



HAL
open science

Bluetongue virus: European Community proficiency test (2007) to evaluate ELISA, RT-PCR detection methods with special reference to pooling of samples

C.A. Batten, A.J. Swain, K. Bachanek-Bankowska, A. Bin-Tarif, C.A.L. Oura

► **To cite this version:**

C.A. Batten, A.J. Swain, K. Bachanek-Bankowska, A. Bin-Tarif, C.A.L. Oura. Bluetongue virus: European Community proficiency test (2007) to evaluate ELISA, RT-PCR detection methods with special reference to pooling of samples. *Veterinary Microbiology*, 2009, 135 (3-4), pp.380. 10.1016/j.vetmic.2008.09.080 . hal-00532515

HAL Id: hal-00532515

<https://hal.science/hal-00532515>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Bluetongue virus: European Community proficiency test (2007) to evaluate ELISA, RT-PCR detection methods with special reference to pooling of samples

Authors: C.A. Batten, A.J. Swain, K. Bachanek-Bankowska, A. Bin-Tarif, C.A.L. Oura



PII: S0378-1135(08)00470-7
DOI: doi:10.1016/j.vetmic.2008.09.080
Reference: VETMIC 4223

To appear in: *VETMIC*

Received date: 10-6-2008
Revised date: 26-8-2008
Accepted date: 29-9-2008

Please cite this article as: Batten, C.A., Swain, A.J., Bachanek-Bankowska, K., Bin-Tarif, A., Oura, C.A.L., Bluetongue virus: European Community proficiency test (2007) to evaluate ELISA, RT-PCR detection methods with special reference to pooling of samples, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2008.09.080

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Bluetongue virus: European Community proficiency test (2007) to evaluate**
2 **ELISA and RT-PCR detection methods with special reference to pooling of**
3 **samples.**

4 Batten, C.A.*, Swain, A.J., Bachanek-Bankowska, K., Bin-Tarif, A., and Oura, C.A.L.
5 Institute for Animal Health, Ash Road, Pirbright, Woking, GU24 0NF, UK.

6
7 *Corresponding Author:

8 Dr Carrie A Batten,

9 Institute for Animal Health,

10 Ash road,

11 Pirbright,

12 Woking,

13 GU24 0NF,

14 UK.

15 Tel: +44 (0) 1483 231146

16 Fax : +44 (0) 1483 235745

17 Email: carrie.batten@bbsrc.ac.uk

18

19

20

21

22

23

24

25

26

27 **Abstract:**

28 Bluetongue virus European Community national reference laboratories (BTV-EC-
29 NRLs) participated in an inter-laboratory proficiency test in 2007. The aim of the
30 inter-laboratory proficiency test was to determine the ability of laboratories to detect
31 antibodies to a series of BTV serotypes by cELISA and to detect viral RNA in
32 animals infected with the European strain of BTV-8 by RT-PCR. Both serum and
33 EDTA blood sample were diluted in order to determine the sensitivity of the assays.
34 All the cELISAs were 'fit-for purpose' to detect antibodies to the common BTV
35 serotypes circulating in Europe and the real time RT-PCR assays were all capable of
36 detecting BTV-8 RNA albeit with varying sensitivities. There were however
37 inconsistencies in the ability of the gel-based PCR assays to detect BTV RNA. In
38 addition, samples taken on the first day of viraemia and at the peak of viraemia from
39 animals experimentally infected with BTV-8, were diluted to determine if the diluting
40 of samples affected the ability of the of the Shaw *et al* (2007) RT-PCR assay to detect
41 BTV-RNA at these time-points. Results indicated that, if samples were taken at the
42 onset of viraemia, diluting at 1/5 resulted in a reduced ability of the assay to detect
43 BTV RNA in the diluted compared to the neat samples. Diluting samples taken at the
44 peak of viraemia at 1/10 however resulted in no loss in sensitivity.

