Use of a Rapid Acetoin Test in the Identification of Staphylococci and Micrococci

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In 269 parallel acetoin tests upon 212 strains of staphylococci and micrococci, 227 were positive by the 14-day tube method and 213 by a 2-day paper-disk method. Both tests showed the same percentage error in primary results.

Acetoin production is an important differential feature in Baird-Parker's identification system for staphylococci and micrococci (1). Although taxonomists have reason to doubt the generic division used in this classification, it remains currently accepted (4) and widely used (3). The 14-day incubation period specified by Baird-Parker for the conventional tube acetoin test makes the system somewhat slow. Various methods have been described for speeding up the collection of identification test data in bacteriology, and paper-disk systems are among the most rapid and economical (2).

We recently had cause to classify 212 strains of commensal and reference cocci and took the opportunity to compare the results of the conventional acetoin test with those of a rapid, paper-disk method.

Strains were inoculated and tested in the prescribed manner (1) and also patch-inoculated onto agar containing: tryptone, yeast extract, glucose (TYG) (Oxoid L42), 2%; yeast

extract (Oxoid L21), 0.2%; glucose, 1%; Oxoid agar No. 1, 1%; pH 7.2. After 48 h of incubation at 30 C, a 1-cm disk of Whatman 3M paper freshly soaked in 10% sodium pyruvate solution was placed on each growth patch, and the plates were reincubated for 3 h. Acetoin production was detected by spotting one drop of 40% potassium hydroxide, one drop of 1% creatine, and one drop of 1% alpha-naphthol (alcoholic) onto each disk and observing for a pink-red color change for 1 h at room temperature.

The results for the 212 strains classified into their respective subgroups are shown in Table 1. Of the 269 tests performed, 57 were repetitions. For example, the one strain of subgroup S3 gave a positive tube test but negative disk test when first tested, but two further tests by both methods were all negative; a similar phenomenon occurred in four strains of subgroup M6, which were retested only once. In the 212 primary tests, there were 33 discord-

TABLE 1. Results of 269 parallel disk and tube acetoin tests performed on 212 strains classified according to Baird-Parker (1)

Genus	Subgroup	No. of strains	No. of tests	Positive tube test	Positive disk tests
<i>Staphylococcus</i>	S1	31	48	47	45
	S2	9	12	11	10
	S3	1	3	1	0
	S4	9	11	10	8
	S5	12	15	13	15
	S6	26	32	27	31
<i>Micrococcus</i>	M1	15	17	16	16
	M2	32	37	34	31
	M3	44	49	47	42
	M4	15	18	17	15
	M5	5	7	0	0
	M6	12	17	4	0
	M7	1	3	0	0

ancies in the results of the two acetoin tests. Twelve of these were in the staphylococcal subgroups and consisted of the one S3 strain (see above) in which a false-positive tube test occurred, and eleven strains of the other five subgroups in seven of which false-negative tube tests occurred and in four of which false-negative disk tests occurred. In the micrococcus subgroups M1 to M4, thirteen false-negative disk tests and four false-negative tube tests occurred see also four false-positive tube tests in M6 strains above). In these 33 cases, therefore, the tube test proved correct 17 times and the disk test 16 times, indicating equal fallibility (ca. 8%) in both tests over the 212 strains. In view of its simplicity, we recommend the disk test alone or as a readily performed adjunct to the conventional test.

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REPRINT REQUESTS

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LITERATURE CITED

1. Baird-Parker, A. C. 1966. Methods for classifying staphylococci and micrococci, p. 59-64. *In* B. M. Gibbs and F. A. Skinner (ed.), Identification methods for microbiologists, Part A. Academic Press, Inc., New York.
2. Clarke, P. H., and K. J. Steel, 1966. Rapid and simple biochemical tests for bacterial identification, p. 111-115. *In* B. M. Gibbs and F. A. Skinner (ed.), Identification methods for microbiologists, Part A. Academic Press Inc. New York.
3. Noble, W. C. 1969. Distribution of the Micrococcaceae. *Brit. J. Derm.* **81**: Suppl. 1, p. 27-32.
4. Subcommittee on Taxonomy of Staphylococci and Micrococci. 1971. Minutes of meeting. *Int. J. Syst. Bacteriol.* **21**:161-163.