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# Differentiation of vancomycin-resistant enterococci using enterococcus differential medium

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The genus *Enterococcus* includes over 20 species.[1] Increasing numbers of enterococci are becoming resistant to glycopeptide antibiotics. *Enterococcus faecium* and, to a lesser extent, *E. faecalis* are of greater epidemiological importance,[2] as they harbour transferable *vanA* and *vanB* genes. Other enterococci such as *E. gallinarum* and *E. casseliflavus / E. flavescens* do not normally cause human disease but commonly demonstrate intrinsic low-level glycopeptide resistance.[3] Furthermore, they are often isolated as a result of active surveillance procedures.

Knowledge of the type of resistance is critical for infection control purposes and, in a non-molecular laboratory, necessitates accurate identification. Accurate identification is also required to determine whether changes in glycopeptide resistance rate reflect the dissemination of resistance determinants among enterococci in general or a rise in the relative importance of *E. faecium*, the strain in which glycopeptide resistance is most common.

The Health Protection Agency (HPA) standard method for identification of enterococci[4] recommends the use of 'a commercial kit'. Along with the API Rapid ID 32 Strep (bioMerieux, Basingstoke, UK), BBL Crystal Gram Positive (Becton Dickinson, Oxford, UK) and BBL Crystal Rapid Gram Positive (Becton Dickinson), the API 20 Strep system (bioMerieux) is often used to identify enterococci to species level. However, Reed *et al*.[5] used API 20 Strep and misidentified nine out of 12 *E. gallinarum* isolates as *E. faecium*. Similarly, Winston *et al*.[6] used this system to identify 46 enterococcal isolates with low-level vancomycin resistance (predominantly *E. gallinarum*) and misidentified 42 as *E. faecium*. The correct identification was obtained only when additional tests were employed.

*E. faecium* and *E. faecalis* are non-motile, whereas *E. gallinarum* and *E. casseliflavus / E. flavescens* are considered to be motile.[7] Furthermore, most isolates of *E. casseliflavus / E. flavescens* have a distinct yellow pigment. Collins *et al*. [8] used acid production from methyl-α-D-glucopyranoside (MGP) to identify certain streptococci as enterococci. Devriese *et al*.[9] confirmed that the acidification of MGP differentiated *E. casseliflavus* and *E. gallinarum* from *E. faecium*. Turenne *et al*.[7] examined 33 isolates identified as *E. faecium* by conventional methods and showed that, of the 11 that sequenced as *E. casseliflavus* or *E. gallinarum*, all were MGP-positive, while the *E. faecium* were MGP-negative. The MGP test has been evaluated by workers in Brazil,[10] the USA[11] and Canada,[12] with similar results.

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**Table 1**. Typing results using API 20 Strep and MGP fermentation.

| Species identification | Number of isolates | API 20 Strep identification | | | Fermentation of MGP |
|---|---|---|---|---|---|
| | | *E. faecium* | *E. faecalis* | *E. gallinarum* | |
| *E. faecium* | 20 | 20 | 0 | 0 | 0 |
| *E. faecalis* | 6 | 0 | 6 | 0 | 0 |
| *E. gallinarum* | 11 | 5 | 2 | 4 | 10 |
| *E. casseliflavus* | 6 | 2 | 1 | 3 | 6 |

A recent UK NEQAS distribution (specimen 6945, distribution 1728, March 2004) included *E. gallinarum*. Using API 20 Strep, the authors incorrectly identified this isolate as *E. faecium* and this prompted them to re-evaluate the identification methods used and to examine the usefulness of the MGP test for routine use in the UK.

The strain collection of 47 enterococci comprised *E. faecalis* ($n$=6), *E. faecium* ($n$=20), *E. gallinarum* ($n$=11), *E. casseliflavus* ($n$=6) and a single strain each of *E. avium*, *E. durans, E. hirae* and *E. saccharolyticus*. Identification was by biochemistry (rapid ID 32 Strep), motility, pigment production and ability to grow at 45°C for *E. faecalis* ($n$=3), *E. faecium* ($n$=9), *E. casseliflavus* ($n$=5), *E. gallinarum* ($n$=3); pulsed-field gel electrophoresis (PFGE) for *E. gallinarum* ($n$=5), *E. faecalis* ($n$=2) and *E. faecium* ($n$=11). Stock culture strains of *E. gallinarum* (NCTC 11428 [ATCC 35038], ATCC 49573, ATCC 400425), *E. casseliflavus* (ATCC 700327), *E. faecalis* (ATCC 29212 [NCTC 12697]), *E. avium* (ATCC 14025), *E. durans* (ATCC 49135), *E. hirae* (ATCC 8043) and *E. saccharolyticus* (ATCC 43076) were also studied.

Strains were identified using API 20 Strep according to the manufacturer's instructions. A suspension equivalent to a McFarland 0.5 opacity standard was prepared in sterile water and 20 µL of this suspension was used to inoculate a tube of enterococcus differential medium (MGP with bromothymol blue indicator; Mast, Bootle, UK). An uninoculated tube was used as a control. Following incubation at 37°C in air for 18 h, the broths were examined for a colour change (green to yellow) that signified acidification of MGP.

