Diagnostic differences between real-time RT-PCR and virus isolation tests on cattle probang samples following infection with FMDV
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Introduction
Foot and mouth disease virus (FMDV) causes a highly contagious viral disease in cloven-hooved animals (Alexandersen et al., 2003). Our previous studies on FMDV have shown that the diagnostic performance of real-time RT-PCR was equivalent to or possibly better than virus isolation in oesophageal-pharyngeal fluid samples (probang samples) and suitable as a screening test for such animals. Both these tests are also of equivalent value during the acute phase of disease where viral loads in these samples are at their peaks. This report highlights an elevated incidence of mismatches between these tests for probang samples taken between 6 and 21 days post-infection (dpi) from experimentally infected cattle.

Materials and Methods
A total of 230 probang samples were collected from 16 cattle infected with type O UKG 34/2001 at various intervals after infection as shown in Fig. 2. Thirty two probang samples collected from naive cattle were used as negative controls. All the collected samples were tested in parallel by real-time RT-PCR as previously described (Zhang et al., 2003) and virus isolation assays in primary bovine thyroid (BTY) cells as previously described (Snowdon, 1966).

Total nucleic acids were extracted from samples by using the automated MagNA Pure LC robotic system (Roche). Primers and probe were directed to the conserved sequences in the IRES of FMDV and PCR was carried out in a GeneAmp 5700 Sequence Detection System (Applied Biosystems).

Fig. 2. shows the experimental design in which the 16 cattle were distributed across 4 high security boxes such that each box contained 4 animals. Animal VD35, VD 36, VD49, VD50 were removed after seven days.

Conclusion
We report a significant level of mismatch between real time RT-PCR and infectivity assays for probang samples taken between 6 and 21 dpi. While factors such as sample quality may play a role, stage of the disease is most likely to be a critical factor.

Acknowledgements
We thank Colin Randall and Malcolm Turner for animal care. This work was supported by the Department for Environment, Food and Rural Affairs (DEFRA, SE2920), UK.

Reference