Commercialisation of a real-time RT-PCR assay for the pan-detection of Bluetongue virus

MedReoNet - Lisbon 2009

Andrew Shaw

a.shaw@vet.gla.ac.uk

The Demand

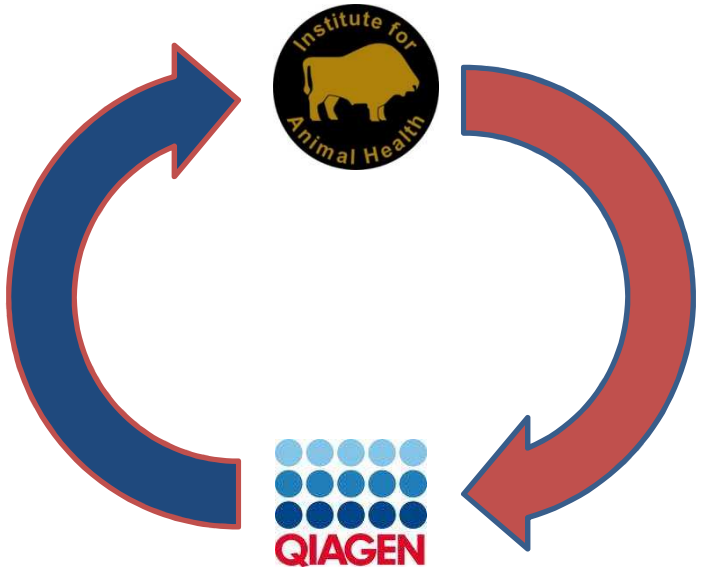
- Multiple laboratories performing tests to detect the same target
- A kit format allows inter- and intra-laboratory standardisation
- A simplified, easy-to-use protocol allows straightforward establishment of the assay
- Quality control is provided by the manufacturer
- High throughput testing puts high demands on procedures

....inevitable commercial interest

Defining responsibilities

Qiagen

- Adaptation to Qiagen chemistry
- Provision of trial kits
- Adjustment of chemistry
- Quantitative analysis of kit performance



IAH

- Testing on field samples
- Testing on different platforms
- ‘field’ input/consultancy
- Testing of samples unavailable to Qiagen

Initial testing

The Ring Trial Samples:

11 positive samples covering 5 serotypes

2 negative samples

2/11 positive ring trial samples produced No Ct result

(Ct 32.13 [BTV-8] and 28.62 [BTV-1] with Shaw *et al*.)

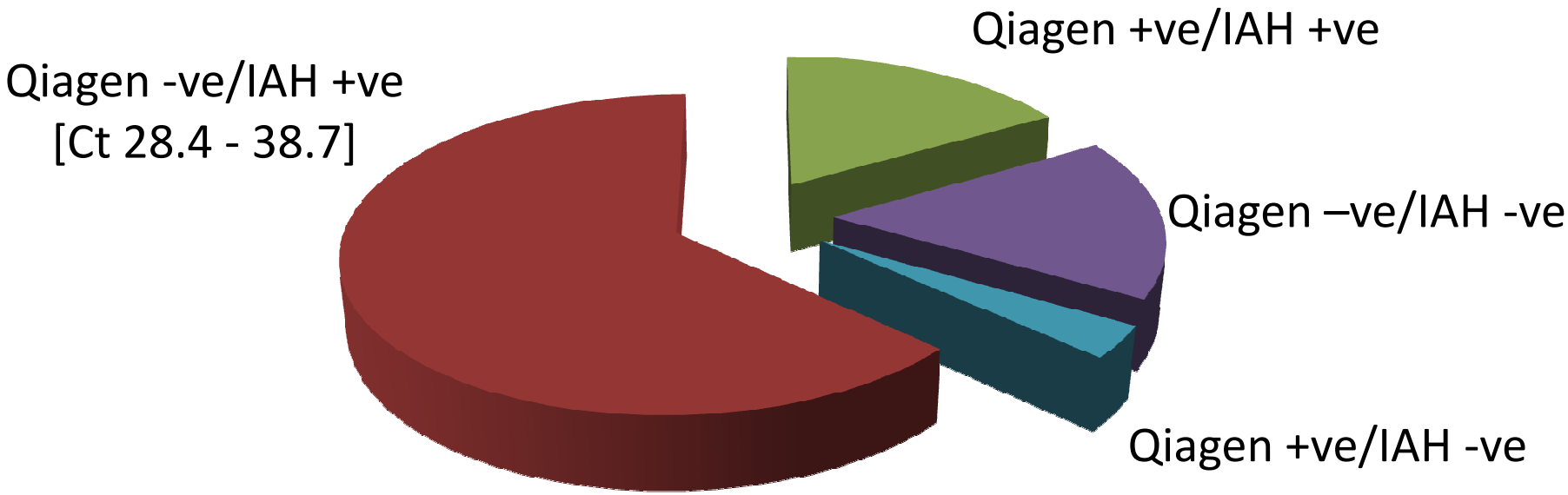
Not consistent – other BTV-1 and BTV-8 samples were easily detected

