



Clinical and analytical performance of the BD Onclarity™ HPV assay for detection of CIN2+ lesions on SurePath samples



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ABSTRACT

Background: The novel BD Onclarity™ HPV assay (Onclarity) on the BD Viper™ LT system (BD Diagnostics, Sparks, MD), detects E6/E7 DNA from 13 high-risk HPV genotypes and HPV66. We compared the analytical and clinical performance of the Onclarity Assay to that of Hybrid Capture 2 and LINEAR ARRAY using adjudicated histological outcomes from Danish women referred for colposcopy.

Methods: 276 women from Copenhagen, Denmark were referred for colposcopy with abnormal cytology and/or a positive HPV test. Two samples for HPV analysis were taken in BD SurePath™ and in the BD cervical brush diluent (CBD) media. ClinicalTrials.gov identifier: NCT01671462, Ethical Approval: H-4-2012-070.

Results: Histology was normal in 84 (31%) women, 70 (26%) had CIN1, 47 (17%) CIN2, and 68 (25%) had CIN3. The Onclarity assay detected 67 out of 68 (99%) ≥ CIN3 and 113/115 (98%) ≥ CIN2. The specificities for < CIN2 were 21%, 17%, and 22%, for HC2, Onclarity and LA, respectively.

Conclusion: Overall, the Onclarity HPV assay performed well on SurePath LBC and CBD media, with clinical sensitivity and specificity matching those of HC2 and LA.

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1. Introduction

The link between cervical cancer, the cervical cancer precursors of cervical intraepithelial neoplasia (CIN2 and CIN3), cervical cancer and persistent infection with certain Human Papilloma Virus (HPV) genotypes is well established [1]. More than 200 genotypes are currently identified [2], of which approximately 40 can infect the mucosa of the genital tract [3], with 13 identified as High Risk (hr) with respect to oncogenicity [4,5].

Today liquid based cytology or Pap smears constitute the diagnostic choice for cervical screening, often with HPV testing as reflex. This however will change shortly to HPV testing as the primary choice for cervical screening given HPV DNA tests higher sensitivity for detection of high-grade CIN and cervical cancer and its superior quality assurance and quality controls measures as compared to cytology [6,7].

Furthermore, in countries like Denmark systematic HPV vaccinated birth cohorts are entering organized screening programs

from 2016–17, which challenges cytology further by the substantially reduced abnormality rate because of the vaccination [8]. Together, this emphasizes the need to exchange cytology for HPV screening within a very short timeframe.

The majority of molecular HPV assays have so far been developed based on consensus primers targeting the L1 region of the HPV viral genome. L1 based amplification assays have been shown to have clinical performance compatible to the clinically validated HC2 assay [9–12] but there are certain limitations to the use of a consensus L1 primer approach namely the inconsistency in detecting mixed infections of multiple genotypes. Moreover, consensus assays with no or limited genotyping only allows for a limited risk stratification of HPV positive women, most notably for HPV16 and 18. Incidence of these genotypes is expected to be reduced in immunized women [8,13]. Alternatively assays allowing for genotype specific amplification with broader genotyping capability could offer a viable alternative.

The BD Onclarity™ HPV Assay (Onclarity, BD Diagnostics, Sparks, MD) is a novel Real Time PCR assay. The assay simultaneously detects 13 hrHPV genotypes and HPV66 (Class 2B, possibly carcinogenic) [3]; provides genotyping information on six individual

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genotypes (HPV 16, 18, 31, 45, 51 and 52) and reports the remaining eight HPV genotypes in three distinct groups (33/58, 56/59/66, and 35/39/68). The assay has an internal human beta globin (HBB) gene control for sample sufficiency and assay performance, which is important for laboratory quality control (QA/QC). The Onclarity assay is the first clinical HPV assay specifically detecting HPV DNA *E6* and *E7* genes. The design includes multiple, individual real-time PCR target amplifications [14,15]. Furthermore, by choosing non-*L1* regions as the analytic target, any discussion of the argued rare *L1* deleted infections can be disregarded [16,17].

This study was designed to clinically evaluate the performance of the Onclarity assay on the fully-automated BD VIPER LT System in a Danish population of women referred to colposcopy. We used fresh cervical SurePath samples and samples collected in a novel BD transport medium. The clinical performance of Onclarity was determined relative to the well-studied Hybrid Capture 2 (HC2), the full genotyping assay LINEAR ARRAY (LA) as well as adjudicated histology.

2. Material and methods

2.1. Settings

The Department of Pathology, Copenhagen University Hospital, Hvidovre, is the largest cervical screening laboratory in Denmark, which, annually evaluates up to 160,000 cervical SurePath samples from women living in the Capital Region of Denmark. The laboratory handles all cervical cytology regardless of the reason for sample-taking. Since the 1960's Copenhagen has been covered by an organized cervical cancer screening program [8]. The current call/recall program targets women 23–49 year of age for screening every three years and women aged 50–65 years of age every five years.

