Detection of high-risk HPV in FFPE specimens of various tumors using the BD Onclarity™ HPV Assay

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ABSTRACT

Analysis of high-risk HPV status on formalin-fixed paraffin-embedded (FFPE) tissue material is valuable for cervical-, head and neck-, anogenital- and other types of cancer, but commercial HPV assays have been developed specifically for cervix swab cells. We evaluated the BD Onclarity™ HPV Assay for the detection of high-risk HPV on an assortment of relevant FFPE tissues with known HPV status. Detection of high-risk HPV types using the BD Onclarity™ HPV Assay in FFPE specimens was easy and accurate.

1. Introduction

HPV infections are the cause of a proportion of cancers of the head and neck, including the oropharynx, larynx and nasopharynx [1, 2], and in the anogenital region, including anus, penis, and vagina and vulva [2, 3]. HPV positivity has also been described for cancers in other tissues such as lung [4]. The impact of carcinogenic infections to the global burden of cancer has been estimated to be over 15% of all cancers worldwide, with high-risk HPV (hr-HPV) as a major contributor [5]. The number of new cancer cases attributable to a hr-HPV infection is over 600,000 a year [2, 5]. Moreover, hr-HPV plays a major role in the development of cervical cancer, which is the second most common cancer in women worldwide with approximately half a million new cases each year. Over 90% of squamous cervical cancers have been shown to contain HPV DNA [6]. For cervical cancer prevention, recent guidelines strongly recommend periodic primary hr-HPV-based screening on cells taken by a swab from the cervix over standard cytology-based screening [7]. Tissue material of cervix-, head and neck-, anogenital-, lung- and other cancers is routinely processed as formalin-fixed paraffin-embedded (FFPE) material for histological diagnostics. An easy method for the detection of hr-HPV in FFPE material therefore is valued. Since commercial assays for the analysis of hr-HPV types have been designed for hr-HPV detection in unprocessed cervical swab samples, a standard diagnostic method to detect hr-HPV in FFPE material is lacking [8]. DNA isolated from cytology specimens generally is of much better quality compared to DNA isolated from FFPE. Amplification of HPV sequences from FFPE specimens therefore is more challenging, as formalin fixation causes extensive DNA damage, including cross-linking and fragmentation which may impact assay performance.

Whereas robust validation guidelines exist for HPV testing on cervical cells specimens for use in screening [9, 10] no such rules exist for HPV testing on histological specimens. A limited number of studies have described the use of the BD Onclarity™ HPV Assay on FFPE specimens [11–14] but none of these studies used FFPE specimens with a known HPV status, but rather compared the BD Onclarity™ HPV Assay analysis of the FFPE specimen with the HPV status of a paired cytology specimen. In addition, these four studies all focused exclusively on cervical material.

Here, we report the use of the BD Onclarity™ HPV Assay on formalin-fixed paraffin-embedded tissue material of cervical- and a variety of relevant other tissue types with known hr-HPV status as determined by reference laboratories.

