# Principles of microscopy, culture and serology-based diagnostics

Peter A Riley

## Abstract

The increasing rates of antibiotic resistance justify the need for microbiological investigations of patients with infections. Despite rapid advances in molecular diagnostics, traditional methods of microscopy, culture and serology still play a major role in the diagnosis of infectious diseases. Developments in differential media and the introduction of automated techniques for identification, susceptibility testing and serological diagnosis have improved efficiency. Many specimens are now examined using a combination of these older techniques and newer molecular methods. This article gives an overview of the use of microscopy, culture, antimicrobial susceptibility testing, serology and antigen detection techniques, with examples of how these methods are combined in the analysis of specimens.

**Keywords** Antibiotic resistance; communicable diseases; microbiology; MRCP; serology; virology

## Key points

- Microscopy can be used to visualize microbes directly in specimens
- A variety of different culture media can be employed to detect specific pathogens
- Cultures can be incubated at different temperatures and in different atmospheres to target specific pathogens
- Microbes can be identified by their microscopic, biochemical and antigenic properties
- Antimicrobial susceptibility testing guides therapy but also provides information on rates of resistance
- Serology can be used to detect antigens and antibodies
- Presence of immunoglobulin M (IgM) antibodies indicates current or recent infection
- Presence of IgG antibodies, in the absence of IgM antibodies, indicates past infection

## Introduction

Diagnostic medical microbiology is undergoing rapid change as advances in molecular technology, including whole-genome sequencing, become cheaper and more widely available. Merging of smaller laboratories to form large laboratories serving several hospitals creates economies of scale. These foster the introduction of automation, with some larger laboratories now able to provide a core service 24 hours a day, 7 days a week for common specimen types.

However, the backbone of most diagnostic work still relies on the older techniques of microscopy, culture, antimicrobial susceptibility testing and serology; these are efficient and comparatively cheap, and sometimes still provide more information than molecular methods. These traditional techniques can be mixed with molecular techniques in order improve diagnostic accuracy and reduce turn-around times. This article focuses on these older established techniques, but should be read in conjunction with the article on molecular-based diagnostics.

## Purpose of microbiology tests

There are many reasons why it is important to microbiologically confirm the diagnosis of an infection. The increasingly worrying rise of antimicrobial resistance justifies microbiological investigation, providing useful therapeutic and prognostic information to benefit this patient as well as other patients. It can provide information about epidemiology, assist infection prevention and control, contribute to wider surveillance of antibiotic resistance and guide important public health actions, such as determining prophylaxis after exposure to particular pathogens, such as *Neisseria meningitidis*.

## Variety of tests

Diagnostic laboratories can analyse specimens for the bacteria, viruses, fungi and parasites. A large variety of different techniques have been developed; some are reliable for certain pathogens but not others, but similar methods are often used. Many specimens are analysed using a combination of different techniques; increasingly, many of these are automated or use molecular methods such as polymerase chain reaction (PCR) or whole-genome sequencing. Some methods provide useful results within minutes or hours; others take days or weeks. All good accredited laboratories provide detailed information for users on test availability and turn-around time, and a good working knowledge of these can be invaluable to clinicians faced with investigating patients with infections.[1]

Figures 1 and 2 show the analytical journey of two types of specimen (cerebrospinal fluid (CSF) and faeces, respectively), indicating the typical variety of tests, information generated and timescale of analysis.

## Microscopy

Many forms of microscopy are used at various times during the analytical journey (e.g. directly on a specimen or to characterize microbes after culture).