2.2. Sample population

276 women from the Copenhagen area referred to colposcopy due to abnormal cytology and/or routine HPV positive samples in concordance with national guidelines were enrolled in the study with informed consent between December 2012 and May 2013. Two hospitals and seven gynecologist clinics were used for recruitment. All women meeting the study inclusion criterion attending these sites in the recruitment period were asked for participation. Exclusion criteria were; previous treatment for CIN; conization, LEEP, laser surgery or cryosurgery treatment, known pregnancy, partial or complete hysterectomy, and application of chemical compounds to the cervix 24 hours prior to study entry. Three women were excluded due to noncompliance with the study protocol. An additional four women were excluded as the HPV result was not available on all samples. No randomization was conducted upon enrollment; all enrolled women received the same diagnostic work-up. All 269 included women had four samples taken upon gynecology examination prior to acidic acid application to exclude any influence on subsequent analysis. A SurePath™ liquid based cytology (LBC) and a novel cervical brush diluent (CBD) transport medium sample followed by colposcopy with subsequent biopsy and ECC taken. Women with negative colposcopy had four-punch random clockwise biopsies, which is routine practice in Denmark. The SurePath sample was used for LBC evaluation, and the residual vial material was used for Onclarity testing as well as LA HPV genotyping. The residual pellet material from the SurePath LBC processing (the post-quot material) was used for HC2 HPV testing. The CBD medium sample was used solely for Onclarity CBD HPV testing. The age span of the included women was 18–74, mean 32.5 years, median 30 years, IQR 26 to 36.

2.3. Cytology

Routine cytology evaluation of SurePath samples was undertaken first by FocalPoint™ Slide Profiler (BD, Burlington, NC). HPV analysis was done after cytology evaluation; hence the cyto-screener was blinded to the HPV result. Cytology slides were read by cyto-screener using FocalPoint™ GS Imaging System (BD), all findings were adjudicated by a pathologist, which had access to the HPV result upon adjudication. Cytology was reported using the Bethesda 2001 system. Cytology at referral was retrieved from the Danish national PatoBank.

2.4. Histology

In the Danish screening program, women with atypical squamous cell of undetermined significance (ASCUS) aged ≥ 30 years with a positive HPV test are referred to colposcopy and biopsies, as are women with high-grade squamous intraepithelial lesions (HSIL), atypical squamous cells – cannot exclude HSIL (ASC-H), atypical glandular cells (AGC) or cytological signs of carcinoma and women with continued ASCUS and low-grade SIL (LSIL) cytological diagnoses. All histology slides were read upfront by the same expert pathologist (JJ). Histology was subsequently adjudicated by a pathologist from the same laboratory (MF) and upon disagreement the slide was discussed by the two pathologists and the agreed diagnosis was used as the final diagnosis. The highest adjudicated diagnosis found in either the biopsy or ECC sample was used as the reported diagnosis. After adjudication of all 269 samples, 12 women had a histological diagnosis of CIN not otherwise specified (NOS); these 12 samples were regarded as less than CIN2 in the specificity calculations.

2.5. Hybrid Capture 2 HPV DNA testing

Hybrid Capture 2 (HC2) analysis was done on the post-quot SurePath pellet material from the cytology procedure. HC2 is a DNA/RNA hybridization assay detecting 13 hrHPV genotypes as a pooled result (16, 18, 31, 33, 39, 45, 51, 52, 56, 58, 59 & 68). The post-quot samples were denatured manually prior to HC2 analysis on the automated Rapid Capture System (RCS) or manually according to manufacturer's protocol (Qiagen Gaithersburg, MD, USA). The HC2 assay has no internal control for sample sufficiency, but contains a batch control for assay performance. Post-quot samples with no visual cellular pellet upon centrifugation and a subsequent negative HPV result after HC2 testing were reported as invalids. There was an average of 17 days from receiving the sample to denaturation for HC2. Samples were denatured 1–2 days before test on the RCS.

2.6. LINEAR ARRAY HPV Genotyping test

The LINEAR ARRAY (LA) is a full genotyping assay that detects 14 hrHPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 & 68, defined here by us for comparison with Onclarity) and 23 potential, possible high and low risk genotypes (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, CP6108). The assay has an internal human beta globin control for sample sufficiency and assay performance. An aliquot of 0.5 ml of SurePath residual material was spun down (14,000 rpm, 5 min), supernatant removed and pellet resuspended in a mix of 180 μ l phosphate buffered saline (10x conc. pH 7.4, Pharmacy product) and 20 μ l Proteinase K (recombinant, PCR grade, Roche Diagnostics). Samples were subsequently vortexed and incubated one hour at 56 °C and one hour at 90 °C. DNA was purified using MagNa Pure LC 96 (Roche Diagnostics, Pleasanton, CA) with MagNa Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics). The LA included controls were extracted using AmpliLute Liquid Media Extraction Kit (Roche Diagnostics). 4 μ l of the purified control DNA and 12.5 μ l MagnaPure

purified sample DNA respectively, was used for each PCR reaction (final volume 50 μ l). Using LINEAR ARRAY HPV genotyping test, amplification was performed on Biosystems Gold Plate 96 well GeneAmp PCR system 9700. 25 μ l of the PCR reaction was used for LA testing using the LINEAR ARRAY detection kit, which was performed according to manufacturer's protocol. Hybridization strips were read by two people for adjudication. Unfortunately the LA package insert does not provide any information on how to read the hybridization strips, here all bands which had the same intensity as the lower band of internal human beta globin control was included as positives, in addition borderline bands were included as positive if they were part of a multiple infection.

