SERELISA™ ParaTB Mono Indirect

zoetis

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ENGLISH

MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS ANTIBODY TEST KIT

In-vitro diagnostic kit for the detection of *Mycobacterium avium paratuberculosis* (ParaTB) in individual bovine serum, plasma and milk samples and in individual caprine serum.

GENERAL INFORMATION AND INTENDED USES

SERELISA™ ParaTB Ab Mono Indirect uses an immunoenzymatic technique for the detection of antibodies to ParaTB in individual bovine serum, plasma and milk samples and in individual caprine serum samples. The principle of the test is as follows: Samples obtained from cattle or goats exposed to ParaTB contain specific anti-ParaTB antibodies. Samples diluted in Sample Diluent (containing *Mycobacterium phlei* extracts) are added to antigen coated wells. Specific antibody in the serum forms an antibody-antigen complex with the antigen bound to the plate. After incubation and washing the plate, a monoclonal anti-bovine IgG HRP peroxidase conjugate is added to each well. If ParaTB specific antibodies are present in the sample, the conjugate will bind with the antigen/antibody complex formed at the previous step. After a brief incubation period, the unbound conjugate is removed by a second wash step. Substrate, which contains a chromogen (ABTS), is added to each well. Chromogen color change (from clear to green-blue) occurs in the presence of the peroxidase enzyme. After the substrate has incubated, Stop Solution is added to each well to terminate the reaction and the plate is read using an ELISA plate reader at **405-410 nm**. Comparison of the assay results from unknown samples with those of known positive and negative controls provides the basis for determination of sample status in comparison to a sample/positive (S/P) cutoff.

KIT COMPOSITION AND CONSERVATION

SERELISA™ ParaTB Ab Mono Indirect contains materials sufficient to test a maximum of 460 samples.

ITEM	REAGENT NATURE	VOLUME	RECONSTITUTION AND CONSERVATION
A	5 microplates containing 12 strips of 1 X 8 wells coated with *M. a. paratuberculosis* antigen	5 x 96 wells	Remaining wells may be used for up to 3 months after the pouch is first opened, provided the plate and desiccant are resealed in the pouch and stored at 2 °C – 7 °C.
SD	Sample Diluent; preserved with phenol and gentamicin sulfate	1 x 100 mL	Ready to use
CONTROL -	40X Negative control; preserved with Microcide III	1 x 200 µL	Dilute to 1X in Sample Diluent. Use within 8 hours of dilution.
CONTROL +	40X Positive control	1 x 200 µL	Dilute to 1X in Sample Diluent. Use within 8 hours of dilution.
C	100X HRP Conjugate	1 x 800 µL	Dilute to 1X in Conjugate Diluent. Use within 2 hours of dilution.
CD	Conjugate Diluent; preserved with phenol and gentamicin sulfate	1 x 75 mL	Ready to use
W	20X Wash Solution; preserved with Imidazole	1 x 200 mL	Dilute to 1X in deionized or reverse osmosis water. Diluted Wash Solution can be stored at 18 °C - 26 °C and used for up to 3 months following dilution.
ABTS	Substrate	1 x 100 mL	Ready to use

S	5X Stop Solution (5% SDS)	1 x 25 mL	Dilute to 1X in deionized or reverse osmosis water. Diluted Stop Solution can be stored at 18 °C - 26 °C and used for up to 3 months following dilution.

REAGENTS REQUIRED TO PERFORM 92 TESTS*

a) 1 ParaTB Antigen coated microplate
b) 20 mL Sample Diluent
c) 10 µL 40X Negative Control
d) 10 µL 40X Positive Control
e) 110 µL 100X HRP Conjugate
f) 11 mL Conjugate Diluent
g) 20 mL 20X Wash
h) 11 mL ABTS Substrate
i) 2.5 mL 5X Stop

** Volumes required are applicable when 92 samples are run within one single plate.*

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

a) High precision multiple delivery pipetting devices (i.e. 1-20 µL. Measurement deviation must be ≤ 10 % for volumes ≤ 10 µL and ≤ 5 % for all other volumes)
b) 0.2 mL, 1.0 mL, and 5.0 mL pipettes and tips
c) 8 or 12 channel pipettes (or automated device)
d) 2 graduated cylinders (100 mL and 1000 mL)
e) 1 mL or 5 mL borosilicate glass test tubes
f) Uncoated low binding 96 well microplates
g) Deionized or reverse osmosis water
h) Microplate reader with **405-410 nm** filter
i) Microplate washing apparatus
j) Waste container with bleach or other oxidizing agent

WARNINGS TO THE USERS OF REAGENTS AND ANTIGEN COATED MICROPLATES

- Handle all reagents and samples as biohazardous material. It is recommended to dispose reagents and contaminated material according to the applicable regulations.
- Wear suitable protective clothing.
- Take care not to contaminate any test reagents with samples or bacterial agents.
- If the humidity indicator of a microplate exhibits a pink color, the microplate should not be used.
- The best results are achieved by following the protocols as they are described below, using good, safe laboratory techniques.
- Never add water to the microplates, conjugate, controls, or substrate.
- Do not use this kit after the expiration date.
- **NEVER PIPETTE BY MOUTH.** Harmful if swallowed.
- For veterinary use only.
- *In vitro* use only.

