the distal ileum and colon and is much more virulent than salmonella: as few as 100 organisms are necessary for disease as opposed to the 10 000 organisms required with salmonella or *V. cholerae*. More selective media such as xylose-lysine deoxycholate may be used to isolate shigella or salmonella from faecal specimens.

Five major non-enterobacteriaceae also inhabit the enteric tract. *Vibrio cholera* causes cholera and is a comma shaped, oxidase positive Gram negative bacillus and its characteristic appearance can help make a presumptive diagnosis. Campylobacter are also comma or S shaped, oxidase positive, and often interpreted as cocccobacilli on Gram stain. *Campylobacter jejuni* causes enterocolitis while *Campylobacter intestinalis* causes bacteraemia; the two are differentiated by nalidixic acid sensitivity. *Helicobacter pylori*, the cause of gastritis and peptic ulcer disease, is urose positive and may be demonstrated by Giemsa staining of gastric biopsies. Anaerobic Gram negative bacilli such as *Bacteroides fragilis* are abundant in the human colon whereas *Fusobacterium spp* and others are normal flora in the human oral cavity.

**Respiratory tract**

Of the six serotypes of *H influenzae* (a-f), type b (Hib) causes the majority of invasive disease such as meningitis and epiglottitis. The *H influenzae* species involved in acute otitis media and sinusitis are largely unencapsulated and, therefore, non-typable strains. The incidence of invasive Hib disease has declined dramatically since the introduction of the polyribosylriboside phosphate vaccine in April 1985. Depending on local patterns, 10% to 40% of *H influenzae* isolates produce β-lactamases. Latex particle agglutination for detection of capsular antigen in the cerebrospinal fluid is available, but antigen detection in the serum and urine can be unreliable due to asymptomatic nasopharyngeal carriage. Cultures of *H influenzae* require the growth factors haemin (X) and/or nicotinamide adenine diphosphate (V) provided by heated blood agar.

*Legionella spp* are bacilli that stain faintly Gram negative with the standard Gram stain and biopsy specimens do not stain with haematoxylin and eosin, requiring the use of the Dietzler silver impregnation stain. Because these organisms require high concentrations of iron and cyanide to grow, *Legionella pneumophila*...
fails to grow on ordinary media and is cultured on buffered charcoal yeast extract medium or investigated directly by immunofluorescence. The majority of human disease is caused by *L. pneumophila* serogroup 1, which can be detected in the urine by radioimmunoassay, enzyme immunoassay, or serologically. Most species produce some β-lactamases.

*Bordetella pertussis*, the cause of whooping cough, occurs as Gram negative cocccobacilli singly or in pairs. *Bordetella pertussis* can best be isolated from nasopharyngeal swabs (calcium alginate) obtained during the cattarrhal stage when the organisms attach to the ciliated epithelium of the upper respiratory tract and cause decreased cilia activity and epithelial cell death. The special medium used for culture isolation in the past, Bordet-Gengou medium, has now been replaced with Regan-Lowe agar, a half strength charcoal agar with horse blood and cephalexin. Direct fluorescent antibody staining is also used, but is less sensitive than culture. No single serological test is diagnostic of pertussis. A profound leukocytosis, with up to 70% lymphocytes, can be seen. These are generally "typical" lymphocytes, as opposed to the classic "atypical" lymphocytes seen in Epstein-Barr virus infections.

Pseudomonas and related species include bacteria that are ubiquitous, some of which are important pathogens. *Pseudomonas aeruginosa* causes a wide variety of infections, including wound infections, urinary tract infections, and septicemia. *Pseudomonas aeruginosa* is a non-lactose fermenter, oxidase positive, and isolates can be classified as smooth, rough, or mucoid based on their appearance on agar. The mucoid strains isolated from patients with cystic fibrosis produce alginate, a polysaccharide polymer with antiphagocytic activity. All cystic fibrosis produce alginate, a polysaccharide polymer with antiphagocytic activity. All mucoid strains are resistant to infection by the organism from the tick vector is usually positive, but culture of *B. burgdorferi* is rarely positive, but culture of the organism from the tick vector is usually positive. Diagnosis of Lyme disease is made with serological tests, most commonly enzyme immunoassay, and due to the concern for cross reactivity with other spirochetal antibodies a second step using western immunoblot is now advocated for verification of enzyme immunoassay results. Cultures for *B. burgdorferi* are rarely positive, but culture of the organism from the tick vector is usually positive. *Borrelia recurrentis* can be seen in Giemsa stains of blood films from infected patients.

