

## Clinical Validation of the BD Onclarity™ HPV Assay Using a Non-Inferiority Test

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### Abstract

The clinical performance of The BD Onclarity™ HPV Assay, a novel type-specific real-time E6/E7-based PCR assay, was evaluated for clinical and analytical performance using the Meijer et al. international guidelines for validation of high-risk HPV tests. Assay performance was found to be similar to the reference method, Hybrid Capture 2 (HC2, QIAGEN) using PreservCyt® Specimens (Hologic®, Inc.). In addition, the fully automated assay was found to have excellent intra- and inter-laboratory reproducibility (98.6% (kappa=0.967) and 98.4% (kappa=0.962), respectively). These data show that the BD Onclarity™ HPV Assay fulfills the clinical validation requirements for a HPV based cervical cancer screening assay (Meijer et al. International journal of cancer 2009; 124: 516-520.).

### Introduction

Molecular HPV testing is routinely used for cervical disease management and HPV testing as a primary screen with reflex to cytology, HPV 16/18 genotyping or other triage method is becoming increasingly accepted as the optimal modality for cervical screening [1-3]. There are a number of different molecular methods used to detect HPV and it is critical that any new HPV test undergoes robust technical evaluation and clinical validation before being used routinely for cervical disease management. With this in mind, an international panel of experts on cervical cancer screening proposed a standardized non-inferiority metric to adjudicate whether an HPV test conformed to acceptable standards of clinical performance. This metric states that clinical validation of a candidate high-risk HPV DNA assay for screening is performed through cross-sectional clinical equivalence analysis of the assay relative to an established, clinically validated reference assay (i.e. high-risk HPV Hybrid Capture 2 (HC2) or GP5+/6+-PCR). Accordingly, candidate assays should demonstrate clinical non-inferiority to HC2 or GP5+/6+-PCR with a relative sensitivity for CIN2+ of at least 90% and a relative specificity for CIN2+ of at least 98%. The candidate assay must also demonstrate intra-laboratory reproducibility and inter-laboratory agreement (i.e. both showing a percentage of agreement with a lower confidence bound not less than 87% (kappa value of at least 0.5)) [4]. Recently a number of different molecular HPV test methods have been subjected to this test criterion and it has become the de facto standard by which new tests are assessed [5-7].

Since the introduction of the HC2 assay, the majority of HPV assays have been developed using polymerase chain reaction (PCR) technology that utilizes consensus PCR primers targeting the L1 region of the virus genome [6-8]. While L1-based amplification assays have been shown to have clinical performance comparable to clinically validated assays such as HC2 [9,10], there are certain technical limitations to the use of a consensus primer approach, namely, inconsistency in detecting low copy number infections within mixed infections [11-13], and the potential risk of missing late stage cancers due to deletion of the L1 target region [14,15]. As an alternative to HPV assays that involve L1 consensus primers, amplification assays that incorporate type specific PCR designs may improve the specificity of detection by returning only

positive results where the presence of HPV is above the established, validated cut-off [12,13]. By choosing non L1 regions, any risk of not detecting rare L1 deleted events can be addressed.

The BD Onclarity™ HPV Assay is a Real-Time PCR assay that utilizes specific E6 and E7 gene target regions [16]. It simultaneously detects all 14 high-risk HPV types, provides genotyping information on 6 individual genotypes (HPV 16, 18, 31, 45, 51 and 52) and reports the remaining HPV types in three distinct groups: 33\_58; 56\_59\_66 and 35\_39\_68. The assay also detects the human beta goblin gene which provides a control for both sample and process adequacy. The clinical performance of the assay versus other commercially available assays has previously been described [9,10,16,17]. Here, we analyze the performance of the new BD Onclarity™ HPV Assay relative to HC2 using previously described referral and screening populations [9,10] and combine these data with the analytical performance of the assay on the fully integrated Viper™ LT platform in order to measure the performance of the assay according to the non-inferiority criteria described earlier [4].