2.7. BD Onclarity™ HPV Assay

The Cervical Brush Diluent (CBD) transport sample and an aliquot of the SurePath sample were used for BD Onclarity HPV testing. The CBD transport sample did not require any pre-handling before Onclarity HPV testing and was stored refrigerated or frozen prior to testing. For the SurePath sample; 0.5 ml material was taken from the SurePath vial prior to the SurePath cytology procedure. The SurePath vial was vortexed vigorously and 0.5 ml was aliquoted into a BD HPV liquid based cytology (LBC) diluent medium tube containing 1.7 ml sample medium. The aliquoted LBC samples were inverted 3–4 times and then stored refrigerated or frozen until Onclarity testing on the BD Viper LT system. Prior to Onclarity testing on the Viper platform, the samples (both LBC and CBD) including the positive and negative controls (included in the kit) were pre-warmed for 30 min at 120 °C on the BD pre-warm heater station (model 443159). The pre-warmed samples were manually loaded into the automated Viper LT platform. At the first automated step, the pre-warmed material was transferred to a preloaded extraction tube that contains ferric oxide particle. A high pH is used to lyse the cells and free the DNA into the solution, and magnets are used to hold the bound DNA during aspiration of waste. After washing and high pH elution, neutralization buffer is added to the DNA to bring the pH of the extracted solution to optimal pH for PCR amplification. The VIPER LT instrument transfers the extracted sample material, positive and negative controls to manually assembled amplification micro wells, after which real-time PCR analysis was performed and detected by the integrated VIPER LT reader. The assay has an internal control (human beta globin, HBB) in each well, which provides a control for both sample and process adequacy. The Onclarity is an E6-E7 DNA based Real Time assay that detects 16, 18, 31, 45, 51, 52 as single genotypes and the remaining eight genotypes in three groups (33/58, 56/59/66, 35/39/68).

2.8. Statistical analysis

HPV positive outcomes with Onclarity were defined in accordance with the manufacturer threshold. hrHPV genotypes by LA were reported as the presence of one or more of the 14 detected HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, 66 & 68) as detected by Onclarity and non-hrHPV if one or more of the remaining 23 potential, possible high and low risk genotypes were present without any hrHPV infection. HC2 positive samples were reported for those with a relative light unit per cut-off (RLU/co) value ≥ 1 , no retest range was used. Cytology was considered abnormal if \geq ASCUS was reported. The histology diagnosis CIN, not otherwise specified (CIN NOS), was considered in the CIN1 group for the specificity calculations. The 95% confidence intervals (CI) for sensitivity and specificity were calculated using binomial distribution. We calculated the relative specificity for CLART and HC2 by comparing to LA and relative specificity for CLART and LA by comparing to HC2. The 95% CI for relative sensitivity and specificity were calculated assuming that their algorithms were approximately

normal distributed. Finally, we applied McNemar's Odd Ratio to the results as presented in [Tables 2](#) and [3](#) using HC2 and LA as reference test when compared to Onclarity LBC and CBD. Onclarity LBC was used as reference test when compared to Onclarity CBD. HC2 was used as a reference test when compared to LA.

2.9. Ethical approval

The study was part of a large Viper CE IVD trial (ClinicalTrials.gov identifier: NCT01671462) that had approval from the Danish Capital Regional ethical committee, H-4-2012-070. All included women gave signed informed consent prior to the collection of samples. All women in the study were followed up and managed according to Danish national guidelines, independent of the study protocol.

3. Results

276 women referred to colposcopy, based on abnormal cytology and/or positive HPV, were enrolled in the study; seven women were excluded from the study. The majority of the included women, 257 (96%), were between 23–65 years, which is the target age of the Danish screening program ([Table 1](#)). Of the 269 women, 68 (25%) had normal cytology, 33 (12%) ASCUS, 31 (12%) LSIL, 9 ASC-H (3%) and 124 (46%) HSIL, 2 with cytological signs of cancer (0.7%) and 2 samples (0.7%) had inadequate cytology. The Onclarity test results were similar by cytology category regardless of media type. No statistical differences were observed between all three assays for cytology at referral and cytology at colposcopy ([Table 1](#)). Onclarity testing on LBC and CBD respectively found 234 (87%) and 240 (89%) positive samples, which was similar to findings by HC2 (87%) and LA (85%) ([Table 1](#)).

3.1. Agreement

The overall agreement between Onclarity Assay on LBC and HC2 was 92% (Kappa 0.639) and between Onclarity Assay on CBD and HC2 90% (Kappa 0.539). Agreement with LA was slightly higher, 96% to Onclarity Assay on LBC (Kappa: 0.861) and 95% to Onclarity Assay on CBD (Kappa: 0.752) ([Table 3](#)).

The Onclarity assay detects six HPV genotypes as single results and the remaining eight in three groups. When compared to the genotyping of LA, including only the 14 hrHPV genotypes, the Onclarity assay found similar amounts of HPV genotypes in each of the Viper genotype or genotype groups ([Table 2](#)). The agreement between Onclarity Assay LBC and LA for HPV 16, 18, 31, 45 and 51 was very good (Kappa value above 0.9). Agreement for genotype HPV52 was still good between Onclarity and LA (Kappa value 0.863) although the Onclarity detects HPV52 individually whereas LA detects this genotype using a consensus primer targeting in fact four HPV genotypes. Furthermore agreement among the three Onclarity genotype groups and LA was good (Kappa values above 0.8). For Onclarity Assay on LBC, 189 out of the 234 positive samples (80%), had an identical hrHPV genotype distribution as observed by LA.

