Implementation of medium- and high-throughput automated solutions to meet the BTV testing challenge

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Introduction

Bluetongue disease (BT), caused by the bluetongue virus serotype 8 (BTV-8), was unknown in the EU before 2006. After a major epidemic beginning in August 2006 in Netherlands, Belgium, Germany, Luxemburg, and the north of France, the disease spread across Europe and is currently present in the Spain, Italy, the Czech Republic, Denmark, and England. The disease has caused considerable socioeconomic concern and is an issue of major importance in the international trade of animals and animal products.

To minimize the risk of infected animals moving into disease-free areas, reliable diagnostic tools are essential. Since 2006, the French National Reference Laboratory (AFSSA, Maisons-Alfort) has been using a real-time reverse-transcription polymerase chain reaction (RtRT-PCR) assay for the rapid detection of BT virus in susceptible animals. The sensitivity and specificity of this "in-house" RtRT-PCR assay has been validated in a European inter-laboratory comparison for the detection of BT viral RNA. In 2007, the National Reference Laboratory decentralized this assay to allow diagnostics on a larger scale. Analyses from 3 sessions of inter-laboratory comparison demonstrated that the 58 participants provided satisfactory repeatability estimates and concordant status for all samples.

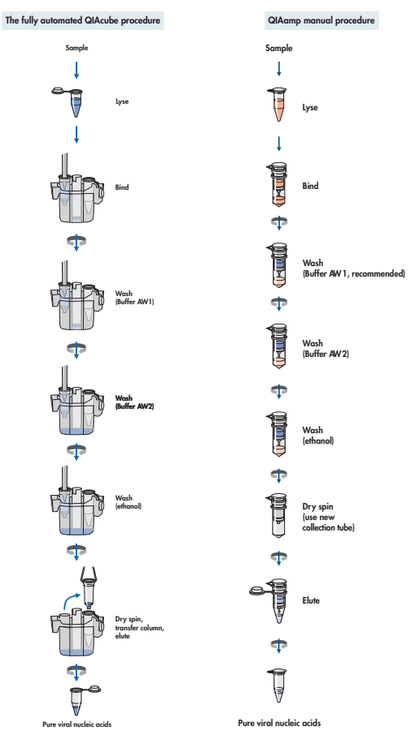
In addition to the assay, upstream extraction of RNA from complex samples such as whole blood must be established for different throughput needs. A number of RNA extraction methods have been validated and will be commercialized in France for diagnosis based on the real-time RT-PCR assay. Results are presented here for the development of an existing low-throughput manual procedure to establish standardized medium- and high-throughput procedures that require minimal human interactions using different automated systems.

Materials and methods

For medium-throughput processing (see flowchart, right), 100 µl whole blood from cattle was diluted with 40 µl PBS. RNA was extracted from 11 samples previously used for national ring test using the manual QIAamp® Viral RNA Mini Kit following the standard protocol (**Manual**) and in two runs of the automated QIAcube® "Body Fluid" protocol (**QIAcube**).

For high-throughput processing on the BioRobot® Universal System, 70 µl whole blood from cattle was processed using the QIAamp Virus BioRobot MDx Kit and a high-throughput, 96-well customized protocol available from QIAGEN (**BioRobot Universal**). Results are shown for 14 of the 103 samples prepared using several extraction conditions that gave acceptable results.

PCR was performed using the AFSSA protocol using a β-actin internal control. BTV viral RNA was detected with RtRT-PCR according to Toussaint *et al.*



Results for medium-throughput extraction

Assay:	βactin (internal control)			VP1 (target)			
Method:	Manual	QIAcube		Manual	QIAcube		
Sample		Test 1	Test 2		Test 1	Test 2	Result
1	18.82	23.45	19.18	35.77	37.35	36.63	Positive
2	17.79	19.08	20.00	30.30	30.28	33.53	Positive
3	17.70	18.82	18.81	30.67	30.34	31.06	Positive
4	18.12	18.90	18.68	33.74	33.71	34.03	Positive
5	17.94	19.06	19.91	29.32	29.27	29.46	Positive
6	18.00	18.74	20.87	ND	ND	ND	Negative
7	17.50	18.87	20.95	ND	ND	ND	Negative
8	18.33	20.49	19.34	30.29	30.03	30.28	Positive
9	17.06	18.46	18.98	25.63	25.35	26.12	Positive
10	17.54	18.35	19.24	30.12	29.90	29.93	Positive
11	18.42	19.74	20.93	29.01	28.77	29.07	Positive
Average	17.93	19.45	19.72	—	—	—	—
CV	0.49	1.46	0.87	—	—	—	—
Negative control	ND	ND	ND	ND	ND	ND	Control ok
No template control	ND	ND	ND	ND	ND	ND	Control ok
Positive control	ND	ND	ND	21.02	20.88	21.17	Control ok
Positive control	ND	ND	ND	30.01	30.94	31.15	Control ok

The QIAcube.

