

Prionics USA, Inc.

Parachek the original Johne's ELISA test kit



Tom Kellner, D.V.M.

Manager: Diagnostics

Prionics USA, Inc.

Prionics Core Products

- TSE
- Paratuberculosis (Johne's Disease)
- Tuberculosis
- BVDV
- Typifix
- Bolus / Implants
- Other PrioCHECK ELISA Products (FMD, CSF, Salmonella, BLV...)
- forensiX

PARACHEK®

“The Original Johne’s absorbed ELISA”

2 plate kit



30 plate kit



Commercial History

- Developed in 1991 by CSL/ Melbourne Australia
- 1st USDA license (serum) and use in the US (until 1997)
- 2nd USDA license (serum) in 2000 (Biocor/CSL).
- Collaboration with Antel Bio (2000)
- USDA license for sheep and goats (2003)
- Biocor → Pfizer Animal Health (2003) → Prionics (2005).
- USDA license for milk (2006)
- Parachek 2 (2010)

PARACHEK[®] - Test Principle



Indirect enzyme immunoassay to detect antibodies to *M. paratuberculosis* in serum

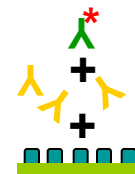
① Milk or blood collection:



② Milk or Serum absorption:

Cross-reactive antibodies are removed with *M. phlei* absorbing antigen

③ Parachek[®] EIA:



HRP labelled α -bovine Ig

Diluted serum samples

M. paratuberculosis antigens

④ Test interpretation:

ANIMAL STATUS

Negative \leq Cutoff*

Positive $>$ Cutoff*

* Mean of the negative controls plus 0.100

Step by Step presentation



**Serum Absorption
for samples and
controls (30min)**



**Plate incubation
(30min)**



**Plate wash step (6
washes)**



**Conjugate
incubation (30min)**



**Plate wash step (6
washes)**



Add Substrate



**Add Stop Solution
when OD of
positive controls
is 0.35-0.40**



**Read OD results
(450 nm filter),
2-20 min after stop**

Option for high throughput automation
Stop after 30 min.

Package Insert

Mycobacterium paratuberculosis Test Kit for Cattle PARACHEK® 2

An *in vitro* diagnostic test kit for detection of antibodies to *Mycobacterium avium* subsp. *paratuberculosis* in serum of cattle, sheep and goat and milk of cattle

184 Maximum Test Samples – Two (2) Microplate Test Kit
2760 Maximum Test Samples – Thirty (30) Microplate Test Kit

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Version 1.0_e

Package Insert

For *in vitro* veterinary diagnostic laboratory use only.
Store at 5±3°C
Product No.: 63327/63328

The protocol for milk samples has been optimized and validated by Antel BioSystem Inc.

Introduction

Johne's disease is a chronic, debilitating enteritis of ruminants caused by infection with *Mycobacterium paratuberculosis*.

During the active stage of infection and prior to onset of clinical disease, animals generally develop antibodies to *M. paratuberculosis* antigens. Uninfected animals lack specific antibodies to *M. paratuberculosis*, but may have cross-reacting antibodies to other mycobacteria. These cross reacting antibodies can be removed by absorption of serum with *M. phlei* prior to commencement of the EIA¹.

The automated version of the PARACHEK® 2 is based on the original Johne's absorbed EIA and shows the same high performance. It detects antibodies to *M. paratuberculosis* in serum and milk and may be used as a specific test for Johne's disease in cattle. This original PARACHEK® 2 has been evaluated on animals from Johne's disease-endemic and Johne's disease-free regions of Australia² and the United States³. The test specificities were 99% or greater for cattle, sheep and goats. Calculations of sensitivity were affected by the history of the cattle herd under test. However, the EIA detected up to 80% of animals before onset of clinical disease and 60-65% of faecal shedders were EIA positive on or before first detection of *M. paratuberculosis* in their faeces². For sheep, the test sensitivity estimates were 38-44%⁴ and for goats, the sensitivity ranged from 65-88%⁵.

The test should permit epidemiological studies and be a useful tool in the management and control of Johne's disease in cattle, goat and sheep.

appropriate. Chemical hazard data are available in section "Safety Regulations and R&S Statements".