		IAH	
		+	-
Qiagen	+	9	0
	-	2	2

Weak positive samples

- Weak RNA samples (extracted from EDTA treated blood) tested during routine import screening were re-tested using the Qiagen assay

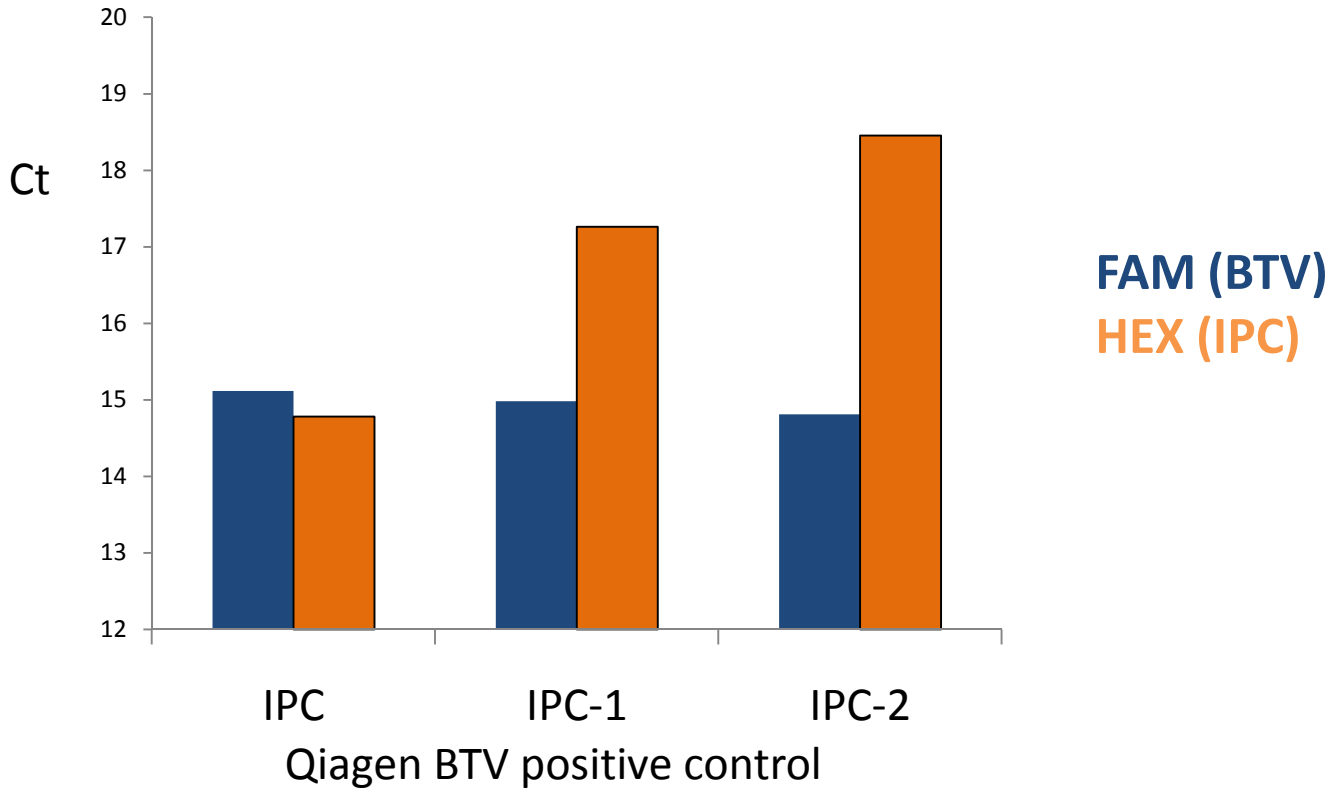
- In line with the previous data, 63% of the samples returned a No Ct result



Does the IPC impact upon detection?

