

INSTRUCTIONS FOR USE

Reagent kit for qualitative and quantitative human papillomavirus (HPV) DNA detection and differentiation of low carcinogenic risk 6, 11, 44 genotypes and high carcinogenic risk 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types by multiplex PCR-RT "HPV-test"

IVD

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List of abbreviations

The following abbreviations and designations are used in this instruction:

| | |
|------|---------------------------|
| PCR | polymerase chain reaction |
| DNA | deoxyribonucleic acid |
| HPV | human papillomavirus |
| SVC | sampling volume control |
| NC | negative control sample |
| PC | positive control sample |
| SenC | sensitivity control |
| SC | specificity control |
| SS | standard sample |

Introduction

The human papillomavirus is a serious problem associated with the growth and high prevalence of malignant neoplasms, high mortality, disability, and a significant decrease in reproductive ability among both the female and male populations.

Target analyte: specific regions of human papillomavirus types genomic DNA, detected with HPV-test reagent kit.

The test material is DNA samples isolated from clinical material – epithelial cells scrapings from the mucous membrane of the cervical canal, urethra, vagina, biopsy, prostate secretions, ejaculate, urine.

The scientific validity of the target analyte lies in its specificity (DNA sequence uniqueness) in relation to the genomes of human papillomavirus types – 6, 11, 44, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82.

Papillomaviruses are divided into two groups according to their ability to cause malignant degeneration of the epithelium: high (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82 types) and low (6, 11, 44 types) oncogenic risk.

In HPV-test system, gene fragments were used to differentiate types: for 6 – *E7*, 11 – *E5B*, 44 – *E4*, 16 – *E1*, 18 – *E5*, 26 – *E2*, 31 – *E6*, 33 – *E5*, 35 – *E2*, 39 – *E4*, 45 – *L1*, 51 – *E1*, 52 – *E7*, 68 – *E2*, 56 – *E2*, 58 – *L2*, 59 – *E2*, 66 – *E6*, 53 – *E6*, 73 – *E1*, 82 – *E7*. The choice is determined by the relative conservation of regions for one genotype representatives. It should be noted that different test systems may use different regions to differentiate types.

The scope of the reagent kit is clinical laboratory testing of infectious diseases.

Indications and contraindications for use

ATTENTION! Diagnosis and treatment should be determined by a specialist doctor.

A reagent kit is used in clinical laboratory testing for detection, typing and quantitation of human papillomavirus DNA in human clinical material (epithelial cells scrapings from the mucous membrane of the cervical canal, urethra, vagina, biopsy, prostate secretions, ejaculate, urine).

There are no contraindications to use, except in cases when the material cannot be taken for medical reasons.

Population, demographic aspects of the kit use: population, demographic aspects of the reagent kit use have not been identified.

Sterility: the product is not sterile.

1. Intended use

Intended use: HPV-test reagent kit is designed for qualitative and quantitative human papillomavirus (HPV) DNA detection and differentiation of low carcinogenic risk 6, 11, 44 genotypes and high carcinogenic risk 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 genotypes in the following configuration forms:

1. HPV-test-21 - 6, 11, 44, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 HPV types;
2. HPV-test-16/18 - 16, 18 HPV types;
3. HPV-test-31/33 - 31, 33 HPV types;
4. HPV-test-6/11 - 6, 11 HPV types;
5. HPV-test-14 - 16, 18, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66 HPV types;

by multiplex polymerase chain reaction with hybridization-fluorescence detection in a DNA sample isolated from clinical material (epithelial cells scrapings from the mucous membrane of the cervical canal, urethra, vagina, biopsy, prostate secretion, ejaculate, urine) for HPV types differential diagnosis and risk evaluation of HPV-associated neoplasms (cervical cancer, rectum cancer, anogenital cancer).

Functional purpose. The results obtained can be used for HPV types differential diagnosis and risk evaluation of developing tumors associated with HPV (cervical cancer, rectal cancer, anogenital cancer). The results obtained can be used for HPV types differential diagnosis and risk evaluation of HPV-associated neoplasms (cervical cancer, rectal cancer, anogenital cancer).

Diagnosis and treatment should be determined by a specialist doctor in combination with clinical observations, medical history and epidemiological information.

Potential consumers of a kit

The kit is intended for research use only.

Method principle

Method

Real-time multiplex polymerase chain reaction with hybridization-fluorescence detection.

Test sample type

Test material is DNA samples isolated from clinical material – epithelial cells scrapings from the mucous membrane of the cervical canal, urethra, vagina, biopsy, prostate secretions, ejaculate, urine.

Detection principle

Qualitative and quantitative HPV DNA detection by real-time multiplex PCR in a DNA sample isolated from clinical material includes three stages:

1. PCR preparation;
2. DNA PCR amplification and hybridization-fluorescence detection of amplification products in real time;
3. Result interpretation.

Amplification reactions of gene regions are carried out with DNA samples with primers specific to these DNA regions and DNA probes in the reaction buffer.

PCR buffer contains all the basic reagents, including thermally stable DNA polymerase with a "hot start", dNTP, uracil DNA glycosidase and an optimized buffer. The presence of the uracil-DNA glycosidase enzyme prevents false positive results when contaminated with amplification products. The enzyme is completely inactivated during the first DNA denaturation cycle and does not interfere with the current reaction products amplification.

The primer mix contains fluorescently labelled oligonucleotide probes, which hybridise with the complementary region of the amplified DNA target and are destroyed by *Taq*-polymerase, as a result the fluorescent dye and the quencher are separated and the fluorescence intensity increases. This allows register the accumulation of a specific amplification product by measuring the fluorescent signal intensity in real time.

The kit contains reagents for multiplex detection of highly specific HPV DNA regions of 6, 11, 44, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types, as well as a human ALB gene specific fragment (for sampling volume control, hereinafter - SVC) (Table 1).

Table 1 – Primer mixes components (included in the kit) and the corresponding target

| Primer mix | The channel corresponding to the fluorophore | | | |
|-----------------------------------|--|----------------|-------------|-----------------------------|
| | FAM/ Green | ROX/ Orange | Cy5/ Red | HEX/ Yellow |
| HPV-test-21 configuration form | | | | |
| HPV-No.1 | 6 | 11 | 44 | SVC (human <i>ALB</i> gene) |
| HPV-No.2 | 16 | 18 | 26 | SVC (human <i>ALB</i> gene) |
| HPV-No.3 | 31 | 33 | 35 | SVC (human <i>ALB</i> gene) |
| HPV-No.4 | 39 | 45 | 51 | SVC (human <i>ALB</i> gene) |
| HPV-No.5 | 52 | 68 | 56 | SVC (human <i>ALB</i> gene) |
| HPV-No.6 | 58 | 59 | 66 | SVC (human <i>ALB</i> gene) |
| HPV-No.7 | 53 | 73 | 82 | SVC (human <i>ALB</i> gene) |
| HPV-test-16/18 configuration form | | | | |
| HPV-16/18 | 16 | 18 | – | SVC (human <i>ALB</i> gene) |
| HPV-test-31/33 configuration form | | | | |
| HPV-31/33 | 31 | 33 | – | SVC (human <i>ALB</i> gene) |

| HPV-test-6/11 configuration form | | | | |
|----------------------------------|----|----|----|-----------------------------|
| HPV-6/11 | 6 | 11 | – | SVC (human <i>ALB</i> gene) |
| HPV-test-14 configuration form | | | | |
| HPV-16/18 | 16 | 18 | – | SVC (human <i>ALB</i> gene) |
| HPV-No.3 | 31 | 33 | 35 | SVC (human <i>ALB</i> gene) |
| HPV-No.4 | 39 | 45 | 51 | SVC (human <i>ALB</i> gene) |
| HPV-No.5 | 52 | 68 | 56 | SVC (human <i>ALB</i> gene) |
| HPV-No.6 | 58 | 59 | 66 | SVC (human <i>ALB</i> gene) |

SVC allows to confirm the fact of material collection from a person, evaluate the quality, DNA isolation effectiveness and inhibitors possible presence in the sample, which can lead to false negative results.

Method limitations

A possible reason for obtaining a false positive result is contamination at DNA isolation or multiplex PCR reaction stage. A false positive result can be detected with a negative control sample.

The reagent kit cannot be used after expiry date.

Do not use the reagent kit if the inner package is damaged, or the reagent appearance does not match the description.

A reagent kit transported or stored in violation of the temperature regime cannot be used.

The clinical diagnosis cannot be based only on the assay results with this kit. For diagnostic purposes, the results should be used in combination with other data: symptoms, the common clinical picture, the results from other test systems, and the therapy used.

Multiplex PCR reaction time ranges from 60 to 80 minutes (excluding sample preparation), depending on the cycler used.

3. Reagent kit components

Configuration form

HPV-test reagent kit is available in five configuration forms:

1. HPV-test-21 for 96 reactions of each multiplex (HPV-No.1 (6, 11, 44), HPV-No.2 (16, 18, 26), HPV-No.3 (31, 33, 35), HPV-No.4 (39, 45, 51), HPV-No.5 (52, 68, 56), HPV-No.6 (58, 59, 66), HPV-No.7 (53, 73, 82));
2. HPV-test-16/18 for 96 reactions with HPV-16/18 multiplex (16, 18);
3. HPV-test-31/33 for 96 reactions with HPV-31/33 multiplex (31, 33);
4. HPV-test-6/11 for 96 reactions with HPV-6/11 multiplex (6, 11);
5. HPV-test-14 for 96 reactions of each multiplex (HPV-16/18 (16, 18), HPV-No.3 (31, 33, 35), HPV-No.4 (39, 45, 51), HPV-No.5 (52, 68, 56), HPV-No.6 (58, 59, 66)).

Test sample number for quantitative analysis (when using plate-type cyclers for 96 wells):

1. HPV-test-21 is designed to assay 90 test samples, calibration samples, negative and positive control samples with seven consecutive runs of the cycler, each run with one of seven multiplexes. In one cycler run using simultaneously all seven multiplexes, it is designed for the assay of 48 test samples (7 samples per run), calibration samples, negative and positive control samples with seven runs of the cycler using seven multiplexes (Fig. 2). In a single run, it is designed for 13 test samples (1 sample per run) with calibration samples, negative and positive control samples in each run.

2. HPV test-16/18 is designed to assay 90 clinical test samples, calibration samples, negative and positive control samples with simultaneous placing in a cycler. In a single run, it is designed for 13 test samples (1 sample per run) with calibration samples, negative and positive control samples in each run.

3. HPV-test-31/33 is designed to assay 90 clinical test samples, calibration samples, negative and positive control samples with

simultaneous placing in a cycler. In a single run, it is designed for 13 test samples (1 sample per run) with calibration samples, negative and positive control samples in each run.

4. HPV-test-6/11 is designed to assay 90 clinical test samples, calibration samples, negative and positive control samples with simultaneous placing in a cycler. In a single run, it is designed for 13 test samples (1 sample per run) with calibration samples, negative and positive control samples in each run.

5. HPV-test-14 is designed to assay 90 test samples, calibration samples, negative and positive control samples with five consecutive runs of the cycler, each run with one of five multiplexes. With one run of the cycler using all five multiplexes, it is designed for 65 test samples (13 samples per run), calibration samples, negative and positive control samples with six runs of the cycler using five multiplexes. In a single run, it is designed for 13 test samples (1 sample per run) with calibration samples, negative and positive control samples in each run.

Test sample number for qualitative analysis (when using plate-type cyclers for 96 wells):

1. HPV-test-21 is designed to assay 94 test samples, negative and positive control samples with seven consecutive cycler runs, each run with one of seven multiplexes. With one cycler run using all seven multiplexes, it is designed for 80 test samples (11 samples per run), negative and positive control samples with seven runs of the cycler using seven multiplexes. In a single run, it is designed for 32 test samples (1 sample per run) with negative and positive control samples in each run.

2. HPV test-16/18 is designed to assay 94 clinical test samples, negative and positive control samples with simultaneous placing in a cycler. In a single run, it is designed for 32 test samples (1 sample per run) with negative and positive control samples in each run.

3. HPV test-31/33 is designed to test 90 test clinical samples, negative and positive control samples with simultaneous placing in a cycler. In a single run, it is designed for 32 test samples (1 sample per run) with negative and positive control samples in each run.

4. HPV test-6/11 is designed to assay 94 clinical test samples, negative and positive control samples with simultaneous placing in a cycler. In a single run, it is designed for 32 test samples (1 sample per run) with negative and positive control samples in each run.

5. HPV-test-14 is designed to assay 94 test samples, negative and

positive control samples with seven consecutive runs of the cycler, each run with one of five multiplexes. With one cycler run using all five multiplexes, it is designed for 85 test samples (17 samples per run), negative and positive control samples with five runs of the cycler using five multiplexes. In a single run, it is designed for 32 test samples (1 sample per run) with negative and positive control samples in each run.

Kit components

Table 2 – HPV-test-21 components

| No. | Reagent name | Short name on the tube lid | Description | Quantity, volume |
|-----|----------------------------------|----------------------------|------------------------------|-----------------------|
| 1 | PCR buffer | PCR-b | Transparent colorless liquid | 2 tubes, 1350 µl each |
| 2 | Primer Mix HPV-No.1 (6, 11, 44) | 6, 11, 44 | Transparent blue liquid | 1 tube, 960 µl |
| 3 | Primer Mix HPV-No.2 (16, 18, 26) | 16, 18, 26 | Transparent blue liquid | 1 tube, 960 µl |
| 4 | Primer Mix HPV-No.3 (31, 33, 35) | 31, 33, 35 | Transparent blue liquid | 1 tube, 960 µl |
| 5 | Primer Mix HPV-No.4 (39, 45, 51) | 39, 45, 51 | Transparent blue liquid | 1 tube, 960 µl |
| 6 | Primer Mix HPV-No.5 (52, 68, 56) | 52, 68, 56 | Transparent blue liquid | 1 tube, 960 µl |
| 7 | Primer Mix HPV-No.6 (58, 59, 66) | 58, 59, 66 | Transparent colorless liquid | 1 tube, 960 µl |
| 8 | Primer Mix HPV-No.7 (53, 73, 82) | 53, 73, 82 | Transparent colorless liquid | 1 tube, 960 µl |
| 9 | CS1 | CS1 | Transparent colorless liquid | 1 tube, 1100 µl |
| 10 | CS2 | CS2 | Transparent colorless liquid | 1 tube, 1100 µl |
| 11 | PC | PC | Transparent colorless liquid | 1 tube, 1350 µl |
| 12 | NC | NC | Transparent colorless liquid | 1 tube, 1800 µl |

Table 3 - HPV-test-16/18 components

| No. | Reagent name | Short name on the tube lid | Description | Quantity, volume |
|-----|-------------------------------|----------------------------|------------------------------|------------------|
| 1 | PCR buffer | PCR-b | Transparent colorless liquid | 1 tube, 390 µl |
| 2 | Primer mix HPV-16/18 (16, 18) | 16, 18 | Transparent blue liquid | 1 tube, 960 µl |
| 3 | CS1 | CS1 | Transparent colorless liquid | 1 tube, 160 µl |
| 4 | CS2 | CS2 | Transparent colorless liquid | 1 tube, 160 µl |
| 5 | PC | PC | Transparent colorless Liquid | 1 tube, 200 µl |
| 6 | NC | NC | Transparent colorless liquid | 1 tube, 1800 µl |

Table 4 – HPV-test-31/33 components

| No. | Reagent name | Short name on the tube lid | Description | Quantity, volume |
|-----|-------------------------------|----------------------------|------------------------------|------------------|
| 1 | PCR buffer | PCR-b | Transparent colorless liquid | 1 tube, 390 µl |
| 2 | Primer Mix HPV-31/33 (31, 33) | 31, 33 | Transparent blue liquid | 1 tube, 960 µl |
| 3 | CS1 | CS1 | Transparent colorless Liquid | 1 tube, 160 µl |
| 4 | CS2 | CS2 | Transparent colorless Liquid | 1 tube, 160 µl |
| 5 | PC | PC | Transparent colorless liquid | 1 tube, 200 µl |
| 6 | NC | NC | Transparent colorless liquid | 1 tube, 1800 µl |

Table 5 – HPV-test-6/11 components

| No. | Reagent name | Short name on the tube lid | Description | Quantity, volume |
|-----|-----------------------------|----------------------------|------------------------------|------------------|
| 1 | PCR buffer | PCR-b | Transparent colorless liquid | 1 tube, 390 µl |
| 2 | Primer Mix HPV-6/11 (6, 11) | 6, 11 | Transparent blue liquid | 1 tube, 960 µl |
| 3 | CS1 | CS1 | Transparent colorless liquid | 1 tube, 160 µl |
| 4 | CS2 | CS2 | Transparent colorless liquid | 1 tube, 160 µl |
| 5 | PC | PC | Transparent colorless liquid | 1 tube, 200 µl |
| 6 | NC | NC | Transparent colorless liquid | 1 tube, 1800 µl |

Table 6 –HPV-test-14 components

| No. | Reagent name | Short name on the tube lid | Description | Quantity, volume |
|-----|----------------------------------|----------------------------|------------------------------|----------------------|
| 1 | PCR buffer | PCR-b | Transparent colorless liquid | 2 tubes, 960 µl each |
| 2 | Primer Mix HPV-16/18 (16, 18) | 16, 18 | Transparent blue liquid | 1 tube, 960 µl |
| 3 | Primer Mix HPV-No.3 (31, 33, 35) | 31, 33, 35 | Transparent blue liquid | 1 tube, 960 µl |
| 4 | Primer Mix HPV-No.4 (39, 45, 51) | 39, 45, 51 | Transparent blue liquid | 1 tube, 960 µl |
| 5 | Primer Mix HPV-No.5 (52, 68, 56) | 52, 68, 56 | Transparent blue liquid | 1 tube, 960 µl |
| 6 | Primer Mix HPV-No.6 (58, 59, 66) | 58, 59, 66 | Transparent blue liquid | 1 tube, 960 µl |
| 7 | CS1 | CS1 | Transparent colorless liquid | 1 tube, 800 µl |

| | | | | |
|----|-----|-----|------------------------------|----------------------|
| 8 | CS2 | CS2 | Transparent colorless liquid | 1 tube, 800 μ l |
| 9 | PC | PC | Transparent colorless liquid | 1 tube, 960 μ l |
| 10 | NC | NC | Transparent colorless liquid | 1 tube, 1800 μ l |

PCR buffer is ready for use, it is a buffer containing all the basic reagents, including a thermally stable DNA polymerase with a "hot start", deoxynucleotide triphosphates (dNTP), uracil DNA glycosylase and a PCR-optimized buffer.

Primer mix HPV-No.1 (6, 11, 44) is ready for use, it contains a multiplex mixture of primers and probes in deionized water free of DNase and RNase:

1. Primers and a probe to HPV 6 genomic DNA specific region. Detection is carried out on the FAM channel.

2. Primers and a probe to HPV 11 genomic DNA specific region. Detection is carried out on the ROX channel.

3. Primers and a probe to HPV 44 genomic DNA specific region. Detection is carried out on the Su5/Red channel.

4. Primers and a probe to SVC. Detection is carried out on the HEX channel.

Primer mix HPV-No.2 (16, 18, 26) is ready for use, it contains a multiplex mixture of primers and probes in deionized water free of DNase and RNase:

1. Primers and a probe to HPV 16 genomic DNA specific region. Detection is carried out on the FAM channel.

2. Primers and a probe to HPV 18 genomic DNA specific region. Detection is carried out on the ROX channel.

3. Primers and a probe to HPV 26 genomic DNA specific region. Detection is carried out on the Su5/Red channel.

4. Primers and a probe to SVC. Detection is carried out on the HEX channel.

Primer mix HPV-No.3 (31, 33, 35) is ready for use, it contains a multiplex mixture of primers and probes in deionized water free of DNase and RNase:

1. Primers and a probe to HPV 31 genomic DNA specific region.

Detection is carried out on the FAM channel.

2. Primers and a probe to HPV 33 genomic DNA specific region. Detection is carried out on the ROX channel.

3. Primers and a probe to HPV 35 genomic DNA specific region. Detection is carried out on the Su5/Red channel.

4. Primers and a probe to SVC. Detection is carried out on the HEX channel.

Primer mix HPV-No.4 (39, 45, 51) is ready for use, it contains a multiplex mixture of primers and probes in deionized water free of DNase and RNase:

1. Primers and a probe to HPV 39 genomic DNA specific region. Detection is carried out on the FAM channel.

2. Primers and a probe to HPV 45 genomic DNA specific region. Detection is carried out on the ROX channel.

3. Primers and a probe to HPV 51 genomic DNA specific region. Detection is carried out on the Su5/Red channel.

4. Primers and a probe to SVC. Detection is carried out on the HEX channel.

Primer mix HPV- No.5 (52, 68, 56) is ready to use, it contains a multiplex mixture of primers and probes in deionized water free of DNase and RNase:

1. Primers and a probe to HPV 52 genomic DNA specific region. Detection is carried out on the FAM channel.

2. Primers and a probe to HPV 68 genomic DNA specific region. Detection is carried out on the ROX channel.

3. Primers and a probe to HPV 56 genomic DNA specific region. Detection is carried out on the Su5/Red channel.

4. Primers and a probe to SVC. Detection is carried out on the HEX channel.

Primer mix HPV-No.6 (58, 59, 66) is ready to use, it contains a multiplex mixture of primers and probes in deionized water free of DNase and RNase:

1. Primers and a probe to HPV 58 genomic DNA specific region. Detection is carried out on the FAM channel.

2. Primers and a probe to HPV 59 genomic DNA specific region. Detection is carried out on the ROX channel.

3. Primers and a probe to HPV 66 genomic DNA specific region. Detection is carried out on the Su5/Red channel.

4. Primers and a probe to SVC. Detection is carried out on the HEX channel.

Primer mix HPV-No.7 (53, 73, 82) is ready to use, it contains a multiplex mixture of primers and probes in deionized water free of DNase and RNase:

1. Primers and a probe to HPV 53 genomic DNA specific region. Detection is carried out on the FAM channel.

2. Primers and a probe to HPV 73 genomic DNA specific region. Detection is carried out on the ROX channel.

3. Primers and a probe to HPV 82 genomic DNA specific region. Detection is carried out on the Su5/Red channel.

4. Primers and a probe to SVC. Detection is carried out on the HEX channel.

Primer mix HPV-16/18 (16, 18) is ready for use, it contains a multiplex mixture of primers and probes in deionized water free of DNase and RNase:

1. Primers and a probe to HPV 16 genomic DNA specific region. Detection is carried out on the FAM channel.

2. Primers and a probe to HPV 18 genomic DNA specific region. Detection is carried out on the ROX channel.

3. Primers and a probe to SVC. Detection is carried out on the HEX channel.

Primer mix HPV-31/33 (31, 33) is ready for use, it contains a multiplex mixture of primers and probes in deionized water free of DNase and RNase:

1. Primers and a probe to HPV 31 genomic DNA specific region. Detection is carried out on the FAM channel.

2. Primers and a probe to HPV 33 genomic DNA specific region. Detection is carried out on the ROX channel.

3. Primers and a probe to SVC. Detection is carried out on the HEX channel.

Primer Mix HPV-6/11 (6, 11) is ready for use, it contains a multiplex mixture of primers and probes in deionized water free of DNase and RNase:

1. Primers and a probe to HPV 6 genomic DNA specific region. Detection is carried out on the FAM channel.

2. Primers and a probe to HPV 11 genomic DNA specific region. Detection is carried out on the ROX channel.

3. Primers and a probe to SVC. Detection is carried out on the HEX channel.

Calibration sample CS1 is ready for use, it contains specific fragments of HPV DNA genomes 6, 11, 44, 16, 18, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types detected by the reagent kit (types kit in different configuration forms corresponds to the detected target analytes) with 1×10^6 copies/ml concentration in a 10% TE buffer (1 mM Tris, 0.1 mM EDTA) and contains 0.05% sodium azide as a preservative.

