Isolation and identification of anaerobes in the routine diagnostic laboratory - an EQA provider's perspective

by Christine Walton and Dr. Val Hall

Anaerobic bacteria cause significant human infections that require specific therapy, therefore, the isolation and identification of anaerobes is as important as for any other pathogen. Individual laboratories can monitor their technical ability with specimens containing anaerobes through the use of quality control and by participating in an external quality assessment (EQA) scheme.

Conventional methods for isolating and identifying anaerobes involve culture, time consuming biochemical testing under strict anaerobic conditions, and in some cases, gas liquid chromatography (GLC) and molecular typing before definitive identification can be made. These procedures are costly and often are not practical for many clinical microbiology laboratories. However, it could be argued that simply detecting anaerobes in clinical specimens and performing some basic identification tests can provide the clinician with sufficient information to start appropriate patient management. In this mini-review, participant performance with EQA specimens containing anaerobe strains is assessed, and advice is provided by the ARL, Cardiff on simple techniques to improve the recovery of anaerobes.

EQA participant base

There are currently over 850 laboratories from 26 countries [Table 1] participating in the United Kingdom National External Quality Assessment Service (UK NEQAS) microbiology scheme for general bacteriology. Clinical diagnostic microbiology laboratories form the main participant base but a small number of veterinary, commercial in vitro diagnostic manufacturers and central laboratories also participate in the scheme. Distributions comprise three lyophilised specimens for bacterial isolation and identification, which are despatched twelve times per year. Until March 2003, the distribution also contained a fourth specimen for antimicrobial susceptibility testing (AST) but since April 2004, AST is organised as a separate scheme.

Simulated specimens containing anaerobes

A request form sent with the specimens specified the site of isolation and relevant clinical details that would assist in a diagnostic investigation. Some of the specimens distributed for bacterial identification comprised mixtures of bacteria chosen to simulate the flora of the site of isolation specified on the request form. Other specimens consisted of pure cultures when this would be the expected finding from the site of isolation. Participants were expected to examine the specimens using their normal routine methods and return their results within three weeks. Over the last 10 years, 36 specimens were distributed containing anaerobic organisms including *Actinomyces, bacteroides, clostridia, fusobacteria, peptostreptococci, prevotella and porphyromonas* [Table 2].

**Table 2. Anaerobic organisms sent out in the General Bacteriology scheme over the last 10 years.**

<table>
<thead>
<tr>
<th>Source of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Anaerobe Reference Laboratory (ARL) of the National Public Health Service for Wales (formerly the Public Health Laboratory Service Anaerobe Reference Unit) supplies strains of anaerobic bacteria upon request to UK NEQAS. Strains are selected from recent isolates from clinical laboratories throughout the UK, and referred to the ARL for confirmation of identity. Each is tested to ensure, as far as possible, that it exhibits growth, morphological and principal biochemical characteristics typical of the designated species.</td>
</tr>
</tbody>
</table>

**Information provided to participants**

Within 24 hours of the close of a distribution period, the intended results are posted on the website [www.ukneqasmicro.org.uk]. Subsequently, a report is sent to participants, which includes an analysis of individual performance with the current specimens and a cumulative analysis of performance with all specimens in the general bacteriology scheme sent out in the previous six months. In addition, where there were more than 10 laboratories in any one country, a country performance analysis is provided.

**Scoring of results**

The purpose of the scheme is to help participants to monitor their own performance and take action where needed. Generally a four point system is used with scores of 2 awarded for correct results, 1 for partially correct results, 0 for wrong results and failure to return results and -1 for a grossly misleading result. Specimens are not scored if less than 80% of referee laboratories, those with the highest score over the previous 12 month period, obtain the correct result.