### Materials and Methods

#### Sample collection

The clinical specificity analysis was performed on a subset of a screening population of 6000 women who attended for a routine screening at St. Mary's Hospital in London-all samples were been

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in PreservCyt® [9]. The final selection included 4,599 cervical scrapes from women 30 years and above where CIN2+ had not been detected through standard of care. The clinical sensitivity analysis was performed on a subset of a previously described population of 1,099 women referred to the colposcopy clinics at the Hammersmith and St. Mary's Hospitals in London, United Kingdom [10]. The final selection included a set of 156 cervical scrapes from women 30 years and above with a confirmed CIN2+ diagnosis. These patient results were used to construct 2x2 tables for relative sensitivity and specificity and were used to calculate the non-inferiority test statistic (T) as previously described [4]. All testing associated with clinical performance assessment was performed previously at the Wolfson Institute, London and the data were used to perform the non-inferiority test [9,4,10].

### BD Onclarity testing for sensitivity and specificity Non-inferiority analysis

Clinical specimens were collected and tested as previously described using a semi-automated workflow on the Viper™ XTR [10]. A fully integrated process workflow was subsequently developed on the Viper™ LT platform and this instrument was used to perform the intra- and inter-laboratory reproducibility studies described here. In order to confirm that the semi-automated Viper™ XTR workflow is equivalent to the Viper™ LT workflow, split samples from a sub-set of the clinical specimens used in the non-inferiority test were run on both systems and the results were found to be equivalent (Supplemental information). The clinical performance data and the reproducibility data were therefore combined and analyzed for acceptance of the non-inferiority guidelines [4]. All comparative testing was done using sample aliquots from the same patients and all testing was blinded to both HC2 and pathology results.

### BD Onclarity reproducibility testing

The intra- and inter-reproducibility studies were performed using de-identified unselected residual PreservCyt® specimens from the Danish Cervical Cancer Screening Program and were performed at the Department of Pathology, Copenhagen University Hospital, Hvidovre and at the Scottish HPV Reference Laboratory, Edinburgh. A total of 984 total residual PreservCyt® specimens were initially screened to identify the required number of positive samples for the reproducibility study. Of these, 512 samples were tested using the HC2 assay (Qiagen, Gaithersburg, US), and a total of 147 HC2 positive and 365 HC2 negative specimens constituted the sample-set for reproducibility testing. Both BD Onclarity testing and HC2 testing were performed according to manufacturer's recommendations; however no retest value was used for the HC2. For the BD Onclarity™ PCR test (cat. No 442946), the Viper LT instrument was used, whereas HC2 was performed after manual conversion (cat. No 5127-1220), using the Rapid Capture System (QIAGEN, Gaithersburg, US) in accordance with the manufacturer's specifications.

### Results

In women ≥ 30 years, the sensitivity for CIN2+ of the HC2 and BD assays was 94.2% (95% CI=89.3, 97.3) and 93.0% (95% CI=87.7, 96.4), and the specificity for HC2 and BD was 88.8% (95% CI=87.9, 89.7) and 87.7% (95% CI=86.8, 88.7), respectively. These results were not statistically different (*p*-value=0.644 for sensitivity and *p*-value=0.112 for specificity). The clinical sensitivity and specificity of the BD Onclarity™ assay was compared to that of the Qiagen HC2 test using the non-inferiority score test outlined in the guidelines, [4]. The sensitivity threshold for CIN2+ was set to 90% relative to hc2 and the

specificity threshold was set to 98% relative to HC2. The BD Onclarity test was found to be non-inferior to that of HC2 with greater than 95% confidence (*p*-value=0.00085 and *p*-value=0.02164, respectively) (Table 1).

