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For Peer Review

**Performance of the Abbott RealTime High Risk HPV test in women
with abnormal smears**

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Abstract

HPV DNA testing is known to be much more sensitive than cytology, but less specific. A range of HPV and related tests in 858 women referred for colposcopy because of an abnormal smear were evaluated to compare the performances of these tests. This paper compared the Abbott test to other tests which had been previously evaluated. This test was a real true test for 14 high risk HPV types. The Abbott test was found to be highly sensitive for cervical intraepithelial neoplasia grade 3 or worse (CIN3+) (98.9%) with a specificity of 31.5%. These numbers were comparable with the Qiagen HC2 test, the Roche Linear Array and Amplicor tests, and the Gen-Probe APTIMA test. Differences between these tests appeared to be related mostly to the choice of cutoff level. An added feature of the Abbott test was the provision of type specific results for HPV 16 and 18.

Key Words

HPV testing

Cervical Screening

Abbott RealTime High Risk HPV Test

Sensitivity

Specificity

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Introduction

High risk (HR) HPV is a necessary factor for the development of cervical cancer (Walboomers et al. [1999]) but the presence of HR HPV DNA does not lead invariably to disease. Recently it was found that the detection of HR HPV provides high sensitivity but has lower specificity than cytology for the identification of high grade cervical lesions in a screening population in the UK (Cuzick et al. [2003]) and this finding has been replicated in several other studies (Cuzick et al. [2006], Almonte et al. [2007], Bulkmand et al. [2007], Mayrand et al. [2007], Naucler et al [2007], Kotaniemi-Talonen et al. [2008], Ronco et al. [2008]). In addition, several studies have shown that HR HPV DNA positive women are significantly more likely to develop high grade cervical intraepithelial neoplasia (CIN) than women with a negative HPV DNA test (Bulkmand et al. [2005], Khan et al. [2005]) and that a negative HPV test offers a longer period of low risk (protection) of developing high grade cervical intraepithelial neoplasia (CIN) than a negative cytology result (Cuzick et al. [2008], Dillner et al. [2008]). If testing for HR HPV DNA is to be used as a primary cervical screening test, refinements or additional tests are highly desirable to improve specificity while retaining its very high sensitivity.

The introduction of a liquid-based medium for collection of cytological specimens has allowed other molecular techniques to be evaluated more easily as the sole initial test, as an adjunctive test with cytology or as a triage test in women with minimal cytologic abnormalities. In a previous report we compared directly the sensitivity and specificity of a range of tests from the same sample for the detection of high grade cervical intraepithelial

Abbott RealTime High Risk HPV

neoplasia (CIN) in a population referred to colposcopy because of abnormal cytology (Szarewski et al. [2008]). In this report the Abbott RealTime which detects High Risk (HR) HPV test is evaluated, which detects fourteen high risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) with type specific detection for types 16, 18, and detection of the other twelve types as a group. In addition, a separate (fourth) channel is used to detect beta-globin as an internal control for sample adequacy. Although designed as a qualitative test utilizing real-time PCR technology, the test also provides cycle number that is generally indicative of the amount of viral DNA present in the sample. Thus high risk HPV detection and HPV16, HPV18 typing results are provided for each sample in addition to the check for sample cellularity. All the tests were compared against the gold standard of histopathology.

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Methods

The study population comprised of women who had been referred to the colposcopy clinics at the Hammersmith and St. Mary’s Hospitals in London between August 2005 and January 2007. While not a screening population, the advantage was a broad range of outcomes and a high disease rate, which would enable accurate evaluation of sensitivity and specificity in a relatively small number of samples. Women were eligible if they had been referred as a result of one or more abnormal cervical smears, were not pregnant, had not been treated previously for cervical intraepithelial neoplasia (CIN), nor had a hysterectomy. All women received a patient information sheet explaining the study and provided written consent. Approvals were obtained from the relevant Local Research Ethics Committees.

