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Detection of high-risk HPV in FFPE specimens of various tumours 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

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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 HVP 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 4800R 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, 31, 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

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[a]	HPV18
6	Vulvar biopsy	14,9	Negative	Negative
7	Bronchus biopsy (metastasis cervix carcinoma)	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	FAILURE
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.

Table 2
Analysis using the BD Onclarity HPV assay of 41 specimens of FFPE tissues that previously tested positive for HPV by reference labs. A fully concordant positive result was observed for a total of 38/41 specimens, (sensitivity 93%). The specimens were tested in two separate cohorts, i.e. cohort 1 (specimens 1–22) and cohort 2 (specimens 23–41) (see methods for details).

Sample number	Tissue tested	Tissue surface (mm2)	HPV type reference	HPV type BD Onclarity HPV assay
1	Uterus cervix extirpation	203,9	HPV16	HPV16
2	Larynx biopsy	97,5	HPV16	HPV16
3	Cervix biopsy	36,1	HPV16	HPV16
4	Lung biopy (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	Tonsil biopsy	21,6	HPV18	HPV18
8	Cervix biopsy	58,6	HPV18	HPV18
9	Tonsil 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	HPV59	P2 (56/59/66)
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 curretage	35	HPV18	HPV18
26	Tonsil 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	63,6	HPV33	P1 (33/58)
30	Cervix biopsy	12,9	HPV18	HPV18
31	Tonsil 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

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. The concordance of these 33 true-negative FFPE specimens was 100%, resulting in 100% specificity in this series (Table 3). Of the 41 specimens that previously tested hr-HPV positive, 38 showed a fully concordant result (Table 2). HPV45 and HPV52 were however not detected by the BD Onclarity™ HPV Assay in two specimens previously tested positive for these HPV types, respectively (Table 2, specimens 6 and 41). The HPV45 positive specimen was subsequently re-tested in another lab which confirmed the presence of HPV45 (Table 2, specimen 6). Because both the initial result and the multiplex HPV assay result identified HPV45 we considered the specimen positive for HPV45 and our BD Onclarity™ HPV Assay analysis a false-negative. The reference HPV multiplex qPCR assay used to detect HPV52 in specimen 41 (Table 2) had shown that the specimen was also positive for HPV6 and HPV11, both in high viral loads. This specimen was retested with the same HPV assay yielding the same result. In this case, the presence of high viral loads of low risk HPV types may have affected the detection of hr-HPV in either the BD Onclarity™ HPV Assay or in the reference HPV assay. This specimen was considered discordant. Similarly, no HPV56 was detected by the BD Onclarity™ HPV Assay in specimen 31 that was shown to be positive for HPV16 in a high viral load and in which HPV56 was only detected in a low viral load (Table 2). Conceivably, the detection of HPV56 by the BD Onclarity™ HPV Assay was hampered by the presence of an excess of HPV16, or detection of HPV56 by the reference HPV assay was the result of slight cross-reactivity. Nevertheless, we considered the HPV result of this specimen discordant.

Taken together, we obtained fully concordant results with 38 out of the 41 hr-HPV positive specimens tested with the BD Onclarity™ HPV Assay. The concordance of the positive FFPE specimens therefore was 39 out of 42, including the false-negative specimen from Table 1, resulting in a sensitivity of 93% in this series. With 72/75 concordant specimens the accuracy of the BD Onclarity™ HPV Assay for FFPE tissues was 96%

Table 3
Statistical analysis on the performance of the BD Onclarity HPV Assay on FFPE specimens resulting in a sensitivity of 93%, a specificity of 100% and a Cohen's kappa coefficient of 0,92.

	ReferenceHr-HPV+	ReferencHr-HPV-	Total
Onclarity Hr-HPV+	39	0	39
Onclarity Hr-HPV-	3	33	36
Total	42	33	75

Table 4
In-run and between run reproducibility. Analysis was performed using the BD Onclarity assay on a FFPE specimen with a sized piece of HPV16 positive tissue and constant tissue size throughout the paraffin block. For between run-reproducibility the sample was analyzed three times in separate runs (sample 1 of run 2 was used as second sample). For in-run reproducibility the sample was analyzed three times in the same run.

Reproducibility between-runs		
	org.	VIPER FFPE
Analysis 1	HPV16	HPV16 Cp = 26,6
Analysis 2 (sample 1)	HPV16	HPV16 Cp = 25,6
Analysis 3	HPV16	HPV16 Cp = 26,0

Reproducibility in-run		
	org.	VIPER FFPE
Analysis 2, sample 1	HPV16	HPV16 Cp = 25,6
Analysis 2, sample 2	HPV16	HPV16 Cp = 26,0
Analysis 2, sample 3	HPV16	HPV16 Cp = 26,1

Table 5
Minimal cell surface. Analysis was performed using the BD Onclarity HPV assay on dilution series of a FFPE specimen with HPV16, HPV18 and HPV45 positive tissue. Cell surface of the original tissue is given between brackets.

