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LATE-PCR and the BioSeeq: Maximum Information from a Field Based Instrument

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in collaboration with Smiths Detection, Inc., Watford, UK

LATE-PCR and PrimeSafe™, two technologies invented in my laboratory, make it easier to construct multi-plexed single-tube assays which can be probed at low temperature with either sequence specific or mis-match tolerant probes. We are now using these methods to construct highly informative assays for several infectious diseases of global importance, including Foot and Mouth Disease (FMDV) and Avian Influenza (AI). These assays are designed to run on either standard laboratory equipment or a sophisticated portable instrument from Smiths Detection, Inc., known as the BioSeeq Portable Veterinary Laboratory. The FMDV assay is a duplex assay designed to detect virtually all strains of this RNA virus. It includes both an external RNA control and an internal DNA control to guard against both false negatives. All targets are detected at end-point. The AI assay is a highly multi-plex assay composed of seven pairs of primers and ten fluorescent probes. It tests for both low/high pathogenic variants of Avian Influenza subtypes H5 and H7, together with their N subtypes and also detects Newcastle Virus, if Avian Influenza is not present. An internal DNA standard and an external RNA control are included to guard against false negatives. Depending on which virus is present, sets of 1-3 single-stranded DNA target sequences are amplified and detected via hybridization to their specific probes. Four colors are read at end-point by dropping the temperature to 70°, 50°, and 35° degrees C. The BioSeeq Portable Veterinary Laboratory is a light weight thermal cycler which can carry out up to five independent reactions in parallel. Each reaction is controlled by insertion of a sample preparation device that contains all of the protocols, reagents, and mechanisms needed for automated nucleic acid extraction, purification, reverse transcription, and amplification with minimum user training or bias. Several forms of telecommunications guarantee the resulting information can be relayed from virtually anywhere on earth to virtually anywhere on earth as needed.