

研究用

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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 These classical techniques have rapidly been replaced by the ELISA technology because of its simplicity, and the limited requirements in laboratory equipment.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

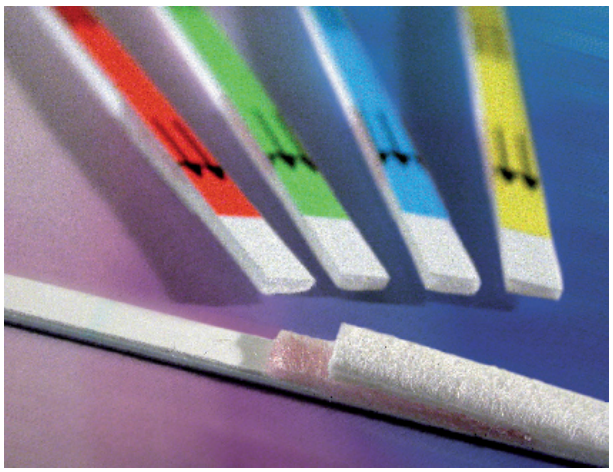
The kit is designed to detect *Cryptosporidium* in young ruminants stool. It also could be used with other species.

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

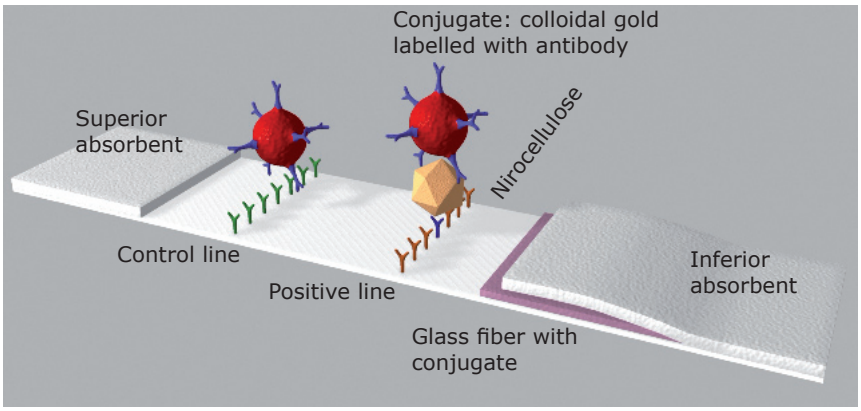
Minimal hands-on-time
Room temperature incubation
Results available in 10 minutes.





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Example of results

Flotation

Crypto STRIPS BIO K 155

	+	-	
+	32	3	35
-	2	63	65
	34	66	100

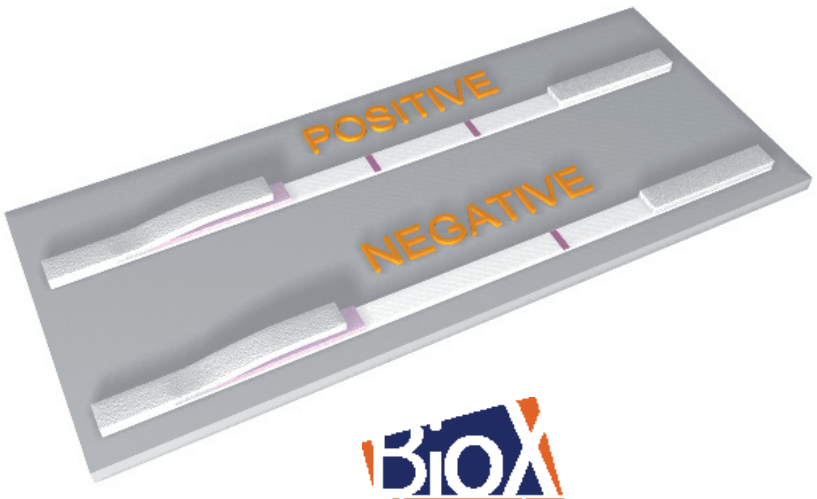
Specificity: 95.5 %
Sensitivity: 94.1 %

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

Crypto STRIPS BIO K 155

	+	-	
+	47	5	52
-	13	70	83
	60	75	135

Specificity: 93.3 %
Sensitivity: 78.3 %



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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. Nydam, Frames Jamieson, Andrew S. Peregrine

Veterinary parasitoloy 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

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DETECTION OF ENTEROPATHOGENS INVOLVED IN CALF NEONATAL DIARRHOEA:
VALIDATION OF ELISAs AND LATERAL FLOW IMMUNOASSAYS AS COMPARED WITH REFERENCE METHODS

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Key words: Calves; neonatal diarrhoea; diagnosis; enteropathogens; ELISA;lateral flow immunochromatography; PCR

1. Introduction and Objectives

Several pathogens play a role in calf neonatal diarrhoea. The major enteropathogens involved are *Escherichia coli* F5⁺/K99 (*E. coli*), *Cryptosporidium parvum*, bovine enteric coronavirus, bovine rotavirus and bovine viral diarrhoea virus. In our laboratory different methods – e.g. selective culture for *E. Coli* F5/K99, microscopic examination of faecal smears 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 At necropsy rectal contents were sampled from calves between 0 and 6 weeks of age with diarrhoea (n=216). Samples were investigated by routine procedures and then stored at -20 °C to enable batchwise testing.

