Fully Automated Genomic DNA Isolation from Cervix Scrapes for Human Papilloma Virus (HPV) Detection

Within the approximately 130 identified types of human papillomavirus (HPV), several HPV types are considered as "high risk types" based on their association with cervical cancer. Accordingly, HPV testing has been considered as cervical cancer screening tool. For this, an automated DNA extraction has been established using the HAMILTON MICROLAB® STAR and MACHEREY-NAGEL´s NucleoMag 96 Tissue kit to allow PCR-based detection and typing of HPV. Reliability and process control are major issues for automated genomic DNA isolation from cervix swab samples. The new automated solution from HAMILTON on the MICROLAB® STAR liquid handling robot using Nucleo-Mag technology from MACHEREY-NAGEL features DNA isolation using a monitored air displacement pipetting principle that allows the detection of incorrect aspiration (e.g. due to clots), and a contamination-free washing of the magnetic beads. HAMILTON's Barcode identification of all labware and reagents and sample tracking ensures a secure data handling. The system processes 96 samples within approx. 3 hours (15 min hands-on-time).

Features and Benefits

- Hands-on time is reduced to a few minutes per 96 DNA extractions
- User friendly loading routine
- Flexible sample number: The number of samples to be processed can be individually adjusted

Figure 1: *A MICROLAB® STAR, equipped with 4 individual 1000µl channels was used in this study.*

Equipment and Material

Equipment

- MICROLAB® STAR equipped with 4 channels, built-in robotic plate-handler (CO-RE Grip) and autoload function to read barcodes from sample tubes
- Functional modules on the MICROLAB® STAR include a plate shaker (Variomag® Teleshake), a MultiFlex Heating Module for lysis incubation and the magnetic separator NucleoMag SEP from MACHEREY-NAGEL

Reagents

- NucleoMag 96 Tissue (from MACHEREY-NAGEL GmbH, Dueren, Germany)

Application Software

The protocol was implemented with the MICROLAB® Vector software. All labware definitions and liquid classes are included in the method.

Kit Description

The NucleoMag 96 Tissue kit uses paramagnetic beads with a high binding capacity for DNA. Following lysis, DNA is bound to the magnetic beads. With subsequent wash steps, impurities and contaminants are removed. After removal of the final wash buffer, DNA can be eluted and directly used for further downstream reactions.

Protocol

- Resuspend the cell-pellet in 100µl T1 buffer manually and transfer it to a deep well reaction plate or a tube on the sample carrier for autoloading onto the robot.
- Add lysis buffer and proteinase K
- Incubate at 56°C for 15 min
- Add binding buffer and magnetic beads and shake at room temperature for 5 min
- Separate and remove supernatant
- Add 600µl wash buffer 1 and shake at room temperature for 2 min
- Separate and remove supernatant
- Add 600µl wash buffer 2 and shake at room temperature for 2 min

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Protocol (continued)

- Separate and remove supernatant
- Add 900µl wash buffer 3 and remove it directly
- Add elution buffer (50 – 200 µl) and shake for 5 min
- Separate and transfer eluate to elution tubes and elution plate

Validation

Different experiments were carried out using the beta-globin PCR and the GP5+/6+ PCR as tools. The experiments were compared to DNA isolations obtained with the EasyMag (Biomerieux).

Results

Yield and Quality

The automated method on the MICROLAB® STAR resulted in DNA yields with concentrations of up to 80ng/ul. Also a robot protocol using half the number of beads as stated above resulted in sufficient DNA for PCR testing. The extracted DNA was clean (OD 260/280 between 1.4-1.8) and of high molecular weight. However, the DNA yield per specimen could vary strongly due to the large variation of cell numbers in cervical scrapes (Figure 2).

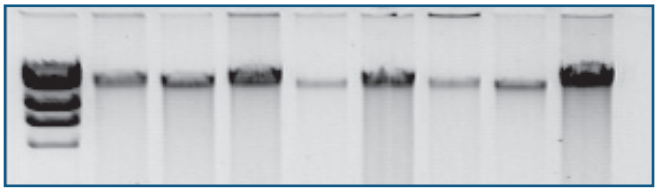

Figure 2: *Agrarose gel electrophoresis of isolated DNA. Randomly selected samples were analyzed on a 1 % TAE-agarose gel (ethidium bromide staining). DNA size standard: Lambda Hind III.*

PCR detection

PCR results comparable to the EasyMag method were obtained for both beta-globin PCR (Figure 3) and GP5+/6+ PCR (data not shown).

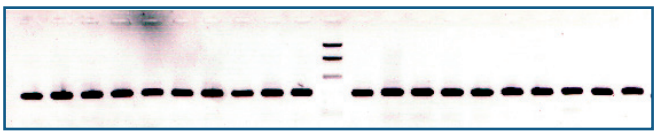

Figure 3: *beta-globin PCR amplification of purified DNA samples. Randomly selected samples of purified DNA were subjected to a beta globin PCR in order to check quality and quantity of purified DNA. All samples were amplified.*

Throughput and Capacity

The system can process 3 runs of 96 cervical scrapes samples (pre-treated) within a working day (8 hours).

Discussion

The automated method described above performs successfully DNA isolation from cervical smears and delivers good results in terms of DNA yield and quality. Outstanding features are the relatively high-throughput of the system with only little hands-on time, the easy way to handle the robot and the flexibility of the method.

Acknowledgements

We would like to thank Rick van Andel and René Pol, Department of Patholgoy, VUmc Amsterdam, The Netherlands, and Thomas Zinn of Macherey Nagel GmbH & Co. KG for co-developing this method and kindly providing the data used in this Application Note.

Literature:

MV Jacobs, PJ Snijders, AJ van den Brule, TJ Helmerhorst, CJ Meijer and JM Walboomers:

A General Primer GP5+/GP6+-Mediated PCR-Enzyme Immunoassay Method for Rapid Detection of 14 High-Risk and 6 Low-Risk Human Papillomavirus Genotypes in Cervical Scrapings

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