
# Stark Genotyping HPV Molecular Diagnostic Kit

IVD

CE

Contents

## Description

The Stark Genotyping HPV Molecular Diagnostic Kit utilizes in vitro nucleic acid TaqMan assay technology, employing polymerase chain reaction for the genotyping detection of 14 high-risk and two low-risk types of HPV DNA in cervical or vaginal specimens. The high-risk HPV types detected include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68, alongside low-risk types 6 and 11. This test is intended exclusively for professional use by trained and validated laboratory personnel. The Kit employs a three-Channels Multiplex Real-Time PCR system and includes an internal control to enhance isolation quality and prevent false PCR inhibition results.

## Intended Use

The Stark HPV Genotyping Molecular Diagnostic Kit (Real-Time PCR) is intended for in vitro diagnostic use. This Kit is designed for the qualitative detection and genotyping of human papillomavirus (HPV) DNA in cervical or vaginal specimens. Results obtained are for professional use only by trained and validated laboratory personnel. This Kit aids in identifying HPV infections, assisting healthcare professionals in making informed decisions regarding patient management and treatment strategies. Careful adherence to provided instructions is crucial to ensure accurate and reliable results.

## Kit Contents

| Ingredients | 25 Preps (REF: ST242001) |
|---|---|
| Q-ROMAX, 4X | 600µl |
| Pro 1 HPV Mix | 350µl |
| Pro 2 HPV Mix | 350µl |
| Pro 3 HPV Mix | 350µl |
| Pro 4 HPV Mix | 350µl |
| Positive Control | 150µl |
| Water (PCR Grade) | 150µl |

## Storage

Ensure all reagents are stored away from direct light in darkness at temperatures ranging between -20°C to -25°C. Avoid frequent freeze-thaw cycles. When stored as directed, all reagents maintain stability until the expiration date indicated on the Kit box.

## Guarantee and Warranty

CARBON Technologies LLC assures the efficiency of all manufactured Kits and reagents. For guidance on selecting the appropriate Kits for your requirements, please reach out to our technical support team. Should the products fail to meet your satisfaction for reasons other than misuse, please contact our technical support team. In the event of issues stemming from the manufacturing process, CARBON Technologies LLC will promptly replace the Kit.

## Warning and Precautions

- This Kit is for in vitro diagnostic use only.
- Material Safety Data Sheets (MSDS) for all products and reagents are available online at www.carbontechnologiesco.com.
- Adhere to laboratory safety protocols diligently.
- Familiarize yourself thoroughly with the guidelines before usage.
- Prohibit eating, drinking, smoking, chewing gum, applying cosmetics, or taking medicine in laboratories where hazardous materials and human samples are handled.
- Treat all patient samples and positive controls as potentially infectious.

- Use the Stark Genotyping HPV Molecular Diagnostic Kit under the supervision of a physician for emergency and in vitro diagnostic purposes.
- Ensure all steps of procedures, including sampling, storage, shipping, and laboratory tests, comply with biosafety and molecular laboratory management standards.
- Equip clinical laboratories with instruments and operators in accordance with the regulations of the Ministry of Health.
- Any alteration or replacement of Kit contents may affect functionality and contravene product licensing.
- Utilize sterile and DNase-RNase-free pipette tips and microtubes to prevent contamination. Change filter pipette tips after each substance or sample addition.
- Dispose of waste in line with biosafety guidelines. Regularly sanitize desks and laboratory instruments with 70% Ethanol or 10% Sodium Hypochlorite.
- Shield Pro Mixes from sunlight exposure.
- Clean and disinfect specimen spills promptly using suitable disinfectants adhering to national and local regulations.
- Dispose of all specimens, reagents, and potentially contaminated materials following national and local regulations.
- The hazard and precautionary statements provided pertain to the components of the Stark Genotyping HPV Molecular Diagnostic Kit.

### Quality Control

The Stark Genotyping HPV Molecular Diagnostic Kit undergoes rigorous testing against predetermined experiments on a lot-to-lot basis to ensure consistent product quality. Accessible results of these experiments are obtainable online by referencing the REF and Lot numbers at www.carbontechnologiesco.com.

### Materials Required (but Not Provided)

- DNase-RNase-free microtubes (1.5ml)
- PCR microtube 0.1ml or 0.2ml strip
- Various models of pipettes and pipette tips (10µl, 100µl, and 1000µl filter pipette tips)
- Surface sanitizing solution
- Disposable Powder-Free gloves and surgical gown
- Three-Channels Multiplex Real-Time PCR Instrument (with green, yellow, and orange channels)
- Vortex
- Cool box

### Procedures

The Stark Genotyping HPV Molecular Diagnostic Kit employs a polymerase chain reaction of Real-Time PCR for its testing procedure. Specifically designed for genotyping molecular diagnosis of L1, E1, E2, E6, and E7 genes of the Human Papillomavirus (HPV), this Kit facilitates nucleic acid isolation using the DNall VirAll Kit or other approved Kits. Verified sample combinations are then added to the master mix primer/probe mix to initiate the reaction. Additionally, the Stark Genotyping HPV Molecular Diagnostic Kit incorporates a second heterologous amplification system to identify potential PCR inhibition. This is detected as an internal control (IC) in the fluorescence channel Cycling Yellow of Real-Time PCR instruments. Through its sampling mechanism, the quality of sample isolation and PCR reaction process can be monitored and controlled to prevent false-negative results. The assay demonstrates a Limit of Detection (LoD) of 5 Copies/5µl for types 16, 18, and 45, 50 Copies/5µl for types 6/11, 51, 56/66, 33/52/58, 35, 59, and 39, and 500 Copies/5µl for types 68 and 31.

### Applications

The Stark Genotyping HPV Molecular Diagnostic Kit technology is an in vitro nucleic acid TaqMan assay employing signal amplification through polymerase chain reaction and fluorescent probes (ROX/Texas Red, Yakima Yellow, and FAM). It is utilized for the genotyping detection of 14 high-risk and two low-risk types of HPV DNA in cervical or vaginal specimens. The high-risk HPV types detected include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68, along with low-risk types 6 and 11. This diagnostic test Kit is applicable in human cervical screening, cytology samples, urine, and paraffin-embedded tissue analysis. Utilizing the polymerase chain reaction (PCR), the Kit is configured with three-Channels Multiplex Real-Time PCR instruments.

### Features

**Table 1**: Stark Genotyping HPV Molecular Diagnostic Kit features and specifications

| Technology | Real-Time PCR |
|---|---|
| Type of Analysis | Genotyping |
| Target Sequence | L1, E1, E2, E6 and E7 genes |
| Analytical Specificity | high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68<br>low-risk HPV types 6 and 11 |
| Analytical Sensitivity (LOD) | for types 16, 18, 45 is 5 Copies/5µl, for types 6/11, 51, 56/66, 33/52/58, 35, 59, 39 is 50 Copies/5µl and for types 68, 31 is 500 Copies/5µl. |
| Diagnostic Specificity | 100% |
| Diagnostic Sensitivity | 98.52% |
| Extraction/Inhibition Control | PCR inhibition and DNA extraction efficiency control |
| Validated Specimen | Cervical screening, Pap smear, Urine and Paraffin-embedded tissue sample |
| Storage | -20 ± 5°C |
| Validated Extraction Method | DNall VirAll Kit |
| Instruments | Rotor-Gene Q, 2plex, Corbett Rotor-Gene 3000&6000, Mic qPCR Cycler, StepOne and StepOne plus Applied Biosystem |
| Required Detection Channels | Green-Yellow-Orange |

### Recommended Starting Material

- Before commencing any tests, ensure each component is thawed, vortexed, and briefly centrifuged. Avoid subjecting the components to repeated freeze-thaw cycles.
- Treat all samples as infectious and biohazardous, following safe laboratory procedures.
- Collect samples from the cervix using a brush or swab, cytology samples, urine, or paraffin-embedded tissue.

### Storage and Sample Preparation

Cervical screening, Pap smear, Urine, and Paraffin-embedded tissue samples:

Fresh specimens should either be processed immediately according to the sample procedure outlined in the Sample Processing Protocol section or stored frozen at -20°C. Frozen samples must be brought to room temperature before initiating sample processing. Sample Pre-treatment decontaminates the specimen and prepares it for extraction.

### DNA Isolation

For nucleic acid isolation, utilize the DNall VirAll Kit or other approved Kits.

### Before Starting

Remove each component from the Kit and place them on the benchtop. Allow the reagents to equilibrate to room temperature, then briefly vortex each tube for later use.

### Buffer Preparation

Take out each component from the Kit and allow the reagents to equilibrate to room temperature. Before use, briefly vortex the components. The total volume of isolated nucleic acid should be 5µl. Refer to Table 1 for buffer preparation and Table 2 for PCR run instructions.

**Table 2**: Preparation of components per single reaction

| Components | Volume |
|---|---|
| Q-ROMAX, 4X | 6µl |
| Pro 1 HPV or Pro 2 HPV or Pro 3 HPV or Pro 4 HPV Mix | 14µl |
| Isolated DNA | 5µl |

### Pathogenicity

Human papillomaviruses (HPVs) are small double-stranded DNA viruses that infect the cutaneous and mucosal epithelium. Infection by specific HPV types has been associated with the development of cervical carcinoma. HPV targets epithelial cells undergoing terminal differentiation and employs multiple mechanisms to override normal differentiation regulation, producing progeny virions. This leads to morphological changes resulting in noncancerous tumors in the skin, commonly referred to as "Papilloma".

Human papillomavirus comprises 150 identified strains categorized into various types. Approximately 75% of papilloma types target epithelial cells (cutaneous types), while the remaining 25% (around four types) target mucosal epithelial tissues, identified as mucosal or genital types. Cutaneous types mainly cause noncancerous epithelial warts in different skin areas, particularly in the lips' epithelial cells, while mucosal types induce genital warts and cancerous tumors, especially cervical cancer.

### Low-risk HPV

Low-risk HPV strains include types that cause noncancerous and low-risk warts in genital areas of both women and men. In women, they predominantly manifest in internal genitalia, such as the vagina and cervix. There are 12 types of papillomaviruses with low cancer risk that can induce warts and morphological changes in the genital area: 6, 11, 40, 42, 43, 44, 53, 54, 61, 72, 73, and 81. Types 6 and 11 are responsible for 90% of genital warts.

### High-risk HPV

High-risk HPV can lead to cancers of the cervix, vagina, and vulva in women; penis in men; anus; and the back of the throat, including the base of the tongue and tonsils (oropharynx), in both men and women. There are 14 types of high-risk HPV viruses, including: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Approximately 70% of reported cervix cancers are caused by HPV 16 and 18. Additionally, HPV 16 is responsible for 95% of oral, vaginal, and anal cancers. HPV 31 and 45 are considered high-risk cancer strains, contributing to 5%-10% of cervix cancers.

### Workstation Preparation

Before usage, all work surfaces, pipettes, and other supplies must be thoroughly cleaned and sanitized. To minimize

the risk of nucleic acid contamination, utilize sanitizers such as 70% Ethanol or 10% Sodium Hypochlorite.

