1. Introduction
Foot-and-mouth disease virus (FMDV) is an OIE list A pathogen causing a highly contagious disease of susceptible cloven-footed livestock. Outbreaks of FMD can result in devastating losses to the agricultural industry as a consequence of mass culling and international trade restrictions. In light of the rapid spread of FMDV, fast and effective laboratory diagnosis is vital so that appropriate control restrictions can be imposed. During the UK 2001 epidemic, over 16,200 samples were tested for the presence of FMDV by the Pirbright Laboratory, predominantly by the combined use of virus isolation (VI) and antigen detection ELISA. Detection of FMDV by VI is a reliable and sensitive method (1). However, it is slow, taking up to 4 days to declare a negative result. In contrast, the antigen detection ELISA is able to quickly detect strong positives and has the advantage of identifying the serotype of virus involved, although its sensitivity is too low for a negative result to be definitive (1). The serotyping information from the ELISA can be further enhanced using sequencing and analysis of FMDV genomic fragments (2). Reverse-transcriptase polymerase chain reaction (RT-PCR) provides a suitable alternative approach for the laboratory detection of FMDV. A fluorogenic (TaqMan®) RT-PCR based on amplification of a fragment of the 5' untranslated region (5' UTR) of the FMDV genome has proved sensitive and robust for routine use (3). In the event of a future FMDV outbreak in the UK, it is likely that more emphasis will be placed on RT-PCR for laboratory diagnosis. With this in mind, the aim of this study was to integrate RT-PCR and sequence analysis into existing methods for the detection and classification of FMDV.

2. Material and methods
A comparative assessment of the three detection methods was performed on submissions (n = 722) from the 2001 UK epidemic and samples submitted to the WRL from overseas. Briefly, suspensions of vesicular epithelium (ES) from suspect cases were tested for the presence of FMDV using three complementary approaches: antigen ELISA, VI in cell culture (using primary calf thyroid cell cultures or a permanent line of IB-RS-2 cells) or fully automated TaqMan® RT-PCR. Antigen ELISA was also used to confirm the identity of the agent causing any recognisable cytopathic effect in cell cultures. Original samples of oesophageal-pharyngeal scrapings ("probangs"), blood or milk cannot be tested directly by ELISA, although they are suitable to be submitted for analysis by TaqMan® RT-PCR or VI. The entire VPI (approximately 600 bp) of selected positive samples were sequenced (Beckman-Coulter CEQ 8000). As shown in Figure 1, this fragment was generated by RT-PCR using the same RNA (or cDNA) prepared using the robotic Magna Pure LC (Roche). Sequences were assembled using SEQMAN (DNASTAR) and analysis (pairwise comparison to a database containing approximately 2000 FMDV sequences) was performed using in-house software.

3. Results
There was a broad agreement between VI and TaqMan® RT-PCR methods for detection of FMDV for the 722 samples (ES, probang and blood). As an example, 62% (151/245) of ES tested by VI were positive while 66% (161/245) were positive by TaqMan® RT-PCR (13 samples positive by RT-PCR were negative by VI and 3 samples were negative by RT-PCR but positive by VI). The combined use of ELISA and VI detected FMDV in 153 ES samples: 80% (123/153) of these were positive by ELISA alone while 97% (149/153) were positive by the TaqMan® RT-PCR.

![Figure 1: Overview of integrated approach to FMDV laboratory diagnosis. The approximate timings for individual steps are indicated.](image-url)

4. Discussion
With this approach, it is possible to report on the detection and classification of FMDV within 24 hours of receipt of a sample. Furthermore, since the performance of the TaqMan® RT-PCR is similar to VI, it may also now be possible to clear a negative sample within 24 hours. This compares with 4 days when VI is used alone.

5. Conclusion
The work described here provides an integrated approach for robotic sample handling and automated RT-PCR and sequencing that can be used for the rapid detection and subsequent classification of FMDV present in suspect samples submitted to reference laboratories.

6. Acknowledgements
This work was supported by the Department for Environment, Food & Rural Affairs (DEFRA), UK. The authors acknowledge the assistance of Ian Gumm and Robert Armstrong during ISO 9001 accreditation of these diagnostic assays.

7. References