

## **LATE-PCR for Detection and Analysis of Infectious Diseases**

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LATE-PCR, invented in our laboratory, is an advanced form of asymmetric PCR that uses two primers of unequal concentration and differing melting temperatures to generate double-stranded DNA amplicons exponentially, followed by linear amplification of one strand of each amplicon. One of the two primers can also be used to synthesize cDNA from RNA prior to the start of amplification, thus allowing for RT-PCR. PrimeSafe™, also invented in our laboratory, is a PCR additive that enhances construction of single-tube multiplexed assays comprised of several target sequences from a bacterial or viral genome. These assays can detect as little as a single DNA or cDNA molecule. The resulting single-stranded DNA amplicons are either quantified at end-point by use of multiple sequence-specific probes, or mis-match tolerant probes, or via our convenient Dilute-‘N’-Go sequencing protocol.

We are employing these technologies to detect and analyze animal and human infectious diseases in the laboratory or in the field using the BioSeq, a novel point-of-care device developed by Smiths Detection, Inc. Our Avian Influenza assay, for instance, detects both low/high pathogenic variants of both subtypes H5 and H7 and their N subtypes, plus Newcastle Virus, if Avian Influenza is not present. An internal DNA standard and an external RNA control, guard against false negatives. Depending on which virus is present, sets of 1-3 single-stranded cDNA target sequences are amplified and detected based on their patterns of hybridization to ten fluorescent probes in four colors.

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