APPLIED MICROBIOLOGY, June 1969, p. 923-924
Vol. 17, No. 6
Copyright © 1969 American Society for Microbiology
Printed in U.S.A.

Evaluation of Two Spot-Indole Reagents

B. L. LOWRANCE, P. REICH, AND W. H. TRAUB

Departments of Microbiology and Pathology, Bowman Gray School of Medicine, Winston-Salem, North Carolina 27103

Received for publication 18 February 1969

Two spot-indole reagents, *p*-dimethylaminobenzaldehyde (DMABA) and *p*-dimethylaminocinnamaldehyde, were evaluated quantitatively. Although fourfold less sensitive, DMABA proved to be more stable and economical.

Certain *Enterobacteriaceae*, as well as other facultatively or obligatively anaerobic bacteria, are capable of decomposing tryptophan with the resultant production of indole, pyruvate, and ammonia; this is the so-called tryptophanase reaction (5). The indole test, as is well known, is a commonly performed taxonomic test in clinical microbiology laboratories.

Several years ago a spot-indole test, using *p*-dimethyl-aminobenzaldehyde (DMABA) in aqueous HCl, was introduced (4); brief reference was made to the more sensitive *p*-dimethylamino-cinnamaldehyde (DMACA; 1), the vinyl analogue of DMABA. We used the latter of these two spot-indole reagents. It was noted that tube-indole-positive (2) strains of *Escherichia coli* that had been grown on blood-agar gave rise to a green, a bluish-green, or an intense ink-blue color when tested with this reagent in spot-test plates. On the other hand, strains of indole-negative species of *Enterobacteriaceae* gave either a yellow or a red color with this test. These observations prompted us to determine what compounds are responsible for these various color changes and to find out which of the two spot-indole reagents is the more sensitive. Indole, indole-3-pyruvic acid, and tryptophan were purchased from General Biochemicals, Chagrin Falls, Ohio. Aqueous stock solutions of these three compounds were prepared to yield, per ml, 1,000 μg of indole, 1,000 μg of indole-3-pyruvic acid, and 5,000 μg of tryptophan. A series of twofold dilutions was made of each stock solution. One-milliliter amounts of each appropriate dilution were tested with 0.1 ml of Kovacs indole reagent (2), 0.1 ml of 1 or 5% DMABA (Eastman Organic Chemicals, Rochester, N.Y.) in 10% (1.13 N) aqueous HCl, 0.1 ml of 1 or 5% DMABA (Fisher Scientific Co., Fair Lawn, N.J.) in 10% aqueous HCl, and 1 and 5% DMACA (Aldrich Chemical Co., Inc., Milwaukee, Wis.) in 10% aqueous HCl, respectively, in tubes (13 by 100 mm). Any color change observed within 20 min was recorded. One loopful (approximately 0.03 ml) of each dilution of the various compounds examined was transferred to a plate (100 by 15 mm) containing Whatman no. 1 filter paper (90 MM) soaked with either DMABA or DMACA reagent (1 ml); any change in color within 10 sec was also recorded.

It was found that neither indole-3-pyruvic acid nor tryptophan reacted with Kovacs indole reagent or DMABA as tested by the tube and spot-plate methods. Indole-3-pyruvic acid, furthermore, did not produce any change in color with DMACA. It is known (3) that strains of *Proteus* and *Providencia* are capable of converting tryptophan into indole-3-pyruvic acid. Therefore, it was attempted to determine whether either DMABA or DMACA is capable of detecting this substance, and if so at what concentration. As stated above, the results were invariably negative.

The results obtained with Kovacs indole reagent, 1 and 5% DMABA, and 1 and 5% DMACA with regard to the quantitative detection of indole can be summarized as follows. Kovacs reagent detected 0.1 μg of indole, per ml, whereas 1% DMABA gave a positive reaction with 0.4 μg of indole per ml in tubes. The 1% DMACA reagent, however, detected 0.1 μg of indole per ml, sometimes as little as 0.05 μg of indole per ml, suggesting that this reagent is four times as sensitive as DMABA on a weight-for-weight basis.