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

2.2 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 is presented in table 1. For *E. coli* F5/K99, 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 fast tests then 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 pathogens associated with neonatal diarrhoea in calves, displayed as κ-values (Kappa)

		Reference method			
		E. coli K99	bovine rotavirus	bovine coronavirus	Cryptosporidium parvum
BIO K 348	ELISA kit A	0.93	0.80	0.55	0.81
	ELISA kit B	0.96	0.72	0.54	0.70
BIO K 156	Fast test kit C	0.89	0.91	0.37	0.85
	Fast test kit D	0.91	0.72	0.05	0.73

For coronavirus all positive samples in ELISA kit A wer confirmed by PCR, whereas ELISA kit B scored some fals positives. In the comparative study on a subset of 100 sample PCR scored 26 samples positive for coronavirus, of which 12 an 14 samples scored positive in ELISA kits A and B, respectivel Fast test C was as sensitive as ELISA kit A, but scored a additional 14 samples positive, discrepant, however, from th additional PCR positives. Fast test D only scored 1 sampl positive.

Fig. 1 shows the numbers of samples for each pathogen detected b ELISA kit A (four pathogens) or routine methods for BVDV an Salmonella typhimurium/dublin. Fig. 2 demonstrates detection c more than one pathogen in 25 % of the samples.

Fig. 1 Frequency distribution of defined enteropathogens in faecal samples of young calves with diarrhoea

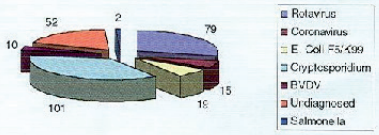
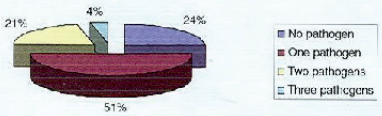


Fig. 2 Simultaneous detection of enteropathogens in faecal samples of young calves with diarrhoea



4

Discussion and Conclusions

Hardly any literature is available concerning diagnosti performance of commercially available ELISA kits and lateral flo kits for detection of the major enteropathogens involved in cal neonatal diarrhoea (2, 3). All kits showed satisfactory diagnosti performance for detection of E. coli K99, bovine rotavirus an cryptosporidium parvum, with kits A and C showing the highes kappa-values. For detection of bovine coronavirus, kit D faile almost completely, whereas kappa-values of the other kits wer rather poor. The reference test, however, was PCR. Considering th relative low detection limits of PCRs in general, the clinica significance of these PCR results remain to be seen (1).

Also the significance of – frequently occurring – combinations o enteropathogens in calf neonatal diarrhoea may cause a headach for the veterinary practitioner.

5. References

1. Kapil,S., Trent,A.M. and Goyal,S.M., 1990. Excretion and persistence o bovine coronavirus in neonatal calves. Arch.Virol., 115 (1-2): 127-132
2. Khattar,S. and Pandey,R., 1990. A comparison of four methods fo detecting rotavirus in faeces of bovine calves. J.Diarrhoeal Dis.Res., (1-2): 31-33. 3. Trotz-Williams,L.G.,Martin,S.W., Martin,D. Duffield,T., et al., 2005. Multiattribute evaluation of two simple tests fo the detection of *Cryptosporidium parvum* in calf faeces. Vet. Parasitol

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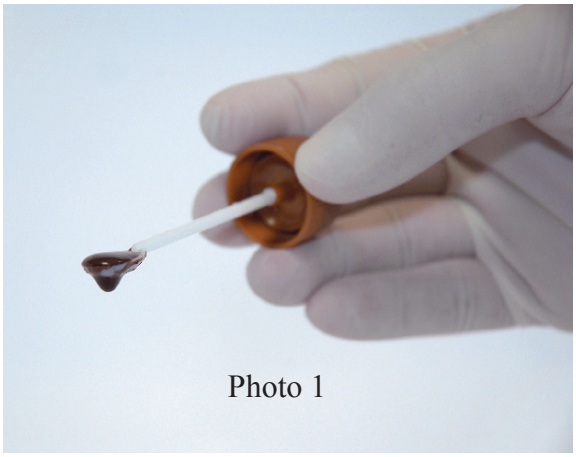
TEST STRIPS FOR THE DETECTION OF CRYPTOSPORIDIUM

