Commercialisation of a Real-Time RT-PCR Assay for the Pan-Detection of Bluetongue Virus

A.E. Shaw[1*] H. Marqardt[2]
A. Lukas[2] P.P.C. Mertens[1]

The arrival of bluetongue virus serotype 8 (BTV-8) in Northern Europe (2006), followed by BTV-1, 6 and 11 (2008-09), has resulted in an unprecedented epidemiological situation, which (like the earlier situation in Southern Europe and the Mediterranean region) requires rapid and accurate diagnosis to monitor and help control virus transmission and spread.

Reverse-transcription polymerase chain reaction (RT-PCR) assays and molecular sequencing have increasingly become accepted as front line tools for the analysis and investigation of BTV outbreaks. The increasing number of BTV sequences that are now available provides a basis for molecular epidemiology studies to characterize new incursions into Europe and neighbouring regions, for real-time tracing of virus movement and development of additional diagnostic tools. The resolution and therefore the impact of molecular epidemiology will inevitably increase still further as more sequence data become available.

However, although most individual BTV isolates from a single outbreak will not be fully sequenced, it is still important to identify infected animals as rapidly as possible in order to help control disease spread. Real-time RT-PCR is a very rapid, high throughput and effective method for detection of viral ribonucleic acid (RNA) (and thus infection) in blood / tissue samples, cell cultures and vector insects. Real-time RT-PCR is not affected by the immune status of the animal, or by vaccination with inactivated BTV vaccines. It has therefore become very important for investigation of suspected clinical cases, as well as screening animals for importation. In order to be fully effective, these diagnostic capabilities must also be concordant across different laboratories.

An assay developed at the Institute for Animal Health (IAH), has recently been commercialized in collaboration with Qiagen. Pre-production test kits were initially assessed without denaturation of RNA samples and their sensitivity was below that of the original IAH assay. The assay in this format also failed to detect several weak positive blood samples received by the Community Reference Laboratory at Pirbright, despite an internal positive control signal confirming the absence of inhibition. Heat denaturation of these weak positive samples, before adding the mastermix, increased detection sensitivity of these samples. A denaturation step was therefore included in all further experiments. These data indicate that BTV RNA present in non-clinical, convalescent cases is double stranded and is therefore derived primarily from viral particles, not from actively replicating viral mRNA.

The sensitivity and specificity of the optimized assay were subsequently evaluated. Probit analysis using *in vitro* transcribed RNA copies indicated that the limit of detection was 0.85 copy per microlitre of sample, equal to 8.5 copies per reaction. Further testing showed that assay specificity equalled that of the original IAH assay. It efficiently detected all 24 established BTV types with no detection of non-BTV RNAs, including genetically and clinically related viruses (epizootic haemorrhagic disease, African horse sickness, foot-and-mouth disease, and vesicular stomatitis viruses), or the ruminant hosts. A 100% concordance was observed with the IAH assay when a batch of samples from Libya were tested using the optimized Qiagen assay.

This represents a commercially available assay that is fully compatible with different high throughput systems. This assay potentially allows greater concordance between different laboratories that will integrate well with molecular epidemiology investigations of virus origin and movement.

Keywords: *Bluetongue virus* – Diagnosis – PCR.

1. Institute for Animal Health, Pirbright, Ash Road, Woking Surrey, GU24 0NF, United Kingdom.
2. QIAGEN, Hamburg, Germany.

* Corresponding author
Tel.: +44 14 83 23 10 94; Fax: +44 14 83 23 24 48
E-mail: andrew.shaw@bbsrc.ac.uk