

RESEARCH COMMUNICATION

THE USE OF MESO-ERYTHRITOL SENSITIVITY DISCS IN THE TYPING OF BRUCELLA STRAINS

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ABSTRACT

PIETERSON, P. M., TE BRUGGE, LESLEY A. & HERR, S., 1987. The use of meso-erythritol sensitivity discs in the typing of *Brucella* strains. *Onderstepoort Journal of Veterinary Research*, 54, 651-652 (1987).

Sensitivity discs containing 1 and 2 mg meso-erythritol were found to give comparable results to the use of meso-erythritol incorporated into growth medium at 1 and 2 mg/ml. The discs proved easy and efficient when used in a disc ring together with benzyl penicillin and streptomycin sulphate discs.

INTRODUCTION

*Brucella* species show limited activity in conventional tests used to identify bacteria, and the identification of various biotypes within the species is done on the basis of specific combinations of characteristics (Corbel, Gill & Thomas, 1983). It is sometimes also necessary to differentiate between virulent field strains and the strains used in vaccines, such as *B. abortus* strain 19 (S19) and *B. melitensis* strain Rev 1 (Rev 1) (Corbel *et al.*, 1983). Among the tests used for this purpose are sensitivity to benzyl penicillin, streptomycin sulphate and meso-erythritol. The use of sensitivity test discs containing benzyl penicillin at 5 and 10 i.u. per disc, and streptomycin sulphate at 5 µg per disc is common practice (Corbel *et al.*, 1983). No discs containing meso-erythritol were available, as this substance is usually incorporated into a nutrient medium at 1 and 2 mg/ml medium (Corbel *et al.*, 1983). The addition of any such test substrate to a medium is subject to error, and each batch of medium made must be tested. This type of medium also has a limited shelf-life. It was felt that if discs containing meso-erythritol, similar to those containing the antibiotics, could be obtained, the typing process could be expedited, and fewer errors made.

MATERIALS AND METHODS

**Strains used.** Reference strains NCTC 10093 (*Brucella abortus* biotype 1), NCTC 8038 (*B. abortus* strain 19), NCTC 10512 (*B. ovis*) and NCTC 10094 (*B. melitensis* biotype 1) were used. Local strains were the S19 and Rev 1 strains used at the Veterinary Research Institute, Onderstepoort, for vaccine production, and field isolates of *B. abortus* biotype 1 (2 isolates), *B. abortus* biotype 2, S19, an erythritol resistant S19<sup>1</sup> and Rev 1.

**Media.** All typing was done on serum dextrose agar (SDA) (Corbel *et al.*, 1983). For penicillin sensitivity, benzyl penicillin was added to give final concentrations of 5 and 10 i.u./ml respectively. For streptomycin sensitivity, the final concentration was 5 µg/ml medium. For meso-erythritol sensitivity, the final concentrations were 1 mg/ml and 2 mg/ml respectively.

SDA without additives was used for the sensitivity test discs and for control cultures of each strain.

**Sensitivity test discs<sup>2</sup>.** These were specially manufactured. The ring of discs consists of 2 discs (No. 1 & 2)

containing 1 and 2 mg meso-erythritol respectively, 2 discs (No. 3 & 4) containing 5 and 10 i.u. benzyl penicillin respectively, 1 disc (No. 5) containing 5 µg streptomycin sulphate and 1 disc (No. 6) blank to act as a control (Fig. 1 & 2).

**Culture technique.** Forty-eight hour growth of each strain was harvested from a blood tryptose agar plate and suspended in phosphate buffered saline (pH 7.2) to a density of approximately  $8 \times 10^9$  organisms per ml, determined spectrophotometrically (Alton, Jones & Pietz, 1977).

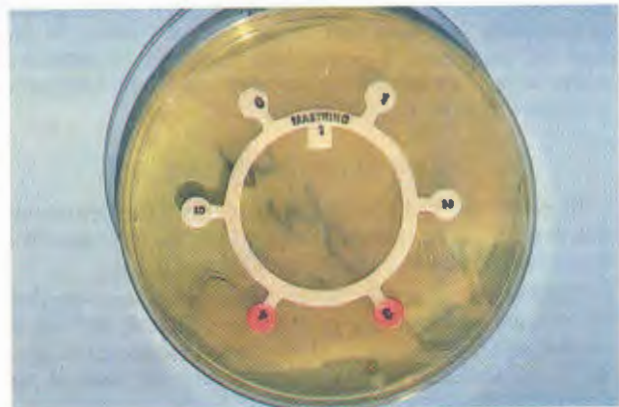


FIG. 1 A culture of *Brucella abortus* biotype 1 field strain showing growth around discs 1 and 2 (meso-erythritol), discs 3 and 4 (penicillin), no growth around discs 5 (streptomycin) and growth around disc 6 (control)