Calibration sample CS2 is ready for use, it contains specific fragments of HPV DNA genomes 6, 11, 44, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types detected by the reagent kit (types kit in different configuration forms corresponds to the detected target analytes) with 1×10^4 copies/ml concentration in a 10% TE buffer (1 mM Tris, 0.1 mM EDTA) and contain 0.05% sodium azide as a preservative.

Positive control sample (PC) is ready for use, it is a mixture of plasmid DNA with amplified DNA fragments synthetic insertions: specific DNA fragments of HPV type 6, 11, 44, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 (types kit in different configuration forms corresponds to the detected target analytes) with 1×10^5 copies/ml concentration and a human *ALB* gene region (used as a sampling volume control (SVC)) with 1×10^5 copies/ml concentration in 10% TE buffer (1 mM Tris, 0.1 mM EDTA) and contains 0.05% sodium azide as a preservative.

Negative control sample (NC) is ready for use, it is deionized water free of DNase.

The kit contains no products for medical use, materials of human or animal origin.

4. Reagent kit characteristics

4.1. Technical and functional characteristics

Table 7 – HPV-test reagent kit

| Indicator name | Characteristics and standards |
|-------------------------------------|-------------------------------|
| 1. Technical characteristics | |
| 1.1. Appearance | |
| 1.1.1 HPV-test-21 | |
| PCR buffer | Transparent colorless liquid |
| Primer mix HPV-No.1 (6, 11, 44) | Transparent blue liquid |
| Primer mix HPV-No.2 (16, 18, 26) | Transparent blue liquid |
| Primer mix HPV-No.3 (31, 33, 35) | Transparent blue liquid |
| Primer mix HPV-No.4 (39, 45, 51) | Transparent blue liquid |
| Primer mix HPV-No.5 (52, 68, 56) | Transparent blue liquid |
| Primer mix HPV-No.6 (58, 59, 66) | Transparent blue liquid |
| Primer mix HPV-No.7 (53, 73, 82) | Transparent blue liquid |
| CS1 | Transparent colorless liquid |
| CS2 | Transparent colorless liquid |
| PC | Transparent colorless liquid |
| NC | Transparent colorless liquid |
| 1.1.2 HPV-test-16/18 | |
| PCR buffer | Transparent colorless liquid |
| Primer mix HPV-16/18 (16, 18) | Transparent blue liquid |
| CS1 | Transparent colorless liquid |
| CS2 | Transparent colorless liquid |
| PC | Transparent colorless liquid |
| NC | Transparent colorless liquid |
| 1.1.3 HPV-test-31/33 | |
| PCR buffer | Transparent colorless liquid |
| Primer mix HPV-31/33 (31, 33) | Transparent blue liquid |
| CS1 | Transparent colorless liquid |
| CS2 | Transparent colorless liquid |
| PC | Transparent colorless liquid |
| NC | Transparent colorless liquid |

| | |
|--|--|
| 1.1.4 HPV-test-6/11 | |
| PCR buffer | Transparent colorless liquid |
| Primer Mix HPV-6/11 (6, 11) | Transparent blue liquid |
| CS1 | Transparent colorless liquid |
| CS2 | Transparent colorless liquid |
| PC | Transparent colorless liquid |
| NC | Transparent colorless liquid |
| 1.1.5 HPV-test-14 | |
| PCR buffer | Transparent colorless liquid |
| Primer mix HPV-16/18 (16, 18) | Transparent blue liquid |
| Primer mix HPV-No.3 (31, 33, 35) | Transparent blue liquid |
| Primer mix HPV-No.4 (39, 45, 51) | Transparent blue liquid |
| Primer mix HPV-No.5 (52, 68, 56) | Transparent blue liquid |
| Primer mix HPV-No.6 (58, 59, 66) | Transparent blue liquid |
| CS1 | Transparent colorless liquid |
| CS2 | Transparent colorless liquid |
| PC | Transparent colorless liquid |
| NC | Transparent colorless liquid |
| 1.2. Completeness | In accordance with clause 1.4 TS 21.20.23-060-97638376-2022 |
| 1.3. Marking | In accordance with clause 1.5 TS 21.20.23-060-97638376-2022 |
| 1.4. Package | In accordance with clause 1.6 TS 21.20.23-060-97638376-2022 |
| 2. Functional characteristics | |
| 2.1 HPV-test-21 | |
| Positive result with PC | Fluorescence signal growth registration in tubes with PC in the channels FAM, ROX, Cy5, HEX $Ct \leq 30$. |
| Negative result with NC | In tubes with NC in the channels FAM, ROX, Cy5 and HEX, Ct is not indicated or >35 . |
| Reaction course in tubes with ESS-SC-HPV | In tubes with ESS-SC-HPV, in the FAM, ROX, Cy5 channels Ct is not indicated or >35 , and in the HEX channel $Ct \leq 30$. |
| Reaction course in tubes with ESS-SenC-6/11/44 | In a tube with ESS-SenC-6/11/44 for the reaction mixture HPV-No.1 (6, 11, 44) in the FAM channel $Ct \leq 35$, ROX $Ct \leq 35$, Cy5 $Ct \leq 35$, and in the HEX channel Ct is not indicated or more than 35 cycle |

| | |
|--|--|
| Reaction course in tubes with ESS-SenC-16/18/26 | In a tube with ESS-SenC-16/18/26 for the reaction mixture HPV- No.2 (16, 18, 26) in the FAM channel $Ct \leq 35$, ROX $Ct \leq 35$, Cy5 $Ct \leq 35$, and in the HEX channel Ct is not indicated or more than 35 cycle |
| Reaction course in tubes with ESS-SenC-31/33/35 | In a tube with ESS-SenC-31/33/35 for the reaction mixture HPV- No.3 (31, 33, 35) in the FAM channel $Ct \leq 35$, ROX $Ct \leq 35$, Cy5 $Ct \leq 35$, and on the HEX channel Ct is not indicated or more than 35 cycle |
| Reaction course in tubes with ESS-SenC-39/45/51 | In a tube with ESS-SenC-39/45/51 for the reaction mixture HPV-No.4 (39, 45, 51) in the FAM channel $Ct \leq 35$, ROX $Ct \leq 35$, Cy5 $Ct \leq 35$, and in the HEX channel Ct is not indicated or more than 35 cycle |
| Reaction course in tubes with ESS-SenC-52/68/56 | In a tube with ESS-SenC-52/68/56 for the reaction mixture HPV-No.5 (52, 68, 56) in the FAM channel $Ct \leq 35$, ROX $Ct \leq 35$, Cy5 $Ct \leq 35$, and in the HEX channel Ct is not indicated or more than 35 cycle |
| Reaction course in tubes with ESS-SenC-58/59/66 | In a tube with ESS-SenC-58/59/66 for the reaction mixture HPV-No.6 (58, 59, 66) in the FAM channel $Ct \leq 35$, ROX $Ct \leq 35$, Cy5 $Ct \leq 35$, and in the HEX channel Ct is not indicated or more than 35 cycle |
| Reaction course in tubes with ESS-SenC-53/73/82 | In a tube with ESS-SenC-53/73/82 for the reaction mixture HPV-No.7 (53, 73, 82) in the FAM channel $Ct \leq 35$, ROX $Ct \leq 35$, Cy5 $Ct \leq 35$, and in the HEX channel Ct is not indicated or more than 35 cycle |
| "Linearity" test | CS1, CS2 and PC correlation coefficient is not less than 0.98 |
| Accuracy test: coefficient of variation (CV) under conditions of repeatability | The coefficient of variation Ct for repeats of each calibration sample CS1 and CS2 under repeatability conditions is not more than 5%. |
| Accuracy of concentration determination test | The obtained HPV DNA concentration value should correspond to the concentration given in the passport of the standard sample (SS) (5.3 \log_{10} copies/ml), with a tolerance of $\pm 0.5 \log_{10}$ copies/ml concentration |
| 2.2 HPV-test-16/18 | |
| Positive result with PC | Fluorescence signal growth registration in tubes with PC in the channels FAM, ROX, HEX $Ct \leq 30$. |
| Negative result with NC | In tubes with NC in the FAM, ROX and HEX channels, Ct is not indicated or > 35 . |
| Reaction course in tubes with ESS-SC-HPV | In tubes with ESS-SC-HPV, Ct is not indicated or > 35 in the FAM and ROX channels, and $Ct \leq 30$ in the HEX channel. |

| | |
|---|---|
| Reaction course in tubes with ESS-SenC-16/18 | In a tube with ESS-SenC-16/18 for the reaction mixture HPV-16/18 (16, 18) in the FAM channel $Ct \leq 35$, ROX $Ct \leq 35$, and the HEX Ct channel is not indicated or more than 35 cycle |
| "Linearity" test | CS1, CS2 and PC correlation coefficient is not less than 0.98 |
| Accuracy test: coefficient of variation (CV) under repeatability conditions | The coefficient of variation Ct for repeats of each calibration sample CS1 and CS2 under repeatability conditions is not more than 5%. |
| Accuracy of concentration determination test | The obtained HPV DNA concentration value should correspond to the concentration given in the passport of the standard sample (SS) ($5.3 \log_{10}$ copies/ml) with a tolerance of $\pm 0.5 \log_{10}$ copies/ml concentration |
| 2.3 HPV-test-31/33 | |
| Positive result with PC | Fluorescence signal growth registration in tubes with PC in the channels FAM, ROX, HEX $Ct \leq 30$. |
| Negative result with NC | In tubes with NC in the FAM, ROX and HEX channels, Ct is not indicated or >35 . |
| Reaction course in tubes with ESS-SC-HPV | In tubes with ESS-SC-HPV, Ct is not indicated or >35 in the FAM and ROX channels, and $Ct \leq 30$ in the HEX channel. |
| Reaction course in tubes with ESS-SenC-31/33 | In a tube with ESS-SenC-31/33 for the reaction mixture HPV-31/33 (31, 33) in the FAM channel $Ct \leq 35$, ROX $Ct \leq 35$, and in the HEX channel Ct is not indicated or more than 35 cycle |
| "Linearity" test | CS1, CS2 and PC correlation coefficient is not less than 0.98 |
| Accuracy test: coefficient of variation (CV) under repeatability conditions | The coefficient of variation Ct for repeats of each calibration sample CS1 and CS2 under repeatability conditions is not more than 5%. |
| Accuracy of concentration determination test | The obtained HPV DNA concentration value should correspond to the concentration given in the passport of the standard sample (SS) ($5.3 \log_{10}$ copies/ml), with a tolerance of $\pm 0.5 \log_{10}$ copies/ml concentration |
| 2.4 Configuration form HPV-test-6/11 | |
| Positive result with PC | Fluorescence signal growth registration in tubes with PC in the channels FAM, ROX, HEX $Ct \leq 30$. |
| Negative result with NC | In tubes with NC in the FAM, ROX and HEX channels, Ct is not indicated or >35 . |

| | |
|---|---|
| Reaction course in tubes with ESS-SC-HPV | In tubes with ESS-SC-HPV, Ct is not indicated or >35 in the FAM and ROX channels, and Ct ≤30 in the HEX channel. |
| Reaction course in tubes with ESS-SenC-6/11 | In a tube with ESS-SenC-6/11 for the reaction mixture HPV-6/11 (6, 11) in the FAM channel Ct ≤ 35, ROX Ct ≤ 35, and in the HEX channel Ct is not indicated or more than 35 cycles |
| "Linearity" test | CS1, CS2 and PC correlation coefficient is not less than 0.98 |
| Accuracy test: coefficient of variation (CV) under repeatability conditions | The coefficient of variation Ct for repeats of each calibration sample CS1 and CS2 under repeatability conditions is not more than 5%. |
| Accuracy of concentration determination test | The obtained HPV DNA concentration value should correspond to the concentration given in the passport of the standard sample (SS) (5.3 log ₁₀ copies/ml), with a tolerance of ±0.5 log ₁₀ copies/ml concentration |
| 2.5 Configuration form HPV-test-14 | |
| Positive result with PC | Fluorescence signal growth registration in tubes with PC in the channels FAM, ROX, Cy5, HEX Ct≤30. |
| Negative result with NC | In tubes with NC in the channels FAM, ROX, Cy5 and HEX, Ct is not indicated or >35. |
| Reaction course in tubes with ESS-SC-HPV | In tubes with ESS-SC-HPV, in the FAM, ROX, Cy5 channels Ct is not indicated or >35, and in the HEX channel Ct ≤30. |
| Reaction course in tubes with ESS-SenC-16/18 | In a tube with ESS-SenC-16/18 for the reaction mixture HPV-16/18 (16, 18) in the FAM channel Ct ≤ 35, ROX Ct ≤ 35, and in the HEX channel Ct is not indicated or more than 35 cycle |
| Reaction course in tubes with ESS-SenC-31/33/35 | In a tube with ESS-SenC-31/33/35 for the reaction mixture HPV-No.3 (31, 33, 35) in the FAM channel Ct ≤ 35, ROX Ct ≤ 35, Cy5 Ct ≤ 35, and in the HEX channel Ct is not indicated or more than 35 cycle |
| Reaction course in tubes with ESS-SenC-39/45/51 | In a tube with ESS-SenC-39/45/51 for the reaction mixture HPV- No.4 (39, 45, 51) in the FAM channel Ct ≤ 35, ROX Ct ≤ 35, Cy5 Ct ≤ 35, and in the HEX channel Ct is not indicated or more than 35 cycles |
| Reaction course in tubes with ESS-SenC-52/68/56 | In a tube with ESS-SenC-52/68/56 for the reaction mixture HPV-No5 (52, 68, 56) in the FAM channel Ct ≤ 35, ROX Ct ≤ 35, Cy5 Ct ≤ 35, and in the HEX channel Ct is not indicated or more than 35 cycle |

| | |
|---|---|
| Reaction course in tubes with ESS-SenC-58/59/66 | In a tube with ESS-SenC-58/59/66 for the reaction mixture HPV-No.6 (58, 59, 66) in the FAM channel Ct ≤ 35, ROX Ct ≤ 35, Cy5 Ct ≤ 35, and in the HEX channel Ct is not indicated or more than 35 cycle |
| "Linearity" test | CS1, CS2 and PC correlation coefficient is not less than 0.98 |
| Accuracy test: coefficient of variation (CV) under repeatability conditions | The coefficient of variation Ct for repeats of each calibration sample CS1 and CS2 under repeatability conditions is not more than 5%. |
| Accuracy of concentration determination test | The obtained HPV DNA concentration value should correspond to the concentration given in the passport of the standard sample (SS) (5.3 log10 copies/ml), with a tolerance of ±0.5 log10 copies/ml concentration |

In case of kit malfunction, deviations in its functioning that may affect safety, changes in product analytical characteristics immediately stop the kit use and inform the manufacturer (see Section 14 of the Instructions).

4.2. Analytical efficiency characteristics

4.2.1. Analytical specificity

| | |
|--|--|
| Configuration form HPV-test-21 | Specific to HPV DNA of 6, 11, 44, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types. If a DNA sample contains <i>Lactobacillus spp.</i> , <i>Staphylococcus aureus</i> , <i>Streptococcus agalactiae</i> , <i>CMV</i> , <i>HHV8</i> , <i>Ureaplasma urealyticum</i> , <i>Ureaplasma parvum</i> , <i>Gardnerella vaginalis</i> , <i>Mycoplasma genitalium</i> , <i>Mycoplasma hominis</i> , <i>Chlamydia trachomatis</i> , <i>Candida albicans</i> , <i>EBV</i> , <i>HHV6</i> , <i>HSV1</i> , <i>HSV2</i> , <i>Varicella Zoster virus</i> there are no nonspecific positive amplification results. |
| Configuration form HPV-test-16/18 | Specific to HPV types 16 and 18 DNA. If a DNA sample contains <i>Lactobacillus spp.</i> , <i>Staphylococcus aureus</i> , <i>Streptococcus agalactiae</i> , <i>CMV</i> , <i>HHV8</i> , <i>Ureaplasma urealyticum</i> , <i>Ureaplasma parvum</i> , <i>Gardnerella vaginalis</i> , <i>Mycoplasma genitalium</i> , <i>Mycoplasma hominis</i> , <i>Chlamydia trachomatis</i> , <i>Candida albicans</i> , <i>EBV</i> , <i>HHV6</i> , <i>HSV1</i> , <i>HSV2</i> , <i>Varicella Zoster virus</i> there are no nonspecific positive amplification results. |

| | |
|---|--|
| <p>Configuration form HPV-test-31/33</p> | <p>Specific to HPV types 31 and 33 DNA. If a DNA sample contains <i>Lactobacillus spp.</i>, <i>Staphylococcus aureus</i>, <i>Streptococcus agalactiae</i>, <i>CMV</i>, <i>HHV8</i>, <i>Ureaplasma urealyticum</i>, <i>Ureaplasma parvum</i>, <i>Gardnerella vaginalis</i>, <i>Mycoplasma genitalium</i>, <i>Mycoplasma hominis</i>, <i>Chlamydia trachomatis</i>, <i>Candida albicans</i>, <i>EBV</i>, <i>HHV6</i>, <i>HSV1</i>, <i>HSV2</i>, <i>Varicella Zoster virus</i> there are no nonspecific positive amplification results.</p> |
| <p>Configuration form HPV-test-6/11</p> | <p>Specific to HPV types 6 and 11 DNA. If a DNA sample contains <i>Lactobacillus spp.</i>, <i>Staphylococcus aureus</i>, <i>Streptococcus agalactiae</i>, <i>CMV</i>, <i>HHV8</i>, <i>Ureaplasma urealyticum</i>, <i>Ureaplasma parvum</i>, <i>Gardnerella vaginalis</i>, <i>Mycoplasma genitalium</i>, <i>Mycoplasma hominis</i>, <i>Chlamydia trachomatis</i>, <i>Candida albicans</i>, <i>EBV</i>, <i>HHV6</i>, <i>HSV1</i>, <i>HSV2</i>, <i>Varicella Zoster virus</i> there are no nonspecific positive amplification results.</p> |
| <p>Configuration form HPV-test-14</p> | <p>Specific to HPV DNA of 16, 18, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66 types. If a DNA sample contains <i>Lactobacillus spp.</i>, <i>Staphylococcus aureus</i>, <i>Streptococcus agalactiae</i>, <i>CMV</i>, <i>HHV8</i>, <i>Ureaplasma urealyticum</i>, <i>Ureaplasma parvum</i>, <i>Gardnerella vaginalis</i>, <i>Mycoplasma genitalium</i>, <i>Mycoplasma hominis</i>, <i>Chlamydia trachomatis</i>, <i>Candida albicans</i>, <i>EBV</i>, <i>HHV6</i>, <i>HSV1</i>, <i>HSV2</i>, <i>Varicella Zoster virus</i> there are no nonspecific positive amplification results.</p> |

4.2.2 Limit of detection (LOD)

In accordance with international recommendations **CLSI EP-17A2**, the limit of detection (LOD) was determined by analyzing **the following standard samples dilutions** in the range of the expected limit of detection – 150, 200, 250, 300, 350, 450, 500 copies/ml:

- WHO International Standard "Collection of WHO International standards for human papillomavirus (HPV) DNA genotypes HPV31, HPV33, HPV45, HPV52, HPV58", NIBSC Code: 19/226 (represents a first international standards collection for HPV DNA genotypes HPV31, HPV33, HPV45, HPV52, HPV58 (NIBSC codes 14/258, 14/260, 14/104, 14/262, 14/264 respectively);

- WHO International Standard "Collection of WHO International standards for human papillomavirus (HPV) HPV16, HPV18, HPV6, HPV11 DNA genotypes", NIBSC Code: 19/224 (represents a first international standards collection for HPV HPV16, HPV18, HPV6, HPV11 DNA genotypes (NIBSC codes 06/202, 06/206, 14/256, 14/100, respectively).

Due to the fact that there are no certified pure SS available in relation to DNA of human papillomavirus (HPV) types 44, 26, 35, 39, 51, 68, 56, 59, 66, 53, 73, 82 so as a retained SS in relation to DNA of human papillomavirus (HPV) types 44, 26, 35, 39, 51, 68, 56, 59, 66, 53, 73, 82 was used isolated from human biological material test analytes DNA:

| No. | Sample name | DNA level, copies/ml | DNA level, log10 copies/ml |
|-----|-------------|----------------------|----------------------------|
| 1 | SS-HPV44 | 67608 | 4.83 |
| 2 | SS-HPV26 | 231847 | 5.37 |
| 3 | SS-HPV 35 | 8102022 | 6.91 |
| 4 | SS-HPV 39 | 11406 | 4.06 |
| 5 | SS-HPV 51 | 1068111 | 6.03 |
| 6 | SS-HPV 68 | 1318281 | 6.12 |
| 7 | SS-HPV 56 | 53342 | 4.73 |
| 8 | SS-HPV 59 | 2628447 | 6.42 |
| 9 | SS-HPV 66 | 265880 | 5.42 |
| 10 | SS-HPV 53 | 3323253 | 6.52 |
| 11 | SS-HPV 73 | 174590 | 5.24 |
| 12 | SS-HPV 82 | 95912 | 4.98 |

Assay results are shown in Table 8.

