Microbe of the month

Breaking The Chain of Infection

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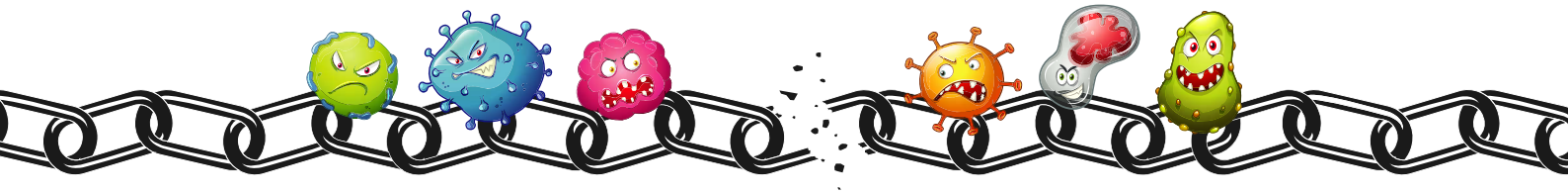
Closing wounds. Together.

JUNE 2019
Newsletter

Compiled by Helen Loudon, Independent IPC Practitioner



Featured this month:

Back to Basics

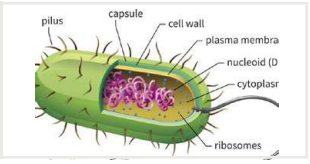
ALL ABOUT BACTERIA
(Part 4) - Insights into microbiology laboratory processes

HELLO READERS! This month's issue (Part 4) concludes the "Back to Basics" series and will describe some fundamental microbiology laboratory processes.

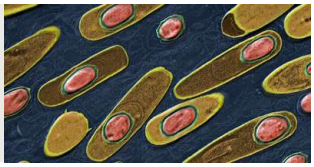
It is hoped that this information will highlight the role of the laboratory in the diagnosis and management of infection, and assist clinicians, pharmacists and wound-care specialists to better interpret laboratory culture reports, so that antimicrobial agents are prescribed appropriately in the context of the patient's overall clinical condition.

The "Back to Basics" series Parts 1 – 3 has covered the following content to date:

March 2019 - Part 1: '**All about Bacteria – structure and virulence strategies**'
- *The purpose-made cell structure and the all-important morphological differences between Gram-positive and Gram-negative bacteria*



April 2019 - Part 2: '**Ensuring survival at all costs**'
- *Exchange of genetic material, bacterial mutation and how bacteria acquire antibiotic resistance; and the role of oxygen and pH in the control of infection in chronic wounds*

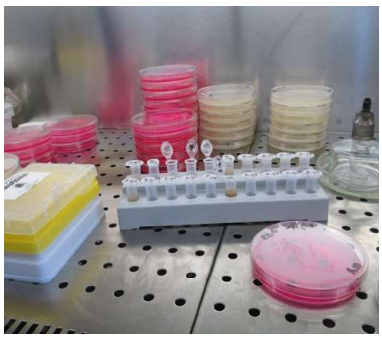


May 2019 - Part 3: '**Mechanisms of antibiotic resistance**'
- *Antibiotic resistance caused by 'selective antibiotic pressure'; and the ability of certain pathogens (e.g., MRSA, E. coli, Pseudomonas and Klebsiella species) to produce beta-lactamase enzymes, which damage the chemical structure of the beta-lactam group of antibiotics and render them useless*

If you have missed any of the preceding issues of Microbe of the Month, please contact your local Essity representative and ensure your email details are on the database for future copies.

Also, be sure not to miss the latest edition of The Link wound management newsletter (published quarterly), which discusses the controversial topic of antiseptics in chronic wound care.

INSIGHTS INTO MEDICAL MICROBIOLOGY LABORATORY PROCESSES



The timely and accurate identification of microorganisms is the primary function of a clinical microbiology laboratory and is accomplished through a constantly-evolving range of laboratory techniques.