### Protocol

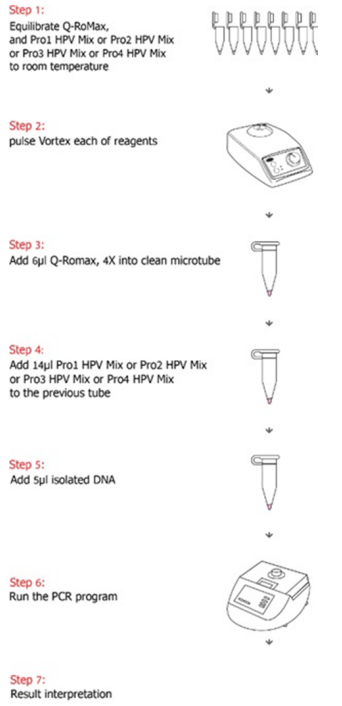


**Figure 1**: preparation of reagents, PCR run, and interpretation of results.

### PCR reaction preparation

**Table 3**: PCR reaction preparation

| Components | Volume |
|---|---|
| Q-ROMAX, 4X | 6µl |
| Pro1 HPV or Pro2 HPV or Pro3 HPV or Pro4 HPV or Mix | 14µl |
| Isolated DNA | 5µl |

### Thermal Profile

**Table 4**: Thermal profile for Stark Genotyping HPV Molecular Diagnostic Kit

| Cycles | Temperature | Incubation Time | Cycle Numbers |
|---|---|---|---|
| Pre-Denaturation | 95 °C | 3 min | 1 |
| Denaturation | 95 °C | 10 sec | 45 |
| Annealing and acquisition on channel Green, Yellow and Orange | 57°C | 30 sec | |

### Results Interpretation

Data analysis for each type should be performed separately using a manual threshold. For the detection of L1, E1, E2, E6, and E7 genes, fluorophores FAM (green), Yakima Yellow (Yellow), and ROX/Texas Red (orange) are utilized for Pro1 HPV Mix to Pro4 HPV Mix. The IC gene in Pro2 HPV Mix is detected using the Yakima Yellow (Yellow) fluorophore.

**Table 5**: Specific fluorescent channels identified for HPV types.

| ProMIX | Green | Yellow | Orange |
|---|---|---|---|
| Pro1 HPV | 45 | 16 | 18 |
| Pro2 HPV | 51 | Internal Control | 56/66 |
| Pro3 HPV | 35/39 | 33/52/58 | 6/11 |
| Pro4 HPV | 31 | 68 | 59 |

### Control Conditions for a Valid PCR Run

A negative control serves as a contamination control. If the magnitude increase of the fluorescence curve in the negative control does not cross the threshold and Ct is less than 35 (Ct<35), it is considered as possible contamination. Strong signals above 35 in the NTC can be PCR artifacts. In such cases, the shape of the curve, typically an S-shaped curve, should be considered for a positive result.

The internal control should yield a positive result for all clinical specimens with Ct ≤ 35, indicating sufficient nucleic acid from the human gene and acceptable sample quality. If the internal control curve has Ct > 37 or lacks Ct, it indicates low sample concentration or inhibitors in the reaction. In such instances, diluting the isolated sample by at least half is recommended. If the test result remains unacceptable upon retesting, obtain a new sample from the patient and repeat the test.

A positive clinical specimen should have Ct ≤ 37 for the gene. If the expected positive reaction, characterized by a typical S-shaped curve, is not achieved, the performed test is deemed unacceptable. Repeat the test following the instructions provided in the Kit catalog.

In case of failure of the positive control, determine the reason, take corrective action, and document the results of the corrective action.

**Table 6**: Control conditions for a valid PCR Run

| ProMIX | ROX/Texas Red | Yakima Yellow | FAM | Results |
|---|---|---|---|---|
| Pro 1 HPV | + | + | + | Positive:45<br>Positive: 16<br>Positive:18 |
| Pro 2 HPV | + | It is not considered | + | Positive:51<br>Positive:56/65 |
| Pro 3 HPV | + | + | + | Positive:35/39<br>Positive:33/52/58<br>Positive:6/11 |
| Pro 4 HPV | + | + | + | Positive:31<br>Positive:68<br>Positive:59 |
| Pro 2 HPV | - | + | - | Negative Result |
| Pro 1 HPV or Pro 2 HPV or Pro 3 HPV or Pro 4 HPV | - | - | - | Invalid results |

Test Limitations

- Low virus titers in patients' specimens, improper transportation, and low-quality DNA isolation can lead to false-negative results.
- All related controls should be checked before result interpretation to ensure reliable results. Failure to do so may compromise the accuracy of the results.
- The limit of detection (LoD) of the present Kit is demonstrated by Ct ≤ 37, and a typical S-shaped curve must appear for all positive specimens.
- Improper storage conditions can result in false-negative results.
- The product should only be used by personnel specially instructed and trained in in-vitro diagnostics, as individual errors can compromise the accuracy of the results.
- Diagnosis of Human papillomavirus infection is made when test results are positive and accompanied by clinical symptoms. Treatment should be conducted based on diagnostic Kit results, medical background, and response to treatment.

Performance Evaluation

- Limit of Detection (LoD) - Analytical Sensitivity

LoD studies were conducted to determine the lowest detectable concentration of HPV DNA, at which approximately 95% of all true positive replicates test positive. The LoD was determined through limiting dilution studies using characterized samples. Analytical sensitivity, considering the purification (DNall VirAll Kit) of the Stark Genotyping HPV Molecular Diagnostic Kit, was determined using a dilution series of standards ranging from 5 to nominal 50 and 500 HPV genome equivalents (GE)/5µl spiked in clinical cervical specimens.

The LoD of each test was confirmed by testing 20 replicates with a dilution series (500, 50, 5 genome equivalents (GE)/5µl) at the tentative limit of detection. The final LoD of each test was determined as the lowest dilution series resulting in positive detection of 19 out of 20 replicates.
The LoD of the Stark Genotyping HPV Molecular Diagnostic Kit was established using the DNall VirAll Kit. Results demonstrated that the LoD of the assay for types 16, 18, and 45 is 5 Copies/5µl, for types 6/11, 51, 56/66, 33/52/58, 35, 59, and 39 is 50 Copies/5µl, and for types 68 and 31 is 500 Copies/5µl.

**Table7**: Detection Results of Stark Genotyping HPV Molecular Diagnostic Kit Using DNall VirAll Kit.

| Test No | (Copies/5µl) | | |
|---|---|---|---|
| | HPV type 16 | | |
| | 500 | 50 | 5 |
| 1 | 32.14 | 34.04 | 36.04 |
| 2 | 32.51 | 35.14 | 36.14 |
| 3 | 32.57 | 35.37 | 36.47 |
| 4 | 32.01 | 34.57 | 35.41 |
| 5 | 32.11 | 34.91 | 34.95 |
| 6 | 31.75 | 34.87 | 35.21 |
| 7 | 32.67 | 34.18 | 36.74 |
| 8 | 32.84 | 35.77 | 37.54 |
| 9 | 31.47 | 35.24 | 36.25 |

| | | | |
|---|---|---|---|
| 10 | 32.55 | 34.44 | 37.64 |
| 11 | 31.94 | 34.15 | Undetermined |
| 12 | 31.27 | 34.27 | 35.73 |
| 13 | 31.53 | 35.04 | 36.28 |
| 14 | 31.84 | 35.21 | 37.42 |
| 15 | 31.95 | 35.11 | 36.08 |
| 16 | 32.01 | 35.29 | 36.47 |
| 17 | 32.43 | 34.77 | 37.51 |
| 18 | 31.47 | 34.69 | 37.69 |
| 19 | 32.63 | 34.94 | 36.73 |
| 20 | 32.77 | 34.23 | 36.47 |
| Positive percentage in each concentration | 100% | 100% | 95% |

| Test No | (Copies/5μl) | | |
|---|---|---|---|
| | HPV type 18 | | |
| | 500 | 50 | 5 |
| 1 | 33.19 | 35.18 | 37.10 |
| 2 | 34.33 | 35.46 | 36.87 |
| 3 | 33.61 | 36.35 | 36.49 |
| 4 | 33.12 | 36.49 | Undetermined |
| 5 | 34.40 | 35.13 | 36.77 |
| 6 | 33.07 | 35.02 | 37.14 |
| 7 | 33.41 | 35.49 | 37.04 |
| 8 | 34.34 | 36.20 | 37.24 |
| 9 | 33.53 | 35.82 | 38.08 |
| 10 | 33.89 | 35.61 | 37.53 |
| 11 | 34.06 | 36.63 | 36.94 |
| 12 | 33.38 | 36.36 | 38.91 |
| 13 | 33.29 | 35.98 | 36.28 |
| 14 | 34.72 | 36.62 | 37.10 |
| 15 | 33.76 | 35.15 | 37.27 |
| 16 | 34.79 | 35.11 | 37.24 |
| 17 | 33.78 | 36.46 | 37.91 |
| 18 | 33.22 | 35.53 | 37.21 |
| 19 | 34.44 | 36.06 | 37.66 |
| 20 | 33.69 | 36.15 | 37.14 |
| Positive percentage in each concentration | 100% | 100% | 95% |

| Test No | (Copies/5μl) | | |
|---|---|---|---|
| | HPV type 45 | | |
| | 500 | 50 | 5 |

| | | | |
|---|---|---|---|
| 1 | 33.19 | 35.18 | 37.10 |
| 2 | 34.33 | 35.46 | 36.87 |
| 3 | 33.61 | 36.35 | 36.49 |
| 4 | 33.12 | 36.49 | Undetermined |
| 5 | 34.40 | 35.13 | 36.77 |
| 6 | 33.07 | 35.02 | 37.14 |
| 7 | 33.41 | 35.49 | 37.04 |
| 8 | 34.34 | 36.20 | 37.24 |
| 9 | 33.53 | 35.82 | 38.08 |
| 10 | 33.89 | 35.61 | 37.53 |
| 11 | 34.06 | 36.63 | 36.94 |
| 12 | 33.38 | 36.36 | 38.91 |
| 13 | 33.29 | 35.98 | 36.28 |
| 14 | 34.72 | 36.62 | 37.10 |
| 15 | 33.76 | 35.15 | 37.27 |
| 16 | 34.79 | 35.11 | 37.24 |
| 17 | 33.78 | 36.46 | 37.91 |
| 18 | 33.22 | 35.53 | 37.21 |
| 19 | 34.44 | 36.06 | 37.66 |
| 20 | 33.69 | 36.15 | 37.14 |
| Positive percentage in each concentration | 100% | 100% | 95% |

| Test No | (Copies/5μl) | | |
|---|---|---|---|
| | HPV type 33/52/58 | | |
| | 500 | 50 | 5 |
| 1 | 36.17 | 37.16 | Undetermined |
| 2 | 35.42 | 38.06 | 39.38 |
| 3 | 35.61 | 37.61 | 39.49 |
| 4 | 35.52 | 37.82 | 39.52 |
| 5 | 35.49 | 38.13 | 41.01 |
| 6 | 36.07 | 38.41 | 41.48 |
| 7 | 35.41 | 37.49 | 41.59 |
| 8 | 35.37 | 37.84 | Undetermined |
| 9 | 35.53 | 37.71 | Undetermined |
| 10 | 35.89 | 37.61 | Undetermined |
| 11 | 36.06 | 37.88 | Undetermined |
| 12 | 35.36 | 37.46 | 40.91 |
| 13 | 35.52 | 38.02 | 41.28 |
| 14 | 35.92 | 37.49 | 41.60 |
| 15 | 35.76 | 37.92 | Undetermined |
| 16 | 35.62 | 37.91 | Undetermined |
| 17 | 36.71 | 37.77 | Undetermined |