Cross reactivity to non-hrHPV genotypes for HC2 accounted for a major proportion of the HC2 POS/Onclarity NEG samples. 9/11 HC2 POS/Onclarity LBC NEG samples reported only non-hrHPV genotypes by LA. In Comparison 0/11 Onclarity LBC POS/HC2 NEG reported only non-hrHPV genotypes by LA. HC2 does not detect HPV66, however only 1/11 Onclarity LBC POS/HC2 NEG sample reported HPV56/59/66 and HPV66 only by Onclarity and LA respectively. 10/11 Onclarity LBC POS/HC2 NEG reported hrHPV by LA. In total HC2 reported hrHPV positive in 19 cases where LA returned a non-hrHPV only result (7%) whereas Onclarity LBC called 3/296.

Within the 115 \geq CIN2, only four samples disagreed between Onclarity on LBC and HC2. Two Onclarity POS/HC2 NEG samples had CIN2 diagnoses and HPV18 by LA. Two Onclarity NEG/HC2 POS

Table 1
Description of the study population.

	Total (column %)	Onclarity LBC (row %)		Onclarity CBD (row %)		HC2 (row %)		LA (row %)	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Low-risk only or negative
Total	269 (100%)	234 (87%)	35 (13%)	240 (89.2%)	29 (10.8%)	234 (87%)	35 (13%)	229 (85.1%)	40 (14.9%)
Age (years)									
< 30	127 (47.2%)	111 (87.4%)	16 (12.6%)	116 (91.3%)	11 (8.7%)	111 (87.4%)	16 (12.6%)	109 (85.8%)	18 (14.2%)
30-39	94 (34.9%)	85 (77.1%)	9 (22.9%)	84 (80.7%)	10 (19.3%)	82 (78.3%)	12 (21.7%)	85 (75.9%)	9 (24.1%)
≥ 40	48 (17.8%)	38 (89.1%)	10 (10.9%)	40 (95.7%)	8 (4.3%)	41 (91.3%)	7 (8.7%)	35 (89.1%)	13 (10.9%)
Cytology at referral									
Normal	17 (6.3%)	15 (88.2%)	2 (11.8%)	15 (88.2%)	2 (11.8%)	13 (76.5%)	4 (23.5%)	2 (11.8%)	15 (88.2%)
ASCUS	83 (30.9%)	64 (77.1%)	19 (22.9%)	67 (80.7%)	16 (19.3%)	65 (78.3%)	18 (21.7%)	63 (75.9%)	20 (24.1%)
LSIL	46 (17.1%)	41 (89.1%)	5 (10.9%)	44 (95.7%)	2 (4.3%)	42 (91.3%)	4 (8.7%)	41 (89.1%)	5 (10.9%)
HSIL+ ^a	123 (45.7%)	114 (92.7%)	9 (7.3%)	114 (92.7%)	9 (7.3%)	114 (92.7%)	9 (7.3%)	110 (89.4%)	13 (10.6%)
Cytology at colposcopy									
Normal	68 (25.3%)	48 (70.6%)	20 (29.4%)	51 (75%)	17 (25%)	42 (61.8%)	26 (38.2%)	45 (66.2%)	23 (33.8%)
ASCUS	33 (12.3%)	29 (87.9%)	4 (12.1%)	30 (90.9%)	3 (9.1%)	30 (90.9%)	3 (9.1%)	29 (87.9%)	4 (12.1%)
LSIL	31 (11.5%)	26 (83.9%)	5 (16.1%)	26 (83.9%)	5 (16.1%)	29 (93.5%)	2 (6.5%)	26 (83.9%)	5 (16.1%)
HSIL+ ^a	135 (50.2%)	131 (97%)	4 (3%)	131 (97%)	4 (3%)	133 (98.5%)	2 (1.5%)	128 (94.8%)	7 (5.2%)
Inadequate	2 (0.7%)	0 (0%)	2 (100%)	2 (100%)	0 (0%)	0 (0%)	2 (100%)	1 (50%)	1 (50%)
Histology at Colposcopy									
Normal	84 (31.2%)	59 (70.2%)	25 (29.8%)	65 (77.4%)	19 (22.6%)	58 (69%)	26 (31%)	58 (69%)	26 (31%)
CIN1	70 (26.0%)	62 (88.6%)	8 (11.4%)	63 (90%)	7 (10%)	63 (90%)	7 (10%)	61 (87.1%)	9 (12.9%)
CIN2	47 (17.5%)	46 (97.7%)	1 (2.1%)	46 (97.7%)	1 (2.1%)	45 (95.7%)	2 (4.3%)	45 (95.7%)	2 (4.3%)
CIN3	68 (25.3%)	67 (98.5%)	1 (1.5%)	66 (97.1%)	2 (2.9%)	68 (100%)	0 (0%)	65 (95.6%)	3 (4.4%)

^a HSIL+: includes: HSIL, ACSH, AGC, AIS.

Table 2
HR genotype distribution of Onclarity and Linear Array.