Prior to colposcopy, a cervical sample was obtained using a Cervex broom and placed in PreservCyt® transport medium. Colposcopy was then performed in the usual manner. The liquid based cytology (LBC) samples were transported to The Doctors’ Laboratory (TDL), where an aliquot was first removed for cytology, processed using the ThinPrep system and returned to the relevant cytopathology departments for reporting. The following tests were performed on remaining aliquots of the material, Hybrid Capture II (Qiagen, Hilden, Germany), Pretest HPV Proofer (Norchip, Klokkearstug, Norway), Amplicor and Linear Array (Roche, Basel, Switzerland), APTIMA (Gen-Probe, San Diego, CA, USA), Clinical-Arrays (Genomica, Madrid, Spain), CINtec p16^{INK4a} (mtm Laboratories, Heidelberg, Germany), and our in-house qPCR test. The characteristics of these tests are summarised in Table 1. Aliquots remained for 858 women, which were used to perform the Abbott test in our lab.

Abbott RealTime High Risk HPV

Abbott RealTime HR HPV is a real-time PCR assay performed on the automated *m2000* system that detects fourteen high-risk HPV types, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 and simultaneously types HPV 16 and HPV 18. The test also features an internal control for specimen cellularity by detecting an endogenous human beta-globin sequence. Through the distinct probe labels and multi-colour detection system, signals for HPV16, HPV18, non-16/18 HR types, and internal control are simultaneously detected and distinguished in a single reaction. 0.4 mL aliquot of the PreservCyt sample is processed and DNA is purified using magnetic microparticle technology. This test amplifies a conserved L1 region of approximately 150 base pairs with a modified GP5+/6+ primer mix. Threshold cycle number of 32 as determined from the amplification curve is the assay cutoff recommended by the manufacturer. One positive and one negative control are included in each run to assess run validity.

Methods for the other assays have been described previously (Szarewski et al. [2008]).

The molecular testing laboratories were blinded to the cytology and histopathology results.

Histopathology was first reported locally and then reviewed centrally by either Dr Hilary Buckley (92% of samples) or Dr. Christine Bergeron (8% of samples), who were blinded to all study test results but did have access to the concurrent cytology. All results are presented based on the reviewed histopathology and the highest grade of abnormality seen in the biopsy or treatment specimen was used.

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Statistical methods

Data entry and statistical analyses were carried out at the CRUK Centre for Epidemiology, Mathematics and Statistics. All statistical analyses were carried out using Stata 9.2 (StataCorp, USA).

It was determined that a minimum of 500 patients would be needed to have 80% power to detect a change in sensitivity from 80% to 90 % assuming a 25% prevalence of CIN2+ and a discordance rate of 3.6% (18/500). This number of subjects would give at least 90% power to detect improvements in specificity from 50% (common in triage) to 75%.

The gold standard was histologically confirmed high grade cervical intraepithelial neoplasia grade 2 or worse (CIN 2+), but calculations were also made for histologically confirmed cervical intraepithelial neoplasia grade 3 or worse (CIN3+). The sensitivity, specificity and positive predictive value (PPV) of each test were computed and 95% confidence intervals were obtained using exact binomial methods when necessary. Additional calculations of sensitivity, specificity and PPV were carried out for different age groups.

Results

Results were previously reported on the 953 women taking part in the study. Of these, sufficient material for the Abbott RealTime HR HPV assay was available for 858 women, for which results are presented here. The histological outcomes for the study are shown in Table 2. A total of 21.1% of the women had cervical intraepithelial neoplasia grade 3 or worse (CIN3+) and an additional 8.7% had cervical intraepithelial neoplasia grade 2 (CIN2), so that overall 29.8% had histologically confirmed high grade disease. Table 3 shows the positivity rates for different combinations of HPV 16, 18 and non-16/18 high risk types. Altogether 643/858 or 74.9% of the women were positive for at least one type. Sensitivity and PPV for the Abbott test for cervical intraepithelial neoplasia grade 3 or worse (CIN3+) and cervical intraepithelial neoplasia grade 2 or worse (CIN2+) are shown in Table 4. The sensitivity and specificity for the Abbott test in comparison to all the other tests are summarised in Figures 1a and 1b. It can be seen that all types together gave a very high sensitivity, both for cervical intraepithelial neoplasia grade 3 or worse (CIN3+) (98.9%, 179/181) and cervical intraepithelial neoplasia grade 2 or worse (CIN2+) (97.7%, 250/256). The positive predictive value (PPV) was higher for HPV16 than other types, being almost 60% for cervical intraepithelial neoplasia grade 2 or worse (CIN2+), whereas the other types (including type 18) had PPVs in the 30-35% range for cervical intraepithelial neoplasia grade 2 or worse (CIN2+). HPV 18 was more predictive for cervical intraepithelial neoplasia grade 3 or worse (CIN3+) (31.5%) than the non-16/18 HR types (21.4%), but still less than for HPV 16 (46.5%). PPVs for HPV 18 were substantially higher in younger women than for those aged 30y or more, but the PPVs for HPV16 and the non-16/18 HR types did not vary appreciably by age.