| | | | |
|---|---|---|---|
| 18 | 36.47 | 37.15 | Undetermined |
| 19 | 36.51 | 38.08 | Undetermined |
| 20 | 36.01 | 38.25 | Undetermined |
| Positive percentage in each concentration | 100% | 100% | 45% |

| Test No | (Copies/5μl) | | |
|---|---|---|---|
| | HPV type 39 | | |
| | 500 | 50 | 5 |
| 1 | 36.61 | 38.11 | Undetermined |
| 2 | 37.37 | Undetermined | Undetermined |
| 3 | 37.88 | 38.15 | Undetermined |
| 4 | 37.02 | 38.19 | Undetermined |
| 5 | 37.59 | 38.72 | Undetermined |
| 6 | 37.07 | 38.02 | Undetermined |
| 7 | 37.41 | 37.82 | 41.04 |
| 8 | 37.92 | 37.60 | Undetermined |
| 9 | 36.53 | 37.42 | 41.58 |
| 10 | 36.89 | 37.72 | 40.83 |
| 11 | 37.06 | 37.53 | Undetermined |
| 12 | 37.41 | 37.73 | 40.91 |
| 13 | 37.28 | 37.92 | 41.48 |
| 14 | 37.01 | 38.16 | Undetermined |
| 15 | 36.03 | 38.35 | Undetermined |
| 16 | 37.38 | 37.91 | Undetermined |
| 17 | 36.79 | 38.12 | Undetermined |
| 18 | 37.12 | 38.02 | 41.31 |
| 19 | 36.66 | 38.27 | Undetermined |
| 20 | 37.50 | 37.85 | Undetermined |
| Positive percentage in each concentration | 100% | 95% | 30% |

| Test No | (Copies/5μl) | | |
|---|---|---|---|
| | HPV type 59 | | |
| | 500 | 50 | 5 |
| 1 | Undetermined | 37.78 | 40.10 |
| 2 | 35.44 | 38.06 | 40.17 |
| 3 | 35.83 | 37.66 | 41.59 |
| 4 | 36.02 | 37.92 | 40.58 |
| 5 | 35.89 | Undetermined | 40.44 |
| 6 | 36.07 | 37.02 | Undetermined |
| 7 | 35.48 | 37.19 | 41.04 |
| 8 | 35.55 | 38.60 | Undetermined |

| | | | |
|---|---|---|---|
| 9 | 35.53 | 38.12 | 40.58 |
| 10 | 35.89 | 37.51 | 41.53 |
| 11 | 36.06 | 38.12 | Undetermined |
| 12 | 36.16 | 38.66 | 41.91 |
| 13 | 36.28 | 37.98 | 42.28 |
| 14 | 35.92 | 37.66 | 40.60 |
| 15 | 35.16 | 38.15 | Undetermined |
| 16 | 35.76 | 39.94 | Undetermined |
| 17 | 35.66 | 38.46 | Undetermined |
| 18 | 36.22 | 39.09 | Undetermined |
| 19 | 36.04 | 37.88 | 40.15 |
| 20 | 36.44 | 37.52 | Undetermined |
| Positive percentage in each concentration | 95% | 95% | 60% |

| Test No | (Copies/5μl) | | |
|---|---|---|---|
| | HPV type 35 | | |
| | 500 | 50 | 5 |
| 1 | 36.27 | 38.28 | 41.01 |
| 2 | 37.20 | 39.06 | 41.52 |
| 3 | 37.61 | 38.15 | 41.49 |
| 4 | 36.02 | 39.79 | 37.04 |
| 5 | Undetermined | 39.13 | 39.22 |
| 6 | 37.07 | 40.02 | Undetermined |
| 7 | 37.40 | 40.49 | 39.04 |
| 8 | 37.37 | 40.60 | Undetermined |
| 9 | 36.53 | 39.42 | 40.58 |
| 10 | 37.89 | Undetermined | Undetermined |
| 11 | 38.06 | 40.63 | Undetermined |
| 12 | 37.36 | 39.86 | 41.21 |
| 13 | 38.18 | 39.98 | Undetermined |
| 14 | 36.92 | 39.56 | 40.10 |
| 15 | 37.76 | 40.15 | Undetermined |
| 16 | 36.66 | 41.01 | Undetermined |
| 17 | 37.72 | 40.46 | Undetermined |
| 18 | 36.21 | 39.33 | Undetermined |
| 19 | 37.44 | 39.06 | Undetermined |
| 20 | 37.31 | 39.09 | Undetermined |
| Positive percentage in each concentration | 95% | 95% | 45% |

| Test No | (Copies/5μl) |
|---|---|
| | HPV type 68 |

| | 500 | 50 | 5 |
|---|---|---|---|
| 1 | 37.27 | Undetermined | Undetermined |
| 2 | 37.12 | Undetermined | Undetermined |
| 3 | 37.71 | 40.13 | Undetermined |
| 4 | 38.92 | 40.49 | Undetermined |
| 5 | Undetermined | 39.81 | Undetermined |
| 6 | 39.07 | 40.21 | Undetermined |
| 7 | 37.41 | Undetermined | Undetermined |
| 8 | 38.37 | 39.21 | Undetermined |
| 9 | 37.13 | 40.12 | Undetermined |
| 10 | 38.89 | Undetermined | Undetermined |
| 11 | 38.12 | Undetermined | Undetermined |
| 12 | 37.31 | 39.56 | 40.91 |
| 13 | 38.28 | 40.18 | 40.32 |
| 14 | 38.92 | Undetermined | 41.04 |
| 15 | 38.16 | Undetermined | Undetermined |
| 16 | 37.23 | Undetermined | Undetermined |
| 17 | 37.72 | Undetermined | Undetermined |
| 18 | 38.12 | Undetermined | 39.78 |
| 19 | 38.54 | Undetermined | 39.17 |
| 20 | 38.29 | Undetermined | Undetermined |
| Positive percentage in each concentration | 95% | 40% | 2%5 |

| Test No | (Copies/5μl) | | |
|---|---|---|---|
| | HPV type 6/11 | | |
| | 500 | 50 | 5 |
| 1 | 35.27 | 37.28 | Undetermined |
| 2 | 35.87 | 38.06 | 41.57 |
| 3 | 35.61 | 37.84 | 41.49 |
| 4 | 36.02 | 37.14 | Undetermined |
| 5 | 35.49 | Undetermined | Undetermined |
| 6 | 37.07 | 37.02 | Undetermined |
| 7 | 35.41 | 37.49 | 40.04 |
| 8 | 35.37 | 38.60 | 40.24 |
| 9 | 36.53 | 37.42 | 40.58 |
| 10 | 35.89 | 38.47 | 41.47 |
| 11 | 37.06 | 39.62 | Undetermined |
| 12 | 35.16 | 36.46 | 40.41 |
| 13 | 35.28 | 38.48 | 41.28 |
| 14 | 35.92 | 38.66 | 40.60 |
| 15 | 36.76 | 38.17 | 40.17 |
| 16 | 36.12 | 37.91 | Undetermined |

| | | | |
|---|---|---|---|
| 17 | 36.04 | 38.74 | 41.28 |
| 18 | 37.01 | 37.19 | 41.41 |
| 19 | 36.57 | 37.11 | 40.65 |
| 20 | 36.77 | 37.25 | Undetermined |
| Positive percentage in each concentration | 100% | 95% | 65 |

| Test No | (Copies/5μl) | | |
|---|---|---|---|
| | HPV type 66/56 | | |
| | 500 | 50 | 5 |
| 1 | 39.71 | Undetermined | Undetermined |
| 2 | 39.30 | 39.06 | Undetermined |
| 3 | 39.41 | 39.35 | Undetermined |
| 4 | 37.02 | 40.49 | Undetermined |
| 5 | 38.49 | 41.13 | 41.34 |
| 6 | 38.64 | 41.02 | Undetermined |
| 7 | 37.14 | 40.49 | 42.05 |
| 8 | 37.37 | 39.60 | Undetermined |
| 9 | 38.53 | 39.42 | 40.28 |
| 10 | 37.89 | 40.61 | Undetermined |
| 11 | 38.06 | 39.48 | Undetermined |
| 12 | 37.36 | 41.46 | 40.91 |
| 13 | 37.78 | 40.48 | 40.28 |
| 14 | 33.92 | 40.66 | 40.60 |
| 15 | 34.76 | 39.15 | Undetermined |
| 16 | 34.76 | 39.97 | 40.38 |
| 17 | 35.72 | 39.47 | Undetermined |
| 18 | 38.71 | 39.41 | 40.25 |
| 19 | 37.97 | 38.67 | 41.25 |
| 20 | 37.57 | 39.24 | Undetermined |
| Positive percentage in each concentration | 100% | 95% | 45% |

| Test No | (Copies/5μl) | | |
|---|---|---|---|
| | HPV type 51 | | |
| | 500 | 50 | 5 |
| 1 | 34.73 | 39.28 | Undetermined |
| 2 | 34.57 | 39.06 | Undetermined |
| 3 | 35.21 | 40.35 | Undetermined |
| 4 | 35.47 | 39.27 | Undetermined |
| 5 | 34.89 | 39.13 | Undetermined |
| 6 | 36.07 | 39.27 | Undetermined |
| 7 | 35.41 | 39.57 | 41.37 |

| | | | |
|---|---|---|---|
| 8 | 35.37 | 39.97 | 40.97 |
| 9 | 34.98 | Undetermined | 40.58 |
| 10 | 35.74 | 39.61 | 40.53 |
| 11 | 35.57 | 39.62 | Undetermined |
| 12 | 36.36 | 39.67 | 40.91 |
| 13 | 35.28 | 39.17 | Undetermined |
| 14 | 34.92 | 39.69 | 39.60 |
| 15 | 34.73 | 39.18 | Undetermined |
| 16 | 34.47 | 39.91 | Undetermined |
| 17 | 35.01 | 40.46 | 39.27 |
| 18 | 37.27 | 40.19 | 40.41 |
| 19 | 36.12 | 39.57 | 40.65 |
| 20 | 35.74 | 39.95 | Undetermined |
| Positive percentage in each concentration | 100% | 95% | 45% |

- **Inclusivity (Analytical Sensitivity)**

The inclusivity of the primer/probe set utilized in the Stark Genotyping HPV Molecular Diagnostic Kit was assessed in silico using HPV sequences obtained from the NCBI database, accessed on September 26, 2021. Sequence alignment analysis of the primer/probe sets targeting the L1, E1, E2, E6, and E7 genes demonstrated 100% inclusivity for HPV sequences identified from patient samples. The table below illustrates representative alignment results for L1, E1, E2, E6, and E7 genes.