FIG. 2 A culture of *Brucella abortus* biotype 1 strain 19 showing no growth around discs 1, 2 (meso-erythritol), 3, 4 (penicillin) and 5 (streptomycin) but growth around disc 6 (control)

<sup>1</sup> Kindly confirmed by The Central Veterinary Laboratory, Weybridge, England

<sup>2</sup> Mastring S OVP 1, Mast Laboratories, Liverpool, England

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TABLE 1 Growth of various *Brucella* strains in the presence of benzyl penicillin, streptomycin sulphate or meso-erythritol, either in the medium or in a sensitivity disc

Strain	Species	Serum dextrose agar plus					Sensitivity discs with						
		P <sup>1</sup> 5	P 10	S <sup>2</sup> 5	E <sup>3</sup> 1	E 2	C <sup>4</sup>	P 5	P 10	S 5	E 1	E 2	C
NCTC 10093	<i>B. abortus</i> biotype 1	+	+	-	+	+	+	+	+	-	+	+	+
NCTC 8038	<i>B. abortus</i> strain 19	-	-	-	+	+	+	-	-	-	+	+	+
NCTC 10512	<i>B. ovis</i>	-	-	-	+	+	+	-	-	-	+	+	+
NCTC 10094	<i>B. melitensis</i> biotype 1	+	+	-	+	+	+	+	+	-	+	+	+
"S19"	<i>B. abortus</i> strain 19	-	-	+	-	-	+	-	-	+	-	-	+
462/2	<i>B. abortus</i> strain 19	-	-	-	-	-	+	-	-	-	-	-	+
325/2	<i>B. abortus</i> strain 19	-	-	-	+	+	+	-	-	-	+	+	+
325/1	<i>B. abortus</i> biotype 1	-	-	-	+	+	+	-	-	-	+	+	+
447	<i>B. abortus</i> biotype 1	+	+	-	+	+	+	+	+	-	+	+	+
325/3	<i>B. abortus</i> biotype 2	-	-	-	+	+	+	-	-	-	+	+	+
60	<i>B. melitensis</i> strain Rev I	-	-	+	+	+	+	-	-	+	+	+	+

<sup>1</sup> P = benzyl penicillin i.u./ml or per disc

<sup>2</sup> S = streptomycin sulphate µg/ml or per disc

<sup>3</sup> E = meso-erythritol mg/ml or per disc

<sup>4</sup> C = control plate or disc

Fifty µl of the suspension was dropped onto each type of medium and onto 2 plain SDA plates, and spread with a sterile glass spreader. A sensitivity test disc ring was placed onto one of the SDA plates, and the other plain SDA was used as a control. Each strain was tested in duplicate on 3 separate occasions.

All cultures were incubated for 48 h in air plus 10 % CO<sub>2</sub> at 37 °C.

**Interpretation:** A strain was regarded as sensitive to the substance added to the medium where growth was absent or markedly less than that of the control culture. In the case of the discs, a strain was regarded as sensitive where a zone of inhibition was present when compared with growth around the control disc (Fig. 1).

#### RESULTS AND DISCUSSION

In each case the results obtained from the sensitivity disc tests were the same as those where the test substance was incorporated in the medium (Table 1).

The consistent results obtained using the meso-erythritol sensitivity test discs provide an efficient and reproducible method of testing for erythritol sensitivity (Fig. 1 & 2). A further advantage to the use of discs is the considerable saving in materials.

Strain 19 is usually sensitive to meso-erythritol (Cor-

bel *et al.*, 1983), and the local vaccine strain is erythritol sensitive. However, the type culture (NCTC 8038) is erythritol-resistant (Thomas, Bracewell & Corbel, 1981), and field isolates of erythritol-resistant Strain 19 have been made overseas (Thomas *et al.*, 1981) and locally. Care must therefore be exercised in placing too much emphasis on the erythritol resistance to designate an isolate as virulent or not. Streptomycin sensitivity is only of importance in differentiating between *B. melitensis* biotype 1 and Rev I (Corbel *et al.*, 1983), and we have noted a variation in the response of S19 to streptomycin sulphate.

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