2. Methods

2.1. BD Onclarity™ HPV Assay

The BD Onclarity™ HPV Assay (BD Diagnostics, Sparks, USA) on the BD Viper™ LT System is an easy-to-use, automated HPV test that is CE-IVD approved for HPV tests on cervix swabs. It is a real-time PCR assay that detects type-specific E6 and E7 genomic DNA that simultaneously
detects all 14 high-risk HPV types, and can provide genotyping information on six individual genotypes (HPV 16, 18, 31, 45, 51 and 52), reporting the remaining HPV types in three distinct groups (33 and 58; 56, 59 and 66; and 35, 39 and 68). For each specimen at least three sections of 3, 10 and 3 μm thick, respectively, were cut and the first and last were HE stained. For some samples one or more additional 10 μm slides were cut after the first 10 μm slide, before the last HE stained 3 μm slide. The surface of the most informative HE (when similar, the first HE was used) was measured using the Digital Pathology Solutions program (Koninklijke Philips N.V., the Netherlands). The 10 μm thick section was combined with 0.5 ml of BD SurePath™ medium and transferred to a BD Onclarity HPV LBC Diluent Tube. The sample was further processed and automatically analyzed in the BD Viper™ LT System with the BD Onclarity™ HPV Assay as described by the manufacturer for routine cervix samples. Briefly, samples were placed in the heating tray for 1 h. After pre-heating the FFPE samples were vortexed for 10 s and placed back on the same spot in the tray before the tray was placed in the Viper machine where it was analyzed in 3 PCR tubes per sample fully automatically with the BD Onclarity™ HPV Assay. In this assay HPV16, HPV18, HPV31, HPV45, HPV51 and HPV52 are all analyzed individually and P1, P2 and P3 in groups. The HPV types detected by this assays are: G1; HPV16, HPV18, HPV45 and internal control (β-globin), G2; P1 = HPV33/58, HPV31, P2 = HPV56/59/66 and internal control (β-globin), G3; HPV51, HPV52, P3 = HPV33/39/68 and internal control (β-globin). A positive signal in P1, P2 or P3 indicates that one or more of the HPV types in that group is positive.

2.2. Sample selection

A retrospective study was performed on 76 FFPE tissue specimens processed from 2015 to 2021 that had previously been tested for the presence of hr-HPV. A total of 35 hr-HPV negative and 41 hr-HPV positive FFPE specimens were thus analyzed with the BD Onclarity™ HPV Assay using the BD Viper™ LT System. Analysis was performed in two cohorts. A first cohort of 36 specimens was comprised of a collection of all available histological FFPE specimens at Isala klinieken Zwolle between 2015 and 2020 that had previously been tested for hr-HPV during routine clinical diagnostics by external reference laboratories. Of these, six HPV positive and six HPV negative specimens were analyzed in a reference lab using three analytically sensitive PCR reactions aimed at detecting HPV16, HPV18 and GP-HPV (detecting most oncogene HPV-types), respectively. One HPV negative and three HPV positive specimens were analyzed using the Cobas 4800 HPV test (Roche). The external method used for HPV analysis of two specimens (including the false-negative sample) was not traceable, those were retested at LabPON Hengelo. In addition, a number of randomly selected, histologically characterized specimens from LabPON Hengelo that had been previously tested using an in-house HPV multiplex PCR assay were included in this cohort. The in-house HPV assay is a multiplex qPCR able to detect the E6/E7 region of 17 hr-HPV genotypes individually (i.e. 16, 18, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82), in addition to two low-risk HPV types, i.e. HPV6 and HPV11.

A second cohort of 40 FFPE specimens was subsequently analyzed with the BD Onclarity™ HPV Assay to further increase the total number of hr-HPV positive and hr-HPV negative FFPE specimens, with the specific aim to increase the representation of specimens positive for hr-HPV types that were underrepresented in the first cohort (e.g. non-HPV16). These specimens were histologically characterized FFPE specimens from LabPON Hengelo that had been tested previously for the presence of hr-HPV and consisted of 21 hr-HPV-negative and 19 hr-HPV-positive specimens. The hr-HPV-negative specimens in this second cohort were selected randomly.

2.3. Reproducibility

Reproducibility testing was performed using uterus extirpation material positive for HPV16 and large enough to allow multiple sections with highly similar quantities of material. Between-run reproducibility was analyzed in three separate runs and in the second of these runs three samples of this specimen were analyzed to evaluate in-run reproducibility.

2.4. Minimal tissue surface area testing

For minimal tissue surface area testing, three specimens were used that tested positive for HPV16, HPV18 and HPV45, respectively. Of these samples a 10 μm slide of was added to a BD Onclarity HPV LBC Diluent Tube with 0,5 ml BD SurePath™ medium, preheated and vortexed briefly. Of the resulting sample, a dilution series was made and pipetted in preheated BD Onclarity HPV LBC Diluent Tubes with 0,5 ml BD SurePath™ medium. Volumes were corrected.

