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SURFACE FIXATION TEST WITH DRIED SERUM AND WHEY FROM *BRUCELLA* INFECTED ANIMALS

by

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INTRODUCTION.

This paper is presented in conjunction with the paper in this volume by the same author, in which the method of transporting rapidly dried serum samples to the laboratory is described. A rapid agglutination test with dried *Brucella* serum absorbed into filter paper is now described. The adopted technique is quite similar to that used in paper chromatography.

The method of surface fixation was first demonstrated by Castañeda in 1950, and its application to dried serum is considered of sufficient interest to be reported.

EQUIPMENT AND MATERIALS.

1. Whatman filter paper n° 1.
2. Wire loop — 3 mm. diameter.
3. Stained antigen, prepared from *Brucella abortus* strain 19 and, stained *intra vitam* by 2, 3, 5 triphenyl tetrazolium chloride.
4. Samples of serum from cows, sheep, goats and swine.
5. Stand to hold the filter paper in saline solution.
6. Tray containing the saline.

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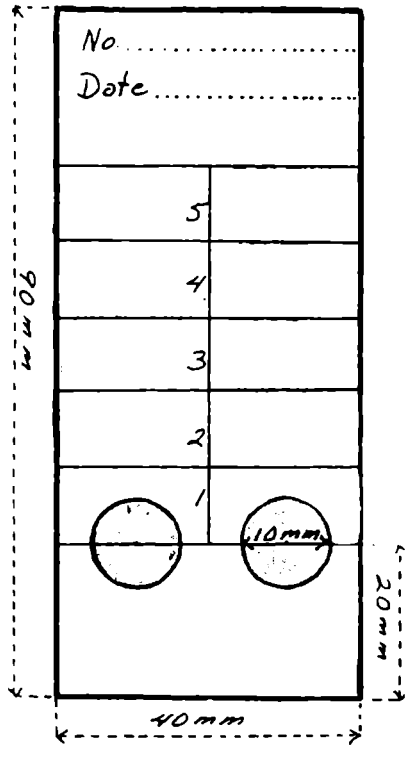
(1) *Bull. Off. int. Epiz.*, 1962, 57 (11-12). 1723-1728.

METHOD AND PREPARATION OF MATERIAL.

1. A piece of filter paper, 40×90 mm. was divided into equal parts longitudinally and subdivided into 10 mm. section horizontally, as shown in Figure I. Two circles each of 10 mm. diameter were marked symmetrically with the centers 20 mm. above the bottom of the paper and 10 mm. from the lateral sides.

Figure 1



2. *Preparation of the antigen.* The colour antigen was prepared by using *Brucella abortus* Strain 19, grown on trytose agar for 72 hours at 37° C. The cultures were washed off in 0.85 percent saline and 1 g of 2, 3, 5 - triphenyl tetrazolium chloride was added to 500 ml of suspension. The flask containing the mixture was agitated gently and put in incubator for 6 hours. By the end of this period the organisms were stained a deep purple colour. The pooled suspension was filtered through a double gauze and heated at

100° C for 30 minutes in order to kill the organisms. It was then cooled and passed through a sterile, non-absorbent cotton to remove small particles of agar or other heatcoagulated proteins. Then the refined sample was washed with saline and centrifuged at 3,000 r.p.m. three timess, until the supernatant fluid became clear. The packed *Brucella* organisms were collected in 0.5 percent phenol in sterile saline. The bacterial suspension was standardized by the Fitch-improved Hopkin's vaccine tube, the reading of which was calculated to give a concentration of 4.5 percent cells by volume, similar to the concentration of standard tube agglutination antigen. The sample was now adjusted to *p*H 7.0 and stored at 4° C. The reaction of the prepared antigen should be equal in sensitivity to standard antigen prepared by the U.S.D.A. (The Agricultural Research Service).

PROCEDURE.

One loopful of saline was put on the right-hand circle and followed by a loopful of the serum : the serum was thus diluted to 1 : 2. One loopful of the same serum was put on the left-hand circle dried inside the limit. After drying, the prepared filter paper would be ready to be mailed to the laboratory for testing. At the laboratory, a loopful of antigen is put on each of the encircled areas, in such a manner that it does not spread out of the marked limit. To avoid any evaporation, the paper was hung immediately on a stand in such a way that the lower tip was immersed in an isotonic solution of NaCl, to a depth of 10 mm. The flow of liquid moved upward over the spots and reached the top of the paper in 25 minutes.

RESULTS.

1. If the serum is positive, the union of antigen and antibody fixes on the surface of the paper, where it leaves a deep red spot in the center of the circle. The antigen is not being washsed upward by the current of saline.

2. If the serum is negative, the antigen is free and most of it is washed upward by the ascending fluid, the vortex of which lies on the upper end of the paper. The spot takes on a light red colour. In the case of an incomplete reaction the ascent would be partial, as a trace of antigen is washed up the middle of the paper.

DISCUSSION.

The resultss of surface-fixation test were confirmed by the agglutination

titers obtained by the rapid plate test, using the standard antigen, and the sensitivity of this technique was proved, since both methods gave similar results. For better interpretation of the results of paper chromatography in terms of the plate test, a table (*Table* I), was developed.

TABLE 1

No. SAMPLES	DISTANCE OF ASCENT OF ANTIGEN					RESULTS
	1	2	3	4	5	
	—	—	—	—	—	Positive.
	±	—	—	—	—	Positive.
	+	±	—	—	—	Suspect.
	+	+	±	—	—	Suspect.
	+	+	+	±	—	Negative.
	+	+	+	+	±	Negative.

The accuracy of this table should be confirmed with the results of tube agglutination test in order to gain still better interpretation.

The procedure for the fixation test on whey is the same as that explained for the dried blood serum. Research on this aspect was continued in conjunction with the whey plate test using the Standard hematoxylin milk-Ring-test antigen, which is now a routine procedure in the Department of Microbiology, School of Veterinary Medicine, University of California at Davis (Cameron, 1956).

The experiment, carried out with a number of samples of whey from cows, sheep and goats on the filter paper, gave similar results as did the fresh whey when plate-tested. This showed also the diagnostic value of the method.

The surface fixation test may be used when the infected sheep and goats are in their lactation period.

It should be pointed out that, occasionally, the high viscosity of serum or whey would restrain the spread of antigen deposited on the dried sopt. For elimination of this problem, a 1 : 2 dilution of the sample is placed on the right-hand side of the filter, as explained above; this allows the antigen to migrate with greater ease. Some factor such as a faulty agglutination phenomenon prozone, which persists in certain serum or whey, seems to disappear in the surface fixation test when a 1 : 2 dilution is used.

Finally, the results from this method should be compared with those from the tube agglutination test in order to ensure that they are in perfect

agreement with results obtained using the coloured antigen prepared from the Standard *Brucella abortus*, Strain 1119-3.

SUMMARY

By diffusion on chromatographic paper, rapid agglutination can be obtained with serum or whey which contains *Brucella* antibodies and *Brucella* antigen, stained with chloride of 2, 3, 5, triphenyl tetrazolium, whose method is described in the paper.

This surface agglutination on the paper was compared with plate agglutination. The resudts were exactly similar and have to be compared with the results by the tube-agglutination test.

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LITERATURE

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