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IMPLEMENTATION VALIDATION OF THE PAPILLOCHECK® (GREINER BIO-ONE) KIT FOR GENOTYPING HUMAN PAPILLOMAVIRUSES (HPV) IN PRESERVCYT LIQUID MEDIUM

Ben Vanmassenhove, Gudrun Alliet (galliet@azdamiaan.be) Az Damiaan, Oostende, Belgium

Az Damiaan
Dicht bij het leven

Introduction and purpose

Validation of the PapilloCheck® kit for the detection of 18 high risk and 6 low risk HPVs.

Material and methods

• DNA from patient samples was extracted using the MagNA Pure platform (Roche DNA I High Performance protocol): 1 ml sample was first concentrated by centrifugation (20 min, 20.000 g). 800 µl supernatant was removed and the remaining 200 µl was used for extraction.
• Due to the low concentration of cell material of the Quality Control for Molecular Diagnostics (QCMD) panel, the whole sample (5 ml) was concentrated (20 min, 4000g).
• DNA was eluted in 110 µl elutionbuffer.
• DNA of the WHO Proficiency Panel 2011 (PP) was already extracted.
• 5 µl DNA was used for the PCR.

The assay was checked for analytical sensitivity, specificity, accuracy and precision following the Belgian guidelines (Raymaekers et al, Acta Clinica Belgica, 2011).

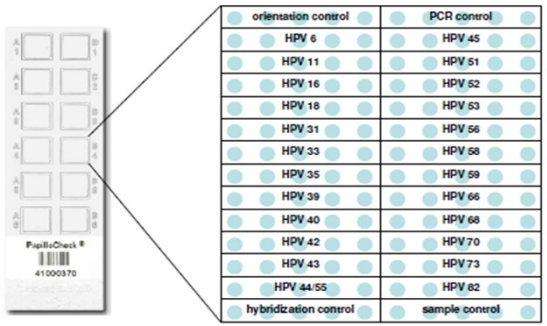

Fig. 1: Layout of the Papill**oCheck**® DNA chip. Each of the 12 wells of a Papill**oCheck**® chip contains a microarray with 28 different probes arranged.

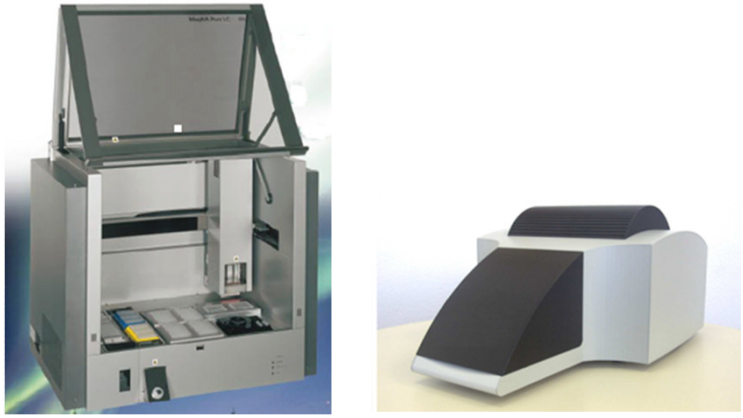

Fig 2: MagNA Pure extraction platform and CheckScanner™. The CheckScanner™ reads the Papill**oCheck®** DNA chip and the CheckReport™ software interprets the Signal to Noise Ratio (SNR) for each probe.

Results (1)

Analytical sensitivity:

• Results of the WHO Proficiency Panel:

5 IU/PCR	50 GE (IU)/PCR	500 GE (IU)/PCR
HPV16	HPV6, HPV11,HPV33, HPV39,HPV45,HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, HPV68	HPV18, HPV31, HPV35

The WHO Proficiency Panel was designed for genotyping needs in HPV vaccinology. The test is not proficient for HPV18 (50 IU/PCR) but according to the manual the LOD for HPV18 is 300 IU/PCR and is in accordance with the clinical needs.

Results (2)

• Results starting from extraction:

A negative PreservCyt specimen was spiked with the WHO-HPV16 DNA standard to determine the limit of detection (LOD with a 95% hit rate) starting from extraction. The lowest concentration was 13.333 international units (IU)/ml, correlating with 120 copies/PCR.

Accuracy:

52 specimens were tested.
• WHO PP2011: 5 out of 43 were false negative (lower than LOD).
• QCMD 2011 panel: 8 out of 9 were typed correctly. HPV45 in ccb10 cells was missed. As HPV45 was 4/4 times detected in the WHO PP, we can suppose that PapilloCheck cannot detect HPV45 in cc10b cells.

Specificity:

the specificity was sufficiently documented by the manufacturer, and was not tested again.

Precision:

One sample positive for HPV31 and HPV51, a second sample with a multiple infection of 3 types: HPV81, HPV33 and HPV73 were extracted in triplicate on 3 different days. All types were detected correctly. This met our validation criteria.

Conclusion

The PapilloCheck® method met all our validation criteria and was implemented in our routine diagnostic laboratory.

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