

Letters to the Editor

Streptococcus milleri found in pulmonary empyemas and abscesses

I was very interested to read the letter of Dr Waitkins *et al* on the importance of *Streptococcus milleri*. We too isolate the organism from cases of empyema from time to time and would agree about the importance of *S milleri* in this context. Although we welcome this reminder about the pathogenicity of the organism, there is one aspect of the letter that could be misleading: *S milleri* apparently requires anaerobic conditions on primary isolation, but it is not an obligate anaerobe. The inclusion of this organism by the authors in the category of anaerobic streptococci implies that it is a true anaerobic coccus¹ and thus susceptible to metronidazole, whereas it is resistant to this agent.

The clinical importance of the non-anaerobic streptococci is not in doubt, but resulting infections must be treated with appropriate antibiotics. Metronidazole is not appropriate in this context and should not be used.

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Reference

¹ Watt B, Jack EP. What are anaerobic cocci? *J Med Microbiol* 1977;10:461-8.

Dr Waitkins replies as follows:

I agree with the comments of Dr Watt. Of course *S milleri* is not strictly an anaerobe. I would, of course, hope that all clinical microbiological colleagues would know that *S milleri* is resistant to metronidazole.

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Detection of rotavirus by latex agglutination

The recent paper by Moosai *et al* concerning the detection of rotavirus by latex aggluti-

nation reported some interesting observations.¹ In common with some previous reports,² which compared latex and electron microscopy, the authors reported an apparent similar specificity and sensitivity for five different methods, including electron microscopy, the reference method used to select the limited number of frozen and stored test samples. Other workers, using larger sample numbers, or samples more representative of those encountered in a routine clinical laboratory failed to obtain these absolute correlations.³

In this laboratory a survey of 120 faecal samples, mainly from infants, tested over two winters by enzyme linked immunoabsorbent assay (ELISA) using a polyclonal antihuman rotavirus antiserum (Dako Ltd, High Wycombe) and by latex agglutination (CRA-latex) using antiserum raised against calf rotavirus isolates (Rotalax, Orion Diagnostics, Finland) produced data to complement those of Moosai *et al*.¹

When CRA-latex was compared with ELISA, which had previously shown good correlations with electron microscopy,³ more than 9% (11 of 120) of our specimens gave conflicting results, and the specimens that reacted only weakly with the latex particles were extremely difficult to interpret, even by our most experienced observers. Immediate retesting of the specimens confirmed the discrepancies, but after freeze thawing three of the nine specimens that were originally positive by ELISA but negative by CRA-latex subsequently became positive by CRA-latex. As treatment with edetic acid or storage coincides with a loss of both infectivity and type specific antigens associated with the outer capsid layer,⁴ an enhanced sensitivity to latex may be due to the exposure of further antigenic determinants after storage and freeze thawing.

Pretreatment of the samples with 0.005M edetic acid, however, showed that they already contained mostly incomplete particles as the sensitivity of the ELISA method was slightly enhanced, but there was no overall qualitative difference to the results. Like Moosai *et al* we concluded that in our hands Rotalax in its present form was unsuitable as a routine screening procedure or as a diagnostic method in a routine clinical laboratory without electron microscopy facilities.

In a second study of 69 samples from infants, however, which compared ELISA with another commercially available latex reagent (RotaScreen, Mercia Brocades, West Byfleet) prepared using human rotavirus isolates (HRA-latex), better correlations were obtained without recourse to modification of the manufacturers' protocol or reagents, and the agglutination was easily read by the naked eye within two minutes. Although there was a similar proportion of positive specimens (37 of 69 compared to 50 of 120) in both studies, only one specimen gave discrepant results (positive by ELISA, negative by LATEX) using HRA-latex, which was also negative by electron microscopy.

A final comparison of 38 specimens using all three methods (Table) included 10 out of 11 which gave discrepant results and 22 of 41 which gave positive results from the first study and confirmed the superiority of HRA-latex compared with CRA-latex. With the exception of one specimen that was singly positive by ELISA, the results given by HRA-latex concurred with those given by ELISA, but 17% of the specimens giving positive results by these methods were persistently negative by CRA-latex. Complete agreement between the use of HRA or CRA in ELISA, with CRA being as sensitive and

Table Comparison of ELISA, HRA-LATEX, and CRA-LATEX

ELISA	HRA-LATEX	CRA-LATEX		
		Positive	Negative	Total
Positive	Positive	25*	5**	30
Negative	Negative	1	6	7
Positive	Negative	0	1***	1
Total		26	12	38

* = Includes three specimens that were positive by CRA-latex only after freeze-thawing.

** = Three of three specimens tested were positive by electron-microscopy at the regional laboratory.

*** = Specimen was negative by electron microscopy at the regional laboratory.

specific as HRA has been reported.⁵ Distinct antigenic differences between rotaviruses from different species, however, result in a variation of cross reactivity to these determinants and reflect reported⁶ differing levels of sensitivity using antisera raised against human or animal rotavirus.

We conclude that HRA-latex is a quick, simple, and sensitive method of detecting rotavirus, particularly in a routine clinical laboratory without electron microscopy facilities.