Repeated tests employing 5% DMABA detected 0.1 μg of indole per ml in tubes. Tests conducted with 5% DMACA (a dark rusty-brown solution) revealed only 0.8 μg of indole per ml, since the color of the material (the addition of 0.1 to 1 ml of distilled water resulted in a golden-brown color) tended to obscure any fine green color that might have indicated a positive test with lower concentrations of indole. Thus, 1% DMACA represents a more optimal concentration of this reagent, and it is at this concentration that DMACA appears to be at least fourfold more sensitive than 1% DMABA.

The spot-indole tests performed with 1%

DMABA and 1% DMACA revealed that the former reagent detected from 6 to 12 μg of indole per ml, whereas the latter reagent gave positive tests with 3 μg of indole per ml. When 5% DMABA or DMACA was used, 3 μg of indole per ml was detected with either of the reagents. It should be stressed that a standard loopful of fluid represents roughly 0.03 ml; therefore, the assumption that the tube-indole test detects smaller amounts of indole than the spot-indole test is misleading because of the difference in the amount of substrate involved (1 ml versus 0.03 ml of substrate).

Large quantities of indole (100 μg/ml in tubes or 1,000 μg/ml in spot plates) gave rise to an intense ink-blue color, whereas successively smaller quantities of indole resulted in a bluish-green, green, and finally yellow color (no change) with the 1% DMACA reagent. Thus, a blue, blue-green, or a green color observed with the 1% DMACA reagent constitutes a positive test for indole.

Tryptophan, when interacting with 1% DMACA, gave rise to a red color at concentrations of 5,000 and 2,500 μg/ml only; smaller concentrations of tryptophan were not detectable with this reagent as determined by the tube method. The spot test with DMACA was invariably negative for all concentrations of tryptophan tested. However, it was previously noted that, when one examined a small piece of blood- or chocolate-agar with this reagent, a red-purple color resulted, suggesting the presence of fairly large amounts of tryptophan in these media. It was felt that this might explain why spreading films of *P. mirabilis* or isolated colonies of other indole-negative *Enterobacteriaceae* sometimes give rise to such a red color when spot-tested with the DMACA reagent, since tryptophan might conceivably have diffused from the agar medium into the surface film of spreading *P. mirabilis* or into the colonies of the coliform organisms. However, our routinely employed blood-agar plates, the constituents of which are Casman base (E. P. Casman, J. Bacteriol. **43:**33) and 5% sheep blood, contain at best approximately 200 μg of tryptophan per ml. Thus it is more probable that the red color obtained with blood-agar on DMACA spot plates is due to a compound present in this medium, the nature of which is presently unknown, rather than to tryptophan itself.

It is of practical interest to note that the DMABA spot-indole reagent is stable for a period of at least 4 months, whereas the DMACA reagent is stable for only 2 months at room temperature.

In summary, the fourfold greater sensitivity of DMACA as compared with DMABA is more than offset by the greater stability and economy of DMABA. For this reason we prefer to use the DMABA reagent (4) for spot-indole tests.

This study was aided by a grant from the United Medical Research Foundation of North Carolina.

LITERATURE CITED

1. Harley-Mason, J., and A. A. P. G. Archer. 1958. Use of *p*-dimethylaminocinnamaldehyde as a spray reagent for indole derivatives on paper chromatograms. Biochem. J. 69:60P.
2. Kovacs, N. 1928. Eine vereinfachte Methode zum Nachweis der Indolbildung durch Bakterien. Z. Immunitatsforsch. 55:311–315.
3. Singer, J., and B. E. Vollani. 1955. An improved ferric chloride test for differentiating Proteus-Providence group from other Enterobacteriaceae. J. Bacteriol. 69:303–306.
4. Vracko, R., and J. C. Sherris. 1963. Indole-spot test in bacteriology. Amer. J. Clin. Pathol. 39:429–432.
5. Wood, W. A., I. C. Gunsalus, and W. W. Umbreit. 1947. Function of pyridoxal phosphate: resolution and purification of the tryptophanase enzyme of Escherichia coli. J. Biol. Chem. 170:313–321.