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this direct assay quality control detect all of these errors. In addition, if the wrong antibiotic is put in a prelabelled plate, the error may be detected if the observed zone size and/or zone edge differ from those that were expected.

The spore suspension is particularly convenient, and it had been hoped that it could be used for the assay of all antibiotics except colistin. However, all three strains of *B. subtilis* tested were found to be resistant to tetracycline as well as colistin and alternative assay organisms were therefore required for these two antibiotics. *E. coli* was selected for the assay of colistin but it was not suitable for the assay of tetracycline owing to the production of very small hazy zones. *Staph. aureus* was therefore selected for the assay of tetracycline. Although agar plugs containing trimethoprim and nalidixic acid caused inhibition zones with *B. subtilis* as indicator organism, *E. coli* was preferred for these antibiotics because the inhibition zones were larger and clearer.

Agar-dilution sensitivity-testing appears to be less subject to error than diffusion methods, and, with the direct assay quality control described here, it achieves a very high degree of reliability.

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Demarcation of antigen preparations on object slides in the immunofluorescent antibody test

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Applications of the immunofluorescent antibody technique are essential in virology, bacteriology, parasitologic serology, and immunohistopathology¹⁻⁶. If background staining is not used the specimens in this technique are often colourless and invisible and therefore difficult to locate on object slides.⁷ To overcome this difficulty we undertook a search for suitable methods for routine and research purposes. To test our method we applied it to the immunofluorescent antibody test using six different antigen preparations.

The antigen preparations were bordered on object slides with rings of paint, 3-7 mm in diameter (Fig. 1a). The paint was a two-component, water-soluble, chloric caoutchouc paint, Akva Epirex (Teknos-Maalit, Takkatie 3, 00370 Helsinki 37, Finland) and it was applied on the slides using a thin-walled metal cylinder of appropriate diameter (Fig. 1b). Object slides, equipped with rings, were used in the investigation of antibody-coated bacteria, toxoplasma and herpes antibodies, and antinuclear and other autoantibodies. Furthermore, the method was utilised in the search for tissue-bound immune reactants in frozen sections of human renal biopsies.

Fig. 1(a) Object slide (2.5 × 7.6 cm) with rings. Bordered by the rings are cryostat sections of rat kidney, stained for photographic purposes with toluidine blue. (b) Application of the rings by labelling with a thin-walled metal cylinder.

Received for publication 14 August 1979

In the preparation of toxoplasma antigen a modification of our own method was used.⁸

The height of the rings depended on the concentration of the paint and on the thickness of the wall of the cylinder used for their application. The paint dried in 12 hours after application. When flat rings were used, it was possible to place cover-slips on the slides. The rings were never seen to produce autofluorescence or to interfere with the fluorescence in the antigen. Once dried, the paint was completely non-toxic, making possible the raising of tissue cultures inside the rings. The rings were stable in absolute acetone, ethanol, ethanol-ether (1:1), methanol, and formaldehyde for at least 2 hours. They detached, in contrast, in absolute ether and in boiling water. The rings also resisted a dry atmosphere at 120°C for at least 2 hours or could be stored at -70°C for at least 12 months.

The reagents used in the immunofluorescent technique are expensive. To minimise their consumption and to be able to locate the antigen preparations on object slides these preparations are usually demarcated using rings made by a diamond marker or glass pencil. Object slides commercially available for this purpose are coated with paint or Teflon, leaving uncoated spots for the application of the antigen preparation. The disadvantage of these slides has been their high price and the instability of the rings or the coating. They detach easily in fixative solutions or they are difficult to prepare. The method described here is simple and inexpensive. It can be used in several applications of the fluorescent antibody test utilising various antigen preparations and concentrated fixatives.

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