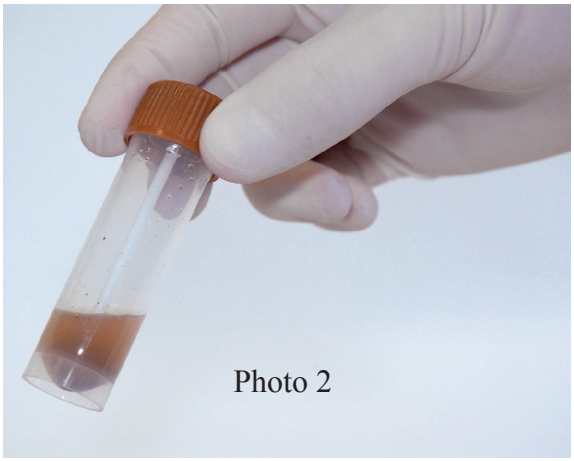
BIO K 155

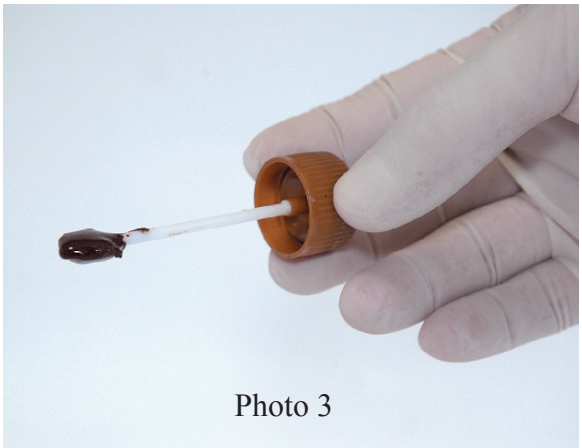
(1 X 10 strips)

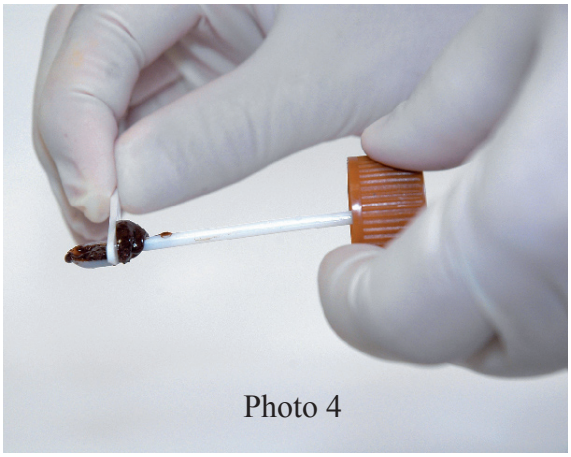
Performing the test.

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.


Photo 1

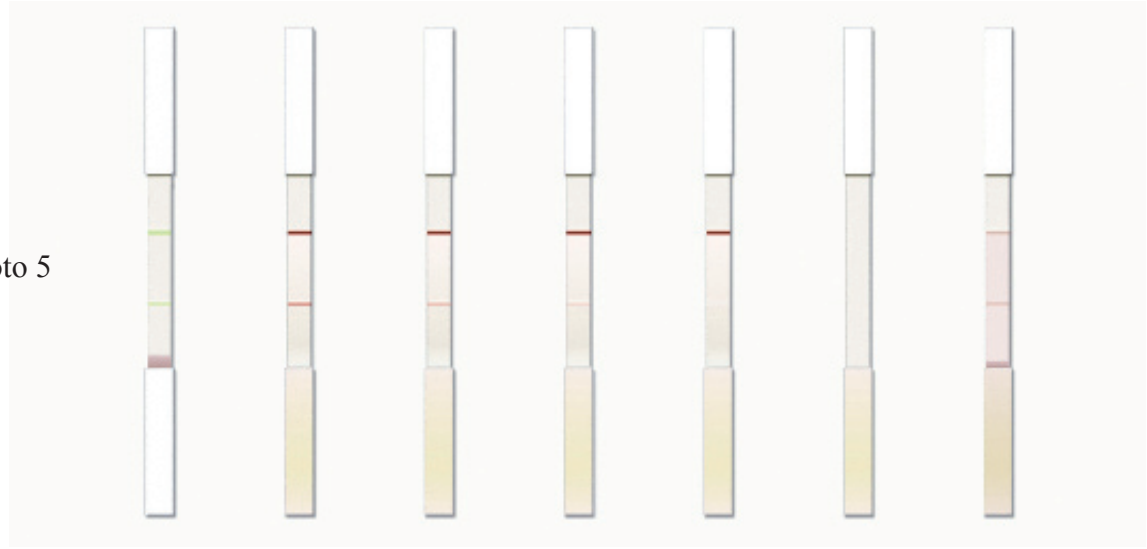

Photo 2


Photo 3


Photo 4

Interpreting the results (Photo 5)

Photo 5



1 line = negative
2 lines = positive

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.

