THE USE OF DIVALENT CATION CHELATING AGENTS
(EDTA/EGTA) TO REDUCE NON-SPECIFIC SERUM PROTEIN
INTERACTION IN ENZYME IMMUNOASSAY

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ABSTRACT
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An indirect enzyme immunoassay (ELISA) for detection of bovine antibody to *Brucella abortus* was
modified by the addition of divalent chelating agents to the serum diluent. This addition resulted in
an increase in specificity from 96.0% in the regular assay to 99.4% in the modified procedure. Of the
15 715 sera initially tested by the indirect ELISA, 691 that had given positive reactions were selected
for retesting in the indirect ELISA with EDTA/EGTA added. The buffered plate antigen test
(BPAT) correctly identified 98.6% of the samples as negative. The addition of chelating agents did
not alter the sensitivity of the indirect ELISA, which correctly classified 609 sera from animals from
which *B. abortus* had been isolated as positive. The sensitivity of the BPAT was 97.8%.

**Keywords:** *Brucella abortus,* cattle, chelating agents, EDTA, EGTA, ELISA, immunoassay, specificity

**Abbreviations:** ABTS, 2,2'-azinobis(3-ethylbenzthiazoline sulphonic acid); BPAT, buffered plate
antigen test; EDTA, ethylenediaminetetraacetic acid disodium salt; EGTA, ethylene
glycol-bis(~/~-aminoethyl ether)-N,N,N',N'-tetraacetic acid; ELISA, enzyme-linked immunosorbent
assay; IgG 1, immuno- globulin G 1; IgM, immunoglobulin M; Tris, tris(hydroxymethyl)aminomethane

INTRODUCTION
Enzyme immunoassays (ELISA) are used to indicate the presence of antibodies to
infectious agents. The most commonly used procedures are the indirect ELISA, which
uses an anti-species globulin, conjugated with an enzyme, as the detection reagent for
antibody bound to immobilized antigen, and the competitive ELISA in which antibody
to the immobilized antigen, conjugated with an enzyme, competes with the test
antibody (Kemeny, 1991).

When measuring antibody to *Brucella abortus* in bovine sera, non-specific
interaction has been a problem in nearly all serological tests. For instance, the tube
agglutination test devised by Wright and Smith (1897) for detection of antibody to *B.
abortus* is a highly sensitive test that measures the IgM isotype of antibody very
efficiently (Nielsen *et al.*, 1992); however, the IgM isotype is also the main contributor
to non-specific agglutination (Nielsen *et al.*, 1981). The complement fixation test (Hill,
1963) gives increased serological specificity, as do precipitation tests (Diaz *et al.*, 1979;
Jones et al., 1980), but neither eliminate the problem.

The buffered plate antigen test (Angus and Barton, 1984) has been used as a screening test for serum antibody to *B. abortus* for several years. It has a number of advantages, including the speed and ease with which it can be performed and its cost. It does, however, also have a number of disadvantages, including subjectivity of assessment of agglutination and its susceptibility to prozoning. Therefore, a number of ELISA formats have been assessed with a view to replacing the BPAT for screening for antibody. Initially, the indirect ELISA was thought to be unsuitable because of its expense, the time involved in setting up, and the low specificity of the test relative to the BPAT.

To overcome these problems, ELISA procedures were evaluated (reviewed by Nielsen et al., 1988; Wright et al., 1990). The indirect format of the ELISA was initially found to have a relatively low specificity compared to the BPAT (Nielsen et al., 1992). Specificity was considerably enhanced by using a more purified lipopolysaccharide preparation as the antigen and a monoclonal antibody specific for the IgG1 isotype, conjugated with enzyme, as the detection reagent (Nielsen et al., 1992). A competitive ELISA (Nielsen et al., 1989) was found to be rather more specific than the indirect ELISA.

The addition of divalent-cation chelating agents to the tube agglutination test reduced non-specific interaction by as much as 80% (Nielsen et al., 1979), but this modification did not decrease the time required for the test and was not widely used. It was decided to investigate the effect of adding divalent-cation chelating agents to the step in the indirect ELISA in which the bovine test antibody interacts with immobilized *B. abortus* lipopolysaccharide antigen.

### MATERIALS AND METHODS

**Sera**

Bovine sera submitted to the diagnostic laboratories for routine brucellosis serology were randomly selected. A total of 15,715 serum samples from Canadian sources (brucellosis free) were tested. In addition, 609 serum samples were obtained from animals from which *Brucella abortus* field strains or strain 19 had been cultured from tissues or secretions.

**Serological tests**

**Buffered plate antigen test:** The BPAT was performed as described by Angus and Barton (1984). Briefly, 30 μl of antigen and 80 μl of test serum were mixed on a glass plate for 30 s and incubated for 8 min at 37°C in a humidified incubator. The final pH of the mixture was 3.65. Agglutination was visually assessed as present or absent.

**Indirect ELISA:** The technique described by Nielsen and colleagues (1992) was used. Briefly, 200 ng of *B. abortus* lipopolysaccharide in 200 μl of 0.06 mol/L carbonate buffer, pH 9.6, was passively immobilized in each well of polystyrene plates (NUNC 2-69620 from Gibco-BRL, Burlington, Ontario, Canada) overnight at 20°C. The plates