CRYPTOSPORIDIUM TEST STRIPS

For all species

BIO K 155

Diarrhoea is a major cause of mortality in young cattle under one month. Bovine neonatal gastroenteritis is a multifactorial disease. It can be caused by viruses: coronavirus or rotavirus, by bacteria: *Salmonella* or *E. coli* F5 (K99), or by protozoan microorganisms such as *Cryptosporidium*.

The diagnosis of the etiological agent of diarrhoea can only be performed in the laboratory because clinical signs do not allow to differentiate between the different microorganisms. It is possible to identify *Cryptosporidium* by means of floating or staining techniques (Ziehl Neelsen modified). However, these techniques are labor intensive and unpracticle. These classical techniques have rapidly been replaced by the ELISA technology because of its simplicity, and the limited requirements in laboratory equipment. Nevertheless, ELISA can be time consuming and expensive especially when small number of analysis has to be performed.

Chromatographic lateral flow immunoassay is becoming the gold standard for gastroenteritis diagnosis because of its simplicity, rapidity, sensitivity and specificity. Laboratory equipment required is limited. Results compared with classical techniques are rather similar in terms of diagnosis and strips are far easier to use.

Use of the kit
The kit is designed to detect *Cryptosporidium* in young ruminants stool. It also could be used with other species.

Reliable Results
The use of monoclonal antibody as conjugate and to capture *Cryptosporidium* ookysts on the strip ensures excellent specificity and very reliable results.

Ease-of-Use
Minimal hands-on-time
Room temperature incubation
Results available in 10 minutes.
Example of results

### Flotation

<table>
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<tr>
<th></th>
<th>+</th>
<th>-</th>
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<tr>
<td>+</td>
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<td>3</td>
<td></td>
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<tr>
<td>-</td>
<td>2</td>
<td>63</td>
<td>65</td>
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<tr>
<td></td>
<td>34</td>
<td>66</td>
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Specificity: 95.5 %
Sensitivity: 94.1 %

### PCR

Trotz-Williams et al. Veterinary parasitology
134 (2005) 15-23

<table>
<thead>
<tr>
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Specificity: 93.3 %
Sensitivity: 78.3 %
Composition of the kit

BIO K 155  CRYPTOSPORIDIUM STRIP KIT

10 spoon vials with dilution buffer
1 vial with 10 Cryptosporidium strips
1 insert

Stability : 1 year between +10°C and +25°C at a dry place

Bibliography

Multiattribute evaluation of two simple tests for the detection of Cryptosporidium parvum in calf faeces
Lise A. Trotz-Williams, S. Wayne Martin, Donald Martin, Todd Duffield, Kenneth E. Leslie, Daryl V. Noydamp, Frames Jamieson, Andrew S. Peregrine
Veterinary parasitology 134 (2005) 15-23

Evaluation of a Bovine Concentrated Lactoserum for Preventing Neonatal Diarrhoea in Belgian Blue Calves
S. Vandeputte, J. Detilleux, S. Carel, B. Bradfer, H. Guyot and F. Rollin
The Open Veterinary Science Journal, 2010, 4, 36-40
DETENTION OF ENTEROPATHOGENS INVOLVED IN CALF NEONATAL DIARRHEA: VALIDATION OF ELISAs AND LATERAL FLOW IMMUNOASSAYS AS COMPARED WITH REFERENCE METHODS


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Key words: Calf; neonatal diarrhea; diagnostic enteropathogens; ELISA; lateral flow immunochromatography; PCR

1. Introduction and Objectives
Several pathogens play a role in calf neonatal diarrhea. The major enteropathogens involved are Escherichia coli F5799 (E. coli), Cryptosporidium parvum, bovine enteric coronavirus, bovine rotavirus and bovine viral diarrhea virus. In our laboratory different methods – e.g. selective culture for E. Coli F5799, microscopic examination of faecal smear for Cryptosporidium parvum, a commercially available latex agglutination test for bovine rotavirus, and a commercially available antigen-detection-ELISA for BVDV are routinely used for detection of these agents. For bovine enteric coronavirus no routine diagnostic method was implemented until now.