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Table 5 indicates the concordance between the Abbott test and the other tests both for all high risk types and types 16 and 18 separately (vs. Linear Array, the PreTect HPV-Proofer Clinical Arrays, and q-PCR). It can be seen that the concordance was highest with Linear Array, but was also quite high with Hybrid Capture II and APTIMA. Poor correlation was seen with the PreTect HPV-Proofer, p16 and Clinical-Arrays. When restricted to types 16 and 18 concordance was again highest with Linear Array, but improved substantially with the PreTect HPV-Proofer and our in-house q-PCR. Of the 27 women with discordant results for HPV 16 between the Abbott assay and the Roche Linear Assay, the 22 women were positive for Linear Assay containing 6 cervical intraepithelial neoplasia grade 2 or worse (CIN2+) cases, while all 5 cases only positive for the Abbott assay had less than cervical intraepithelial neoplasia grade 2 (CIN2). For the 13 women positive for HPV 18 only by Linear Assay, 4 had cervical intraepithelial neoplasia grade grade 2 or worse (CIN2+). No woman was positive for HPV 18 by the Abbott assay, but not the Roche Linear Array.

Figures 2a and 2b show the viral load ROC curves characterizing the effect of cycle number cutoff setting on test performance for the detection of intraepithelial neoplasia grade 3 or worse (CIN3+) and intraepithelial neoplasia grade 2 or worse (CIN2+), respectively. Changing the cutoff of cycle number from ≤ 32 to ≤ 30 would leave the sensitivity unchanged (98.9% for intraepithelial neoplasia grade 3 or worse (CIN3+), 97.7% for intraepithelial neoplasia grade 2 or worse (CIN2+)) but would increase specificity to 35.5% instead of 31.5% for cervical intraepithelial neoplasia grade 3 or worse (CIN3+) and to 39.2% instead of 34.7% for cervical intraepithelial neoplasia grade 2 or worse (CIN2+). The PPV was raised from 27.8% to 29.1% for cervical intraepithelial neoplasia grade 3 or worse (CIN3+) and from 38.9% to 40.6% for cervical intraepithelial neoplasia grade 2 or worse (CIN2+).

Abbott RealTime High Risk HPV

For cervical intraepithelial neoplasia grade 3 or worse (CIN3+) the slope of the ROC curve changed between 24 and 25 cycles. If the cutoff was reduced to ≤ 24.5 , the sensitivity for cervical intraepithelial neoplasia grade 3 or worse (CIN3+) would decrease to 90.1% (84% for cervical intraepithelial neoplasia grade 2 or worse (CIN2+)) while a substantial increase would be observed for both specificity (52.6% for cervical intraepithelial neoplasia grade 3 or worse (CIN3+), 55.3% for cervical intraepithelial neoplasia grade 2 or worse (CIN2+)) and PPV (33.7% for intraepithelial neoplasia grade 3 or worse (CIN3+), 44.4% for cervical intraepithelial neoplasia grade 2 or worse (CIN2+)).

