Design and Construction of an Apparatus for the Growth of Micro Cell Cultures on Standard Glass Microscope Slides and Its Application for Screening Large Numbers of Sera by the Indirect Fluorescent Antibody Technique

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A simple stainless-steel apparatus was designed to contain standard microscope slides on which were grown micro cell cultures in the form of 16 individual monolayers per slide. The application of this apparatus for the screening of serum samples by fluorescent antibody techniques is described.

This paper describes a simple apparatus (U.K. patent application no. 54438/74; patent rights assigned to the National Research Development Corp., London, England) that was designed to facilitate the screening by the indirect fluorescent antibody technique of a large number of sera from cattle, sheep, and goats in Cyprus for evidence of infection with blue tongue virus.

MATERIALS AND METHODS

Construction of apparatus. The top and base plate of the apparatus (Fig. 1 and 2) were cut from 6.35-mm-thick stainless steel (British Standards Specification type EN58J) and machined to size 161.9 by 92.07 mm. Both the top and bottom plates were lapped separately and then lapped together to obtain a match between the contact surfaces. The base plate was made to accommodate five microscope slides (only one is illustrated; C. E. Payne & Son Ltd., London), 76 by 25 by 0.8 to 1.0 mm. The microscope slides were held in place by a brass template (gauge 24, 0.5 mm thick), which was located on the base plate. The base plate was drilled and tapped (International Organization for Standardization metric coarse M6 [6-mm diameter thread]) in four positions for locating studs. The studs, 41.3 mm long, were cut from stainless-steel studding (ISO metric coarse M6). These were screwed into the base plate and fixed into position with a Loctite stud lock (Douglas Kane Group Ltd., Swallow Fields, Welwyn Garden City, Hertfordshire). Twenty holes, 8 mm in diameter, were drilled at various positions on the base plate to allow for examination of the growing cell monolayers. These holes were countersunk at 60° by 12.7 mm in diameter.

Eighty 6.35-mm-diameter holes were drilled through the top plate to give 10 rows of eight holes, with two rows of 16 holes per slide. The holes were set at equal distances apart; the pitch of the holes

was 9.53 mm.

The gasket was cut from a sheet of silicone rubber (Esco Rubber Ltd., London; quality TC 156/4) to the same size as the top and base plate. The holes in the silicone rubber gasket were cut with a cork borer, 6.35 mm in diameter, using the top plate as the template.

Knurled thumbscrews with 19.05-mm-diameter head and a turned shank of 12 mm in diameter, 25 mm long, were machined from a stainless-steel bar (BS spec. EN 58 J).

Assembly of the apparatus. The microscope slides were washed in a detergent (Solgon; Tennants Ltd., Waltham Abbey, Herts), a water softener (Calgon; Robert Womersley & Son, Partridge Green, Sussex), and finally three times in distilled water. Slides that were not used immediately were stored in distilled water. The slides were placed on the base plate in the positions determined by the brass template. The silicone rubber gasket which had previously been sprayed with a Teflon product, Fluoroglide (Chemplast Inc., Wayne, N.J.), was positioned over the slides. The top plate was located over the gasket. The top and bottom plates were sealed to the gasket by applying pressure fingertight, using the knurled thumbscrews. When so assembled, the holes in the top plate formed discrete wells over the glass microscope slides. It was in these wells that the cell cultures were grown. After assembly, the apparatus was sterilized at a temperature of 180 C for 1 h.

Operation of apparatus. Tissue culture cells, BHK-21 (3) or IB-RS-2 (1), were prepared in suspension at the required concentration (5×10^5 /ml). Blue tongue virus was added to the cell suspension at a concentration of 10^5 50% tissue culture infective doses/ml. The cell-virus suspension was added in a volume of 0.1 ml/well. The wells were sealed with Sellotape (Waygood and Peerless Ltd., Croydon, Surrey), and the apparatus was placed at 37 C in an ungassed incubator for the required incubation time. The extent of cytopathic effect in the mono-

FIG. 1. Exploded diagram of the micro cell culture apparatus. Key: 1, Top plate (stainless steel), 161.99 by 92.07 mm, showing 80 6.35-mm-diameter holes arranged in 10 rows of eight holes with two rows per slide. 2, Base plate (stainless steel), 161.9 by 92.07 mm, showing inspection holes for microscope examination of cell cultures. 3, Microscope slide (glass), 76 by 25 by 0.8 to 1.0 mm (only one shown in position). 4, Template (brass) located on the base plate with provision for five slides. 5, Studs (stainless steel), 41.3 mm long, locating the top plate and gasket. 6, Inspection holes, 8.0 mm in diameter, for the examination of the cell cultures. 7, Hole, 6.35 mm in diameter. There are 80; when the plate is assembled, each form discrete wells in which the cell monolayer is grown. 8, Gasket (silicon rubber), 161.9 by 92.07 mm, showing same pattern of holes as top plate. 9, Knurled thumbscrews (stainless steel), 19.05 mm in diameter, with a turned shank (12 mm in diameter by 25 mm long).

layer was monitored by microscope examination of the cultures through the inspection holes.

Fluorescent antibody procedure. The growth medium was removed from the wells, and the infected cell monolayers were washed and then fixed with acetone previously held at -20°C . The staining procedure of Pini et al. (4) was used. The reagents were withdrawn from the wells by the use of Pasteur pipettes and discarded into an appropriate disinfectant. On completion of the test the apparatus was dismantled, the slides were removed, and buffered glycerol was added to the monolayer surface. Each slide was covered with three cover slips (22 by 22

mm), and the microcultures were examined for specific fluorescence.

RESULTS

Micro cell cultures of both BHK-21 and IB-RS-2 were grown using this apparatus (Fig. 3). The apparatus was tested for leaks by (i) filling wells adjacent to others containing cell monolayers with vital stains and (ii) inoculating alternate cell cultures with virus and observing the noninoculated cultures for cytopathic effect. All the materials from which the apparatus

FIG. 2. Assembled micro cell culture apparatus.

FIG. 3. Microscope slide showing the 16 individual monolayers.

was constructed were nontoxic to the tissue culture cells tested and, with the exception of the sealing tape, were re-usable. Specific fluorescence was observed with both direct and indirect techniques. No cross contamination between positive and negative sera was detected by the fluorescent antibody test. This apparatus has been successfully used for other viruses, including foot-and-mouth disease, African swine fever, and epizootic hemorrhagic disease of deer.

DISCUSSION

The established method of growing cell monolayers on glass cover slips for use in fluorescent antibody studies with viruses is both time consuming and wasteful of materials. The use of microplates for diagnostic tests in virology has been widely described (5-7), and several authors (2, 8-10) have realized that the principle can be extended to include the fluorescent antibody test. The apparatus described in this paper was developed to refine the published methods and to permit the fixation and staining of the cell monolayer to be performed *in situ*. We consider that the apparatus is reliable for screening sera by the indirect fluorescent antibody technique for evidence of viral diseases. It may have other applications in microbiology such as parasitology, genetics, and examination of viruses that do not produce a cytopathic effect in tissue culture or where microscope examinations of replicate cell cultures are required.

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