sandwich, in the definition of criteria for judging positivity for a new isolate.

SUMMARY

A new technique is described which allows rapid and easy preparation of replicate tissue culture 'spots' on glass microslides. These can be of any type of tissue culture, contained in glass rings stuck to the microslide with paraffin wax-petroleum jelly mixture. The rings allow easy manipulation of the tissue culture and the presence of up to six replicate spots on a microslide greatly facilitates the identification of unknown virus isolates from tissue culture by the immunofluorescent technique. Rapid and constant reference can be made to control preparations on the same microslide by adjusting only the lateral stage movement controls of the fluorescence microscope.

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Improved optical equipment for immunofluorescence studies[1]

O. M. LIDWELL, C. E. D. TAYLOR, S. P. CLARK, AND G. V. HEIMER *From the Central Public Health Laboratory, Colindale Avenue, London*

Many kinds of optical equipment for immunofluorescence are commercially available. Some are very expensive and, as far as we are aware, most if not all of them require a dark-room for efficient operation. Moreover, when viewing fluorescent structures as small as single bacteria or virus-infected cells, the use of a binocular head on the microscope reduces the brilliance of the image to an unacceptable extent.

In view of these limitations we explored the possibility of designing and building equipment with a more intense source of radiation which should be compact and relatively inexpensive.

Although we considered other types of lamp, a very small mercury vapour arc of high intrinsic brilliance within a quartz envelope seemed to be the most promising. The lamp finally chosen was the HBO100 made by Siemens/Osram, or its near equivalent the PEK110 without starting electrode, manufactured by PEK Labs Inc., California, U.S.A. The main features of this last, taken from the maker's specifications, are as follows:

Lamp operating voltage DC (volts)	20 ± 4
Lamp rated power—DC operation—(watts)	100
Arc size (in.)	0·012 × 0·012 (0·3 × 0·3 mm.)
Average brightness (candlepower/cm.²)	140,000
Average life (hours)	100
Operating position	Horizontal or vertical
Cooling	Convection

[1]All enquiries regarding purchase of this equipment should be addressed to Messrs. Technical and Research Processes Ltd, 5 Buck Street, London, N.W.1, from whom further details including price are available.

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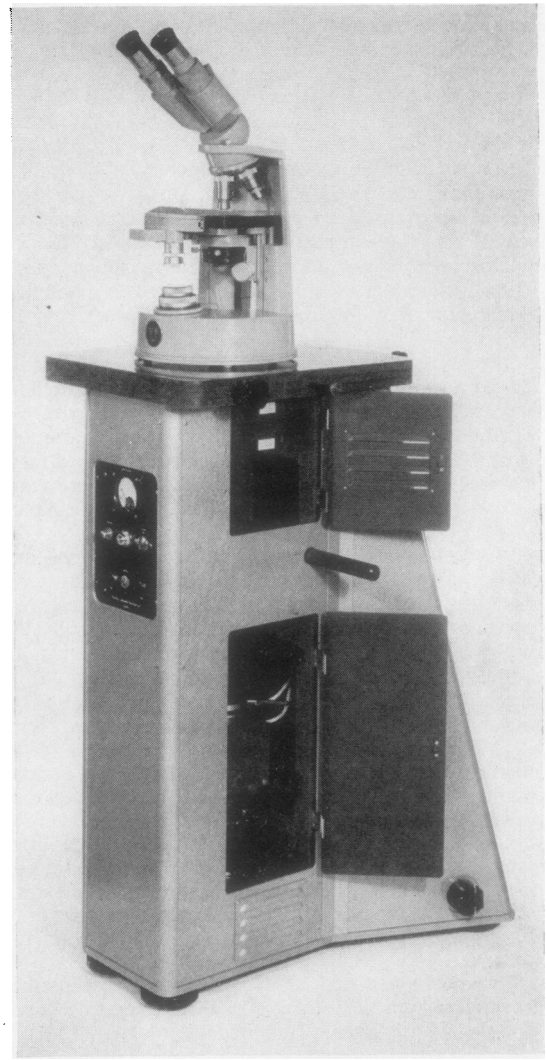