HPV genotype	Onclarity LBC	Onclarity CBD	LA LBC+/LA+	LBC+/LA-	LBC-/LA+	LBC-/LA-	Total agreement	POS agreement	Kappa (95% CI)	Odds ratio (95% CI) ^a	
16	89	90	88	86	3	2	178	98.14	94.51	0.96 (0.92-1.00) P=1.00	1.5 (0.17-17.96) P=1.00
18	22	21	23	22	0	1	246	99.63	95.65	0.98 (0.93-1.00) P=0.0412 ^b	P=1.00 ^b
31	48	50	42	42	6	0	221	97.77	87.50	0.92 (0.86-0.98) P=0.248 ^b	P=0.0412 ^b
45	26	26	29	26	0	3	240	98.88	89.66	0.94 (0.87-1.00) P=0.480 ^b	P=0.248 ^b
51	20	25	22	20	0	2	247	99.26	90.91	0.95 (0.88-1.00) P=0.13	P=0.480 ^b
52	31	32	26	25	6	1	237	97.40	78.13	0.86 (0.76-0.96) P=1.00	6.00 (0.73-276.0) P=0.13
33/58 ^c	42	48	43	39	3	4	223	97.40	84.78	0.90 (0.83-0.97) P=0.061	0.75 (0.11-4.43) P=1.00
56/59/66 ^c	53	53	45	42	11	3	213	94.80	75.00	0.83 (0.74-0.91) P=0.182	3.67 (0.97-20.47) P=0.061
35/39/68 ^c	40	48	35	33	7	2	227	96.65	78.57	0.86 (0.77-0.95) P=0.182	3.50 (0.67-34.53) P=0.182

^a McNemar's odd ratio test with LA as the reference test.

^b The odds ratio and its confidence interval cannot be calculated because one of the discordant values is zero.

^c The genotypes for LA was pooled into three groups and compared to the same groups as detected by Onclarity.

samples had a CIN2 and CIN3 diagnose respectively and concurrent non-hrHPV by LA (both HPV82).

3.2. Clinical outcome and performance

Histology amongst 269 women showed 84 (31%) with normal histology, 70 (26%) with CIN1, 47 (17%) with CIN2 and 68 (25%) with CIN3. In total, 115 women were diagnose with ≥ CIN2 (43%), and of these 68 women had (26%) ≥ CIN3 (see Table 4).

Onclarity Assay LBC found 113 out of 115 (Sensitivity: 98%) ≥ CIN2 cases and 67 out of 68 (Sensitivity: 99%) ≥ CIN3 cases. Onclarity on CBD found 112 out of 115 (Sensitivity: 97%) ≥ CIN2 cases and 66/68 (Sensitivity: 97%) ≥ CIN3 cases. In comparison HC2 found 113/115 (Sensitivity: 98%) ≥ CIN2 cases and 68/68 (100%) ≥ CIN3 cases. When limited to only the 14 HPV genotypes detected by Onclarity, LA detected 110/115 (96%) ≥ CIN2 cases and 65/68 of

≥ CIN3 cases (96%) (See Table 4). There was no statistical difference in sensitivity for either ≥ CIN2 or ≥ CIN3 between Onclarity Assay and reference tests using both media types (See Table 4).

Onclarity reported 33 out of 154 negative < CIN2 samples (specificity: 21%) and 34 out of 201 < CIN3 samples (specificity: 17%). Onclarity using the CBD medium reported 26/154 < CIN2 (specificity: 17%) and 27/201 < CIN3 (specificity: 13%). HC2 reported 33/154 < CIN2 (specificity: 21%) and 35/201 < CIN3 (specificity: 17%). LA detected 35/154 < CIN2 (specificity: 23%) and 37/201 < CIN3 (specificity: 18%) as negative or non-hrHPV only. The CBD media induced a slight reduction in specificity for Onclarity compared to HC2, however the difference were not statistically significant for either < CIN2 (relative specificity 0.79: 95% CI: 0.50-1.25) or < CIN3 (relative specificity: 0.77: 95% CI: 0.49-1.22) (Table 4).

Table 3

Overall agreement between HC2, LA and Onclarity using LBC and CBD collection material.

Assay A	Assay B	A POS/B POS	A NEG/B NEG	A POS/B NEG	A NEG/B POS	Total Agreement	Odd ratio ^a
Onclarity on LBC	HC2	223	24	11	11	247 (91.8%) Kappa: 0.639 95% CI: 0.500–0.778	1,00 95% CI 0,39–2,54 P=0,831
	Onclarity on CBD	232	27	2	8	259 (96.3%) Kappa: 0.823 95% CI: 0.716–0.929	4,00 95% CI 0,8–38,67 P=0,114
	LA	227	33	2	7	260 (96.7%) Kappa: 0.861 95% CI: 0.772–0.950	0,29 95% CI 0,03–1,50 P=0,182
Onclarity on CBD	HC2	224	19	16	10	243 (90.3%) Kappa: 0.539 95% CI: 0.383–0.695	1,60 95% CI 0,68–3,94 P=0,327
	LA	227	27	13	2	254 (94.4%) Kappa: 0.752 95% CI: 0.632–0.871	6,50 95% CI (1,47–59,33) P=0,01
HC2	LA	218	24	16	11	242 (90.0%) Kappa: 0.582 95% CI: 0.440–0.724	0,69 95% CI 0,29–1,58 P=0,441

^a McNemar's odd ratio test. HC2 and LA were used as reference test when compared to Onclarity LBC and CBD. Onclarity LBC was used as reference test when compared to Onclarity CBD. HC2 was used as a reference test when compared to LA.

Table 4

Sensitivity, specificity, for HC2, Onclarity and LA.