Product Numbers

Two (2) Microplate Test Kit: 63327
Thirty (30) Microplate Test Kit: 63328

Component 1

Microtitre Plates coated with *M. paratuberculosis*

63327: 2 x 96 well plates with lids
63328: 30 x 96 well plates with lids
Ready for use.

Component 2

Positive Control

63327: 1 x 0.75 ml
63328: 1 x 2.0 ml
Contains 0.01% w/v thimerosal.
Ready for use.

Component 3

Negative Control

63327: 1 x 0.75 ml
63328: 1 x 2.0 ml
Contains 0.01% w/v thimerosal.
Ready for use.

Component 4

Green Diluent: Sample diluent buffer

Contains 0.01% w/v thimerosal.
63327: 1 x 100 ml
63328: 3 x 500 ml
Ready for use.

Component 5

Wash Buffer – 20x Concentrate

63327: 1 x 100 ml

General Precautions

1. Laboratory Safety

Correct laboratory procedures should be applied at all times.

2. Enzyme Stopping Solution

The Enzyme Stopping Solution is a strong acid. Wipe up spills immediately. Flush the area of the spill with water. If the stopping solution contacts the skin or eyes, flush with copious quantities of water and seek medical attention.

Equipment required but not provided

- Accurate, replaceable-tip variable-volume pipettes (to deliver 475 µl, 25 µl and 10 to 120 µl)
- Graduated 1, 5 and 10 ml pipettes
- Measuring cylinders - 100 ml, 1 litre and 2 litres
- Suitable microtitre plate washer/dispenser
- Suitable microplate shaker
- Multichannel pipette (to deliver 50 and 100 µl)
- Suitable microtitre plate reader. This reader MUST be fitted with filters to read at 450 nm and 620-650 nm.
- Tubes, 1 ml plastic racked in 96 well format or
- 1.2 ml 96-deep well plates.

Preparation of Reagents

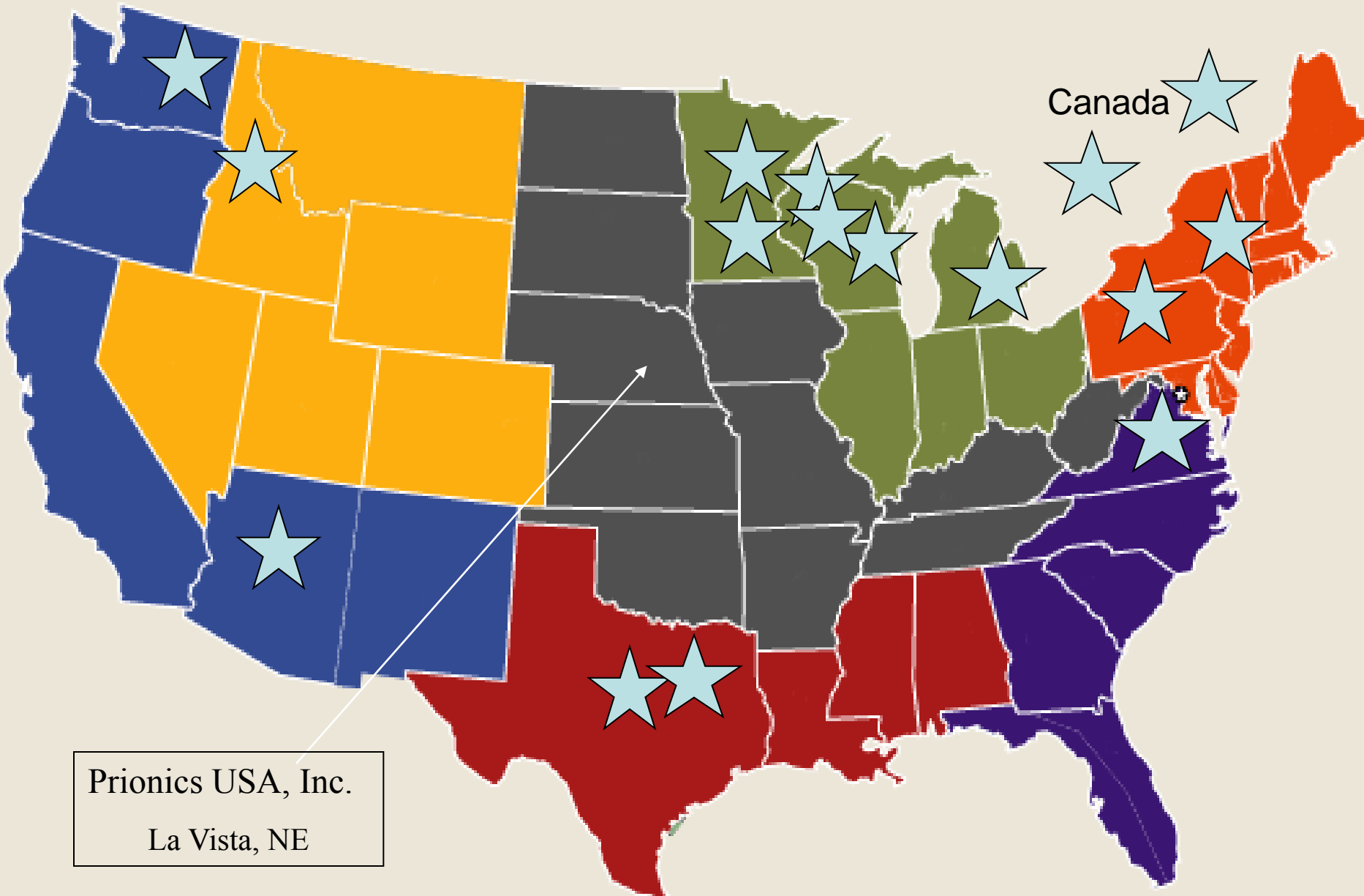
1. Microtitre Plates coated with *M. paratuberculosis*