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Discussion

The Abbott RealTime HR HPV test demonstrated a very high sensitivity for cervical intraepithelial neoplasia grade 2 or worse (CIN2+) and cervical intraepithelial neoplasia grade 3 or worse (CIN3+), in the same range as the Qiagen Hybrid Capture II test, the Roche Amplicor and Linear Array tests, and slightly better than the Gen-Probe APTIMA test. The concordance with these tests was also quite high. The specificity was slightly better than Hybrid Capture II, Linear Array and Amplicor tests, but not as high as the APTIMA test. This suggests the difference between these tests is largely based on the cutoff threshold. The positive predictive value of HPV 16 and to a less extent HPV 18 was greater than for the other high risk types, indicating that this information could be useful for determining who needs immediate referral and who can be safely managed by repeat testing. These results suggest that the Abbott RealTime HR HPV test is a promising candidate for use in primary screening, and further studies are needed to evaluate its performance directly in this context.

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Conflicts of interest

The following companies provided test kits and a financial contribution to the first author's institution to help conduct this study:

Abbott, Qiagen, Gen-Probe Incorporated, Genomica S.A.U, mtm laboratories, NorChip and Roche Diagnostics

References

Almonte M, Ferreccio C, Winkler J, Cuzick J, Tsu V, Robles S, Takahashi R, Sasieni P. 2007. Cervical screening by visual inspection, HPV testing, liquid-based and conventional cytology in Amazonian Peru. *International Journal of Cancer* 121:796–802

Bulkman NW, Berkhof J, Rozendaal L, van Kemenade FJ, Boeke AJ, Bulk S, Voorhorst FJ, Verheijen RH, van Groningen K, Boon ME, Ruitinga W, van Ballegooijen M, Snijders PJ, Meijer CJ. 2007. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. *Lancet* 370(9601):1764-72.

Bulkman NW, Rozendaal L, Voorhorst FJ, Snijders PJ, Meijer CJ. 2005. Long-term protective effect of high risk human papillomavirus testing in population-based cervical screening. *British Journal of Cancer* 92:1800-2.

Cuzick J, Szarewski A, Cubie H, Hulman G, Kitchener H, Luesley D, McGoogan E, Menon U, Terry G, Edwards R, Brooks C, Desai M, Gie C, Ho L, Jacobs I, Pickles C, Sasieni P. 2003. Management of women who test positive for high risk types of human papillomavirus: the HART study. *Lancet* 362:1871-6.

Cuzick J, Clavel C, Petry KU, Meijer CJ, Hoyer H, Ratnam S, Szarewski A, Birembaut P, Kulasingam S, Sasieni P, Iftner T. 2006. Overview of the European and North American studies on HPV testing in primary cervical screening. *International Journal of Cancer* 119:1095–101.

Cuzick J, Szarewski A, Mesher D, Cadman L, Austin J, Perryman K, Ho L, Terry G, Sasieni P, Dina R, Soutter WP. 2008. Long-term follow-up of cervical abnormalities among women screened by HPV testing and cytology - Results from the Hammersmith study. *International Journal of Cancer* 122(10):2294-300.

Dillner J, Rebolj M, Birembaut P, Petry KU, Szarewski A, Munk C, de Sanjose S, Naucler P, Lloveras B, Kjaer S, Cuzick J, van Ballegooijen M, Clavel C, Iftner T; Joint European Cohort Study. 2008. Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. *British Medical Journal* 337:a1754.

Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR, Rush BB, Glass AG, Schiffman M. 2005. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *Journal of the National Cancer Institute* 97:10729.

Kotaniemi-Talonen L, Anttila A, Malila N, Tarkkanen J, Laurila P, Hakama M, Nieminen P. 2008. Screening with a primary human papillomavirus test does not increase detection of cervical cancer and intraepithelial neoplasia 3. *European Journal of Cancer* 44(4):565-71.

Mayrand MH, Duarte-Franco E, Rodrigues I, Walter SD, Hanley J, Ferenczy A, Ratnam S, Coutlée F, Franco EL. 2007. Canadian Cervical Cancer Screening Trial Study Group. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. *New England Journal of Medicine* 357(16):1579-88.