**Table 8**: Alignment test result for different HPV types gene

| Strain- 18 | Target | Accession | % Homology Test Forward primer% | % Homology Test Reverse primer% | % Homology Test Probe% |
|---|---|---|---|---|---|
| Human papillomavirus isolate HPV18 | E1 | KY501976.1 | 100 | 100 | 100 |
| Human papillomavirus isolate HPV18 | E1 | KY501973.1 | 100 | 100 | 100 |
| Human papillomavirus isolate HPV18 P1-20 | E1 | KY501967.1 | 100 | 100 | 100 |
| Human papillomavirus isolate HPV18 P1 | E1 | KY501965.1 | 100 | 100 | 100 |
| Human papillomavirus isolate HPV18 P2 | E1 | KY501971.1 | 100 | 100 | 100 |
| Human papillomavirus isolate HPV18 P1-50 | E1 | KY501970.1 | 100 | 100 | 100 |
| Human papillomavirus isolate HPV18 P2-30 | E1 | KY501974.1 | 100 | 100 | 100 |
| Human papillomavirus isolate HPV18 P1-40 | E1 | KY501969.1 | 100 | 100 | 100 |

This analysis confirms the comprehensive coverage of the primer/probe sets across a diverse range of HPV sequences, ensuring the robust analytical sensitivity of the diagnostic Kit.

| Strain- 45 | Target | Accession | % Homology Test Forward primer% | % Homology Test Reverse primer% | % Homology Test Probe% |
|---|---|---|---|---|---|
| Human papillomavirus type 45 isolate LNS8825361_HPV45 | E1 | LR862061.1.1 | 100 | 100 | 100 |
| Human papillomavirus type 45 isolate Qv34163 | E1 | KC470260.1 | 100 | 100 | 100 |
| Human papillomavirus type 45 isolate Qv30712 | E1 | >KC470259.1.1 | 100 | 100 | 100 |
| Human papillomavirus type 45 isolate Qv20214 | E1 | EF202156.1 | 100 | 100 | 100 |
| Human papillomavirus type 45 isolate LNS8825361_HPV45 | E1 | LR862061.1.1 | 100 | 100 | 100 |
| Human papillomavirus type 45 isolate Qv27565 | E1 | EF202157.1 | 100 | 100 | 100 |
| Human papillomavirus type 45 isolate Qv33330 | E1 | EF202158.1 | 100 | 100 | 100 |
| Human papillomavirus type 45 isolate Qv34178 | E1 | EF202159.1 | 100 | 100 | 100 |

| Strain- 31 | Target | Accession | % Homology Test Forward primer% | % Homology Test Reverse primer% | % Homology Test Probe% |
|---|---|---|---|---|---|
| Human papillomavirus 31 isolate VBD13/14 | E2 | MW814876.1 | 100 | 100 | 100 |
| Human papillomavirus type 31 isolate LNS8465006_HPV31 | E2 | LR862053.1 | 100 | 100 | 100 |
| Human papillomavirus type 31 isolate LNS8357921_HPV31 | E2 | LR862049.1 | 100 | 100 | 100 |
| Human papillomavirus type 31 isolate LNS7548544_HPV31 | E2 | LR862026.1 | 100 | 100 | 100 |
| Human papillomavirus type 31 isolate LNS7384732 | E2 | LR862018.1 | 100 | 100 | 100 |
| Human papillomavirus type 31 isolate LNS7358029 | E2 | LR862015.1 | 100 | 100 | 100 |
| Human papillomavirus type 31 isolate LNS6001593 | E2 | LR861970.1 | 100 | 100 | 100 |
| Human papillomavirus type 31 isolate LNS0937074 | E2 | LR861951.1 | 100 | 100 | 100 |
| Human papillomavirus type 31 isolate IARC366181RW | E2 | MT752571.1 | 100 | 100 | 100 |
| Human papillomavirus type 31 | E2 | MT752570.1 | 100 | 100 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| isolate IARC366076RW | | | | | |
| Human papillomavirus type 31 isolate PAP293744 | E2 | MT752505.1 | 100 | 100 | 100 |
| Human papillomavirus type 31 isolate PAP287325 | E2 | MT752449.1 | 100 | 100 | 100 |

| Strain- 35 | Target | Accession | % Homology Test Forward primer% | % Homology Test Reverse primer% | % Homology Test Probe% |
|---|---|---|---|---|---|
| Human papillomavirus type 35 isolate LNS0838993 | E6/E7 | LR861946.1 | 100 | 100 | 100 |
| Human papillomavirus type 35 isolate PAP294578 | E6/E7 | MT217995.1 | 100 | 100 | 100 |
| Human papillomavirus type 35 isolate PAP282649 | E6/E7 | MT217962.1 | 100 | 100 | 100 |
| Human papillomavirus type 35 isolate PAP163773 | E6/E7 | MT217679.1 | 100 | 100 | 100 |
| Human papillomavirus type 35 isolate PAP162942 | E6/E7 | MT217678.1 | 100 | 100 | 100 |
| Human papillomavirus type 35 isolate PAP145045 | E6/E7 | MT217620.1 | 100 | 100 | 100 |
| Human papillomavirus type 35 isolate IARC1220350CH | E6/E7 | MT217487.1 | 100 | 100 | 100 |
| Human papillomavirus type 35 isolate IARC1101761NI | E6/E7 | MT217470.1 | 100 | 100 | 100 |
| Human papillomavirus type 35 isolate IARC1251067MO | E6/E7 | MT217441.1 | 100 | 100 | 100 |
| Human papillomavirus type 35 isolate IARC166070014RW | E6/E7 | MT217402.1 | 100 | 100 | 100 |
| Human papillomavirus type 35 isolate IARC1110712GU | E6/E7 | MT217344.1 | 100 | 100 | 100 |
| Human papillomavirus type 35 isolate IARC311027GU | E6/E7 | MT217305.1 | 100 | 100 | 100 |

| Strain- 59 | Target | Accession | % Homology Test Forward primer% | % Homology Test Reverse primer% | % Homology Test Probe% |
|---|---|---|---|---|---|
| Human papillomavirus 59 strain kyd-s0359 | E6 | MT783417.1 | 100 | 100 | 100 |
| Human papillomavirus type 59 isolate LNS9786324 | E6 | LR862080.1 | 100 | 100 | 100 |
| Human papillomavirus type 59 isolate LNS3279856 | E6 | LR861868.1 | 100 | 100 | 100 |
| Human papillomavirus type 59 | E6 | KC470263.1 | 100 | 100 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| isolate Qv23880 | | | | | |
| Human papillomavirus type 59 isolate LZod68-59 | E6 | EU918767.1 | 100 | 100 | 100 |
| Human papillomavirus type 59 isolate LNS5859951 | E6 | LR861933.1 | 100 | 100 | 100 |
| Human papilloma virus type 59 | E6 | X77858.1 | 100 | 100 | 100 |
| Human papillomavirus type 59 isolate Qv25652 | E6 | KC470261.1 | 100 | 100 | 100 |
| Human papillomavirus type 59 isolate Qv25808 | E6 | KC470264.1 | 100 | 100 | 100 |
| Human papillomavirus type 59 isolate Qv33993 | E6 | KC470265.1 | 100 | 100 | 100 |
| Human papillomavirus type 59 isolate 32A.59 | E6 | KU298921.1 | 100 | 100 | 100 |
| Human papillomavirus type 59 isolate LNS2917513_HPV59 | E6 | LR861860.1 | 100 | 100 | 100 |

| Strain- 39 | Target | Accession | % Homology Test Forward primer% | % Homology Test Reverse primer% | % Homology Test Probe% |
|---|---|---|---|---|---|
| Human papillomavirus type 39 isolate LNS9068892 | L1 | LR862071.1 | 100 | 100 | 100 |
| Human papillomavirus type 39 isolate LNS8643648 | L1 | LR862054.1 | 100 | 100 | 100 |
| Human papillomavirus type 39 isolate LNS7329845 | L1 | LR862014.1 | 100 | 100 | 100 |
| Human papillomavirus type 39 isolate 39PL03 L1 | L1 | MK344660.1 | 100 | 100 | 100 |
| Human papillomavirus type 39 isolate 39PL02 L1 | L1 | MK344659.1 | 100 | 100 | 100 |
| Human papillomavirus type 39 isolate 16B | L1 | KX514417.1 | 100 | 100 | 100 |
| Human papillomavirus type 39 isolate Tw562 | L1 | KC470243.1 | 100 | 100 | 100 |
| Human papillomavirus type 39 isolate Qv25609 | L1 | KC470242.1 | 100 | 100 | 100 |
| Human papillomavirus type 39 isolate Qv25959 | L1 | KC470240.1 | 100 | 100 | 100 |
| Human papillomavirus type 39 isolate Qv27715 | L1 | KC470237.1 | 100 | 100 | 100 |
| Human papillomavirus type 39 isolate LNS4492831 | L1 | LR861904.1 | 100 | 100 | 100 |
| Human papillomavirus type 39 clone 39L1.A major capsid | L1 | JN104068.1 | 100 | 100 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| protein | | | | | |

| Strain- 6/11 | Target | Accession | % Homology Test Forward primer% | % Homology Test Reverse primer% | % Homology Test Probe% |
|---|---|---|---|---|---|
| Human papillomavirus type 11 isolate HPV11-gw-1108 | L1 | MK463919.1 | 100 | 100 | 100 |
| Human papillomavirus type 11 isolate HPV11-gw-1111 | L1 | MK463922.1 | 100 | 100 | 100 |
| Human papillomavirus type 11 isolate HPV11-gw-1110 | L1 | MK463921.1 | 100 | 100 | 100 |
| Human papillomavirus type 11 isolate HPV11-gw-1109 | L1 | MK463920.1 | 100 | 100 | 100 |
| Human papillomavirus type 6 isolate HPV6-gw-0611 | L1 | MK463909.1 | 100 | 100 | 100 |
| Human papillomavirus type 6 isolate HPV6-gw-0602 | L1 | MK463905.1 | 100 | 100 | 100 |
| Human papillomavirus type 6 isolate ASCUS1/HPV6 | L1 | MK313781.1 | 100 | 100 | 100 |
| Human papillomavirus type 6 isolate CAC1/HPV6 | L1 | MK313778.1 | 100 | 100 | 100 |
| Human papillomavirus type 6 isolate JO-RRP2/HPV6 | L1 | MK313777.1 | 100 | 100 | 100 |
| Human papillomavirus type 6 isolate AO-RRP7/HPV6 | L1 | MK313775.1 | 100 | 100 | 100 |
| Human papillomavirus type 6 isolate AO-RRP5/HPV6 | L1 | MK313773.1 | 100 | 100 | 100 |
| Human papillomavirus type 6 isolate AO-RRP3/HPV6 | L1 | MK313771.1 | 100 | 100 | 100 |
| Human papillomavirus type 6 isolate AO-RRP1/HPV6 | L1 | MK313769.1 | 100 | 100 | 100 |
| Human papillomavirus type 6 isolate 111B | L1 | KX514423.1 | 100 | 100 | 100 |
| Human papillomavirus type 6 isolate J-50 | L1 | KU721785.1 | 100 | 100 | 100 |

| Strain- 51 | Target | Accession | % Homology Test Forward primer% | % Homology Test Reverse primer% | % Homology Test Probe% |
|---|---|---|---|---|---|
| Human papillomavirus type 51 isolate LNS9024319 | L1 | LR862069.1 | 100 | 100 | 100 |
| Human papillomavirus type 51 isolate LNS8077695 | L1 | LR862046.1 | 100 | 100 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| Human papillomavirus type 51 isolate LNS0570411_HPV51 | L1 | LR861939.1 | 100 | 100 | 100 |
| Human papillomavirus type 51 isolate LNS4020261 | L1 | LR861893.1 | 100 | 100 | 100 |
| Human papillomavirus type 51 isolate LNS3425544 | L1 | LR861869.1 | 100 | 100 | 100 |
| Human papillomavirus type 51 isolate LNS9888540_HPV51 | L1 | LR861810.1 | 100 | 100 | 100 |
| Human papillomavirus type 51 isolate CL17 L1 | L1 | MH577961.1 | 100 | 100 | 100 |
| Human papillomavirus type 51 isolate CL16 | L1 | MH577960.1 | 100 | 100 | 100 |
| Human papillomavirus type 51 isolate CL10 | L1 | MH577954.1 | 100 | 100 | 100 |
| Human papillomavirus type 51 isolate 118A.51 | L1 | KU298905.1 | 100 | 100 | 100 |