45

46 Key Words: Bluetongue Virus; Diagnosis; RT-PCR; ELISA; Proficiency test

47

48 **Main text**

49 The Community Reference Laboratory (CRL) for bluetongue (BT) is responsible for
50 sending out an annual inter-laboratory proficiency tests. In September 2007, prior to

51 the BTV-8 outbreak in the UK, the CRL sent out a proficiency test in order to
52 investigate the ability of Bluetongue virus European Community national reference
53 laboratories (BTV-EC-NRLs) to detect antibodies to BTV serotypes present in and
54 around Europe by eELISA. In addition a series of EDTA blood samples from animals
55 infected with BTV-8 were prepared and diluted both to mimic pooling and to
56 determine the analytical sensitivity of routine RT-PCR assays used by the BTV-EC-
57 NRLs.

58 Fifteen randomly coded samples including reference antiserum to eleven BTV
59 serotypes (BTV 1, 2, 3, 4, 8, 9, 10, 11, 15, 16, 23) raised in sheep, two serum samples
60 from BTV-8 experimentally animals 21 dpi and two negative serum samples from
61 cattle and sheep were sent to 27 BTV-EC-NRLs and three other national reference
62 laboratories from non-EC countries. In addition EDTA blood from cattle and sheep
63 infected with BTV-8 in the field were diluted 1/2, 1/5, 1/10, 1/20 and 1/100 in BTV
64 negative blood. In total 12 randomly coded blood samples (including negative cattle
65 and sheep blood) were dispatched to 21 participating BTV-EC-NRLs.

66 Laboratories were asked to use their routine 'in-house' ELISA and RT-PCR
67 assays to analyse the samples in the proficiency panels. A total of six different
68 commercially available ELISAs, seven real-time RT-PCR assays and two
69 conventional gel-based RT-PCR assays were used between the laboratories. In some
70 of the laboratories the samples were tested by more than one assay.

71 Results illustrated that the six commercial ELISA assays (ID-Vet, VMRD,
72 Pourquier, IZS, Ingenasa and BDSL) detected antibodies to the six BTV serotypes
73 previously or currently circulating in Europe (BTV-1, 2, 4, 8, 9 and 16). Five of the
74 assays (ID-Vet, VMRD, Pourquier, Ingenasa and BDSL) detected antibodies to all 11
75 BTV serotypes included in the proficiency test. Only two laboratories used the IZS

76 assay, which failed to detect antiserum against BTV-10 as seen in the 2006 ring trials
77 (Batten *et al*, 2008). The VMRD assay in comparison with the other five assays,
78 appeared to have reduced sensitivity to BTV-16 and BTV-10, however these antisera
79 had a lower antibody titre than the other samples in the panel (data not shown). All the
80 assays detected antibodies in the BTV-8 (21dpi) antiserum and no antibodies were
81 detected in the negative serum.

82 The results of the real-time RT-PCR assays are outlined in Table 1. All of the
83 RT-PCR assays detected BTV-8; however the analytical sensitivity of the assays,
84 measured by their ability to detect BTV RNA in diluted samples varied. Eight
85 laboratories used the Shaw *et al* (2007) assay and this assay consistently detected the
86 1/20 dilution and the majority of laboratories detected the 1/100 dilution. Interestingly
87 two laboratories using the Shaw *et al* (2007) assay only included the UNI primers;
88 however both detected BTV RNA in the 1/100 dilution in cattle. The published Shaw
89 *et al* (2007) assay utilises two primer sets and two probes to ensure that it detects both
90 Eastern and Western BTV topotypes. It should be noted that the BTV-8 serotype used
91 in this proficiency test was a Western topotype which is targeted primarily by the UNI
92 primers. Therefore the CRL recommends that laboratories follow the published
93 protocol using both sets of primers and probes in order to ensure that both Western as
94 well as Eastern BTV topotypes can be detected. Five laboratories used the Toussaint
95 *et al* (2007) assay and five laboratories used the unpublished Hoffmann *et al* assay.
96 Most of the laboratories managed to detect the 1/100 dilution however it appeared that
97 different laboratories were using different cut-offs for both of these assays which
98 could account for some of the inter-laboratory variation. Much of the inter-laboratory
99 variation in analytical sensitivity was most likely due to the fact that different
100 laboratories used different RNA extraction methods; some used robotic extraction

101 methods (Qiagen and Roche) while others used a variety of manual RNA extraction
102 methods.

