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Dimethyl-Alpha-Naphthylamine for the Determination of Bacterial Reduction of Nitrates

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DIMETHYL-ALPHA-NAPHTHYLAMINE FOR THE DETERMINATION OF BACTERIAL REDUCTION OF NITRATES

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The alpha-naphthylamine-sulphanilic acid test is widely employed for the detection of reduction of nitrates to nitrites by bacteria. The test has the disadvantage that the red coloration produced in the presence of the nitrite ion appears for only a few seconds and then fades in cultures of organisms in which a relatively high concentration of nitrite occurs. Frequently in our work it is necessary to test considerable numbers of cultures at one time. Fading makes the results unreliable and cultures having a relatively high concentration of nitrite may be recorded as negative. A second difficulty experienced with the test is the instability of the alpha-naphthylamine solution, resulting in its marked discoloration. When added to the culture, the discolored solution imparts a pink color which may prove confusing when testing cultures having relatively little nitrite present.

Furthermore, the production of H_2S in the culture medium prevents the development of the full coloration of the p-sulfobenzene-azo-alpha-naphthylamine. This correlation has been pointed out by Wallace and Neave [1] who made a study of nitrite tests seeking to avoid the effects due to fading. These workers made a laboratory study of known tests for nitrites and suggested in addition the possible use of dimethylaniline and dimethyl-alpha-naphthylamine.

Germuth [2] in an attempt to find a naphthalene substitution product which would prove superior to alpha-naphthylamine in the estimation of nitrites in waters of known or questionable sources, found that dimethyl-alpha-naphthylamine gave good results.

The use of dimethyl-alpha-naphthylamine in routine work for the detection of nitrate reduction by bacteria has proved decidedly superior to alpha-naphthylamine. The reagents may be added to any number of cultures at one time with no danger of fading before readings can be made. The coloration is permanent. The

1 Wallace, G. I., and Neave, S. L., Jour. Bact. *14*: 377-84 (1927).
2 Germuth, F. G., Ind. and Eng. Chem., *1*: 28-9 (1929).

reagent does not become turbid so that one always works with clear solutions.

However, the dimethyl-alpha-naphthylamine reagent will show darkening upon standing. The discoloration is a dark brown and in no way interferes with the reading of the test.

For use in bacteriology, one cc. of dimethyl-alpha-naphthylamine is dissolved in 5 cc. of methyl alcohol and the volume made up to 100 cc. with 5 N acetic acid. The sulphanilic acid solution is the same as in the standard test: 0.8 grm. sulphanilic acid in 100 cc. of 5 N acetic acid. One cubic centimeter of each solution is added to approximately 5 cc. of the culture. Reduction of nitrate and the presence of nitrite are indicated by the development of a cherry red coloration.

The sensitivity of the dimethyl-alpha-naphthylamine test in culture media is no greater than that of alpha-naphthylamine.

Table I — Comparison of the Dimethyl-alpha-naphthylamine and Alpha-naphthylamine Tests

TREATMENT	α-NAPHTHYLAMINE	DIMETHYL-α-NAPHTHYLAMINE
1:10^2 NO_2	Color fades immediately	Deep red, permanent color
1:2 x 10^2 NO_2	Color fades in 3 seconds	Deep red, permanent color
1:4 x 10^2 NO_2	Color fades in 15 seconds	Deep red, permanent color
1:8 x 10^2 NO_2	Color fades in 30 seconds	Deep red, permanent color
1:10^3 NO_2	Color fades with 1 minute	Deep red, permanent color
1:10^4 NO_2	Deep red, some fading	Deep red, permanent color
1:10^5 NO_2	Deep red color	Deep red, permanent color
1:10^6 NO_2	Pink	Pink, deepening with time
1:10^7 NO_2	Pink	Pink, deepening with time
1:10^8 NO_3	Pink after 30 minutes	Faintly pink after 30 minutes
Esch. coli in 0.2% nitrate broth, 37° C.		
18 hour cultures	Pink	Pink
24 hour cultures	Deep pink	Deep pink
36 hour cultures	Red, fades in 1 minute	Red, permanent
48 hour cultures	Red, fades 4 seconds	Red, permanent
60 hour cultures	Red, fades 3 seconds	Red, permanent

Germuth (1929) states that the color change due to the dimethyl derivative is more rapid. In 0.5% peptone 0.2% nitrate broth we have found little difference. The coloration due to the dimethyl derivative in high dilution of the nitrite ion has proved to be a little slower in developing but shows a greater deepening with time. In routine work the coloration should develop within two or three minutes to be of practical value to the bacteriologist because the significance of any subsequent development of color is doubtful in view of the fact that even sterile tubes of certain nitrate media will show a pink coloration after standing.

We would place the practical limit of sensitiveness of the test at $1:10^7$ dilution of the NO_2 ion as determined in distilled water; coloration to develop to a distinct pink within 3 minutes. Tested with *Aerobacter aerogenes, A. indologenes, A. faeni, A. motorium, A. pectinovorum* and *Escherchia coli*, no culture at any time tested showed the slightest trace of fading of the deep red coloration. The dimethyl-alpha-naphthylamine test has been used on hundreds of culture in this laboratory to determine the presence of the nitrite ion with satisfactory results. In concentrated solutions of nitrites (greater than 1:100) the red coloration fades to an orange. This would certainly never occur in bacterial cultures. Table 1 gives a comparison of the two methods.

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