| Strain- 66 | Target | Accession | % Homology Test Forward primer% | % Homology Test Reverse primer% | % Homology Test Probe% |
|---|---|---|---|---|---|
| Human papillomavirus type 66 isolate NGSk294-66 | E6 | LR861964.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate LNS4474748_HPV66 | E6 | LR861902.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate LNS2720744 | E6 | LR861851.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate 110 | E6 | JN661435.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate 100 | E6 | JN661426.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate 95 | E6 | JN661421.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate 92 | E6 | JN661418.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate 90 | E6 | JN661416.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate Bsb-107 | E6 | HM585479.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 clone Qv11088 | E6 | EF177190.1 | 100 | 100 | 100 |

| Strain- 68 | Target | Accession | % Homology Test Forward primer% | % Homology Test Reverse primer% | % Homology Test Probe% |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Human papillomavirus type 68 isolate 55-86-21 | E6/E7 | MK874671.1 | 100 | 100 | 100 |
| Human papillomavirus type 68 isolate 21-54-34 | E6/E7 | MK874669.1 | 100 | 100 | 100 |
| Human papillomavirus type 68 isolate 21-54-24 | E6/E7 | MK874668.1 | 100 | 100 | 100 |
| Human papillomavirus type 68 isolate 21-54-18 | E6/E7 | MK874666.1 | 100 | 100 | 100 |
| Human papillomavirus type 68 isolate 1-20-9 | E6/E7 | MK874662.1 | 100 | 100 | 100 |
| Human papillomavirus type 68 isolate M180 | E6/E7 | MN047810.1 | 100 | 100 | 100 |
| Human papillomavirus type 68 isolate 268 | E6/E7 | KX514428.1 | 100 | 100 | 100 |
| Human papillomavirus type 68 isolate P141607 | E6/E7 | KU195243.1 | 100 | 100 | 100 |
| Human papillomavirus type 68 isolate Rw826 | E6/E7 | KC470281.1 | 100 | 100 | 100 |
| Human papillomavirus type 68 isolate Qv25395 | E6/E7 | KC470279.1 | 100 | 100 | 100 |

| Strain- 56/66 | Target | Accession | % Homology Test Forward primer% | % Homology Test Reverse primer% | % Homology Test Probe% |
|---|---|---|---|---|---|
| Human papillomavirus type 56 isolate 56SE-23 | E6/E7 | KX645764.1 | 100 | 100 | 100 |
| Human papillomavirus type 56 isolate 56SE-21 | E6/E7 | KX645762.1 | 100 | 100 | 100 |
| Human papillomavirus type 56 isolate 56SE-19 | E6/E7 | KX645760.1 | 100 | 100 | 100 |
| Human papillomavirus type 56 isolate 56SE-09 | E6/E7 | KX645750.1 | 100 | 100 | 100 |
| Human papillomavirus type 56 isolate 60B | E6/E7 | KX514418.1 | 100 | 100 | 100 |
| Human papillomavirus type 56 isolate 87A.56 | E6/E7 | KU298916.1 | 100 | 100 | 100 |
| Human papillomavirus type 56 clone Qv22608 | E6/E7 | EF177179.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate LNS7384732 | E6/E7 | LR862020.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate LNS1839688 | E6/E7 | LR861964.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate LNS5491243 | E6/E7 | LR861922.1 | 100 | 100 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| Human papillomavirus type 66 isolate LNS3785675 | E6/E7 | LR861879.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate 106 | E6/E7 | JN661479.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate 95 | E6/E7 | JN661470.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate 85 | E6/E7 | JN661460.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 isolate Qv32783 | E6/E7 | EF546480.1 | 100 | 100 | 100 |
| Human papillomavirus type 66 clone Qv11088 | E6/E7 | EF177190.1 | 100 | 100 | 100 |

| Strain- 33/52/ 58 | Target | Accession | % Homology Test Forward primer% | % Homology Test Reverse primer% | % Homology Test Probe% |
|---|---|---|---|---|---|
| Human papillomavirus type 33 isolate LNS9453833 | E1/E2 | LR862077.1 | 100 | 100 | 100 |
| Human papillomavirus type 33 isolate LNS3510974 | E1/E2 | LR861872.1 | 100 | 100 | 100 |
| Human papillomavirus type 33 isolate 67B.33 | E1/E2 | KU298894.1 | 100 | 100 | 100 |
| Human papillomavirus type 33 isolate 65C.33 | E1/E2 | KU298893.1 | 100 | 100 | 100 |
| Human papillomavirus type 33 isolate BF375 | E1/E2 | KF436865.1 | 100 | 100 | 100 |
| Human papillomavirus type 33 | E1/E2 | M12732.1 | 100 | 100 | 100 |
| Human papillomavirus type 33 isolate LZcc12-33 | E1/E2 | EU918766.1 | 100 | 100 | 100 |
| Human papillomavirus type 33 isolate Qv32494 | E1/E2 | HQ537688.1 | 100 | 100 | 100 |
| Human papillomavirus 52 isolate PAP3497 | E1/E2 | MZ374448.1 | 100 | 100 | 100 |
| Human papillomavirus 52 isolate PAP1639 | E1/E2 | MZ374436.1 | 100 | 100 | 100 |
| Human papillomavirus 52 isolate PAP1189 | E1/E2 | MZ374435.1 | 100 | 100 | 100 |
| Human papillomavirus 52 isolate PAP2912 | E1/E2 | MZ374431.1 | 100 | 100 | 100 |
| Human papillomavirus 52 isolate PAP2357 | E1/E2 | MZ374425.1 | 100 | 100 | 100 |
| Human papillomavirus 52 isolate PAP2484 | E1/E2 | MZ374419.1 | 100 | 100 | 100 |
| Human papillomavirus 52 | E1/E2 | MZ374415.1 | 100 | 100 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| isolate PAP1917 | | | | | |
| Human papillomavirus 52 isolate PAP2698 | E1/E2 | MZ374413.1 | 100 | 100 | 100 |
| Human papillomavirus type 52 isolate LNS9453833 | E1/E2 | LR862077.1 | 100 | 100 | 100 |
| Human papillomavirus type 52 isolate LNS6759684 | E1/E2 | LR861998.1 | 100 | 100 | 100 |
| Human papillomavirus type 58 isolate LNS6048641 | E1/E2 | LR861973.1 | 100 | 100 | 100 |
| Human papillomavirus type 58 isolate LNS0838993 | E1/E2 | LR861950.1 | 100 | 100 | 100 |
| Human papillomavirus type 58 isolate LNS5390598 | E1/E2 | LR861919.1 | 100 | 100 | 100 |
| Human papillomavirus type 58 isolate LNS4582218 | E1/E2 | LR861906.1 | 100 | 100 | 100 |

As supplemental data, wet testing of inclusivity was conducted using the DNall VirAll Kit. Three HPV-positive specimens from the WHO HPV Lab Net were tested, all of which were confirmed positive by the Stark Genotyping HPV Molecular Diagnostic Kit. Each specimen was diluted to ≤3log10 LOD, ≤2log10 LOD, and ≤1log10 LOD in a negative specimen matrix (Cervical swab) and tested in the tenth replicate. The table below provides details of this testing:

This evaluation confirms the clinical sensitivity of the Stark Genotyping HPV Molecular Diagnostic Kit across a range of dilutions in a negative specimen matrix, demonstrating its ability to accurately detect HPV-positive specimens.

**Table 9**: the results of diagnostic sensitivity of Stark Quantitative HPV Molecular Diagnostic Kit by CARBON Technologies LLC

| Dilution series | Copies/5 µl | Ct |
|---|---|---|
| 3log10> LOD | 50.000 | 24.55 |
| | | 24.25 |
| | | 25.05 |
| | | 25.13 |
| | | 24.71 |
| | | 24.87 |
| | | 24.18 |
| | | 24.46 |
| | | 25.14 |
| | | 25.23 |
| 2log10 > LOD | 5.000 | 28.44 |
| | | 28.48 |
| | | 29.31 |
| | | 28.64 |
| | | 28.78 |
| | | 28.73 |
| | | 29.08 |

| | | |
|---|---|---|
| | | 29.17 |
| | | 29.08 |
| | | 28.87 |
| 1log10 > LOD | 500 | 34.84 |
| | | 33.82 |
| | | 34.52 |
| | | 33.88 |
| | | 34.17 |
| | | 35.27 |
| | | 35.37 |
| | | 35.76 |
| | | 35.78 |
| | | 35.37 |

- **Cross-reactivity (Analytical Specificity)**

The cross-reactivity of the Stark Genotyping HPV Molecular Diagnostic Kit was evaluated through in silico analysis and wet testing of potentially cross-reactive whole pathogens or purified nucleic acid from clinical specimens. No cross-reactivity was detected during either analysis.

In-silico mapping analysis of each primer/probe against several pathogens was conducted using the NCBI nr/nt database, accessed on January 9, 2022, through the online BLASTN 2.10.0+ tool. Representative results of this analysis are provided below the table.

No cross-reactivity was observed for the listed pathogens in either the in-silico or wet-testing experiments, confirming the high analytical specificity of the diagnostic Kit.

**Table 10**: The In-Silico Specificity Analysis of Primer and Probe Set for Other pathogens.

| Strain- 18 Pathogen (Taxonomy ID) | Strain | GenBank Acc# | % Homology Test FP | % Homology Test RP | % Homology Test Probe |
|---|---|---|---|---|---|
| Human immunodeficiency virus | HIV-1 isolate 002211_C07 | MT033226.1 | 59% | 72% | 62% |
| Hepatitis C virus subtype 1a | 10jsszGP017 | JQ303503.1 | 63% | 55% | 62% |
| HSV-1 | s17pp22a | MN159382.1 | 70% | 50% | 66% |
| Hepatitis B virus | isolate 2793 | KF169309.1 | 50% | 66% | 59% |
| HSV-2 | strain 2009-24855_S55_L001 | MH790632.1 | 45% | 61% | 70% |
| Mycoplasma genitalium | M2321 MgPar 9 | CP003773.1 | 45% | 55% | 51% |
| Chlamydia trachomatis | TC0350 | CP042803.1 | 66% | 61% | 77% |
| Streptococcus agalactiae | Sag27 | CP031556.1 | 81% | 77% | 48% |
| Human T-cell leukemia virus | 057-OL | MN453165.1 | 50% | 88% | 68% |

| | | | | | |
|---|---|---|---|---|---|
| type I | | | | | |
| Human gamma herpesvirus | HC-0036 | LR813015.1 | 63% | 63% | 62% |
| Human alpha herpesvirus | s17pp22a | MN159382.1 | 95% | 55% | 66% |
| Human T-lymphotropic virus 2 | HTLV-2/Japan/NIID/001/2020 | K01670.1 | 50% | 44% | 51% |
| Human parvovirus B19 | 911NewF | MN088124.1 | 33% | 55% | 40% |
| JC polyomavirus | UJC005 | MW587996.1 | 72% | 50% | 88% |
| Neisseria gonorrhoeae | TUM16691 | AP023075.1 | 54% | 55% | 44% |
| Trichomonas vaginalis | TVAG_290210 | XM_001312003.1 | 59% | 72% | 62% |

| Strain- 16 Pathogen (Taxonomy ID) | Strain | GenBank Acc# | % Homology Test FP | % Homology Test RP | % Homology Test Probe |
|---|---|---|---|---|---|
| Human immunodeficiency virus | YBF319 | KT198590.1 | 52% | 63% | 65% |
| Hepatitis C virus subtype 1a | HCV-1b/US/BID-V377/2006 | EU256065.1 | 48% | 40% | 55% |
| HSV-1 | 2007-16123 | MG999881.1 | 36% | 33% | 50% |
| Hepatitis B virus | B127W | MH051987.1 | 48% | 40% | 50% |
| HSV-2 | 2006-15095 | MH899846.1 | 55% | 40% | 72% |
| Mycoplasma genitalium | 1206KLM26 MgpC adhesin | MT439445.1 | 44% | 53% | 50% |
| Chlamydia trachomatis | TC0411:TncL2/tetR-104 | CP042798.1 | 75% | 86% | 75% |
| Streptococcus agalactiae | 2013-1366 | CP051844.1 | 70% | 84% | 44% |
| Human T-cell leukemia virus type I | Aus-CS | KF242506.1 | 60% | 45% | 30% |
| Human gammaherpesvirus | 21_AJOUA | MT648662.1 | 55% | 48% | 43% |
| Human alphaherpesvirus | HSV1-ORIGINAL-H1 | MH160367.1 | 36% | 33% | 50% |
| Human T-lymphotropic virus 2 | (HTLV-II AA | X76030.1 | 40% | 72% | 43% |

| | | | | | |
|---|---|---|---|---|---|
| Human parvovirus B19 | HZ13 NS1 | KT389459.1 | 43% | 73% | 75% |
| JC polyomavirus | IRN-26H | MN163027.1 | 70% | 36% | 66% |
| Neisseria gonorrhoeae | NG196 | CP043872.1 | 40% | 40% | 44% |
| Trichomonas vaginalis | TVAG_231320 | XM_001313720.1 | 52% | 46% | 60% |

| Strain- 31 Pathogen (Taxonomy ID) | Strain | GenBank Acc# | % Homology Test FP | % Homology Test RP | % Homology Test Probe |
|---|---|---|---|---|---|
| Human immunodeficiency virus | A09.Q2.20M9D1 | MH264318.1 | 63% | 63% | 68% |
| Hepatitis C virus subtype 1a | AV67 | EU484132.1 | 58% | 75% | 52% |
| HSV-1 | HSV-H1412 | MH999851.1 | 56% | 87% | 45% |
| Hepatitis B virus | 551.31 | KR013975.1 | 70% | 58% | 52% |
| HSV-2 | HSV2_19 | MH697440.1 | 64% | 87% | 52% |
| Mycoplasma genitalium | 1493B26 MgpB | MT439517.1 | 52% | 56% | 42% |
| Chlamydia trachomatis | TC0657:TnxL2/tetR-403 | CP042757.1 | 87% | 42% | 54% |
| Streptococcus agalactiae | CM45/1 | CP088933.1 | 55% | 87% | 62% |
| Human T-cell leukemia virus type I | IR (94) bZIP | MN453136.1 | 57% | 47% | 52% |
| Human gammaherpesvirus | 20_IHOM | MT648661.1 | 78% | 48% | 58% |
| Human alphaherpesvirus | CM1v8 | KX791821.1 | 56% | 87% | 56% |
| Human T-lymphotropic virus 2 | N1232 | KY493238.1 | 50% | 75% | 42% |
| Human parvovirus B19 | NA121 | MG765334.1 | 50% | 56% | 78% |
| JC polyomavirus | SA38527_04 VP1 | EU835186.1 | 42% | 42% | 87% |
| Neisseria gonorrhoeae | SRRSH205 | CP048907.1 | 78% | 68% | 62% |
| Trichomonas vaginalis | TVAG_449190 | XM_001308315.1 | 62% | 63% | 68% |

| Strain- 35 | Strain | GenBank Acc# | % | % | % |
|---|---|---|---|---|---|

| Pathogen (Taxonomy ID) | | | Homology Test FP | Homology Test RP | Homology Test Probe |
|---|---|---|---|---|---|
| Human immunodeficiency virus | OU072 | OK180789.1 | 48% | 44% | 56% |
| Hepatitis C virus subtype 1a | EA03T0 | AM271169.1 | 47% | 39% | 43% |
| HSV-1 | 2011-12741 | MG999893.1 | 43% | 74% | 47% |
| Hepatitis B virus | CHB-47-PS | KY428722.1 | 40% | 37% | 43% |
| HSV-2 | 2012-5447 | MH790637.1 | 56% | 43% | 52% |
| Mycoplasma genitalium | 1491KLM26 | MT439589.1 | 33% | 52% | 44% |
| Chlamydia trachomatis | TC0411: TnxD/tetR-103 | CP042802.1 | 54% | 81% | 48% |
| Streptococcus agalactiae | SA627 | CP019837.1 | 43% | 52% | 33% |
| Human T-cell leukemia virus type I | BHP00140 | MG388047.1 | 52% | 43% | 44% |
| Human gammaherpesvirus | 19_ELTW | MT648660.1 | 40% | 48% | 47% |
| Human alphaherpesvirus | 2011-17239 | MG999896.1 | 43% | 74% | 47% |
| Human T-lymphotropic virus 2 | BBD_7003 | FJ911659.1 | 51% | 29% | 59% |
| Human parvovirus B19 | D1599 NS1 | DQ234775.2 | 40% | 55% | 59% |
| JC polyomavirus | J007 VP1 | MK477564.1 | 37% | 88% | 33% |
| Neisseria gonorrhoeae | TUM19853 | AP023067.1 | 47% | 77% | 52% |
| Trichomonas vaginalis | TVAG_196200 | XM_001312988.1 | 51% | 66% | 52% |

| Strain- 33/52/58 Pathogen (Taxonomy ID) | Strain | GenBank Acc# | % Homology Test FP | % Homology Test RP | % Homology Test Probe |
|---|---|---|---|---|---|
| Human immunodeficiency virus | AA075a_WG7 | JX447542.1 | 63% | 66% | 59% |

| | | | | | |
|---|---|---|---|---|---|
| Hepatitis C virus subtype 1a | PATNO72 | AB285080.1 | 45% | 62% | 41% |
| HSV-1 | 2004-63623 | MG999877.1 | 62% | 59% | 72% |
| Hepatitis B virus | OHBV-HIV137 | MF618348.1 | 45% | 45% | 50% |
| HSV-2 | 2006-18003CAM | MH790593.1 | 41% | 59% | 62% |
| Mycoplasma genitalium | 10467 MgpB | KP318824.1 | 50% | 63% | 40% |
| Chlamydia trachomatis | TC0189:TncL2/tetR-102 | CP042734.1 | 87% | 59% | 63% |
| Streptococcus agalactiae | NEM316 | AL766847.1 | 45% | 87% | 50% |
| Human T-cell leukemia virus type I | BHP00005 | MG388044.1 | 75% | 59% | 54% |
| Human gammaherpesvirus | sLCL-T3.27 | LC573551.1 | 59% | 50% | 45% |
| Human alphaherpesvirus | 2003-15756 | MG999872.1 | 75% | 68% | 62% |
| Human T-lymphotropic virus 2 | NIID18001 | LC440555.1 | 36% | 41% | 31% |
| Human parvovirus B19 | BN58.3 | DQ408302.1 | 54% | 77% | 66% |
| JC polyomavirus | IRN-72H | MN163031.1 | 45% | 37% | 63% |
| Neisseria gonorrhoeae | SS3160 | AP019853.2 | 41% | 58% | 45% |
| Trichomonas vaginalis | TVAG_308110 | XM_001320393.1 | 40% | 58% | 72% |

| Strain- 59 Pathogen (Taxonomy ID) | Strain | GenBank Acc# | % Homology Test FP | % Homology Test RP | % Homology Test Probe |
|---|---|---|---|---|---|
| Human immunodeficiency virus | 505_1982a | MG197155.1 | 60% | 52% | 68% |
| Hepatitis C virus subtype 1a | GZ52540 | KC844051.1 | 35% | 42% | 46% |
| HSV-1 | 11_DOCK8 | MN401208.1 | 64% | 40% | 42% |
| Hepatitis B virus | NOA_142 | MG098582.1 | 48% | 40% | 44% |
| HSV-2 | HJ12 | MN187895.1 | 40% | 36% | 52% |
| Mycoplasma genitalium | M2288 | CP003773.1 | 44% | 56% | 40% |
| Chlamydia | TC0350:TncL2/tetR-936 | CP042803.1 | 44% | 40% | 64% |

| | | | | | |
|---|---|---|---|---|---|
| trachomatis | | | | | |
| Streptococcus agalactiae | NEM316 | AL766845.1 | 56% | 40% | 36% |
| Human T-cell leukemia virus type I | PH1511 | MN781156.1 | 40% | 56% | 40% |
| Human gammaherpesvirus | HKHD138 | MH590507.1 | 64% | 44% | 76% |
| Human alphaherpesvirus | 2007-16123 | MG999881.1 | 64% | 40% | 36% |
| Human T-lymphotropic virus 2 | BBD_3079 | FJ911654.1 | 36% | 40% | 32% |
| Human parvovirus B19 | HZ13 NS1 | KT389459.1 | 40% | 36% | 76% |
| JC polyomavirus | Tn-19 VP1 | JQ433658.1 | 36% | 32% | 48% |
| Neisseria gonorrhoeae | TUM16691 | AP023075.1 | 44% | 76% | 52% |
| Trichomonas vaginalis | TVAG_149710 | XM_001308182.1 | 60% | 52% | 56% |

| Strain- 39 Pathogen (Taxonomy ID) | Strain | GenBank Acc# | % Homology Test FP | % Homology Test RP | % Homology Test Probe |
|---|---|---|---|---|---|
| Human immunodeficiency virus | 505_1982a | MG197155.1 | 60% | 52% | 68% |
| Hepatitis C virus subtype 1a | GZ52540 | KC844051.1 | 35% | 42% | 46% |
| HSV-1 | 11_DOCK8 | MN401208.1 | 64% | 40% | 42% |
| Hepatitis B virus | NOA_142 | MG098582.1 | 48% | 40% | 44% |
| HSV-2 | HJ12 | MN187895.1 | 40% | 36% | 52% |
| Mycoplasma genitalium | M2288 | CP003773.1 | 44% | 56% | 40% |
| Chlamydia trachomatis | TC0350:TncL2/tetR-936 | CP042803.1 | 44% | 40% | 64% |
| Streptococcus agalactiae | NEM316 | AL766845.1 | 56% | 40% | 36% |
| Human T-cell leukemia virus type I | PH1511 | MN781156.1 | 40% | 56% | 40% |
| Human gammaherpesvirus | HKHD138 | MH590507.1 | 64% | 44% | 76% |
| Human alphaherpesvirus | 2007-16123 | MG999881.1 | 64% | 40% | 36% |
| Human | BBD_3079 | FJ911654.1 | 36% | 40% | 32% |