103 The Polci *et al* (2007) and the Jimenez *et al* (2006) assays were used by one
104 laboratory and other unpublished assays were used by a further two laboratories, one
105 of which appeared to be as sensitive as the Shaw *et al* (2007) assay (Table 1). The
106 assay developed by Jimenez-Clavero *et al* (2006) was found to have a reduced
107 sensitivity, although it was able to detect BTV RNA at 1/10 in sheep and 1/100 in
108 cattle, however the cycle threshold (Ct) value in the 1/2 dilutions were high (Ct 36
109 and 37) compared to the other assays.

110 Ten laboratories used conventional RT-PCR assays (Table 2). The nested RT-
111 PCR assay recommended in the OIE manual ((OIE), 2008) was used by four
112 laboratories. BTV was detected down to the 1/20 dilution and was detected in the
113 1/100 dilution by two labs, indicating similar analytical sensitivity to most of the real-
114 time RT-PCR assays. Six laboratories used the Anthony *et al* (2007) assay, however
115 only two labs managed to detect BTV RNA with this assay, both reported that they
116 had non specific bands when they analysed the results by agarose gel electrophoresis.
117 The other four labs failed to consistently amplify any product of the correct size.
118 These inconsistent results were most likely due to the fact that the Anthony *et al*
119 (2007) assay was not sensitive enough to detect BTV RNA in the diluted samples.
120 Due to the higher sensitivity of real-time compared to conventional RT-PCR assays it
121 is advised that all BTV-EC-NRLs develop real-time RT-PCR technology for the
122 detection of BTV RNA in order to increase sensitivity and throughput. The results of
123 the proficiency test showed that there were significant differences in sensitivities
124 when the same real-time RT-PCR assays were used in different laboratories across
125 Europe, this highlighted the need for some of the participating laboratories to optimise

126 the sensitivity of the assay that they are using. Additionally laboratories need to
127 constantly update PCR primers as new topotypes emerge and regularly check for new
128 incursions of other serotypes into Europe.

129 The CRL carried out a study to investigate how the diluting of samples
130 affected the ability of the Shaw *et al* (2007) assay to detect BTV RNA. The aim of
131 this study was to mimic pooling, which can be used to increase the testing through-put
132 of diagnostic samples. It is important to note that pooling has at least two different
133 components: one is the dilution effect and the second is the matrix effect of the
134 multiple samples. In this study we have only investigated the dilution effect. Samples
135 of EDTA blood from BTV-8 experimentally infected sheep and cattle (Darpel *et al*,
136 2007) were collected at different time-points. Samples taken on the first day of
137 viraemia and at the peak of viraemia (as assessed by RT-PCR) were diluted with
138 corresponding species BTV negative blood, to obtain dilutions of 1/5, 1/10, 1/50 and
139 1/100. RNA extraction and real time RT-PCR using the Shaw *et al* (2007) method
140 was performed on each diluted sample in triplicate at the CRL. RT-PCR results
141 indicated that the Ct value for all 8 samples taken on the first day of viraemia that
142 were diluted at 1/5 increased by approximately 2 Cts when compared to the Ct value
143 of neat blood (Table 3a). In one sample (VP75) diluting at 1/5 resulted in the loss of
144 detection of RNA, however the Ct of this sample when tested neat was relatively high
145 (Ct 33) in comparison to the neat Ct values in the other samples (Table 3a). In one of
146 the bovine samples (VP76) the ability of the assay to repeatedly detect RNA in the
147 samples was compromised when diluted 1/5. The Ct value of the neat sample was ~29
148 however diluting 1/5 resulted in increased Ct values (~ 1.5) and viral RNA was only
149 detected in 2 out of 3 triplicate tests. In all samples the ability of the assay to
150 repeatedly detect RNA declined as the samples were further diluted. At 1/50 and