	Onclarity LBC	Onclarity CBD	HC2 LBC	LA LBC
Endpoint \geq CIN2				
Sensitivity (95% CI)	0.98 (0.94–1.0)	0.97 (0.93–1.0)	0.98 (0.94–1.00)	0.96 (0.90–0.99)
Relative Sensitivity vs. HC2	1.0 (0.97–1.03)	0.99 (0.95–1.03)	1.0	0.97 (0.93–1.02)
Specificity	0.21 (0.15–0.29)	0.17 (0.11–0.24)	0.21 (0.15–0.29)	0.23 (0.16–0.30)
Relative specificity vs. HC2	1.0 (0.65–1.53)	0.79 (0.50–1.25)	1.0	1.06 (0.70–1.61)
Endpoint \geq CIN3				
Sensitivity (95% CI)	0.99 (0.92–1.0)	0.97 (0.90–1.0)	1.0 (0.95–1.0)	0.96 (0.88–0.99)
Relative Sensitivity vs. HC2	0.99 (0.96–1.01)	0.97 (0.93–1.01)	1.0	0.96 (0.91–1.01)
Specificity	0.17 (0.12–0.23)	0.13 (0.12–0.23)	0.17 (0.12–0.23)	0.18 (0.13–0.25)
Relative specificity vs. HC2	0.97 (0.63–1.49)	0.77 (0.49–1.22)	1.0	1.06 (0.70–1.61)

4. Discussion

4.1. General findings

The aim of the current study was to evaluate the BD Onclarity™ HPV Assay against HC2 for clinical and analytical performance and Linear Array for analytical genotyping performance. Moreover, this is one of first systematic reports on HPV assay performance comparison on SurePath™™ referral samples. Finally, we evaluated the Onclarity performance on a novel cervical brush transport medium on samples taken in parallel with a regular SurePath sample. All 269 samples taken at colposcopy in the two media types were tested on Onclarity, HC2 and LA and compared to adjudicated histology. HPV positive findings by Onclarity on LBC samples were 234 out of 269 specimens and using the CBD medium 240 out of 269 specimens. This was similar to what was observed using HC2 (234/269) and LA (229/269).

The Onclarity assay is a hrHPV assay with extended genotyping. The value of genotyping is increasingly utilized in clinical screening to risk stratify women based upon the HPV genotype detected. Most recently, the US interim guidelines calls for differential follow up of HPV16 or 18 positive samples [18], and a number of European countries use a similar approach. The genotype dependent risk stratification is founded on the widely recognized knowledge that the 13 hrHPV genotypes overall differs substantially in their oncogenic potential [4,5,19–21]. By utilizing this knowledge differential follow up algorithms can be employed however this requires that the assay used entails genotyping.

The overall concordance on LBC samples between Onclarity and HC2 or LA was found to be substantial or almost perfect (92%, Kappa: 0.639, 95%, kappa: 0.861), respectively. When comparing genotype detection of Onclarity and LA on LBC samples, agreement was substantial for HPV 16, 18, 31, 45 and 51 (kappa values above 0.9), and despite HPV52 being a cross reactive probe for LA [22,23] the agreement was substantial (Kappa value: 0.863). Similar concordances were observed for samples taken in the CBD medium (Table 3). Specimens that tested negative on HC2 but positive on Onclarity were more likely to be positive for hrHPV by LA, whereas HC2 positive and Onclarity negative were more likely to be non-hrHPV by LA. This is in line with previously reported findings [24]. Cross reactivity to non-hrHPV genotypes by HC2 has previously been described in detail on STM stored samples [25–28]. Here 19 out 269 (7%) samples testing positive on HC2 but with non-hrHPV genotypes only by LA (including HPV66). In comparison, only 3 of 269 samples reported Onclarity LBC positive/ LA non-hrHPV (excluding HPV66). Observed discrepancy between Onclarity and HC2 could therefore be attributed to HC2 cross reactivity to non-hrHPV genotypes.

The sensitivity of Onclarity for \geq CIN2 and \geq CIN3 on both LBC and CBD medium were found to be similar to that of HC2 (Table 4). In addition, the specificity for Onclarity on LBC matched that of HC2 and LA. The CBD medium induced a slight reduction in specificity for Onclarity, but the difference was not statistically significant (Table 4).

In this setting an hrHPV positive sample was defined by the 14 HPV genotypes detected by Onclarity. Though HPV66 is not defined by IARC as hrHPV genotype, it was included as a hrHPV genotype in

this analysis. For concordance analysis, the same definition of hrHPV was used to evaluate concordance with LA. Eight LA samples were HPV66 positive, and all were diagnosed with < CIN2. The specificity for > CIN2 for LA was 28% excluding HPV66 as a hrHPV. Hardly surprising, these data indicate that in this particular population the addition of HPV66 as a hrHPV genotype does not provide additional sensitivity for disease.

4.2. Comparison to the literature

The Onclarity Assay has previously been described using ThinPrep and STM samples [9,10,14,15,24,25,29]. In the Predictor 2 study [10] ThinPrep samples from women with abnormal cytology was used to evaluate seven HPV assays including also the previous configuration of the Onclarity Assay. The study reported similar prevalence for Onclarity (82%) and HC2 (86%) in women with abnormal cytology and concordant clinical performances for Onclarity and HC2 (sensitivity: 95%, specificity: 24% and sensitivity: 96%, specificity: 20%, respectively) for \geq CIN2 [10]. Agreement between Onclarity and HC2 was high (90%) and clinical sensitivity for Onclarity was 94%, with a specificity of 26% [29]. Finally, a recent study performed on archived SurePath samples tested on the Viper LT system with Onclarity showed clinical performances comparable to our results [24].