The value of a laboratory test is dependent on several factors; for example, how the sample has been taken, how it has been transported to the laboratory, and the relevance of the clinical information provided with the request. How the test is used in clinical practice is also key. In the wrong setting, a test result may be not only unreliable, but often frankly misleading, resulting in unnecessary or inappropriate antimicrobial prescription.

Many pathogenic microorganisms may be found as part of the normal commensal flora. Isolation of these organisms may not necessarily indicate infection. Likewise, many body sites have normal commensal flora, and samples sent to the laboratory in the absence of signs or symptoms of infection may be difficult to interpret.

It is crucial that clinicians understand the limitations of the tests they order, and assist the laboratory to perform the correct investigations to produce a meaningful result in the context of the patient's clinical condition.

First things first...

1. COMPLETION OF THE LABORATORY REQUEST FORM

Demographic information required	Rationale
Patient's full name, identity number and hospital / clinic reference number (if applicable)	The patient is correctly identified for the laboratory investigations and medical records. A history of hospital admission and interdisciplinary referrals is essential for continuity of care.
Patient's gender	Commensal microbial flora differs between male and female patients at specific anatomical sites.
Patient's age	Extremes of age affect immunocompetency; frail, aged patients from long-term care facilities may be colonised with drug-resistant pathogens.
Date of specimen	The reference point for diagnosing the development of infection or to confirm improvement.
Description of specimen	Mid-stream vs. catheter specimen of urine, sputum vs. endotracheal sputum specimen, superficial wound swab, aspirate of exudate or pus, tissue scraping or biopsy.
Site from which the specimen was obtained	The laboratory technologist must be able to exclude commensal flora or contamination from an adjacent site.
Underlying chronic conditions and/or other wounds	Diabetes, chronic renal or cardiac failure, anaemia, HIV, malignancy, anticoagulants, non-steroidal anti-inflammatory or corticosteroid medication predispose the patient to infection.
Current antibiotic therapy (if applicable)	**NB:** ***The presence of antimicrobial substances in the specimen will affect the quality of the culture.*** Drug-resistant pathogens may be inadvertently cultured as a result of changes in the patient's gut flora from repeated courses of antibiotic therapy.

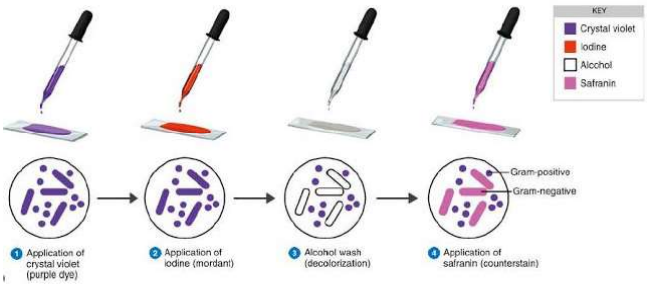
DIRECT MICROSCOPY

Within 24 hours, the 'interim' or provisional microscopy report will comment on the microscopic presence of pus cells (leucocytes), red cells (erythrocytes) and bacteria under a low power magnification field (LPF) or high power magnification field (HPF).
Less than 10 pus cells (< 10/HPF) in a specimen should be considered a normal value; however, the presence of a moderate number (i.e., 6-10 leucocytes/HPF) or numerous (>25 leucocytes/HPF) pus cells on direct microscopy may be suggestive of an inflammatory process or possible infection.

Clinical relevance?

- This is the reason why a wound must be cleansed or irrigated with sterile saline 0.9% solution before a swab for culture is taken. **Swabs taken over slough and old exudate will not provide a representative microbial culture and may lead to misdiagnosis!**
- In the case of urine and sputum specimens, the presence of epithelial cells may be indicative of a contaminated specimen, and the specimen should be collected again.