3. Results

The BD Onclarity™ HPV Assay was applied to a total of 76 FFPE specimens with known hr-HPV status, 35 specimens that were previously tested negative for hr-HPV and 41 specimens that were previously tested positive for hr-HPV16. A total of 41 specimens from LabPON Hengelo that had been tested previously for hr-HPV during routine clinical diagnostics by external reference laboratories. Of these, six HPV positive and six HPV negative specimens were analyzed in a reference lab using three analytically sensitive PCR reactions aimed at detecting HPV16, HPV18 and GP-HPV (detecting most oncogene HPV-types), respectively. One HPV negative and three HPV positive specimens were analyzed using the Cobas 4800 HPV test (Roche). The external method used for HPV analysis of two specimens (including the false-negative sample) was not traceable, those were retested at LabPON Hengelo. In addition, a number of randomly selected, histologically characterized specimens from LabPON Hengelo that had been previously tested using an in-house HPV multiplex PCR assay were included in this cohort. The in-house HPV assay is a multiplex qPCR able to detect the E6/E7 region of 17 hr-HPV genotypes individually (i.e. 16, 18, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82), in addition to two low-risk HPV types, i.e. HPV6 and HPV11.

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2.3. Reproducibility

Reproducibility testing was performed using uterus extirpation

Table 1

Analysis using the BD Onclarity HPV assay of 35 specimens of FFPE tissues that previously tested negative for HPV by reference labs. A concordant result was observed for 33 specimens. Specimen 5 however was false-negative and specimen 31 resulted in a failure, leaving 33 true HPV negative specimens tested (specificity 100%). The specimens were tested in two separate cohorts, i.e. cohort 1 (specimens 1-15) and cohort 2 (specimens 16-35) (see methods for details).

| Sample number | Tissue tested | Tissue surface (mm²) | HPV type reference | HPV type BD Onclarity assay |
|---------------|--------------|----------------------|--------------------|-----------------------------|
| 1             | Cheek biopsy | 3,9                  | Negative            | Negative                    |
| 2             | Cervix biopsy | 3,6                  | Negative            | Negative                    |
| 3             | Cervix biopsy | 72,7                 | Negative            | Negative                    |
| 4             | Labium minus skin biopsy | 17,2 | Negative | Negative |
| 5             | Cervix biopsy | 30,6                 | Negative            | Positive (a)                |
| 6             | Vulvar biopsy | 14,9                 | Negative            | Negative                    |
| 7             | Bronchus biopsy | 11,1                | Negative            | Negative                    |
| 8             | Tongue biopsy | 2,0                  | Negative            | Negative                    |
| 9             | Cervix excision | 21,2                | Negative            | Negative                    |
| 10            | Cervix biopsy | 8,0                  | Negative            | Negative                    |
| 11            | Cervix biopsy | 231,3                | Negative            | Negative                    |
| 12            | Vulvar biopsy | 3,7                  | Negative            | Negative                    |
| 13            | Cervix biopsy | 8,2                  | Negative            | Negative                    |
| 14            | Cervix biopsy | 23,6                 | Negative            | Negative                    |
| 15            | Tonsil biopsy | 14,7                 | Negative            | Negative                    |
| 16            | Liver biopsy | 12,2                 | Negative            | Negative                    |
| 17            | Oropharynx biopsy | 15,7               | Negative            | Negative                    |
| 18            | Preputium resection | 185,3 | Negative | Negative |
| 19            | Vulva biopsy | 9,4                  | Negative            | Negative                    |
| 20            | Cervix biopsy | 29,6                 | Negative            | Negative                    |
| 21            | Cervix biopsy | 32,3                 | Negative            | Negative                    |
| 22            | Skin cheek biopsy | 7,9                | Negative            | Negative                    |
| 23            | Vagina biopsy | 13,8                 | Negative            | Negative                    |
| 24            | Tongue biopsy | 21,8                 | Negative            | Negative                    |
| 25            | Neck biopsy | 15,5                 | Negative            | Negative                    |
| 26            | Tongue biopsy | 26,9                 | Negative            | Negative                    |
| 27            | Anus excision | 36,3                 | Negative            | Negative                    |
| 28            | Perineum biopsy | 7,9                | Negative            | Negative                    |
| 29            | Mouth biopsy | 8,7                  | Negative            | Negative                    |
| 30            | Larynx biopsy | 5,3                  | Negative            | Negative                    |
| 31            | Neck biopsy | 9,2                  | Negative            | FAILURES                    |
| 32            | Pharynx biopsy | 12,4                | Negative            | Negative                    |
| 33            | Oropharynx biopsy | 10,4              | Negative            | Negative                    |
| 34            | Tongue excision | 13,8               | Negative            | Negative                    |
| 35            | Oropharynx biopsy | 13,3              | Negative            | Negative                    |

