NOTES ON THE α-NAPHTHOL MODIFICATION OF THE VOGES-PROSKAUER TEST AND REFERENCE TO THE CREATINE MODIFICATION

By MAXWELL M. BARRITT, B.Sc., Dip.Bact., L.M.S.S.A., M.Sc.,
From the Bacteriological Department of the Liverpool Corporation

The α-naphthol modification of the Voges-Proskauer (V.-P.) test described by Barritt (1936) has proved satisfactory for the examination of the coli-aerogenes group in the hands of several workers, including Harold (1936), Vaughn, Mitchell & Levine (1938), Kluyver & Molt (1939), Iyer & Raghavachari (1938–9), and Batty-Smith (1941).

Batty-Smith in a comparison of this modification (the α-naphthol test) with the creatine modification of O'Meara (1931) found the former more sensitive and highly specific. It had two advantages: speed and definiteness, and he recommended it for wider use. Results of previous comparisons by Harold and Vaughn *et al.* were similar to those of Batty-Smith. Vaughn *et al.* were able to record almost twice as many more positive results in the α-naphthol test with known V.-P. positive cultures after ½ hr. than in the creatine test.

Iyer & Raghavachari found the test to correlate perfectly with the methyl red and indole tests, and to yield definitely positive results where the ordinary V.-P. test and the other modifications were negative or gave only an indefinite reaction.

Barritt stated that very slightly stronger reactions were given by the α-naphthol test if creatine was added, and Batty-Smith and Kluyver & Molt adopted this procedure. The author's conclusion was based on experimental work with acetylmethylcarbinol. This substance in the presence of KOH is oxidized to dicetyl, and it is believed that in turn it condenses with a guanidine group substance in peptone to form a red coloured compound which is indicative of a positive V.-P. result. The writer has found that the intensity of the colour change depends on the brand of peptone used, and deeper reds are obtained with bacto-peptone and Evans's peptone than with either proteose or Witte's peptone. These differences however disappear on the use or addition of creatine, the colour change being then of equal intensity, but are not much affected by increasing the concentrations of the peptones. It is clear that creatine provides a stronger reaction when some peptones are present and possibly on account of differences in their guanidine group content. Hence it is feasible for the reaction in some cultures to be similarly enhanced, but the writer has not observed such when using glucose phosphate broth cultures containing bacto-peptone and reading the results after 1 hr.

There is no doubt, however, that creatine hastens the appearance of the α-naphthol reaction and thereby promotes intensification. So does moderate heat by hastening the oxidation of acetylmethylcarbinol to diacetyl (Kluyver & Molt). Shaking hastens its appearance, and for this purpose Batty-Smith used a Kahn shaking machine. Batty-Smith found that the depth of colour of the positive results was slightly enhanced by the addition of ferric chloride. Werkman (1930) added ferric chloride in the ordinary V.-P. test and believed that it catalysed the oxidation of the acetylmethylcarbinol, but found that this did not occur under normal conditions of the test in the absence of atmospheric oxygen.

During the process of shaking the cultures aeration takes place, but neither of these factors is essential for the α-naphthol reaction, although it is delayed for a few minutes without them. The writer has found that the test can be satisfactorily performed as a *ring test*, by superimposing the α-naphthol solution on the culture after the addition of KOH. By this method, in which there is no shaking or active aeration,

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positive results are often readable for several days. Normally a brown coloration occurs from interaction of the KOH and the α-naphthol solution and eventually masks the positive results. This coloration is delayed in the *ring test* because of the less intimate admixture of these reagents.

The α-naphthol test may be done in a similar way to the creatine test by the addition of some crystals of α-naphthol to the culture, but the amount of KOH should not be altered to correspond. The test is, however, more sensitive when ethyl alcohol is present, either as a solvent for the naphthol or otherwise, and in this respect it differs from the creatine test, for with this I have found the reactions weaker in the presence of alcohol.

Dorner & Hellinger (1935) found that the brand of peptone was of little consequence in the ordinary V.-P. and creatine tests, and among those they used in cultures were Witte's peptone and Difco bacto-peptone. Levine, Epstein & Vaughn (1934) got more V.-P. positive results with a medium containing proteose peptone than with another containing Witte's peptone. Difco laboratories recommended proteose peptone for the V.-P. test and an incubation of 48 hr.

