NOTES

Template Method for the Fluorescent-Antibody Technique

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An easily constructed and inexpensive template has been developed, which enables the fluorescent-antibody technique to be applied to serial dilutions and allows 18 assays on a single microscope slide.

For serological tests using the fluorescent-antibody method there is frequently a need to determine antibody titers. This is generally carried out in tubes, with the serum dilutions being transferred to the appropriate antigen on the microscope slides using a dropping-pipette technique, which is tedious, cumbersome, and time consuming. A method has been developed which enables the fluorescent-antibody method to be applied to serial dilutions with ease, and allows many assays to be done on a single microscope slide.

The method requires an easily constructed and inexpensive template consisting of a Plexiglas block in which three rows of six stainless-steel rods are embedded (Fig. 1). The Plexiglas block measures 60 by 40 by 10 mm. Each steel rod has a 3-mm diameter and protrudes 14 mm from the Plexiglas. The centers of the rods are 9 mm apart, which allows them to fit into the wells of a microtiter plate (Cooke Engineering Co., Alexandria, Va.). This spacing of the rods also allows the template to fit within a 65- by 25-mm microscope slide.

FIG. 1. Template in use with microtiter plate and 18-chamber slide.

The template can be used in four steps of the indirect fluorescent-antibody technique. First it allows preparations of slides with 18 assay chambers. For this, the template is dipped into glycerol and the drops are transferred to a microscope slide, which is then coated with a water-repellent substance such as Teflon. Upon washing the slide, the glycerol is removed, leaving a pattern of 18 clear spots on the slide. Having prepared the slide, 18 antigen smears can be made using the same template. For this, the antigen is prepared in a microtiter plate, picked up with the template and deposited on the slide; due to the uniform dimension of each rod, a constant volume of the antigen suspension is deposited in each spot. Third, serum dilutions prepared in microtiter plates can now be transferred using the same template, again allowing constant volumes for each spot. In the last stage, conjugated antiserum can be transferred to the slide in the same manner. In all cases, the volume of liquid in the microtiter

wells is the 25 μ l normally recommended using this system of microtitrations. The volume of liquid transferred by the template from the microtiter plate to the slide is $4.9 \pm 1.3 \mu$ l.

The method was developed and has now been successfully used in two large studies to determine *Toxoplasma* indirect fluorescent-antibody titrations. The method has been compared with the methylene blue dye test, the complement fixation test, and the direct agglutination test, and the titers obtained with the template method show a good correlation with antibody titers obtained by the other techniques (1). The technique could be used with equal ease for immunofluorescent-antibody techniques using antigens such as *Neisseria gonorrhoeae*, *Salmonella*, and *Escherichia*.

LITERATURE CITED

1. Karim, K. A., and G. B. Ludlam. 1975. The relationship and significance of antibody titres as determined by various serological methods in glandular and ocular toxoplasmosis. *J. Clin. Pathol.* 28:42-49.