NOTES

Simple Method for Immunofluorescent Identification of Mycoplasma Colonies

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General purpose rubber stoppers proved to be a simple, rapid, and satisfactory method of transferring mycoplasma colonies from agar to microscope slides for immunofluorescence.

A number of techniques have been used for examining mycoplasma colonies by immunofluorescence. For instance, colonies may be stained in situ on the agar medium and viewed by incident ultraviolet light (2, 5). This is of value in diagnosis because primary isolates can be stained, but it is extravagant on reagents unless extra time and care is taken to cut and handle colony-bearing agar blocks (1, 10). Background fluorescence can also be a problem, and attempts to circumvent this have included growth and staining of colonies on cellulose acetate membranes (7) or on microscope slides thinly overlaid with agar medium (8).

An alternative way of examining colonies by immunofluorescence is to stain colony impressions on a microscope slide, which may then be viewed with incident or transmitted light. The hot-water fixation technique (4) is commonly used to prepare impressions, although it has been reported to result in reduced specificity of immunofluorescence (9, 10). The method involves exposure of the organisms to a temperature of 80 to 85 C for 30 s or more and this may damage the heat-sensitive antigens (3), leaving only a portion of the colony reactive (10, 11).

This report describes a simple and rapid way of preparing colony impressions in which a rubber stopper is used to transfer antigenic material directly from agar to microscope slide. Fixation and staining can be carried out as desired.

In these studies, general purpose red rubber stoppers (10 mm in diameter) were used. They were washed, boiled in distilled water, and dried in air before use. After use they were soaked in iodophor disinfectant prior to reuse. For colony transfer the bottom of the stopper was gently applied to the selected colonies and then carefully lifted and immediately applied to a microscope slide (Fig. 1-3). Application of

pressure to the stopper was unnecessary, although it was possible to make two or three impressions from one stopper by gradually increasing the pressure for each successive one. The colony impressions were fixed by passing through a luminous flame for two periods of approximately 0.5 s. This short exposure to gentle heat did not appear to damage the antigens.

For indirect immunofluorescence, colonies of each avian mycoplasma serotype and of *Acholeplasma laidlawii* were grown on conventional mycoplasma agar containing 15% swine serum. The type strain (6) or proposed type strain (proposed by the Food and Agriculture Organization/World Health Organization Team for the characterization of avian mycoplasmas) of the organism was used in each case. Tests were conducted with chicken antisera, followed by a horse anti-chicken fluorescein isothiocyanate conjugate (Flow Laboratories, Scotland). Chicken antisera to each serotype were tested against colony impressions of every serotype, and bright fluorescence was obtained with each homologous system. Nonspecific reactions or cross-reactions were not encountered.

For the direct test, antisera to avian serotypes A and S, prepared in rabbits and conjugated with fluorescein isothiocyanate, were used to stain colony impressions of pure and mixed cultures of these serotypes. Bright specific fluorescence was obtained, and mixed cultures were readily recognized (Fig. 4).

The rubber stopper transfer method was compared with the hot-water fixation technique as originally described (4) and as modified (10). It was also compared with the staining of unfixed colonies on agar blocks (10). Transfer of colonies by rubber stopper was considerably simpler and faster than the other methods and in

FIG. 1. Mixed colony impressions of *Mycoplasma gallisepticum* and *M. synoviae* stained with a rabbit anti-*M. gallisepticum* conjugate. $\times 150$.

FIG. 2. Colonies of *M. gallisepticum* on an agar plate (arrows indicate same group of colonies here and in Fig. 3 and 4). $\times 9$.

FIG. 3. Colonies of *M. gallisepticum* on a rubber stopper. $\times 9$.

FIG. 4. Colony impressions of *M. gallisepticum* on a microscope slide. $\times 10$.

our hands yielded better results. All hot-water-fixed colonies gave poor specific fluorescence in comparison. With the agar blocks background fluorescence was a problem, but this did not occur with colony impressions stained with the same reagents.

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