To assess intra and inter-laboratory reproducibility of the BD Onclarity™ HPV Assay, aliquots of 512 residual cervical PreservCyt® samples were used, of which 152 were positive for high-risk HPV infections as determined by the BD Onclarity™ HPV Assay. The HPV prevalence of all 984 samples were 15.4% by BD Onclarity™ HPV Assay, which is similar to previously published prevalence rates on screening samples from Denmark [18,19,20]. Intra-laboratory reproducibility was tested in Copenhagen by comparing the results from two independent tests of each specimen. The resulting overall intra-laboratory reproducibility was 98.6% (505/512; Lower confidence bound=97.5%) with a kappa value of 0.967. The inter-laboratory reproducibility analysis of BD Onclarity™ HPV Assay was determined using a third aliquot of each of the 512 specimens. These were then tested separately in Edinburgh where the operators were blinded to the original results. The inter-laboratory agreement for these specimens was 98.4% (504/512; lower confidence bound=97.2) with a kappa value of 0.962. Re-testing in Edinburgh was performed within 14 days of the original testing in Denmark to minimize any potential influence of storage. For both the intra- and inter-laboratory agreement, the lower confidence bounds were >87%, with kappa values >0.5, and the results thus met the criteria set forth in the guidelines (Table 2).

### Discussion

The BD Onclarity is the first automated PCR based high risk HPV diagnostic assay offering extended genotyping beyond HPV 16 and 18 of high-risk types assessed according to the Meijer criteria. The data show that the assay is highly comparable to HC2 with respect to performance and reproducibility and that the assay fulfills the non-inferiority criteria as described by Meijer et al. [4].

There are limitations to the present study; clinical performance was assessed using previously annotated material (from Predictors Study 2 & 3 [9,10]) where samples were collected in a single PreservCyt® vial and subsequently aliquoted and stored (Predictors 2) or collected in two sequential vials and then aliquoted and stored (Predictors 3) to enable testing to be performed across multiple assays. Therefore, it is possible that the storage of the sample material from Predictors 2 and 3 or the use of dual collection method in Predictors 3 could have had

Relative Sensitivity (≥ CIN2)				
		Hybrid Capture 2		Total
		Positive	Negative	
BD Onclarity™ HPV Assay	Positive	142	3	145
	Negative	5	6	11
	Total	147	9	156
	Test statistic (T)=3.13696; p-value=0.00085			
Relative Specificity (<CIN2)				
		Hybrid Capture 2		Total
		Positive	Negative	
BD Onclarity™ HPV Assay	Positive	411	153	564
	Negative	104	3931	4035
	Total	515	4084	4599
	Test statistic (T)=2.02104; p-value=0.02164			

Table 1: Relative Performance of the BD Onclarity™ HPV Assay versus Hybrid Capture 2.

Intra-laboratory Reproducibility				
Copenhagen laboratory Result 2	Copenhagen laboratory Result 1			Total
		Positive	Negative	
	Positive	147	2	149
	Negative	5	358	363
	Total	152	360	512
<b>Agreement=98.6% (Lower confidence bound=97.5%) and kappa value=0.967</b>				
Inter-laboratory Reproducibility				
Edinburgh Laboratory Result	Copenhagen laboratory Result			Total
		Positive	Negative	
	Positive	145	1	146
	Negative	7	359	366
	Total	152	360	512
<b>Agreement=98.4% (Lower confidence bound=97.2%) and kappa value=0.962</b>				

**Table 2:** Intra-laboratory and Inter-Laboratory Reproducibility of The BD Onclarity™ HPV Assay.

an impact on positivity/performance. In terms of sensitivity, this may not be significant given the overall performance of the assays in both studies with the BD Onclarity™, detecting 100% of CIN2+ disease in women over 30 in Predictors 3 [9]. However, as for any retrospective study impact of storage/sampling technique on specificity is more difficult to quantify.

In addition, the 156 CIN2+ cases on which sensitivity was adjudicated were derived from a colposcopy referral population (Predictors 2), thus we have to extrapolate assay-sensitivity for screen detected CIN2+ as we did not measure this directly. This said, molecular test equivalence was confirmed for both  $\geq$  CIN2 and  $<$ CIN2 and the larger screening ( $<$ CIN2) arm of the study included patient samples that were cytology negative/HPV positive, which is reassuring. In relation to the 4,599 samples relevant to the specificity analysis (derived from Predictors 3) it should also be noted that disease ascertainment was only possible in women who were referred to colposcopy as per standard of care according to the UK Cervical Screening Programme. As Predictors 3 was an anonymized study and HPV testing results did not affect management, disease ascertainment was not performed in the HPV positive/cytology negative group so it is likely that a small proportion of these samples did harbour disease, which would impact the specificity reported above. However, as the sensitivity of Onclarity and HC2 for CIN2+ is equivalent in cytology negative specimens the presence of disease patients in the HPV pos/cytology neg group may have an equivalent impact on the specificity of both assays.