151 1/100 dilutions, detection of viral RNA became problematical and RNA was only
152 detected in the samples with the lowest (neat) Ct values (VP73, VP77 and VP78).
153 These results indicated that if a blood sample taken from an animal at the onset of
154 viraemia was pooled 1/5 or greater there would be the possibility of not detecting the
155 viral RNA in the pooled sample, when it could be detected in the neat samples.
156 Although there is only a short time period of around 1 day when these low levels of
157 viral RNA are present at the start of infection it has been concluded by the EC that
158 pooling samples is an unacceptable risk and therefore pooling of samples for pre-
159 movement RT-PCR testing has not been recommended by the EC. All testing should
160 be performed on neat samples to avoid missing animals in the early stages of infection
161 (CRL annual meeting, Brussels 2007).

162 When blood samples taken at the peak of viraemia were diluted 1:10 (Table
163 3b), detection of viral RNA was not affected. Ct values increased (3 Cts between neat
164 and 1/10), however RNA was detected in all samples tested in triplicate. When diluted
165 1/50, RNA was only detected in 1/3 tests for VP76, however this sample had the
166 lowest neat Ct value (~26). At dilutions of 1/100 viral RNA was not detected in one
167 ovine (VP75) and one bovine (VP78) blood sample and repeatability became
168 inconsistent with two other samples (VP76 and VP79). These samples all had the
169 highest (neat) Ct values. These results indicated that samples taken from animals
170 during the peak of viraemia can be safely pooled up to 1/10 and RNA will be
171 detected. It should be noted that the level of RNA in the blood does wane with time
172 and RNA persists at low levels for up to 200 days post-infection, however, during the
173 late stages of infection (>60 days) animals are not thought to be viraemic ((OIE),
174 2008) and are therefore safe to move. However, it is important to be aware that

175 pooling of samples taken late in infection when Ct values are high may result in a
176 reduction in analytical sensitivity.

177

178 **Acknowledgements**

179 The authors would like to thank Piet van Rijn (CIDC-Lelystad) and Bernd Hoffmann
180 (FLI-Riems) for supplying blood samples from BTV infected animals.

181

182 **References**

183 (OIE), O.I.E., volume 1, 6th Ed 2008, Bluetongue, In: OIE Manual of diagnostic tests
184 and vaccines for terrestrial animals. Paris, pp. 158-174.

185 Anthony, S., Jones, H., Darpel, K.E., Elliott, H., Maan, S., Samuel, A., Mellor, P.S.
186 and Mertens, P.P.C., 2007, A duplex RT-PCR assay for the detection of
187 genome segment 7 (VP7 gene) from 24 BTV serotypes. *journal of virological*
188 *methods* 141, 188-197.

189 Batten, C.A., Bachanek-Bankowska, K., Bin-Tarif, A.1, Kgosana, L., Swain, A.J.,
190 Corteyn, M., Darpel, K., Mellor, P.S, Elliott, H.G. and Oura, C.A.L., 2008,
191 Bluetongue virus: European Community inter-laboratory comparison tests to
192 evaluate ELISA and RT-PCR detection methods. *Veterinary Microbiology*
193 129 80-88.

194 Darpel, K.E., Batten, C.A., Veronesi, E., Shaw, A.E., Anthony, S., Bachanek-
195 Bankowska, K., Kgosana, L., Bin-Tarif, A., Carpenter, S., Muller-Doblies,
196 U.U., Takamatsu, H.H., Mellor, P.S., Mertens, P.P.C and Oura, C.A.L., 2007,
197 A study of British sheep and cattle infected with bluetongue virus serotype 8
198 from the 2006 outbreak in northern Europe. *Veterinary Record*.