Table 8

| Configuration form | Test analyte | Cycler used | Concentration, copies/ml (LOD) with 95% confidence probability | | | |
|-------------------------------|---------------|-------------------|---|---|---|---|
| | | | DNA isolation kit | | | |
| | | | NA-Extra | DNA-sorb-B | PREP-NA/ PREP-NA-PLUS | DNA-Fast |
| HPV-test-21, HPV-test-6/11 | HPV 6 DNA | DTprime | 447 95%CI: 393.32 - 500,68 copies/ml | 436 95%CI: 382.32 - 489,68 copies/ml | 450 95%CI: 396.32 - 503,68 copies/ml | 438 95%CI: 384.32 - 491,68 copies/ml |
| | | CFX 96 | 428 95%CI: 374.32 - 481,68 copies/ml | 454 95%CI: 400.32 - 507,68 copies/ml | 446 95%CI: 392.32 - 499,68 copies/ml | 423 95%CI: 369.32 - 476,68 copies/ml |
| | | Rotor- Gene Q | 420 95%CI: 366.32 - 473,68 copies/ml | 434 95%CI: 380.32 - 487,68 copies/ml | 435 95%CI: 381.32 - 488,68 copies/ml | 430 95%CI: 376.32 - 483,68 copies/ml |
| | | Quant Studio 5 | 436 95%CI: 382.32 - 489,68 copies/ml | 432 95%CI: 378.32 - 485,68 copies/ml | 438 95%CI: 384.32 - 491,68 copies/ml | 441 95%CI: 387.32 - 494,68 copies/ml |
| | | FLUORITE | 453 95%CI: 399.32 - 506,68 copies/ml | 443 95%CI: 389.32 - 496,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml | 435 95%CI: 381.32 - 488,68 copies/ml |
| HPV-test-21, HPV-test-6/11 | DNA 11 HPV | DTprime | 453 95%CI: 399.32 - 506,68 copies/ml | 459 95%CI: 405.32 - 512,68 copies/ml | 455 95%CI: 401.32 - 508,68 copies/ml | 448 95%CI: 394.32 - 501,68 copies/ml |
| | | CFX 96 | 434 95%CI: 380.32 - 487,68 copies/ml | 434 95%CI: 380.32 - 487,68 copies/ml | 449 95%CI: 395.32 - 502,68 copies/ml | 434 95%CI: 380.32 - 487,68 copies/ml |
| | | Rotor- Gene Q | 426 95%CI: 372.32 - 479,68 copies/ml | 426 95%CI: 372.32 - 479,68 copies/ml | 415 95%CI: 361.32 - 468,68 copies/ml | 428 95%CI: 374.32 - 481,68 copies/ml |
| | | Quant Studio 5 | 432 95%CI: 378.32 - 485,68 copies/ml | 452 95%CI: 398.32 - 505,68 copies/ml | 446 95%CI: 392.32 - 499,68 copies/ml | 455 95%CI: 401.32 - 508,68 copies/ml |
| | | FLUORITE | 454 95%CI: 400.32 - 507,68 copies/ml | 436 95%CI: 382.32 - 489,68 copies/ml | 448 95%CI: 394.32 - 501,68 copies/ml | 455 95%CI: 401.32 - 508,68 copies/ml |

| | | | | | | |
|--|------------|----------------|--|--|--|--|
| HPV-test-21 | DNA 44 HPV | DTprime | 424 95%CI: 370.32 - 477,68 copies/ml | 446 95%CI: 392.32 - 499,68 copies/ml | 430 95%CI: 376.32 - 483.68 copies/ml | 426 95%CI: 372.32 - 479,68 copies/ml |
| | | CFX 96 | 428 95%CI: 374.32 - 481,68 copies/ml | 416 95%CI: 362.32 - 469,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml | 428 95%CI: 374.32 - 481,68 copies/ml |
| | | Rotor-Gene Q | 448 95%CI: 394.32 - 501,68 copies/ml | 462 95%CI: 408.32 - 515,68 copies/ml | 416 95%CI: 362.32 - 469,68 copies/ml | 443 95%CI: 389.32 - 496,68 copies/ml |
| | | Quant Studio 5 | 433 95%CI: 379.32 - 486,68 copies/ml | 433 95%CI: 379.32 - 486,68 copies/ml | 427 95%CI: 373.32 - 480,68 copies/ml | 444 95%CI: 390.32 - 497,68 copies/ml |
| | | FLUORITE | 427 95%CI: 373.32 - 480,68 copies/ml | 443 95%CI: 389.32 - 496,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml |
| HPV-test-21, HPV-test-16/18, HPV-test-14 | DNA 16 HPV | DTprime | 422 95%CI: 368.32 - 475,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml | 435 95%CI: 381.32 - 488,68 copies/ml | 453 95%CI: 399.32 - 506,68 copies/ml |
| | | CFX 96 | 457 95%CI: 403.32 - 510,68 copies/ml | 423 95%CI: 369.32 - 476,68 copies/ml | 448 95%CI: 394.32 - 501,68 copies/ml | 436 95%CI: 382.32 - 489,68 copies/ml |
| | | Rotor-Gene Q | 445 95%CI: 391.32 - 498,68 copies/ml | 452 95%CI: 398.32 - 505,68 copies/ml | 443 95%CI: 389.32 - 496,68 copies/ml | 451 95%CI: 397.32 - 504,68 copies/ml |
| | | Quant Studio 5 | 424 95%CI: 370.32 - 477,68 copies/ml | 438 95%CI: 384.32 - 491,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml | 456 95%CI: 402,32 - 509,68 copies/ml |
| | | FLUORITE | 430 95%CI: 376.32 - 483,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml | 448 95%CI: 394.32 - 501,68 copies/ml | 442 95%CI: 388.32 - 495,68 copies/ml |
| HPV-test-21, HPV-test-16/18, HPV-test-14 | HPV 18 DNA | DTprime | 433 95%CI: 379.32 - 486,68 copies/ml | 444 95%CI: 390.32 - 497,68 copies/ml | 434 95%CI: 380.32 - 498,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml |
| | | CFX 96 | 440 95%CI: 386.32 - 493,68 copies/ml | 462 95%CI: 408.32 - 515,68 copies/ml | 456 95%CI: 402.32 - 509,68 copies/ml | 431 95%CI: 377.32 - 484,68 copies/ml |
| | | Rotor-Gene Q | 448 95%CI: 394.32 - 501,68 copies/ml | 430 95%CI: 376.32 - 483,68 copies/ml | 434 95%CI: 380.32 - 487,68 copies/ml | 454 95%CI: 400.32 - 507,68 copies/ml |
| | | Quant Studio 5 | 440 95%CI: 386.32 - 493,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml | 428 95%CI: 374.32 - 481,68 copies/ml | 431 95%CI: 377.32 - 484,68 copies/ml |

| | | | | | | |
|--|---------------|-------------------|---|---|---|--|
| | | FLUORITE | 446 95%CI: 392.32 - 499,68 copies/ml | 419 95%CI: 365.32 - 472,68 copies/ml | 429 95%CI: 375.32 - 482,68 copies/ml | 430 95%CI: 376.32 - 483,68 copies/ml |
| HPV- test-21 | HPV 26 DNA | DTprime | 452 95%CI: 398.32 - 505,68 copies/ml | 430 95%CI: 376.32 - 483,68 copies/ml | 447 95%CI: 393.32 - 500,68 copies/ml | 430 95%CI: 376.32 - 483,68 copies/ml |
| | | CFX 96 | 425 95%CI: 371.32 - 478,68 copies/ml | 440 95%CI: 386.32 - 493,68 copies/ml | 418 95%CI: 364.32 - 471,68 copies/ml | 429 95%CI: 375.32 - 482,68 copies/ml |
| | | Rotor- Gene Q | 441 95%CI: 387.32 - 494,68 copies/ml | 425 95%CI: 371.32 - 478,68 copies/ml | 433 95%CI: 379.32 - 486,68 copies/ml | 447 95%CI: 393.32 - 500,68 copies/ml |
| | | Quant Studio 5 | 456 95%CI: 402.32 - 509,68 copies/ml | 444 95%CI: 390.32 - 497,68 copies/ml | 421 95%CI: 367.32 - 474,68 copies/ml | 460 95%CI: 406.32 - 513,68 copies/ml |
| | | FLUORITE | 434 95%CI: 380.32 - 487,68 copies/ml | 423 95%CI: 369.32 - 476,68 copies/ml | 430 95%CI: 376.32 - 483,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml |
| HPV- test-21, HPV- test- 31/33, HPV- test-14 | HPV 31 DNA | DTprime | 422 95%CI: 368.32 - 475,68 copies/ml | 455 95%CI: 401.32 - 508,68 copies/ml | 432 95%CI: 378.32 - 485,68 copies/ml | 439 95%CI: 385.32 - 492,68 copies/ml |
| | | CFX 96 | 420 95%CI: 366.32 - 473,68 copies/ml | 425 95%CI: 371.32 - 478,68 copies/ml | 450 95%CI: 396.32 - 503,68 copies/ml | 422 95%CI: 368.32 - 475,68 copies/ml |
| | | Rotor- Gene Q | 437 95%CI: 383.32 - 490,68 copies/ml | 452 95%CI: 398.32 - 505,68 copies/ml | 446 95%CI: 392.32 - 499,68 copies/ml | 456 95%CI: 402.32 - 509,68 copies/ml |
| | | Quant Studio 5 | 442 95%CI: 388.32 - 495,68 copies/ml | 428 95%CI: 374.32 - 481,68 copies/ml | 423 95%CI: 369.32 - 476,68 copies/ml | 455 95%CI: 401.32 - 508,68 copies/ml |
| | | FLUORITE | 430 95%CI: 376.32 - 483,68 copies/ml | 453 95%CI: 399.32 - 506,68 copies/ml | 433 95%CI: 379.32 - 486,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml |
| HPV- test-21, HPV- test- 31/33, HPV- test-14 | HPV 33 DNA | DTprime | 437 95%CI: 383.32 -490,68 copies/ml | 439 95%CI: 385.32 - 492,68 copies/ml | 423 95%CI: 369.32 - 476,68 copies/ml | 433 95%CI: 379.32 - 486,68 copies/ml |
| | | CFX 96 | 450 95%CI: 396.32 -503,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml | 456 95%CI: 402.32 - 509,68 copies/ml | 433 95%CI: 379.32 - 486,68 copies/ml |
| | | Rotor- Gene Q | 433 95%CI: 379.32 - 486,68 copies/ml | 439 95%CI: 385.32 - 492,68 copies/ml | 427 95%CI: 373.32 - 480,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml |

| | | | | | | |
|-----------------------------|---------------|----------------|---|---|---|---|
| | | Quant Studio 5 | 439 95%CI: 385.32 - 492,68 copies/ml | 444 95%CI: 390.32 - 497,68 copies/ml | 458 95%CI: 404.32 - 511,68 copies/ml | 430 95%CI: 376.32 - 483,68 copies/ml |
| | | FLUORITE | 434 95%CI: 380.32 - 487,68 copies/ml | 424 95%CI: 370.32 - 477,68 copies/ml | 455 95%CI: 401.32 - 508,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml |
| HPV-test-21, HPV-test-14 | HPV 35 DNA | DTprime | 445 95%CI: 391.32 - 498,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml | 452 95%CI: 398.32 - 505,68 copies/ml | 430 95%CI: 376.32 - 483,68 copies/ml |
| | | CFX 96 | 440 95%CI: 386.32 - 493,68 copies/ml | 443 95%CI: 389.32 - 496,68 copies/ml | 438 95%CI: 384.32 - 491,68 copies/ml | 436 95%CI: 382.32 - 489,68 copies/ml |
| | | Rotor-Gene Q | 434 95%CI: 380.32 - 487,68 copies/ml | 420 95%CI: 366.32 - 473,68 copies/ml | 436 95%CI: 382.32 - 489,68 copies/ml | 438 95%CI: 384.32 - 491,68 copies/ml |
| | | Quant Studio 5 | 448 95%CI: 394.32 - 501,68 copies/ml | 449 95%CI: 395.32 - 502,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml | 444 95%CI: 390.32 - 497,68 copies/ml |
| | | FLUORITE | 445 95%CI: 391.32 - 498,68 copies/ml | 436 95%CI: 382.32 - 489,68 copies/ml | 449 95%CI: 395.32 - 502,68 copies/ml | 421 95%CI: 367.32 - 474,68 copies/ml |
| HPV-test-21, HPV-test-14 | HPV 39 DNA | DTprime | 433 95%CI: 379.32 - 486,68 copies/ml | 433 95%CI: 379.32 - 486,68 copies/ml | 429 95%CI: 375.32 - 482,68 copies/ml | 432 95%CI: 378.32 - 485,68 copies/ml |
| | | CFX 96 | 436 95%CI: 382.32 - 489,68 copies/ml | 455 95%CI: 401.32 - 508,68 copies/ml | 453 95%CI: 399.32 - 506,68 copies/ml | 436 95%CI: 382.32 - 489,68 copies/ml |
| | | Rotor-Gene Q | 446 95%CI: 392.32 - 499,68 copies/ml | 433 95%CI: 379.32 - 486,68 copies/ml | 423 95%CI: 369.32 - 476,68 copies/ml | 436 95%CI: 382.32 - 489,68 copies/ml |
| | | Quant Studio 5 | 452 95%CI: 398.32 - 505,68 copies/ml | 431 95%CI: 377.32 - 484,68 copies/ml | 442 95%CI: 388,32- 495,68 copies/ml | 434 95%CI: 380.32 - 487,68 copies/ml |
| | | FLUORITE | 459 95%CI: 405.32 - 512,68 copies/ml | 429 95%CI: 375.32 - 482,68 copies/ml | 452 95%CI: 398.32 - 505,68 copies/ml | 440 95%CI: 386.32 - 493,68 copies/ml |
| | | DTprime | 444 95%CI: 390.32 - 497,68 copies/ml | 420 95%CI: 366.32 - 473,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml | 442 95%CI: 388.32 - 495,68 copies/ml |

| | | | | | | |
|-----------------------------|---------------|-------------------|---|---|---|--|
| HPV-test-21, HPV-test-14 | HPV 45 DNA | CFX 96 | 435 95%CI: 381.32 - 488,68 copies/ml | 430 95%CI: 376.32 - 483,68 copies/ml | 463 95%CI: 409.32 - 516,68 copies/ml | 464 95%CI: 410.32 - 517,68 copies/ml |
| | | Rotor- Gene Q | 456 95%CI: 402.32 -509,68 copies/ml | 428 95%CI: 374.32 - 481,68 copies/ml | 435 95%CI: 381.32 -488,68 copies/ml | 422 95%CI: 368.32 - 475,68 copies/ml |
| | | Quant Studio 5 | 440 95%CI: 386.32 - 493,68 copies/ml | 429 95%CI: 375.32 - 482,68 copies/ml | 465 95%CI: 411.32 - 518,68 copies/ml | 462 95%CI: 408.32 - 515,68 copies/ml |
| | | FLUORITE | 430 95%CI: 376.32 - 483,68 copies/ml | 423 95%CI: 369.32 - 476,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml | 426 95%CI: 372.32 -479,68 copies/ml |
| HPV-test-21, HPV-test-14 | HPV 51 DNA | DTprime | 435 95%CI: 381.32 - 488,68 copies/ml | 429 95%CI: 375.32 - 482,68 copies/ml | 446 95%CI: 392.32 - 499,68 copies/ml | 429 95%CI: 375.32 - 482,68 copies/ml |
| | | CFX 96 | 441 95%CI: 387.32 -494,68 copies/ml | 433 95%CI: 379.32 - 486,68 copies/ml | 451 95%CI: 397.32 - 504,68 copies/ml | 434 95%CI: 380.32 - 487,68 copies/ml |
| | | Rotor- Gene Q | 427 95%CI: 373.32 - 480,68 copies/ml | 427 95%CI: 373.32 - 480,68 copies/ml | 450 95%CI: 396.32 - 503,68 copies/ml | 429 95%CI: 375.32 - 482,68 copies/ml |
| | | Quant Studio 5 | 437 95%CI: 383.32 -490,68 copies/ml | 454 95%CI: 400.32 - 507,68 copies/ml | 453 95%CI: 399.32 - 506,68 copies/ml | 439 95%CI: 385.32 - 492,68 copies/ml |
| | | FLUORITE | 448 95%CI: 394.32 - 501,68 copies/ml | 435 95%CI: 381.32 - 488,68 copies/ml | 441 95%CI: 387.32 - 494,68 copies/ml | 451 95%CI: 397.32 - 504,68 copies/ml |
| HPV-test-21, HPV-test-14 | HPV 52 DNA | DTprime | 421 95%CI: 367.32 - 474,68 copies/ml | 447 95%CI: 393.32 - 500,68 copies/ml | 424 95%CI: 370.32 - 477,68 copies/ml | 430 95%CI: 376.32 - 483,68 copies/ml |
| | | CFX 96 | 446 95%CI: 392.32 - 499,68 copies/ml | 440 95%CI: 386.32 - 493,68 copies/ml | 436 95%CI: 382.32 -489,68 copies/ml | 452 95%CI: 398.32 - 505,68 copies/ml |
| | | Rotor- Gene Q | 429 95%CI: 375.32 - 482,68 copies/ml | 458 95%CI: 404.32 - 511,68 copies/ml | 460 95%CI: 406.32 - 513,68 copies/ml | 427 95%CI: 373.32 - 480,68 copies/ml |
| | | Quant Studio 5 | 438 95%CI: 384.32 - 491,68 copies/ml | 429 95%CI: 375.32 - 482,68 copies/ml | 434 95%CI: 380.32 - 487,68 copies/ml | 448 95%CI: 394.32 - 501,68 copies/ml |

| | | | | | | |
|-------------------------------------|---------------|-------------------|---|---|--|--|
| | | FLUORITE | 428 95%CI: 374.32 - 481,68 copies/ml | 429 95%CI: 375.32 - 482,68 copies/ml | 454 95%CI: 400.32 - 507,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml |
| HPV- test-21, HPV- test-14 | HPV 68 DNA | DTprime | 427 95%CI: 373.32 - 480,68 copies/ml | 428 95%CI: 374.32 - 481,68 copies/ml | 444 95%CI: 390.32 - 497,68 copies/ml | 439 95%CI: 385.32 - 492,68 copies/ml |
| | | CFX 96 | 435 95%CI: 381.32 - 488,68 copies/ml | 443 95%CI: 389.32 - 496,68 copies/ml | 418 95%CI: 364.32 - 471.68 copies/ml | 446 95%CI: 392.32 - 499,68 copies/ml |
| | | Rotor- Gene Q | 440 95%CI: 386.32 - 493,68 copies/ml | 436 95%CI: 382.32 - 489,68 copies/ml | 432 95%CI: 378.32 - 485,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml |
| | | Quant Studio 5 | 435 95%CI: 381.32 - 488,68 copies/ml | 432 95%CI: 378.32 - 485,68 copies/ml | 426 95%CI: 372.32 - 479,68 copies/ml | 453 95%CI: 399.32 - 506,68 copies/ml |
| | | FLUORITE | 438 95%CI: 384.32 - 491,68 copies/ml | 426 95%CI: 372.32 - 479,68 copies/ml | 434 95%CI: 380.32 - 487,68 copies/ml | 425 95%CI: 371.32 - 478,68 copies/ml |
| HPV- test-21, HPV- test-14 | HPV 56 DNA | DTprime | 449 95%CI: 395.32 - 502,68 copies/ml | 460 95%CI: 406.32 - 513,68 copies/ml | 420 95%CI: 366.32 - 473,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml |
| | | CFX 96 | 449 95%CI: 395.32 - 502,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml | 444 95%CI: 390.32 - 497,68 copies/ml | 425 95%CI: 371.32 - 478,68 copies/ml |
| | | Rotor- Gene Q | 439 95%CI: 385.32 - 492,68 copies/ml | 444 95%CI: 390.32 - 497,68 copies/ml | 426 95%CI: 372.32 - 479,68 copies/ml | 464 95%CI: 410.32 - 517,68 copies/ml |
| | | Quant Studio 5 | 456 95%CI: 402.32 - 509,68 copies/ml | 432 95%CI: 378.32 - 485,68 copies/ml | 421 95%CI: 367.32 - 474,68 copies/ml | 459 95%CI: 405.32 - 512,68 copies/ml |
| | | FLUORITE | 462 95%CI: 408.32 - 515,68 copies/ml | 424 95%CI: 370.32 - 477,68 copies/ml | 458 95%CI: 404.32 - 511,68 copies/ml | 417 95%CI: 363.32 - 470,68 copies/ml |
| HPV- test-21, HPV- test-14 | | DTprime | 449 95%CI: 395.32 - 502,68 copies/ml | 460 95%CI: 406.32 - 513,68 copies/ml | 420 95%CI: 366.32 - 473,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml |
| | | CFX 96 | 449 95%CI: 395.32 - 502,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml | 444 95%CI: 390.32 - 497,68 copies/ml | 425 95%CI: 371.32 - 478,68 copies/ml |

| | | | | | | |
|-------------------------------------|---------------|-------------------|---|---|---|--|
| | HPV 58 DNA | Rotor- Gene Q | 439 95%CI: 385.32 - 492,68 copies/ml | 444 95%CI: 390.32 - 497,68 copies/ml | 426 95%CI: 372.32 - 479,68 copies/ml | 464 95%CI: 410.32 - 517.68 copies/ml |
| | | Quant Studio 5 | 456 95%CI: 402.32 - 509,68 copies/ml | 432 95%CI: 378.32 - 485,68 copies/ml | 421 95%CI: 367.32 - 474,68 copies/ml | 459 95%CI: 405.32 - 512,68 copies/ml |
| | | FLUORITE | 462 95%CI: 408.32 - 515,68 copies/ml | 424 95%CI: 370.32 - 477,68 copies/ml | 458 95%CI: 404.32 - 511.68 copies/ml | 417 95%CI: 363.32 - 470.68 copies/ml |
| HPV- test-21, HPV- test-14 | HPV 59 DNA | DTprime | 448 95%CI: 394.32 - 501,68 copies/ml | 426 95%CI: 372.32 - 479,68 copies/ml | 431 95%CI: 377.32 - 484,68 copies/ml | 422 95%CI: 368.32 - 475,68 copies/ml |
| | | CFX 96 | 438 95%CI: 384.32 - 491,68 copies/ml | 432 95%CI: 378.32 - 485,68 copies/ml | 433 95%CI: 379.32 - 486,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml |
| | | Rotor- Gene Q | 428 95%CI: 374.32 - 481,68 copies/ml | 444 95%CI: 390.32 - 497,68 copies/ml | 434 95%CI: 380.32 - 487,68 copies/ml | 432 95%CI: 378.32 - 485,68 copies/ml |
| | | Quant Studio 5 | 425 95%CI: 371.32 - 478,68 copies/ml | 450 95%CI: 396.32 - 503,68 copies/ml | 436 95%CI: 382.32 - 489,68 copies/ml | 448 95%CI: 394.32 - 501,68 copies/ml |
| | | FLUORITE | 439 95%CI: 385.32 - 492,68 copies/ml | 414 95%CI: 360.32 - 467,68 copies/ml | 447 95%CI: 393.32 - 500,68 copies/ml | 431 95%CI: 377.32 - 484,68 copies/ml |
| HPV- test-21, HPV- test-14 | HPV 66 DNA | DTprime | 444 95%CI: 390.32 - 497.68 copies/ml | 446 95%CI: 392.32 - 499,68 copies/ml | 428 95%CI: 374.32 - 481,68 copies/ml | 452 95%CI: 398.32 - 505,68 copies/ml |
| | | CFX 96 | 420 95%CI: 366.32 - 473,68 copies/ml | 453 95%CI: 399.32 - 506,68 copies/ml | 446 95%CI: 392.32 - 499,68 copies/ml | 460 95%CI: 406.32 - 513,68 copies/ml |
| | | Rotor- Gene Q | 439 95%CI: 385.32 - 492.68 copies/ml | 422 95%CI: 368.32 - 475,68 copies/ml | 443 95%CI: 392.32 - 499,68 copies/ml | 449 95%CI: 395.32 - 502,68 copies/ml |
| | | Quant Studio 5 | 440 95%CI: 386.32 - 493,68 copies/ml | 438 95%CI: 384.32 - 491,68 copies/ml | 443 95%CI: 392.32 - 499,68 copies/ml | 453 95%CI: 399.32 - 506,68 copies/ml |
| | | FLUORITE | 435 95%CI: 381.32 - 488,68 copies/ml | 433 95%CI: 379.32 - 486,68 copies/ml | 428 95%CI: 374.32 - 481,68 copies/ml | 442 95%CI: 388.32 - 495,68 copies/ml |
| | | DTprime | 458 95%CI: 404.32 - 511,68 copies/ml | 438 95%CI: 384.32 - 491,68 copies/ml | 424 95%CI: 370.32 - 477.68 copies/ml | 418 95%CI: 364.32 - 471,68 copies/ml |

| | | | | | | |
|-------------|------------|----------------|---|---|---|---|
| HPV-test-21 | HPV 53 DNA | CFX 96 | 456 95%CI: 402.32 - 509,68 copies/ml | 427 95%CI: 373.32 - 480,68 copies/ml | 428 95%CI: 374.32 - 481,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml |
| | | Rotor-Gene Q | 452 95%CI: 398.32 - 505,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml | 445 95%CI: 391.32 - 498,68 copies/ml | 422 95%CI: 368.32 - 475,68 copies/ml |
| | | Quant Studio 5 | 443 95%CI: 389.32 - 496,68 copies/m | 444 95%CI: 390.32 - 497,68 copies/ml | 456 95%CI: 402.32 - 509,68 copies/ml | 458 95%CI: 404.32 - 511,68 copies/ml |
| | | FLUORITE | 425 95%CI: 371.32 - 478,68 copies/ml | 430 95%CI: 376.32 - 483,68 copies/ml | 444 95%CI: 390.32 - 497,68 copies/ml | 456 95%CI: 402.32 - 509,68 copies/ml |
| HPV-test-21 | HPV 73 DNA | DTprime | 427 95%CI: 373.32 - 480,68 copies/ml | 439 95%CI: 385.32 - 492,68 copies/ml | 451 95%CI: 397.32 - 504,68 copies/ml | 422 95%CI: 368.32 - 475,68 copies/ml |
| | | CFX 96 | 454 95%CI: 400.32 - 507,68 copies/ml | 426 95%CI: 372.32 - 479,68 copies/ml | 449 95%CI: 395.32 - 502,68 copies/ml | 438 95%CI: 368.32 - 475,68 copies/ml |
| | | Rotor-Gene Q | 433 95%CI: 379.32 - 486,68 copies/ml | 437 95%CI: 383.32 - 490,68 copies/ml | 456 95%CI: 402.32 - 509,68 copies/ml | 426 95%CI: 372.32 - 479,68 copies/ml |
| | | Quant Studio 5 | 432 95%CI: 378.32 - 485,68 copies/ml | 456 95%CI: 402.32 - 509,68 copies/ml | 424 95%CI: 370.32 - 477,68 copies/ml | 436 95%CI: 382.32 - 489,68 copies/ml |
| | | FLUORITE | 436 95%CI: 382.32 - 489,68 copies/ml | 453 95%CI: 399.32 - 506,68 copies/ml | 450 95%CI: 396.32 - 503,68 copies/ml | 456 95%CI: 402.32 - 509,68 copies/ml |
| HPV-test-21 | HPV 82 DNA | DTprime | 458 95%CI: 404.32 - 511,68 copies/ml | 419 95%CI: 365.32 - 472,68 copies/ml | 432 95%CI: 378.32 - 485,68 copies/ml | 426 95%CI: 372.32 - 479,68 copies/ml |
| | | CFX 96 | 456 95%CI: 402.32 - 509,68 copies/ml | 459 95%CI: 405.32 - 512,68 copies/ml | 427 95%CI: 373.32 - 480,68 copies/ml | 431 95%CI: 377.32 - 484,68 copies/ml |
| | | Rotor-Gene Q | 433 95%CI: 379.32 - 486,68 copies/ml | 434 95%CI: 380.32 - 487,68 copies/ml | 434 95%CI: 380.32 - 487,68 copies/ml | 456 95%CI: 402.32 - 509,68 copies/ml |
| | | Quant Studio 5 | 458 95%CI: 404.32 - 511,68 copies/ml | 438 95%CI: 384.32 - 491,68 copies/ml | 444 95%CI: 390.32 - 497,68 copies/ml | 449 95%CI: 395.32 - 502,68 copies/ml |
| | | FLUORITE | 418 95%CI: 364.32 - 471,68 copies/ml | 432 95%CI: 378.32 - 485,68 copies/ml | 452 95%CI: 398.32 - 505,68 copies/ml | 438 95%CI: 384.32 - 491,68 copies/ml |

4.2.3 Limit of quantitation (LOQ)

In accordance with international recommendations **CLSI EP-17A2**, the limit of quantitation (LOQ) was determined by analyzing **the following standard samples dilutions** in the range of the expected detection limit – 100, 200, 300, 400, 500, 700, 1000 copies/ μ l:

- WHO International Standard "Collection of WHO International standards for human papillomavirus (HPV) DNA genotypes HPV31, HPV33, HPV45, HPV52, HPV58", NIBSC Code: 19/226 (represents a first international standards collection for HPV DNA genotypes HPV31, HPV33, HPV45, HPV52, HPV58 (NIBSC codes 14/258, 14/260, 14/104, 14/262, 14/264 respectively);

- WHO International Standard "Collection of WHO International standards for human papillomavirus (HPV) HPV16, HPV18, HPV6, HPV11 DNA genotypes", NIBSC Code: 19/224 (represents a first international standards collection for HPV HPV16, HPV18, HPV6, HPV11 DNA genotypes (NIBSC codes 06/202, 06/206, 14/256, 14/100, respectively).

Due to the fact that there are no certified pure SS available **in relation to DNA of human papillomavirus (HPV) types 44, 26, 35, 39, 51, 68, 56, 59, 66, 53, 73, 82** so as a retained SS **in relation to DNA of human papillomavirus (HPV) types 44, 26, 35, 39, 51, 68, 56, 59, 66, 53, 73, 82** was used isolated from human biological material test analytes DNA:

| No. | Sample name | DNA level, copies/ml | DNA level, log ₁₀ copies/ml |
|-----|-------------|----------------------|--|
| 1 | SS-HPV44 | 67608 | 4.83 |
| 2 | SS-HPV26 | 231847 | 5.37 |
| 3 | SS-HPV 35 | 8102022 | 6.91 |
| 4 | SS-HPV 39 | 11406 | 4.06 |
| 5 | SS-HPV 51 | 1068111 | 6.03 |
| 6 | SS-HPV 68 | 1318281 | 6.12 |
| 7 | SS-HPV 56 | 53342 | 4.73 |
| 8 | SS-HPV 59 | 2628447 | 6.42 |
| 9 | SS-HPV 66 | 265880 | 5.42 |
| 10 | SS-HPV 53 | 3323253 | 6.52 |
| 11 | SS-HPV 73 | 174590 | 5.24 |
| 12 | SS-HPV 82 | 95912 | 4.98 |

Assay results are shown in Table 9.

Table 9

| Target analyte (HPV type) | Isolation kit | Cycler | HPV DNA concentrations (LOD) with 95% confidence probability | | Confidence interval with 95% confidence probability | |
|---------------------------|---------------|----------------|--|-----------|---|----------------------------|
| | | | log10 copies/ml | copies/ml | | |
| HPV 6 DNA | NA-EXTRA | Dtprime | 2.872 | 745 | 95%CI: 673 – 816 copies/ml | |
| | | CFX 96 | 2.895 | 785 | 95%CI: 713 – 856 copies/ml | |
| | | Rotor-Gene Q | 2.882 | 762 | 95%CI: 690 – 833 copies/ml | |
| | | Quant Studio 5 | 2.882 | 762 | 95%CI: 690 – 833 copies/ml | |
| | | FLUORITE | 2.877 | 753 | 95%CI: 681 – 824 copies/ml | |
| | DNA-sorb-B | Dtprime | 2.887 | 770 | 95%CI: 698 – 841 copies/ml | |
| | | CFX 96 | 2.877 | 753 | 95%CI: 681 – 824 copies/ml | |
| | | Rotor-Gene Q | 2.886 | 770 | 95%CI: 698 – 841 copies/ml | |
| | | Quant Studio 5 | 2.864 | 730 | 95%CI: 658 – 801 copies/ml | |
| | PREP-NA-PLUS | FLUORITE | 2.881 | 761 | 95%CI: 689 – 832 copies/ml | |
| | | Dtprime | 2.907 | 807 | 95%CI: 735 – 878 copies/ml | |
| | | CFX 96 | 2.903 | 800 | 95%CI: 728 – 871 copies/ml | |
| | | Rotor-Gene Q | 2.869 | 739 | 95%CI: 667 – 810 copies/ml | |
| | | Quant Studio 5 | 2.879 | 757 | 95%CI: 685 – 828 copies/ml | |
| | HPV 11 DNA | NA-EXTRA | FLUORITE | 2.927 | 845 | 95%CI: 773 – 916 copies/ml |
| | | | Dtprime | 2.870 | 741 | 95%CI: 669 – 812 copies/ml |
| CFX 96 | | | 2.898 | 790 | 95%CI: 718 – 861 copies/ml | |
| Rotor-Gene Q | | | 2.861 | 726 | 95%CI: 654 – 797 copies/ml | |
| Quant Studio 5 | | | 2.881 | 760 | 95%CI: 688 – 831 copies/ml | |
| DNA-sorb-B | | FLUORITE | 2.912 | 817 | 95%CI: 745 – 888 copies/ml | |
| | | Dtprime | 2.875 | 751 | 95%CI: 679 – 822 copies/ml | |
| | | CFX 96 | 2.886 | 768 | 95%CI: 609 – 752 copies/ml | |
| | | Rotor-Gene Q | 2.906 | 805 | 95%CI: 733 – 876 copies/ml | |
| | | Quant Studio 5 | 2.873 | 746 | 95%CI: 674 – 817 copies/ml | |
| | | FLUORITE | 2.910 | 812 | 95%CI: 740 – 883 copies/ml | |
| PREP-NA-PLUS | | Dtprime | 2.857 | 719 | 95%CI: 647 – 790 copies/ml | |
| | | CFX 96 | 2.900 | 794 | 95%CI: 722 – 865 copies/ml | |
| | | Rotor-Gene Q | 2.871 | 743 | 95%CI: 671 – 814 copies/ml | |
| | | Quant Studio 5 | 2.889 | 775 | 95%CI: 703 – 846 copies/ml | |
| | | | FLUORITE | 2.910 | 812 | 95%CI: 740 – 883 copies/ml |

| | | | | | |
|---------------|------------------|----------------|-------|-----|----------------------------|
| HPV 44 DNA | NA- EXTRA | Dtprime | 2.907 | 807 | 95%CI: 735 – 878 copies/ml |
| | | CFX 96 | 2.903 | 800 | 95%CI: 728 – 871 copies/ml |
| | | Rotor-Gene Q | 2.869 | 739 | 95%CI: 667 – 810 copies/ml |
| | | Quant Studio 5 | 2.879 | 757 | 95%CI: 685 – 828 copies/ml |
| | | FLUORITE | 2.927 | 845 | 95%CI: 773 – 916 copies/ml |
| | DNA- sorb-B | Dtprime | 2.913 | 819 | 95%CI: 747 – 890 copies/ml |
| | | CFX 96 | 2.873 | 746 | 95%CI: 674 – 817 copies/ml |
| | | Rotor-Gene Q | 2.892 | 779 | 95%CI: 707 – 850 copies/ml |
| | | Quant Studio 5 | 2.888 | 773 | 95%CI: 701 – 844 copies/ml |
| | | FLUORITE | 2.874 | 749 | 95%CI: 677 – 820 copies/ml |
| | PREP-NA- PLUS | Dtprime | 2.933 | 858 | 95%CI: 786 – 929 copies/ml |
| | | CFX 96 | 2.864 | 732 | 95%CI: 660 – 803 copies/ml |
| | | Rotor-Gene Q | 2.871 | 742 | 95%CI: 670 – 813 copies/ml |
| | | Quant Studio 5 | 2.895 | 785 | 95%CI: 713 – 856 copies/ml |
| | | FLUORITE | 2.879 | 757 | 95%CI: 685 – 828 copies/ml |
| HPV 16 DNA | NA- EXTRA | Dtprime | 2.857 | 719 | 95%CI: 647 – 790 copies/ml |
| | | CFX 96 | 2.900 | 794 | 95%CI: 722 – 865 copies/ml |
| | | Rotor-Gene Q | 2.871 | 743 | 95%CI: 671 – 814 copies/ml |
| | | Quant Studio 5 | 2.889 | 775 | 95%CI: 703 – 846 copies/ml |
| | | FLUORITE | 2.910 | 812 | 95%CI: 740 – 883 copies/ml |
| | DNA- sorb-B | Dtprime | 2.875 | 749 | 95%CI: 677 – 820 copies/ml |
| | | CFX 96 | 2.924 | 840 | 95%CI: 768 – 911 copies/ml |
| | | Rotor-Gene Q | 2.862 | 728 | 95%CI: 656 – 799 copies/ml |
| | | Quant Studio 5 | 2.841 | 693 | 95%CI: 621 – 764 copies/ml |
| | | FLUORITE | 2.872 | 745 | 95%CI: 673 – 816 copies/ml |
| | PREP-NA- PLUS | Dtprime | 2.855 | 715 | 95%CI: 643 – 786 copies/ml |
| | | CFX 96 | 2.867 | 736 | 95%CI: 664 – 807 copies/ml |
| | | Rotor-Gene Q | 2.901 | 797 | 95%CI: 725 – 868 copies/ml |
| | | Quant Studio 5 | 2.905 | 804 | 95%CI: 732 – 875 copies/ml |
| | | FLUORITE | 2.900 | 794 | 95%CI: 722 – 865 copies/ml |
| HPV 18 DNA | NA- EXTRA | Dtprime | 2.933 | 858 | 95%CI: 786 – 929 copies/ml |
| | | CFX 96 | 2.864 | 732 | 95%CI: 660 – 803 copies/ml |
| | | Rotor-Gene Q | 2.871 | 742 | 95%CI: 670 – 813 copies/ml |
| | | Quant Studio 5 | 2.895 | 785 | 95%CI: 713 – 856 copies/ml |
| | | FLUORITE | 2.879 | 757 | 95%CI: 685 – 828 copies/ml |
| | DNA- sorb-B | Dtprime | 2.905 | 803 | 95%CI: 731 – 874 copies/ml |
| | | CFX 96 | 2.848 | 705 | 95%CI: 633 – 776 copies/ml |
| | | Rotor-Gene Q | 2.867 | 735 | 95%CI: 663 – 806 copies/ml |
| | | Quant Studio 5 | 2.866 | 735 | 95%CI: 663 – 806 copies/ml |
| | | FLUORITE | 2.896 | 787 | 95%CI: 715 – 858 copies/ml |
| | PREP-NA- PLUS | Dtprime | 2.866 | 735 | 95%CI: 663 – 806 copies/ml |
| | | CFX 96 | 2.854 | 715 | 95%CI: 643 – 786 copies/ml |
| | | Rotor-Gene Q | 2.860 | 724 | 95%CI: 652 – 795 copies/ml |
| | | Quant Studio 5 | 2.884 | 766 | 95%CI: 694 – 837 copies/ml |
| | | FLUORITE | 2.857 | 720 | 95%CI: 648 – 791 copies/ml |

| | | | | | |
|------------------|------------------|----------------|---------|-------|----------------------------|
| HPV 26 DNA | NA- EXTRA | Dtprime | 2.855 | 715 | 95%CI: 643 – 786 copies/ml |
| | | CFX 96 | 2.867 | 736 | 95%CI: 664 – 807 copies/ml |
| | | Rotor-Gene Q | 2.901 | 797 | 95%CI: 725 – 868 copies/ml |
| | | Quant Studio 5 | 2.905 | 804 | 95%CI: 732 – 875 copies/ml |
| | | FLUORITE | 2.900 | 794 | 95%CI: 722 – 865 copies/ml |
| | DNA- sorb-B | Dtprime | 2.900 | 794 | 95%CI: 722 – 865 copies/ml |
| | | CFX 96 | 2.876 | 751 | 95%CI: 679 – 822 copies/ml |
| | | Rotor-Gene Q | 2.904 | 802 | 95%CI: 730 – 873 copies/ml |
| | | Quant Studio 5 | 2.888 | 773 | 95%CI: 701 – 844 copies/ml |
| | | FLUORITE | 2.905 | 804 | 95%CI: 732 – 875 copies/ml |
| | PREP-NA- PLUS | Dtprime | 2.941 | 872 | 95%CI: 800 – 943 copies/ml |
| | | CFX 96 | 2.888 | 772 | 95%CI: 700 – 843 copies/ml |
| | | Rotor-Gene Q | 2.881 | 761 | 95%CI: 689 – 832 copies/ml |
| | | Quant Studio 5 | 2.911 | 815 | 95%CI: 743 – 886 copies/ml |
| | | FLUORITE | 2.869 | 739 | 95%CI: 667 – 810 copies/ml |
| | HPV 31 DNA | NA- EXTRA | Dtprime | 2.872 | 745 |
| CFX 96 | | | 2.895 | 785 | 95%CI: 713 – 856 copies/ml |
| Rotor-Gene Q | | | 2.882 | 762 | 95%CI: 690 – 833 copies/ml |
| Quant Studio 5 | | | 2.882 | 762 | 95%CI: 690 – 833 copies/ml |
| FLUORITE | | | 2.877 | 753 | 95%CI: 681 – 824 copies/ml |
| DNA- sorb-B | | Dtprime | 2.887 | 770 | 95%CI: 698 – 841 copies/ml |
| | | CFX 96 | 2.877 | 753 | 95%CI: 681 – 824 copies/ml |
| | | Rotor-Gene Q | 2.886 | 770 | 95%CI: 698 – 841 copies/ml |
| | | Quant Studio 5 | 2.864 | 730 | 95%CI: 658 – 801 copies/ml |
| | | FLUORITE | 2.881 | 761 | 95%CI: 689 – 832 copies/ml |
| PREP-NA- PLUS | | Dtprime | 2.907 | 807 | 95%CI: 735 – 878 copies/ml |
| | | CFX 96 | 2.903 | 800 | 95%CI: 728 – 871 copies/ml |
| | | Rotor-Gene Q | 2.869 | 739 | 95%CI: 667 – 810 copies/ml |
| | | Quant Studio 5 | 2.879 | 757 | 95%CI: 685 – 828 copies/ml |
| | | FLUORITE | 2.927 | 845 | 95%CI: 773 – 916 copies/ml |
| HPV 33 DNA | | NA- EXTRA | Dtprime | 2.870 | 741 |
| | CFX 96 | | 2.898 | 790 | 95%CI: 718 – 861 copies/ml |
| | Rotor-Gene Q | | 2.861 | 726 | 95%CI: 654 – 797 copies/ml |
| | Quant Studio 5 | | 2.881 | 760 | 95%CI: 688 – 831 copies/ml |
| | FLUORITE | | 2.912 | 817 | 95%CI: 745 – 888 copies/ml |
| | DNA- sorb-B | Dtprime | 2.875 | 751 | 95%CI: 679 – 822 copies/ml |
| | | CFX 96 | 2.886 | 768 | 95%CI: 609 – 752 copies/ml |
| | | Rotor-Gene Q | 2.906 | 805 | 95%CI: 733 – 876 copies/ml |
| | | Quant Studio 5 | 2.873 | 746 | 95%CI: 674 – 817 copies/ml |
| | | FLUORITE | 2.910 | 812 | 95%CI: 740 – 883 copies/ml |
| | PREP- NA-PLUS | Dtprime | 2.857 | 719 | 95%CI: 647 – 790 copies/ml |
| | | CFX 96 | 2.900 | 794 | 95%CI: 722 – 865 copies/ml |
| | | Rotor-Gene Q | 2.871 | 743 | 95%CI: 671 – 814 copies/ml |
| | | Quant Studio 5 | 2.889 | 775 | 95%CI: 703 – 846 copies/ml |
| | | FLUORITE | 2.910 | 812 | 95%CI: 740 – 883 copies/ml |

| | | | | | |
|---------------|------------------|----------------|-------|-----------------------------|-----------------------------|
| HPV 35 DNA | NA- EXTRA | Dtprime | 2.907 | 807 | 95% CI: 735 – 878 copies/ml |
| | | CFX 96 | 2.903 | 800 | 95% CI: 728 – 871 copies/ml |
| | | Rotor-Gene Q | 2.869 | 739 | 95% CI: 667 – 810 copies/ml |
| | | Quant Studio 5 | 2.879 | 757 | 95% CI: 685 – 828 copies/ml |
| | | FLUORITE | 2.927 | 845 | 95% CI: 773 – 916 copies/ml |
| | DNA- sorb-B | Dtprime | 2.913 | 819 | 95% CI: 747– 890 copies/ml |
| | | CFX 96 | 2.873 | 746 | 95% CI: 674 – 817 copies/ml |
| | | Rotor-Gene Q | 2.892 | 779 | 95% CI: 707 – 850 copies/ml |
| | | Quant Studio 5 | 2.888 | 773 | 95% CI: 701 – 844 copies/ml |
| | | FLUORITE | 2.874 | 749 | 95% CI: 677 – 820 copies/ml |
| | PREP-NA- PLUS | Dtprime | 2.933 | 858 | 95% CI: 786 – 929 copies/ml |
| | | CFX 96 | 2.864 | 732 | 95% CI: 660 – 803 copies/ml |
| | | Rotor-Gene Q | 2.871 | 742 | 95% CI: 670 – 813 copies/ml |
| | | Quant Studio 5 | 2.895 | 785 | 95% CI: 713 – 856 copies/ml |
| FLUORITE | | 2.879 | 757 | 95% CI: 685 – 828 copies/ml | |
| HPV 39 DNA | NA- EXTRA | Dtprime | 2.857 | 719 | 95% CI: 647 – 790 copies/ml |
| | | CFX 96 | 2.900 | 794 | 95% CI: 722 – 865 copies/ml |
| | | Rotor-Gene Q | 2.871 | 743 | 95% CI: 671 – 814 copies/ml |
| | | Quant Studio 5 | 2.889 | 775 | 95% CI: 703 – 846 copies/ml |
| | | FLUORITE | 2.910 | 812 | 95% CI: 740 – 883 copies/ml |
| | DNA- sorb-B | Dtprime | 2.875 | 749 | 95% CI: 677 – 820 copies/ml |
| | | CFX 96 | 2.924 | 840 | 95% CI: 768 – 911 copies/ml |
| | | Rotor-Gene Q | 2.862 | 728 | 95% CI: 656 – 799 copies/ml |
| | | Quant Studio 5 | 2.841 | 693 | 95% CI: 621 – 764 copies/ml |
| | | FLUORITE | 2.872 | 745 | 95% CI: 673 – 816 copies/ml |
| | PREP-NA- PLUS | Dtprime | 2.855 | 715 | 95% CI: 643 – 786 copies/ml |
| | | CFX 96 | 2.867 | 736 | 95% CI: 664 – 807 copies/ml |
| | | Rotor-Gene Q | 2.901 | 797 | 95% CI: 725 – 868 copies/ml |
| | | Quant Studio 5 | 2.905 | 804 | 95% CI: 732 – 875 copies/ml |
| FLUORITE | | 2.900 | 794 | 95% CI: 722 – 865 copies/ml | |
| HPV 45 DNA | NA- EXTRA | Dtprime | 2.933 | 858 | 95% CI: 786 – 929 copies/ml |
| | | CFX 96 | 2.864 | 732 | 95% CI: 660 – 803 copies/ml |
| | | Rotor-Gene Q | 2.871 | 742 | 95% CI: 670 – 813 copies/ml |
| | | Quant Studio 5 | 2.895 | 785 | 95% CI: 713 – 856 copies/ml |
| | | FLUORITE | 2.879 | 757 | 95% CI: 685 – 828 copies/ml |
| | DNA- sorb-B | Dtprime | 2.905 | 803 | 95% CI: 731– 874 copies/ml |
| | | CFX 96 | 2.848 | 705 | 95% CI: 633 – 776 copies/ml |
| | | Rotor-Gene Q | 2.867 | 735 | 95% CI: 663 – 806 copies/ml |
| | | Quant Studio 5 | 2.866 | 735 | 95% CI: 663 – 806 copies/ml |
| | | FLUORITE | 2.896 | 787 | 95% CI: 715 – 858 copies/ml |
| | PREP-NA- PLUS | Dtprime | 2.866 | 735 | 95% CI: 663 – 806 copies/ml |
| | | CFX 96 | 2.854 | 715 | 95% CI: 643 – 786 copies/ml |
| | | Rotor-Gene Q | 2.860 | 724 | 95% CI: 652 – 795 copies/ml |
| | | Quant Studio 5 | 2.884 | 766 | 95% CI: 694 – 837 copies/ml |
| FLUORITE | | 2.857 | 720 | 95% CI: 648 – 791 copies/ml | |

| | | | | | | |
|----------------|------------------|------------------|--------------|----------------------------|----------------------------|----------------------------|
| HPV 51 DNA | NA- EXTRA | Dtprime | 2.855 | 715 | 95%CI: 643 – 786 copies/ml | |
| | | CFX 96 | 2.867 | 736 | 95%CI: 664 – 807 copies/ml | |
| | | Rotor-Gene Q | 2.901 | 797 | 95%CI: 725 – 868 copies/ml | |
| | | Quant Studio 5 | 2.905 | 804 | 95%CI: 732 – 875 copies/ml | |
| | | FLUORITE | 2.900 | 794 | 95%CI: 722 – 865 copies/ml | |
| | | Dtprime | 2.900 | 794 | 95%CI: 722 – 865 copies/ml | |
| HPV 52 DNA | DNA- sorb-B | CFX 96 | 2.876 | 751 | 95%CI: 679 – 822 copies/ml | |
| | | Rotor-Gene Q | 2.904 | 802 | 95%CI: 730 – 873 copies/ml | |
| | | Quant Studio 5 | 2.888 | 773 | 95%CI: 701 – 844 copies/ml | |
| | | FLUORITE | 2.905 | 804 | 95%CI: 732 – 875 copies/ml | |
| | PREP-NA- PLUS | Dtprime | 2.941 | 872 | 95%CI: 800 – 943 copies/ml | |
| | | CFX 96 | 2.888 | 772 | 95%CI: 700 – 843 copies/ml | |
| | | Rotor-Gene Q | 2.881 | 761 | 95%CI: 689 – 832 copies/ml | |
| | | Quant Studio 5 | 2.911 | 815 | 95%CI: 743 – 886 copies/ml | |
| | HPV 52 DNA | NA- EXTRA | FLUORITE | 2.869 | 739 | 95%CI: 667 – 810 copies/ml |
| | | | Dtprime | 2.872 | 745 | 95%CI: 673 – 816 copies/ml |
| | | | CFX 96 | 2.895 | 785 | 95%CI: 713 – 856 copies/ml |
| | | | Rotor-Gene Q | 2.882 | 762 | 95%CI: 690 – 833 copies/ml |
| Quant Studio 5 | | | 2.882 | 762 | 95%CI: 690 – 833 copies/ml | |
| DNA- sorb-B | | FLUORITE | 2.877 | 753 | 95%CI: 681 – 824 copies/ml | |
| | | Dtprime | 2.887 | 770 | 95%CI: 698 – 841 copies/ml | |
| | | CFX 96 | 2.877 | 753 | 95%CI: 681 – 824 copies/ml | |
| | | Rotor-Gene Q | 2.886 | 770 | 95%CI: 698 – 841 copies/ml | |
| | | Quant Studio 5 | 2.864 | 730 | 95%CI: 658 – 801 copies/ml | |
| | | FLUORITE | 2.881 | 761 | 95%CI: 689 – 832 copies/ml | |
| | | PREP-NA- PLUS | Dtprime | 2.907 | 807 | 95%CI: 735 – 878 copies/ml |
| CFX 96 | 2.903 | | 800 | 95%CI: 728 – 871 copies/ml | | |
| Rotor-Gene Q | 2.869 | | 739 | 95%CI: 667 – 810 copies/ml | | |
| Quant Studio 5 | 2.879 | | 757 | 95%CI: 685 – 828 copies/ml | | |
| FLUORITE | 2.927 | | 845 | 95%CI: 773 – 916 copies/ml | | |
| HPV 68 DNA | NA- EXTRA | Dtprime | 2.870 | 741 | 95%CI: 669 – 812 copies/ml | |
| | | CFX 96 | 2.898 | 790 | 95%CI: 718 – 861 copies/ml | |
| | | Rotor-Gene Q | 2.861 | 726 | 95%CI: 654 – 797 copies/ml | |
| | | Quant Studio 5 | 2.881 | 760 | 95%CI: 688 – 831 copies/ml | |
| | | FLUORITE | 2.912 | 817 | 95%CI: 745 – 888 copies/ml | |
| | DNA- sorb-B | Dtprime | 2.875 | 751 | 95%CI: 679 – 822 copies/ml | |
| | | CFX 96 | 2.886 | 768 | 95%CI: 609 – 752 copies/ml | |
| | | Rotor-Gene Q | 2.906 | 805 | 95%CI: 733 – 876 copies/ml | |
| | | Quant Studio 5 | 2.873 | 746 | 95%CI: 674 – 817 copies/ml | |
| | | FLUORITE | 2.910 | 812 | 95%CI: 740 – 883 copies/ml | |
| | PREP-NA- PLUS | Dtprime | 2.857 | 719 | 95%CI: 647 – 790 copies/ml | |
| | | CFX 96 | 2.900 | 794 | 95%CI: 722 – 865 copies/ml | |
| | | Rotor-Gene Q | 2.871 | 743 | 95%CI: 671 – 814 copies/ml | |
| | | Quant Studio 5 | 2.889 | 775 | 95%CI: 703 – 846 copies/ml | |
| | | FLUORITE | 2.910 | 812 | 95%CI: 740 – 883 copies/ml | |

| | | | | | |
|------------|--------------|----------------|-------|-----|----------------------------|
| HPV 56 DNA | NA-EXTRA | Dtprime | 2.907 | 807 | 95%CI: 735 – 878 copies/ml |
| | | CFX 96 | 2.903 | 800 | 95%CI: 728 – 871 copies/ml |
| | | Rotor-Gene Q | 2.869 | 739 | 95%CI: 667 – 810 copies/ml |
| | | Quant Studio 5 | 2.879 | 757 | 95%CI: 685 – 828 copies/ml |
| | | FLUORITE | 2.927 | 845 | 95%CI: 773 – 916 copies/ml |
| | DNA-sorb-B | Dtprime | 2.913 | 819 | 95%CI: 747 – 890 copies/ml |
| | | CFX 96 | 2.873 | 746 | 95%CI: 674 – 817 copies/ml |
| | | Rotor-Gene Q | 2.892 | 779 | 95%CI: 707 – 850 copies/ml |
| | | Quant Studio 5 | 2.888 | 773 | 95%CI: 701 – 844 copies/ml |
| | | FLUORITE | 2.874 | 749 | 95%CI: 677 – 820 copies/ml |
| | PREP-NA-PLUS | Dtprime | 2.933 | 858 | 95%CI: 786 – 929 copies/ml |
| | | CFX 96 | 2.864 | 732 | 95%CI: 660 – 803 copies/ml |
| | | Rotor-Gene Q | 2.871 | 742 | 95%CI: 670 – 813 copies/ml |
| | | Quant Studio 5 | 2.895 | 785 | 95%CI: 713 – 856 copies/ml |
| | | FLUORITE | 2.879 | 757 | 95%CI: 685 – 828 copies/ml |
| HPV 58 DNA | NA-EXTRA | Dtprime | 2.857 | 719 | 95%CI: 647 – 790 copies/ml |
| | | CFX 96 | 2.900 | 794 | 95%CI: 722 – 865 copies/ml |
| | | Rotor-Gene Q | 2.871 | 743 | 95%CI: 671 – 814 copies/ml |
| | | Quant Studio 5 | 2.889 | 775 | 95%CI: 703 – 846 copies/ml |
| | | FLUORITE | 2.910 | 812 | 95%CI: 740 – 883 copies/ml |
| | DNA-sorb-B | Dtprime | 2.875 | 749 | 95%CI: 677 – 820 copies/ml |
| | | CFX 96 | 2.924 | 840 | 95%CI: 768 – 911 copies/ml |
| | | Rotor-Gene Q | 2.862 | 728 | 95%CI: 656 – 799 copies/ml |
| | | Quant Studio 5 | 2.841 | 693 | 95%CI: 621 – 764 copies/ml |
| | | FLUORITE | 2.872 | 745 | 95%CI: 673 – 816 copies/ml |
| | PREP-NA-PLUS | Dtprime | 2.855 | 715 | 95%CI: 643 – 786 copies/ml |
| | | CFX 96 | 2.867 | 736 | 95%CI: 664 – 807 copies/ml |
| | | Rotor-Gene Q | 2.901 | 797 | 95%CI: 725 – 868 copies/ml |
| | | Quant Studio 5 | 2.905 | 804 | 95%CI: 732 – 875 copies/ml |
| | | FLUORITE | 2.900 | 794 | 95%CI: 722 – 865 copies/ml |
| HPV 59 DNA | NA-EXTRA | Dtprime | 2.933 | 858 | 95%CI: 786 – 929 copies/ml |
| | | CFX 96 | 2.864 | 732 | 95%CI: 660 – 803 copies/ml |
| | | Rotor-Gene Q | 2.871 | 742 | 95%CI: 670 – 813 copies/ml |
| | | Quant Studio 5 | 2.895 | 785 | 95%CI: 713 – 856 copies/ml |
| | | FLUORITE | 2.879 | 757 | 95%CI: 685 – 828 copies/ml |
| | DNA-sorb-B | Dtprime | 2.905 | 803 | 95%CI: 731 – 874 copies/ml |
| | | CFX 96 | 2.848 | 705 | 95%CI: 633 – 776 copies/ml |
| | | Rotor-Gene Q | 2.867 | 735 | 95%CI: 663 – 806 copies/ml |
| | | Quant Studio 5 | 2.866 | 735 | 95%CI: 663 – 806 copies/ml |
| | | FLUORITE | 2.896 | 787 | 95%CI: 715 – 858 copies/ml |
| | PREP-NA-PLUS | Dtprime | 2.866 | 735 | 95%CI: 663 – 806 copies/ml |
| | | CFX 96 | 2.854 | 715 | 95%CI: 643 – 786 copies/ml |
| | | Rotor-Gene Q | 2.860 | 724 | 95%CI: 652 – 795 copies/ml |
| | | Quant Studio 5 | 2.884 | 766 | 95%CI: 694 – 837 copies/ml |
| | | FLUORITE | 2.857 | 720 | 95%CI: 648 – 791 copies/ml |

| | | | | | |
|---------------|------------------|----------------|-------|-----|----------------------------|
| HPV 66 DNA | NA- EXTRA | Dtprime | 2.855 | 715 | 95%CI: 643 – 786 copies/ml |
| | | CFX 96 | 2.867 | 736 | 95%CI: 664 – 807 copies/ml |
| | | Rotor-Gene Q | 2.901 | 797 | 95%CI: 725 – 868 copies/ml |
| | | Quant Studio 5 | 2.905 | 804 | 95%CI: 732 – 875 copies/ml |
| | | FLUORITE | 2.900 | 794 | 95%CI: 722 – 865 copies/ml |
| | DNA- sorb-B | Dtprime | 2.900 | 794 | 95%CI: 722 – 865 copies/ml |
| | | CFX 96 | 2.876 | 751 | 95%CI: 679 – 822 copies/ml |
| | | Rotor-Gene Q | 2.904 | 802 | 95%CI: 730 – 873 copies/ml |
| | | Quant Studio 5 | 2.888 | 773 | 95%CI: 701 – 844 copies/ml |
| | | FLUORITE | 2.905 | 804 | 95%CI: 732 – 875 copies/ml |
| | PREP-NA- PLUS | Dtprime | 2.941 | 872 | 95%CI: 800 – 943 copies/ml |
| | | CFX 96 | 2.888 | 772 | 95%CI: 700 – 843 copies/ml |
| | | Rotor-Gene Q | 2.881 | 761 | 95%CI: 689 – 832 copies/ml |
| | | Quant Studio 5 | 2.911 | 815 | 95%CI: 743 – 886 copies/ml |
| | | FLUORITE | 2.869 | 739 | 95%CI: 667 – 810 copies/ml |
| HPV 53 DNA | NA- EXTRA | Dtprime | 2.872 | 745 | 95%CI: 673 – 816 copies/ml |
| | | CFX 96 | 2.895 | 785 | 95%CI: 713 – 856 copies/ml |
| | | Rotor-Gene Q | 2.882 | 762 | 95%CI: 690 – 833 copies/ml |
| | | Quant Studio 5 | 2.882 | 762 | 95%CI: 690 – 833 copies/ml |
| | | FLUORITE | 2.877 | 753 | 95%CI: 681 – 824 copies/ml |
| | DNA- sorb-B | Dtprime | 2.887 | 770 | 95%CI: 698 – 841 copies/ml |
| | | CFX 96 | 2.877 | 753 | 95%CI: 681 – 824 copies/ml |
| | | Rotor-Gene Q | 2.886 | 770 | 95%CI: 698 – 841 copies/ml |
| | | Quant Studio 5 | 2.864 | 730 | 95%CI: 658 – 801 copies/ml |
| | | FLUORITE | 2.881 | 761 | 95%CI: 689 – 832 copies/ml |
| | PREP-NA- PLUS | Dtprime | 2.907 | 807 | 95%CI: 735 – 878 copies/ml |
| | | CFX 96 | 2.903 | 800 | 95%CI: 728 – 871 copies/ml |
| | | Rotor-Gene Q | 2.869 | 739 | 95%CI: 667 – 810 copies/ml |
| | | Quant Studio 5 | 2.879 | 757 | 95%CI: 685 – 828 copies/ml |
| | | FLUORITE | 2.927 | 845 | 95%CI: 773 – 916 copies/ml |
| HPV 73 DNA | NA- EXTRA | Dtprime | 2.870 | 741 | 95%CI: 669 – 812 copies/ml |
| | | CFX 96 | 2.898 | 790 | 95%CI: 718 – 861 copies/ml |
| | | Rotor-Gene Q | 2.861 | 726 | 95%CI: 654 – 797 copies/ml |
| | | Quant Studio 5 | 2.881 | 760 | 95%CI: 688 – 831 copies/ml |
| | | FLUORITE | 2.912 | 817 | 95%CI: 745 – 888 copies/ml |
| | DNA- sorb-B | Dtprime | 2.875 | 751 | 95%CI: 679 – 822 copies/ml |
| | | CFX 96 | 2.886 | 768 | 95%CI: 609 – 752 copies/ml |
| | | Rotor-Gene Q | 2.906 | 805 | 95%CI: 733 – 876 copies/ml |
| | | Quant Studio 5 | 2.873 | 746 | 95%CI: 674 – 817 copies/ml |
| | | FLUORITE | 2.910 | 812 | 95%CI: 740 – 883 copies/ml |
| | PREP-NA- PLUS | Dtprime | 2.857 | 719 | 95%CI: 647 – 790 copies/ml |
| | | CFX 96 | 2.900 | 794 | 95%CI: 722 – 865 copies/ml |
| | | Rotor-Gene Q | 2.871 | 743 | 95%CI: 671 – 814 copies/ml |
| | | Quant Studio 5 | 2.889 | 775 | 95%CI: 703 – 846 copies/ml |
| | | FLUORITE | 2.910 | 812 | 95%CI: 740 – 883 copies/ml |

| | | | | | |
|---------------|------------------|----------------|-------|-----|----------------------------|
| HPV 82 DNA | NA- EXTRA | Dtprime | 2.907 | 807 | 95%CI: 735 – 878 copies/ml |
| | | CFX 96 | 2.903 | 800 | 95%CI: 728 – 871 copies/ml |
| | | Rotor-Gene Q | 2.869 | 739 | 95%CI: 667 – 810 copies/ml |
| | | Quant Studio 5 | 2.879 | 757 | 95%CI: 685 – 828 copies/ml |
| | | FLUORITE | 2.927 | 845 | 95%CI: 773 – 916 copies/ml |
| | DNA- sorb-B | Dtprime | 2.913 | 819 | 95%CI: 747- 890 copies/ml |
| | | CFX 96 | 2.873 | 746 | 95%CI: 674 – 817 copies/ml |
| | | Rotor-Gene Q | 2.892 | 779 | 95%CI: 707 – 850 copies/ml |
| | | Quant Studio 5 | 2.888 | 773 | 95%CI: 701 – 844 copies/ml |
| | | FLUORITE | 2.874 | 749 | 95%CI: 677 – 820 copies/ml |
| | PREP-NA- PLUS | Dtprime | 2.933 | 858 | 95%CI: 786 – 929 copies/ml |
| | | CFX 96 | 2.864 | 732 | 95%CI: 660 – 803 copies/ml |
| | | Rotor-Gene Q | 2.871 | 742 | 95%CI: 670 – 813 copies/ml |
| | | Quant Studio 5 | 2.895 | 785 | 95%CI: 713 – 856 copies/ml |
| | | FLUORITE | 2.879 | 757 | 95%CI: 685 – 828 copies/ml |

4.2.4 Linear measurement range

HPV-test reagent kit linearity was determined by testing a series of dilutions, which included 10 panel samples covering the expected linear range. Panel samples were prepared by diluting the **following standard samples**:

- WHO International Standard "Collection of WHO International standards for human papillomavirus (HPV) DNA genotypes HPV31, HPV33, HPV45, HPV52, HPV58", NIBSC Code: 19/226 (represents a first international standards collection for HPV DNA genotypes HPV31, HPV33, HPV45, HPV52, HPV58 (NIBSC codes 14/258, 14/260, 14/104, 14/262, 14/264 respectively);

- WHO International Standard "Collection of WHO International standards for human papillomavirus (HPV) HPV16, HPV18, HPV6, HPV11 DNA genotypes", NIBSC Code: 19/224 (represents a first international standards collection for HPV HPV16, HPV18, HPV6, HPV11 DNA genotypes (NIBSC codes 06/202, 06/206, 14/256, 14/100, respectively).

Due to the fact that there are no certified pure SS available **in relation to DNA of human papillomavirus (HPV) types 44, 26, 35, 39, 51, 68, 56, 59, 66, 53, 73, 82** so as a retained SS **in relation to DNA of human papillomavirus (HPV) types 44, 26, 35, 39, 51, 68, 56, 59, 66, 53, 73, 82** was used isolated from human biological material test analytes DNA:

| No. | Sample name | DNA level, copies/ml | DNA level, log ₁₀ copies/ml |
|-----|-------------|----------------------|--|
| 1 | SS-HPV44 | 67608 | 4.83 |
| 2 | SS-HPV26 | 231847 | 5.37 |
| 3 | SS-HPV 35 | 8102022 | 6.91 |
| 4 | SS-HPV 39 | 11406 | 4.06 |
| 5 | SS-HPV 51 | 1068111 | 6.03 |
| 6 | SS-HPV 68 | 1318281 | 6.12 |
| 7 | SS-HPV 56 | 53342 | 4.73 |
| 8 | SS-HPV 59 | 2628447 | 6.42 |
| 9 | SS-HPV 66 | 265880 | 5.42 |
| 10 | SS-HPV 53 | 3323253 | 6.52 |
| 11 | SS-HPV 73 | 174590 | 5.24 |
| 12 | SS-HPV 82 | 95912 | 4.98 |

Based on the linear range assay results, it can be concluded that for 100 µl samples, the assay results with HPV-test reagent kit are linear in the range 1×10^3 - 2×10^9 copies/ml and show a maximum deviation from the regression line not more than $\pm 0.22 \log_{10}$.

4.2.5 Accuracy under repeatability and reproducibility conditions:

1. Coefficient of variation under the kit repeatability conditions is not more than 6%;
2. Coefficient of variation under the kit reproducibility conditions is not more than 7%.

4.2.6. Calibration metrological traceability and the assigned value of end-user calibrators - PC, CS1, CS2, included in HPV-test reagent kit, and used calibrators **ESS-SenC-6/11/44, ESS-SenC-16/18/26, ESS-SenC-31/33/35, ESS-SenC-39/45/51, ESS-SenC-52/68/56, ESS-SenC-58/59/66, ESS-SenC-53/73/82, ESS-SenC-16/18, ESS-SenC-31/33, ESS-SenC-6/11, ESS-HPV** in accordance with ISO 17511:2020.

The calibration hierarchy in relation to human papillomavirus (HPV) DNA 6, 11, 16, 18 types was carried out with an internationally accepted calibrator determining the measured value (clause 5.5, ISO 17511:2020):

- WHO International Standard "Collection of WHO International Standards for human papillomavirus (HPV) HPV16, HPV18, HPV6,

HPV11 DNA genotypes", NIBSC Code: 19/224, consisting of standard samples:

- HPV16 DNA (06/202) is 5×10^6 IU per ampoule;
- HPV18 DNA (06/206) is 5×10^6 IU per ampoule;
- HPV6 DNA (14/256) is 1×10^7 IU per ampoule;
- HPV11 DNA (14/100) is 1×10^7 IU per ampoule.

The calibration hierarchy in relation to human papillomavirus (HPV) DNA 31, 33, 45, 52, 58 types was carried out with an internationally accepted calibrator determining the measured value (clause 5.5, ISO 17511:2020):

- WHO International Standard "Collection of WHO International Standards for human papillomavirus (HPV) HPV31, HPV33, HPV45, HPV52, HPV58 DNA genotypes", NIBSC Code: 19/224, consisting of standard samples:

HPV31 (14/258) DNA is 1.6×10^7 International Units (IU) per ampoule;

- HPV33 DNA (14/260) is 1.6×10^7 IU per ampoule;
- HPV45 DNA (14/104) is 1×10^7 IU per ampoule;
- HPV52 DNA (14/262) is 7.9×10^6 IU per ampoule;
- HPV58 DNA (14/264) is 7.9×10^6 IU per ampoule.

The calibration hierarchy in relation to human papillomavirus (HPV) DNA 44, 26, 35, 39, 51, 68, 56, 59, 66, 53, 73, 82 types was carried out in accordance with clause 5.7, ISO 17511:2020 for measurements with metrological traceability only to internal randomly detected manufacturer's standard samples.

As a retained SS in **relation to human papillomavirus (HPV) DNA types 44, 26, 35, 39, 51, 68, 56, 59, 66, 53, 73, 82** was used isolated from human biological material test analytes DNA:

| No. | Sample name | DNA level, copies/ml | DNA level, log ₁₀ copies/ml |
|-----|-------------|----------------------|--|
| 1 | SS-HPV44 | 67608 | 4.83 |
| 2 | SS-HPV26 | 231847 | 5.37 |
| 3 | SS-HPV 35 | 8102022 | 6.91 |
| 4 | SS-HPV 39 | 11406 | 4.06 |
| 5 | SS-HPV 51 | 1068111 | 6.03 |
| 6 | SS-HPV 68 | 1318281 | 6.12 |
| 7 | SS-HPV 56 | 53342 | 4.73 |
| 8 | SS-HPV 59 | 2628447 | 6.42 |
| 9 | SS-HPV 66 | 265880 | 5.42 |
| 10 | SS-HPV 53 | 3323253 | 6.52 |

| | | | |
|----|-----------|--------|------|
| 11 | SS-HPV 73 | 174590 | 5.24 |
| 12 | SS-HPV 82 | 95912 | 4.98 |

The common calibration hierarchy with indicated measurement uncertainty at each stage is shown in Table 10.

Table 10

| Analyte | Sample | Sample type | Assigned concentration Value | Measurement uncertainty | Combined standard uncertainty | Combined expanded uncertainty |
|-----------|--|---------------------------------------|--------------------------------|--------------------------------|-------------------------------|-------------------------------|
| HPV16 DNA | Internationally accepted HPV16 DNA calibrator (06/202) (from NIBSC Collection: 19/224) | International conventional calibrator | 5×10^6 IU per ampoule | $u_{m,3}(\text{HPV16}) = 0,05$ | $u(y) = 0,09$ | $U(y) = 0,18$ |
| | ESS-SenC-16/18/26 | Calibrator used | 500 copies/ml | $u_{p,4}(\text{HPV16}) = 0,04$ | | |
| | ESS-SenC-16/18 | | 500 copies/ml | | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1×10^6 copies/ml | $u_{p,5}(\text{HPV16}) = 0,05$ | | |
| | CS2 | | 1×10^4 copies/ml | $u_{cal}(\text{HPV16}) = 0,08$ | | |
| | PC | | 1×10^5 copies/ml | | | |
| HPV18 DNA | Internationally accepted HPV18 DNA calibrator (06/206) (from NIBSC Collection: 19/224) | International conventional calibrator | 5×10^6 IU per ampoule | $u_{m,3}(\text{HPV18}) = 0,06$ | $u(y) = 0,11$ | $U(y) = 0,22$ |
| | ESS-SenC-16/18/26 | Calibrator used | 500 copies/ml | $u_{p,4}(\text{HPV18}) = 0,04$ | | |
| | ESS-SenC-16/18 | | 500 copies/ml | | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1×10^6 copies/ml | $u_{p,5}(\text{HPV18}) = 0,07$ | | |
| | CS2 | | 1×10^4 copies/ml | $u_{cal}(\text{HPV18}) = 0,10$ | | |
| | PC | | 1×10^5 copies/ml | | | |

| | | | | | | |
|--------------|--|---------------------------------------|-------------------------------------|--|---------------|---------------|
| HPV6 DNA | Internationally accepted HPV6 DNA calibrator (14/256) (from NIBSC Collection: 19/224) | International conventional calibrator | 1×10^{17} IU per ampoule | $u_{m.3}(\text{HPV6}) = 0,05$ | $u(y) = 0,08$ | $U(y) = 0,16$ |
| | ESS-SenC-6/11/44 | Calibrator used | 500 copies/ml | $u_{p.4}(\text{HPV6}) = 0,03$ | | |
| | ESS-SenC-6/11 | | 500 copies/ml | | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1×10^6 copies/ml | $u_{p.5}(\text{HPV6}) = 0,04$ $u_{cal}(\text{HPV6}) = 0,07$ | | |
| | CS2 | | 1×10^4 copies/ml | | | |
| | PC | | 1×10^3 copies/ml | | | |
| HPV11 DNA | Internationally accepted HPV11 DNA calibrator (14/100) (from NIBSC Collection: 19/224) | International conventional calibrator | 1×10^{17} IU per ampoule | $u_{m.3}(\text{HPV11}) = 0,04$ | $u(y) = 0,09$ | $U(y) = 0,18$ |
| | ESS-SenC-6/11/44 | Calibrator used | 500 copies/ml | $u_{p.4}(\text{HPV11}) = 0,05$ | | |
| | ESS-SenC-6/11 | | 500 copies/ml | | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1×10^6 copies/ml | $u_{p.5}(\text{HPV11}) = 0,04$ $u_{cal}(\text{HPV11}) = 0,08$ | | |
| | CS2 | | 1×10^4 copies/ml | | | |
| PC | 1×10^5 copies/ml | | | | | |
| HPV31 DNA | Internationally accepted HPV31 DNA calibrator (14/258) (from NIBSC Collection: 19/226) | International conventional calibrator | $1,6 \times 10^{17}$ IU per ampoule | $u_{m.3}(\text{HPV31}) = 0,03$ | $u(y) = 0,08$ | $U(y) = 0,16$ |
| | ESS-SenC-31/33/35 | Calibrator used | 500 copies/ml | $u_{p.4}(\text{HPV31}) = 0,04$ | | |
| | ESS-SenC-31/33 | | 500 copies/ml | | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| CS1 | | 1×10^6 copies/ml | $u_{p.5}(\text{HPV31}) = 0,04$ | | | |

| | | | | | | |
|-----------|--|---------------------------------------|--------------------------------------|-------------------------|---------------|---------------|
| | CS2 | End user IVD kit calibrator | 1x10 ⁴ copies/ml | $u_{cal}(HPV31) = 0,06$ | | |
| | PC | | 1x10 ⁵ copies/ml | | | |
| HPV33 DNA | Internationally accepted HPV33 DNA calibrator (14/260) (from NIBSC Collection: 19/226) | International conventional calibrator | 1,6 x 10 ⁷ IU per ampoule | $u_{m.3}(HPV33) = 0,05$ | $u(y) = 0,09$ | $U(y) = 0,18$ |
| | ESS-SenC-31/33/35 | Calibrator used | 500 copies/ml | $u_{p.4}(HPV33) = 0,04$ | | |
| | ESS-SenC-31/33 | | 500 copies/ml | | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1x10 ⁶ copies/ml | | | |
| | CS2 | | 1x10 ⁴ copies/ml | $u_{p.5}(HPV33) = 0,04$ | | |
| | PC | | 1x10 ⁵ copies/ml | $u_{cal}(HPV33) = 0,08$ | | |
| HPV45 DNA | Internationally accepted HPV45 DNA calibrator (14/104) (from NIBSC Collection: 19/226) | International conventional calibrator | 1 x 10 ⁷ IU per ampoule | $u_{m.3}(HPV45) = 0,03$ | $u(y) = 0,10$ | $U(y) = 0,20$ |
| | ESS-SenC-39/45/51 | Calibrator used | 500 copies/ml | $u_{p.4}(HPV45) = 0,05$ | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1x10 ⁶ copies/ml | $u_{p.5}(HPV45) = 0,05$ | | |
| | CS2 | | 1x10 ⁴ copies/ml | $u_{cal}(HPV45) = 0,08$ | | |
| | PC | | 1x10 ⁵ copies/ml | | | |
| HPV52 DNA | Internationally accepted HPV52 DNA calibrator (14/262) (from NIBSC Collection: 19/226) | International conventional calibrator | 7,9 x 10 ⁶ IU per ampoule | $u_{m.3}(HPV52) = 0,03$ | $u(y) = 0,10$ | $U(y) = 0,20$ |
| | ESS-SenC-52/68/56 | Calibrator used | 500 copies/ml | $u_{p.4}(HPV52) = 0,06$ | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | | 1x10 ⁶ copies/ml | $u_{p.5}(HPV52) = 0,06$ | | |

| | | | | | | |
|-----------|---|---------------------------------------|----------------------------------|-------------------------|---------------|---------------|
| | CS2 | End user IVD kit calibrator | 1×10^4 copies/ml | $u_{cal}(HPV52) = 0,09$ | | |
| | PC | | 1×10^5 copies/ml | | | |
| HPV58 DNA | Internationally accepted HPV58 DNA calibrator (14/264) (from NIBSC Collections: 19/226) | International conventional calibrator | $7,9 \times 10^6$ IU per ampoule | $u_{m.3}(HPV58) = 0,06$ | $u(y) = 0,09$ | $U(y) = 0,18$ |
| | ESS-SenC-58/59/66 | Calibrator used | 500 copies/ml | $u_{p.4}(HPV58) = 0,03$ | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1×10^6 copies/ml | $u_{p.5}(HPV58) = 0,05$ | | |
| | CS2 | | 1×10^4 copies/ml | | | |
| | PC | | 1×10^5 copies/ml | $u_{cal}(HPV58) = 0,08$ | | |
| HPV44 DNA | SS-HPV44 | Manufacturer's retained SS | $4.83 \log_{10}$ copies/ml | $u_{m.3}(HPV44) = 0,06$ | $u(y) = 0,09$ | $U(y) = 0,18$ |
| | ESS-SenC-6/11/44 | Calibrator used | 500 copies/ml | $u_{p.4}(HPV44) = 0,05$ | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1×10^6 copies/ml | $u_{p.5}(HPV44) = 0,01$ | | |
| | CS2 | | 1×10^4 copies/ml | | | |
| | PC | | 1×10^5 copies/ml | | | |
| HPV26 DNA | SS-HPV26 | Manufacturer's retained SS | $5.37 \log_{10}$ copies/ml | $u_{m.3}(HPV26) = 0,06$ | $u(y) = 0,11$ | $U(y) = 0,22$ |
| | ESS-SenC-16/18/26 | Calibrator used | 500 copies/ml | $u_{p.4}(HPV26) = 0,05$ | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1×10^6 copies/ml | $u_{p.5}(HPV26) = 0,05$ | | |
| | CS2 | | 1×10^4 copies/ml | | | |
| | PC | | 1×10^5 copies/ml | | | |
| HPV35 DNA | SS-HPV35 | Manufacturer's retained SS | $6.91 \log_{10}$ copies/ml | $u_{m.3}(HPV35) = 0,09$ | $u(y) = 0,13$ | $U(y) = 0,26$ |
| | ESS-SenC-31/33/35 | Calibrator used | 500 copies/ml | $u_{p.4}(HPV35) = 0,05$ | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1×10^6 copies/ml | $u_{p.5}(HPV35) = 0,06$ | | |
| | CS2 | | 1×10^4 copies/ml | | | |

| | | | | | | |
|-----------|---------------------------|-----------------------------|--------------------------------|--|---------------|---------------|
| | PC | | 1×10^5 copies/ml | | | |
| HPV39 DNA | SS-HPV39 | Manufacturer's retained SS | $4.06 \log_{10}$ copies/ml | $u_{m.3}(\text{HPV39}) = 0,04$ | $u(y) = 0,08$ | $U(y) = 0,16$ |
| | ESS-SenC-39/45/51 | Calibrator used | 500 copies/ml | $u_{p.4}(\text{HPV39}) = 0,01$ | | |
| | ESS-HPV | | $200,000$ copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1×10^6 copies/ml | $u_{p.5}(\text{HPV39}) = 0,04$ $u_{cal}(\text{HPV39}) = 0,06$ | | |
| | CS2 | | 1×10^4 copies/ml | | | |
| PC | 1×10^5 copies/ml | | | | | |
| HPV51 DNA | SS-HPV51 | Manufacturer's retained SS | $6.03 \log_{10}$ copies/ml | $u_{m.3}(\text{HPV51}) = 0,07$ | $u(y) = 0,12$ | $U(y) = 0,24$ |
| | ESS-SenC-39/45/51 | Calibrator used | 500 copies/ml | $u_{p.4}(\text{HPV51}) = 0,06$ | | |
| | ESS-HPV | | $200,000$ copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1×10^6 copies/ml | $u_{p.5}(\text{HPV51}) = 0,04$ $u_{cal}(\text{HPV51}) = 0,10$ | | |
| | CS2 | | 1×10^4 copies/ml | | | |
| PC | 1×10^5 copies/ml | | | | | |
| HPV68 DNA | SS-HPV68 | Manufacturer's retained SS | $6.12 \log_{10}$ copies/ml | $u_{m.3}(\text{HPV68}) = 0,08$ | $u(y) = 0,12$ | $U(y) = 0,24$ |
| | ESS-SenC-52/68/56 | Calibrator used | 500 copies/ml | $u_{p.4}(\text{HPV68}) = 0,05$ | | |
| | ESS-HPV | | $200,000$ copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1×10^6 copies/ml | $u_{p.5}(\text{HPV68}) = 0,05$ $u_{cal}(\text{HPV68}) = 0,11$ | | |
| | CS2 | | 1×10^4 copies/ml | | | |
| PC | 1×10^5 copies/ml | | | | | |
| HPV56 DNA | SS-HPV56 | Manufacturer's retained SS | $4.73 \log_{10}$ copies/ml | $u_{m.3}(\text{HPV56}) = 0,06$ | $u(y) = 0,09$ | $U(y) = 0,18$ |
| | ESS-SenC-52/68/56 | Calibrator used | 500 copies/ml | $u_{p.4}(\text{HPV56}) = 0,05$ | | |
| | ESS-HPV | | $200,000$ copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1×10^6 copies/ml | $u_{p.5}(\text{HPV56}) = 0,01$ | | |
| | CS2 | | 1×10^4 copies/ml | | | |
| PC | 1×10^5 copies/ml | | $u_{cal}(\text{HPV56}) = 0,08$ | | | |

| | | | | | | |
|--------------|-------------------|-----------------------------|----------------------------------|--|---------------|---------------|
| HPV59 DNA | SS-HPV59 | Manufacturer's retained SS | 6.42 log ₁₀ copies/ml | $u_{m.3}(\text{HPV59}) = 0,06$ | $u(y) = 0,12$ | $U(y) = 0,24$ |
| | ESS-SenC-58/59/66 | Calibrator used | 500 copies/ml | $u_{p.4}(\text{HPV59}) = 0,04$ | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1x10 ⁶ copies/ml | $u_{p.5}(\text{HPV59}) = 0,07$ $u_{cal}(\text{HPV59}) = 0,10$ | | |
| | CS2 | | 1x10 ⁴ copies/ml | | | |
| | PC | | 1x10 ⁵ copies/ml | | | |
| HPV66 DNA | SS-HPV66 | Manufacturer's retained SS | 5.42 log ₁₀ copies/ml | $u_{m.3}(\text{HPV66}) = 0,05$ | $u(y) = 0,09$ | $U(y) = 0,18$ |
| | ESS-SenC-58/59/66 | Calibrator used | 500 copies/ml | $u_{p.4}(\text{HPV66}) = 0,04$ | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1x10 ⁶ copies/ml | $u_{p.5}(\text{HPV66}) = 0,03$ $u_{cal}(\text{HPV66}) = 0,07$ | | |
| | CS2 | | 1x10 ⁴ copies/ml | | | |
| | PC | | 1x10 ⁵ copies/ml | | | |
| HPV53 DNA | SS-HPV53 | Manufacturer's retained SS | 6.52 log ₁₀ copies/ml | $u_{m.3}(\text{HPV53}) = 0,05$ | $u(y) = 0,09$ | $U(y) = 0,18$ |
| | ESS-SenC-53/73/82 | Calibrator used | 500 copies/ml | $u_{p.4}(\text{HPV53}) = 0,02$ | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1x10 ⁶ copies/ml | $u_{p.5}(\text{HPV53}) = 0,05$ $u_{cal}(\text{HPV53}) = 0,07$ | | |
| | CS2 | | 1x10 ⁴ copies/ml | | | |
| | PC | | 1x10 ⁵ copies/ml | | | |
| HPV73 DNA | SS-HPV73 | Manufacturer's retained SS | 5.24 log ₁₀ copies/ml | $u_{m.3}(\text{HPV73}) = 0,06$ | $u(y) = 0,10$ | $U(y) = 0,20$ |
| | ESS-SenC-53/73/82 | Calibrator used | 500 copies/ml | $u_{p.4}(\text{HPV73}) = 0,03$ | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1x10 ⁶ copies/ml | $u_{p.5}(\text{HPV73}) = 0,05$ $u_{cal}(\text{HPV73}) = 0,08$ | | |
| | CS2 | | 1x10 ⁴ copies/ml | | | |
| | PC | | 1x10 ⁵ copies/ml | | | |

| | | | | | | |
|--------------|-------------------|-----------------------------|---------------------------|--|---------------|---------------|
| HPV82 DNA | SS-HPV82 | Manufacturer's retained SS | 4,98 log10 copies/ml | $u_{m.3}(\text{HPV82}) = 0,07$ | $u(y) = 0,10$ | $U(y) = 0,20$ |
| | ESS-SenC-53/73/82 | Calibrator used | 500 copies/ml | $u_{p.4}(\text{HPV82}) = 0,03$ | | |
| | ESS-HPV | | 200,000 copies/ml | | | |
| | CS1 | End user IVD kit calibrator | 1×10^6 copies/ml | $u_{p.5}(\text{HPV82}) = 0,02$ $u_{cal}(\text{HPV82}) = 0,08$ | | |
| | CS2 | | 1×10^4 copies/ml | | | |
| | PC | | 1×10^3 copies/ml | | | |

$U_{max}(y) = 2 \log_{10}$ copies/ml can be taken as the upper limit of the medical decision threshold.

Table 10 shows that in accordance with clause 4.7.1(c), ISO 17511:2020, the combined standard measurement uncertainty of the value assigned to the end user IVD kit calibrators (CS1, CS2, PC) u_{cal} does not exceed the permissible proportion of specification $U_{max}(y)$ for the IVD kit, taking into account a coverage factor k ($k = 2$, for an approximate 95% confidence level):

$$u_{cal} \leq \frac{1}{2} U_{max}(y) = 1$$

And in accordance with clause 4.1(c), ISO 17511:2020, the estimated combined extended measurement uncertainty $U(y)$ does not exceed the maximum permissible measurement uncertainty $U_{max}(y)$:

$$U(y) \leq U_{max}(y) = 2$$

4.2.7 Interfering substances influence and restrictions on the test material use

Potentially interfering substances influence on HPV-test reagent kit performance has been tested for potentially interfering substances that may occur during HPV-test reagent kit normal use, and presumably affect the reagent kit ability to produce reliable results.

Interfering substances can originate from the following external and internal sources:

1) substances used in the patient's treatment (for example, medicines);

2) substances found in specific samples types – in this case, clinical sample contamination with blood hemoglobin can inhibit PCR if not sufficiently purified during the DNA isolation procedure;

Tested interfering substances concentrations are shown in Table 11.
Table 11 – Interfering substances

| Interfering substances | Maximum permissible concentration |
|--|-----------------------------------|
| Endogenous interfering substances | |
| Hemoglobin | 100 mg/ml |
| Mucin | 50 mg/ml |
| Exogenous interfering substances | |
| Chlorhexidine | 0.05 g/ml |
| Miramistin (benzyltrimethyl[3-(myristoylamino)-propyl]ammonium chloride monohydrate) | 0.1 mcg/ml |
| Clotrimazole | 200 mg/ml |

Limitations on the test material use:

- the test material shall not be used in case of storage and transportation condition violation (temperature, duration, multiple freezing-thawing);

- it is not allowed to use samples contaminated with extraneous biological material.

4.2.6 Biological reference intervals

The biological reference interval for human papillomavirus (HPV) DNA levels of low carcinogenic risk 6, 11, 44 types and high carcinogenic risk types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 73, 82 types among 796 patients aged 2 to 73 years, as determined by clinical trial results, is 3.36 to 6.98 log₁₀ copies/mL. The CMV DNA median concentration in the sample is 5.20 log₁₀ copies/ml.

4.3. Characteristics of clinical efficiency

For clinical assays 796 samples of human clinical material (91

- biopsy, 135 vaginal mucosal swabs, 145 urine, 71 prostate secretions, 135 urethral scrapings, 147 cervical canal scrapings, 72 ejaculate) were used from patients aged 2 to 73 years diagnosed with human papillomavirus (HPV) infection, without regard to the disease form and stage in all population groups.

International guideline CLSI EP09-A3 recommends that clinical assays be conducted on at least 40 clinical samples. **In order to conduct assays on a larger sample size, in accordance with CLSI EP09-A3 recommendations, each sample was tested in 4 repeats** starting from the DNA isolation procedure.

Each sample was tested in four repeats with “Reagent kit for qualitative and quantitative human papillomavirus (HPV) DNA detection and differentiation of low carcinogenic risk 6, 11, 44 genotypes and high carcinogenic risk 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types by multiplex PCR-RT “HPV-test” according to TS 21.20.23-060-97638376-2022”, produced by TestGene LLC and a comparison kit:

- Reagent kit for detection, typing and quantification of human papillomavirus by PCR (HPV QUANT) according to TS 9398-032-46482062-2019, produced by NPO DNA Technology LLC, Russia, (Registration certificate No.FSR 2010/08811 dated 12.03.2020), version HPV QUANT-21, packaging A.

Correct operation proof for the test kit was result matching.

The PCR assay was performed with cyclers recommended by the test reagent kit manufacturer:

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia);
- CFX 96 cycler (Bio-Rad, USA);
- Rotor-Gene Q cycler (Qiagen, Germany);

- QuantStudio 5 cycler (Thermo Fisher Scientific, USA);
- FLUORITE cycler (Xian TianLong Science and Technology Co, China).

When testing **159 human clinical material samples** (25 - cervical scrapings, 23 - urethral scrapings, 24 - vaginal swabs, 20 - biopsy, 21 - prostate secretion, 20 - ejaculate, 26 - urine), **that did not contain HPV DNA of the tested types**, but with confirmed positive presence of the following microorganisms genomic NA: *Lactobacillus spp*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *CMV*, *HHV8*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Gardnerella vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Chlamydia trachomatis*, *Candida albicans*, *EBV*, *HHV6*, *HSV1*, *HSV2*, *Varicella Zoster virus*, no cross-reactivity was observed.

Diagnostic characteristics confidence intervals (CI) will be calculated using the Clopper and Pearson Confidence Interval (Clopper-Pearson Confidence Interval; Clopper, C., & Pearson, E. (1934). The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. *Biometrika*, 26(4), 404-413. doi:10.2307/2331986). The test kit diagnostic characteristics were calculated with a 95% confidence probability.

4.3.1 The diagnostic characteristics assay results based on clinical material samples are shown in Table 12.

| Test material type | Test analyte | Number of observations with positive samples | Number of observations with negative samples | Diagnostic sensitivity with a 95% confidence level | Diagnostic specificity with a 95% confidence level |
|--------------------|--------------|--|--|--|--|
| | HPV 6 | 48 | 640 | 100 % (95% CI: 92,60%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 11 | 48 | 640 | 100 % (95% CI: 92,60%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 44 | 56 | 632 | 100 % (95% CI: 93,62%-100%) | 100 % (95% CI: 99,42%-100%) |
| | HPV 16 | 84 | 604 | 100 % (95% CI: 95,70%-100%) | 100 % (95% CI: 99,39%-100%) |
| | HPV 18 | 44 | 644 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 26 | 48 | 640 | 100 % (95% CI: 92,60%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 31 | 76 | 612 | 100 % (95% CI: 95,26%-100%) | 100 % (95% CI: 99,40%-100%) |

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|-----------------------------|--------|-----|-----|--------------------------------|--------------------------------|
| Cervical scraping | HPV 33 | 84 | 604 | 100 % (95% CI: 95,70%-100%) | 100 % (95% CI: 99,39%-100%) |
| | HPV 35 | 40 | 648 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 39 | 40 | 648 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 45 | 40 | 648 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 51 | 40 | 648 | 100% (95% CI: 91.19%-100%) | 100% (95% CI: 99,43%-100%) |
| | HPV 52 | 56 | 632 | 100% (95% CI: 93.62%-100%) | 100 % (95% CI: 99,42%-100%) |
| | HPV 68 | 40 | 648 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 56 | 56 | 632 | 100% (95% CI: 93.62%-100%) | 100 % (95% CI: 99,42%-100%) |
| | HPV 58 | 48 | 640 | 100 % (95% CI: 92,60%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 59 | 56 | 632 | 100% (95% CI: 93.62%-100%) | 100 % (95% CI: 99,42%-100%) |
| | HPV 66 | 100 | 588 | 100 % (95% CI: 92,60%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 53 | 48 | 640 | 100 % (95% CI: 96,38%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 73 | 44 | 644 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 82 | 36 | 652 | 100 % (95% CI: 90,26%-100%) | 100 % (95% CI: 99,44%-100%) |
| Scraping from urethra | HPV 6 | 52 | 580 | 100 % (95% CI: 93,15%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 11 | 60 | 572 | 100% (95% CI: 94.04%-100%) | 100 % (95% CI: 99,36%-100%) |
| | HPV 44 | 52 | 580 | 100 % (95% CI: 93,15%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 16 | 72 | 560 | 100% (95% CI: 95.01%-100%) | 100 % (95% CI: 99,34%-100%) |
| | HPV 18 | 40 | 592 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,38%-100%) |
| | HPV 26 | 44 | 588 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 31 | 44 | 588 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 33 | 68 | 564 | 100% (95% CI: 94.72%-100%) | 100 % (95% CI: 99,35%-100%) |
| | HPV 35 | 40 | 592 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,38%-100%) |
| | HPV 39 | 36 | 596 | 100 % (95% CI: 90,26%-100%) | 100 % (95% CI: 99,38%-100%) |
| | HPV 45 | 40 | 592 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,38%-100%) |

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|---------------|--------|-----|-----------------------------|-----------------------------|-----------------------------|
| | HPV 51 | 40 | 592 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,38%-100%) |
| | HPV 52 | 48 | 584 | 100 % (95% CI: 96,38%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 68 | 36 | 596 | 100 % (95% CI: 90,26%-100%) | 100 % (95% CI: 99,38%-100%) |
| | HPV 56 | 52 | 580 | 100 % (95% CI: 93,15%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 58 | 40 | 592 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,38%-100%) |
| | HPV 59 | 60 | 572 | 100% (95% CI: 94.04%-100%) | 100 % (95% CI: 99,36%-100%) |
| | HPV 66 | 104 | 528 | 100 % (95% CI: 96,52%-100%) | 100 % (95% CI: 99,30%-100%) |
| | HPV 53 | 44 | 588 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 73 | 44 | 588 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 82 | 40 | 592 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,38%-100%) |
| Vaginal swabs | HPV 6 | 48 | 588 | 100 % (95% CI: 96,38%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 11 | 56 | 580 | 100% (95% CI: 93.62%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 44 | 56 | 580 | 100% (95% CI: 93.62%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 16 | 72 | 564 | 100% (95% CI: 95.01%-100%) | 100 % (95% CI: 99,35%-100%) |
| | HPV 18 | 40 | 596 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,38%-100%) |
| | HPV 26 | 48 | 588 | 100 % (95% CI: 96,38%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 31 | 44 | 592 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,38%-100%) |
| | HPV 33 | 68 | 568 | 100% (95% CI: 94.72%-100%) | 100 % (95% CI: 99,35%-100%) |
| | HPV 35 | 36 | 600 | 100 % (95% CI: 90,26%-100%) | 100 % (95% CI: 99,39%-100%) |
| | HPV 39 | 36 | 600 | 100 % (95% CI: 90,26%-100%) | 100 % (95% CI: 99,39%-100%) |
| | HPV 45 | 40 | 596 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,38%-100%) |
| | HPV 51 | 36 | 600 | 100 % (95% CI: 90,26%-100%) | 100 % (95% CI: 99,39%-100%) |
| | HPV 52 | 48 | 588 | 100 % (95% CI: 96,38%-100%) | 100 % (95% CI: 99,37%-100%) |
| | HPV 68 | 36 | 600 | 100 % (95% CI: 90,26%-100%) | 100 % (95% CI: 99,39%-100%) |
| HPV 56 | 52 | 584 | 100 % (95% CI: 93,15%-100%) | 100 % (95% CI: 99,37%-100%) | |

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|--------|--------|-----|-----------------------------|-----------------------------|-----------------------------|
| | HPV 58 | 44 | 592 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,38%-100%) |
| | HPV 59 | 60 | 576 | 100% (95% CI: 94.04%-100%) | 100 % (95% CI: 99,36%-100%) |
| | HPV 66 | 108 | 528 | 100 % (95% CI: 96,64%-100%) | 100 % (95% CI: 99,30%-100%) |
| | HPV 53 | 44 | 592 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,38%-100%) |
| | HPV 73 | 44 | 592 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,38%-100%) |
| | HPV 82 | 44 | 592 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,38%-100%) |
| Biopsy | HPV 6 | 52 | 392 | 100 % (95% CI: 93,15%-100%) | 100 % (95% CI: 99,06%-100%) |
| | HPV 11 | 48 | 396 | 100 % (95% CI: 96,38%-100%) | 100 % (95% CI: 99,07%-100%) |
| | HPV 44 | 36 | 408 | 100 % (95% CI: 90,26%-100%) | 100 % (95% CI: 99,10%-100%) |
| | HPV 16 | 44 | 400 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,08%-100%) |
| | HPV 18 | 36 | 408 | 100 % (95% CI: 90,26%-100%) | 100 % (95% CI: 99,10%-100%) |
| | HPV 26 | 32 | 412 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 99,11%-100%) |
| | HPV 31 | 48 | 396 | 100 % (95% CI: 96,38%-100%) | 100 % (95% CI: 99,07%-100%) |
| | HPV 33 | 56 | 388 | 100% (95% CI: 93.62%-100%) | 100 % (95% CI: 99,05%-100%) |
| | HPV 35 | 40 | 404 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,09%-100%) |
| | HPV 39 | 28 | 416 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 99,12%-100%) |
| | HPV 45 | 28 | 416 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 99,12%-100%) |
| | HPV 51 | 28 | 416 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 99,12%-100%) |
| | HPV 52 | 40 | 404 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,09%-100%) |
| | HPV 68 | 28 | 416 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 99,12%-100%) |
| | HPV 56 | 32 | 412 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 99,11%-100%) |
| | HPV 58 | 36 | 408 | 100 % (95% CI: 90,26%-100%) | 100 % (95% CI: 99,10%-100%) |
| | HPV 59 | 36 | 408 | 100 % (95% CI: 90,26%-100%) | 100 % (95% CI: 99,10%-100%) |
| | HPV 66 | 60 | 384 | 100% (95% CI: 94.04%-100%) | 100 % (95% CI: 99,04%-100%) |
| HPV 53 | 36 | 408 | 100 % (95% CI: 90,26%-100%) | 100 % (95% CI: 99,10%-100%) | |

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|--------------------|--------|-----|-----------------------------|-----------------------------|------------------------------|
| | HPV 73 | 40 | 404 | 100% (95% CI: 91,19%-100%) | 100 % (95% CI: 99,09% -100%) |
| | HPV 82 | 32 | 412 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 99,11%-100%) |
| Prostate secretion | HPV 6 | 36 | 332 | 100 % (95% CI: 90,26%-100%) | 100 % (95% CI: 98,90%-100%) |
| | HPV 11 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 44 | 28 | 340 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 98,92%-100%) |
| | HPV 16 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 18 | 28 | 340 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 98,92%-100%) |
| | HPV 26 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 31 | 40 | 328 | 100% (95% CI: 91,19%-100%) | 100 % (95% CI: 98,88%-100%) |
| | HPV 33 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 35 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 39 | 28 | 340 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 98,92%-100%) |
| | HPV 45 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 51 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 52 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 68 | 28 | 340 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 98,92%-100%) |
| | HPV 56 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 58 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 59 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 66 | 44 | 324 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 98,87%-100%) |
| | HPV 53 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 73 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| HPV 82 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) | |
| Ejaculate | HPV 6 | 52 | 316 | 100 % (95% CI: 93,15%-100%) | 100 % (95% CI: 98,84%-100%) |
| | HPV 11 | 44 | 324 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 98,87%-100%) |

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|-------|--------|----|-----|-----------------------------|-----------------------------|
| | HPV 44 | 28 | 340 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 98,92%-100%) |
| | HPV 16 | 28 | 340 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 98,92%-100%) |
| | HPV 18 | 24 | 344 | 100 % (95% CI: 85,75%-100%) | 100 % (95% CI: 98,93%-100%) |
| | HPV 26 | 28 | 340 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 98,92%-100%) |
| | HPV 31 | 48 | 320 | 100 % (95% CI: 96,38%-100%) | 100 % (95% CI: 98,85%-100%) |
| | HPV 33 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 35 | 28 | 340 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 98,92%-100%) |
| | HPV 39 | 32 | 336 | 100 % (95% CI: 89,11%-100%) | 100 % (95% CI: 98,91%-100%) |
| | HPV 45 | 28 | 340 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 98,92%-100%) |
| | HPV 51 | 40 | 328 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 98,88%-100%) |
| | HPV 52 | 24 | 344 | 100 % (95% CI: 85,75%-100%) | 100 % (95% CI: 98,93%-100%) |
| | HPV 68 | 28 | 340 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 98,92%-100%) |
| | HPV 56 | 24 | 344 | 100 % (95% CI: 85,75%-100%) | 100 % (95% CI: 98,93%-100%) |
| | HPV 58 | 24 | 344 | 100 % (95% CI: 85,75%-100%) | 100 % (95% CI: 98,93%-100%) |
| | HPV 59 | 40 | 328 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 98,88%-100%) |
| | HPV 66 | 48 | 320 | 100 % (95% CI: 96,38%-100%) | 100 % (95% CI: 98,85%-100%) |
| | HPV 53 | 28 | 340 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 98,92%-100%) |
| | HPV 73 | 44 | 324 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 98,87%-100%) |
| | HPV 82 | 28 | 340 | 100 % (95% CI: 87,66%-100%) | 100 % (95% CI: 98,92%-100%) |
| Urine | HPV 6 | 60 | 624 | 100% (95% CI: 94.04%-100%) | 100 % (95% CI: 99,41%-100%) |
| | HPV 11 | 48 | 636 | 100 % (95% CI: 96,38%-100%) | 100 % (95% CI: 99,42%-100%) |
| | HPV 44 | 68 | 616 | 100% (95% CI: 94.72%-100%) | 100 % (95% CI: 99,40%-100%) |
| | HPV 16 | 60 | 624 | 100% (95% CI: 94.04%-100%) | 100 % (95% CI: 99,41%-100%) |
| | HPV 18 | 44 | 640 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 26 | 40 | 644 | 100% (95% CI: 91.19%-100%) | 100 % (95% CI: 99,43%-100%) |

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|--|--------|-----|-----|-----------------------------|-----------------------------|
| | HPV 31 | 44 | 640 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 33 | 80 | 604 | 100% (95% CI: 95,49%-100%) | 100 % (95% CI: 99,39%-100%) |
| | HPV 35 | 40 | 644 | 100% (95% CI: 91,19%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 39 | 40 | 644 | 100% (95% CI: 91,19%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 45 | 48 | 636 | 100 % (95% CI: 96,38%-100%) | 100 % (95% CI: 99,42%-100%) |
| | HPV 51 | 44 | 640 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 52 | 52 | 632 | 100 % (95% CI: 93,15%-100%) | 100 % (95% CI: 99,42%-100%) |
| | HPV 68 | 44 | 640 | 100 % (95% CI: 91,96%-100%) | 100 % (95% CI: 99,43%-100%) |
| | HPV 56 | 52 | 632 | 100 % (95% CI: 93,15%-100%) | 100 % (95% CI: 99,42%-100%) |
| | HPV 58 | 48 | 636 | 100 % (95% CI: 96,38%-100%) | 100 % (95% CI: 99,42%-100%) |
| | HPV 59 | 56 | 628 | 100% (95% CI: 93,62%-100%) | 100 % (95% CI: 99,41%-100%) |
| | HPV 66 | 132 | 552 | 100 % (95% CI: 97,24%-100%) | 100 % (95% CI: 99,33%-100%) |
| | HPV 53 | 56 | 628 | 100% (95% CI: 93,62%-100%) | 100 % (95% CI: 99,41%-100%) |
| | HPV 73 | 48 | 636 | 100 % (95% CI: 96,38%-100%) | 100 % (95% CI: 99,42%-100%) |
| | HPV 82 | 40 | 644 | 100% (95% CI: 91,19%-100%) | 100 % (95% CI: 99,43%-100%) |

4.3.2 Method comparison: accuracy

The data obtained by testing **796 samples of human clinical material** (91 biopsy, 135 vaginal swabs, 145 urine, 71 prostate secretions, 135 urethral scrapings, 147 cervical scrapings, 72 - ejaculate), from patients aged from 2 to 73 years diagnosed with human papillomavirus (HPV) infection, without regard to the disease form and stage in all population groups, allow to conclude on reliable conformity of the results of quantitative HPV DNA concentration detection of 6, 11, 44, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types in clinical samples obtained with the **test kit** “Reagent kit for qualitative and quantitative human papillomavirus (HPV) DNA detection and differentiation of low carcinogenic risk 6, 11, 44 genotypes and high carcinogenic risk 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types by multiplex PCR-RT “HPV-test” according to TS 21.20.23-060- 97638376-2022”, produced by TestGene LLC and a

comparison kit:

- Reagent kit for detection, typing and quantification of human papillomavirus by PCR (HPV QUANT) according to TS 9398-032-46482062-2019, produced by NPO DNA Technology LLC, Russia, (Registration certificate No. FSR 2010/08811 dated 12.03.2020),

when performing a PCR test with cyclers:

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia), registration certificate No. FSR 2011/10228 dated March 03, 2011;

- CFX 96 cycler (Bio-Rad, USA), registration certificate No. FSZ 2008/03399 dated June 21, 2016;

- Rotor-Gene Q cycler (Qiagen, Germany), registration certificate No. FSZ 2010/07595 dated August 10, 2010;

- QuantStudio 5 cycler (Thermo Fisher Scientific, USA), registration certificate No. RZN 2019/8446 dated June 06, 2019;

- FLUORITE cycler (Xian TianLong Science and Technology Co, China, RU No.RZN 2022/16415 dated January 24, 2022).

The systematic error in measuring the logarithm of HPV DNA concentration of 6, 11, 44, 16, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types does not exceed 3 %

4.3.3 Detection results of the interlot correlation

To determine the interlot correlation of measurement results in clinical samples in accordance with the international guidelines CLSI EP09-A3, a scatter diagram of the dependent variable X - tested types HPV DNA concentration was drawn using each configuration form HPV-test kit of **one lot**, and Y - tested types HPV DNA concentration using each configuration form HPV-test kit of the **other lot**.

The obtained data allow to conclude on the reliable conformity of the results of quantitative human papillomavirus (HPV) DNA concentration detection of low carcinogenic risk 6, 11, 44 types and high carcinogenic risk 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types by HPV-test kit in the following configuration forms:

1. HPV-test-21 - HPV 6, 11, 44, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types;

2. HPV-test-16/18 - HPV 16, 18 types;

3. HPV-test-31/33 - HPV 31, 33 types;

4. HPV-test-6/11 - HPV 6, 11 types;

5. HPV-test-14 - HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66 types;

when using different lots and performing PCR test using **cyclers**:

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia), registration certificate No. FSR 2011/10228 dated March 03, 2011;
- CFX 96 cycler (Bio-Rad, USA), registration certificate No. FSZ 2008/03399 dated June 21, 2016;
- Rotor-Gene Q cycler (Qiagen, Germany); registration certificate No. FSZ 2010/07595 dated 10.08.2010;
- QuantStudio 5 cycler (Thermo Fisher Scientific, USA), registration certificate No. RZN 2019/8446 dated June 06, 2019;
- FLUORITE cycler (Xian TianLong Science and Technology Co, China, RU No.RZN 2022/16415 dated January 24, 2022).

The correlation coefficient R² during each used amplifiers testing was more than 0.98. In accordance with CLSI EP09-A3 recommendations, using the regression and correlation method, it can be concluded that the results of **HPV DNA concentration detection of 6, 11, 44, 16, 18, 26, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types** obtained using **two different HPV-test' tested kit lots in each configuration form**, manufactured by TestGene LLC, **are highly correlated.**

5. Risks associated with the reagent kit use

The borderline risk zone includes hazards:

1. Loss of functional properties of the reagents included in the kit due to transportation, storage or use under inappropriate conditions;
2. Clinical material contamination with inhibitory substances in concentrations exceeding the permissible ones;
3. Contamination of reaction mixtures and test DNA samples with contents from the PC tube or amplification products;
4. Testing with a poor quality DNA sample (low concentration and/or poor purification);
5. Failure to comply with the requirements for sample preparation, testing and disposal due to work with unqualified personnel;
6. The use of an unsuitable kit (use after the expiry or in case of damaged package).

The cumulative residual risk of using a kit "Reagent kit for qualitative and quantitative human papillomavirus (HPV) DNA detection and differentiation of low carcinogenic risk 6, 11, 44 genotypes and high carcinogenic risk 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types by multiplex PCR-RT "HPV-test" according to TS 21.20.23-060-97638376- 2022 " is acceptable, the benefit of its use

exceeds the risk.

6. Safety precautions

All components and reagents included in HPV-test reagent kit belong to low-hazard substances. Precautions against any special, unusual environmental risks when using or selling the product are not provided.

The reagents included in HPV-test kit have low vapor pressure and exclude the possibility of inhalation poisoning.

The reagents included in HPV-test kit are non-toxic, since they are prepared by mixing individual non-toxic components.

Work with material infected or suspected of infection is carried out in accordance with the requirements of sanitary and epidemiological requirements for the prevention of infectious diseases, requirements of organization of the work of laboratories using nucleic acid amplification methods when working with material containing microorganisms I– IV pathogenicity group.

It is necessary to simultaneously ensure and comply with the biological safety rules and the requirements for the organization and conduct of these works by personnel in order to prevent premises and equipment contamination of with nucleic acids and (or) amplicons of the test samples.

The work should be carried out in a laboratory performing clinical material molecular-biological (PCR) testing in accordance with sanitary and epidemiological requirements.

The following requirements should always be met when working:

- remove unused reagents in accordance with sanitary and epidemiological requirements for the management of medical waste;

ATTENTION! When removing waste after amplification (tubes containing PCR products), it is unacceptable to open the tubes and spray the contents, as this may lead to contamination of the laboratory area, equipment and reagents with PCR products;

1. use the kit strictly for its intended purpose, according to these instructions;

2. allow only specially trained personnel to work with the kit (a specialist with higher medical education who has been trained in licensed specialization courses for working with PBA of pathogenicity groups III-IV and PCR diagnostics, as well as a laboratory assistant with secondary specialized medical education);

3. do not use the kit after the expiry date;

4. avoid contact with skin, eyes and mucous membranes. In case of contact, rinse immediately the affected area with water and seek medical assistance.

The necessary precautions regarding the influence of magnetic fields, external electrical influences, electrostatic discharges, pressure or pressure changes, overload, sources of thermal inflammation are not provided.

The kit contains no substances of human or animal origin with a potential infectious nature, therefore, precautions against any special, unusual risks during product use or sale are not provided.

7. Required equipment and materials

Work with a reagent kit for multiplex PCR HPV-test is carried out in the working area 3 (for preparation of reactions).

1. Class II and III biosafety cabinet (for example, microbiological safety boxes BMB-II-Laminar-C according to TS 32.50.50-010-51495026-2020 , manufactured by ZAO Laminar Systems, RU No. FSR 2012/13259 dated 29.07.2021 or a box for sterile work DNA/RNA UV-Cleaner Box UVC/T-M-AR, Biosan, Latvia, RU No. RZN 2023/19369 dated 18.01.2023);

2. Vortex (for example, Microspin 12 high-speed mini-centrifuge, BIOSAN SIA, Latvia, RU No. FSZ 2011/10116 dated 11.07.2011 or Vortex Centrifuge CM-70M, manufactured by SIA ELMI, Latvia, RU No. RZN 2016/4616 dated 31.05.2023);

3. Variable volume dispensers, allowing to take liquid volumes of 0.5–10 µl, 10-100 µl or 20-200 µl, 100-1000 µl (for example, Eppendorf Research Plus, Germany, RU No. FSZ 2011/11028 dated 15.11.2011 or Biohit, Finland, RU No. FSZ 2012/12201 dated 18.05.2012);

4. Refrigerator from 2°C to 8°C with a freezer less than -16 °C; (for example, a combined laboratory refrigerator XL-250 POZIS, XL-250-1 POZIS according to TS 9452-203-07503307-2012, manufactured by AO POSiS, RU No. RZN 2016/4043 dated 08.05.2019);

5. Cycler ¹ with real-time fluorescence detection on channels corresponding to FAM, HEX fluorophores:

- CFX96 (BioRad, USA, RU No. FSZ 2008/03399 dated 21.06.2016),
- DTprime (NPO DNA Technology LLC, Russia, RU No. FSR 2011/10229 dated 03.03.2011),

- Rotor-Gene Q (Qiagen, Germany), registration certificate No. FSZ 2010/07595 dated 10.08.2010;
- QuantStudio 5 (Thermo Fisher Scientific, USA, RU No. RZN 2019/8446 dated 06.06.2019),
- FLUORITE (Xian TianLong Science and Technology Co, China, RU No. RZN 2022/16415 dated 24.01.2022).

Materials and reagents not included in the product:

ATTENTION! When working with DNA, it is necessary to use only disposable sterile plastic consumables with "DNase-free" label.

1. Disposable pipette tips with aerosol barrier up to 1000 µl, 200 µl, 20 µl and 10 µl (Axygen, USA, RU No. FSZ 2012/12077 dated 27.02.2014);

2. 1.5 or 2.0 ml disposable sterile tubes of the Eppendorf type ml ("Axygen", USA, RU No. FSZ 2012/11892 dated 26.08.2014);

3. Thin-walled disposable tubes with an optically transparent lid for PCR (Axygen, USA, RU No. FSZ 2012/11892 dated 26.08.2014):

- 0.2 ml PCR tubes,
- 0.1 or 0.2 ml PCR tubes in strips,
- PCR plates with an optically transparent film.

4. Disposable gown and disposable gloves without talcum powder;

5. Container with disinfectant solution;

6. Test tube rack for 0.2 ml tubes or for stripped 0.2 ml tubes;

7. Kit for DNA isolation from clinical material (see Section 8.2)

8. When taking scrapings of urogenital tract mucous membranes:

- sterile saline solution (0.9% NaCl)
- or transport medium "Reagent for clinical material transportation and storage "Transport medium with mucolytic (TMM)" according to TS 9398-098-01897593-2009", produced by the FBIS the Central Research Institute of Epidemiology of Rospotrebnadzor, Russia (registration certificate No. FSR 2009/05514 dated 13.03.2019)

- or DNA-Fast reagent for DNA collecting, transporting and isolating from clinical material according to TS 21.20.23-016- 97638376-2019, manufactured by TestGene LLC, Russia (registration certificate No. RZN 2021/14885 dated 05.06.2023)

¹ Cyclers should be maintained, calibrated and used according to the manufacturer's recommendations. Use of this kit in an uncalibrated device may affect the reagent kit performance.

9. When carrying out a test using urine as a transport medium for addition to the urine cellular sediment, it is recommended to use the "Reagent for transporting and storing clinical material "Transport medium with mucolytic (TMM)" according to TS 9398-098-01897593-2009", produced by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia (registration certificate No. FSR 2009/05514 dated 13.03.2019).

8. Test samples

Test sample types

Test material is DNA samples isolated from clinical material - epithelial cell scrapings from cervical mucosa, urethra, vagina, biopsy, prostate secretion, ejaculate, urine.

8.1. Clinical material collection

Clinical material sampling and its packaging is carried out by an employee of a medical organization trained in the requirements and rules of biological safety when working and collecting material suspected of infection with microorganisms of the pathogenicity group III.

Material sampling for assay

Scrapings from the urogenital tract (cervical canal, urethra, vagina) mucous membranes.

The material is collected using special sterile disposable instruments – urogenital probes, cytobrushes or gynaecological swab, depending on the clinical material source. After taking the clinical material, immerse the working part of the probe into a 1.5–2.0 ml test tube containing 300-500 ml of sterile saline solution or transport medium intended for biomaterial samples transportation and storage for PCR test or reagent for nucleic acid isolation (e.g. DNA-Fast, RU No. RZN 2021/14885 dated 05.06.2023, produced by TestGene LLC). If the reagent kit instructions allow, leave the working part of the probe in the tube with transport medium by breaking it off in the notch area. If there is no notch, or if leaving the probe is not provided by the instruction, immerse the working part of the probe into the medium and, pressing it against the inner wall of the tube, rotate the probe for 5-10 seconds, after which the probe is removed and the tube is tightly closed. Before carrying out the nucleic acid isolation procedure, precipitate drops from the tube walls and the lid by centrifugation (1 500 – 3 000 g), then gently mix the tube contents on the vortex, avoiding

splashing and getting material on the lid.

Peculiarities of collecting material from the urethra

Women should not perform genital hygiene and vaginal douching on the examination eve. To obtain an objective result, it is necessary that the test material contains as many epithelial cells as possible and a minimum amount of mucus and blood admixture. Incorrect biomaterial collection can lead to an unreliable result and therefore the need to repeat the biomaterial collection.

Before taking the biomaterial, it is recommended to refrain from urinating for 1.5–2.0 hours. Immediately before taking the biomaterial, it is necessary to treat the outer urethra opening with a tampon, which can be moistened with sterile saline solution.

If there is purulent discharge, it is recommended to take a scraping 15–20 minutes after urination, in the absence of discharge, it is necessary to massage the urethra with a probe to take biomaterial. In women, the probe is inserted into the urethra to 1.0–1.5 cm depth, in children, the material for the essay is taken only from the external urethra opening.

Peculiarities of collecting material from the vagina

The material should be taken before the manual examination. The speculum before manipulation can be moistened with hot water, the use of antiseptics for speculum treatment is contraindicated. The scraping is taken from the posterolateral vaginal wall. In girls, the material is taken from the mucous membrane of the vaginal vestibule and, in some cases, from the posterior vaginal wall through the hymenal rings.

Peculiarities of collecting material from the cervical canal

Before material collecting, it is necessary to remove the mucus with a cotton swab and then treat the cervix with a sterile saline solution. The probe is inserted into the cervical canal to 0.5–1.5 cm depth. Carefully remove the probe, avoiding the vaginal walls.

Method limitation is the local use of medications, ultrasound with a vaginal sensor less than 24 hours before the assay.

Urine

For assay, the first morning urine portion is collected in an amount of at least 20–30 ml in a special dry sterile 50 ml container.

Sample pre-processing

Shake the urine container. Transfer 1 ml of urine, using a filter pipette tip, into sterile 1.5 ml tubes, centrifuge for 5 min at 10,000 g, if large salt amounts are present, resuspend only the top layer of the salt sediment in 1 ml volume, and then concentrate again. Using a vacuum

aspirator with a trap flask, the supernatant is completely removed without capturing the sediment. Add the transport medium to the sediment to 0.2 ml final volume, mix the contents thoroughly on the vortex.

