TECHNICAL METHODS

A Simple and Rapid Technique for the Identification of *Proteus-Providence* Strains

U. BACHRACH

*From the Department of Clinical Microbiology,
Hebrew University—Hadassah Medical School,
Jerusalem, Israel*

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In a previous communication it was proposed to use a filter paper strip impregnated with urea for the detection of urease activity of *Proteus* strains (Bachrach and Wormser, 1959). Urease activity is not limited to *Proteus* strains, for other bacteria, such as *Corynebacterium pseudodiphtheriticum* (cf. Breed, Murray, and Smith, 1957) and some *Klebsiella* strains, are also active (Epstein, 1959). On the other hand, *Providence* strains, which are included in the genus *Proteus*, are urease negative (Shaw and Clarke, 1955). The urease test is therefore not a reliable method for the identification of bacteria belonging to the *Proteus-Providence* group.

An additional test has been added to our filter paper strip technique to make it a reliable method for the identification of bacteria belonging to the *Proteus-Providence* group. The additional test is based on the well-known fact that these strains rapidly deaminate tryptophan to indolepyruvic acid, which can be detected by ferric chloride (Singer and Volcani, 1955; Falkow, 1957).

Reagents

Impregnation Solution.—Dissolve 1.0 g. urea, 0.1 g. 1-tryptophan, and 0.1 g. phenolphthalein in 10 ml. 96% ethyl alcohol.

Saline Solution.—Make up 8.5 g. sodium chloride in 100 ml. distilled water.

Dilute Hydrochloric Acid Solution.—Add 1 ml. concentrated hydrochloric acid to 100 ml. distilled water.

Sodium Hydroxide Solution.—Add 0.4 g. sodium hydroxide to 100 ml. distilled water.

Hydrochloric Acid Solution (50%).—Add 50 ml. concentrated hydrochloric acid to 50 ml. distilled water.

Ferric Chloride Solution.—Dissolve 10 g. ferric chloride in 100 ml. distilled water.

Technique

Sodium hydroxide solution is added drop by drop to the impregnation solution until the indicator turns pink. Diluted hydrochloric acid is then added till the

solution becomes just colourless. Whatman No. 3 filter paper is soaked with the colourless impregnation solution, dried at room temperature, and cut into strips (15 by 5 mm.) which are kept in a dry test-tube.

The bacterial strain to be tested is grown on a liquid or solid medium. If a liquid medium is used, the cells are harvested by centrifugation (2,500 r.p.m.) and the supernatant discarded. Bacteria grown on solid media are scraped off with a bacteriological needle and suspended in 0.1 ml. saline solution in a small test-tube (9 by 100 mm.). After immersing the impregnated filter paper strip in the bacterial suspension, the test-tube is placed in a 37° C. water-bath and examined after one to two hours. A red coloration of the filter paper strip indicates the presence of urease in the bacterial cells.

The presence of tryptophan deaminase activity is demonstrated by the addition of one drop of the 50% hydrochloric acid solution and one drop of the ferric chloride solution to the filter paper still immersed in the bacterial suspension. A brown colour appears within two minutes, when the result is positive.

Results

Various *Enterobacteriaceae* were examined by our modified filter paper strip technique. Only *Proteus* and *Providence* strains deaminated tryptophan (Table I). Other bacteria, such as *Shigellae*, *Salmonellae*,

TABLE I
BEHAVIOUR OF VARIOUS BACTERIA EXAMINED BY
FILTER PAPER STRIP TECHNIQUE

Organism	No. of Strains	Filter Paper Test			
		Urease		Tryptophan Deaminase	
		Positive	Negative	Positive	Negative
<i>Proteus</i>	32	32		32	
<i>Providence</i> .. .	20		20	20	
<i>Salmonella</i> .. .	23		23		23
<i>Shigella</i>	22		22		22
<i>Escherichia</i> .. .	20		20		20
<i>Klebsiella</i>	5	1	4		5
<i>Bethesda-Ballerup</i>	13		13		13

Escherichiae, and *Klebsiellae*, gave negative results. As was to be expected, *Proteus* strains were urease positive when tested by the filter paper strip technique, whereas bacteria of the *Providence* group were negative. One of the *Klebsiella* strains examined was urease positive (Table I).