In the original paper on the α-naphthol test the writer recommended, in the examination of the coli-aerogenes group, bacto-peptone and an incubation of 3 days, but has now found proteose peptone to be more suitable for this incubation period. Even better results have been obtained with a 1–2 day incubation and the use of bacto-peptone or Evans's peptone, results being superior to those with proteose peptone after 1–3 days. Attention is drawn to the recommendation made by Difco laboratories because the writer has found a greater number of positive results to be yielded by cultures containing proteose peptone after 3 days than after 48 hr. Batty-Smith used this peptone for the α-naphthol test and a 2-day incubation period, but contended, by comparison with the creatine test, that more positive results might have been obtained after 3 days. His contention, therefore, which seemed justified, is independently supported.

The writer has compared four peptones in broth cultures of twenty strains of *Bact. aerogenes* of 1–3 days' incubation. The sensitiveness of the α-naphthol test permitted tests to be done on dilutions of these cultures, and if sensitiveness may be recorded in terms of the mean of the highest dilutions yielding positive results it was of the following order:

(1) Bacto-peptone (1–2 days).
(2) Evans's peptone (2 days); Evans's peptone (1 day).
(3) Proteose peptone (3 days).
(4) Bacto-peptone (3 days); Witte's-peptone (3 days).
(5) Proteose peptone (1–2 days); Evans's peptone (3 days).
(6) Witte's peptone (1–2 days).

These results not only indicate that some peptones are to be preferred to others in cultures for the α-naphthol test, but that the optimum incubation period for testing may be different for cultures containing different peptones. (It is noteworthy that Vaughn *et al.*, in a comparison of the α-naphthol test with the creatine and ordinary V.-P. tests, used bacto-peptone and an incubation period of 1–2 days.)

In the creatine test a similar comparison was made, the results being read after 1 hr. This test was insufficiently sensitive to be done on dilutions of cultures, but the results with undiluted cultures were largely in accord with those in the α-naphthol test. Some reactions were faint and a few negative, and corresponded in the α-naphthol test with results of cultures that were positive only when in low dilution. Evans's peptone proved particularly suitable for the creatine test, especially with cultures of 1–2 days' incubation. Witte's peptone by comparison was unsatisfactory, the reactions of many cultures being very faint and of two of them negative after 1–2 days' incubation. One culture with proteose peptone was negative after 1 day but positive in 3 days, and in another a reaction the reverse of this was observed.

The writer is able to confirm the findings of Batty-Smith that 2–5 min., as recommended by the Ministry of Health (1939), is inadequate for the creatine test. Vaughn *et al.* stated that the reaction is not complete until 4 hr., and this is the period Batty-Smith used in comparing the test with the α-naphthol test after 1 hr. Iyer & Raghavachari used one broth tube for both the methyl red and α-naphthol tests with satisfactory results. The methyl red result was recorded and the KOH and α-naphthol then added. The writer, however, finds that a brownish colour is often imparted to the broth by this procedure, which although readily permitting the detection of average positive results renders that of some of the weak reactions difficult or impossible.

Kluyver & Molt found approximately the first 10 c.c. of distillates of 100 c.c. cultures of *Bact. coli* to yield positive results in the α-naphthol test, and in one instance a weak positive in the creatine test, and they confirmed the significance of these reactions by detecting diacetyl in the distillates on chemical analysis.

By modifying the α-naphthol test faintly positive results may be obtained with some *Bact. coli*. This can be done by using a more and sufficiently concentrated solution of α-naphthol whereby the sensitiveness of the test is raised; conversely it may be lowered by using less α-naphthol. The amounts of the constituents in the α-naphthol tests (1936) are 0·6 c.c. of a 5% alcoholic solution of α-naphthol and 0·2 c.c. of KOH (40%) to 1 c.c. of culture. With these amounts the test is believed to have the highest possible sensitiveness consistent with specificity for application to the coli-aerogenes group of bacteria.

After this paper was accepted for publication the author has noted that Vaughn and Levine (1943), in a study of the intermediates of the coli-aerogenes group, used 1–5 day cultures grown in Difco M.R.–V.-P. medium at 30° C. for the α-naphthol test. Results were read after ½ hour and again within 6 hours. Difco M.R.–V.-P. medium contains proteose peptone.

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