

# Cost-effective screening of genital HPV infections in a Hungarian automated high throughput laboratory

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## Introduction

Diagnosis of anogenital HPV infection, a causative agent of cervical cancer, is an important component of cervical cancer protocols. It improves the sensitivity of cytology. The development of cost-effective and high throughput laboratory techniques for HPV diagnostics as a screening method is an important requirement.

Genoid Molecular Diagnostic Laboratory examines the sexually transmitted disease (STD) samples collected from all around the country by PCR. Samples from abroad arrive in liquid based cytology collection tubes transported at room temperature. A single sampling is enough to perform the following tests: CT, NG, MG, UU, HSV, HPV, TV, GV.

## Abstract

Our laboratory developed a high throughput PCR based technology for the diagnostic of sexually transmitted diseases (STDs), exemplified by the Full Spectrum HPV PCR Kit. Briefly, total sample DNAs are prepared in a 96 well format by a TECAN RSP150 robotic device. Based on the database and the requested test from each sample, microorganisms-specific PCR plates are generated and set up in a 96 well format by the same TECAN RSP150 robotic device. In case of HPV our laboratory developed an automated format of the Full Spectrum HPV PCR Kit. The test is the first PCR-based test with balanced detection sensitivity for all (over 50) genital HPV types, including all high- and low-risk genital types. We used the system to detect HPV in over fifty thousand clinical samples successfully.

<b>Sample identification, preparation</b>	Vaginal, cervical, urethral, anal, pharyngeal samples, urine or tissue biopsy can be processed through the system. Following sample identification by bar code reading, the requested examinations are recorded in the database.		Preanalytical steps of sample preparation differ by the type of sample and transportation system and are carried out manually. All samples are proteinase K digested and subsequent steps carried out by robotic means use this clarified form as the starting material.	Internal control (IC). IC is an artificial PCR template mix which signals the presence of inhibitors or any kind of technical failures during the whole process. It is added to the samples with the proteinase K.
<b>DNA preparation</b>	The layout of a 96 well preparation plate is generated from the database, the layout of plates being adjusted by different (already digested) sample types. Every sample gets a "preparation processed" flag after DNA extraction is completed.		Total sample DNAs are prepared in a 96 well format by the TECAN RSP150 robotic device with a modified silica-based extraction method. Briefly the proteinase K treated samples are loaded into the robot in a predefined layout (see above) and then the extraction solutions are used on a 96 well FiltrEx Glass Fiber Filter plate to prepare total DNA. The DNA plates are stored at -20°C.	Negative control of DNA preparation. Tubes containing only proteinase-K solution with IC mix are prepared in parallel with the samples in the 96 well plate. Four of these controls are prepared in parallel in a 96 well plate as preparation controls for any kind of contamination during DNA preparation.
<b>PCR reaction</b>	Based on the database and the requested tests from each sample, microorganisms-specific PCR plates are generated and set up in a 96 well format by the same TECAN RSP150 robotic device. It allows collection of DNAs from different DNA plates onto one PCR plate. The database software generates a specific robotic script to carry out the cherry picking (reformatting) process in the TECAN RSP150.		For HPV our laboratory developed an automated format of the Full Spectrum HPV PCR Kit (GenoID).	NTC: negative control of PCR reaction. It contains only the PCR mix and no DNA. We use four NTCs/96 well PCR plate. PK: positive control of PCR. It is the cloned DNA of the microorganisms. The primer set of the PCR mix is amplifying the cloned region of the PK plasmid giving a positive result controlling the PCR reaction. We use four PKs/96 well PCR plate.
<b>Detection, Validation, Documentation</b>	The PCR products are detected and the reaction is validated, evaluated and documented. Results are recorded in the database and finally printed.		We detect PCR products either by agarose gel electrophoresis or by the more sensitive and specific solid phase hybridization, where the PCR products are hybridized with labelled specific probes. Our laboratory uses an automated version of the Full Spectrum HPV hybridization kit (GenoID) on a Tecan Miniprep Robotic device. This device prepares 96 well hybridization plates and performs the hybridization steps. Briefly, all HPV samples are hybridized with the probe mixtures yielding high risk, low risk, general positivity/negativity as results. In the next step 14 high risk HPVs are typed by individual hybridization probes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68).	Hybridization positive control: A standard amount of a PCR product yielding a constant hybridization signal to validate the reaction. Hybridization negative control: It does not contain any PCR products yielding a background hybridization signal.
<b>HPV typing and Hybridisation</b>		<b>Conclusions</b> There is a growing demand for a cost effective and specific HPV diagnostic and typing method. Our laboratory developed the cost-effective screening network of genital HPVs and other STD infections: <ul style="list-style-type: none"> <li>• Nation-wide sample collection service</li> <li>• One sample—all STDs by PCR</li> <li>• Automated, 96 well plate-based high quality DNA preparation</li> <li>• Microorganisms-specific 96 well PCR plates (automated reformatting)</li> <li>• Automated PCR and hybridization steps</li> <li>• Computerized documentation, sample control</li> <li>• Internal, positive and negative controls ensuring quality and reliability.</li> </ul>		

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