Letters to the Editor

Recovery of spores from impregnated filter paper

Filter paper discs impregnated with spores are commonly used for testing various sterilising procedures. Kelsey (1961) and, more recently, Everall and Morris (1978) claimed that the quantitative recovery of spores from such discs was difficult, and the latter workers described a method of achieving it by means of a macerating machine. In this laboratory the task is accomplished by a simple hand shaking procedure, which takes 15 seconds or less.

The following is an illustrative experiment. Antibiotic assay discs (Whatman, 6 mm) were inoculated with single drops of a dilute suspension of spores of Bacillus subtilis var globigii. The spores were suspended in distilled water and dispersed by a syringe-pipette (Annear, 1956), using drop volumes of 0.02 ml. The discs were dried at 56°C for 24 hours. Counts of the inoculum were made by delivering single drops into 20-ml volumes of 1/4 strength broth (Oxoid Nutrient Broth No. 2). Four drops (0.1 ml) of this suspension were spread on the surface of dried nutrient agar plates (Oxoid Blood Agar Base No. 2) for viable counts.

The spores were recovered from a disc by shaking in 2 ml of eluent (1/4 strength broth) in a 20-ml screw-capped bottle together with about 10 small beads (3 mm diam). Vigorous shaking for 15 seconds or less resulted in maceration of the disc into a homogenous suspension of fine fibres. The volume was then made up to 20 ml, and within a minute or two the fibres had sedimented, leaving clear supernatant fluid. Counts were made from this fluid as for the inoculum. All plates were incubated at 37°C overnight.

The results from duplicate plates of three sets of counts were as follows:

Inoculum (1) 92, 62 (2) 71, 92 (3) 69, 94
Total = 480
Discs (1) 72, 66 (2) 68, 76 (3) 89, 87
Total = 458

Analysis of these figures reveals no significant difference between the inoculum and recovery ($x^2 = 0.52, P = 0.45$); complete release and recovery of the spores has thus been demonstrated.

Similar results were obtained from heavier inocula (100 × that reported above) of the same organism and also for heavy inocula of spores of Bacillus stearothermophilus.

The factors, apart from shaking, that facilitate the ready liberation of the spores from the paper are the disparity of the container and eluent volumes during shaking, the use of beads, and, possibly, the surface active properties of the eluent.

Microscopy may indicate organisms adhering to fibres, but the results here show that the adhesion is not a firm one and that the spores are homogeneously distributed throughout the eluting fluid and fine fibres after shaking.

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References


A non-specific agglutination problem in the rubella haemagglutination-inhibition test using trypsinised human red cells

The use of trypsinised human red blood cells in testing for rubella antibodies by haemagglutination-inhibition (HI) was reported by Biddle (1971), and subsequently their advantages over day-old chick cells were emphasised (Quirin et al., 1972). Both these reports advocated the use of group O Rh-negative cells to avoid agglutination of the cells by anti-D, the predominant immune rhesus antibody at that time.

During the past four years we have used trypsinised group O Rh-negative cells for rubella HI tests by the method described by Grist et al. (1974). Of 20000 antenatal sera tested, one showed non-specific agglutination of the trypsinised group O Rh-negative cells, thereby masking the presence of any rubella HI antibody. After adsorption of the treated serum with 10% trypsinised group O Rh-negative cells at 37°C for one hour, the supernatant still contained the non-specific agglutinin; a further adsorption also failed to remove the agglutinin. Failure to adsorb this agglutinin suggested that it might be an immune rhesus antibody reacting with the Rh-negative cells, possibly anti-C. The haematology department confirmed that this woman's serum did contain anti-C detectable by enzyme and indirect anti-human globulin techniques. To obtain a valid rubella HI test result it was therefore necessary to use cells compatible with the patient's serum or to use chick cells, the latter being uneconomic for only one test.

Screening of the group O Rh-positive blood stock was carried out by the haematology staff, and a compatible cell type group O RrRr was selected. A portion of this pilot tube blood was trypsinised and used in a rubella HI test on the patient's serum. The rubella HI antibody titre was found to be 32 with no non-specific agglutination of the red cells.

It seems therefore that when antibodies other than anti-D cause non-specific agglutination, which cannot be removed by adsorption with the test cells, the use of cells compatible with the patient's serum may solve the problem. Also, since the rhesus isoimmunisation scheme has effected a marked decrease in the incidence of anti-D it seems probable that in rubella HI tests Rh-positive human red blood cells may now be as free of non-specific agglutination as Rh-negative cells.

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References


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