FIG. 1. *The equipment*

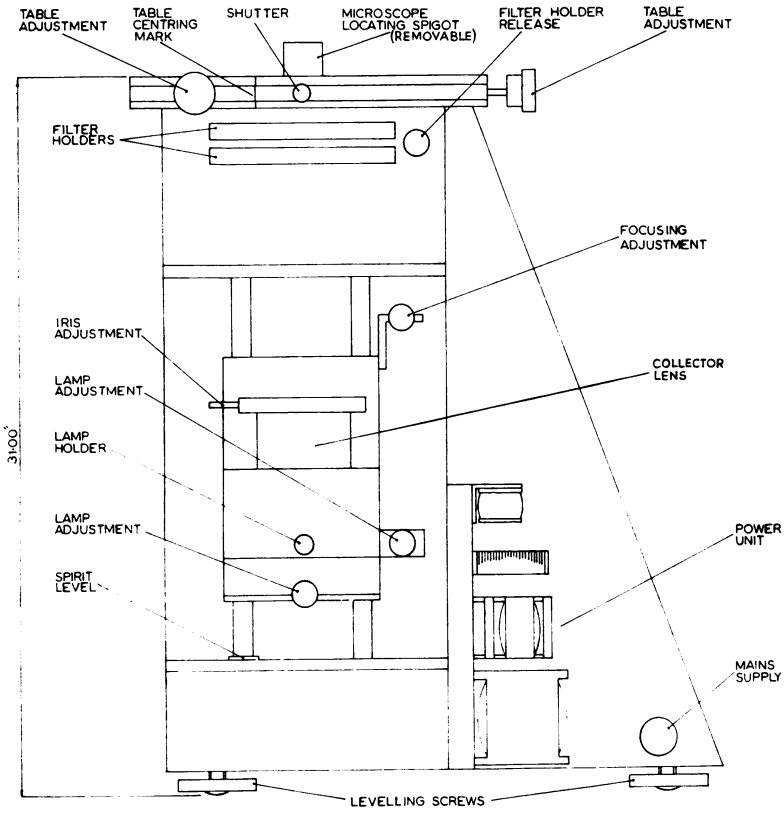

FIG. 2. *Drawing showing the position of the various components.*

From preliminary experiments, it was apparent that an optical bench built with a high degree of precision was required to ensure sufficiently accurate optical alignment when using such a small light source. The choice of collector lens also proved to be particularly critical. The work here has been done with a Carl Zeiss glass collector of focal length 33·8 mm. incorporating an iris diaphragm. The rear component of this is 36 mm. in diameter. When the necessary conditions were fulfilled the results were highly satisfactory.

The prototype equipment, designed to explore a variety of configurations, was large and cumbersome. For further development Messrs. Technical and Research Processes Ltd., who have had many years of experience manufacturing miniature multichannel recording equipment incorporating similar light sources, were approached with a view to producing a more compact and conveniently designed version of the experimental equipment. The result of the ensuing collaboration is illustrated in Figure 1. Figure 2 is an outline drawing showing the positions of the various components.

Essentially the apparatus consists of a pedestal 30 in. in height, on which can be placed any suitable microscope. The lamp, collector lens, exciter filters, and power unit are all contained in the pedestal, on the front of which is a small control panel for the operation of the lamp. The panel carries the main switch for the electrical supply, a striker button for starting, a control resistance to adjust and set the current for the individual lamp, and a meter which indicates the correct wattage. The optical components can all be adjusted through doors on the sides of the pedestal. A removable rear panel allows access to the power unit which can be removed readily if the need arises. Exciter filters, either singly or combined in pairs, can be selected by means of two rotatable discs each holding a maximum of five circular glass filters 32 mm. in diameter. The platform, on which the microscope rests, incorporates a shutter to intercept the light path through a central aperture as required and is movable in a horizontal plane in all directions, to simplify alignment of the optical axis of the microscope with the light beam. This procedure is assisted by temporarily removing the microscope and placing a plane mirror, reflecting surface downwards, over the aperture in the platform. With the diphragm on the collector lens almost closed, the position of the mercury vapour arc is then adjusted, by means of the fine controls provided, until the image of the light source is reflected back on to the centre of the diaphragm, *i.e.*, the upward and downward light paths coincide and are normal to the surface of the platform. The microscope is then placed in position and the mirror transferred to the specimen

