Arch. Inst. Razi, 1965, 17, 43 - 46

THE AGGLUTINATION TEST WITH BRUCELLA SERA ABSORBED INTO FILTER PAPER

by

F. ENTESSAR *

INTRODUCTION.

Rapid shipping of blood samples from the field to the laboratory for brucellosis testing is a serious problem in many countries because of transportation difficulties. Spoilage of samples, occurring especially in summer, interferes with the sensitivity of the laboratory test.

A method of testing sera, absorbed into filter paper, was developed to overcome this difficulty. A report on this very practical procedure, suitable for areas where transportation difficulties exist, is given below.

MATERIAL AND METHODS.

Serum samples from cattle, sheep, goats and swine, either from experimental animals or from blood submitted for diagnosis, were used. Agglutination titers were previously determined in all the samples by the conventional testing method with the standard tube test antigen, prepared by the U.S. Department of Agricultural Research Service.

Each serum sample was absorbed in a strip of N 2 Whatman filter paper, 10 mm wide and 64 mm long (fig. I). It was determined that each 8 mm of

---

1. Dr. ENTESSAR is Chief of the Microbiology Section of Razi Institute, Hessarak, Teheran, Iran. While conducting this work, he was an F.A.O. Fellow at the University of California, Department of Microbiology, School of Veterinary Medicine, Davis, California.

Bull. Off. int. Epiz., 1962, 57 (11-12). 1711-1715.

the strip of this paper holds 0.01 ml of serum. Each sample was identified with a note written with an ordinary black pencil (fig. II) on the reverse side of the paper, including the date of bleeding. The paper must be completely saturated with serum. This is achieved by immersing both ends of the strip in the serum. Strips are then allowed to dry.

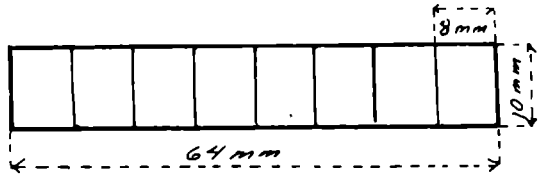

Figure I

Figure II

Two strips are required for each sample. Filter paper sheets with printed strip patterns as shown in figure I should be supplied by a central laboratory in order that the method may be uniform.

When high titration is desired, the serum is diluted to 1 : 16 per volume with saline solution, and then paper of the same dimension is dipped into this dilution.

Test procedure in the laboratory.

1. Cut the filter paper sample by the lines, and drop with forceps 8, 4, 2 and 1 sections into the tubes test 1, 2, 3 and 4, respectively.

2. Add 2 ml of antigen emulsion to each tube, such as used in the classical system; the dilution of sera will range from 1 : 25 to 1 :200 (fig. H).

3. Keep the rack with the test tubes at room temperature for 30 minutes, then shake gently. (Shaking should mix the remaining serum from the paper with the antigen solution), and incubate at 37° C or 48 hours.

4. Remove the tubes from the incubator and read the reaction while holding the tubes and shaking them slowly in front of a lamp.

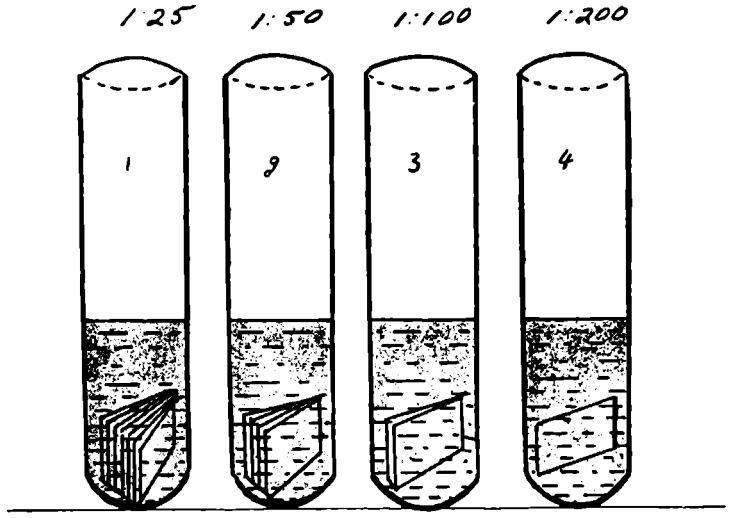

Figure III

Results.

A series of positive bovine, caprine, ovine and porcine serum samples with titers ranging from 1 : 25 to 1 : 200 were examined and tested after being absorbed into filter paper and desiccated. A parallel control series was set up and tested by the conventional tube method for fresh sera. Comparative reading of reactions after 48 hours showed that the desiccated sera gave results similar to those from the routine agglutination test.

The occasional occurrence of prozone inhibition can be removed by heating the serum as follows : add 1 ml of saline solution to each tube with the filter paper, put into water bath at 56° C for 15 minutes, then add 1 ml of twice concentrated antigen, and incubate at 37° C as above.

Whey, absorbed into filter paper, can be used instead of serum in the test. Cameron et al (1960) found that the whey test was as efficient as the blood serum test in indentifying the infected animals in brucellosis eradication programs.

Many positive samples of whey from cows, sheep and goats were exa-

mined by this method and gave similar results as the routine tube agglutination tests.

Discussion.

By keeping dried samples of serum under the influence of heat and sunlight for some time, the potency of the contained agglutinins, may become changed and a really positive reaction may not be achieved. The dried samples should, therefore, be protected from such harmful agents. They should be kept in coloured envelopes at moderate temperature.

Our preliminary investigation showed that the dried samples did not lose their agglutinin level when stored at laboratory temperature for two months. Further experiments on stability of dried specimens are necessary to determine the practical value of this method and period of time during which the sensitivity of dried sample is preserved.

SUMMARY

The absorption of serum for test on a slip of filter paper gets over the difficulty in sending samples to the laboratory for the serological diagnosis of brucellosis. A given amount of the serum is absorbed on to a fixed area of filter paper. The agglutination test is carried out at the laboratory by placing the filter paper in an emulsion of antigen.

A series of specimens of positive serum from cattle, goats, sheep and pigs were examined by this method. The results were exactly similar to those obtained by using the ordinary tube-agglutination method. Whey from milk can be used instead of the serum from blood.

Further work is necessary to find out the practical value of the method and the exact time during which agglutinins in serum dried by this method, are preserved.

LITERATURE CONSULTED

Agricultural Research Service, Animal Division Eradication Disease.

Laboratory Service, Beltsville, Md. *Brucella Abortus Antigen.* Revised, August 1959.

Cameron (H. S.), Kendrick (J. W.), Merriman (R. W.). — *J. Amer. vet. med. Ass.,* 1956, 129, 19.

Gregory (T.S.J.). — *J. comp. Path.,* 1953, 63, 171.