

ALLEGATO A

IDEXX Paratuberculosis Screening

English version

Mycobacterium paratuberculosis Antibody Test Kit

For veterinary use only.

Name and Intended Use

IDEXX Paratuberculosis Screening is IDEXX's enzyme immunoassay for the detection of Antibodies directed against *Mycobacterium avium* spp *paratuberculosis* (MAP) in bovine individual serum, plasma and milk samples, and in individual serum and plasma samples from small ruminants.

General Information

Paratuberculosis, or Johne's disease, is a chronic enteritis of ruminants, for which main clinical signs are abundant diarrhoea and weight loss. The infection is due to an acid-fast bacillus: *Mycobacterium paratuberculosis*. Usually animals are infected during the first year of their life by ingesting food contaminated with faeces from other infected animals. Because the incubation period can be many months to several years, the disease is not manifested clinically until the animal becomes a young adult. There is no effective treatment and the infected animal ultimately dies from the disease. The method outlined in the kit is very similar to that described in the 5b/009 recommendations (Manual Of Recommended Diagnostic Techniques And Requirements For Biological Products" Volume III-Paratuberculosis 5b/009, Paris: World Organization for Animal Health, 1989-91). The antigen coated in the bottom of the wells is a protoplasmic extract of *Mycobacterium paratuberculosis*. Samples are first incubated with an extract of *Mycobacterium phlei* in order to neutralize any possible cross-reactions with atypical mycobacteria.

Descriptions and Principles

Microplates are coated with MAP Antigen. Samples to be tested are pre-incubated with *Mycobacterium phlei* extract in order to bind unspecific Antibodies. Then samples are transferred and incubated in the wells of coated microplate. Upon incubation of the test sample in the coated wells, MAP specific Antibodies form a Antibody-Antigen immune-complex. After washing away unbound material, an anti-ruminant Antibody enzyme Conjugate is added which binds to any Antigen-Antibody immune-complex. Unbound conjugate is washed away and enzyme substrate (TMB) is added. In presence of the enzyme, the Substrate is oxidized and develops a blue compound becoming yellow after blocking. Subsequent color development is directly related to the amount of Antibody to MAP present in the test sample. The result is obtained by comparing the sample Optical Density with the Positive Control mean Optical Density.