Medical microbiology – specimen collection

Proper handling of microbiology specimens is vital to obtaining the best results.

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Arderne Forder graduated MB ChB at the University of Cape Town and completed his specialist training in medical microbiology at the same institution. He was appointed Head and Professor of the Department of Medical Microbiology (UCT) in 1983 and Deputy Dean of the Faculty of Medicine in 1989, a position he held until his retirement in 1997. He is now a part-time consultant and lecturer in the Department of Medical Microbiology, Medical School, University of Stellenbosch as well as a part-time consultant at a firm of private pathologists. Professor Forder's main interests revolve around hospital-acquired infections, antibiotics and their use, sterilisation and disinfection, hospital air-conditioning, clinical and applied microbiology and hospital design. Hospital design includes that of intensive care units, operating theatres and central sterile facilities.

'Specimens submitted for microbiological testing require proper handling from the time of *collection* through all stages of *transport, storage and processing*. Issues common to all clinical specimens submitted for microbiological testing include not only proper identification, but also *collection* techniques that maximize recovery of microbiological pathogens and minimize contamination. For specimens like sputum & urine, the relative proportions of micro-organisms present in vivo, must be preserved or culture results can be misleading. If specimens are handled properly, culture results are easier to interpret, patient care is improved and costs are potentially decreased. In addition emphasis has now been placed on modifying traditional practices to decrease or eliminate unnecessary work, increase laboratory efficiency and make microbiological testing more cost effective.'

– Weinstein & Reller, 1996

There is no doubt that proper handling of specimens is crucial for obtaining microbiological test results that are both timely and clinically relevant. Proper handling of specimens is also one of the most important factors – along with appropriate use of tests – in maximising the cost-effectiveness and clinical relevance of microbiological testing.

It is vital that the clinical microbiologist liaise closely with clinical colleagues in the wards and ICUs if patients are to receive the best possible care. There needs to be open communication and co-operation between the partners. Each needs to learn from the other.

Clinical microbiologists feel that their clinical colleagues have the right to assume that the microbiology results are accurate, significant and clinically relevant. Microbiologists are also aware that reporting misleading or accurate but insignificant information can be as harmful as reporting incorrect results.

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It is essential to appreciate that a laboratory report is only as good as the specimen collected. We would much prefer quality to quantity when it comes to specimen type.

Sadly, it is also important to indicate that litigation has now entered the sphere of microbiological diagnosis and patient treatment. A poorly collected specimen with an incorrect answer and inappropriate treatment resulting in patient dissatisfaction, is now cause for legal examination and action.

It is hoped that this brief overview of proper specimen collection and evaluation will engender in all a proper sense of responsibility and in the end better patient diagnosis and care.

Basic issues

Collection of specimens

Specimens must be collected with the use of strict aseptic techniques from anatomical sites most likely to yield pathogenic organisms. Laboratories often receive specimens collected from inappropriate sites, e.g. sinus tract specimens from patients suspected of having chronic osteomyelitis, surface swabs from decubitus or diabetic foot ulcers, and so on.

Specimens should be collected in such a way that contamination by indigenous flora is minimised. This is of vital importance for cultures of blood, bone or other tissues or fluids in which infection can be caused by indigenous flora and for specimens collected from sites of putative infection, that are contiguous to, or immediately adjacent to, cutaneous or mucosal surfaces.

Sufficient material must be submitted for cultures and other tests. Elaborate tests cannot be carried out on a poorly taken swab or a few drops of fluid. Volume, while important for all specimens, is crucial for blood and for mycobacterial and fungal cultures of CSF and urine.

Whenever possible tissue or fluid should be submitted for culture. Swab specimens are often far from ideal.

NB: While swabs have the advantage of being convenient and easy to use, they limit the volume of specimens that can be collected,

they compromise the direct Gram stain, they become easily contaminated and they can adversely affect recovery of certain micro-organisms.

Although occasions do occur when collection of tissue or fluid is not possible and swabs must be used, this should be an infrequent event in routine patient care.

Persons collecting specimens should provide complete information on specimen request forms. Important information includes:

- the specific site(s) from which the specimen(s) were collected
- whether the patient was receiving antibiotics prior to the specimen being collected or at the time the specimen was collected
- specific pathogens that are being sought
- the methods by which the specimen was collected
- whether the patient may be infected with pathogens known to be dangerous to laboratory staff.

