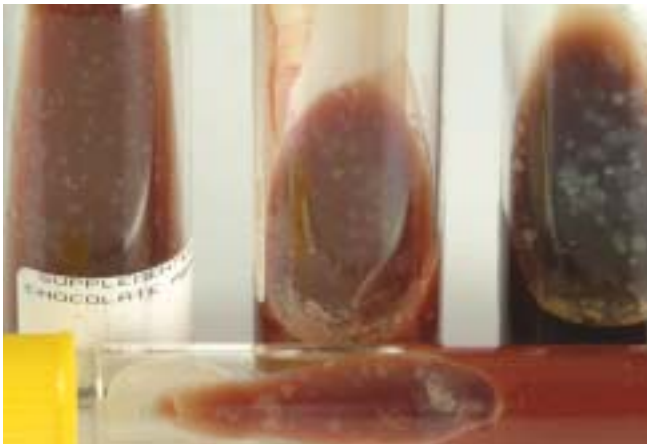
M. haemophilium on various FAC slopes.



M. haemophilium on chocolate agar and B83 slopes.

Unfortunately, the Australian clinical data presented here are skewed, with half the isolates coming from WA and several deficiencies in isolation media and techniques noted.

Appropriate *M. haemophilum* media, set up at 30-32˚C, should be used for most tissues types: blood, bone marrow, skin, tendons, joints, bone, bursa, lymphatic & the like. In immunocompromised individuals, it requires a specific 30-32˚C culture of pulmonary samples. Recovery media requires rigorous quality assurance and users should be encouraged to use standardised formulations.

The AMRLN will continue to review the *M. haemophilum* story for Australia and a better demographic picture will emerge of this most interesting *Mycobacterium* species. (The author would be grateful for strains and details of future cases).

References

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2. Cousins DV, Francis BR & Gow BL. Advantages of a new agar medium in the primary isolation of *Mycobacterium bovis*. *Vet Micro* 1989; 20:89-95.

Diagnosis of mycobacteria in the routine diagnostic laboratory

Introduction

Non-tuberculous mycobacteria (NTM) have become increasingly important over the last 20 years. These mycobacteria are classified as 'rapid growers' (growth in 7 days or less) or slow growers (growth of isolated colonies in more than 7 days).

The rapidly growing mycobacteria (RGM) are the group of mycobacteria which are being recognised in cultures performed in routine diagnostic microbiology laboratories. The increase in notification rate of RGM is partially due to a heightened alertness for mycobacteria by clinicians and laboratory scientists and technicians. However, diagnostic delays remain relatively common, with mycobacteria being considered only following failed treatment with empiric therapy.

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In immunocompetent patients, the most commonly encountered rapidly growing mycobacteria include *Mycobacterium chelonae, M. abscessus,* and the *M. fortuitum* complex which are often implicated in skin and soft tissue infections associated with penetrating injuries, catheter associated or iatrogenic infections. *M. mucogenicum,* which is resistant to the activity of many disinfectants, is seen in catheter associated infections. In immuno-compromised patients or patients with underlying lung pathology, e.g. cystic fibrosis disease, RGM can cause severe disseminated pulmonary disease.

M. abscessus is the most predominantly isolated pathogen among the latter patient group. Other clinically significant mycobacteria which can be isolated in the routine bacteriology laboratory include *M. haemophilum* and *M. marinum.* It is

important to be able to recognise and differentiate mycobacteria from other aerobic actinomycetes, including *Streptomyces, Nocardia, Gordona, Rhodococcus,* as both the clinical significance and treatment differs.

Laboratory indicators of possible mycobacterial infection

Gram stain

The Gram stain can often provide the first clue to the presence of mycobacteria. Weakly stained Gram-positive organisms or stippled Gram-positive bacilli may be indicators of the presence of mycobacteria. Occasionally, ghost cells or unstained areas in the shape of organisms may be suggestive of mycobacteria. The presence of pus cells in the absence of other bacteria can also be a clue to the possible presence of mycobacteria.

