

Laboratory Technology

Exploring HPV Genotyping

By Jin Han, PhD

Traditionally, cervical cancer prevention programs to detect human papillomavirus (HPV) have relied on cytological testing using the Papanicolaou (Pap) smear test. However, such screening methods can be expensive, prone to error and logistically difficult to set up, so the direct detection of HPV in cervical specimens is necessary as an alternative or complement to population-based cytological screening.

Testing Problems

Seegene Inc. has moved to automate a nucleic acid–based detection method that provides rapid results on a subtyping-specificity level and shows how the system can be applied to clinical HPV genotyping testing. Seegene’s dual priming oligonucleotide (DPO) technology-based multiplex system is combined with the automated high-throughput detection platform. The underlying assay works from cervical specimens for HPV screening and genotyping without extensive hybridization steps for detection. It is simpler and more efficient than miniaturizing complex procedures.

Aside from traditional Pap smears based on cytological morphology, commercially available products to diagnose HPV are based on probe-hybridization method. However, these methods may detect HPV types that aren’t intentionally targeted. Sequence variation within the probe binding sites and non-specific binding between the probe and non-targeted mismatched DNA are well recognized sources of error if nucleic acid hybridization is relied upon for viral genotyping.

In addition to the high false-positive rates, current methods also employ multiple complicated steps to get results and readout of results can be subjective for the method in which the interpretation of results relies on colorimetric probe detection that can be differently interpreted by various observers.

A New Approach

Scientists at Seegene developed the Seeplex[®] HPV 18-plex Genotyping Test, in which the detection of pathogens relies solely on specific PCR amplification that can be directly read using automated fluorescent capillary electrophoresis and GeneScan software analysis (Applied Biosystems) without complicated, false-generating probe-hybridization steps. In PCR applications of this platform, automated sequencer, one dye (FAM)-labeled DPO primer and one unlabeled DPO primer are used to amplify targets specifically and a small portion of the amplified product is combined with a dye-labeled size standard and electrophoresed on an automated sequencer, where the fluorescent product is sized and quantified.

The DPO system utilizes primers that contain two separate priming regions connected by a polydeoxyinosine linker, allowing it to overcome the most difficult problems with conventional multiplex PCR—incompatible primer sets and high background amplification/detection. In conventional multiplex PCR, each amplification target has a relatively narrow range for optimal conditions, such as annealing temperature and salt concentration. In the Seeplex test, optimization is easy to achieve since all 19 primer pairs (including internal control) are designed using DPO technology with a wider

optimal range of annealing temperature, tolerance to high G/C contents in primer targeting regions and salt concentration. Using cloned HPV plasmid standards as control, the test shows excellent type specificity.

System Benefits

The system is amenable to a high-throughput system. Seegene replaced agarose gel-based detection systems by automated capillary electrophoresis type. Ordinary agarose-type detection systems are not suitable for use in a high-throughput manner, particularly when rapid turnaround is desired. Without an improved detection system, the agarose gel-based method lacks applications in the diagnostic lab since clinical labs will not run agarose gels to visualize thousands of amplified PCR products. Additionally, documentation of correct molecular weight of amplified PCR products may be problematic without some sort of objective output in agarose gel-based detection system. In this system, results can be interpreted automatically by setting the panel for the automatic size calling of PCR amplicons corresponding to each HPV type.

The DPO-based HPV genotyping system presents an innovative, accurate and rapid detection method. The platforms provide high sensitivity combined with subtyping specificity. The high specificity of the Seplex HPV 18-plex Genotyping Test should be considered a useful epidemiological tool for prevalence studies as well as pre- and post-prophylactic vaccine development for analysis of HPV infections.

Dr. Han is principal investigator/team leader, R&D Molecular Diagnostics, Seegene Inc.

PHOTO CAPTION

The Seplex[®] 18-plex HPV Genotyping Test from Seegene Inc.

PULL QUOTE

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