Overall, Onclarity performs similar to HC2 in ThinPrep and SurePath stored samples on multiple referral patient cohorts. Onclarity brings the added clinical information of genotyping to the table compared to the “positive/negative” result of HC2. One element stands out in our study compared to previous publications in that our test material were fresh samples from a routine work flow. All samples here were sent to one laboratory and tested by the same routine staff thereby minimizing any potential laboratory-to-laboratory performance variability. Moreover, our routine diagnostic procedures requires that LA strips were read by two persons for adjudication and the subsequent genotype comparison between the Onclarity and LA were performed on mutually detected genotypes only. Finally, where the majority of studies focused on ThinPrep stored samples, we evaluated the performance on SurePath stored samples as well as on a novel virus transport medium taken in parallel. This study provides important performance data on SurePath stored samples not previously reported for the Onclarity HPV assay. However the weakness of our study is that using only referral population samples, true primary screening performance cannot be assessed in this study design. This has recently been reported for ThinPrep stored samples through the Predictor 3 study the Onclarity Assay [9], however a similar study has not yet been performed on SurePath samples.

4.3. Conclusion

Onclarity performed well on SurePath and on the new novel cervical brush transport medium samples from women referred to colposcopy. Clinical performance was similar to that of HC2. On prospective collected samples, the Onclarity Assay had equal high sensitivity and specificity for \geq CIN2 and \geq CIN3 compared to HC2, but with the added power of extended hrHPV genotyping concordant to that of LA. Finally, the Onclarity is fully automated for ease of use. Overall, the BD Onclarity HPV Assay is a strong HPV candidate for use in clinical cervical cancer screening pending further studies ongoing to validate its clinical performance also outside the high prevalence referral population used here.

Competing interests

All authors have attended meetings with manufacturers of HPV assays. DME received honoraria from Genomica and Qiagen for

lectures. JB has in the past served as paid advisor to Roche and Genomica, and received honoraria from Hologic/Gen-Probe, Roche, Qiagen, Genomica, and BD Diagnostics for lectures. None of the authors were compensated for their work on this project, holds stock, or received bonuses from any of the manufacturers.

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Contributors

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REFERENCES

- [1] H. zur Hausen, Papillomaviruses in the causation of human cancers—a brief historical account, *Virology* 384 (2) (2009) 260–265.
- [2] H.U. Bernard, R.D. Burk, Z. Chen, K. van Doorslaer, H. zur Hausen, E.M. de Villiers, Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments, *Virology* 401 (1) (2010) 70–79.
- [3] V. Bouvard, R. Baan, K. Straif, Y. Grosse, B. Secretan, F. El Ghissassi, L. Benbrahim-Tallaa, N. Guha, C. Freeman, L. Galichet, et al., A review of human carcinogens—Part B: biological agents, *Lancet Oncol.* 10 (4) (2009) 321–322.
- [4] M. Arbyn, P.J. Snijders, C.J. Meijer, J. Berkhof, K. Cuschieri, B.J. Kocjan, M. Poljak, Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? *Clin. Microbiol. Infect.: Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.* 21 (9) (2015) 817–826.
- [5] Cancer IAFro: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. In., vol. 100B. Lyon, France: WHO Press; 2012.
- [6] R.S. Kelly, J. Patnick, H.C. Kitchener, S.M. Moss, HPV testing as a triage for borderline or mild dyskaryosis on cervical cytology: results from the Sentinel Sites study, *Br. J. Cancer* 105 (7) (2011) 983–988.
- [7] M. Arbyn, G. Ronco, A. Anttila, C.J. Meijer, M. Poljak, G. Ogilvie, G. Koliopoulos, P. Naucler, R. Sankaranarayanan, J. Peto, Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer, *Vaccine* 30 (Suppl 5) (2012) F88–F99.
- [8] E. Lyng, C. Rygaard, M.V. Baillet, P.A. Dugue, B.B. Sander, J. Bonde, M. Rebolj, Cervical cancer screening at crossroads, *APMIS: Acta Pathol., Microbiol. Et. Immunol. Scand.* 122 (8) (2014) 667–673.
- [9] J. Cuzick, L. Cadman, D. Mesher, J. Austin, L. Ashdown-Barr, L. Ho, G. Terry, S. Liddle, C. Wright, D. Lyons, et al., Comparing the performance of six human papillomavirus tests in a screening population, *Br. J. Cancer* 108 (4) (2013) 908–913.
- [10] A. Szarewski, D. Mesher, L. Cadman, J. Austin, L. Ashdown-Barr, L. Ho, G. Terry, S. Liddle, M. Young, M. Stoler, et al., Comparison of seven tests for high-grade cervical intraepithelial neoplasia in women with abnormal smears: the Predictors 2 study, *J. Clin. Microbiol.* 50 (6) (2012) 1867–1873.
- [11] J.T. Cox, P.E. Castle, C.M. Behrens, A. Sharma, T.C. Wright Jr., J. Cuzick, Comparison of cervical cancer screening strategies incorporating different combinations of cytology, HPV testing, and genotyping for HPV 16/18: results from the ATHENA HPV study, *Am. J. Obstet. Gynecol.* 208 (3) (2013) 184 e181 184 e111.
- [12] T.C. Wright Jr., M.H. Stoler, C.M. Behrens, R. Apple, T. Derion, T.L. Wright, The ATHENA human papillomavirus study: design, methods, and baseline results, *Am. Journal. Obstet. Gynecol.* 206 (1) (2012) 46 e41 46 e11.