**SPIROCHETES**

Spirochetes are spiral, motile organisms that are not easily cultivated in the routine laboratory. The three genera of importance are *Borrelia*, *Treponema*, and *Leptospira*. *Borrelia burgdorferi* causes Lyme disease, while *Borrelia recurrentis* and *Borrelia hermsii* cause relapsing fever, so named for its antigenic variation during relapses of the illness. Diagnosis of Lyme disease is made with serological tests, most commonly enzyme immunoassay, and due to the concern for cross reactivity with other spirochetal antibodies a second step using western immunoblot is now advocated for verification of enzyme immunoassay results. Cultures for *B. burgdorferi* are rarely positive, but culture of the organism from the tick vector is usually positive. *Borrelia recurrentis* can be seen in Giemsa stains of blood films from infected patients.

*Treponema pallidum*, the cause of syphilis, may be identified as tightly wound spirochetes using dark field microscopy since only non-pathogenic treponemes have ever been grown in culture. Generally serological tests are used in the diagnosis of syphilis with non-treponemal antigens such as cardiolipin from beef heart reacting with serum antibodies (called reagins). Floculation tests like the Venereal Disease Research Laboratory and rapid plasma reagin detect these antibodies. *Treponema pallidum* in treponemal specific tests react with immunofluorescence in the fluorescent treponemal antibody absorbed test or haemagglutination in the microtitre haemagglutination assay. Whereas a non-treponemal test usually becomes non-reactive
after successful therapy, treponemal tests remain reactive for life despite successful therapy.

*Leptospira interrogans*, the cause of leptospirosis, is occasionally isolated from blood and urine in special cultures, but diagnosis is made through a marked rise in enzyme immunoassay or agglutination antibodies.

**OBLIGATE INTRACELLULAR ORGANISMS**

These bacteria lack some of the mechanism for production of energy and therefore grow only inside host cells. Their cell walls resemble Gram negative bacteria, but lack muramic acid. *Chlamydia trachomatis* is the most common chlamydial pathogen and new nucleic acid amplification using ligase chain reactions is more sensitive than cell culture and detects antigen in the urine. Diagnosis of rickettsiae is usually made serologically and Rocky Mountain spotted fever (*Rickettsia rickettsii*) is best detected through indirect fluorescent antibody and indirect haemagglutination, but antibodies are detected 7–10 days after illness. No microbiological test is readily available for rapid diagnosis early in the illness; the polymerase chain reaction has been used during the acute phase. This test, while specific, is insensitive and performs only slightly better on skin biopsies than blood specimens.1

**ORGANISMS WITH NO CELL WALL**

Mycoplasmas are small, non-motile, freeliving organisms that lack a cell wall, which means there are no Gram stain results and antibiotics that inhibit cell wall synthesis (for example, penicillins and cephalosporins) are ineffective. The majority of infections caused by *Mycoplasma pneumoniae* include pneumonia and rickettsiosis, while *Mycoplasma hominis* can cause urethritis, postpartum infection, and pelvic inflammatory disease. Mycoplasmas are slow growing so diagnosis is made serologically. In children cold agglutinins, immune globulin M autoantibodies against type O red blood cells that agglutinate at 4°C but not at 37°C, are not as reliable as in adults. Ureaplasma can be distinguished from mycoplasma by its ability to produce urease.

**Conclusion**

The clinical bacteriology laboratory can be pivotal in guiding clinicians to make a rapid diagnosis and initiate appropriate treatment. The Gram stain is the microbiologists’ century old quintessential first line diagnostic tool allowing preliminary identification of bacteria. Housestaff physicians should receive formal training in the interpretation of the Gram stain and other basic clinical bacteriological tests. A more rigorous and confident use of clinical microbiological knowledge may allow greater precision in diagnosis and focused narrow spectrum antibiotic treatment, thus curbing the growing trend of inappropriate antibiotic use in the current era of increased antimicrobial resistance.