Refer to the end of this insert for reagent hazard and precaution statements. Also reference the Safety Data Sheet for additional details.

NOTE: STORE ALL REAGENTS PROVIDED IN THE KIT AT 2 – 7 °C. REAGENTS SHOULD NOT BE FROZEN. ALLOW COMPONENTS TO COME TO TEMPERATURE (18 °C - 26 °C) FOR AT LEAST ONE HOUR BEFORE STARTING.

SAMPLE COLLECTION

- Follow proper sample collection procedures.
- Store samples as follows:
  - Serum and plasma up to four days at 2 °C – 7 °C or -20 °C for longer.
  - Bovine milk samples should be skimmed before testing (either overnight decantation or low-speed centrifugation). Store up to five days at 2 °C to 7 °C or at -20 °C for longer. Do not use colostrum or

milk that has been collected during the first week of lactation. Any alteration of the milk sample aspect such as observed in certain clinical mastitis should lead to discarding the sample.

- Test only good quality samples (i.e.avoid bacterial contamination, heavy hemolysis or lipemia). When in doubt, obtain a better quality sample.

SAMPLE AND CONTROL DILUTION PROCEDURE

Dilute samples using the Sample Diluent provided in a clean, uncoated 96 well microplate (Sample Dilution Microplate) or using vials or microfuges tubes. Samples should be completely thawed and thoroughly mixed before diluting. The following table describes the sample and control dilution procedure using a sample dilution microplate.

SAMPLE DILUTION PROTOCOL FOR SERUM OR PLASMA:

STEP	UNITS	MATERIAL	LOCATION	FINAL DILUTION	NOTES
1)	195 µL	Sample Diluent	Each well	N/A	
2)	5 µL	Sample	Add into all wells excluding the control wells (A1, A2, B1, B2)	1:40	Mix well. Discard pipette tips after each sample.
3)	5 µL	40X Negative Control	Into wells A1 and A2	1:40	Mix well.
4)	5 µL	40X Positive Control	Into wells B1 and B2	1:40	Mix well.
5)	Allow all diluted controls and samples to equilibrate for 5 minutes before transferring to the ELISA microplate				
6)	Proceed to STEP 7.				

SAMPLE DILUTION PROTOCOL FOR MILK:

STEP	UNITS	MATERIAL	LOCATION	FINAL DILUTION	NOTES
1)	75 µL	Sample Diluent	Add into all wells excluding the control wells (A1, A2, B1, B2)	N/A	
2)	195 µL	Sample Diluent	Into wells A1, A2, B1 and B2	N/A	
3)	75 µL	Sample	Add into all wells excluding the control wells (A1, A2, B1, B2)	1:2	Mix well. Discard pipette tips after each sample.
4)	5 µL	40X Negative Control	Into wells A1 and A2	1:40	Mix well.
5)	5 µL	40X Positive Control	Into wells B1 and B2	1:40	Mix well.
6)	Allow all diluted controls and samples to equilibrate for 5 minutes before transferring to the ELISA microplate				

PREPARATION OF 1X CONJUGATE, 1X WASH AND 1X STOP SOLUTIONS

STEP	UNITS	MATERIAL	LOCATION	NOTES
1X CONJUGATE SOLUTION				
7)	10.89 mL	Conjugate Diluent	Clean test tube or bottle	Mix well. 1:100 final dilution.
8)	110 µL	100X Conjugate		
1X WASH SOLUTION				
9)	20 mL	20X Wash	Microplate washing bottle or apparatus	Mix well. 1:20 final dilution
10)	380 mL	Deionized or reverse osmosis water		
1X STOP SOLUTION				

11)	2.5 mL	5X Stop	Clean tube or bottle	Warm 5X Stop Solution to room temperature or to 37 °C and mix to dissolve any precipitates.
12)	10 mL	Deionized or reverse osmosis water		Mix well. 1:5 final dilution

ELISA TEST PROCEDURE

STEP	UNITS	MATERIAL	LOCATION	NOTES
a)	Remove the test microplate (or the required number of strips if whole microplate is not needed) from the protective bag. Return any unused wells to a pouch with desiccant and properly seal for storage.			
b)	100 µL	Diluted controls and samples (Step 5)	Add into appropriate microplate wells	Change pipette tips for each new sample
c)	Incubate microplate for 30 minutes (± 5 min) at 18 °C - 26 °C.			