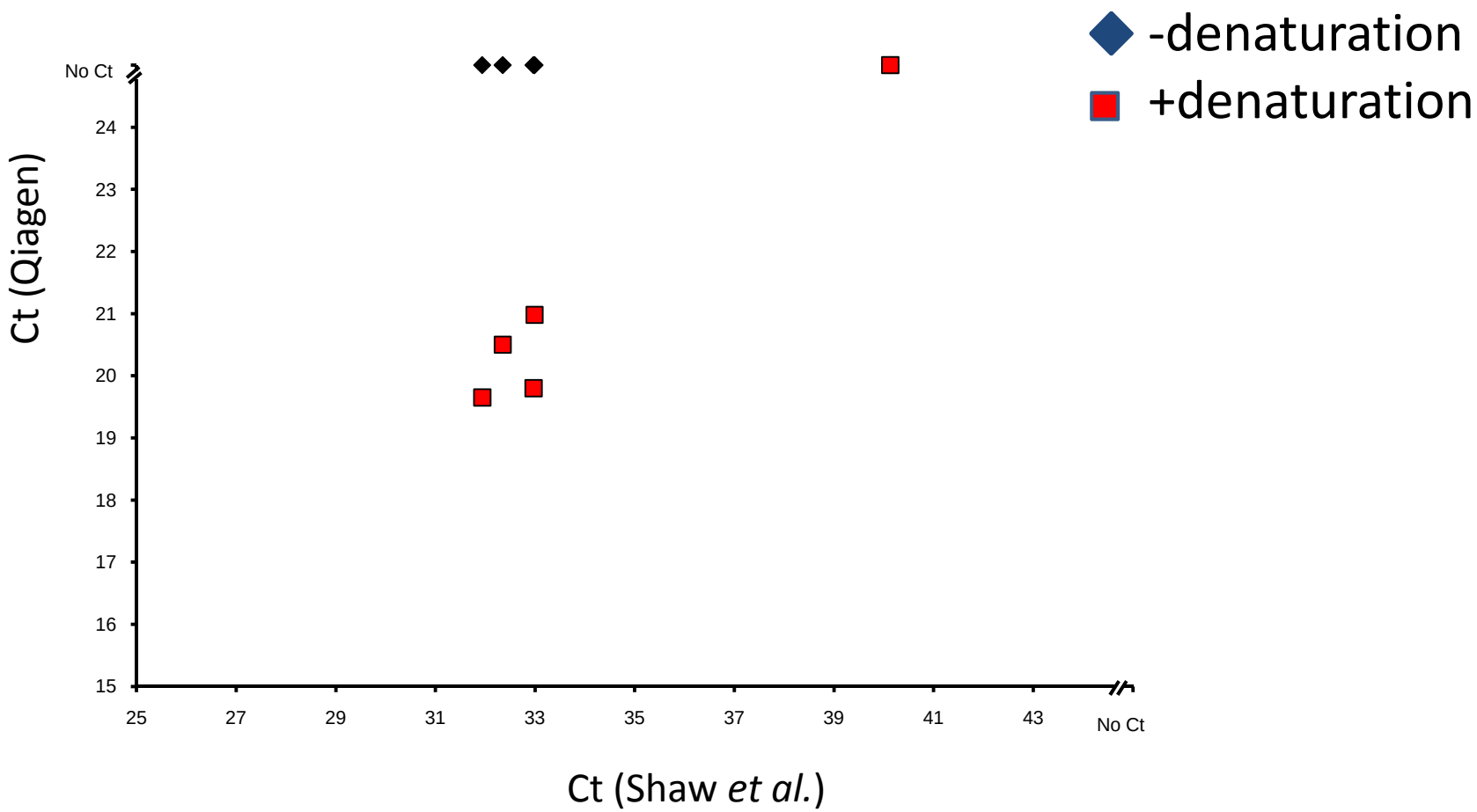
- BTV-16 vaccine strain RNA and the Qiagen BTV positive control RNA was tested with different dilutions of the IPC

