INTERPRETATION

The following criteria must be met with in order to validate the analysis (otherwise, the assay must be repeated):

- The negative control wells must be colorless or pale blue.
- The positive control wells must be dark blue.

Interpretation:

If the blue color is darker than the one obtained for the corresponding negative control, the sample is positive for this pathogen. Otherwise, the sample is negative.

BIBLIOGRAPHY

1. Thorns CJ, Bell MM, Chasey D, Chesham J, Roeder PL. (1992) Development of monoclonal antibody ELISA for simultaneous detection of bovine coronavirus, rotavirus serogroup A, and *Escherichia coli* K99 antigen in feces of calves. *Am J Vet Res*, **53**:36-43.

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Rotavirus, Coronavirus, *Escherichia coli* K99 Antigen Test Kit (ELISA)
Pathasure® Enteritis
Insert

2009-02-02

This kit is an immunoenzymatic assay intended for the detection of rotavirus, coronavirus and *Escherichia coli* (*E. coli*) K99 antigens in bovine feces.

Diarrhea problems in the calf are often caused by rotavirus, coronavirus, or *E. coli* K99. These problems can lead to important economical losses since affected animals generally show reduced weight gain, sometimes resulting in death. Quick identification of the pathogen involved helps in disease control. It is recommended to test the feces of at least 3 calves per infected herd throughout symptom evolution.

PRINCIPLE OF THE TEST

Feces samples are diluted and incubated in wells coated with a mixture of 3 antibodies (Abs) specific to rotavirus, coronavirus, and *E. coli* K99. If one of these pathogens is in the feces samples, it will bind to its specific Ab already in the wells. After several washes to eliminate unbound substances, a conjugate (an Ab coupled to an enzyme) targeted at either rotavirus, coronavirus, or *E. coli* K99 is added. After incubation, excess of this conjugate is eliminated by a second wash and its attachment to the specific pathogen is revealed with a chromogenous substrate. Following this incubation, the enzyme, if present, reacts with the substrate and a blue color develops. The intensity of the color allows the determination of the type of sample tested. A negative sample will show a weak reaction (colorless, pale blue) whereas a positive sample will show a reaction stronger than the one for the negative control (darker blue than negative control).

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MATERIAL

Materials required but not provided:

- Purified water
- Test tubes for sample dilution
- Containers for dilution of other solutions

Reagents provided with the kit:

Components	Quantity
• Strips of 8 wells coated with a mix of 3 Abs specific to rotavirus, coronavirus, and *E. coli* K99	12
• Ready-to-use positive control (dark yellow)	2.0 mL
• Ready-to-use negative control (pale yellow)	2.0 mL
• Ready-to-use dilution buffer	200 mL
• Concentrated wash solution (50X)	20 mL
• Ready-to-use anti-rotavirus conjugate (red)	8.5 mL
• Ready-to-use anti-coronavirus conjugate (blue)	8.5 mL
• Ready-to-use anti-*E. coli* K99 conjugate (green)	8.5 mL
• Substrate A	14 mL
• Substrate B	4 mL
• 3 mL pipette*	1
• 1 mL pipettes	30

* Can be used for the preparation of the wash solution or for the distribution of the dilution buffer in the tubes. Pipette should be rinsed with water after each use.

PRECAUTIONS

- For *in vitro* veterinary use only.
- The materials used in this kit must be considered as infectious. Therefore, all waste must be decontaminated before being discarded.
- Do not use the kit after the expiry date indicated on the package.
- Do not mix the reagents from different serial numbers.
- The sensitivity and specificity of this test are guaranteed only if the procedures are strictly observed. It is strongly recommended not to test more than 6 strips at the same time.
- Do not expose substrate B to either light or oxidizing agent. Always keep the substrate in a plastic container. This solution might cause skin or eye irritation.
- Keep all reagents at 2-7°C and bring to room temperature before use.

EXECUTION

A. Preparation of wash solution

After homogenizing the concentrated wash solution, dilute at 1/50 with purified water (e.g., 10 mL 50X concentrated wash solution in 490 mL purified water).

B. Sample preparation

Dilute feces samples in dilution buffer at 1/10 (e.g., 0.5 mL feces sample in 4.5 mL dilution buffer). Make sure you use new material (pipettes, tubes) for each feces sample. Also make sure each dilution is properly mixed before being distributed into the wells (only supernatants must be distributed). Feces sample can be kept at 2-7°C up to 72 hours before diluting them. Once diluted, they can be kept at 2-7°C up to 36 hours before being tested.

C. Test procedures

Bring all reagents to room temperature and mix well manually before use. Two controls are supplied with this kit. They must be used every time an assay is performed.

1. Make a schematic representation of the plate and the distribution of controls and samples.
2. Select the necessary number of strips by breaking the plastic between the strips. Three wells are needed for each sample and control (1 well for each pathogen).
3. Dispense 2 drops ready-to-use positive control into wells A1, A2, and A3. For a better distribution, hold the dropper bottle vertically above the wells. The drops have to fall freely into the well without touching the sides of the well.
4. Dispense 2 drops ready-to-use negative control into wells B1, B2, and B3.
5. Dispense 2 drops diluted samples (see section B) into wells C1/C2/C3, D1/D2/D3, …
6. Incubate at 23 ± 2°C for 30 minutes.
7. Empty the plate contents in a sink and wash each well 5 times with 1X wash solution (see section A). Throw away all liquid contained in the plate after each wash. After the last wash, dry the plate by tapping it on absorbent paper. Do not scrub the inside of the well.
8. Dispense 2 drops ready-to-use red conjugate (anti-rotavirus) into each well of column 1.
9. Dispense 2 drops ready-to-use blue conjugate (anti-coronavirus) into each well of column 2
10. Dispense 2 drops ready-to-use green conjugate (anti-*E. coli* K99) into each well of column 3.
11. Incubate at 23 ± 2°C for 30 minutes.
12. Repeat step 7.
13. Dispense 2 drops substrate A into each well.
14. Dispense 1 drop substrate B into each well.
15. Stir the plate to mix the content.
16. Incubate, away from light, at 23 ± 2°C for 15 minutes.
17. Stir the plate and read the results.