- 199 Jimenez-Clavero, M.A., Agüero, M., San Miguel, E., Mayoral, T., Lopez, M.C.,
200 Ruano, M.J., Romero, E., Monaco, F., Polci, A., Savini, G., Gomez-Tejedor,
201 C., 2006, High throughput detection of bluetongue virus by a new real-time
202 fluorogenic reverse transcription-polymerase chain reaction: application on
203 clinical samples from current Mediterranean outbreaks. *J Vet Diagn Invest* 18,
204 7-17.
- 205 Polci, A., Cammà, C, Serini, S., Di Gialleonardo, L., Monaco, F., Savini, G. , 2007,
206 Real-time Polymerase chain reaction to detect bluetongue virus in blood
207 samples. *Vet Italia* 43, 77-87.
- 208 Shaw. A.E., M., P., Alpar, H.O., Anthony, S., Darpel, K.E., Batten, C.A., Carpenter,
209 S., Jones, H., Oura, C.A.L., King, D.P., Elliott, H., Mellor, P.S. and Mertens,
210 P.P.C., 2007, Development and validation of a real-time RT-PCR assay to
211 detect genome bluetongue virus segment 1. *Journal of virological methods*.
- 212 Toussaint, J.F., Sailleau, C., Breard, E., Zientara, S., De Clercq, K., 2007, Bluetongue
213 virus detection by two real-time RT-qPCRs targeting two different genomic
214 segments. *Journal of virological methods* 140, 115-123.
- 215
- 216

Table 1: Comparison of real-time RT-PCR results generated from 26 participating laboratories.

Real-time RT-PCR from different labs vs BTV-8												
	Sheep neg	Sheep 1/2	Sheep 1/5	Sheep 1/10	Sheep 1/20	Sheep 1/100	Cattle neg	Cattle 1/2	Cattle 1/5	Cattle 1/10	Cattle 1/20	Cattle 1/100
	>50	25	26	27	28	30	>50	24	26	27	27	30
	no Ct	35	35	37	38	38	no Ct	33	36	37	36	no Ct
	no Ct	27	28	29	29	32	no Ct	25	27	27	28	31
	no Ct	33	35	no Ct	38	no Ct	no Ct	35	33	34	37	no Ct
Shaw <i>et al.</i> , 2007 (10 labs)	41	36	36	39	39	41	no Ct	33	36	36	38	no Ct
	no Ct	28	29	31	30	no Ct	no Ct	27	30	29	30	no Ct
	no Ct	31	32	33	34	39	no Ct	30	31	33	33	36
	no Ct	27	28	29	31	33	no Ct	26	28	30	31	33
*	no Ct	30	31	31	33	36	no Ct	29	29	30	32	34
*	no Ct	30	33	32	33	no Ct	no Ct	29	31	34	33	35
	no Ct	35	35	36	39	40	no Ct	35	36	37	38	no Ct
	no Ct	32	34	35	37	39	no Ct	31	33	33	35	38
Toussaint <i>et al.</i> , 2007 (5 labs)	no Ct	32	37	35	35	no Ct	no Ct	31	32	33	37	36
	no Ct	33	34	36	36	37	no Ct	32	35	33	37	38
	no Ct	31	33	34	35	37	no Ct	31	32	33	35	37
	no Ct	31	32	33	33	40	no Ct	30	32	33	34	35
	no Ct	34	34	36	35	37	no Ct	30	32	33	35	no Ct
Hoffmann <i>et al.</i> , (5 labs)	no Ct	30	31	32	33	inc	no Ct	29	30	32	32	inc
	no Ct	26	27	28	30	32	no Ct	25	29	28	29	31
	no Ct	28	29	31	32	34	no Ct	28	29	30	31	34
	>45	29	31	32	33	36	>45	29	30	31	32	33
SANCO (2 labs)	>45	35	37	37	38	39	>45	34	36	35	35	37
	no Ct	36	38	39	no Ct	no Ct	no Ct	37	38	39	41	41
Jimenez-Clavero <i>et al.</i> , 2006 (1 lab)												
	39	32	33	33	35	37	42	31	32	34	34	36
Polci <i>et al.</i> , 2007 (1 lab)												
	no Ct	30	31	30	no Ct	no Ct	no Ct	30	30	30	32	no Ct
Unpublished (2 labs)	no Ct	26	31	32	33	35	no Ct	33	31	31	33	35

* two laboratories only used the UNI primers from the Shaw *et al* (2007) assay.