- The mean increase in Ct value is 0.89



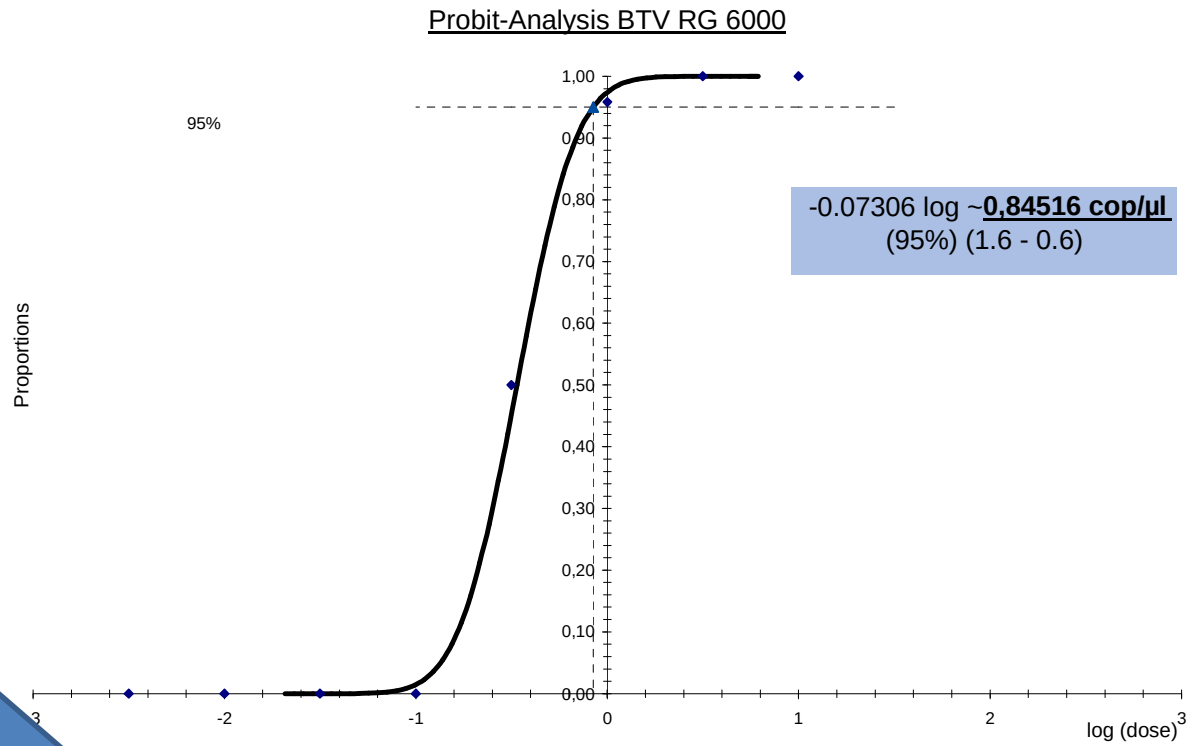
Denaturation is required

- Weak-positive BTV-11 blood samples from Belgium were tested using the Qiagen assay with and without heat denaturation of the RNA samples (95°C for five minutes)



Assay sensitivity

‘proportion of samples detected’