It is recommended to use ‘Reagent for transport and storage of clinical material “Transport medium with mucolytic (TMM)” according to TS 9398-098-01897593-2009’, produced by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia as a transport medium for addition to the urine cellular sediment.

Prostate secretion

Before taking the prostate secretion, the head of the penis is treated with a sterile cotton swab moistened with saline solution. Prostate secretion is taken after a preliminary prostate massage through the rectum. The doctor performs a pressure massage with several vigorous movements from the base to the top. Then the prostatic secretion is squeezed out of the cavernous part and collected in a sterile container (wide-mouth vessels, tubes).

Peculiarities of ejaculate collection

Before collecting the ejaculate, sexual abstinence is recommended for three days before the assay. Before collecting the ejaculate, the patient urinates in the toilet, completely emptying the bladder. After urination, the patient should wash hands thoroughly with soap and perform genital hygiene with soap and water. The foreskin and the head of the penis must be gently drained with a sterile tissue. The ejaculate is obtained by masturbation and collected in a 60 ml sterile container. The ejaculate container is hermetically sealed with a lid and labeled.

Peculiarities of biopsy collection

Biopsy samples should be placed in disposable vacuum tubes with EDTA preservative or disposable 1.5 ml screw-top tubes containing no more than 0.5 ml sterile saline solution.

Conditions of transportation, storage and disposal of initial clinical material:

- at a temperature from 2 to 8°C – up to 24 hours,
- at a temperature from -18 to -22°C – up to one month.

ATTENTION! Avoid repeated freezing and thawing of samples.

8.2. Procedure for collection of human DNA sample

For DNA isolation **from epithelial cell scrapings from the mucous membrane of the cervical canal, urethra, vagina, prostate secretion, urine** during quantitative and qualitative assay, it is recommended to use:

- Reagent kit for DNA/RNA isolation from the clinical material NA-EXTRA according to TS 21.20.23-013-97638376-2019, manufactured by TestGene LLC, Russia (registration certificate No. RZN 2021/15428 dated 05.06.2023);

For DNA isolation **from the ejaculate** during quantitative and qualitative assay, it is recommended to use:

- Reagent kit for DNA isolation from the clinical material DNA-sorb-B according to TS 9398-003-01897593-2009, produced by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia, (registration certificate No. FSR 2009/05220 dated 05.03.2019);

For DNA isolation **from the ejaculate** during quantitative and qualitative assay, it is recommended to use:

- Reagent kit for nucleic acids isolation (PREP-NA/PREP-NA-PLUS) according to TS 9398-035-46482062-2009, manufactured by NPO DNA Technology LLC, Russia, (registration certificate No. FSR 2010/08867 dated 13.10.2016);

For DNA isolation **from epithelial cell scrapings from the mucous membrane of the cervical canal, urethra, vagina, prostate secretions, urine** during qualitative assay, it is recommended to use:

- Reagent for DNA collection, transportation and isolation from clinical material DNA-Fast according to TS 21.20.23-016-97638376-2019, manufactured by TestGene LLC, Russia (registration certificate No. RZN 2021/14885 dated 05.06.2023).

During DNA isolation procedure, the protocol and instructions of the reagent kit used must be strictly followed.

Test DNA samples storage conditions are according to the instructions for DNA isolation kits and reagents.

9. Kit components preparation for testing

Installation, assembling, adjustment, calibration of the kit for commissioning is not required.

ATTENTION! When working with DNA, it is necessary to use only disposable sterile plastic consumables with "DNase-free" label. It is mandatory to use a separate pipette tip with an aerosol barrier for each

reaction component.

ATTENTION! The reaction mixture components should be mixed immediately before the analysis.

Before preparing the reaction mixtures, it is necessary to wet clean the PCR box, as well as the equipment and materials contained in it, using disinfectants suitable for use in PCR laboratories, turn on the UV lamp for 20-30 minutes. Before the test, it is necessary to defrost the kit components at room temperature.

1. Thoroughly mix the contents of the tubes with the isolated DNA, PCR buffer, primer mixes, CS1, CS2, NC and PC turning upside down each tube 10 times or mixing on a vortex at low speed for 3-5 seconds, then precipitate drops from the tube lids by short centrifugation.

2.1 **For quantitative assay**, select the required number of 0.1-0.2 ml tubes for PCR based on the calculation for each multiplex used: the test samples number is $2 + 1 \times \text{PC} + 1 \times \text{NC} + 2 \times \text{CS1} + 2 \times \text{CS2}$ (Table 13).

Table 13 – Labeling principle of test tubes for quantitative assay

| | Test samples | | | CS1 | | CS2 | | PC | NC |
|---------------------|--------------|---|---|-----|---|-----|---|----|----|
| | 1 | 2 | n | | | | | | |
| Primer mix HPV-No.1 | + | + | + | + | + | + | + | + | + |
| Primer mix HPV-No.2 | + | + | + | + | + | + | + | + | + |
| Primer mix HPV-No.3 | + | + | + | + | + | + | + | + | + |
| Primer mix HPV-No.4 | + | + | + | + | + | + | + | + | + |
| Primer mix HPV-No.5 | + | + | + | + | + | + | + | + | + |
| Primer mix HPV-No.6 | + | + | + | + | + | + | + | + | + |
| Primer mix HPV-No.7 | + | + | + | + | + | + | + | + | + |

2.2 **For qualitative assay**, select the required number of 0.1-0.2 ml PCR tubes for each multiplex used: test samples number is $^2 + 1 \times \text{PC} + 1 \times \text{NC}$ (Table 14).

Table 14 – Labeling principle of test tubes for qualitative assay

| | Test samples | | | PC | NC |
|---------------------|--------------|---|---|----|----|
| | 1 | 2 | n | | |
| Primer mix HPV-No.1 | + | + | + | + | + |
| Primer mix HPV-No.2 | + | + | + | + | + |
| Primer mix HPV-No.3 | + | + | + | + | + |
| Primer mix HPV-No.4 | + | + | + | + | + |
| Primer mix HPV-No.5 | + | + | + | + | + |
| Primer mix HPV-No.6 | + | + | + | + | + |
| Primer mix HPV-No.7 | + | + | + | + | + |

² To improve accuracy, it is recommended to analyze each sample twice.

10.1. Quantitative testing procedure

PCR assay consists of the following stages:

1. PCR preparation;
2. DNA PCR amplification and hybridization-fluorescence detection of amplification products in real time;
3. Result interpretation.

A) PCR Preparation

(carried out in pre-PCR area – a room for reagent dispensing and preparation for PCR amplification)

Total reaction volume is 25 µl.

ATTENTION! It is forbidden to change the reaction volume. If the volume is changed, the method sensitivity decreases dramatically!

To prepare a reaction mixture for 1 reaction, you need:

1. PCR buffer – 4 µl,
2. A corresponding primer mix (HPV-No.1 (6, 11, 44), HPV-No.2 (16, 18, 26), HPV-No.3 (31, 33, 35), HPV-No.4 (39, 45, 51), HPV-No.5 (52, 68, 56), HPV-No.6 (58, 59, 66), HPV-No.7 (53, 73, 82) – 10 µl,
3. A sample (DNA, PC, CS1, CS2, NC test sample) is 6 µl.

It is necessary to prepare reaction tubes in the following order:

1. Mark 0.2 ml PCR tubes. For each multiplex, the required number of tubes for the test samples is taken + 1 x PC + 2 x CS1 + 2 x CS2 + 1 x NC.
2. Add 4 µl of PCR buffer³ to each tube.
3. Add 10 µl of primer mixes into the test tubes for the corresponding multiplexes.
4. Add 6 µl of isolated DNA to the corresponding test tubes for the test samples. DNA is not added to PC and NC tubes.
5. Add 6 µl of CS1 to the corresponding 2 tubes of each multiplex used.
6. Add 6 µl of CS2 to the corresponding 2 tubes of each multiplex used.
7. Add 6 µl of PC to the appropriate tubes of each multiplex used.

³ It is recommended to first prepare a mixture of primer mix and PCR buffer for each multiplex in a separate 1.5-2.0 ml tube at the rate of: (n+7) x 4 µl of PCR buffer + (n+7)x10 µl of the corresponding primer mix, where n is the number of samples. Mix on a vortex, precipitate the drops by short centrifugation and add 14 µl to the PCR tubes for the corresponding multiplex.

8. Add 6 µl of NC to the corresponding tubes of each multiplex used.
9. Centrifuge the tubes for 1-3 seconds on a vortex microcentrifuge to precipitate drops from the walls.

10.2. Qualitative testing procedure

PCR assay consists of the following stages:

1. PCR preparation;
2. DNA PCR amplification and hybridization-fluorescence detection of amplification products in real time;
3. Result interpretation.

A) PCR Preparation

(carried out in pre-PCR area – a room for reagent dispensing and preparation for PCR amplification.

Total reaction volume is 25 µl.

ATTENTION! It is forbidden to change the reaction volume. If the volume is changed, the method sensitivity decreases dramatically!

To prepare a reaction mixture based on 1 reaction, you need:

1. PCR buffer – 4 µl,
2. An appropriate primer mix (HPV-No.1 (6, 11, 44), HPV-No.2 (16, 18, 26), HPV-No.3 (31, 33, 35), HPV-No.4 (39, 45, 51), HPV-No.5 (52, 68, 56), HPV-No.6 (58, 59, 66), HPV-No.7 (53, 73, 82) – 10 µl.
3. A sample (DNA, PC, NC test sample) is 6 µl.

It is necessary to prepare reaction tubes in the following order:

1. Mark PCR 0.2 ml tubes. For each multiplex, the required number of test tubes for the test samples is taken + 1 x PC + 1 x NC.
2. Add 4 µl of PCR buffer⁴ to each tube.
3. Add 10 µl of primer mixes into the test tubes for the corresponding multiplexes.
4. Add 6 µl of isolated DNA to the corresponding test tubes for the test samples. DNA is not added to the PC and NC tubes.

⁴ It is recommended to first prepare a mixture of primer mix and PCR buffer for each multiplex in a separate 1.5-2.0 ml tube at the rate of: (n+3)x4 µl of PCR buffer + (n+3)x10 µl of the corresponding primer mix, where n is the number of samples. Mix on a vortex, precipitate the drops by short centrifugation and add 14 µl to the PCR tubes for the corresponding multiplex.

5. Add 6 µl of PC to the corresponding tubes of each multiplex used.
6. Add 6 µl of PC to the corresponding tubes of each multiplex used.
7. Centrifuge the tubes for 1-3 seconds on a vortex microcentrifuge to precipitate drops from the walls.

DNA PCR amplification and hybridization-fluorescence detection of amplification products in real time;

(carried out in pre-PCR area – a room for PCR amplification)

1. Place the test tubes in the reaction module of the real-time PCR device. It is recommended to place the test tubes in the center of the thermoblock to evenly press the test tubes with a heating lid.

2. Program the device to perform the corresponding program for the fluorescent signal amplification and detection, following the instructions for the device used. The PCR protocol for DTprime cyclers (NPO DNA Technology LLC, Russia), QuantStudio 5 (Thermo Fisher Scientific, USA), Rotor-Gene Q (Qiagen, Germany), CFX 96 (Bio-Rad, USA), FLUORITE (Xian TianLong Science and Technology Co, China) is shown in Table 15.

Table 15 – PCR protocol for HPV-test

| Stage | Temperature, °C | Time, min:sec | Detection channels | Total cycles |
|-------|-----------------|---------------|-----------------------------|--------------|
| 1 | 95 | 02:00 | - | 1 |
| 2 | 95 | 00:15 | - | 5 |
| | 64 | 00:20 | | |
| 3 | 95 | 00:15 | - | 40 |
| | 64 | 00:20 | FAM, ROX, HEX, Cy5 | |

3. Specify samples number and identifiers, mark the tubes location on the thermoblock matrix of in accordance with their placement.

4. Make sure that FAM, HEX, ROX, Cy5 detection channels are involved in the optical measurement parameters of the amplification program.

5. Start PCR with fluorescent signal detection.

6. At the end of the program, start analyzing the results.

11. Result registration and interpretation

Results registration is carried out automatically upon PCR completion with the used device software.

Recommendations on setting the threshold line

For cyclers of any models, the threshold line is set individually for each detection channel at a level corresponding to 5-20% of the maximum fluorescence level obtained for a positive control sample in the last amplification cycle.

ATTENTION! If a cycler Rotor-Gene is used, activate the functions Dynamic Tube, Noise slope correction, set 15% value in the section Outlier Removal.

In the case of using QuantStudio 5 cycler, it is necessary to adjust the optical filters before starting the amplification protocol (VIC signal may be registered by the ROX channel in case of $\Delta Rn > 100\ 000$, which may lead to false positive results)! To do this, press Action button in Method tab, then in the pop-up menu select Optical filter settings, in PCR Filter section leave only the following filter combinations: x1 - m1, x2 - m2, x4 - m4, x5 - m5, x6 - m6.

The result interpretation is performed using the Ct values of the FAM, ROX, HEX, Cy5 channels (Table 1).

First, the reaction progress and Ct values in the control samples are evaluated. Test samples results interpretation begins only after the correct PC and NC passage.

Result interpretation in control samples

The following results should be obtained for NC and PC (Table 16).

Table 16 – Test results for NC and PC

| Control sample | The selected fluorophore | | | |
|--------------------------------|--------------------------|---------------------|---------------------|---------------------|
| | FAM | HEX | ROX | Cy5 |
| For HPV-test-21 multiplexes | | | | |
| NC | Ct>35 or missing | Ct>35 or missing | Ct>35 or missing | Ct>35 or missing |
| PC | Ct ≤30 | Ct ≤30 | Ct ≤30 | Ct ≤30 |
| CS1 and CS2 ⁵ | E = 90–110% | E = 90–110% | E = 90–110% | E = 90–110% |
| For HPV-test-16/18 multiplexes | | | | |
| NC | Ct>35 or missing | Ct>35 or missing | Ct>35 or missing | - |

| | | | | |
|--------------------------------|----------------------|----------------------|----------------------|----------------------|
| PC | Ct ≤30 | Ct ≤30 | Ct ≤30 | - |
| CS1 and CS2 ⁵ | E = 90–110% | E = 90–110% | E = 90–110% | - |
| For HPV-test-31/33 multiplexes | | | | |
| NC | Ct >35 or missing | Ct >35 or missing | Ct >35 or missing | - |
| PC | Ct ≤30 | Ct ≤30 | Ct ≤30 | - |
| CS1 and CS2 ⁵ | E = 90–110% | E = 90–110% | E = 90–110% | - |
| For HPV test-6/11 multiplexes | | | | |
| NC | Ct >35 or missing | Ct >35 or missing | Ct >35 or missing | - |
| PC | Ct ≤30 | Ct ≤30 | Ct ≤30 | - |
| CS1 and CS2 ⁵ | E = 90–110% | E = 90–110% | E = 90–110% | - |
| For HPV-test-14 multiplexes | | | | |
| NC | Ct >35 or missing | Ct >35 or missing | Ct >35 or missing | Ct >35 or missing |
| PC | Ct ≤30 | Ct ≤30 | Ct ≤30 | Ct ≤30 |
| CS1 and CS2 ⁵ | E = 90–110% | E = 90–110% | E = 90–110% | E = 90–110% |

Designation: E – PCR efficiency.

When obtaining values for NC that differ from those indicated in Table 16, the results of the entire series are considered unreliable. In this case, special measures should be taken to eliminate possible contamination.

When obtaining values for PC that differ from those indicated in Table 16, repeated amplification of the entire sample batch is required. When reobtaining values for PC that differ from those indicated in Table 16, it is necessary to replace the reagents.

⁵ For quantitative assay only.

Result interpretation in control samples

Result interpretation principles for the tested clinical samples are shown in Table 17.

Table 17 – Result interpretation principle

| Multiplex | FAM | ROX | Cy5 | HEX | Result |
|-----------|-----------------------|-----|--------------------------|---|--------|
| HPV-No.1 | 6 | 11 | 44 | SVC | |
| HPV-No.2 | 16 | 18 | 26 | SVC | |
| HPV-No.3 | 31 | 33 | 35 | SVC | |
| HPV-No.4 | 39 | 45 | 51 | SVC | |
| HPV-No.5 | 52 | 68 | 56 | SVC | |
| HPV-No.6 | 58 | 59 | 66 | SVC | |
| HPV-No.7 | 53 | 73 | 82 | SVC | |
| HPV-16/18 | 16 | 18 | – | SVC | |
| HPV-31/33 | 31 | 33 | – | SVC | |
| HPV-6/11 | 6 | 11 | – | SVC | |
| | Ct missing or Ct > 35 | | Ct ≤ 35 | DNA of the corresponding HPV genotype not detected or below the detection limit | |
| | Ct ≤ 35 | | not indicated | DNA of the corresponding HPV genotype is detected | |
| | Ct missing or Ct > 35 | | Ct > 35 or missing | Invalid result | |

Low DNA concentration, the presence of inhibitors in the DNA preparation obtained from clinical material, incorrect test protocol implementation, non-compliance with the PCR temperature regime, etc. may be the cause of invalid results. In case of an invalid result, a conclusion is not issued, it is necessary to recollect the patient's biomaterial and reperform the test.

ATTENTION! Only when the interpretation conditions in Table 17 are met, proceed to the evaluation of the quantitative assays (if necessary).

Quantitative assay result interpretation in the tested clinical samples

Result interpretation is carried out automatically using the software supplied with the detection cycler used, or manually. Based on the obtained Ct values for calibration samples and their concentrations, it is necessary to draw a calibration line. CS1 contains all target analytes (HPV genotypes and human genomic DNA) at 10^6 copies/ml concentration; CS2 – 10^4 copies/ml.

PCR efficiency should be in the range of 90 to 110%; otherwise, it is necessary to reperform the test, starting from the DNA isolation stage. If several primer mixes are present in one run, calibrators must be assigned to each genotype separately. Accordingly, individual genotypes HPV concentrations must be produced using calibrators of the corresponding multiplex. Thus, for example, samples with reaction mixture No.1 are analyzed first with the corresponding calibrators assignment, then with reaction mixture No.2, and so on. When using a calibration line, HPV DNA of each detected genotype and human DNA absolute concentrations in copies/mL are calculated.

The values obtained are used to calculate HPV DNA amount. Measurement accuracy: ± 0.5 lg concentration.

If target analyte concentration is between 1×10^3 and 2×10^9 copies/ml, the exact concentration in copies/ml is indicated. If the concentration is less or more than the specified range, the results are "concentration less than 1000 copies/ml" or "concentration more than 2×10^9 copies/ml", respectively, without specifying the exact value.

The obtained exact concentration values can also be used to calculate HPV DNA amount per 10^5 human cells (relative concentration), according to the formula:

$$C_{rel} = \frac{\text{the number of HPV DNA copies in ml}}{\text{the number of human DNA copies in ml}} \times 2 \times 10^5$$

Diagnostic value of the obtained test result:

The obtained results can be used for detection and typing of HPV DNA of 6, 11, 44, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 68, 56, 58, 59, 66, 53, 73, 82 types (type set may differ in different configuration forms) in human biological material.

To calculate clinically significant HPV concentrations of high carcinogenic risk for women, it is recommended to use the following indicators ⁶:

- from 10^7 copies/ml or more – high HPV load (risk of cervical cancer);
- 10^5 – 10^7 copies/ml - moderate HPV load (CIN presence);
- less than 10^5 copies/ml is a clinically insignificant HPV amount.

To calculate clinically significant HPV concentrations of high carcinogenic risk for males, it is recommended to use the following indicators ⁷:

- from 10^5 copies /ml or more – clinically highly significant viral load, risk of progression;
- 10^3 – 10^5 copies /ml - clinically significant viral load, indicates the virus persistence;
- less than 10^3 copies/ml is a clinically insignificant viral load.

Diagnosis and treatment should be determined by a specialist doctor in combination with clinical observations, medical history and epidemiological information.

12. Storage, transportation and usage conditions

Storage

HPV-test reagent kit in the manufacturer's package should be stored at the temperature from -16 to -24°C during the entire kit shelf life. It is allowed to store at a temperature from 2 to 8°C up to 14 days.

It is not allowed to freeze/thaw HPV-test kit more than 10 times.

After opening store the reagents under the same conditions as before opening.

A reagent kit stored in violation of the regulated regime cannot be used.

⁶ In accordance with the clinical recommendations of the Ministry of Health of the Russian Federation "Benign and precancerous diseases of the cervix from the perspective of cancer prevention". Moscow, 2017.

⁷ In accordance with the clinical recommendations of the Ministry of Health of the Russian Federation "Penile cancer". 2020.

Transportation

HPV-test reagent kit should be transported by all types of covered vehicles in accordance with transportation rules applicable to this transport type.

Transport HPV-test reagent kit at temperatures from -18 to -22°C during the entire kit shelf life. Transportation is allowed at temperatures from 2 to 8°C up to 14 days.

Atmospheric pressure is not controlled, as it does not affect the product quality.

To ensure compliance with transportation conditions throughout the entire transportation period, a reagent kit is placed in a reusable polyurethane foam thermal container for temporary storage and transportation with prepared ice packs. The type, volume and quantity of ice packs placed in a thermal container with transported reagent kits, as well as the volume of the thermal container are selected depending on the duration and transportation conditions.

Reagent kits transported in violation of the temperature regime cannot be used.

Shelf life

HPV-test reagent kit shelf life is 12 months from the acceptance date of the manufacturer's QCD (Quality Control Dept) at a temperature from -16 to -24°C, if all transportation and storage conditions are met. A reagent kit with an expired shelf life cannot be used.

Shelf life of the opened kit components

12 months from the date of acceptance of the manufacturer's QCD, if stored at temperatures from -16 to -24°C.

Shelf life of the reaction mixture prepared for PCR analysis (stage "PCR preparation")

One hour at a temperature from 2 to 8 °C under conditions that prevent the components from drying out, as well as extraneous biological material contamination.

13. Disposal

Reagent kits that have become unusable including the ones with expired shelf life, are subject to disposal in accordance with sanitary and epidemiological requirements for the management of medical waste.

According to medical waste classification the kits belong to Class A (epidemiologically safe waste, which is similar in composition to solid household waste).

Unused reagents are collected in a single-use labeled packaging of any color (except yellow and red) in accordance with sanitary and epidemiological requirements for the management of medical waste.

Used tubes and materials are disposed of in accordance with the requirements for disinfection, pre-sterilization, cleaning and sterilization of medical devices.

Liquid components (reagents, chemical agents) are disposed by draining into a sewer with a reagent preliminary dilution with tap water 1:100 and removing the packages remains as industrial or household garbage.

HPV-test reagent kit consumer package is subject to mechanical destruction with the removal of residues as industrial or household waste.

Personnel destroying a reagent kit must comply with the safety rules of a particular destruction method.

14. Warranty, contacts

The manufacturer guarantees HPV-test kit compliance with the TS while meeting the established requirements for transport, storage and operation.

If you have any complaints about the quality of the kits, please contact:

Limited Liability Company Testgene (Testgene LLC)

9, 44th Ingenuity proezd, office 13, Ulyanovsk, 432072, Russia

Phone number: +7 499 705 03 75









www.testgene.com

Technical Support Service:

Phone number: +7 927 981 58 81

E-mail: help@testgen.ru

Label symbols

| Symbol | Explanation |
|---|--|
|  | Please refer to the instructions for use |
|  | Kit for in vitro diagnostics |
|  | Temperature range |
|  | Batch code |
|  | Use before... |
|  | Production date |
|  | Fragile, handle with care |
|  | "Top" symbol shows the correct position of the load in space. The arrows point to the top of the content. Transport packaging with this symbol must not be turned over or piled on its side. It must be stored and transported upright only. |