SOME RESULTS OF THE TETRAZOLIUM REDUCTION TEST TO DIFFERENTIATE LANCEFIELD GROUP D STREPTOCOCCI

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SUMMARY

Because of the easy visual assessment, the number of equivocal results was reduced in the tetrazolium reduction test for distinguishing between *Str. faecalis* and *Str. faecium* by using a stab inoculation method instead of surface sowing or growth in liquid.

All 12 strains designated *Str. faecalis* by other workers were positive by the stab method, whereas one was negative by both broth and plate techniques. Fifteen of 16 strains of *Str. faecium* were negative by the stab method, 12 by plate method and 15 by broth method. One of 3 strains of *Str. durans* was positive by all methods and 2 were negative by all methods. All 5 strains of *Str. bovis* were positive by the stab method and 4 were negative by both broth and plate methods.

Twenty of 31 Queensland strains of *Str. faecalis* were positive by all methods, 2 by broth and plate but not stab, 2 by stab only, and 7 were negative by all methods. One of 35 Queensland strains of *Str. faecium* was positive by all methods, 3 were positive by broth and plate but not stab, 6 were negative by broth and plate but positive by stab method, and 27 were negative by all methods.

I. INTRODUCTION

Tetrazolium reduction as a means of differentiating *Streptococcus faecalis* from *Str. faecium* was first introduced by Barnes (1956). She described two methods of using this test, a liquid medium and a solid medium, which were called T.G. media. She stated that for the broth medium, positive reactions were a bright red colour in the butanol layer and negative reactions were either colourless or pale pink, and for the agar medium isolated colonies of *Str. faecalis* were deep red and those of *Str. faecium* were colourless.

Morelis and Colobert (1958) used the agar medium and described difficulties in interpreting the results. They stated that it was common to see quite large colonies with a red centre and a pink border, and that on the same plate, if large colonies were close together they were usually white or pink, but if they were widely separated, they usually had a red centre or were red all over. If the pH was altered very slightly, the white colonies were changed into red colonies, and vice versa.

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Mieth (1960) also used the agar medium. He described the colonies produced by *Str. faecalis* as large, flat, dark red with a paler margin, and a conspicuous metallic sheen, seen clearly in daylight; those produced by *Str. faecium* as colourless or pink, and if a faint metallic sheen was present, rod-like structures of formazan could be seen under a stereo-microscope; those produced by *Str. bovis* and *Str. durans* were similar to those of *Str. faecium* but very little growth occurred; those of atypical enterococci were stated to differ from the above but were not described.

Langston, Gutierrez, and Bouma (1960), Lake, Diebel, and Niven (1957) and Buttiaux (1958) used tetrazolium reduction but did not comment on the reading of results.

Bartley and Slanetz (1960), Kenner, Clark, and Kabler (1960/61) and Muller (1961) used selective media which incorporated tetrazolium for isolating enterococci from faeces, sewage and water.

This paper describes the results using deep and surface growth on agar and growth in liquid medium to determine tetrazolium reduction.

II. MATERIALS AND METHODS

The strains used were those described previously (Elder and Simmons 1963*a*), excluding one strain of *Str. bovis,* plus some strains obtained from other workers. The full list appears in Table 1.

The broth medium (pH 6·0) was prepared and used as described by Barnes (1956), being dispensed in 1-oz screw-capped bottles. The tetrazolium compound used was 2,3,5-triphenyl tetrazolium chloride. The agar medium (pH 6·0) was prepared and used in petri plates of 100-mm dia. as described by Barnes (1956) and was also dispensed in 5-ml quantities in 1-oz screw-capped bottles to form a column of media about 15 mm deep (stab culture).

To differentiate between the different types of media they will be referred to as broth, plate and stab respectively.

All were inoculated using a loopful of an 18-hr broth culture of the streptococci. The inoculum was streaked out on the agar plate to give individual colonies and the agar medium in bottles was inoculated by pushing the loop into the centre of the medium.