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3 Naucner P, Ryd W, Törnberg S, Strand A, Wadell G, Elfgrén K, Rådborg T, Strander B,
4 Forslund O, Hansson BG, Rylander E, Dillner J. 2007. Human papillomavirus and
5 Papanicolaou tests to screen for cervical cancer. *New England Journal of Medicine*
6 357(16):1589-97.
7

8
9 Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Dalla Palma P, Del Mistro A, Gillio-Tos
10 A, Minucci D, Naldoni C, Rizzolo R, Schincaglia P, Volante R, Zappa M, Zorzi M, Cuzick J,
11 Segnan N. 2008. New Technologies for Cervical Cancer Screening Working Group. Results
12 at recruitment from a randomized controlled trial comparing human papillomavirus testing
13 alone with conventional cytology as the primary cervical cancer screening test. *Journal of the*
14 *National Cancer Institute* 100(7):492-501.
15
16

17
18 Szarewski A, Ambrosine L, Cadman L, Austin J, Ho L, Terry G, Liddle S, Dina R,
19 McCarthy J, Buckley H, Bergeron C, Soutter P, Lyons D, Cuzick J. 2008. Comparison of
20 Predictors for High-Grade Cervical Intraepithelial Neoplasia in Women with Abnormal
21 Smears. *Cancer Epidemiology Biomarkers & Prevention* 17(11):3033-42.
22

23
24 Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ,
25 Peto J, Meijer CJ, Muñoz N. 1999. Human papillomavirus is a necessary cause of invasive
26 cervical cancer worldwide. *The Journal of Pathology* 189:12-9.
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Table 1. Characteristics of the different HPV tests employed in this study.

Test Manufacturer	Target	Types Detected	Methodology
Hybrid Capture II Qiagen	DNA	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	Signal amplification by hybridization to a cocktail of RNA probes chemiluminescent detection
Amplicor Roche Diagnostics	DNA	As above	Consensus PCR 40 cycles
Linear Array Roche Diagnostics	Type-specific DNA	37 types including those above	PcR with PGMY primers 40 cycles Type-specific oligonucleotide probes colorimetric detection
Clinical Arrays Genomica	Type-specific DNA	35 types including those above	MY09/11 Primers PCR with 45 cycles Low density microarray colorimetric detection
PreTect HPV-Proofer Norchip	Type-specific E6/E7 mTNA	16, 18, 31, 33, 35	Multiplex NASBA isothermal amplification real time detection
APTIMA Gen-Probe	Consensus E6/E7 mRNA	13 types listed above plus 66	Target Capture (Magnetic Particles Transcription mediated Amplification (Isothermal) Hybridization protection
RealTime HR HPV Abbott	Consensus and Limited type-specific DNA	14 types above plus type- specific for HPV 16+18	RealTime PcR of LI region Threshold cycle 32 multi- colour detection
p16 ^{INK4a} mtm Laboratories	p16 protein	-	Immunohistochemistry with mouse monochloral antibody to p16 ^{INK4a} with second anti-mouse antibody and DAB chromogen detection

Table 2. Distribution of worst reviewed histological outcomes in the study.

Worst histology (reviewed)	Freq.	%
Normal colposcopy / Negative biopsy	386	45.0
Unsatisfactory	9	1.0
HPV only / Borderline	127	14.8
CIN 1 / CIN ungradable / CGIN low grade	80	9.3
CIN 2	75	8.7
CIN 3	165	19.2
CGIN high grade	4	0.5
Invasive carcinoma	12	1.4
Total	858	100.0

CIN – cervical intraepithelial neoplasia

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Table 3. Prevalence of the different HPV types using the Abbott RealTime HR HPV test.

