Sputum for Microscopy, Culture and Sensitivity (MC&S) is one of the commonest investigations processed by most microbiology laboratories. This is not surprising, since Lower Respiratory Tract Infection (LRTI) is a relatively common infectious disease suspected or encountered in clinical practice, and expectorated sputum has always seemed to be a logical choice as a diagnostic specimen, for obvious reasons: it is relatively easily obtainable, non-invasive and therefore procured without any risk to the patient.

On the laboratory side, the investigation itself, which entails a Gram stain microscopy, followed by culture for commonly encountered rapidly growing bacterial species, is fairly basic and straightforward, with an average turn-around time of around 48 hours.

The Gram stain results are often used by the clinician as a guide to initiate empiric antibiotic therapy, which may or may not be altered depending on the results of culture. Thus many important therapeutic decisions are made daily based on this simple investigation, which in turn impacts significantly on overall treatment costs and outcomes.

In the light of this, it is pertinent to ask the question: just how useful, reliable and accurate is a Sputum MC&S investigation?

The answer, unfortunately, is that it is far from ideal. There are a number of pitfalls in the processing of specimens and interpretation of results.

If this is not appreciated by the clinician (and by the performing microbiology laboratory), it could impact negatively on patient management and overall costs, not to mention the impact on related issues such as antibiotic overuse and misuse and its attendant consequences, such as selection of antibiotic resistant organisms.

The sensitivity and specificity of Sputum MC&S has always been an area of controversy. The sentiment amongst many infectious disease physicians and pulmonologists is that often enough the results are unhelpful, or worse still, actually misleading.

There are several possible reasons for this. For a start, up to 40% of patients with LRTI fail to produce an adequate sputum sample. However, the biggest problem by far is the fact that sputum, even if expectorated in adequate quantities, almost invariably becomes contaminated with saliva as it passes through the heavily contaminated oropharyngeal region en-route to the exterior.

To maximize or to optimize the diagnostic usefulness of a test result, the only samples that should be processed fully by the laboratory are:

- Those that are truly representative of the lower respiratory tract, and/or
- Those that have a minimum of salivary contamination

This requires that the performing lab assess the quality of the sputum using one or other grading system. In general, the number of neutrophils and epithelial cells is quantitated under low power microscopy (X100), and only specimens that have more than 10 neutrophils and/or less than 10 epithelial cells are considered suitable for processing. Those that do not fulfill these criteria should as a rule be discarded.

While most laboratories do perform some form of quality assessment and report on it, the policy of discarding of unsuitable specimens for further processing is unfortunately almost never practised.

A few specific issues regarding Microscopy and Culture separately are discussed briefly below:

**Sputum Gram-stain microscopy:**

This is often the only stain that is routinely performed on an MC&S request.

The obvious drawback is that only common bacterial pathogens take up the Gram stain, and there are important pathogens that will not be visualized unless additional (and often much more expensive) stains are performed. Examples of such organisms include Mycobacteria, Pneumocystis and Legionella. Other organisms such as the Mycoplasma and Chlamydia are practically unstainable.

Because of the huge burden of Mycobacterial disease locally, many laboratories now include an acid-fast stain (ZN or Auramine) as part of routine microscopy.

Outside of this, it is obviously not feasible or justifiable to routinely perform additional stains to detect less common organisms.
But even for those organisms that are easily visualized, there are problems with the sensitivity of Gram stain results. For example, in proven pneumococcal or *Haemophilus influenzae* pneumonia, the causative organism can be visualized and identified with confidence on microscopy in less than 50% of cases on average.

Specificity is yet another problem: often enough organisms that are seen on microscopy have nothing to do with the aetiology of the pneumonia. The indiscriminate reporting by the lab of all organisms seen on microscopy (which appears to be the norm), and/or over-interpretation of Gram stain results by the clinician has the potential to impact adversely on patient management.

This is true especially for Gram-positive cocci, which are invariably present to a varying extent in almost every sputum specimen. This is mostly due to the contamination of sputum by the large number of streptococci that normally colonise the oropharynx as part of the normal flora.

As a rule, the presence of Gram-positive cocci should only be reported by the lab (or acted upon by the clinician) if there is good reason to do so e.g. if exceptionally large numbers are seen (‘wall-to-wall’ on microscopy), or if they clearly resemble pneumococci morphologically.

It is the failure to observe these basic principles that result in many unnecessary or inappropriate antibiotic scripts for presumed Staphylococcal or pneumococcal pneumonia.

**Sputum culture:**

Routine sputum culture suffers mostly from the same limitations that are mentioned above for sputum Gram stain microscopy. For example, only common bacterial pathogens are routinely recovered, and the same range of organisms that cannot be visualized microscopically, cannot be cultured either, unless special procedures are used, which is not routinely feasible.

The sensitivity of sputum culture is just as problematic as microscopy. For example, sputum cultures are positive in less than about half of the cases in proven cases of pneumonia caused by *Streptococcus pneumoniae* and *Haemophilus influenzae*, e.g. sputum culture is negative but blood culture is positive for pneumococcus, or large numbers of typical gram-positive diplococci (pneumococci) are observed on sputum microscopy, yet fail to grow on culture.

The specificity of sputum culture is also bedevilled by oropharyngeal contamination. As is the case with sputum microscopy, indiscriminate reporting by the lab of all organisms grown on culture, and/or over-interpretation of culture results by the clinician (regardless of sputum quality), has the potential to impact adversely on patient management.

A lack of correlation between the findings of sputum culture, and the results of more definitive tests such as blood cultures and serologic investigations, has been noted in a number of studies. In summary, the sensitivity and specificity of sputum culture under routine laboratory conditions is far from ideal. In fact, even under ideal conditions, it may not be that reliable. On top of this, if the pitfalls mentioned above are not adequately addressed, the laboratory results could very easily be a source of misinformation for the clinician.

Is there anything that could be done to improve the diagnostic accuracy of a sputum MC&S investigation? The following simple tips might go a long way to circumvent some of these problems, and are strongly recommended:

1. Brushing of the teeth or even a simple mouthwash with water dramatically reduces the salivary bacterial concentration, and hence subsequent specimen contamination. Patients should be instructed to do this prior to sputum production.
2. As far as possible, try to obtain an early morning specimen, because the pooling of secretions overnight increases pathogen concentration, and the chances of recovering it on specimen processing.
3. If a patient is unable to produce an adequate sputum sample, try to obtain an induced sample after inhalation of nebulised saline. Remember, no specimen at all is better than a poor specimen that yields potentially misleading results.
4. Finally, interpret MC&S results critically, especially since laboratories in general have a tendency to report microscopy and culture results indiscriminately. In particular, if the specimen has a low Bartlett score* and/or there are >10 epithelial cells per low power field treat results with great circumspection.

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*B one of the most popular microscopic scoring systems used by laboratories to assess the quality or appropriateness of an expectorated sputum sample, and it is based mainly on the ratio of neutrophils to epithelial cells in the sample

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Bibliography: