

Discussion

The procedures described simplify the measurement of chemotaxis. Elimination of visual counting facilitates replication of the procedure and allows a larger number of samples to be tested in a short time.

We had anticipated that counting all the cells crossing the membrane rather than a random sample of them would reduce the variance of the test readings. This did not prove to be the case, however; most of this variance seemed to be due to differences between the filters. Cell concentrations of 1×10^6 are used routinely because they produce visually acceptable numbers of cells on the collecting filters. Counting cells by chemical means permits use of a much wider range of polymorphonuclear leucocyte concentrations, especially physiological ones. Because chemotactic responses are affected by the cell density, it is important to use physiological concentrations.⁶

The DNA method gave higher estimates of cell numbers than visual counts of the membranes. One explanation for this is that cells may drop off the collecting membranes during the process of fixation and staining. It is likely that the DNA method also measures cells that disintegrate after transverse the membrane and thus are not visible to the staining.

A portion of the polymorphonuclear leucocyte DNA producing fluorescence enhancement appears to be susceptible to cellular nucleases. It is impor-

tant to measure fluorescence soon after the collecting membranes are sonicated. Several hours delay can be tolerated, however, if the membranes are not sonicated immediately on removal from the chemotaxis chambers.

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Requests for reprints to: Dr H Elliott Larson, Division of Communicable Diseases, Watford Road, Harrow, Middlesex HA1 3UJ, England.

Rapid identification of *Clostridium difficile* by direct detection of volatile organic acids from primary isolation media

T MAKIN *Department of Medical Microbiology, Royal Liverpool Hospital, Liverpool, Merseyside*

The importance of *Clostridium difficile* in pseudomembranous colitis¹⁻⁵ and its increasing role in antibiotic-associated diarrhoea⁶⁻⁹ have encouraged many laboratories to screen routinely for the organism in all samples of faeces.

Isolation of *C difficile* has been greatly facilitated by the development of more effective selective media.¹⁰ Identification by conventional biochemical

techniques, however, is often confounded by the variable and fastidious nature of the organism. Detection by gas chromatography of characteristic volatile organic acids in broth cultures of *C difficile* provides a more reliable method of identification, but entails a delay in reporting of 24-48 h because of the need to incubate broth cultures.

An observation that the same volatile organic acids are present in plugs of agar removed from beneath suspected colonies of *C difficile* provides the basis for the rapid method described.

Material and methods

All samples of faeces received for bacteriological investigations were inoculated on to cycloserine-cefoxitin fructose agar (CCFA) (Oxoid Ltd). Cultures were incubated at 37°C in an atmosphere of CO₂(5%), H₂(5%), and N₂(90%) for up to 48 h.

A hollow glass tube (approximate internal diameter 4 mm) was pushed through suspected colonies of *C difficile* and into the underlying agar. A rubber

teat fitted to the glass tube was used to create a partial vacuum, which aided the removal of the plug of agar. Before the plug was removed, a sample of each colony was inoculated into Robertson's cooked meat broth and incubated at 37°C for 48 h.

Acidified ether extracts of the agar plugs were analysed by gas liquid chromatography.¹¹ The same procedure was performed on 0.5 ml of cooked meat broth after incubation for 48 h.

Results

During an outbreak of pseudomembranous colitis a total of 186 samples of faeces were screened for the presence of *C difficile*.

Ninety four colonies morphologically resembling *C difficile* were isolated from 74 of the faecal samples inoculated on to CCFA. Seventy two of the isolates were identified as *C difficile* by gas chromatography on cooked meat broth.

In all cases the plug method detected the same volatile organic acids in similar quantities to those found in the corresponding cooked meat broth.

Volatile organic acids detected under the sampled colonies of *C difficile* were unaffected by the presence of other bacterial species which were capable of growing on the agar.

Discussion

Since the causative role of *C difficile* in pseudomembranous colitis has been established,¹⁻⁵ and its contribution to antibiotic-associated diarrhoea is recognised,⁶⁻⁹ it has become increasingly important to examine faeces routinely for the presence of *C difficile*. The severe nature of these conditions and their rapid response to treatment^{12 13} underline the need for methods which rapidly identify the aetiological agent.

Detection of volatile organic acids from a plug of agar beneath suspect colonies on solid primary selective medium has proved to be a rapid and reliable alternative method for identifying *C difficile*. In each of 72 cases the plug method successfully identified colonies that were subsequently confirmed as *C difficile* by gas chromatography performed on pure broth cultures.

In contrast to other rapid methods,^{14 15} the plug method (a) avoids the need to add precursor metabolites to the primary selective medium, (b) is based on an established method, and (c) adopts the

same material and operating conditions as those used in gas chromatography on broth cultures.

By identifying *C difficile* on primary selective medium the plug method allows a report on the identity of the suspect colony to be given 24-48 h earlier.

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Requests for reprints to: Mr T Makin, Department of Medical Microbiology, Royal Liverpool Hospital, Prescot Street, Liverpool L7 8XP, England.