| | | | | | |
|---|---|---|---|---|---|
| T-lymphotropic virus 2 | | | | | |
| Human parvovirus B19 | HZ13 NS1 | KT389459.1 | 40% | 36% | 76% |
| JC polyomavirus | Tn-19 VP1 | JQ433658.1 | 36% | 32% | 48% |
| Neisseria gonorrhoeae | TUM16691 | AP023075.1 | 44% | 76% | 52% |
| Trichomonas vaginalis | TVAG_149710 | XM_001308182.1 | 60% | 52% | 56% |

| Strain- 66 Pathogen (Taxonomy ID) | Strain | GenBank Acc# | % Homology Test FP | % Homology Test RP | % Homology Test Probe |
|---|---|---|---|---|---|
| Human immunodeficiency virus | 2303-08PT46 | MH832382.1 | 50% | 35% | 46% |
| Hepatitis C virus subtype 1a | EG06T1ak | AM708703.1 | 35% | 42% | 39% |
| HSV-1 | H166 | KM222726.1 | 35% | 39% | 53% |
| Hepatitis B virus | 124_CA_Kal | MH580631.1 | 39% | 35% | 42% |
| HSV-2 | HJ12 | MN187895.1 | 35% | 50% | 57% |
| Mycoplasma genitalium | 199 MgPar 7 | EF117299.2 | 57% | 42% | 39% |
| Chlamydia trachomatis | KU2043c768-137 | CP042789.1 | 32% | 39% | 35% |
| Streptococcus agalactiae | NY84115 | JF270524.1 | 35% | 39% | 42% |
| Human T-cell leukemia virus type I | Gab1014FC | EU444097.1 | 80% | 50% | 38% |
| Human gammaherpesvirus | DOCK8 | MN401207.1 | 35% | 42% | 53% |
| Human alphaherpesvirus | E25 | HM585506.2 | 50% | 57% | 42% |
| Human T-lymphotropic virus 2 | HTLV-2/Japan/NIID/001/2020 | LC534557.1 | 57% | 50% | 73% |
| Human parvovirus B19 | BX2 | MH201456.1 | 34% | 30% | 65% |
| JC polyomavirus | VP1 | EF369493.1 | 61% | 69% | 42% |
| Neisseria gonorrhoeae | SW0236 | CP061485.1 | 61% | 53% | 30% |
| Trichomonas vaginalis | TVAG_402520 | XM_001580933.1 | 42% | 69% | 50% |

| Strain- 68 Pathogen (Taxonomy ID) | Strain | GenBank Acc# | % Homology Test FP | % Homology Test RP | % Homology Test Probe |
|---|---|---|---|---|---|
| Human immunodeficiency virus | BA855 | MK732623.1 | 57% | 76% | 50% |
| Hepatitis C virus subtype 1a | 9419KBS/12 | MH627077.1 | 50% | 53% | 50% |
| HSV-1 | DOCK8 | MN401208.1 | 46% | 61% | 73% |
| Hepatitis B virus | BB1618958 | MH247244.1 | 69% | 42% | 57% |
| HSV-2 | HJ12 | MN187895.1 | 61% | | |
| Mycoplasma genitalium | TC0350:TncL2/tetR-936 | CP042803.1 | 35% | 39% | 57% |
| Chlamydia trachomatis | 01173 | CP053027.1 | 42% | 69% | 42% |
| Streptococcus agalactiae | IL1657 | KF202322.1 | 61% | 36% | 34% |
| Human T-cell leukemia virus type I | IT1036 | KF202320.1 | 34% | 32% | 38% |
| Human gammaherpesvirus | HKHD44 | MH590413.1 | 42% | 38% | 69% |
| Human alphaherpesvirus | BB1618958_p | MH247244.1 | 30% | 61% | 50% |
| Human T-lymphotropic virus 2 | HTLV-2/Japan/NIID/001/2020 | LC534557.1 | 69% | 34% | 30% |
| Human parvovirus B19 | R0416 | DQ234769.2 | 38% | 50% | 42% |
| JC polyomavirus | JCV144CSFRRC | JF425684.1 | 65% | 46% | 57% |
| Neisseria gonorrhoeae | TUM16691 | AP023075.1 | 42% | 65% | 46% |
| Trichomonas vaginalis | TVAG_454270 | XM_001329699.1 | 46% | 50% | 42% |

| Strain- 6/11 Pathogen (Taxonomy ID) | Strain | GenBank Acc# | % Homology Test FP | % Homology Test RP | % Homology Test Probe |
|---|---|---|---|---|---|
| Human immunodeficiency virus | 7864_6 | MW189577.1 | 54% | 41% | 50% |
| Hepatitis C virus subtype 1a | 1b.NR.022 | KM580649.1 | 66% | 50% | 54% |

| | | | | | |
|---|---|---|---|---|---|
| HSV-1 | Sc16 | MN159383.1 | 54% | 45% | 50% |
| Hepatitis B virus | cxx16 | MZ244221.1 | 50% | 41% | |
| HSV-2 | HJ12 | MN187895.1 | 45% | 45% | 54% |
| Mycoplasma genitalium | M2288 | CP003773.1 | 62% | 54% | 41% |
| Chlamydia trachomatis | TC0693:TncL2/tetR-3509 | CP042693.1 | 50% | | 41% |
| Streptococcus agalactiae | QMA0271 | CP029632.1 | 50% | 54% | 66% |
| Human T-cell leukemia virus type I | mel5 | L02534.1 | 54% | 41% | 45% |
| Human gammaherpesvirus | RK_LCL_L3 | MG298914.1 | 66% | 45% | 54% |
| Human alphaherpesvirus | McKrae | MN136524.1 | 37% | 50% | 70% |
| Human T-lymphotropic virus 2 | HTLV-2/Japan/NIID/001/2020 | LC534557.1 | 70% | 37% | 66% |
| Human parvovirus B19 | 057214 | JN211130.1 | 29% | 62% | 45% |
| JC polyomavirus | NIID11-53 | LC164352.1 | 70% | 33% | 37% |
| Neisseria gonorrhoeae | TUM16691 | AP023075.1 | 66% | 37% | 45% |
| Trichomonas vaginalis | TVAG_439770 | XM_001306803.1 | 62% | 29% | 37% |

| Strain- 56/66 Pathogen (Taxonomy ID) | Strain | GenBank Acc# | % Homology Test FP | % Homology Test RP | % Homology Test Probe |
|---|---|---|---|---|---|
| Human immunodeficiency virus | RF (HAT-3) | M17451.1 | 83% | 50% | 58% |
| Hepatitis C virus subtype 1a | Pt9.Seg8-Con | KX084702.1 | 50% | 75% | 34% |
| HSV-1 | 2339/2009 | JQ352258.1 | 45% | 70% | 66% |
| Hepatitis B virus | HBV_NWFD_AHB111 | MZ090870.1 | 75% | 54% | 58% |
| HSV-2 | M2288 | CP003773.1 | 58% | 50% | |
| Mycoplasma genitalium | TC0411: TnxD/tetR-103 | CP042802.1 | 75% | 62% | 58% |
| Chlamydia trachomatis | TC0411: TnxD/tetR-103 | CP042802.1 | 50% | 75% | 45% |

| | | | | | |
|---|---|---|---|---|---|
| Streptococcus agalactiae | SG-M25 | CP021867.1 | 58% | 54% | 41% |
| Human T-cell leukemia virus type I | AC038 | LC192501.1 | 41% | 54% | 58% |
| Human gammaherpesvirus | eBL-Tumor-0014 | LR813032.1 | 30% | 41% | |
| Human alphaherpesvirus | 2339/2009 | JQ352258.1 | 62% | 56% | 50% |
| Human T-lymphotropic virus 2 | HTLV-2/Japan/NIID/001/2020 | LC534557.1 | 41% | 50% | 45% |
| Human parvovirus B19 | HZ92 | KT389469.1 | 34% | 30% | 58% |
| JC polyomavirus | RKAB15 LTAg | LC650363.1 | 35% | 42% | 61% |
| Neisseria gonorrhoeae | FA6140 | CP012027.1 | 34% | 62% | 58% |
| Trichomonas vaginalis | TVAG_480160 | XM_001307664.1 | 50% | 30% | 62% |

| Pathogen / 51 (Taxonomy ID) | Strain | GenBank Acc# | % Homology Test FP | % Homology Test RP | % Homology Test Probe |
|---|---|---|---|---|---|
| Human immunodeficiency virus | 20263v08 | MN791407.1 | 54% | 65% | 50% |
| Hepatitis C virus subtype 1a | HCV-1b/US/BID-V152/2003 | EU155224.2 | 45% | 50% | 50% |
| Hepatitis B virus | 94T.13. | KR812069.1 | 54% | 50% | 54% |
| HSV-1 | 2018-5971 | MH813987.1 | 87% | 45% | 59% |
| Human papillomavirus | NCI_230248 | MG849960.1 | 40% | 45% | 45% |
| HSV-2 | 1996-45091 | MH790670.1 | 66% | 83% | 45% |
| Mycoplasma genitalium | M6285 MgPa | GU226202.1 | 59% | 45% | 41% |
| Chlamydia trachomatis | TC0411: TnxD/tetR-296 | CP042800.1 | 50% | 63% | 81% |
| Streptococcus agalactiae | SG-M25 | CP021867.1 | 68% | 65% | 83% |
| Human T-cell leukemia virus type I | 012BR_HAM107 | KF797884.1 | 41% | 50% | 40% |
| Human gammaherpesvirus | HC-0020 | LR813006.1 | 54% | 85% | 58% |

| | | | | | |
|---|---|---|---|---|---|
| Human alphaherpesvirus | Y033 UL40 | AY240581.1 | 45% | 87% | 50% |
| Human T-lymphotropic virus 2 | NIID18002 | LC440556.1 | 40% | 45% | 45% |
| Human parvovirus B19 | CAEN.FRA 21.09/1 | FN669504.1 | 37% | 40% | 45% |
| JC polyomavirus | PE-21 | AB081027.1 | 33% | 45% | 40% |
| Neisseria gonorrhoeae | AUSMDU00010541 | CP045832.1 | 63% | 55% | 45% |
| Trichomonas vaginalis | TVAG_308030 | XM_001579636.1 | 59% | 55% | 50% |

- **Cross-Reactivity (Clinical Specificity)** of the Stark Genotyping HPV Molecular Diagnostic Kit

To assess the clinical specificity of the Kit, nucleic acid from other pathogens was tested in a matrix of negative samples (Cervical negative swab) diluted to a specific concentration. These samples were then extracted and tested using the Stark Genotyping HPV Molecular Diagnostic Kit. No cross-reactivity was observed for the pathogens listed in the following table:

This testing confirms the high clinical specificity of the Stark Genotyping HPV Molecular Diagnostic Kit, indicating its ability to accurately detect HPV without interference from other pathogens.