III. RESULTS

Using the broth, difficulty was experienced in reading the results. Some strains gave a bright red colour, others a colourless reaction, but between the two extremes there was a gradation of colour so that the decision to class the result as positive or negative was not easy.

Surface colonies were white, pink or red, or had red centres with white or pink margins. Sometimes all colonial colour variants were seen on one plate inoculated with a single strain.

TABLE 1

RESULTS OF BROTH, PLATE AND STAB METHODS OF TETRAZOLIUM REDUCTION ON 48 OVERSEAS STRAINS

Species	Strain	+++*	−++	−−+	−+−	−−−
Str. faecalis	S161	X				
Str. faecalis	B65	X				
Str. faecalis	B8	X				
Str. faecalis	N83	X				
Str. faecalis	EB/F/30/82	X				
Str. faecalis var. *zymogenes* ..	N37	X				
Str. faecalis	GB112	X				
Str. faecalis var. *liquefaciens* ..	N97	X				
Str. faecalis	N161	X				
Str. faecalis	GB122			X		
Str. faecalis	D36	X				
Str. faecalis	EB/F/30/39	X				
Str. bovis	S112			X		
Str. bovis	H6			X		
Str. bovis	S194			X		
Str. bovis	S182	X				
Str. bovis	TM/C/33			X		
Str. faecium	N55					X
Str. faecium	P1/12				X	
Str. faecium	CH1				X	
Str. faecium	N/H4					X
Str. faecium	N/GE4B				X	
Str. faecium	S98					X
Str. faecium	H24				X	
Str. faecium	P3					X
Str. faecium	P14/6	X				
Str. faecium	P6/4					X
Str. faecium	N205					X
Str. faecium	N/R64					X
Str. faecium	P17					X
Str. faecium	N/H2					X
Str. faecium	HGH511					X
Str. faecium	S748					X
Unclassified	B26	X				
Unclassified	N42	X				
Unclassified	B74	X				
Unclassified	L6		X			
Unclassified type 1	CH12					X
Unclassified type 1	P20/5					X
Unclassified type 1	P16/5					X
Unclassified type 1	P/17/8					X
Str. durans	N16					X
Str. durans	H2	X				
Str. durans	C3					X
Not designated	CN2626	X				
Not designated	CN2625	X				
Not designated	C1	X				
Not designated	CN2530	X				
		21	1	5	4	17

* Broth result first; plate result second; stab result third

When stab cultures were used, 114 strains gave either a very distinct red colour along the stab line, most readily seen by holding the tube upside down, or no change in colour. With 12 strains of *Str. faecalis,* including the varieties *zymogenes* and *liquefaciens,* the stab method gave similar results to the other methods except for strain *Str. faecalis* GB122, which gave the presumably correct positive result with the stab method but was negative by both broth and plate methods (Table 1).

Of the 5 strains of *Str. bovis,* all were positive by stab, but 4 of them were negative by both broth and plate. Sixteen strains of *Str. faecium* were all negative by the stab method except one (P14/6), although 4 were classed as positive by the plate method. Strain P14/6 was positive by both broth and plate methods.

TABLE 2

RESULTS OF BROTH, PLATE AND STAB METHODS OF TETRAZOLIUM REDUCTION FOR 31 QUEENSLAND STRAINS OF *Str. faecalis*

Species*	Strain No.	+++†	++−	−−+	−−−
Str. faecalis	1				X
Str. faecalis	2				X
Str. faecalis	6				X
Str. faecalis var. *liquefaciens*	7				X
Str. faecalis	10				X
Str. faecalis	11			X	
Str. faecalis	12	X			
Str. faecalis	15	X			
Str. faecalis	17	X			
Str. faecalis var. *liquefaciens*	20	X			
Str. faecalis	22	X			
Str. faecalis	23				X
Str. faecalis	24	X			
Str. faecalis	25		X		
Str. faecalis var. *zymogenes*	27	X			
Str. faecalis var. *zymogenes*	28	X			
Str. faecalis	30	X			
Str. faecalis var. *liquefaciens*	31	X			
Str. faecalis var. *liquefaciens*	33		X		
Str. faecalis	45				X
Str. faecalis	46	X			
Str. faecalis var. *zymogenes*	60	X			
Str. faecalis var. *liquefaciens*	65	X			
Str. faecalis	70	X			
Str. faecalis	71	X			
Str. faecalis	72	X			
Str. faecalis	74	X			
Str. faecalis var. *liquefaciens*	76	X			
Str. faecalis	77			X	
Str. faecalis var. *liquefaciens*	84	X			
Str. faecalis var. *zymogenes*	88	X			
		20	2	2	7

