THE DIFFERENTIATION OF PATHOGENIC AND NON-
PATHOGENIC STAPHYLOCOCCI BY ESTIMATION OF
THE REDUCTION OF AMIDO-BLUE-BLACK
AND METHYLENE BLUE*

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ABSTRACT. The methylene blue reduction of pathogenic and nonpathogenic staphylococci was tested in the culture medium, recommended by the IAMS Subcommittee for Staphylococci and Micrococci, for differentiating staphylococci and micrococci; the medium was used without the addition of agar or sugar. Coagulase-positive staphylococci reduced methylene blue within 30 minutes whereas the majority of nonpathogenic staphylococci and micrococci reduced methylene blue in a longer period of time. Amido-blue-black could be used as a means of differentiating pathogenic staphylococci from coagulase-negative staphylococci and micrococci.

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In order to distinguish pathogenic from nonpathogenic staphylococci a standardized coagulase test is applied. Another test is that of anaerobic mannitol fermentation. Despite these two procedures it would be advantageous in case of doubt to make use of still another standard test. Shaw, Stitt and Cowan (1951) tested the reduction of methylene blue as a method for the differential diagnosis of pathogenic from nonpathogenic staphylococci. The authors stated that pathogenic as well as nonpathogenic staphylococci reduced methylene blue after 2 hours, so they attributed no importance to this test. On the other hand, Moreira-Jacob reported at the

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Vith International Congress on Microbiology in Rome that the reduction test was a useful method for the differentiation of staphylococci. Moreira-Jacob determined the time required to reduce methylene blue and stated that pathogenic staphylococci decolorized methylene blue more quickly than nonpathogenic staphylococci. He used normal broth as liquid medium.

Our tests of this result have shown that the time for reduction largely depends on the culture medium; glucose-containing media are particularly unsuitable. A definite result of the reduction time suitable for use in differential diagnosis seemed only to be possible by use of a standard medium. During the last Subcommittee meeting in Brno, 1964, a culture medium was recommended for the differentiation of staphylococci and micrococci on the basis of anaerobic dextrose fermentation (Recommendations of Subcommittee, 1965). We decided to use the same medium culture without agar and without dextrose for testing reduction of methylene blue and amido-blue-black.

Method. The strains to be tested were inoculated on tryptone-yeast-agar (Recommendations of Subcommittee, 1965) and incubated for 24 hours at 37°C. One loop from this culture was transferred to 5 ml of the liquid standard medium and incubated for 24 hours at 37°C. They were then cooled to room temperature and mixed with 0.1 ml of a 1% aqueous methylene blue solution. Immediately the tubes were placed into a water bath of 37°C and examined frequently to determine the time for the complete decolorization of the methylene blue; a 3-5 mm blue ring on the surface of the culture was disregarded in reading the results.

There were tested 107 strains of coagulase-positive staphylococci, 30 of coagulase-negative staphylococci, and 39 strains of micrococci including 21 from Dr. Baird-Parker. Results are shown in Table 1.

All 107 strains of coagulase positive staphylococci were decolorized in 30 minutes; none of the 40 strains of coagulase-negative staphylococci were decolorized in 25 minutes, 2 in 30 minutes and all within 120 minutes. None of the 39 strains of micrococci decolorized in less than 50 minutes, and 34 did not decolorize in 120 minutes.

Using the standard medium, the methylene blue reduction may serve as an additional technique for differentiating pathogenic staphylococci and coagulase-negative staphylococci as well as micrococci. Errors are possible if the

Table 1. Reduction of methylene blue.

Minutes	Coagulase + staphylococci 107	Coagulase - staphylococci 40	Micrococci 39
10	5	-	-
15	27	-	-
20	35	-	-
25	30	-	-
30	10	2	-
40	-	2	-
50	-	8	2
60	-	4	-
90	-	14	2
120	-	3	1
120	-	7	34 ø

34 ø = no reaction in 2 hours

addition of methylene blue is performed under different temperatures and if the culture medium is shaken during the reduction time.

Use of amido-blue-black instead of methylene blue as an indicator gave no useful results. The reaction time even with the addition of only 5 ml of a 0.5% amido-blue-black solution to 1000 ml of medium was too slow. The colour change from blue to violet even after 5 days incubation is of no use for characterizing coagulase-positive and -negative staphylococci or micrococci. Amido-blue-black is reduced within 5 days by 99 of 100 pathogenic staphylococci strains. However, 13 strains of 40 coagulase-negative strains and 2 micrococci strains also reduced amido-blue-black within this time.

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