The BD Onclarity HPV assay's ability to offer extended genotyping provides a number of potential benefits. Firstly, it enables one to discriminate the different high-risk HPV types which can help in patient specific risk-stratification. Secondly, providing information on the 13 IARC HR-HPV genotypes plus HPV66 allows surveillance, relevant to vaccine monitoring to be embedded in the regular screening activity, potentially creating a cost benefit synergy for the clinical laboratories, and health care authorities. Finally, extended genotyping enables longitudinal genotype specific assessments of persistence in women attending the cervical cancer screening programs. The accumulation of accurate population-based genotyping information will increase our clinical knowledge and potentially allow more precise risk stratification of the individual women. This should inform future guidelines that will help balance the burden of repeat testing against risk of CIN3+ disease.

In conclusion, the BD Onclarity™ HPV Assay was shown to be clinically comparable to the clinically validated HC2 assay. It is the first fully automated assay offering extended genotyping to meet the criteria. Since the assay satisfies the criteria for cross-sectional clinical equivalence and reproducibility according to the international guidelines, the BD Onclarity™ HPV Assay can be considered clinically validated for cervical screening purposes.

## Sources of Funding

Equipment, reagents and support for this study was provided by Becton Dickinson and Company.

## Potential Conflicts of Interest

Ditte Ejegod has received honoraria from Genomica SAU and Qiagen Ltd. for lectures. Jesper Bonde has served as a paid advisor to Roche Molecular Systems in the past and Genomica SAU, and received honoraria from Hologic/Gen-Probe, Roche, Qiagen and Genomica and BD Diagnostics for lectures. Jesper Bonde has received funding and/or consumables to carry out assay evaluations from Hologic/Gen-Probe, Qiagen, Roche, Genomica SAU and BD Diagnostics.

Kate Cuschieri has received project funding and/or consumables to carry out assay evaluations from Hologic/Gen-Probe, Qiagen, Roche, NorChip, GSK, Cepheid & Abbott Molecular and has been a member of the Gen-Probe Advisory Board in the past.

Jack Cuzick has received honoraria from Qiagen, Roche, Abbott, Gen-Probe/Hologic, Becton Dickinson as a consultant, lecturer or advisory board member.

Jack Cuzick's Institution has received support and materials from Qiagen, Roche, Abbott, Hologic/Gen-Probe, Becton Dickinson, mtm, Genomica, Genera, Trovogene and Oncohealth to perform the PREDICTORS series of evaluations.

Neither Ditte Ejegod, Jesper Bonde, Itziar Serrano, Kate S. Cuschieri, Amar S. Ahmad or Jack Cuzick holds stock, or received bonuses from Becton Dickinson and Company

William A. Nussbaumer, and Laurence M. Vaughan are employed by Becton Dickinson and Company, the sponsor of the study.

All authors had full access to all of the data in the study.

## Ethical Considerations

### Predictors 2

The study population comprised 1,099 women who had been referred to the colposcopy clinics at the Hammersmith and St. Mary's Hospitals in London, United Kingdom, between September 2007 and October 2009 because of abnormal screening smears. All women received a patient information sheet explaining the study and provided written consent. Approvals were obtained from the relevant local research ethics committees.

### Predictors 3

Residual material was used from the liquid-based cytology PreservCyt samples from 6000 women who attended for a routine 3 or 5 yearly (depending on age) screening smear, and whose samples were sent to the cytology laboratory at St. Mary's Hospital, London. Consent was deemed not to be necessary, as the women were not going to be contacted with their result, nor would it be used to influence their management.

## Danish reproducibility cohort

All reproducibility samples were obtained as residual material that would otherwise have been discarded. According to Danish regulations of biomedical research, published on 5 May 2011 in the Guidelines about Notification etc. of a Biomedical Research Project to the Committee System on Biomedical Research Ethics No. 9154 section 2.5, quality development studies do not require ethical approval.

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