Evaluation of three methods using deoxyribonuclease production as a screening test for *Serratia marcescens*

W. A. BLACK, R. HODGSON, AND ANN McKECHNIE

From the University Department of Bacteriology, Royal Infirmary, Glasgow

SYNOPSIS Deoxyribonuclease (DNase) produced by *Serratia marcescens* is a characteristic feature which is useful in distinguishing this organism from closely related members of the Enterobacteriaceae. In an assessment of three methods of performing the DNase test as a screening procedure for *Serratia marcescens*, the conclusion was reached that the standard test was most suitable for use in the clinical bacteriology laboratory.

Since it is now well established that *Serratia marcescens* must be accepted as a pathogen (Ewing, Johnson, and Davis, 1962; Dodson, 1968; Alexander, Reichenbach, and Merendino, 1969) and that only 26.6% of strains are capable of producing pigment (Ewing *et al.*, 1962), it has become increasingly important in recent years for the bacteriology laboratory positively to identify *Serratia marcescens* in clinical material and to distinguish this organism from closely related non-pigmented members of the *Klebsiella-Enterobacter-Serratia* (K-E-S) group of the Enterobacteriaceae. In practice, this differentiation is usually made on the ability of *Serratia marcescens* to liquefy gelatin rapidly, and on its inability to ferment arabinose, raffinose, or rhamnose (Edwards and Ewing, 1962). The demonstration, however, that deoxyribonuclease (DNase) production is a characteristic of *Serratia marcescens* but not of the other members of the K-E-S division (Rothberg and Swartz, 1965; Elston and Elston, 1968) represented a major advance in the biochemical identification of this group since it meant that, for the first time, there was available a test which was sufficiently specific and straightforward to be of value as a screening procedure. Recently some modifications of the original test have been suggested (Schreier, 1969; Smith, Hancock, and Rhoden, 1969), and, since in this laboratory for the past few months we have been engaged in determining the exact incidence of *Serratia marcescens* in our clinical material (Black and Hodgson, 1970), it was thought that this presented an ideal opportunity for comparing the usefulness of the original test and these two modifications in the bacteriological identification of *Serratia marcescens*.

Received for publication 20 August 1970.

Materials and Methods

The survey was carried out in a Glasgow 800-bed teaching hospital over a four-month period during which a total of 497 Gram-negative bacilli isolated from 20,500 clinical specimens were tested for DNase production by the methods to be described.

The organisms included 221 strains which appeared as non-lactose fermenters on MacConkey's medium and which were fully identified biochemically (Black and Hodgson, 1970). The remaining 276 organisms, which were lactose-fermenters on MacConkey's medium, were screened initially for DNase production, the intention being to investigate fully any strains giving a positive or weak positive result.

Three methods of detecting DNase production by the test organisms were studied:

1 THE STANDARD TEST (OXOID)

The standard test, based on the method of Jeffries, Holtman, and Guse (1957), was carried out using Oxoid DNase agar (CM 321). After incubation, the surface of the plates was flooded with 1N HCl which forms a cloudy precipitate with the DNA in the medium. Production of DNase results in hydrolysis of the DNA into nucleotide fractions which are not precipitated by acid, this being demonstrated by a clear zone surrounding the DNase producing colonies.

2 SCHREIER'S MODIFICATION (TOLUIDINE BLUE)

The second test was based on the modification proposed by Schreier (1969). Toluidine blue (G. T. Gurr Ltd, London, NW9) was added to Oxoid DNase

agar to give a final dye concentration of 0.01%, the resulting dye-DNA complex producing a clear blue medium. If DNase is produced by the organism, this complex is broken down and the zone surrounding the colony changes colour from clear blue to rose-pink.

3 MODIFICATION OF SMITH *et al* (1969) (METHYL GREEN)

The third test was essentially that described by Smith *et al* (1969). Methyl green (G. T. Gurr Ltd, London, NW9) was added to Oxoid DNase agar to give what was found to be a final optimal dye concentration of 0.02%. The dye combines with the DNA to form a stable complex producing a green medium at pH 7.5, hydrolysis of the complex by DNase resulting in freeing of the methyl green with the production of a clear zone surrounding the colonies producing the enzyme.

Neither of these modifications requires the addition of 1N HCl to the plates after incubation.

