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DIGESTIVE ELISA KIT

Diagnosis test for bovines.

BIO K 348/2 - BIO K 348/5

Diarrhoea is a major cause of mortality in young cattle under six months. Bovine neonatal gastroenteritis is a multifactorial disease. It can be caused by viruses (coronavirus or rotavirus), by bacteria: (*Salmonella*, pathogenic strains of *E. coli*) or by protozoa such as *Cryptosporidium*. Coronavirus and rotavirus are often associated with episodes of neonatal diarrhoea. *Cryptosporidium* is also frequently isolated in faeces, where it can be present in very high quantities. It can persist for a long period in the environment. F5-positive enterotoxigenic *E. coli* is frequently isolated in under-three-day-old calves, particularly in colostrum-deprived calves or in calves fed colostrum that is free of anti- *E. coli* F5 + specific antibody. The diagnosis of the etiological agent of diarrhoea can be performed only in the laboratory because the clinical signs do not suffice to distinguish between these different microorganisms. It is possible to identify these agents by means of different techniques, including culture, staining, electron microscopy and floating techniques. However, these techniques are labour intensive, impractical and time consuming. These classical techniques have rapidly been replaced by the ELISA technology because of its simplicity and limited laboratory equipment requirements. The sensitivity and specificity of the ELISA technique for detecting these pathogens is at least as good as that of the more classic techniques, and the results are very similar. The ELISA technique is rapid and reliable and is particularly suited to the analysis of large numbers of samples.

Reliable Results

The use of monoclonal antibody as conjugate ensures excellent specificity and very reliable results.

Ease-of-Use

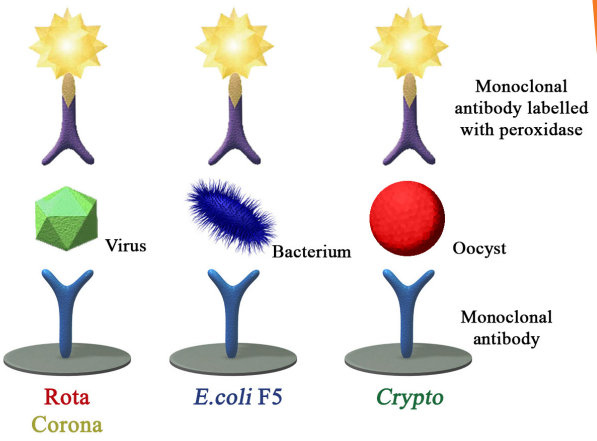
Minimal hands-on-time
Room temperature incubation
Results available in 140 minutes.
All reagents are ready to use.

Flexibility

Results can be read visually or spectrophotometrically.

EIA Procedure

1- Microplate coated with monoclonal antibodies
2- Add samples and positive controls.
Incubate 1 hour at 21°C +/- 3°C
Wash
3- Add conjugates.
Incubate 1 hour at 21°C +/- 3°C .
Wash
4- Add TMB (chromogen)
Wait 10 minutes.
Add stop solution.
Read at 450 nm



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Example of results for Rotavirus

dsRNA electrophoresis on PAGE
(Silver staining)

ELISA BIO K 348

	+	-	
+	49	0	49
-	1	40	41
	50	40	90

Specificity: 100 %
Sensitivity: 98 %

Days after birth

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Calf 1	Crypto						+	+	+	+	+	+	+	+	+	+	
	Rota			+	+	+	+										
	Diarrhoea							+	+	+	+	+			+		
Calf 2	Crypto							+	+	+	+	+	+	+	+	+	
	Rota							+	+	+	+						
	Diarrhoea								+	+			+			+	

Detectability

The kit gives a positive signal with a minimum of 40,000 $TCID_{50}$

Example of results for Coronavirus

Electron microscopy

ELISA BIO K 348

	+	-	
+	21	4	25
-	1	35	36
	22	39	61

Specificity: 90 %
Sensitivity: 95 %

RT-PCR

ELISA BIO K 348

	+	-	
+	14	1	15
-	4	77	81
	18	78	96

Specificity: 98.7 %
Sensitivity: 77.8 %

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Days after birth

Calf 1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Corona											+	+	+	+		
Rota											+	+	+	+	+	
Crypto					+	+	+	+	+	+	+	+	+	+		
Diarrhoea								+				+	+	+	+	

