Journal of Clinical Virology 53 (2012) 121–124

Contents lists available at SciVerse ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Comparison of Abbott RealTime High Risk HPV and Hybrid Capture 2 for the detection of high-risk HPV DNA in a referral population setting

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ARTICLE INFO

Article history:
Received 10 August 2011
Received in revised form 28 October 2011
Accepted 30 October 2011

Keywords:
HPV testing
Real time PCR
HPV16/18 typing
Referral population

ABSTRACT

Background: The Abbott RealTime High Risk HPV assay (ART) is an automated multiplex real-time PCR test for detection of DNA from 14 high risk (HR) HPV types in cervical specimens and simultaneous distinction of HPV16 and HPV18 from other HR-HPV.
Objectives: To evaluate the performance of the ART assay in specimens referred for HPV testing to our laboratory (referral population) by comparison with historical data from HC2 and INNO-LiPA as well as histological status, if available.
Study design: 412 cervical specimens were collected from women between 18 and 70 years of age: 301 previously tested by HC2 without clinical data and 111 previously tested by HC2 and INNO-LiPA with histological diagnosis of CIN3+.
Results: Our study demonstrated good overall agreement between ART, HC2 and INNO-LiPA. In the group of the CIN3+ specimens HR-HPV was detected by ART in 93.07% (95% CI: 88.12–98.02), while HR-HPV detection rates with HC2 and INNO-LiPA were 91.09% (95% CI: 85.53–96.65) and 95.05% (95% CI: 90.82–99.28), respectively. The typing capability of ART for HPV16, HPV18 and a pool of twelve other HR-HPV types was investigated by comparison with INNO-LiPA demonstrating high overall assay concordance (89.81%; *k* 0.87).
Conclusions: The Abbott RealTime assay showed similar clinical performance for detection of CIN3+ compared with HC2. The high level of automation and ability to identify HPV16, HPV18 and other HR-HPV make this assay a very attractive option for HR-HPV testing, potentially improving patient management by risk stratification of cytological abnormal populations.

1. Background

Recently established guidelines recommend HPV DNA testing in order to improve the efficacy of primary cytological screening programs or triage tests.[1–7]

The High Risk Hybrid Capture 2 assay has shown high sensitivity for the detection of cervical intraepithelial neoplasia of grade 2 and worse (CIN2+)[8] and it was recently recommended to be used as a benchmark for performance assessment of new candidate HPV tests for primary cervical cancer screening in women of 30 years and older.[9] HPV typing assays such as INNO-LiPA HPV (Innogenetics) and Linear Array (Roche) are commonly used in the follow up of persistent infections to monitor the presence of specific HPV genotypes.[10]

The Abbott RealTime High Risk HPV assay (ART) is a new real-time multiplex PCR able to detect 14 HR-HPV types and simultaneously type HPV16 and HPV18. Since 70% of cervical carcinoma are known to be caused by HPV16 and HPV18, the new assay could be a useful tool both for primary screening and for the triage of women with abnormal cytology.[11]

2. Objectives

The aim of the present study was to evaluate the performance of the ART assay by analyzing archival material from diagnostic

Abbreviations: HPV, human papillomaviruses; HR, high risk; LR, low risk; pHR, probable high risk; ndR, non-determined risk; HSIL, high-grade squamous intraepithelial lesions; CIN, cervical intraepithelial neoplasia; IA1, microinvasive squamous cell carcinomas; ART, Abbott RealTime High Risk HPV assay; HC2, Hybrid Capture 2; INNO-LiPA, INNO-LiPA HPV Genotyping Extra; LA, Linear Array; ACOG, American congress of obstetricians and gynecologists.

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doi:10.1016/j.jcv.2011.10.016

cervical specimens of a referral population, providing that all data would be kept anonymous. The results were compared with historical data by Hybrid Capture 2 High Risk HPV DNA (HC2) and INNO-LiPA HPV Genotyping Extra (INNO-LiPA).

3. Study design

3.1. Study population

The study population consisted of 412 cytological cervical specimens, collected in PreservCyt Solution (Hologic, Marlborough, MA, USA) between March 2008 and February 2009 from women between 18 and 70 years of age (median 38.4 years). Specimens had been referred for investigation of their HPV status based on previously observed cytological or histological abnormalities (referral population). Two groups were available:

Group 1: 301 specimens previously tested by HC2. Clinical data had not been provided by the referring sites. Specimens displaying discordant results between ART and HC2 were further analyzed by INNO-LiPA, to ascertain a consensus result (2 out of 3 positive or negative test results) and to assess the sensitivity and specificity of the tests.

Group 2: 111 specimens from women with pap-test diagnosis of high-grade squamous intraepithelial lesions (HSIL) and histological diagnosis on punch biopsy of cervical intraepithelial neoplasia grade 3 and higher (CIN3+), performed about one month before conisation. A surgical specimen was analyzed at conisation time and concomitantly HPV test was performed by HC2 and INNO-LiPA. Cone histology consisted of 10 cases with CIN2, 97 CIN3 and four microinvasive squamous cell carcinomas (IA1). Histological diagnosis was confirmed based on pathology consensus review of tissue samples.