The objectives of this study were to evaluate two commercially available antigen-detection-ELISA kits and two lateral flow immunochromatography tests (on site tests) for the detection of four of the above mentioned pathogens.

2. Materials and Methods
2.1 Samples
All necropsy rectal contents were sampled from calves between 0 and 6 weeks of age with diarrhea (n=216). Samples were investigated by routine procedures and then stored at -20 °C to enable backwasting testing.

2.2 ELISAs
Samples were tested in two different ELISA kits according to the instructions of the manufacturer. Samples positive for bovine coronavirus in one or both ELISAs were tested by a coronavirus-specific PCR for confirmation.

2.3 Lateral flow immunochromatography tests
A subset of 100 samples with a more or less equal distribution of positive results for the four pathogens of interest, were tested by two lateral flow strip tests (C and D). Tests A and C were produced by the same manufacturer. All samples of this subset were also tested for bovine coronavirus by PCR.

3. Results
Agreement was assessed in table 1. For E. coli F5799, the number of positives in the reference test and other tests was comparable. For rotavirus and cryptosporidium, slightly more samples were positive in ELISAs and slightly less samples were positive in the tests than in the reference tests. Agreement between ELISA tests was also good, and correlation coefficients between ELISA results were high for the four enteropathogens evaluated.

Table 1. Level of agreement between different tests for four enteropathogens associated with neonatal diarrhea in calves, displayed as x-values (Kappa)

<table>
<thead>
<tr>
<th>Reference method</th>
<th>E. coli F5799</th>
<th>bovine rotavirus</th>
<th>bovine coronavirus</th>
<th>Cryptosporidium parvum</th>
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<tr>
<td>BID K 348</td>
<td></td>
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<tr>
<td>ELISA kit A</td>
<td>0.93</td>
<td>0.80</td>
<td>0.55</td>
<td>0.81</td>
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<td>ELISA kit B</td>
<td>0.96</td>
<td>0.72</td>
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<td>BID K 156</td>
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<td></td>
<td></td>
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<tr>
<td>Test kit C</td>
<td>0.89</td>
<td>0.91</td>
<td>0.37</td>
<td>0.85</td>
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<tr>
<td>Test kit D</td>
<td>0.91</td>
<td>0.72</td>
<td>0.05</td>
<td>0.73</td>
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</tbody>
</table>

4. Discussion and Conclusions
Hardly any literature is available concerning diagnostic performance of commercially available ELISA kits and lateral flow kits for detection of the major enteropathogens involved in calf neonatal diarrhea (2, 3). All kits showed satisfactory diagnostic performance for detection of E. coli F5799, bovine rotavirus and Cryptosporidium parvum, with kits A and C showing the highest kappa-values. For detection of bovine coronavirus, kit D fails almost completely, whereas kappa-values of the other kits were rather poor. The reference test, however, was PCR. Considering the relative low detection limits of PCR in general, the clinical significance of these PCR results remain to be seen (1).

Also the significance of – frequently occurring – combinations of enteropathogens in calf neonatal diarrhea may cause a headache for the veterinary practitioner.

5. References
Performing the test.

Take a sample of stool using the enclosed measuring spoon. If the samples are liquid, collect one level spoonful (Photo 1) and dilute with the liquid contained in the bottle (Photo 2).

Homogenize well, taking care to prevent foam formation.

If the samples are solid (Photo 3), remove the excess amount with a spatula or clean object (Photos 4), then dilute and homogenize as indicated above.

Plunge a strip, with the arrow pointing down, into the liquid. The red portion of the strip must not be immersed in the liquid. Wait at most 10 minutes and interpret the result by comparing it with Photo 5.

To safeguard the reagents’ stability, take care to close the tube containing the strips immediately.
Precautions for use

- If the reagent is to work properly, the red portion of the strip must not be below the surface of the liquid at any time. To prevent this happening, avoid producing foam when diluting the sample.
- The test sample must not be too concentrated. Do not make up a sample that is greater than the volume of one level spoonful.
- Wear gloves when carrying out the test.
- The kit must be kept at room temperature in a dry place. Close the tube immediately after use, for humidity greatly alters the strips’ stability.

1 line = negative
2 lines = positive