**Review of participant performance: results and discussion**

The overall level of participant performance with specimens containing anaerobes distributed in the last 10 years was not as good as with specimens containing other commonly isolated pathogens. On average, 80% of participants reported the correct result for scored specimens containing anaerobes (n=36), which compares with 93% for all other identification specimens (n=278) in the general bacteriology scheme over the same period. A comparison of the level of performance for EQA specimens containing common anaerobes, with specimens containing other pathogens commonly encountered in the clinical diagnostic laboratory is shown in table 3.
The level of performance seen with *Clostridium* species was generally better than with other anaerobes, such as *Prevotella* species or *Bacteroides* species. On average, participants achieved a success rate of 78% for *Clostridium* species (n=11), within a range of 65% for a specimen containing *C. tetani* to greater than 90% for specimens containing *C. perfringens*, *C. septicum* and *C. novyi*. Performance rates for other anaerobes varied considerably and revealed a 97% success rate for a distribution of *Actinobacillus israelii*, 52% for a *Prevotella intermedia* and 56% for a *Fusobacterium nucleatum* [Figure 1]. Commercial kits which yield a pre-formed enzyme profile (PEP) have made identification of Gram positive anaerobic cocci (GPAC) much simpler; they enable the routine laboratory to identify most of their GPAC without the aid of GLC. However, it is notable that a single kit is expected to identify all of the many genera of anaerobes whereas other kits are designed specifically for streptococci, enterobacteriaceae, corynebacteria, etc. Further limitations are that manufacturers' databases do not include all clinically relevant anaerobes and have not kept abreast of recent taxonomic changes, some species are poorly differentiated and reactions may be difficult to interpret. Also, where the user is unfamiliar with cell and colony morphology there is 'blind-faith' in the result obtained from the kit.

With a little experience and familiarity with the morphology of common isolates, a few simple tests together with a good laboratory manual may be useful as supplements or alternatives to kit-based systems for the confident identification of anaerobes.

Participant success rate with specimens containing *Actinobacillus israelii* ranged from 59% to 86%. Technical problems frequently arise from the unusual growth characteristics of these organisms [1]. Slow growth and exacting nutritional and atmospheric requirements may result in false negative test reactions. Furthermore, it may be impossible to obtain a smooth suspension of these organisms which tend to form gritty deposits. These factors may be significant to the poor performance of commercial kit identifications. It is often necessary to make a series of subcultures of isolates before they will grow readily in vitro and perform reliably in tests.

For isolation of relevant anaerobes from clinical (or NEQAS) material, specimens can be handled on the open bench, but culture plates must be incubated anaerobically as soon as practicable. Arguably, leaving plates on the bench for long periods prior to incubation is the major cause of culture-failure. Initial incubation of cultures for 48 hours without exposure to air is also important, with a further 3 days incubation for slow-growing and black-pigmented anaerobes, and up to 10 days for *Actinomyces* species.

### Summary and conclusion
Isolation of anaerobes from NEQAS samples should not present problems given that strains are drawn from those isolated initially from clinical material in routine laboratories using standard practices. However, some anaerobes are more nutritional and/or atmospherically demanding than others. Key elements for successful isolation are media, anaerobiosis and the incubation protocol.

### Table 3. Participant performance with common anaerobes compared to performance with other pathogens commonly encountered in the routine diagnostic microbiology laboratory.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>% participants obtaining a correct result (n= total no. specimens)</th>
<th>Other pathogens</th>
<th>% participants obtaining a correct result (n= total no. specimens)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Actinobacillus israelii</em></td>
<td>75% (n=5)</td>
<td><em>Staphylococcus aureus</em></td>
<td>96% (n=8)</td>
</tr>
<tr>
<td><em>Bacteroides fragilis</em></td>
<td>82% (n=2)</td>
<td><em>Haemophilus influenzae</em></td>
<td>92% (n=10)</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>87% (n=3)</td>
<td><em>Neisseria meningitidis</em></td>
<td>97% (n=10)</td>
</tr>
<tr>
<td><em>C. difficile</em></td>
<td>87% (n=9)</td>
<td><em>Salmonella enteritidis</em></td>
<td>98% (n=8)</td>
</tr>
<tr>
<td><em>Prevotella spp.</em></td>
<td>61% (n=2)</td>
<td><em>Haemolytic Strept. Gp A</em></td>
<td>97% (n=6)</td>
</tr>
<tr>
<td><em>Peptostreptococcus spp.</em></td>
<td>97% (n=1)</td>
<td><em>Streptococcus pneumoniae</em></td>
<td>98% (n=12)</td>
</tr>
</tbody>
</table>

### Table 4. Participant performance with specimens containing two anaerobes.

<table>
<thead>
<tr>
<th>Year of distribution</th>
<th>Organisms</th>
<th>Specimen type</th>
<th>Percentage of participants reporting a fully correct result (n= total no. reporting)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td><em>Prevotella intermedia</em> and <em>Peptostreptococcus aneurobus</em></td>
<td>Blood</td>
<td>45% (n=632)</td>
</tr>
<tr>
<td>2000</td>
<td><em>Prevotella intermedia</em> and <em>Peptostreptococcus aneurobus</em></td>
<td>Pus</td>
<td>76% (n=675)</td>
</tr>
</tbody>
</table>

### References

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