- [13] H.A. Cubie, K. Cuschieri, Understanding HPV tests and their appropriate applications, *Cytopathology* 24 (5) (2013) 289–308.
- [14] K. Cuschieri, D.T. Geraets, C. Moore, W. Quint, E. Duvall, M. Arbyn, Clinical and Analytical Performance of the Onclarity HPV Assay Using the VALGENT Framework, *J. Clin. Microbiol.* 53 (10) (2015) 3272–3279.
- [15] D.M. Ejegod, I. Serrano, K.S. Cuschieri, W.A. Nussbaumer, L.M. Vaughan, A. S. Ahmad, J. Cuzick, J. Bonde, Clinical validation of the BD Onclarity HPV assay using a non-inferior test, *J. Med. Microbiol. Diagn.* (2013), <http://dx.doi.org/10.4172/2161-0703.S-003>.
- [16] C.E. Depuydt, G.A. Boulet, C.A. Horvath, I.H. Benoy, A.J. Vereecken, J.J. Bogers, Comparison of MY09/11 consensus PCR and type-specific PCRs in the detection of oncogenic HPV types, *J. Cell. Mol. Med.* 11 (4) (2007) 881–891.
- [17] W.A. Tjalma, C.E. Depuydt, Cervical cancer screening: which HPV test should be used-L1 or E6/E7? *Eur. J. Obstet. Gynecol. Reprod. Biol.* 170 (1) (2013) 45–46.
- [18] W.K. Huh, K.A. Ault, D. Chelmow, D.D. Davey, R.A. Goulart, F.A. Garcia, W. K. Kinney, L.S. Massad, E.J. Mayeaux, D. Saslow, et al., Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance, *Gynecol. Oncol.* 136 (2) (2015) 178–182.
- [19] S.K. Kjaer, K. Frederiksen, C. Munk, T. Iftner, Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence, *J. Natl. Cancer Inst.* 102 (19) (2010) 1478–1488.
- [20] M. Schiffman, A.G. Glass, N. Wentzensen, B.B. Rush, P.E. Castle, D.R. Scott, J. Buckland, M.E. Sherman, G. Rydzak, P. Kirk, et al., A long-term prospective study of type-specific human papillomavirus infection and risk of cervical neoplasia among 20,000 women in the Portland Kaiser Cohort Study, *Cancer Epidemiol. Biomarkers Prev.: Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* 20 (7) (2011) 1398–1409.
- [21] C.M. Wheeler, W.C. Hunt, N.E. Joste, C.R. Key, W.G. Quint, P.E. Castle, Human papillomavirus genotype distributions: implications for vaccination and cancer screening in the United States, *J. Natl. Cancer Inst.* 101 (7) (2009) 475–487.
- [22] J.M. Onyekwuluje, M. Steinau, D.C. Swan, E.R. Unger, A real-time PCR assay for HPV52 detection and viral load quantification, *Clin. Lab.* 58 (1–2) (2012) 61–66.
- [23] Roche Molecular System I: Linear Array HPV Genotyping Test Package Insert. 2013.
- [24] T.C. Wright Jr., M.H. Stoler, P.M. Agreda, G.H. Beitman, E.C. Gutierrez, J.M. Harris, K.R. Koch, M. Kuebler, W.D. LaViers, B.L. Legendre Jr., et al., Clinical performance of the BD Onclarity HPV assay using an adjudicated cohort of BD SurePath liquid-based cytology specimens, *Am. J. Clin. Pathol.* 142 (1) (2014) 43–50.
- [25] P.E. Castle, E.C. Gutierrez, S.V. Leitch, C.E. Maus, R.A. McMillian, W.A. Nussbaumer, L.M. Vaughan, C.M. Wheeler, P.E. Gravitt, M. Schiffman, Evaluation of a new DNA test for detection of carcinogenic human papillomavirus, *J. Clin. Microbiol.* 49 (8) (2011) 3029–3032.
- [26] P.E. Castle, M. Schiffman, R.D. Burk, S. Wacholder, A. Hildesheim, R. Herrero, M. C. Bratti, M.E. Sherman, A. Lorincz, Restricted cross-reactivity of hybrid capture 2 with nononcogenic human papillomavirus types, *Cancer Epidemiol. Biomarkers Prev.: Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* 11 (11) (2002) 1394–1399.
- [27] P.E. Castle, D. Solomon, C.M. Wheeler, P.E. Gravitt, S. Wacholder, M. Schiffman, Human papillomavirus genotype specificity of hybrid capture 2, *J. Clin. Microbiol.* 46 (8) (2008) 2595–2604.
- [28] A.T. Hesselink, N.W. Bulkman, J. Berkhof, A.T. Lorincz, C.J. Meijer, P.J. Snijders, Cross-sectional comparison of an automated hybrid capture 2 assay and the consensus GP5+/6+ PCR method in a population-based cervical screening program, *J. Clin. Microbiol.* 44 (10) (2006) 3680–3685.
- [29] D. Mesher, A. Szarewski, L. Cadman, J. Austin, L. Ashdown-Barr, L. Ho, G. Terry, M. Young, M. Stoler, C. Bergeron, et al., Comparison of human papillomavirus testing strategies for triage of women referred with low-grade cytological abnormalities, *Eur. J. Cancer* (2013).