**Table 12**: Investigation of the cross-reactivity of the HPV using Stark Genotyping HPV Molecular Diagnostic Kit

| Virus/Bacteria/Parasite | Source/ Sample type | Ct Value |
|---|---|---|
| Human immunodeficiency virus-1 | Clinical sample | -/- |
| Hepatitis C virus | Clinical sample | -/- |
| Cytomegalovirus | Clinical sample | -/- |
| Herpes simplex virus type 1 | Clinical sample | -/- |
| Herpes simplex virus type 2 | Clinical sample | -/- |
| Human papillomavirus | Clinical sample | -/- |
| Epstein-Barr virus | Clinical sample | -/- |
| Adenovirus | Clinical sample | -/- |
| Influenza A | Clinical sample | -/- |
| Influenza B | Clinical sample | -/- |
| Legionella pneumophila | Clinical sample | -/- |
| Cryptococcus neoformans | Clinical sample | -/- |
| Chlamydia pneumonia | Clinical sample | -/- |
| Streptococcus pneumonia | Clinical sample | -/- |
| Respiratory Syncytial Virus | Clinical sample | -/- |
| Mycoplasma pneumonia | Clinical sample | -/- |
| Streptococcus pyogenes | Clinical sample | -/- |
| Mycobacterium tuberculosis | Clinical sample | -/- |
| 10 Pooled human genomes | Clinical sample | -/- |

- **Clinical Evaluation**

The clinical performance of the Stark Genotyping HPV Molecular Diagnostic Kit was assessed using 206 cervical specimens, liquid-based cytology specimens, urine, and paraffin-embedded tissue collected from patients suspected of HPV infection. The comparator methods utilized were the HPV 3.5 LCD-Array Kits (Chipron GmbH) and the Cobas® HPV test (Roche), both of which have received CE-IVD certification. The extraction method employed was the DNall VirAll Kit.

The results of the clinical evaluation are summarized in the analysis, demonstrating a Positive Percent Agreement (PPA) of 96.47% and Negative Percent Agreement (NPA) of 100% when compared to the HPV 3.5 LCD-Array Kits (Chipron GmbH). Similarly, when compared to the Cobas® HPV test (Roche), the Stark Genotyping HPV Molecular Diagnostic Kit exhibited a PPA of 94.4% and NPA of 100%.

**Table 13**: Results of the Stark Genotyping HPV Molecular Diagnostic Kit (CARBON Technologies LLC) alongside the HPV 3.5 LCD-Array Kits (Chipron GmbH) and Cobas® HPV test (Roche).

| Number | HPV 3.5 LCD-Array Kits (Chipron GmbH) | Stark Genotyping HPV Molecular Diagnostic Kit (CARBON Technologies LLC) |
|---|---|---|
| | Result | Result |
| 1 | 11 | 6/11 |
| 2 | 6/16 | 6/11, 16 |
| 3 | 51 | - |
| 4 | 16 | 16 |
| 5 | 51 | 51 |
| 6 | 6 | 6/11 33/52/58 |
| 7 | 6/59 | 6/11 |
| 8 | 16/18 | 16/18 |
| 9 | 16 | 16 |
| 10 | 45 | 45 |
| 11 | 31 | 31 |
| 12 | 45 | 45 |
| 13 | 45 | 45 |
| 14 | 45 | 45 |
| 15 | 18 | 18 |
| 16 | 18 | 18 |
| 17 | 18 | 18 |
| 18 | 45 | 45 |
| 19 | 45 | 45 |
| 20 | 16 | 16 |
| 21 | 16 | 16 |
| 22 | 16 | 16 |
| 23 | 16 | 16 |
| 24 | 16 | 16 |
| 25 | 16 | 16 |
| 26 | 16 | 16 |
| 27 | 51 | 51 |
| 28 | 51 | 51 |
| 29 | 51 | 51 |

| | | |
|---|---|---|
| 30 | 51 | 51 |
| 31 | 51 | 51 |
| 32 | 56 | 56/66 |
| 33 | 56 | 56/66 |
| 34 | 56 | 56/66 |
| 35 | 56 | 56/66 |
| 36 | 56 | 56/66 |
| 37 | 56 | 56/66 |
| 38 | 56 | 56/66 |
| 39 | 66 | 56/66 |
| 40 | 66 | 56/66 |
| 41 | 66 | 56/66 |
| 42 | 66 | 56/66 |
| 43 | 66 | 56/66 |
| 44 | 66 | 56/66 |
| 45 | 66 | 56/66 |
| 46 | 35 | 35/39 |
| 47 | 35 | 35/39 |
| 48 | 35 | 35/39 |
| 49 | 35 | 35/39 |
| 50 | 35 | 35/39 |
| 51 | 35 | 35/39 |
| 52 | 35 | 35/39 |
| 53 | 35 | - |
| 54 | 59 | 59 |
| 55 | 59 | 59 |
| 56 | 59 | 59 |
| 57 | 59 | 59 |
| 58 | 59 | 59 |
| 59 | 59 | 59 |
| 60 | 59 | - |
| 61 | 6 | 6/11 |
| 62 | 6 | 6/11 |
| 63 | 11 | 6/11 |
| 64 | 11 | 6/11 |
| 65 | 11 | 6/11 |
| 66 | 52 | 33/52/58 |
| 67 | 52 | 33/52/58 |
| 68 | 52 | 33/52/58 |
| 69 | 52 | 33/52/58 |
| 70 | 52 | 33/52/58 |
| 71 | 52 | 33/52/58 |
| 72 | 58 | 33/52/58 |

| | | |
|---|---|---|
| 73 | 58 | 33/52/58 |
| 74 | 58 | 33/52/58 |
| 75 | 58 | 33/52/58 |
| 76 | 58 | 33/52/58 |
| 77 | 58/33 | 33/52/58 |
| 78 | 58/33 | 33/52/58 |
| 79 | 33 | 33/52/58 |
| 80 | 33 | 33/52/58 |
| 81 | 33 | 33/52/58 |
| 82 | 33 | 33/52/58 |
| | | |
| Number | Cobas® HPV test (Roche) | Stark Genotyping HPV Molecular Diagnostic Kit (CARBON Technologies LLC) |
| 1 | 16 | 16 |
| 2 | 16 | 16 |
| 3 | 16 | 16 |
| 4 | 16 | 16 |
| 5 | 16 | 16 |
| 6 | 16 | 16 |
| 7 | 16 | 16 |
| 8 | 16 | 16 |
| 9 | 16 | 16 |
| 10 | 16 | 16 |
| 11 | 16 | 16 |
| 12 | 16 | - |
| 13 | 18 | 18 |
| 14 | 18 | 18 |
| 15 | 18 | 18 |
| 16 | 18 | 18 |
| 17 | 18 | 18 |
| 18 | 18 | 18 |
| 19 | 18 | 18 |
| 20 | 18 | 18 |
| 21 | 18 | 18 |
| 22 | 18 | - |
| 23 | High Risk | 33/52/58, 56/66 |
| 24 | High Risk | 6/11, 33/52/58, 51 |
| 25 | High Risk | 6/11, 16, 51 |
| 26 | High Risk | 6/11, 51 |
| 27 | High Risk | 6/11, 33/52/58, 68, 51 |
| 28 | High Risk | 33/52/58, 51 |
| 29 | High Risk | 59 |
| 30 | High Risk | 33/52/58, 51 |

| | | |
|---|---|---|
| 31 | High Risk | 33/52/58, 68, 51 |
| 32 | High Risk | 39, 33/52/58, 35, 68 |
| 33 | High Risk | 68, 51, 56/66 |
| 34 | High Risk | 51, 56/66 |

- **The results of Clinical Evaluation**

**Table 14**: Clinical Evaluation of Stark Genotyping HPV Molecular Diagnostic Kit compared to the HPV 3.5 LCD-Array Kits (Chipron GmbH)

| Test | | HPV 3.5 LCD-Array Kits (Chipron GmbH) | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| Stark Genotyping HPV Molecular Diagnostic Kit (CARBON Technologies LLC) | Positive | 82 | 0 | 82 |
| | Negative | 3 | 100 | 103 |
| Total | | 85 | 100 | 185 |

- Positive Agreement Rate: 82 ÷ 85 × 100% = 96.47%
- Negative Agreement Rate: 100 ÷ 100 × 100% = 100%
- Overall rates of agreement: (100 + 82) ÷ (82 + 0 + 100 + 3) × 100% = 98.37%

These rates indicate the level of agreement between the Stark Genotyping HPV Molecular Diagnostic Kit and the HPV 3.5 LCD-Array Kits by Chipron GmbH in detecting HPV infections, demonstrating high levels of concordance between the two diagnostic methods.

**Table 15**: Clinical Evaluation of Stark Genotyping HPV Molecular Diagnostic Kit compared to the Cobas® HPV test (Roche)

| Test | | Cobas® HPV test (Roche) | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| Stark Genotyping HPV Molecular Diagnostic Kit (CARBON Technologies LLC) | Positive | 34 | 0 | 34 |
| | Negative | 2 | 100 | 102 |
| Total | | 36 | 100 | 136 |

- Positive Agreement Rate: 34 ÷ 36 × 100% = 94.44%
- Negative Agreement Rate: 100 ÷ 100 × 100% = 100%
- Overall rates of agreement: (100 + 34) ÷ (34 + 0 + 100 + 2) × 100% = 98.52%

These rates indicate the level of agreement between the Stark Genotyping HPV Molecular Diagnostic Kit and the Cobas® HPV test by Roche in detecting HPV infections, demonstrating high levels of concordance between the two diagnostic methods.

### Symbols

| | |
|---|---|
|  | Manufacturer |
|  | Date of manufacture |
|  | Use-by date |
|  | Contains sufficient for <n> tests |
|  | Consult instructions for use |
|  | Biological risks |
|  | Temperature limit |
|  | CE Marking |
|  | EU Representative |
|  | In Vitro diagnostic medical device |
|  | Catalogue Number |
|  | Lot Number |

### Troubleshooting

For troubleshooting assistance with the Stark Genotyping HPV Molecular Diagnostic Kit, please refer to the following guidelines. Our ROJE Technical Support Team is available to address any further questions or concerns you may have:

| Problems | Possible Causes | Action |
|---|---|---|
| No fluorescent signal is detected in any samples, including positive control. | Error in the preparation of the master mixture | Verify each component and ensure the volumes of reagent dispensed during the preparation of the master mixture are correct. Repeat PCR mixture preparation. |
| | Instrument settings error | Verify the Real-time PCR instrument settings are correct. |
| If the fluorescent signal is detected in a negative control reaction | Contamination of the extraction/preparation area | Clean surfaces and instruments with aqueous detergents, wash lab coats and replace test tubes and tips in use. |
| | PCR tube not properly sealed | Ensure plates are sealed correctly |
| If the fluorescent signal does not display the sigmoidal characteristic | Components degraded | Use a new batch. |
| | Poor quality of DNA samples carrying interferences | Repeat the test with the neat extracted DNA and 1:2 dilution of the extracted DNA. |

| | | |
|---|---|---|
| | PCR equipment failure | Repeat the test or contact the equipment supplier |

### Technical assistance

For technical assistance, CARBON Technologies LLC guarantees your complete satisfaction. Our ROJE technical support team is comprised of highly trained and experienced scientists who are capable of troubleshooting most problems you may encounter. Our technical support team can offer expert advice to help you select the most suitable product for your needs.

You can contact our technical support team at any time through the following methods:

- By phone or fax: +96897058350
- Directly submit your questions to the CARBON Technologies technical support team through our website: www.carbontechnologiesco.com
- Send your questions via email to: technicalsupport@carbontechnologiesco.com

Rest assured, our team is dedicated to providing prompt and effective assistance to address any inquiries or issues you may have. We are committed to ensuring your satisfaction and success in using our products.

### References

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After-sale Service Center: Carbon Technologies LLC

CE
IVD

Release Date: ... ... ... Date of Manufacture: ... ... ...