Tissue surface mm^2	Specimen 1 HPV16 (213 mm^2)	Specimen 2 HPV18 (109,6 mm^2)	Specimen 3 HPV45 (45,5 mm^2)
	Result	Result	Result
15	**HPV16**	**HPV18**	**HPV45**
10	**HPV16**	**HPV18**	**HPV45**
5	**HPV16**	**HPV18**	**HPV45**
2	**HPV16**	**HPV18**	Negative
1	**HPV16**	Negative	Failure

with a Kappa coefficient of 0.92, indicating very good agreement (Table 3). Good in-run reproducibility and between-run reproducibility was found when a HPV16 positive specimen was analyzed in triplo in the same run (Cp value 25,9 ± 0,21) and in three separate runs (Cp value 26,1 ± 0,41), respectively (Table 4).

Notably, the HPV-negative sample series included many more specimens with a low tissue surface (<15 mm^2) than the series with HPV-positive specimens. To exclude the possibility of false negative results due to low tissue surface areas, the lower limit of the tissue surface area for an accurate HPV detection was determined. The minimal cell surface required to get a positive result was determined by diluting HPV16, HPV18 and HPV45 positive samples to a cell surface area expected to give failures or false-negative results (Table 5). The minimal tissue surface area was determined to be 5 mm^2.

4. Discussion

The BD Onclarity™ HPV Assay is an automatized assay designed for detection of high-risk HPV in cervix swabs and was used to detect hr-HPV in FFPE tissues. The analyzed material was mainly derived from cervical- and head and neck cancer, but also lymph node, skin, vulval, vaginal, cheek and lung tissues were included (Tables 1 and 2). The assay can detect HPV types 16, 18, 31, 45, 51 and 52 individually and HPV33/58 (P1), HPV56/59/66 (P2), HPV35/39/68 (P3) combined. A positive signal in P1, P2 or P3 shows that at least one of the HPV types in the corresponding groups is present. Despite the fact that the assay was designed for cervix swabs, detection of high-risk HPV in FFPE material using the BD Onclarity™ HPV Assay run on the BD Viper™ LT System was very easy. By simply adding a single 10 μm FFPE section and 0,5 ml BD SurePath™ medium to a Diluent Tube and running the samples exactly as done for routine cervix swabs we were able to analyze 75 out of 76 specimens, with two failures of which one specimen was successfully repeated. We had concordant results for 96% of all 75 samples analyzed, a sensitivity of 93% and a specificity of 100% in this series (Table 3). This is similar to results by others [11–14]. The assay failed to detect HPV45 in one specimen. Presence of HPV45 was indeed confirmed in this specimen by another method (Table 2, sample 6). HPV45 was detected in two other specimens in our cohort, so HPV45 is not typically missed by the BD Onclarity™ HPV Assay in FFPE specimens. Additionally, HPV56 and HPV52 were not detected in two specimens (Table 2; 32 and 41, respectively). In both cases however additional HPV types were present in relatively high viral loads that may have affected the detection of HPV52 and HPV56 that were detected in relative low viral loads. The difference in detection of these low viral loads of hr-HPV may have been the result of slight cross-reactivity in the reference HPV assay or of some interference in the BD Onclarity™ HPV Assay. On the other hand, we did detect HPV18 using the BD Onclarity™ HPV Assay, that was previously missed. This specimen was indeed found to contain HPV18 after testing with another method (Table 1, sample 5).

The BD Onclarity™ HPV Assay has a build-in test for the presence of sufficient DNA (internal control β-globin) in each reaction tube. Analysis of too little material (i.e. small surface area) is expected to result in a failure rather than a false negative result when FFPE tissue with a small surface is analyzed. However, minimal surface area testing by diluting material of HPV16, HPV18 and HPV45 positive specimens revealed that surface areas smaller than 2 mm^2 may result in false-negative results instead of test failures, as exemplified by the results obtained with HPV18 (1 mm^2) and HPV45 (2 mm^2). HPV positivity was detected in all these three materials with tissue surface areas of 5 mm^2 and higher, whereas HPV16 and HPV18 could also be detected with lower tissue surface areas (Table 5). Therefore, the lower limit of tissue surface area needed for reliable detection of hr-HPV with the BD Onclarity™ HPV Assay is 5 mm^2. In HPV negative samples with a lower surface area false-negativity cannot be ruled out.

