Technical method

Fig. 3  Modified apparatus: (1) Pump, (2) Internal trap, (3) Modified boss, (4) Cannula, (5) 4-way connector (converted to 3-way), (6) External water trap, (7) Intermittent interruptor valve ("breathing"), (8) Air control valve, (9) Formaldehyde 36% solution, (10) Vent, (11) Heater, (12) Lid seal, (13) Lid handle, (14) Plug.

Fig. 4  Original circulation (key as in Fig. 3 except: (3) Unmodified boss, (5) 4-way connector.

increases available ventilatory capacity, effort, and reserve. The pump, in this circulation, has a considerable excess of capacity, thus facilitating inflation and fixation. Many lungs in which there is increased rigidity or which are otherwise pathological can be satisfactorily inflated and fixed. Relatively normal lungs are satisfactorily fixed in approximately 3½ hours at operational temperature. Siting the apparatus in a well ventilated room during normal operation seems unnecessary, as fume production is negligible. Fixation is entirely satisfactory for normal histological procedures.

References


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Letters to the Editor

A rapid method for identification of surface antigens on fixed cells using monoclonal antibodies

A relatively large number of cells is necessary for the identification of surface antigens using live cell staining. The following method has been developed to allow testing with multiple antibodies in cases where only a small number of cells has been obtained and also to allow testing of imprints from lymph nodes, trephine biopsies etc, especially where efforts to obtain cell suspensions have failed or resulted in loss of the cell population one most wishes to examine.

Material and methods

Cytocentrifuge preparations of live cell suspensions from peripheral blood or tissues, or imprints from tissues, are either processed immediately or wrapped in tinfoil and stored at −20°C to −70°C. The latter samples are allowed to equilibrate with room temperature before the foil is removed. In the case of imprints the area to be tested is chosen by examining a Leishman stained consecutive imprint and marked with a diamond. An indirect
antibody method is used and the stages are as follows:

1. Slides are fixed in Baker's formalin for 4 min and washed in phosphate-buffered saline (PBS).
2. Monoclonal antibody (15 μl) is applied to a 5 mm area, and the slides are left at room temperature in a humid chamber for 30 min.
3. They are washed in four changes of PBS over a period of 20 min.
4. Fluorescein isothiocyanate-labelled goat antimouse IgG (15 μl) (Meloy Laboratories) is applied and incubated as before for 30 min. A duplicate slide, to which primary antiserum has not been applied, is similarly treated.
5. Washing is repeated as in stage 3.
6. Slides are mounted in Bacto FA mounting fluid pH 7-2 (Difco) and sealed with Glyceel (Searle Diagnostic).

The preparations are examined immediately using either a Leitz Ortholux I microscope with 200 W mercury lamp or a Leitz Ortholux II with 50 W mercury lamp, or can be stored at +4°C for a considerable length of time, at least two weeks, before examination.

Results and comment

Examples of our results are shown in Figures (a) and (b). A ring-like pattern of fluorescence is obtained at this magnification. Figure (a) is an OKT8-reacted cytopsin of peripheral blood lymphoid cells from a known case of T prolymphocytic leukaemia of suppressor type, whose lymphocytes had been kept in liquid nitrogen after her demise in 1979. Live cell staining showed 58% of cells to be positive with OKT8 while only 2-5% of cells were positive with OKT4. Figure (b) is an OKT8-reacted imprint from a lymph node “replaced” by a diffuse lymphocytic lymphoma of Mx immunoglobulin type showing positive suppressor T cells scattered through the negative larger lymphoma cells.

We have found this method satisfactory with the following antibodies: OKT3, OKT4, OKT6, OKT8, OKIal (Ortho Diagnostic Systems) and with anti J5 or CALLA which detects the common acute lymphoblastic leukaemia antigen (Coulter Clone). It is easy and quick to perform and the results are rapidly and reliably interpretable. In addition to its diagnostic advantages, the use of this method allows a panel of slides to be kept at −70°C and therefore readily available for control of future tests.

Letters to the Editor

(a) OKT8 reacted cytopsin from case of suppressor T. PLL. × 800.

(b) OKT8 reacted imprint from diffuse lymphocytic lymphoma. × 800.

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Faecal carriage of group B streptococci

The results of a study conducted at this laboratory show that, contrary to the findings of Islam and Thomas, only type II, but a wide range of serotypes of group B streptococci (GBS) can be isolated from adult faeces. These include types I and III which are the serotypes most commonly implicated in neonatal infection.

Routine faecal samples (175) were studied. The first 94 specimens were homogenised in glycerol broth and stored at −20°C for up to three months, while the rest were dealt with immediately. A faecal suspension was made of 1 g of stool in 9 ml of glycerol broth or Ringer’s solution (1/10). Two further tenfold dilutions were made in Ringer’s solution (1/100 and 1/1000) and 200 μl volumes of these served as inocula for surface viable counts. Two media were used: Islam’s starch serum agar and blood agar (Columbia agar base + 5% horse blood), both made selective by the addition of nalidixic acid 7.5 mg/l, polymyxin B 17 000 U/l and neomycin 2-125 mg/l (Oxoid Streptococcus Selective Supplement). In addition 1 ml of the initial faecal suspension was inoculated into Todd Hewitt Broth (BBL) containing the same antibiotic supplement, for enrichment culture, and after overnight incubation at 37°C this was subcultured on to the same media. All plates were incubated overnight anaerobically at 37°C using the Gaspak system (BBL). Orange colonies on the Islam’s medium and β-haemolytic colonies on blood agar were serogrouped by coagglutination with the Phadebact system (Pharmacia). All isolates were confirmed as GBS and serotyped at the Streptococcus Reference Unit, Colindale.

Out of a total of 17 GBS isolated, 16 were detected by enrichment as compared to only 10 by direct culture. Viable counts in these 10 samples were in the range 4 × 10⁹ to 2.5 × 10¹⁰/g. Seven specimens however, had counts of < 10⁹/g. The quantitative technique could not detect counts below 5 × 10⁸/g which suggests that in the seven carriers detected by enrichment alone viable counts were below this level. The overall isolation rate of 9-7% (Table 1) is comparable with previous studies: Islam and Thomas (4%), and Easmon et al (6%), although there was a much higher isolation rate from fresh samples (16%) as compared to frozen ones (4-3%). The numbers involved were too small for statistical significance, but it is possible that freezing may have reduced the recovery rate of GBS, especially as viable counts tended to be low.

A wide range of serotypes were detected (Table 2). Only two out of 15 isolates from adult faeces were type II strains, whereas 10 were either type I or III. The only other
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