Development of a Template for Use in Immunofluorescent Identification of Mycoplasmas

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A template for securing blocks of agar onto glass slides is described. Use of the template allows efficient handling as well as thorough washing of agar blocks during the immunofluorescence staining process.

The fluorescent-antibody technique remains the most reliable diagnostic tool for the identification of *Mycoplasma* isolates. Both direct and indirect procedures (7, 11) have found wide application in the species characterization of isolates from bovine (1), caprine (13), avian (9), porcine (8), and human (12) sources.

The use of immunofluorescence in the identification of mycoplasmas was initially applied to colonies fixed on glass for transmitted light illumination (5, 10). With the subsequent introduction of "epi" or incident light illumination (6), several different modifications have been described to identify mixed cultures and cloned isolates (4, 13) directly on the surface of the agar medium. Immune staining of *Mycoplasma* colonies on whole agar plates (3, 6) necessitates the use of large quantities of reagents. This disadvantage has been obviated by staining colonies in situ on blocks of agar fixed onto glass slides with wax (1) or by lucite cylinders (2). However, these procedures share similar disadvantages in that the agar blocks frequently become dislodged from the slide during the process of washing and other manipulations that are imperative in immunofluorescence staining procedures.

In this communication, we describe a template made of Plexiglas that serves to hold the blocks of agar firmly onto the glass slide throughout the staining process. The Plexiglas template (Fig. 1A) is 5.5 cm long, 2.5 cm wide, and 2 mm thick and fits exactly over the transparent surface of a standard frosted microscope slide. Each template bears eight square slots. The rim on the lower surface of each slot is 7 mm from side to side and tapers to provide a rim of 6 mm on the top surface. This arrangement provides that blocks of agar become firmly lodged between the glass slide and template.

For indirect immunofluorescence, several bovine strains of mycoplasmas were grown on heart infusion agar (11) supplemented with 20% swine serum. After incubation for 2 days at 37°C in an

atmosphere of 10% CO₂, the plates were examined under a stereoscopic microscope. Areas with discrete colonies were demarcated, and blocks of agar with colonies were removed by cutting with a sterile scalpel blade (Sterisharps 11). The blocks were placed on a microscope slide and trimmed to form an angle on the sides. The blocks should be approximately 6 by 6 mm on the top surface, with a slightly wider base (Fig. 1B). After the blocks of agar were positioned on the slide, the template was fitted with the slots on the template corresponding to each of the agar blocks, thus providing firm positioning of the agar blocks on the slide. The template was fastened onto the slide with a few wraps of adhesive tape (Fig. 1C).

The slides were positioned on a horizontal slide tray (four slides per tray) and placed in a glass staining dish with a magnetic stirrer (3.5 cm) at the bottom. To provide free movement of the magnetic stirrer, the slide tray was kept slightly elevated by means of a glass rim at the bottom of the staining dish. Magnetic stirring was maintained at high speed during all of the washing procedures.

The blocks were initially washed with phosphate buffer, pH 7.2, for 20 to 30 min. The slides were removed and the excess buffer was mopped by gently touching the rim of each block with a clean blotting paper. A drop of antiserum was placed on the surface of each block, and the slides were placed in a moist chamber for 30 min. The slides were washed twice for 10 min in fresh phosphate buffer and dried as before. A drop of fluorescein isothiocyanate-conjugated globulin was placed on each block, and the slides were placed in the moist chamber for a further 30 min. The slides were washed in two changes of phosphate buffer for 10 min and dried. To eliminate background fluorescence and provide contrast, the blocks were counterstained with a drop of 0.001% Evans blue for 30 s and washed once in fresh phosphate buffer for 5 to 10 min.

FIG. 1. (A) Plexiglas template. (B) Glass microscope slide with blocks of agar in position. (C) Plexiglas template fastened onto the slide with a few wraps of adhesive tape.

The adhesive tape was removed while maintaining the template in position with the agar blocks within each slot. The stained preparations were examined under a Zeiss Universal microscope (4).

In our studies on mycoplasmosis, the fixation

of blocks of agar onto glass slides has been accomplished with a high degree of success by using the Plexiglas template. Besides economical usage of reagents, the technique allows secure fixation of agar blocks throughout the entire staining procedure despite the turbulence created by high-speed gyration of the magnetic stirrer. This permits a rigorous washing procedure, sufficient for elimination of nonspecific fluorescence.

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