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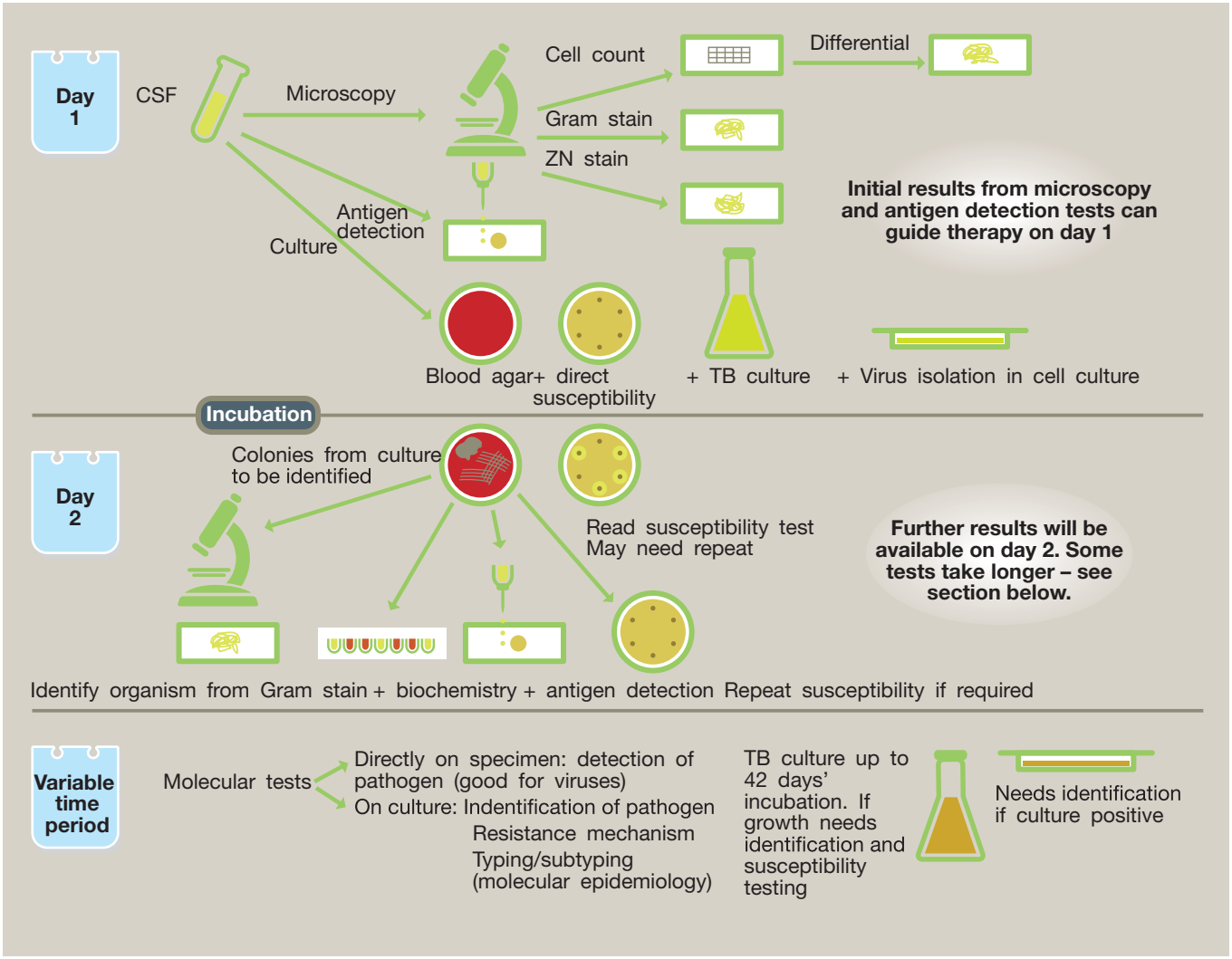


**Figure 1** Microbiological analysis of CSF showing a combination of microscopy, culture, biochemical identification and antigen detection techniques. Many laboratories employ molecular tests, for example PCR or MALDI-TOF, for identification of bacteria from cultures or direct detection of microbes in the specimen. TB, tuberculosis; ZN, Ziehl–Neelsen.

### Light microscopy (up to ×1000 magnification)

**Bright-field microscopy** uses visible light as a source of illumination. Unstained or wet microscopy is useful for counting white cells and red cells in liquid specimens such as urine, or for detecting and identifying ova or cysts of parasites in other specimens. Potassium hydroxide-digested specimens of nail and hair can be examined for fungal hyphae. In laboratories that process large numbers of urine specimens, flow cytometry is an efficient alternative.

Specimens can be stained to visualize and characterize bacteria more easily using, for example, Gram staining, or the Ziehl–Neelsen technique if mycobacteria are suspected. Gram-stained smears of bacterial colonies from culture plates can also be examined by this method. Lactophenol cotton blue staining is useful for characterizing and identifying fungi from preparations made from cultures. Useful information regarding infectious aetiology can also often be obtained by examining tissue specimens under light microscopy using a variety of different 'special stains'.

**Dark-field microscopy** causes the specimen to appear light against a dark background. This is useful for detecting organisms that are not easily seen by bright-field microscopy or cannot be easily stained, such as *Treponema pallidum* – the cause of syphilis – obtained from primary lesions. This can be performed in the clinic as a near-patient test.

**Fluorescence microscopy** relies on the principle that fluorescent dyes (fluorochromes) absorb invisible short-wavelength (ultraviolet) light and give off visible light. Mycobacteria can be stained with auramine O, which is very useful for detecting mycobacteria directly in specimens. Other fluorochromes include calcofluor white, which binds chitin and is useful for detecting fungi. Immunofluorescence uses fluorochrome-labelled antibodies that bind to specific antigens (e.g. viral antigens). Respiratory samples can be examined to detect the presence of respiratory viruses in respiratory tract epithelial cells, although molecular methods such as PCR have replaced these tests in most laboratories.




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