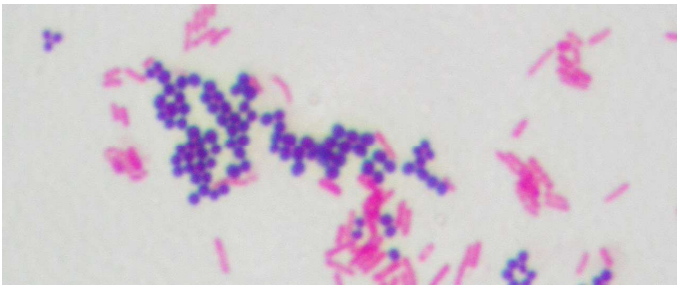
THE GRAM STAIN



Because bacteria are colourless and usually invisible to light microscopy, colourful stains have been developed to visualize them. The Gram stain involves applying a sample from an infected area or a sample of bacteria grown in culture onto a glass slide; ***this is an important first step in the preliminary identification of bacterial organisms under the microscope***.

Gram staining differentiates bacteria into two main groups (Gram-positive and Gram-negative) by the chemical and physical properties of their cell walls.

Gram-positive bacteria retain the primary crystal violet dye and stain a dark purple colour. Gram-negative bacteria, when



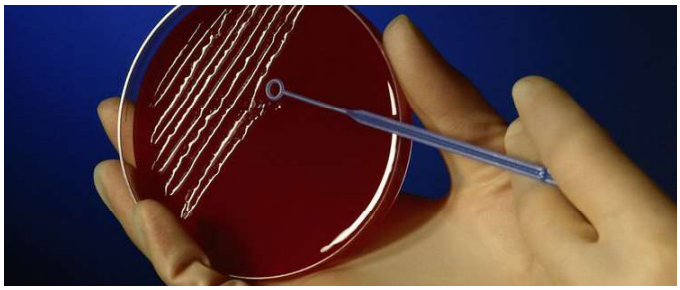
Microscopic image illustrating the visual appearance of Gram-positive cocci and Gram-negative bacilli after Gram staining

decolourized with 95% alcohol, lose the primary stain and take up the counterstain (safranin) which gives them a pink colour under the microscope.

Clinical relevance?

- The choice of empirical antibiotic therapy (based upon an 'educated guess' as to the most likely pathogen involved, pending definitive laboratory results) will be determined by whether Gram-positive or Gram-negative bacteria are identified in the preliminary microscopy report.

CULTURE AND THE PRELIMINARY CULTURE REPORT (24 hours)



A preliminary culture report will be available 48 hours after the specimen was submitted, depending on how quickly the microorganism/s grow on the culture plates.

Counting microbial colonies may be performed manually using a pen and a click-counter, or from digital photographs enabled by microbiological software. The latter approach saves time and is more objective as it will also provide information on other features such as the size and colour of the bacterial colonies.

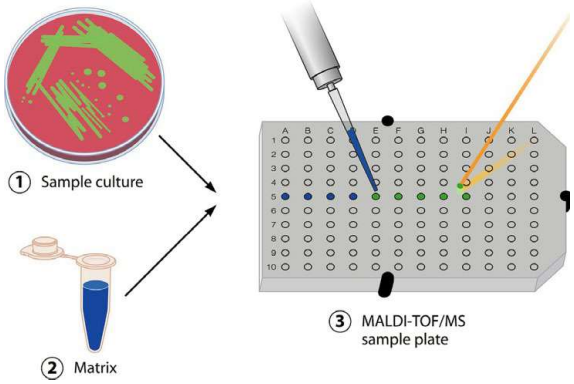
Laboratory results are often expressed ***semi-quantitatively***; i.e., the culture will be described as 'scanty' (+), 'moderate' (++) or 'profuse' (+++) growth.

Quantitative laboratory analysis is slightly more detailed; for example, 'scanty' (+) growth will be described as 10^3 /CFUs (i.e., 10 000 colony forming units/mL), moderate (++) growth as 10^4 CFUs/mL and profuse (+++) growth as $>10^5$ CFUs/mL (i.e., more than 100 000 colony forming units/mL) depending on which quadrant of the inoculated media demonstrates bacterial growth.

Clinical relevance?

- Quantitative cultures assist clinicians in determining the threshold above which the bacterial burden of a culture may be of clinical significance
- If a specimen result returns as 'no growth', this could be because the microorganisms died en route to the laboratory due to delays or an improper transport medium; or the resulting culture was too scanty to be considered meaningful.
- Occasionally, 'fastidious' or 'fussy' organisms are cultured after a prolonged period (i.e., up to 5-7 days afterwards) – these bacteria have specific nutritional requirements which are not met by the standard culture medium.
- Finally, the culture result will also indicate whether the organisms were cultured under aerobic or anaerobic conditions.

MALDI-TOF MASS SPECTOMETRY

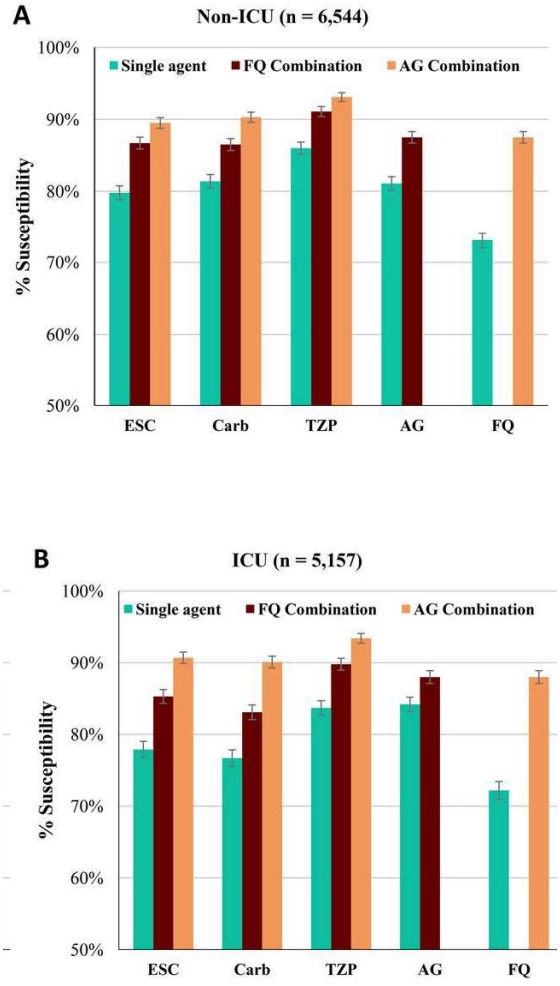


MALDI-TOF ('matrix-assisted laser desorption / ionization time-of-flight') mass spectrometry is a versatile analytical technique to detect and characterise mixtures of organic molecules. In microbiology, it is being used as a rapid, accurate and cost-effective method for the identification of microorganisms (bacteria, fungi and viruses).

Bacterial or fungal growth is isolated from plated culture media (or can be concentrated from broth culture by centrifugation in specific cases) and applied directly onto the MALDI test plate. Samples are then overlaid with matrix and dried. The plate is subsequently loaded into the MALDI-TOF MS instrument and analysed by the software system, allowing rapid identification of the organism.

Within less than a decade, MALDI-TOF mass spectrometry has become the gold standard for microbial identification in clinical microbiology laboratories. Besides more rapid turnaround times for the identification of microorganisms and the typing of single strains and antibiotic resistance, this technology has enabled timeous and more-accurately targeted antimicrobial therapy in critically-ill patients.

THE ANTIMICROBIAL SUSCEPTIBILITY REPORT ('antibiogram')

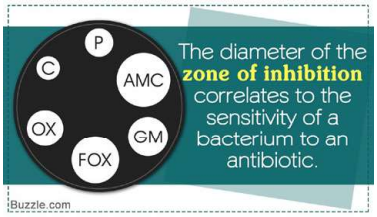


This report reflects the outcome of an in *vitro* laboratory process called the Kirby-Bauer or '*disc diffusion method*', and is used to ***test the susceptibility of bacterial strains to multiple antimicrobial agents***.