3.2. HPV DNA detection assays

3.2.1. Hybrid Capture 2 High-Risk HPV DNA (Qiagen, Hilden, Germany)

Samples were pretreated with the HC2 Sample Conversion Kit. The HC2 is a nucleic acid hybridization technique able to detect 13 HR-HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68.

3.2.2. Abbott RealTime High Risk HPV (Abbot, Wiesbaden, Germany)

The ART assay is an automated qualitative assay for the detection of 14 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and simultaneous identification of HPV16 and HPV18. DNA-extraction, amplification and detection are carried out on the Abbott *m*2000 System.[12]

3.2.3. INNO-LiPA HPV Genotyping Extra test (Innogenetics, Ghent, Belgium)

The INNO-LiPA assay performed from DNA extracted by NucliSENSE EasyMag system on the EasyMag Extraction Platform (bioMèrieux, Marcy l'Etoile, France) allows for detection of 28 different HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, 73, 74, 82, 69/71).

3.3. Statistical analysis

Proportions are presented with 95% confidence intervals (95% CI), estimated by standard methods.[13] Cohen's kappa (*k*) was calculated to determine the level of chance-adjusted agreement between pairs of assay methods.[14]

Table 1
Analysis of 27 specimens with discordant results between ART and HC2 (*Group 1*) by INNO-LiPA.

No. specimen	HC2	ART HPV16/HPV18/ Other HR	INNO-LiPA HR/pHR-HPV[a]	INNO-LiPA LR/ndR HPV[b]
1	pos	neg	**16 52 68**	11
2	pos	neg	53 **66 68**	neg
3	pos	neg *[HR: ct 32.75]*[c]	**51 68**	neg
4	pos	neg *[HR: ct 35.54]*[c]	**58**	neg
5	pos	neg	**66**	neg
6	pos	neg	**68**	neg
7	pos	neg	**68**	neg
8	pos	neg	53	neg
9	pos	neg	53	74
10	pos	neg	82	neg
11	neg	pos (16)	**16**	neg
12	neg	pos (16)	**16**	neg
13	neg	pos (16)	**16**	6
14	neg	pos (16)	**16**	74
15	neg	pos (HR)	**51**	neg
16	neg	pos (HR)	**52**	6
17	neg	pos (HR)	**52**	neg
18	pos	neg *[HR: ct 32.75]*[c]	neg	6
19	pos	neg	neg	44
20	pos	neg	neg	69/71
21	pos	neg	neg	69/71
22	pos	neg	neg	74
23	pos	neg	neg	neg
24	pos	neg	neg	neg
25	neg	pos (HR)	neg	54
26	neg	pos (HR)	neg	neg
27	neg	pos (HR)	neg	neg

[a] HR-HPV (high risk HPV): genotypes **16**, **18**, **31**, **33**, **35**, **39**, **45**, **51**, **52**, **56**, **58**, **59**, **68**, 73, 82; pHR-HPV (probable high risk HPV): genotypes 26, 53, **66**. Only "bold" genotypes are detected by Abbott RealTi*m*e HR-HPV assay (ART).
[b] LR HPV (low risk HPV): genotypes 6, 11, 40, 43, 44, 54, 70; nd HPV (non determined risk HPV): genotypes 69/71 and 74.
[c] Square brackets: Ct values above the manufacturer's fixed assay cut off cycle.

4. Results

Group 1: The numbers of concordant positive and negative samples, between ART and HC2, were 171 and 103, respectively, giving an overall concordance rate of 91.03% (*k* 0.81).

Further analysis by INNO-LiPA of the 27 discordant samples (Table 1) revealed the presence of targeted HR-HPV genotypes in seven samples negative with ART and positive by HC2; two of these samples showed amplification beyond the cycle number cut off of the test. Non-targeted HPV genotypes were identified with INNO-LiPA in three samples negative for HR-HPV with ART and positive with HC2. ART results were confirmed for seven samples positive with ART and negative with HC2 and for seven negative with ART and positive with HC2. Three samples positive with ART were negative with HC2 and INNO-LiPA.

After analysis of discordant results, sensitivity and specificity of ART were 94.0% (95% CI: 89.72–98.31) and 98.4% (95% CI: 96.54–100.00), while of HC2 were 94.0% (95% CI: 89.63–98.30) and 94.1% (95% CI: 90.64–97.46).

Group 2: The concordance between ART and HC2 was 90.99% (*k* 0.59) (Table 2). Ten specimens proved discordant between both tests: the four HC2 positive and ART negative were two CIN2 and two CIN3 at cone histology, the six HC2 negative and ART positive were two CIN2 and four CIN3. Considering only the 101 CIN3+, the concordance between ART and HC2 was 94.06% (*k* 0.59).

Further analysis of the 19 CIN2+ specimens with negative results with one or more assays (Table 3), showed concordance of negative results in three out of eight CIN2 and in two out of eleven CIN3 at cone histology.