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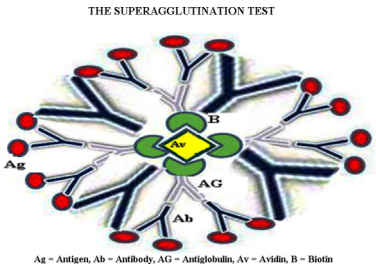
Simple solutions to false results with plate/slide agglutination tests in diagnosis of infectious diseases of man and animals

Hari Mohan Saxena *, Shubhada Chothe, Paviter Kaur

Department of Veterinary Microbiology, College of Veterinary Science, Guru Angad Dev, Veterinary and Animal Sciences University (GADVASU), Ludhiana 141004, India

Superagglutination test

GRAPHICAL ABSTRACT



ABSTRACT

We have developed a new Superagglutination test for serodiagnosis of infectious diseases. It differs from conventional plate/slide agglutination tests (PAT/SAT) by three additional steps: prior staining of serum antibody by adding a dye and addition of diluted biotinylated antiglobulin and avidin in sequence after mixing the antigen with the test serum. The new steps circumvent the problems of false positive and false negative results of PAT/SAT. In serodiagnosis of brucellosis, Superagglutination test had higher positive predictive value and specificity than Rose Bengal Plate Test (RBPT) and Standard Tube Agglutination Test (STAT) and higher negative predictive value and sensitivity than RBPT, STAT, ELISA and Complement Fixation Test (CFT).

- Superagglutination is a simple, accurate and economic screening test for infections.
- More specificity, sensitivity, positive & negative predictive value than RBPT, STAT.
- More sensitivity, negative predictive value than ELISA and Complement Fixation Test.

* Corresponding author.
E-mail address: drhmsaxena@gmail.com (H.M. Saxena).

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Background

In many countries, the standard Plate Agglutination Test is the routine screening test for human and animal brucellosis. RBPT is a variant of plate/slide agglutination test where killed *Brucella* organisms stained with Rose Bengal dye are used as antigen for detection of antibodies in the serum. The RBPT is a quick, cheap and effective test for the diagnosis of brucellosis. However, it may give false negative results [1,2]. Many factors affect the RBPT reactions and their reading. Some people are able to see the finer agglutination while many others cannot. This causes variation in results. Although the International Office of Epizootics has recommended the RBPT as one of the tests for the diagnosis of bovine brucellosis [3], some authors [4] have reported unacceptable rate of false negatives with the RBPT. Very low concentration of antibodies may not give visible agglutination. False positive results may arise due to the inability to differentiate non-specific aggregates of antigen particles alone from the true agglutinates comprising both antigen and antibody. False negative results may be due to a small clump size in sera with low titers of antibodies. False negative reactions are believed to occur mostly due to prozoning. The lack of agglutination at high concentrations of antigen or antibodies is called the Prozone effect. In Prozone, very small complexes are formed that do not clump to form visible agglutination. Prozoning may often lead to a false negative reaction in RBPT when sera of high antibody titers are tested against it. It has been suggested [5,6] that in order to get a better diagnosis of *Brucella* infection, a combination of RBPT and ELISA should be used, especially in case of samples found negative by either RBPT or STAT used alone or in combination.

Method details

Guidelines of the Institutional Animal Ethics Committee were followed in the study. Cattle and buffalo serum samples were derived from the animals in veterinary clinics, dairy farms and animal shelters in and around Ludhiana. All the animals were of age two years or more. Brucellosis suspected herds were selected for sampling primarily based on the history of abortions in the herd while normal healthy animals were sampled from the herds of the university dairy farm without the history of abortions and with repeatedly Rose Bengal Plate Test (RBPT) negative status. The new Superagglutination test and common serological tests i.e. the RBPT, STAT, ELISA and CFT were applied on all the serum samples (Table 1).

In the conventional RBPT, equal volumes (5 μl of each) of RBPT colored antigen (IVRI, Izatnagar, India) and test serum are mixed on a clean glass slide with the help of a clean sterilized toothpick. The slide is observed after 2 min for the formation of clumps. The formation of clear clumps is considered a positive test while the absence of clear clumps is considered a negative reaction. However, we modified the RBPT by incorporating the following additional steps in the RBPT. The modified RBPT as given below is named as the Superagglutination test [7,8].

Table 1
Number of positive and negative samples in each of the test conducted.

Test conducted	Number of samples		
	Positive	Negative	Total
Superagglutination	104	96	200
RBPT	97	103	200
STAT	119	81	200
iELISA	75	125	200
CFT	86	114	200