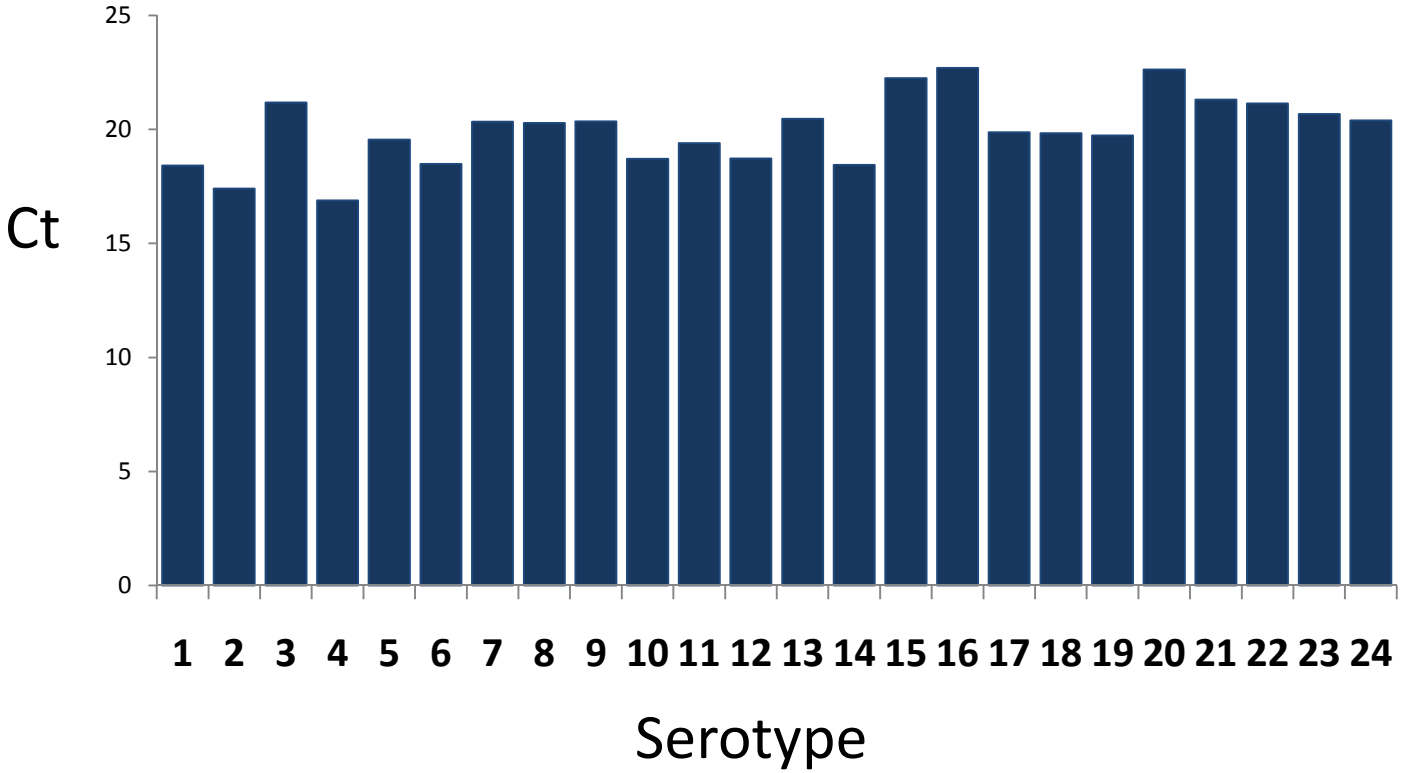
‘Copies’

8.5 copies/reaction

Performed by Qiagen, Hamburg

Detection of serotypes 1-24

- The reference strains of BTV-1-BTV-24 were extracted on the Roche MagNA Pure and tested using the *Cador* BTV RT-PCR kit
- Thus far, diagnostic sensitivity appears to be similar to the 'homebrew' assay



Assay Specificity

- Three classes of non-BTV sample were tested to determine assay specificity:

Genetically related	Clinically related	Host species
AHSV	FMDV A	Bovine epithelium
EHDV-1 USA	FMDV O	Bovine blood
EHDV Ibaraki	FMDV Asia1	Ovine blood
	FMDV C	
	FMDV SAT1	
	FMDV SAT2	
	BVDV 1 NADL	
	BVDV 2 890	
	BDV	
	VSV-NJ	

Libyan samples

- Blood samples were received from Libya derived from animals with
- suspected BTV

- 11 samples were positive in the CRL, Pirbright, and were subsequently ‘typed’ as BTV-9

- The remaining RNA was tested using the *cador* BTV RT-PCR kit, resulting in 100% concordance

		IAH	
		+	-
Qiagen	+	11	0
	-	0	2

Acknowledgements

- Molecular Research Lab, IAH Pirbright
- CRL for BTV, IAH Pirbright
- WRL for FMDV, IAH Pirbright
- Collaborators at Qiagen, Hilden and Hamburg