Type	No. positive	%positive
HPV16 alone	126	14.7
HPV16 any	243	28.3
HPV 18 alone	25	2.9
HPV 18 any	73	8.5
Other HR alone	344	40.1
Other HR any	486	56.6
HPV16 & 18 (w/o others)	6	0.7
HPV16 & other HR (w/o 18)	100	11.6
HPV18 & other HR (w/o 16)	31	3.6
All 3 groups	11	1.3
Any high risk type	643	74.9

Abbott RealTime High Risk HPV

Table 4. PPV and sensitivity of Abbott RealTime HR HPV test and PPV of type-specific detection for a) cervical intraepithelial neoplasia grade 3 or worse (CIN3+) and b) cervical intraepithelial neoplasia grade 2 or worse (CIN2+), overall and according to age (<30, ≥30). Numbers for sensitivity add up to more than 100% due to multiple types.

a) CIN3+ (n = 181)				
	Age	No. with disease & positive / No. positive for type	PPV	Sensitivity
Abbott RealTime HR HPV	All	179 / 643	27.8	98.9
	< 30	93 / 356	26.1	97.9
	≥ 30	86 / 287	30.0	100.0
HPV16	All	113 / 243	46.5	—
	< 30	64 / 142	45.1	—
	≥ 30	49 / 101	48.5	—
HPV18	All	23 / 73	31.5	—
	< 30	16 / 40	40.0	—
	≥ 30	7 / 33	21.2	—
All other HR types*	All	104 / 486	21.4	—
	< 30	55 / 282	19.5	—
	≥ 30	49 / 204	24.0	—
b) CIN2+ (n = 256)				
	Age	No. with disease & positive / No. positive for type	PPV	Sensitivity
Abbott RealTime HR HPV	All	250 / 643	38.9	97.7
	< 30	133 / 356	37.4	97.1
	≥ 30	117 / 287	40.8	98.3
HPV16	All	140 / 243	57.6	—
	< 30	77 / 142	54.2	—
	≥ 30	63 / 101	62.4	—
HPV18	All	25 / 73	34.2	—
	< 30	16 / 40	40.0	—
	≥ 30	9 / 33	27.3	—
All other HR types*	All	159 / 486	32.7	—
	< 30	89 / 282	31.6	—
	≥ 30	70 / 204	34.3	—

* HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

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Table 5. Summary of discordant results between Abbott RealTime HR HPV test and the other tests for all individuals. The table shows the discordant results between two tests (A,B): A (positive for the test in the first column, negative for Abbott RealTime HR HPV test), B (negative for the test in the first column, positive for Abbott RealTime HR HPV test) for all HR HPV types (second column), HPV16 (third column) and HPV18 (fourth column), and the value of Cohen’s kappa statistic. For all comparisons, $p < 0.0001$.

	Abbott RealTime HR HPV		
	HR HPV types	HPV16	HPV18
Hybrid Capture II	(62,20)* $\kappa=0.73$		
Amplicor	(98,11) $\kappa=0.61$		
PreTect HPV-Proofer	(4,284) $\kappa=0.36$	(7,36) $\kappa=0.87$	(0,21) $\kappa=0.81$
APTIMA	(29,77) $\kappa=0.69$		
CINtec p16^{INK4a} Cytology	(25,160) $\kappa=0.27$		
Linear Array	(36,31) $\kappa=0.78$	(22,5) $\kappa=0.92$	(13,0) $\kappa=0.91$
Clinical-Arrays	(72,118) $\kappa=0.30$	(126,25) $\kappa=0.52$	(79,18) $\kappa=0.38$
q-PCR	(46,139) $\kappa=0.49$	(26,17) $\kappa=0.87$	(45,4) $\kappa=0.80$

* eg (62 individuals positive for Hybrid Capture II and negative for Abbott RealTime HR HPV, 20 individuals negative for Hybrid Capture II and positive for Abbott RealTime HR HPV).

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Figure 1a, 1b. Summary of comparative sensitivity and specificity for cervical intraepithelial neoplasia grade 3 or worse (CIN3+) (a) and cervical intraepithelial neoplasia grade 2 or worse (CIN2+) (b) for all tests evaluated in the study

a) CIN3+

b) CIN2+

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Figure 2a, 2b. Viral load Receiver Operating Characteristic (ROC) curves of different cycle number assay cutoff settings for detection of cervical intraepithelial neoplasia grade 3 or worse (CIN3+) (a) and cervical intraepithelial neoplasia grade 2 or worse (CIN2+) (b)

a) CIN3+

b) CIN2+