stage. The image of the light source is now restored to the centre of the diaphragm, by adjusting the microscope axis so as to bring this normal to the platform. The light beam is finally centred by lateral adjustment of the platform.

The microscope illustrated is the Vickers Patholette, the hole in the centre of the base of which facilitates optical alignment. Using this or any other standard microscope fitted with a suitable dark ground substage condenser and binocular angled head, immunofluorescence of bacteria in clinical specimens as well as virus-infected cells in culture can be viewed in ordinary room lighting. As far as we are aware, no other equipment at present commercially available allows satisfactory viewing of immunofluorescence preparations of micro-organisms in ordinary room lighting and with a binocular head on the microscope.

Fading of fluorescent images occurs with all forms of exciting radiation. The more intense this is the faster does fading occur. With the HBO100 or PEK110 lamp used in the equipment described, the rate of fading of the fluorescence is not so rapid as to constitute a serious inconvenience. Using a 50× fluorite objective lens with a 12× eye-piece, adequate photographic exposure of black and white film of ASA rating 125 has been obtained in about 30 seconds.

Use of barium sulphate as a continuous marker for faeces

MATTHEW DICK *From the Metabolic Unit, University College Hospital, London*

The advantages of using continuous marking of faeces in metabolic balance studies is well established (Rose, 1964), the most common method being the chromium sesquioxide of Anderson and Weinbren (1961). This paper describes a new method using barium sulphate which requires no special apparatus or powerful and potentially dangerous oxidizing agents.

REAGENTS

ALKALINE E.D.T.A. NaOH 2·5%, E.D.T.A. (disodium salt) 5% in water

METHYL RED Saturated solution in 50% alcohol

AMMONIUM SULPHATE A 5% solution of $(NH_4)_2SO_4$ in 0·5 NHCl

METHODS

In our first trials, barium sulphate B.P. was administered in capsules, originally of 0·5 g. four times daily with the main meals (12 g. per six-day period). Later 8 g. per six-day period, spread out as before, was used as this quantity was more suitable for the capsule packing machine in the hospital pharmacy, use of which avoided the labour of filling by hand. $BaSO_4$ was given during the five-day equilibration period before the first carmine marker was taken, and continued until the last marker was passed. Collection of stools, homogenization, drying, and ashing were carried out in the normal manner, similar to that described by Rose (1964). The samples taken from the homogenate should be judged to contain about 100 to 300 mg. of $BaSO_4$.

The ashed sample in a silica beaker is dissolved in 10 ml. of 30% HCl with warming; to this 20 ml. of $(NH_4)_2SO_4$ solution is added and water to about 40 ml. The sample is mixed, allowed to stand for a few minutes, then the fluid (and fine $BaSO_4$) decanted into a 50 ml. centrifuge tube which is centrifuged at 3,000 r.p.m. for five minutes. The supernatant is discarded and the $BaSO_4$ returned to the original beaker by washing with water, 100 ml., alkaline E.D.T.A. added, and the beaker placed on a steam bath with occasional stirring until solution of the $BaSO_4$ is complete (about one hour). Meanwhile, 50 ml. alkaline E.D.T.A. is added to the centrifuge tube in order to dissolve any very fine $BaSO_4$ remaining.

The contents of the beaker are filtered through Whatman no. 54 paper into a conical flask, the E.D.T.A. solution in the centrifuge tube being then transferred to the beaker which is rewarmed on the steam bath for about 15 minutes, then filtered into the same flask and

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