

A HIGHLY MULTIPLEXED SINGLE-TUBE RT-LATE-PCR ASSAY FOR THE DETECTION OF H5N1 AND OTHER SUBTYPES OF INFLUENZA VIRUS IN THE LAB OR THE FIELD

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Introduction: Our goal over the past two years has been to use LATE-PCR to construct a highly multiplexed single-tube assay that can rapidly distinguish between several high-pathogenic (HPA) and low-pathogenic (LPA) subtypes of Avian Influenza (AI), as well as Newcastle Disease Virus (NDV) if AI is not present.

Materials & Methods: LATE-PCR (1-3), invented in our laboratory, is an advanced form of asymmetric PCR which uses a limiting primer and an excess primer to efficiently generate single-stranded DNA products. Eight pairs of such primers were constructed to achieve optimal reverse transcription and amplification of synthetic RNA targets corresponding to the following influenza virus genes: H5, H7, N1, HPA-H5 or LPA-H5, HPA-H7 or LPA-H7 and M, to an NDV gene, and to an RNA control sequence. All primers were added together to the assay and tested on virus-like combinations of the above synthetic RNAs. The resulting amplicons, different for each virus-like combination, were detected at end-point using ten fluorescent probes, in four colors, which hybridized to their corresponding targets at either of two low temperatures. Scatter among replicate assays was virtually eliminated by use of a novel reagent (also invented in our laboratory) that suppresses mis-priming.

Results: These experiments demonstrate that a single-tube RT-LATE-PCR assay can reliably generate fifteen possible patterns of signals in four colors that are indicative of the presence of a particular subtype/viral strain. The presence of an internal control in all tests rules out false negatives. This assay takes about 90 minutes to run. Results are quantitative over several orders of magnitude.

Conclusions: Tests of our highly informative LATE-PCR assay with viral RNA samples are now underway using standard laboratory equipment. These tests will soon also be available on the BioSeeq Portable Veterinary Diagnostics Laboratory, a pen-side testing system from Smiths Detection Diagnostics which includes an automatic sample preparation and analysis capability in one unit (see additional abstract at this meeting).

References:

1. US Pat No. 7,198,897 issued April 3, 2007, European Patent: 02 795963.4 granted May 7, 2008.
2. Sanchez JA, Pierce KE, Rice JE, Wangh LJ: Linear-after-the-exponential (LATE)-PCR: an advanced method of asymmetric PCR and its uses in quantitative real-time analysis. Proc Natl Acad Sci U S A. 2004, 101:1933-1938.
3. Pierce KE, Sanchez JA, Rice JE, Wangh LJ: Linear-After-The-Exponential (LATE)-PCR: primer design criteria for high yields of specific single-stranded DNA and improved real-time detection. Proc Natl Acad Sci U S A 2005, 102:8609-8614.

Titulo: PRUEBA DE ENSAYO ALTAMENTE MÚLTIPLE DE RT-LATE-PCR EN UNA REACCIÓN ÚNICA PARA LA DETECCIÓN DE H5N1 Y OTROS SUB-TIPOS DE VIRUS DE INFLUENZA EN EL LABORATORIO O EN EL CAMPO

Palabras claves: H5N1, influenza aviar, cepa altamente patogénica, cepa bajamente patogénica, enfermedad de Newcastle, LATE-PCR, prueba de ensayo en una reacción única, termociclador portátil, reacción en cadena de la polimerasa con transcripción inversa (RT-PCR), prueba de corral (pen-side tests), reacción en cadena de la polimerasa múltiple.