For each of the three media the test was performed in the same way, namely, a pure culture of the test organism was spot-inoculated on to the DNase test plates using a straight wire and the plates were incubated for about 18 hours at 37°C. Using this method, it is possible to test up to 52 colonies on any one plate. A pigmented strain of *Serratia marcescens* and a strain of *Escherichia coli* which did not produce DNase were included as controls on every plate.

Results

These are shown in detail in Tables I and II where it can be seen that a total of 21 organisms gave either a positive (+) or a weak positive (+/-) reaction on at least one test medium.

Of the 15 positive results obtained using the standard (Oxoid) test medium, 12 positives were given by *Serratia marcescens*, two by *Proteus vulgaris*, and one by *Aeromonas formicans*. Using the toluidine blue and methyl green methods, the positive results obtained corresponded exactly with those given by the standard techniques, except that one of the *Proteus* species (no. 164) yielded only a weak positive reaction when tested on these modified media.

Apart from *Proteus vulgaris* no. 164, weak positive results were given by six organisms, namely, two *Proteus mirabilis* species, two *Alkaligenes* species, and two species of *Enterobacter liquefaciens*. As shown in Table I, with the exception of one of the strains of *Enterobacter liquefaciens* (no. 95) which was negative by the standard technique but weakly positive by the other methods, weak positive results were given either by the standard test alone or by all three methods.

Discussion

Before any test gains widespread acceptance as a

Reference Number	Identity	Deoxyribonuclease Production		
		Standard Method (Oxoid)	Toluidine Blue Modification	Methyl Green Modification
7	<i>Alkaligenes bronchisepticus</i>	+/-	+/-	+/-
45	<i>Serratia marcescens</i>	+	+	+
55	<i>Proteus mirabilis</i>	+/-	-	-
63	<i>Serratia marcescens</i>	+	+	+
66	<i>Alkaligenes bronchisepticus</i>	+/-	-	-
78	<i>Serratia marcescens</i>	+	+	+
95	<i>Enterobacter liquefaciens</i>	-	+/-	+/-
113	<i>Enterobacter liquefaciens</i>	+/-	+/-	+/-
122	<i>Proteus mirabilis</i>	+/-	-	-
137 ¹	<i>Serratia marcescens</i>	+	+	+
164	<i>Proteus vulgaris</i>	+	+/-	+/-
166 ¹	<i>Serratia marcescens</i>	+	+	+
177 ¹	<i>Serratia marcescens</i>	+	+	+
183 ¹	<i>Serratia marcescens</i>	+	+	+
194	<i>Serratia marcescens</i>	+	+	+
198 ²	<i>Serratia marcescens</i>	+	+	+
200	<i>Serratia marcescens</i>	+	+	+
213	<i>Serratia marcescens</i>	+	+	+
216 ²	<i>Serratia marcescens</i>	+	+	+
244	<i>Aeromonas formicans</i>	+	+	+
245	<i>Proteus vulgaris</i>	+	+	+

Table I Organisms producing deoxyribonuclease on the three test media

¹Isolated from the same patient (McL) on different occasions

²Isolated from the same patient (McK) on different occasions

+ Zone of clearing greater than 2 mm from edge of colony

+/- Zone of clearing less than 2 mm from edge of colony

- No zone of clearing

Group	Number of Isolates	Deoxyribonuclease Activity								
		Standard Method (Oxoid)			Toluidine Blue Modification			Methyl Green Modification		
		+	+/-	-	+	+/-	-	+	+/-	-
<i>Serratia marcescens</i>	12	12	—	—	12	—	—	12	—	—
<i>Enterobacter cloacae</i>	19	—	—	19	—	—	19	—	—	19
<i>Enterobacter hafniae</i>	6	—	—	6	—	—	6	—	—	6
<i>Enterobacter aerogenes</i>	5	—	—	5	—	—	5	—	—	5
<i>Enterobacter liquefaciens</i>	1	—	—	1	—	1	—	—	1	—
<i>Klebsiella</i>	13	—	—	13	—	—	13	—	—	13
<i>Esch. coli</i>	62	—	—	62	—	—	62	—	—	62
<i>Proteus</i>	40	2	2	36	1	1	38	1	1	38
<i>Providencia</i>	5	—	—	5	—	—	5	—	—	5
<i>Salmonella typhimurium</i>	4	—	—	4	—	—	4	—	—	4
<i>Pseudomonas</i>	15	—	—	15	—	—	15	—	—	15
<i>Alkaligenes</i>	12	—	2	10	—	1	11	—	1	11
<i>Acinetobacter</i>	16	—	—	16	—	—	16	—	—	16
<i>Aeromonas</i>	2	1	—	1	1	—	1	1	—	1
<i>Flavobacterium</i>	2	—	—	2	—	—	2	—	—	2
Unclassified	7 ¹	—	—	7	—	—	7	—	—	7
Total (all non-lactose fermenters on MacConkey)	221	15	4	202	14	3	204	14	3	204
Miscellaneous Gram-negative bacilli (all lactose fermenters on MacConkey)	276	—	1 ²	275	—	1 ²	275	—	1 ²	275

