Feasibility of the Abbott HR HPV assay on three LBC media

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Introduction

• HPV DNA testing is known as more sensitive for detecting high grade disease (CIN2+) than cytology, but less specific.

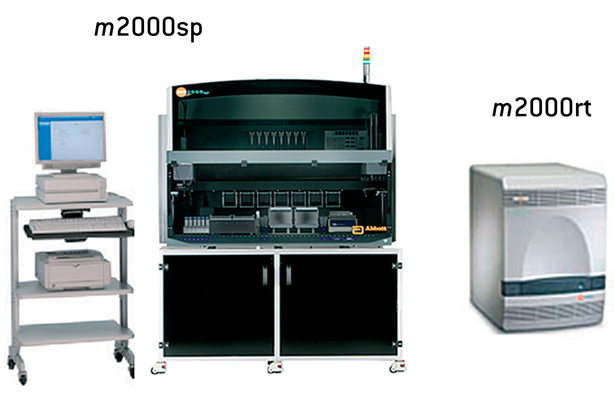
• High risk HPV DNA screening is recommended for triage of patients demonstrating an ASC-US (Atypical Squamous Cells of Undetermined Significance) pap-smear result.

• The aim of this study was to evaluate the feasibility of the recently launched Abbott RealTime HR HPV assay (HRHPV), automated on the m2000 platform, for detection of HPV16, HPV18 and other 12 high risk HPV in cervical samples taken on three liquid-based cytology (LBC) media commonly used in France, CytoScreen (SeroA), EasyFix (Labonord), and SurePath (Tripath Imaging). HRHPV has already been validated on the ThinPrep (Cytyc/Hologic) LBC medium.

Methods

• A total of 146 cervical samples with an ASC-US cytology result, previously tested with Hybrid-Capture 2 (HC2) (Digene/Qiagen), were selected and tested with HRHPV assay; 50 samples taken on EasyFix LBC medium, 50 on CytoScreen and 46 on SurePath .

• Sample preparation for HC2 was performed according to the manufacturer's recommendations for SurePath medium, or as described previously for EasyFix and CytoScreen media, with Proteinase K digestion or/and washings and centrifugations. HC2 was performed according to the manufacturer's recommendations.



ABBOTT M2000 system

• HRHPV was performed using the automated Abbott 2000sp and m2000rt instruments by loading samples directly. HRHPV detects 14 high risk types with modified GP 5+/6+ primers and 14 type specific probes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). It differentiates HPV 16 and 18 from the other high risk types, and detects an endogenous human beta globin sequence as internal control for validating cell adequacy, sample DNA extraction and amplification efficiency.

• Discrepant samples were tested with the Roche HPV Linear Array (LA) using extracted DNA from Abbott m2000sp.

Results

• Results are summarized in tables 1 to 5.

• All samples gave a positive result for internal control.

• Sixty-nine samples were positive with HRHPV; 9 with HPV16, 11 with HPV16 + other high risk HPV types, 1 with HPV18 + other, 48 with only other high risk HPV types.

• Overall correlation between HRHPV and HC2 was 87%.

• None of the 58 HC2 negative samples tested positive with HRHPV.

• Nineteen samples (13%) were positive with HC2 and negative with HRHPV.

• Among these 19 samples,

– 6 were negative with the LA genotyping assay.

– 9 contained low risk HPV and/or HPV of undetermined risk.

– 4 were positive for high risk HPV DNA by LA and 3 out of these 4 were amplified with HRHPV but beyond cutoff (32 cycles)

• No discrepancy could be related to a particular LBC medium

All samples N=146

Abbott RealTime \ HC2	+	-
+	69	0
-	19	58

TABLE 1

EasyFix samples N=50

Abbott RealTime \ HC2	+	-
+	22	0
-	8	20

TABLE 2

CytoScreen samples N=50

Abbott RealTime \ HC2	+	-
+	32	0
-	6	12

TABLE 3

SurePath samples N=46

Abbott RealTime \ HC2	+	-
+	15	0
-	5	26

TABLE 4

Resolution of discrepant results

Sample ID	LBC	HC2 Result	HR HPV Result	Target crossing point	Internal control crossing point	Linear Array Result
285207	Easyfix	1,44	Not Detected	no	19,18	66,84
266036	CytoScreen	3,01	Not Detected	32,15	26,92	35,627 53
141185	CytoScreen	1,84	Not Detected	33,79	27,14	58
201859	SurePath	4,11	Not Detected	32,43	23,64	27
279167	Easyfix	33,12	Not Detected	no	24,28	Negative
295243	Easyfix	3,47	Not Detected	no	21,44	Negative
294480	Easyfix	1,52	Not Detected	no	19	Negative
243527	CytoScreen	1,30	Not Detected	no	25,72	Negative
143478	CytoScreen	1,26	Not Detected	no	27,13	Negative
137379	SurePath	14,30	Not Detected	no	24,51	Negative
294930	Easyfix	21,14	Not Detected	no	21,02	53,61,62,83,CP6108
278971	Easyfix	3,36	Not Detected	no	20,84	42
265086	Easyfix	2,91	Not Detected	no	21,69	42,55,83,84
301535	Easyfix	1,44	Not Detected	no	23,18	42
125164	CytoScreen	3,13	Not Detected	no	25,09	IS39
125213	CytoScreen	1,25	Not Detected	no	28,5	67
193518	SurePath	344,57	Not Detected	no	23,45	53,84
286529	SurePath	2,04	Not Detected	no	21,78	53
367624	SurePath	4,01	Not Detected	no	27,14	67

TABLE 5

Conclusions

The Abbott HRHPV assay demonstrated its feasibility on CytoScreen, EasyFix and Surepath LBC media.

The use of a co-amplified internal control allowed the assessment of DNA extraction and PCR efficiency : All samples were validated for the presence of sufficient human DNA, and no PCR inhibition was detected in these alcohol-rich media.

Nineteen discrepancies between the two techniques were investigated by a Linear Array genotyping test and led to a good concordance between HRHPV and LA.

The absence of amplification by HRHPV of low risk HPV DNA detected by HC2 is consistent with what is known of the possible detection of some low risk HPV with HC2 due to cross-hybridization, particularly when a low positive signal is obtained, as it was here for 7 out of 9 discrepant samples.

Four positive samples, all of them with a low signal (≤ 4) with HC2 , and also positive with the LA genotyping assay with the presence of high risk HPV types, were negative with HRHPV. Three out of these 4 were amplified but below the 32 cycles cut-off.

These findings on lower positivity signals are consistent with the known possibility of improvement of the clinical specificity of HPV DNA tests for detecting CIN2+ by raising the detection threshold. Increasing the HC2 positivity cut-off from 1 to 2-3 has been regularly proposed to increase its clinical specificity, and the HRHPV cut-off has been optimized by the manufacturer during internal studies to increase its specificity for detecting CIN2+.

The absence of follow-up histology data for the patients included in this small study strongly limited the possibility of evaluation.

Further correlations studies are therefore needed on routine unselected ASC-US samples taken on various LBC media to evaluate the clinical sensitivity and specificity of the HRHPV versus HC2 and other HPV DNA tests for detecting high risk HPV in these media and predicting CIN2+.

Aknowledgments : HRHPV DNA tests were performed by Abbott Molecular. LA test was performed by Dr.Sam Ratnam at Public Health Laboratory, St. John's, Newfoundland, Canada