Such information is necessary to ensure that specimens are processed promptly, that appropriate cultures are performed, that the test processing is appropriate for the method of specimen collection (e.g. urine obtained via suprapubic aspiration v. a midstream specimen).

Request forms should specify whether separate specimens have been submitted for culture and for cytopathological or histopathological examination, or whether laboratory staff must divide the specimen. A consultation between microbiologist and pathologist can often yield valuable clues as to the patient's diagnosis.

Selecting a representative specimen

This may appear simple, but many specimens arrive daily at the laboratory inappropriately selected and usually on swabs. Laboratory data generated from these specimens can be misleading and can result in an erroneous diagnosis and inappropriate therapy.

Examples of inappropriately collected samples:

- *Wound specimen/swabs* – this on a label/request form is inadequate. The anatomical site must always be reported. The specimen choice is the advancing margin of the lesion, not a superficial sample from the surface of the lesion. Record whether the wound is superficial or deep. Exudate alone is usually not adequate for cultures and often contains confusing commensal flora.
- *'Ear' specimens.* 'Ear' usually indicates a specimen from a patient suffering from otitis media, in which case a swab is not the specimen of choice, but rather fluid obtained by tympanocentesis. Use a small swab ('Calgie' type swab) only if the tympanic membrane is ruptured and then only after thorough cleaning of the outer ear and external canal.
- *Sputum specimens.* Sputum may not be the specimen of choice for diagnosing bacterial pneumonia. A blood culture or bronchoalveolar lavage is more likely to provide the causative organism with a higher degree of confidence. All sputum specimens are to some degree contaminated with oropharyngeal flora. However, with proper instruction a patient can often provide a good lower respiratory tract specimen suitable for culture. A Gram stain can be very useful in determining the presence of a single morphological type as well as the presence of abundant pus cells.

Most specimens requiring prolonged storage before processing should be refrigerated. This maintains the viability of pathogens and preserves them in their relative proportions.

Transport of specimens

All general specimens should be transported in sterile specimen containers directly to the laboratory. If there is to be any delay in getting the specimen to the laboratory, appropriate transport media should be used.

Specimens for culture should be transported to the laboratory as promptly as possible for processing. Unavoidable delays must be minimised. Most specimens can be transported at room temperature.

Storage of specimens

Most specimens requiring prolonged storage before processing should be refrigerated. This maintains the viability of pathogens and preserves them in their relative proportions. This latter factor is crucial when semi-quantitative cultures are necessary for interpretation of results. Refrigeration also minimises the growth of contaminants.

Specimens that *should not* be refrigerated include:

- blood – should be left at room temperature or in an incubator at 5°C
- cerebrospinal fluid – transport at room temperature
- *Neisseria* species – transport rapidly to the laboratory.

Guidelines

Written guidelines should be available for proper specimen collection, transport and storage. The guidelines should be complete, explicit and up to date, and prepared by laboratory staff.

Rejection criteria

These should be specified for specimens that are collected, transported or stored under improper conditions prior to processing, e.g.:

- unlabelled or improperly labelled specimens
- specimens received in leaking, cracked or broken containers
- specimens obviously contaminated
- unpreserved specimens received >12 hours after collection
- specimens not appropriate for a particular test.

There is no benefit – rather, there is a potential for harm to patients when specimens that have been improperly collected or improperly transported are processed and test results are reported.

Correct labelling is of particular importance for ensuring that patient misidentification does not occur and that appropriate testing is performed.

In all instances the physician who ordered the test should be notified when a specimen is rejected.

Recommended reading

Carroll K, Reimer L. Microbiology and lab diagnosis of upper respiratory tract infections. *Clin Infect Dis* 1996; 23: 442-448.

Hines J, Nachamkin I. Effective use of the clinical microbiology laboratory for diagnosing diarrhoeal diseases. *Clin Infect Dis* 1996; 23: 1292-1301.

Weinstein MP. Current blood culture methods and systems: clinical concepts, technology and interpretation of results. *Clin Infect Dis* 1996; 23: 40-46.

Wilson ML. General principles of specimen collection and transport. *Clin Infect Dis* 1996; 22: 766-777.