Discussion

The development of a technique demonstrating both tryptophan deaminase and urease activities in one test is thus of great help in the identification of *Proteus-Providence* strains.

As has already been pointed out, bacteria grown on various culture media may be employed in the filter paper strip test, which has the advantage of being simple and rapid (Bachrach and Wormser, 1959). The test is very sensitive, and pure culture is not required. Furthermore, the filter paper strip, which is easily prepared at low cost, may be kept for a long period without deterioration.

The use of filter papers for the identification of bacteria has already been suggested by different authors. Knox (1949) placed filter paper strips impregnated with carbohydrates on inoculated agar plates and noted the subsequent change of the indicator. Paper discs containing culture media were employed by Snyder (1954) for the differentiation of bacteria. Similar techniques were described by Sanders, Faber, and Cook (1957) and Cook and Pelczar (1958).

Summary

A filter paper strip impregnated with urea, phenolphthalein, and tryptophan was used in the identification of bacteria of the *Proteus-Providence* group.

Bacterial cells were suspended in 0.1 ml. saline, and the impregnated filter paper strip inserted into the suspension. After one to two hours of incubation at 37° C., urease activity was detected by a change of the colour of the indicator to pink.

Tryptophan deaminase activity was demonstrated by the addition of one drop of hydrochloric acid and ferric chloride solutions. A brown coloration indicated a positive reaction.

Of all *Enterobacteriaceae* tested, only *Proteus-Providence* strains gave positive results.

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A New Method of Demonstrating Capsulated *Bacillus anthracis*

M. A. SOLTYS

*From the Department of Animal Pathology,
University of Cambridge*

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In order to show the capsules of anthrax bacilli, Heim (1901) and M'Fadyean (1903) stained them by 1% aqueous solution of methylene blue. According to M'Fadyean (1903, 1909) films made from blood or tissue of an animal which died from anthrax should be gently heated, or preferably only dried in order not to destroy the capsule. Such films, stained by 1% methylene blue for about three to five minutes and examined microscopically, showed blue-stained bacilli, surrounded by a deposit of granular or amorphous material scattered among the bacilli coloured reddish purple, representing the debris of imperfectly fixed capsules.

The slight fixation by heat results of course in a danger of infection from the slide. Despite the precautions normally taken to prevent infection with *Bacillus anthracis*, a laboratory worker sometimes becomes infected from slides. Soltys (1948) reported anthrax in a laboratory worker infected from a stained film. He showed that ordinary fixation and staining of films of anthrax bacilli have no bactericidal effect on anthrax bacilli. Even films containing non-sporulating forms, made from the infected tissue, after being only gently heated to preserve the capsule are still dangerous, as such films left for some time will sporulate.

In order to kill the organism, but at the same time to preserve the capsule, various chemicals which kill spores of *B. anthracis* were tested. Of all chemical compounds tested, Zenker's solution used for the fixation of tissue for histological studies provided the best results. It was found that once the preparation was fixed with Zenker's solution it could be stained by any available stain at any time. The organism takes on the colour of the stain, while the capsule remains as a well-defined clear zone. If films fixed by Zenker's solution are stained by methylene blue, the capsule is stained faintly purple. Films should be washed with water after being fixed by Zenker's solution. This method has been used by the writer since 1948 in his classes for students and also for routine diagnostic work, and has proved to be far superior to the old method recommended by M'Fadyean. In addition to being a much better method it is completely safe, as anthrax bacilli are killed.

In order to show that slides containing both capsulated bacilli and sporulating bacilli were safe