Understanding the spectrum of infections caused by mycobacteria and the patient groups most at risk, combined with a heightened alertness to the possible presence of mycobacteria, will inevitably result in additional isolations. In cases where mycobacteria are suspected, additional staining using both the standard ZN and the modified ZN stain should be performed.

Although most rapidly growing mycobacteria exhibit a degree of acid fastness, many species stain weakly (i.e. some cells are decolorised) compared to *M. tuberculosis.* The application of the Modified ZN using gentle decolorising (1% sulphuric acid without alcohol) allows for any RGM to be more easily recognised. The absence of branching in mycobacteria combined with the ZN staining reaction can be useful in discriminating RGM from other aerobic actinomycetes.

Specimen type

Lymph nodes when submitted for bacteriological examination should also be sent for mycobacterial culture. *M. haemophilum* is an intermediate growing mycobacterium that can readily be isolated from subcutaneous ulcers and lymph nodes in a routine bacteriology laboratory. *M. haemophilum* is a haemin dependant organism which requires a temperature of 32˚C for optimal growth and prefers a humid environment. Tissue or swabs from wounds of the hands or feet may suggest penetrating injury with environmental contamination.

If water is a possible source of infection, *M. marinum* should be considered and appropriate culture conditions set up. *M. marinum* is an intermediate growing organism which requires lower temperatures for growth.

Both *M. marinum* and *M. haemophilum* could be isolated in the routine laboratory more frequently if primary samples were inoculated onto chocolate agar and incubated for 10-14 days at 32˚C or less. Dehydration of media can lead to decreased sensitivity of culture. The drying of plates can be minimised by incubating the plates in a sealed plastic bag or by inoculating chocolate agar slopes. If a 30-32˚C incubator is not available, culture plates/slopes can be left at room temperature on the bench.

Patient history

A common complaint from microbiology laboratories is the lack of relevant clinical information on request forms accompanying specimens for culture. Although clinicians should be encouraged to provide all relevant clinical notes, clues that mycobacteria may be associated with a particular infection can be recognised in less specific terms such as chronic wound or ulcer, multiple lesions, discharging sinuses or trauma associated with environmental contamination as in gardening or aquatic injuries. Any indications that a patient may be immunosuppressed, including dialysis, diabetes, corticosteroid therapy, or malignancy should also trigger an alertness to the possibility of mycobacteria.

Recognition of mycobacteria on culture

Rapid growing mycobacteria usually appear as small white or grey colonies on blood agar or chocolate agar and typically require at least 3 days' incubation at 35˚C. The texture of the rapidly growing mycobacteria varies from smooth or mucoid colonies to rough and friable. Mature colonies often have irregular edges and folded surfaces but do not show the aerial mycelia or 'bloom' characteristic of many *Nocardia sp.*

Most RGM are not inhibited by colistin and can be isolated on selective agars such as blood agar incorporating naladixic acid and colistin or *Burkholderia cepacia* selective media using polymixin B and ticarcillin as selective agents. All presumptive cultures of RGM should be stained by Gram stain ZN and Modified ZN stains prior to referring to a reference centre for identification.

Although routine diagnostic laboratories have the capacity for isolating RGM, it is recommended that samples containing AFB on direct microscopy or samples from unusual or unexplained infections should also be referred to a mycobacterium reference laboratory so that all mycobacterial species can be excluded. Primary samples can be mixed or contaminated with normal regional flora and may require decontamination with sodium hydroxide prior to culture. Alternatively, the mycobacteria can be selectively cultured in broth with antibiotic supplements. Speciation of isolated mycobacteria has traditionally relied on a battery of phenotypic characteristics; however, molecular based identification strategies are increasingly being applied to yield unequivocal identifications.

Clarification

In the July issue of MA, it was stated that "EML was Australia's only NATA accredited food allergen testing laboratory". EML has NATA accreditation for peanut allergen testing, which at present is the only type of food allergen testing available for accreditation by NATA.