The cultured strain is spread evenly across the surface of several petri dishes or plates which contain a variety of nutrient media designed to encourage microbial growth. Several circular paper discs, each impregnated with a different antibiotic, are evenly deposited over the surface of the plate, and gently pushed down into the agar to make contact with the bacteria. The plates are then incubated overnight.

The antimicrobial agents subsequently diffuse into the surrounding nutrient medium, and following overnight incubation, a circular area devoid of bacterial growth will surround each paper disc. ***This bare area is called the 'zone of inhibition'***.

The diameter of the zone of inhibition for each disc is measured and compared to a control chart to determine if the bacterial strain tested is resistant (R), intermediate (I), or sensitive (S) to each of the different antibiotics.
Therefore, a larger zone of inhibition would mean the bacterial strain is more sensitive or susceptible to the antibiotic on the test disc.



Clinical relevance?

- The antibiogram is an essential tool for any clinician faced with treating an infection empirically, until the results of the culture and susceptibility testing are confirmed.

- **NB**: The antibiotics on the susceptibility report are listed in their respective categories and not in order of prescribing preference. Just because the antibiogram indicates that the bacterial strain is susceptible (S) *it does not necessarily infer that the antimicrobial agent is appropriate in that instance, or that antibiotic therapy should be prescribed.*
- Where an MIC value is indicated on a culture and sensitivity report, it informs the doctor that the microorganism displays features of antimicrobial resistance and assists with the calculation of a therapeutic dosage.

Lessons learned for infection prevention and control

1. Careful history taking and physical examination may yield clues to the source of infection and help guide subsequent microbiological evaluation.

2. Strict adherence to laboratory guidelines for correct specimen collection is crucial to avoid contamination and inaccurate culture results.

3. Where possible, specimens should always be collected prior to the commencement of antibiotic therapy.

4. Gram stain of specimens from sites of possible infection assists with the early and appropriate prescribing of antibiotics in critically-ill patients.

5. The diagnosis of infection *should never* be made based on a culture result alone, but rather on a holistic assessment of the patient's clinical condition and the presence of local or systemic signs and symptoms of infection.

6. Additional supporting laboratory investigations for the diagnosis of infection include full blood count (FBC) and differential leucocyte count, renal function (urea, creatinine), glycated haemoglobin (HbA1c), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), procalcitonin (PCT) and blood culture (for pyrexia >38 °C).

7. Practitioners have a responsibility to use laboratory facilities and techniques correctly. This will improve patient safety and clinical outcomes, ensure more accurate reporting and diagnosis, and when antimicrobial therapy is indicated, encourage informed antimicrobial stewardship practices to minimise the risk of drug resistance.

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Your comments or suggestions for future topics?

1. Biemer, J. (1973). Antimicrobial susceptibility testing by the Kirby-Bauer Disc diffusion method. Annals of Clinical Laboratory Science, 3(2). http://www.annclinlabsci.org/content/3/2/135.full.pdf **2.** Boundless Microbiology. (2016). Kirby-Bauer Disk Susceptibility Test. Boundless Microbiology. https://www.boundless.com/microbiology/textbooks/boundlessmicrobiology-textbook/antimicrobial-drugs-13/measuring-drug-susceptibility-157/kirby-bauer-disk-susceptibility-test-791-6152/ **3.** British Society for Antimicrobial Therapy (2018 E-Book). Antimicrobial Stewardship – in collaboration with the European Society of Clinical Microbiology and Infectious Diseases. **4.** Gardner, S., Frantz, R., Hillis, S.L., Park, H., Scherubel, M. (2007). Diagnostic Validity of Semi-quantitative Swab Cultures. Wounds, 19(2),31-38.