Allow plates to equilibrate to room temperature (22±3°C) for at least 30 minutes before unsealing

- Package Inserts (PIs) are in Prionics format

Milk Testing

- August 2000 – Development
- 2006 USDA License for Parachek Milk Test
- Setup of Testing Laboratories and infrastructure throughout the US and Canada.
- Improvements in Uploading Data into Data Processing Centers
- Quality Monitoring: Proficiency Testing through QCS



Canada

Prionics USA, Inc.

La Vista, NE

Our Focus is on.....

- Quality Service (both technical and customer support)
- Product quality and consistency
- Uniform testing and consistency
- Continuous Improvements in our products, lab testing, data management....etc.
- New products: Development of milk platform.

PARACHEK 2[®]

“The Original Johne’s absorbed ELISA”

2 plate kit



30 plate kit



“Parachek 2 is Parachek with additional flexibility in testing”

PARACHEK® & PARACHEK® 2

| | Current PARACHEK | PARACHEK® 2 |
|--------------------------|--|--|
| Samples | Serum: 25 µl, 1:20 with Green Diluent | Serum: ≥10 µl , 1:20 with Green Diluent Milk: ≥ 100 µl , 1:2 with Green Diluent |
| Preabsorption | Milk: 200 µl, 1:2 with Green Diluent Incubation 30-60 min | Incubation 30 min – 24 h |
| Washing | 6x , manually | 6x, automated plate washer recomm. |
| Sample incubation | Incubation 30 min | unchanged |
| Conjugate | 1+100, use immediately Incubation 30 min | 1+100, stability for 8h at 5°C ± 3°C Incubate for 30 min |
| Substrate | 2 components | One component |
| Stopping Reading | Kinetic measurement (620 nm) until Pos control 0.35-0.40. Stop. Read (450 nm) within 2-20 min after termination | Kinetic and End-point 1) Kinetic measurement (620 nm) until Pos control 0.35-0.40. Stop. 2) 30 min incubation. Stop. Read (450 nm) with reference wavelength (620 nm) within 2-60 min after termination |
| Validity criteria | Pos Control 0.9-1.2 Neg Control < 0.15 | Pos Control > 0.9 end-point 0.90 – 1.20 kinetic Neg Control < 0.2 end-point |

Summary

- Parachek, the original Johne's ELISA test has been commercially available since 1991. Long track record. Benchmark in Johne's testing.
- Parachek milk ELISA was developed in 2000
- USDA license for Parachek in milk in 2006.
- Prionics is focused and committed on providing:
 - strong customer and technical support
 - Product consistency and Quality
 - Continuous improvement to simplify processes
- Parachek 2

Questions?

