A SIMPLE METHOD FOR STAINING THE CAPSULES OF BACTERIA

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The possession of mucinous envelopes by certain bacteria, apart from their purely morphological interest, has a great value in the identification of such organisms and many methods have been devised for their demonstration. The number of such methods however argues against any one being completely satisfactory.

The introduction of a new technique into this already overcrowded field can be justified only if it offers distinct advantages over any or all of the older methods. The staining of capsules as a rule, particularly in inexperienced hands, is time consuming and attended by many failures, and a new method to be acceptable should as far as possible eliminate those factors which experience has shown to be the most general cause of failure.

These factors may be the uncertain or variable quality of the diluent employed, the impossibility of giving absolute directions as to the amount of heat required in fixation or in staining, or the use of fixing agents, such as strong chemicals, that in themselves tend to shrink the capsule.

The search for a method that would overcome these difficulties led to the employment of an artificial menstruum prepared from Nutrose\(^1\) (as directed below), the abandonment of heat as a fixing agent and as an aid in staining, and the use of weak solu-

\(^1\) Nutrose (sodium caseinate) is a proprietary food preparation made in Germany and, as under present conditions it may be unobtainable, a fairly efficient substitute for the 3 per cent Nutrose solution may be made from milk by the following procedure: Render milk as nearly fat free as possible by means of centrifugation, add 1 per cent of 2 N sodium hydrate and bring to a boil, after cooling add ether and shake. After a few minutes decant off the ether. The remaining opalescent fluid can then be employed in place of the Nutrose solution.
tions of lactic acid as precipitating agents. Thin films made from an emulsion of organisms in 3 per cent Nutrose solution are precipitated immediately by dilutions of lactic acid of a strength of 0.1 of 1 per cent causing a perfect fixation of the organisms and capsules.

Experience has shown that 0.25 to 0.5 of 1 per cent of lactic acid gives the best results, that it is perfectly feasible to introduce the dye employed directly into the fixing solution, thus simplifying the technique, and that the addition of acetic acid in a strength of 1–10,000 improves the definition.

*Method of preparing the reagents employed*

*Solution 1.* To be used as a diluent.

Three grams of Nutrose are sifted into 100 cc. of distilled water and heated to 100°C. in the Arnold sterilizer for one hour.

Add 5 cc. of 2 per cent aqueous solution of carbolic acid to serve as a preservative. Decant into test tubes and allow to settle.

Employ the supernatant fluid as the diluent.

(Since the supernatant fluid tends to become thinner by constant precipitation of the Nutrose the solution should occasionally be reboiled.)

*Solution 2.* Fixing and staining solution.

2 per cent aqueous solution of carbolic acid, 100 cc.
Concentrated lactic acid, 0.25–0.5 cc.
1 per cent acetic acid, 1 cc.
Saturated alcoholic solution of basic fuchsin, 1 cc.
Carbol-fuchsin (old), 1 cc.

(This solution must be kept tightly corked, and then keeps very well.)

Experience has shown that in the above solution the addition of old and fresh fuchsin as given makes a better product than an increase in the amount of either one alone.

*Technique of staining*

1. Employ the Nutrose solution (no. 1) as a diluent, emulsifying the bacteria in one or two loopfuls and then spreading in as thin a film as possible with the loop. The use of the edge of a slide in spreading the film, as in blood work, is not to be recommended.
2. Allow to dry in air.
3. Cover the film with the fixative and staining solution (no. 2) and allow to act for thirty to forty-five seconds.
4. Wash quickly in water, dry and examine.
These steps take about one and half minutes.

In examining the films the best pictures are usually found in the thinnest portions near the edges.

The use of fuchsin as a dye has proven to be generally satisfactory for a routine stain but any of the more diffuse basic dyes may be employed. Methyl violet added in 1 per cent of a saturated alcoholic solution gives probably the best pictures due to the failure of the Nutrose film to stain, the organisms appearing as blue black bodies surrounded by delicate violet capsules. This dye, however, tends to precipitate out of the mixture in a few days and the preparations are apt to fade.

The above method has been in use in this laboratory for three years and has been used in the class room during this period with very satisfactory results, being taught in conjunction with one of the older methods so that a comparison of results on a very large number of preparations was possible. It rapidly became the method of choice by students due to the simplicity of the technique and the very high percentage of successful results secured. The results of preparations made by this method are shown in figures 1 to 6.

Since the presence of a demonstrable capsule has such differential value, a method of this kind to be acceptable must fail to show such a structure on those organisms which are commonly held to be non-capsulated.

In the course of an investigation along another line this method has been tried on streptococci, staphylococci, members of the Gram negative coccus group, and many flagellate bacteria and has in no instance shown a capsule on these organisms although a similar structure may be demonstrated on all by means of a special technique which will be the subject of a subsequent report.

The advantages claimed for the above method of capsule staining are:

1. Standardization of the reagents employed.
2. The elimination of personal judgment as a factor by precise instructions as to the various steps.
3. Simplicity of technique.
4. Reliability of results.
FIG. 1. Pneumococcus
FIG. 2. Streptococcus mucosus
FIG. 3. Pneumobacillus of Friedländer and Bacillus pyocyaneus
FIG. 4. Bacillus aerogenes
FIG. 5. Bacillus aerogenes
FIG. 6. Micrococcus tetragenus showing the indefinite outlines of the gelatinous envelope

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