All 20 isolates of *E. faecium* and all six isolates of *E. faecalis* identified correctly using API 20 Strep and none fermented MGP. Of the 11 *E. gallinarum* isolates, four identified correctly using API 20 Strep, five identified as *E. faecium* and two identified as *E. faecalis* (Table 1). All isolates of *E. gallinarum* (except NCTC 11428 [ATCC 35038]) fermented MGP. The six isolates of *E. casseliflavus* identified using API 20 Strep as *E. faecalis, E. faecium* ($n$=2) and *E. gallinarum* ($n$=3). Despite high probabilities to the contrary, the API identification software often suggested 'possibility of *E. casseliflavus*'. All fermented MGP.

Fermentation of MGP reliably separates *E. faecalis* and *E. faecium* from *E. gallinarum* and *E. casseliflavus*. Together, these four strains constitute the most commonly isolated entrococci. Most strains belonging to the *E. avium* group (*E. avium, E. pseudoavium, E. raffinosus, E. malodoratus*) and the newer strains of *E. columbae, E. sulfureus, E. dispar* and *E. saccharolyticus* used in this study also ferment MGP.[13] The single strains of *E. avium* and *E. hirae* did not ferment MGP (data not shown) but these are rarely implicated in human disease.

The isolate of *E. gallinarum* that did not ferment MGP (NCTC 11428 / ATCC 35038) was identified as *E. faecalis* using API 20 Strep, and, interestingly, this has been classified as such by Coleman (data in United Kingdom National Culture Collection).

The National Glycopeptide-Resistant Enterococcal Bacteraemia Surveillance Working Group[14] recommends that identification of enterococci should be based on the use of commercial kits. However, it highlights the suspect accuracy with species other than *E. faecalis* and *E. faecium*, and calls for further studies with a range of enterococcal species. A recent UK NEQAS distribution (specimen 7199, distribution 1811, September 2004) again highlighted the difficulty that routine laboratories experience when attempting to identify enterococci with low-level resistance to vancomycin.

The MGP test is a simple, effective and inexpensive adjunct to commercial kits such as the API 20 Strep system and is essential for the accurate differentiation of enterococci with transferable glycopeptide resistance from those with intrinsic resistance. As a result of this group's findings, enterococcus differential medium is now available commercially in the UK.

***Take home messages***

- Strains of *Enterococcus faecium* and *E. faecalis* pose infection control problems, as their glycopeptide resistance may be transferable.
- Other species of enterococci are intrinsically resistant to glycopeptides.
- Some commercial kits cannot reliably identify strains of enterococci other than *E. faecalis* and *E. faecium*.
- The MGP test is a useful adjunct to commercial systems and reliably discerns enterococci with intrinsic resistance from those with transferable resistance.

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# Peroxidase activity and nuclear density analysis (PANDA) in the diagnosis of haematological malignancy

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Diagnosis of haematological malignancy relies on the assessment of cellular morphology and immunophenotype (having largely replaced cytochemistry), cytogenetic, molecular, and clinical features. Usually, however, an abnormal full blood count (FBC) is the first laboratory indication that haematological malignancy may be present.

Modern haematology analysers measure an increasing number of parameters in addition to traditional indices in a variety of different ways, depending on the manufacturer. Awareness of these parameters on front-line laboratory instruments, and their ability to offer additional diagnostic clues, is a growing area of interest. The Advia 120 haematology analyser (Bayer Diagnostics, Newbury, UK) uses a combination of cytochemistry and light-scatter measurements to derive its peroxidase activity (PA) and nuclear density (ND) analysis (PANDA) cytograms.

Peroxidase activity is measured using the peroxidase channel. In a heated reaction chamber, red blood cells are lysed with a surfactant, and the white blood cells are fixed using formaldehyde. In the presence of hydrogen peroxide and the chromogen 4-chloro-1-naphthol, cells containing myeloperoxidase form a dark precipitate and are characterised by their light-scatter and light-absorption properties.

Nuclear density is derived from the basophil/nuclear lobularity channel. In a heated reaction chamber, phthalic acid strips the cytoplasm from white blood cells (except basophils). Two-angle light scatter is then used to determine cell size and nuclear density. Together, PA and ND are used to derive the white blood cell (WBC) count and the WBC differential.

In a recent study by d'Onofrio,[1] PA and ND cytograms were used to assess the utility of these parameters to assist in the diagnosis and classification of haematological malignancy, leading to the construction of a PANDA pre-classification grid comprising seven PA and two ND categories (Fig. 1). One hundred and eighty cases were studied, including examples of acute leukaemia, chronic lymphoproliferative and myeloproliferative disorders, as well as cases of infectious mononucleosis and peroxidase-deficient neutrophils (both of which also have abnormal cytograms). With some variation in respective categories, overall accuracy of classification using the PANDA grid was reported to be 91.1%.

Use of pattern-recognition software on the next generation of laboratory computer systems seems increasingly likely. Rather than using relatively simple analyser flagging, future computer systems may integrate and assess parameters such as the PANDA profiles, and even alert the operator to possible diagnoses for further investigation.

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