* Strains designations are based on results published elsewhere (Elder and Simmons 1963*d*)

† Broth result first; plate result second; stab result third

One *Str. durans* was positive by all methods and 2 were negative by all methods.

All strains labelled unclassified type 1 were negative by all methods.

Fifty-three of the Queensland strains gave similar results for all methods (Tables 2 and 3). Five strains were positive by both broth and plate method but negative by the stab method, and 8 were negative by both the broth and plate methods and positive by the stab method.

TABLE 3

RESULTS OF BROTH, PLATE AND STAB METHODS OF TETRAZOLIUM REDUCTION FOR 35 QUEENSLAND STRAINS OF *Str. faecium*

Species*	Strain No.	+++†	++−	−−+	−−−
Str. faecium	3		X		
Str. faecium	4				X
Str. faecium	5				X
Str. faecium	8				X
Str. faecium	9				X
Str. faecium	13			X	
Str. faecium	14			X	
Str. faecium	16				X
Str. faecium	18				X
Str. faecium	19				X
Str. faecium	26				X
Str. faecium	29		X		
Str. faecium	32				X
Str. faecium	41				X
Str. faecium	42				X
Str. faecium	43				X
Str. faecium	44			X	
Str. faecium	47			X	
Str. faecium	48				X
Str. faecium	49				X
Str. faecium	50				X
Str. faecium	51			X	
Str. faecium	52				X
Str. faecium	53				X
Str. faecium	54	X			
Str. faecium	55				X
Str. faecium	61				X
Str. faecium	62		X		
Str. faecium	63				X
Str. faecium	73				X
Str. faecium	75			X	
Str. faecium	80				X
Str. faecium	81				X
Str. faecium	87				X
Str. faecium	89				X
		1	3	6	25

* Strains designations are based on results published elsewhere (Elder and Simmons 1963*d*)

† Broth result first; plate result second; stab result third

IV. DISCUSSION

The main criterion for a satisfactory test based on a colour reaction is the absence of intermediate colours if the test is to be read visually. In addition, a test used for the classification of bacteria should give reproducible results.

The difficulty experienced by Morelis and Colobert (1958) and ourselves detracts from the value of the tetrazolium reduction test when done by either the broth or the plate method described by Barnes (1956), although in Tables 1-3 this is not expressed, as the result is assessed as either positive or negative.

The stab method as described in this paper materially assisted in the visual assessment as to whether or not the strains of enterococci reduced tetrazolium, and in this regard it was better than either the broth or the plate method. Somerson and Morton (1953) reported that the reduction of tetrazolium compounds was only visible when human strains of Mycoplasma were incubated anaerobically. The stab method of inoculation may result in lowered oxygen tension, bringing about increased colour visibility when streptococci capable of reducing tetrazolium were present. The stab method has not been tried using media of different pH, as it is considered desirable to standardize the technique using pH 6·0 as recommended by Barnes (personal communication).

The results on strains of *Str. bovis* are of interest, as with the stab method all were positive whereas with broth and plate methods 4 were negative. *Str. bovis* may be readily identified from the enterococci using the criteria proposed by Sherman (1937), but if the stab result obtained with these 5 strains applied to all strains of *Str. bovis* the test may be of some use in its rapid identification.

The results indicate that 4 strains of *Str. faecium* gave a positive plate result whereas the broth and stab methods were negative as recorded by Barnes (1956).

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