Detectability

The kit gives a positive signal with a minimum of 100,000 $TCID_{50}$

Example of results for *E. coli* F5

Isolated strain on Minca	ELISA (BIO K 348) O.D.	Strips (BIO K 154)	PCR (314 bp)
16179	2,1	+	+
7951	1,2	+	+
6785	0,06	-	-
2180	1,55	+	+
785	0,069	-	-
03-029429	1,94	+	+
03-005404/1	0,061	-	-

PCR

ELISA BIO K 348

	+	-	
+	20	0	20
-	2	64	66
	22	64	86

Specificity: 100 %
Sensitivity: 90.9 %

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Example of results for *Cryptosporidium*

Flotation

ELISA BIO K 348

	+	-	
+	33	6	39
-	1	60	61
	34	66	100

Specificity: 90.9 %
Sensitivity: 97.1 %

Days after birth

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Calf 1	Crypto						+	+	+	+	+	+	+	+	+			
	Rota																	
	Diarrhoea						+	+	+	+	+	+	+					
Calf 2	Crypto							+	+	+	+	+	+	+	+	+	+	+
	Rota					+	+											
	Diarrhoea					+	+	+	+	+	+	+	+	+	+		+	

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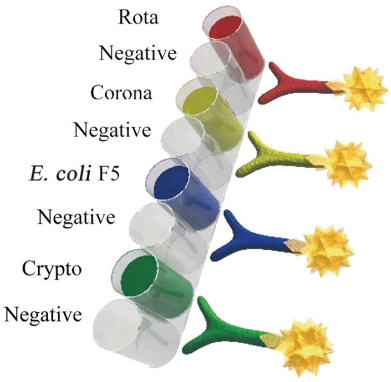
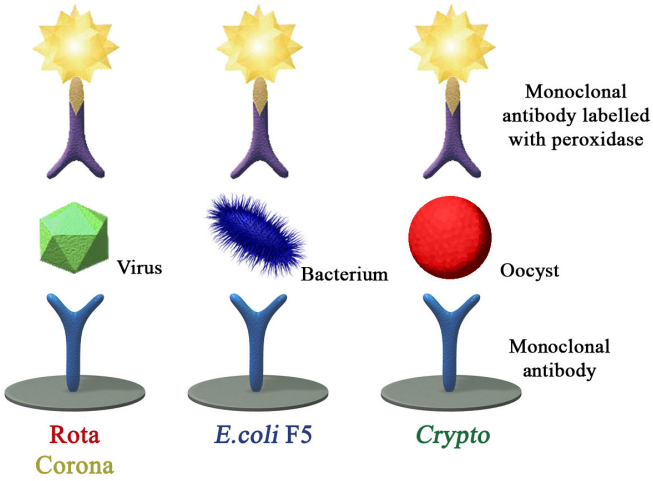
Composition of the kit

BIO-X DIGESTIVE ELISA KIT : BIO K 348

	BIO K 348/2	BIO K 348/5
Microplates	2	5
Washing solution	1 X 100 ml (20 X)	1 X 250 ml (20 X)
Dilution buffer	1 X 50 ml (5 X)	1 X 50 ml (5 X)
Conjugate	4 X 6 ml (1 X)	4 X 6 ml (1 X)
Control antigen	1 X 4 ml (1 X)	1 X 10 ml (1 X)
Single component TMB	1 X 25 ml (1 X)	1 X 55 ml (1 X)
Stopping solution	1 X 15 ml (1 X)	1 X 30 ml (1 X)





Bibliography

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, respectively 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

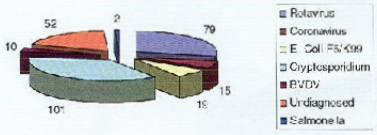
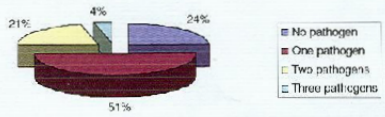


Fig. 2 Simultaneous detection of enteropathogens in faecal samples of young calves with diarrhoea



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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 headache 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., 8 (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