WASH PROCEDURE

STEP	UNITS	MATERIAL	LOCATION	NOTES
d)	Either manually or with an automated washer, discard or aspirate solution from all wells into an appropriate waste container.			**Wash process is a critical step for an ELISA. Please follow steps d through i.**
e)	300 µL	Diluted Wash Solution (Steps 9-10)	Each test well	
f)	Allow Wash solution to soak in wells for 10-15 seconds			
g)	Discard or aspirate solution from all wells into an appropriate waste container.			
h)	**Repeat wash procedure (steps e-g) 2 more times.**			
i)	Invert and blot onto absorbent pad to remove remaining liquid after final wash.			

ADDITION OF 1X CONJUGATE, SUBSTRATE, AND 1X STOP SOLUTION

STEP	UNITS	MATERIAL	LOCATION	NOTES
j)	100 µL	1X Conjugate Solution (steps 7-8)	Each test well	Discard pipette tips.
k)	Incubate for 30 minutes (± 5 min) at 18 °C - 26 °C.			
l)	Follow the **WASH PROCEDURE** above (steps d to i).			
m)	100 µL	Substrate	Each test well	Discard pipette tips.
n)	Incubate for 15 minutes (± 1 min) at 18 °C - 26 °C.			
o)	100 µL	1X Stop Solution (Steps 11-12)	Each test well	Discard pipette tips.
p)	Gently tap to mix contents within the wells. Read the microplate using an ELISA microplate reader set at a single wavelength of 405 or 410 nm. Allow bubbles to dissipate before reading.			

RESULTS

ASSAY CONTROL VALUES, VALID ELISA RESULTS:

Negative Control average optical density (OD) is ≤ 0.25.
Positive Control average OD is between 0.40 and 1.20.
If either value is out of range, the test results should be considered invalid and the samples should be retested.

MANUAL PROCESSING OF DATA

a) Calculate the average Negative Control absorbance optical density (OD) using the values of wells A1 and A2. Calculate the average Positive Control OD using values obtained from wells B1 and B2. Record both averages.
b) Subtract the average Negative Control OD from the average Positive Control OD. The difference is the Corrected Positive Control.

c) Calculate a sample to Positive (S/P) ratio by subtracting the average Negative Control OD from each sample OD and dividing the difference by the corrected Positive Control. Use the following equation format:

$$S/P = \frac{(SAMPLE\ OD) - (AVERAGE\ NEGATIVE\ CONTROL\ OD)}{CORRECTED\ POSITIVE\ CONTROL}$$

EXAMPLE:

Example Positive Control ODs:
0.585 and 0.605
Average = (0.585 + 0.605) / 2 = 0.595

Corrected Positive Control:
(0.595) – (0.067) = 0.528

Example Negative Control ODs:
0.072 and 0.062
Average = (0.072 + 0.062) / 2 = 0.067

Example S/P value calculation:
OD of sample = 0.560
[(0.560) – (0.067)] / 0.528 = 0.934

INTERPRETATION OF RESULTS
The SERELISA™ ParaTB Ab Mono Indirect S/P values should be interpreted using the following ranges:

For bovine serum or plasma and caprine serum:

a) Negative: Serum or plasma samples with S/P value < 0.70 are presumed negative for antibody.
b) Positive: Serum or plasma samples with a ParaTB S/P ratio value ≥ 0.70 are presumed positive for antibody.

The SERELISA™ ParaTB Ab Mono Indirect S/P values should be interpreted using the following ranges:

For bovine milk:

a) Negative: Bovine milk samples with a ParaTB S/P ratio value < 0.40 are presumed negative for antibody.
b) Positive: Bovine milk samples with a ParaTB S/P ratio value ≥ 0.40 are presumed positive for antibody.

The complex pathophysiology and immune response to *Mycobacterium avium* subspecies *paratuberculosis* is limiting the ability to determine the infection status of individual cattle utilizing single serologic tests. SERELISA™ ParaTB Ab Mono Indirect is intended for use as a part of a *Mycobacterium avium* subspecies *paratuberculosis* control program in conjunction with additional testing methods such as fecal culture or fecal PCR or combining results with herd level status to determine the MAP infection of individual animals. The milk test is not intended to be used as a confirmatory test for individual animals.

Any test should be interpreted in the context of all available individual and herd clinical, historical and epidemiological information relevant to the animal(s) under test. Further confirmatory testing may be required in certain circumstances.

Reviews for MAP testing strategies for herd and individual animals are available to clarify advantages and limitations of individual tests, and to aid in test deployment for specific herd goals.

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M.T. Collins Diagnosis of paratuberculosis Vet Clin Food Anim, 27 (2011), pp. 581-591

Diagnosis and Control of Johne's Disease National Research Council (US) Committee on Diagnosis and Control of Johne's Disease. Washington (DC): National Academies Press (US); 2003.