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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References

[1] K.J.W. Chang Sing Pang, T. Mur, L. Collins, S.R. Rao, D.L. Faden, Human papillomavirus in sinonasal squamous cell carcinoma: a systematic review and meta-analysis, Cancers 13 (2021) 45, https://doi.org/10.3390/cancers13010045.
[2] D. Forman, C. de Martel, C.J. Lacey, I. Soerjomataram, J. Lortet-Tieulent, L. Bruni, J. Vignat, J. Ferlay, F. Bray, M. Plummer, S. Franceschi, Global burden of human papillomavirus and related diseases, Vaccine Volume 30 (Supplement 5) (20 November 2012) F12–F23, https://doi.org/10.1016/j.vaccine.2012.07.055.
[3] S. Bryan, C. Barbara, J. Thomas, A. Olaitan, HPV vaccine in the treatment of usual type vulval and vaginal intraepithelial neoplasia: a systematic review, BMC Wom. Health 19 (2019) 3, https://doi.org/10.1186/s12905-018-0707-9.
[4] J. Karnosky, W. Dietmaier, H. Knuettel, V. Freigang, M. Koch, F. Koll, F. Zeman, C. Schulz, HPV and lung cancer: a systematic review and meta-analysis, Cancer Rep. (2021) e1350, https://doi.org/10.1002/cnr2.1350.
[5] M. Plummer, C. de Martel, J. Vignat, J. Ferlay, F. Bray, S. Franceschi, Global burden of cancers attributable to infections in 2012: a synthetic analysis, Lancet Global Health 4 (2016) e609–e616, https://doi.org/10.1016/S2214-109X(16)30143-7.
[6] S.E. Waggoner, Cervical cancer, Lancet 361 (2003) 2217–2225, https://doi.org/10.1016/S0140-6736(03)13778-6.
[7] P.J. Maver, M. Poljak. Primary HPV-based cervical cancer screening in Europe: implementation status, challenges, and future plans.https://doi.org/10.1016/j.cmi.2019.09.006.
[8] F. Bussu, C. Ragin, P. Boscolo-Rizzo, D. Rizzo, R. Gallus, G. Delogu, P. Morbini, M. Tommasino, HPV as a marker for molecular characterization in head and neck oncology: looking for a standardization of clinical use and of detection method(s) in clinical practice, Head Neck 41 (2019) 1104–1111, https://doi.org/10.1002/hed.25591.
[9] Arbyn, et al., VALGENT: a protocol for clinical validation of human papillomavirus assays, J. Clin. Virol. 76 (Suppl 1) (2016 Mar) S14–S21, https://doi.org/10.1016/j.jcv.2015.09.014.
[10] Arbyn, et al., List of human papillomavirus assays suitable for primary cervical cancer screening, 2020, Clin. Microbiol. Infect. 27 (8) (2021 Aug) 1083–1095, https://doi.org/10.1016/j.cmi.2021.04.031.
[11] F. Bottari, R. Passerini, G. Renne, M.E. Guerrieri, M.T. Sandri, A. Li, A. Orlandini, A.D. Lacobone, Onclarity performance in human papillomavirus DNA detection in formalin-fixed paraffin-embedded cervical samples, J. Low. Genit. Tract Dis. 25 (3) (2021 Jul 1) 216–220, https://doi.org/10.1097/LGT.0000000000000613.
[12] F.A. Castro, J. Koshiol, W. Quint, C.M. Wheeler, M.L. Gillison, L.M. Vaughan, B. Kleter, L.- J van Doorn, A.K. Chaturvedi, A. Hildesheim, M. Schiffman, S. S. Wang, R.E. Zuna, J.L. Walker, S.T. Dunn, N. Wentzensen, Detection of HPV DNA in paraffin-embedded cervical samples: a comparison of four genotyping methods, BMC Infect. Dis. 15 (2015 Nov 25) 544, https://doi.org/10.1186/s12879-015-1281-5.
[13] G. Kir, H. Gunel, Z.C. Olgun, W.G. McCluggage, High-risk human papillomavirus (HPV) detection in formalin-fixed paraffin-embedded cervical tissues: performances of Aptima HPV assay and Beckton Dickinson (BD) Onclarity assay, J. Clin. Pathol. (2021) 1–7, https://doi.org/10.1136/jclinpath-2021-207657, 0.
[14] M.L. Nogueira Dias Genta, T.R. Martins, R.V. Mendoza Lopez, J.C. Sadalla, J.P. M. de Carvalho, E.C. Baracat, J.E. Levi, J.P. Carvalho, Multiple HPV genotype infection impact on invasive cervical cancer presentation and survival, PLoS One 12 (8) (2017), e0182854, https://doi.org/10.1371/journal.pone.0182854.
[15] E. Tumban, A current update on human papillomavirus-associated head and neck cancers, Viruses 11 (2019) 922, https://doi.org/10.3390/v11100922.
[16] T. Mpunga, et al., Human papillomavirus genotypes in cervical and other HPV-related anogenital cancer in Rwanda, according to HIV status, Int. J. Cancer 146 (2020) 1514–1522.
[17] M. Guardado-Estrada, et al., The distribution of high-risk human papillomaviruses is different in young and old patients with cervical cancer, PLoS One 9 (10) (2014 Oct 8), e109406, https://doi.org/10.1371/journal.pone.0109406.