Table 2: Comparison of gel-based RT-PCR results generated from ten participating laboratories.

		Gel-based PCR from different labs vs BTV-8											
		Sheep neg	Sheep 1/2	Sheep 1/5	Sheep 1/10	Sheep 1/20	Sheep 1/100	Cattle neg	Cattle 1/2	Cattle 1/5	Cattle 1/10	Cattle 1/20	Cattle 1/100
Anthony <i>et al.</i> , 2007 (6 labs)		N	inc	inc	inc	inc	inc	inc	inc	inc	inc	inc	inc
		N	N	N	N	N	N	N	N	N	N	N	N
		N	N	N	N	N	N	N	N	N	N	N	N
		N	inc	P	P	P	P	P	P	P	P	P	P
		N	P	P	N	P	N	N	P	P	P	P	N
		N	N	N	N	N	N	N	N	N	N	N	N
OIE method (4 labs)		N	P	P	P	P	P	N	P	P	P	P	P
		N	P	P	P	P	N	N	P	P	P	P	N
		N	P	P	P	P	N	N	P	P	P	P	N
		n/s	P	P	P	P	inc	N	P	P	P	P	P

P: Positive

N: negative

Inc: inconclusive

Table 3: RT-PCR results of ovine (VP72-75) and bovine (VP76-79) blood taken on the first day of viraemia (3a) and blood taken during the peak of viraemia (3b) diluted in BTV negative blood.

3a

Animal ID	Dilution	neat		1/5		1/10		1/50		1/100	
		mean Ct value	detection	mean Ct value	detection	mean Ct value	detection	mean Ct value	detection	mean Ct value	detection
VP 72	3dpi	28.96	3/3	31.09	3/3	30.99	3/3	No Ct	0/3	36.27	1/3
VP 73	3dpi	27.59	3/3	29.92	3/3	32.64	3/3	34.43	2/3	39.08	1/3
VP 74	3dpi	29.12	3/3	31.32	3/3	32.91	1/3	No Ct	0/3	No Ct	0/3
VP 75	3dpi	33.75	3/3	No Ct	0/3	No Ct	0/3	No Ct	0/3	No Ct	0/3
VP 76	3dpi	29.36	3/3	31.8	2/3	33.09	1/3	No Ct	0/3	No Ct	0/3
VP 77	6dpi	25.25	3/3	27.98	3/3	28.73	3/3	32.63	1/3	33.79	1/3
VP 78	6dpi	27.78	3/3	28.97	3/3	30.33	3/3	33.87	1/3	No Ct	0/3
VP 79	3dpi	30.12	3/3	34.32	3/3	34.15	1/3	No Ct	0/3	No Ct	0/3

3b

Animal ID	Dilution	Peak of viraemia	neat		1/5		1/10		1/50		1/100	
			mean Ct value	detection	mean Ct value	detection	mean Ct value	detection	mean Ct value	detection	mean Ct value	detection
VP 72	4dpi	22.75	3/3	24.74	3/3	25.68	3/3	28.00	3/3	29.26	3/3	
VP 73	4dpi	22.57	3/3	23.09	3/3	24.54	3/3	26.82	3/3	27.89	3/3	
VP 74	4dpi	24.40	3/3	26.26	3/3	27.36	3/3	29.40	3/3	30.39	3/3	
VP 75	4dpi	26.47	3/3	28.57	3/3	30.06	3/3	32.89	3/3	No Ct	0/3	
VP 76	8dpi	26.72	3/3	29.41	3/3	30.78	3/3	34.91	1/3	39.99	1/3	
VP 77	7dpi	23.03	2/3	26.44	3/3	27.35	3/3	30.17	3/3	31.27	3/3	
VP 78	7dpi	25.34	3/3	26.91	3/3	27.95	3/3	30.95	3/3	No Ct	0/3	
VP 79	8dpi	25.98	3/3	27.66	3/3	29.83	3/3	32.46	3/3	34.33	2/3	

dpi: days post infection