Evaluation of a New Incubation-Type Indole Strip Test

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A total of 189 of Enterobacteriaceae and three strains of Clostridium perfringens were tested with a new incubation-type indole paper strip. This 4-hr incubation test gave the same results as a standard 48-hr indole test, except with some strains of Proteus rettgeri. Results with P. rettgeri varied somewhat according to the medium upon which the organisms had been grown.

Rapid tests which are useful in the identification of microorganisms in the clinical laboratory have been studied extensively. Many of these tests are available to the clinical microbiologist either through preparation in the laboratory or through commercial sources. Matsen and Sherris (1) evaluated seven paper strip tests and determined that the indole strip gave false-negative readings with some Proteus strains. This paper presents results obtained from evaluation of a new indole paper reagent strip which requires a 4-hr incubation period.

### Table 1. Comparison of two methods of determining indole production

| Organism                  | No. tested | No. positive | 1% Tryptone with Kovacs' reagent | Source of inoculum for incubation-type indole strip | Direct indole strip |
|---------------------------|------------|--------------|----------------------------------|----------------------------------------------------|--------------------|
|                           |            |              | 24 hr 48 hr                      | SB^a EMB^b Mac^c TSI^d                            |                    |
| Escherichia coli          | 20         | 20           | ND*                              | 20 20 20 20                                        | 20                 |
| Klebsiella                | 20         | 8            | 8                                | 8 8 8 8                                           | 8                  |
| Enterobacter              | 20         | 4            | 6                                | 6 6 6 6                                          | 6                  |
| Serratia (two pigmented) | 10         | 2            | 2                                | 2 2 2 2                                           | 2                  |
| Shigella                  | 10         | 0            | 0                                | 0 0 0 0                                           | 0^f                |
| Salmonella                | 10         | 0            | 0                                | 0 0 0 0                                           | 0                  |
| Arizona                   | 10         | 0            | 0                                | 0 0 0 0                                           | 0                  |
| Citrobacter               | 10         | 0            | 0                                | 0 0 0 0                                           | 0                  |
| Edwardsiella              | 10         | 10           | ND                               | 10 10 10 10                                        | 10                 |
| Proteus mirabilis         | 19         | 0            | 0                                | 0 0 0 0                                           | 0                  |
| Proteus vulgaris          | 21         | 21           | ND                               | 21 21 21 21                                        | 21                 |
| Proteus morganii          | 9          | 7            | 9                                | 9 9 9 9                                           | 9                  |
| Proteus rettgeri          | 10         | 10           | ND                               | 7 (1±) 6 (2±) 4 (2±)                                | 0                  |
| Providence                | 10         | 10           | ND                               | 10 10 10 10                                        | 10                 |
| Clostridium perfringens   | 3          | 0            | 0                                | ND ND ND                                           | 0                  |

* Sheep blood-agar 5% (Trypticase Soy Agar base, BBL).
^a Levine Eosin Methylene Blue agar (BBL).
^b MacConkey Agar (BBL).
^c Triple Sugar Iron Agar (Difco)
^d Not done
^f Two pigmented strains were not tested.

Escherichia coli, Klebsiella, Enterobacter, and Proteus mirabilis strains studied were recent clinical isolates; the remaining organisms tested were clinical isolates which had been stored on agar.
slants for various lengths of time. All the strains had been isolated from different patients. These organisms were all tested for indole production after overnight growth at 35°C on four different media. A large loopful of growth was emulsified in 0.3 ml of sterile 0.85% NaCl. The incubation-type indole strips (kindly supplied by General Diagnostic Division, Warner Chilcott Laboratories, Morris Plains, N.J.) were placed in the suspensions, and the tubes were incubated in a water bath at 37°C for 4 hr. The tubes were then tilted to wet the reagent portion of the strip; a red color indicated the presence of indole. PathoTec indole strips (Warner Chilcott Laboratories) were tested by rubbing a loopful of overnight growth from sheep blood-agar onto the reagent zone of the strips. The strains were also inoculated into broth containing 1% tryptone (Difco) and 0.5% NaCl; after incubation for 24 and 48 hr at 35°C, indole was detected with Kovacs’ reagent.

Accurate results (Table 1) were obtained with the incubation-type paper strips except in the case of P. rettgeri. With these organisms, results were better when the inoculum was taken from sheep blood-agar, but even then two strains were negative and one was equivocal. However, one of these strains was very slimy, and it was difficult to pick up a loopful for the test. This is undoubtedly why these strips gave a false-negative for this particular strain. The direct-type indole strips gave erratic results with P. vulgaris, P. rettgeri, and one strain each of Providence and Enterobacter. These tests were repeated by other laboratory personnel on an unknown basis with the same results. These results with the direct strips were not as good as those previously obtained (1), which lends question to the reproducibility of the direct-type strips.

Two red-pigmented strains of Serratia marcescens did turn the reagent zone a pink color, but it was obvious this color came from the organisms. A red color was also obtained with these organisms in the standard indole test.

Although lactose-fermenting organisms from the differential media gave a red-colored suspension, this did not interfere with the test. The reagent portion of the paper strip usually turned a green color with organisms not producing indole, whereas indole-positive organisms turned the strip a darker pink color. Lactose-negative organisms on eosin methylene blue usually gave a blue suspension, but this did not interfere. The incubation-type indole strips were very easy to read, with no question as to what was positive and what was negative. Usually the positives were evident within 2 hr, and a tinge of dark pink could be seen at the bottom of the reagent zone.

In conclusion, the incubation-type strips gave better results than the direct indole strips, and they should be a helpful tool for the clinical microbiologist interested in rapid results. They would be especially helpful in differentiating swarming Proteus species on a primary isolation plate.

LITERATURE CITED

1. Matsen, J. M., and J. C. Sherris. 1969. Comparative study of the efficacy of seven paper-reagent strips and conventional biochemical tests in identifying gram-negative organisms. Appl. Microbiol. 18:452–457.