ND: not detected.

Results for high-throughput extraction

Assay:	β-actin (internal control)			VP1 (target)			
Method:	Manual	BioRobot		Manual	BioRobot		
Sample		Test 1	Test 2		Test 1	Test 2	Result
19	20.77	20.89	21.20	27.87	26.57	27.11	Positive
184	19.57	19.75	20.04	33.25	33.82	32.58	Positive
197	19.21	20.58	20.68	31.76	31.28	30.74	Positive
198	19.5	21.89	22.28	34.43	34.05	35.00	Positive
203	19.34	21.48	22.23	32.8	32.31	32.60	Positive
204	19.46	20.91	21.38	33.72	32.21	32.36	Positive
208	19.01	21.79	22.51	39.14	38.23	39.39	Positive
220	19.09	21.26	21.04	ND	ND	ND	Negative
221	18.61	20.13	20.00	ND	ND	ND	Negative
222	19.24	21.21	21.46	ND	ND	ND	Negative
291	20.22	21.66	21.69	32.24	32.33	31.54	Positive
299	20.73	20.86	21.18	31.09	30.84	30.31	Positive
301	21.33	21.20	21.45	31.28	29.88	29.90	Positive
302	23.96	22.12	22.41	31.01	30.41	29.85	Positive
Average	20.00	21.12	21.40	—	—	—	—
CV	1.38	0.67	0.80	—	—	—	—
Negative control	ND	ND	ND	ND	ND	ND	Control ok
No template control	ND	ND	ND	ND	ND	ND	Control ok

The BioRobot Universal System.

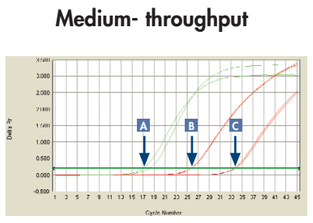
ND: not detected.

Comparison of manual and automated extraction

Medium- throughput

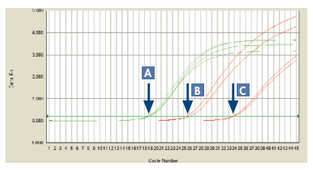
Manual

A β actin: C_T = 17–18
B N 9: C_T = 25.7
C N 4: C_T = 33.6



QIAcube

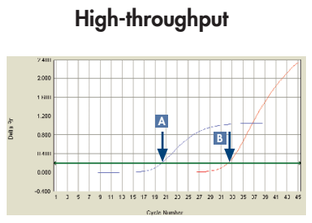
A β actin: C_T = 17–18
B N 9: C_T = 25.7
C N 4: C_T = 33.9



High-throughput

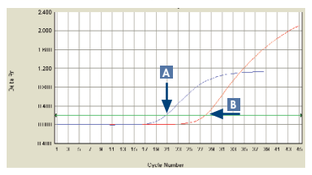
Manual

A β actin: C_T = 20.2
B N 291: C_T = 32.2



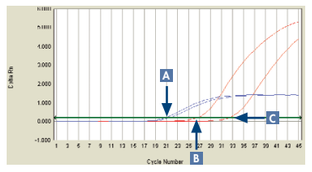
Manual

A β actin: C_T = 20.8
B N 19: C_T = 27.9



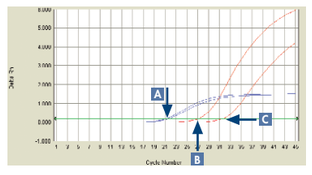
BioRobot Universal

A β actin: C_T = 21
B N 19: C_T = 26.6
C N 291: C_T = 32.3



BioRobot Universal

A β actin: C_T = 21
B N 19: C_T = 27.1
C N 291: C_T = 31.5



No difference in sensitivity and specificity of the assay was observed. The implementation of different automated procedures increased the throughput capacity to approximately 470 samples/day and facilitated standardization and tracking of results. This method has been successfully transferred into routine workflows and provides the necessary flexibility for different throughput needs.

Discussion and Conclusions

Sample preparation is a critical step in detection of BT viral RNA by RtRT-PCR. We have established automated procedures for sample preparation and successfully implemented these procedures to manage testing during the BT outbreak in France. In the meantime, more than 14,000 blood samples have been processed successfully using the described automated sample preparation methods. The availability of 2 different throughput platforms based on the same purification technology provides testing laboratories reliable sample preparation with the necessary flexibility to meet changing throughput needs.

Acknowledgements
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References
Toussaint *et al.*, 2007. Bluetongue virus detection by two real time RT-qPCRs targeting two different genomic segments. *J. Virolog. Methods* 140, 115

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