(a) HPV18 positive when retested by another lab.
tested hr-HPV positive. Thirty-three out of the 35 hr-HPV negative specimens also tested negative with the BD Onclarity™ HPV Assay (Table 1). One hr-HPV negative specimen however tested HPV18 positive and another specimen was reported as a failure by the assay. The HPV18 positive specimen was consequently retested in another lab and was confirmed to be HPV18 positive, supporting the HPV positive result of the BD Onclarity™ HPV Assay. We concluded that the original HPV negative status of this specimen was a false-negative, leaving 34 true-negative FFPE specimens tested of which 33 gave a valid result by the assay. For the concordance of the positive FFPE specimens therefore was 39 out of 41, including the false-negative specimen from Table 1, resulting in a sensitivity of 93% in this series. With 72/75 concordant specimens out of 42, including the false-negative specimen from Table 1, resulting in a sensitivity of 93%, a specificity of 100% and a Cohen’s kappa coefficient of 0.92. Statistical analysis on the performance of the BD Onclarity HPV Assay on FFPE tissues was 96%. 

Table 2

| Sample number | Tissue tested | Tissue surface (mm²) | HPV type reference | HPV type BD Onclarity HPV assay |
|---------------|---------------|----------------------|--------------------|--------------------------------|
| 1             | Uterus cervix excision | 203,9 | HPV16 | HPV16 |
| 2             | Larynx biopsy     | 97,5 | HPV16 | HPV16 |
| 3             | Cervix biopsy     | 36,1 | HPV16 | HPV16 |
| 4             | Lang biopsy (metastasis cervix carcinoma) | 32,0 | HPV18 | HPV18 |
| 5             | Lymph node excision (metastasis SCC, primary unknown) | 82,7 | HPV16 | HPV16 |
| 6             | Cervix excision   | 88,6 | HPV45 | Negative |
| 7             | Tomil biopsy      | 21,6 | HPV18 | HPV18 |
| 8             | Cervix biopsy     | 58,6 | HPV18 | HPV18 |
| 9             | Tomil biopsy      | 69,1 | HPV16 | HPV16 |
| 10            | Cervix biopsy     | 52,5 | HPV16 | HPV16 |
| 11            | Vaginal biopsy    | 15,8 | HPV45 | HPV45 |
| 12            | Cervix biopsy     | 45,4 | HPV45 | HPV45 |
| 13            | Cervix biopsy     | 109,6 | HPV18 | HPV18 |
| 14            | Cervix loop excision | 98,8 | HPV18 | HPV18 |
| 15            | Cervix loop excision | 67,0 | HPV16 | HPV16 |
| 16            | Cervix biopsy     | 32,3 | HPV16 | HPV16 |
| 17            | Endometrial curettage | 160,8 | HPV16 | HPV16 |
| 18            | Cervix curettage  | 278,5 | HPV16 | HPV16 |
| 19            | Cervix loop excision | 149,9 | HPV16 | HPV16 |
| 20            | Cervix biopsy     | 14,6 | HPV16 | HPV16 |
| 21            | Cervix resection  | 490,0 | HPV16 | HPV16 |
| 22            | Cervix loop excision | 153,0 | HPV16 | HPV16 |
| 23            | Skin penis biopsy | 6,9 | HPV16, HPV31 | HPV16, HPV31 |
| 24            | Cervix biopsy     | 18,5 | HPV18 | HPV18 |
| 25            | Cervix curettage  | 35 | HPV18 | HPV18 |
| 26            | Tomil biopsy      | 86,1 | HPV33 | P1 (33/58) |
| 27            | Cervix loop excision | 144,1 | HPV18 | HPV18 |
| 28            | Cervix excision   | 119,4 | HPV18 | HPV18 |
| 29            | Anus biopsy       | 62,6 | HPV33 | P1 (33/58) |
| 30            | Cervix biopsy     | 12,9 | HPV18 | HPV18 |
| 31            | Tomil biopsy      | 24,9 | HPV33 | P1 (33/58) |
| 32            | Vulva excision    | 101,5 | HPV16, HPV56 | HPV16 |
| 33            | Mouth biopsy      | 15,7 | HPV35 | P3 (35/39/68) |
| 34            | Anus excision     | 158,6 | HPV52 | HPV52 |
| 35            | Oropharynx biopsy | 23,5 | HPV33 | P1 (33/58) |
| 36            | Palatum molle biopsy | 23,1 | HPV33 | P1 (33/58) |
| 37            | Neck excision     | 51,2 | HPV59 | P2 (56/59/66) |
| 38            | Cervix biopsy     | 29,6 | HPV18 | HPV18 |
| 39            | Cervix biopsy     | 46,5 | HPV31 | HPV31 |
| 40            | Rectum biopsy     | 12,3 | HPV18, HPV52 | HPV18, HPV52 |
| 41            | Mons pubis biopsy | 110,2 | HPV52 | Negative |