Table II Screening of 497 Gram-negative bacilli for deoxyribonuclease production

¹Mostly members of the Achromobacteraceae which did not readily fit into a genus²No. 113 in Table I

screening method it has to satisfy certain basic criteria. First of all the expected result must be of sufficient value to justify the extra effort expended; secondly, it must be a sensitive test, so as to eliminate any possibility of false-negative results; thirdly, it must be specific, for, if too many false positives are collected, the value of the screen is minimized; and lastly, it must be technically simple to perform so as to make its use applicable to the busy routine laboratory. The three methods of detecting production of DNase will therefore be considered with these four criteria in mind.

Several recent publications have emphasized the role of *Serratia marcescens* as a pathogen, especially in debilitated patients (Dodson, 1968; Altmeir, Culbertson, Fullen, and McDonough, 1969). It has, moreover, been encountered in hospital as a contaminant of peritoneal dialysis fluid (McCracken and Lipscomb, 1965), of banked blood (Black, Pollock, and Batchelor, 1967), and of ultrasonic nebulizers (Ringrose, McKown, Felton, Barclay, Muchmore, and Rhodes, 1968); in all of these instances, severe, and in two of the cases fatal, infection of patients followed. Despite the fact that this organism does not appear to be so common in this country as in the USA (Black and Hodgson, 1970), it has been implicated in two fatalities in the UK in recent years (McCracken and Lipscomb, 1965; Black *et al.*, 1967) and, with the rising incidence in Gram-negative infections in hospitals (Barber, 1961;

Barrett, Casey, and Finland, 1968; DuPont and Spink, 1969), there is little doubt that it will be seen more and more frequently. It is felt, therefore, that it is justifiable to advocate the DNase test as one of the essential items in any set of screening procedures for Gram-negative bacilli, particularly those appearing as non-lactose fermenters, as it is otherwise very difficult to separate *Serratia marcescens* from the closely related *Enterobacter* species.

The sensitivity of all three techniques is shown to be satisfactory in Table I, *Serratia marcescens* giving a strong positive reaction in every case.

If positive reactions alone are considered, then the specificity of the test is constant for all three methods except that only the Oxoid method gave a positive reaction for *Proteus* no. 164. The fact that three organisms apart from *Serratia*, namely, two *Proteus* species and one *Aeromonas*, gave positive reactions does not seriously detract from the value of the test as these organisms were very easily distinguishable from *Serratia* by other means. As distinct from the positive reactions, the production of weak positive reactions varied with the media and, as is shown in Table I, the standard (Oxoid) method was, apart from the results given by *Enterobacter liquefaciens* no. 95, more sensitive but less specific than the two modifications. Once again, the production of weak positive reactions by *Proteus* and *Alkaligenes* species is relatively unimportant but it was surprising to find this type of

reaction given by both *Enterobacter liquefaciens* strains tested as this has not been mentioned by other workers (Rothberg and Swartz, 1965; Elston and Elston, 1968). These weak positive reactions were, however, easily distinguishable from those given by *Serratia marcescens*.

For the purposes of this study, the standard test was found to be preferable to the two modifications although it was appreciated that the latter may have a part to play in other circumstances (Smith *et al.*, 1969). Our experience with the methyl green medium of Smith *et al.* (1969) is that it is technically difficult to prepare and that DNase production is shown much less clearly than by either of the other two methods. Although the toluidine blue modification of Schreier (1969) gave excellent results which were easy to interpret, we were unable to agree with her that this test was superior to the standard method. Some allowance, however, can possibly be made for the fact that we obtained our commercial media from different manufacturers.