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References

- Moosai RB, Alcock R, Bell TM, *et al*. Detection of rotavirus by a latex agglutination test, Rotalex: comparison with electron microscopy, immunofluorescence, polyacrylamide gel electrophoresis, and enzyme linked immunosorbent assay. *J Clin Pathol* 1985;38:694-700.
- Sanekata T, Yoshida Y, Okada H. Detection of rotavirus in faeces by latex agglutination. *J Immun Methods* 1981;41:377-85.
- Grauballe PC, Vestergaard BF, Meyling A, Gerner J. Optimised enzyme-linked immunosorbent assay for detection of human and bovine rotavirus in stools. *J Med Virol* 1981;7:29-40.
- Shirley JA, Beards GM, Thouless ME, Flewett TH. The influence of divalent cations on the stability of human rotavirus. (*Arch Virol* 1981;67:1-9.
- Sarkkinen HK. Human rotavirus antigen detection by enzyme-immunoassay with antisera against Nebraska calf diarrhoea virus. *J Clin Pathol* 1981;34:680-5.
- Yolken R, Wyatt RG, Kapikian AZ. ELISA for Rotavirus. *Lancet* 1977;ii:819.

Tests for detecting rubella specific IgM

Drs Cubie and Edmond recently reported their experience with five different tests to detect rubella specific IgM.¹ We tried a new enzyme linked immunosorbent assay (ELISA) kit for the detection of rubella IgM antibodies, which was prepared by the Division of Microbiological Reagents and Quality Control (DMRQC) of the Public Health Laboratory Service and compared it with Rubenz M II (Northumbria Biologicals

Ltd). The protocols for these two assays were very similar. Both tests used a μ chain capture technique and monoclonal anti-rubella immunoglobulin conjugated to horseradish peroxidase. The objective of the trial was to seek advantages in terms of sensitivity and ease of use over the current method (fractionation on sucrose density gradient followed by haemagglutination inhibition).

Material and methods

Sera were selected on the basis of the rubella IgM titre by haemagglutination inhibition after fractionation: there were five categories (titres shown in parentheses); high (≥ 64), intermediate (16-32), low (4-8), equivocal (2), and negative (< 2). A serum with an IgM titre of 512 was diluted in negative serum to find the dilution at which it became non-reactive in each assay. Inadequate serum samples prevented repetition of the fractionation technique. The sucrose density gradient and haemagglutination inhibition method was refined to be as rapid, uncomplicated, and sensitive as possible. Discontinuous sucrose gradients were prepared in five steps (range 37.5%-12.5% w/v sucrose in dextrose gelatin veronal buffer), and 250 μ l samples of serum were immediately layered on top and the gradients centrifuged at 35,000 rpm (125,000 $\times g$) for 16-18 hours. The fractions containing IgM were titrated from 1/2 against 4 HAU₅₀ of rubella haemagglutinin (DMRQC) with and without treatment with 2 mercapto ethanol (Sigma) for one hour at 37°C.

Rubenz M II was used in accordance with the manufacturer's instructions and differed from the test described by Cubie and Edmond in that phosphate buffered saline-Tween was used for the control well in place of "negative" antigen and that the cut off level for a positive result was >80% of the net value obtained with the 5 unit control serum. A reading of 50-80% of the 5 unit value was regarded as an equivocal result. The technique used in the DMRQC test has been described elsewhere.²

Plates were read on a Titertek Multiskan spectrophotometer, and in the case of the DMRQC assay results were interpreted by an interfaced BBC B microcomputer using Plateskan 2 software (IQ Bio Ltd) to plot the control sera (100, 30, 10, 3 arbitrary units) on to a standard curve, which calibrated the values of the test sera in arbitrary units (au). The Table shows the IgM values expressed in au of sera tested in the various categories. The test could be read reliably by comparing the visual or spectrophotometric absorbance

values with the 10 au control serum used as a cut off. Values between 3 and 10 au were regarded as equivocal for the purposes of this trial.

The arbitrary unit standards for rubella specific IgM were originally established by Mortimer *et al* for the M-antibody capture radioimmunoassay (MACRIA)³ and the DMRQC ELISA is based on the MACRIA, but the DMRQC chose to use a nominal value of 10 au for the cut off level; hence all values of rubella specific IgM obtained with the DMRQC ELISA had to be divided by three for comparison with results of Rubenz M II, which uses units directly based on MACRIA standards.

Results

Accurate calibration of the Rubenz M results was impossible with the control sera provided, and the format of the test (strips of 12 wells) did not lend itself to the structure of the computer program. Crude estimates of the unit measurement of samples were prepared from a manually drawn curve.

Eighteen sera were positive according to the criteria of both ELISAs. Of the remainder, seven were unreactive in the DMRQC assay, and a further six sera that gave equivocal values did not react in Rubenz M II. The estimated unit measurements of samples by Rubenz M II were lower by a factor three than the results in au obtained with the DMRQC assay, but this was expected. These and the results of tests on dilutions of the strongly positive serum showed that the two assays had identical sensitivity, which was broadly equivalent to the sucrose density gradient and haemagglutination inhibition method. Of nine sera that gave equivocal results by sucrose density gradient and haemagglutination inhibition, one was reactive in both ELISAs and one equivocal in both. These values accorded with the eventual interpretation of the patient's rubella state, which had been reached on the basis of clinical information and reference tests performed elsewhere. Thus we concluded that there was no clear advantage to be obtained by the use of either assay.

Conclusion

With respect to the fractionation technique no advantage was found (in terms of time and effort) for using the ELISAs over the sucrose density gradient and haemagglutination inhibition method. All three methods are designed to be set up one and a half to two and a half hours before the