Table 3

|                | Reference HPV+ | Reference HPV- | Total |
|----------------|----------------|----------------|-------|
| Onclarity HPV+ | 39             | 0              | 39    |
| Onclarity HPV- | 3              | 33             | 36    |
| Total          | 42             | 33             | 75    |
The BD Onclarity™ HPV Assay in FFPE specimen of a variety of tissue types known to be targets of HPV infection. The unbiased selection of hr-HPV positive FFPE specimens tested in cohort 1 was mostly positive for HPV16, followed by HPV18, HPV45 and in one case HPV59 (P2). Generally, HPV16 infections are the most prevalent in all types of tumor, followed by HPV18, and the other hr-HPV types are usually present in fewer cases [4,15–17]. This is in line with our results. Which non-HPV16/18 types are present in FFPE material depends on the tumor type and on the geographical location of the HPV positive patients [4,15–17]. Because HPV16 was easily detected in cohort 1, HPV16 was excluded from the selection of hr-HPV positive specimens in cohort 2. The combined results of cohorts 1 and 2 verified that the BD Onclarity™ HPV Assay was able to detect positive signals in FFPE specimens in the channels detecting HPV16, HPV18, HPV45, HPV31, HPV52, P1, P2 and P3. As a HPV51 positive FFPE specimen was not available, we have not been able to verify the detection channel for HPV51 in the BD Onclarity™ HPV Assay using FFPE specimens.

In conclusion, the ease of testing and the high accuracy shows that the BD Onclarity™ HPV Assay is a convenient and reliable test for the detection of hr-HPV in FFPE specimens of a variety of tissue types known to be targets of HPV infection.

**Ethics**

The Daily Board of the Medical Ethics Committee Isala Zwolle The Netherlands, has reviewed the above mentioned research proposal. As a result of this review, the Committee informs you that the rules laid down in the Medical Research Involving Human Subjects Act (also known by its Dutch abbreviation WMO), do not apply to this research.

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**Author contributions**

Robert van der Geize: Methodology, Writing-Reviewing and Editing. Natalie Methorst: Investigation. Maarten Niemantsverdriet: Conceptualization, Supervision, Writing-first draft, Writing-Reviewing and Editing.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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