In the final analysis, our preference for the standard method was based on the fact that the medium was easy to prepare and the results were clear-cut. Criticism of this technique has been based on the fact that results are irreproducible and equivocal (Schreier, 1969) and that, because IN HCl has to be added to the test plate, this precludes further incubation (Smith *et al.*, 1969). As already stated, we cannot agree with Schreier on the first point and, as regards the second, if the DNase test is to be used as a screening procedure for *Serratia* in the identification of pure isolates of Gram-negative bacilli then the question of reincubation is irrelevant as large colonies are invariably produced by the Enterobacteriaceae after 18 hours' incubation.

In conclusion, therefore, it is felt that the DNase test for *Serratia* is of considerable value and that, if the standard method is adopted, it is well within the

scope of every laboratory interested in the identification of Gram-negative bacilli.

References

- Alexander, R. H., Reichenbach, D. D., and Merendino, K. A. (1969). *Serratia marcescens* endocarditis. *Arch. Surg.*, **98**, 287-291.
- Altemeier, W. A., Culbertson, W. R., Fullen, W. D., and McDonough, J. J. (1969). *Serratia marcescens* septicaemia. *Arch. Surg.*, **99**, 232-238.
- Barber, M. (1961). Hospital infection yesterday and today. *J. clin. Path.*, **14**, 2-10.
- Barrett, F. F., Casey, J. I., and Finland, M. (1968). Infections and antibiotic use among patients at Boston City Hospital, February, 1967. *New Engl. J. Med.*, **278**, 5-9.
- Black, W. A., and Hodgson, R. (1970). Search for *serratia*. (In preparation.)
- Black, W. A., Pollock, A., and Batchelor, E. L. (1967). Fatal transfusion reaction due to *Serratia marcescens*. *J. clin. Path.*, **20**, 883-886.
- Dodson, W. H. (1968). *Serratia marcescens* septicaemia. *Arch. intern. Med.*, **121**, 145-150.
- DuPont, H. L., and Spink, W. W. (1969). Infections due to Gram-negative organisms: An analysis of 860 patients with bacteremia at the University of Minnesota Medical Center, 1958-1966. *Medicine (Baltimore)*, **48**, 307-332.
- Edwards, P. R., and Ewing, W. H. (1962). *Identification of Enterobacteriaceae*, 2nd ed. Burgess, Minneapolis.
- Elston, H. R., and Elston, J. H. (1968). Further use of deoxyribonuclease in a screening test for *serratia*. *J. clin. Path.*, **21**, 210-212.
- Ewing, W. H., Johnson, J. G., and Davis, B. R. (1962). *The Occurrence of Serratia marcescens in Nosocomial Infections*. US Department of Health, Education and Welfare, Washington, DC.
- Jeffries, C. D., Holtman, D. F., and Guse, D. G. (1957). Rapid method for determining the activity of microorganisms on nucleic acids. *J. Bact.*, **73**, 590-591.
- McCracken, A. W., and Lipscomb, F. E. (1965). *Serratia marcescens* infection complicating peritoneal dialysis. *Brit. med. J.*, **1**, 1536-1537.
- Ringrose, R. E., McKown, B., Felton, F. G., Barclay, B. O., Muchmore, H. G., and Rhodes, E. R. (1968). A hospital outbreak of *Serratia marcescens* associated with ultrasonic nebulisers. *Ann. intern. Med.*, **69**, 719-729.
- Rothberg, N. W., and Swartz, M. N. (1965). Extracellular deoxyribonucleases in members of the family enterobacteriaceae. *J. Bact.*, **90**, 294-295.
- Schreier, J. B. (1969). Modification of deoxyribonuclease test medium for rapid identification of *Serratia marcescens*. *Amer. J. clin. Path.*, **51**, 711-716.
- Smith, P. B., Hancock, G. A., and Rhoden, D. L. (1969). Improved medium for detecting deoxyribonuclease-producing bacteria. *Appl. Microbiol.*, **18**, 991-993.