Prevo-Check®

Rapid test for the qualitative detection
of antibodies against HPV 16 L1 in whole blood and serum

REF LFT-001 CE IVD

Abviris Deutschland GmbH

INTENDED USE

The Prevo-Check® is an IVD rapid test for the qualitative detection of antibodies against HPV 16 L1 in whole blood and serum. The test is used as an adjunctive aid for the early detection of HPV16 induced carcinomas in the head and neck area and anogenital region.

Introduction

Human Papilloma viruses (HPV) represent a group of viruses of more than 120 different types. They infect the epithelial layer of the skin or other mucosal membranes e.g. in the oral cavity and can cause uncontrolled growth and proliferation of infected cells. Some HPV subtypes can cause malignant alterations. Head and neck carcinomas are the most common group of cancer diseases caused by HPV.

The yearly number of HPV-induced squamous cell carcinomas cancer is increasing. HPV-induced tumors typically present in certain areas of the body depending on patient gender. While females show tumors in the anogenital region more frequently, males present more tumors in the head-neck area. HPV-induced tumors in the head-neck area, especially in the oropharyngeal region, are the most frequent HPV-induced cancer type in the US and other developed countries. Early detection of these cancers is indicative of better treatment prognosis and outcomes.

The presence of increased HPV16 L1 antibodies is a highly-specific indicator for altered cells in patients which have not been vaccinated against HPV. An elevated level of these antibodies is linked to the presence of precancerous lesions or tumors.

While serological methods for the early detection of these HPV-induced tumors did not exist in the past, Abviris Deutschland has developed a rapid test for the detection of HPV16 L1 antibodies in blood or serum.

The cut off value of the Prevo-Check® is adjusted to detect relevant antibody levels. The high quality performance data are based on the use of the special antibody clone anti-HPV16 L1 DRH1. This antibody is directed against a protein which is produced only by cells in which HPV16 has actively affected the cell division. This means that the antibody is only present if a sub clinical HPV16 infection has progressed to a cancer pre-stage or carcinoma.

PRINCIPLE OF THE TEST

In a first step, the sample is mixed with an HPV-reagent. If HPV16-antibodies are present in the sample, a complex of HPV-reagent and HPV16 antibodies is formed and inactivates the HPV-reagent. Otherwise the HPV-reagent remains reactive.

In the second step this mixture is dropped on the sample well of the cassette and migrates chromatographically on the membrane to the reaction zone. During that process, the mixture flows over a pad containing gold labelled antibodies. If the HPV-reagent is reactive after step 1, a complex with the gold-labelled antibodies of the pad is formed. This complex will be caught by immobilized HPV16 antibodies at the test result line. If the test result line is formed, the result is negative; no HPV16 antibodies were detected. If the HPV-reagent was inactivated by HPV16 antibodies (step 1), no test line is formed in the reaction zone; the result is positive. A positive result indicates the presence of HPV16 L1 antibodies. Another independent reaction at the control line indicates that the test has been performed properly. The presence of this control line serves as verification that sufficient volume has been applied and that proper flow was obtained. The control line should always appear, regardless whether HPV16 L1 antibodies were detected or not.

SETUP OF THE DEVICE

The mould contains a test strip. The sample well (S) indicates the lower end of the strip. This is the area where the sample-reagent-mix is applied. The result window contains a test zone (T) and the control zone (C). The result in the test zone (T) indicates whether HPV16 L1 antibodies are present in the patient sample or not.

STORAGE AND STABILITY

The test kit should be stored at 2-30°C. The test´s expiration date is printed on the sealed pouch and on the box label. The test should be performed right after opening of the pouch. Under these conditions, the test is stable until the stated expiry date. Make sure to protect the test components against contamination. Do not use the device or reagents if there are signs of microbial contamination. Biological contamination of tubes or reagents can lead to false results.

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MATERIALS

Reagents and materials supplied

- 4 tubes with each 120 µl HPV-reagent (HPV 16-antibody-specific surface protein, non-infectious, in buffer (containing 0.09% sodium azide))
- 4 test cassettes, single pouched
- 8 pipettes
- 1 tube holder
- 1 instruction for use

Materials required but not provided

- Timer

PRECAUTIONS

- For professional use only.
- For single use only.
- Do not freeze components of the test kit.
- Do not use after expiration (see pouch and box label).
- Store the test kit at 2-30°C.
- Do not eat, drink or smoke in area where specimens or kits are handled.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- The test device is humidity sensitive. Therefore do not use test if the sealed pouch is damaged. The device should remain in the sealed pouch until use.
- Do not mix HPV reagents or devices of different lots. The HPV reagent should be used exclusively with the corresponding lot of test devices.
- Bring all reagents to room temperature (15-30°C) before use.
- Do not touch the reaction zone of the device to avoid contamination.
- Do not spill the specimen into the reaction zone.
- Avoid cross-contamination of specimens by using a new specimen container and specimen pipette for each specimen.
- Do not use more than the required amount of liquid.
- The buffer is slightly caustic. Clean with copious amounts of water in case of contact with skin or eyes
- The buffer contains sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. While disposing of these solutions, always flush with copious amounts of water to prevent metal azide formation.
- Treat all specimens as if they contain infectious agents.
- Standard guidelines for handling infectious agents and chemical reagents should be applied throughout all procedures. All contaminated waste should be properly disposed.
- The used test should be discarded according to local requirements.
- Humidity and high temperature can adversely affect results.

SPECIMEN COLLECTION, HANDLING AND STORAGE

The test can be performed with whole blood (capillary and venous) or serum. The sample collection and handling of the samples is described below:

Whole Blood from oral contact bleeding

1. Fresh blood from the contact bleeding (e.g. during professional tooth cleaning) must be collected with the pipette which is included in the test kit. Dripping blood can also be used. Avoid contact with the tongue and cheek.
2. Use the sample immediately to avoid clotting, following the test procedure. Do not freeze whole blood samples.

Capillary blood/ Whole blood (e.g. fingertip or earlobe)

1. Disinfect the puncture site with alcohol or another disinfectant. Massage the hand in the direction of the fingertip without touching the puncture site.
2. Prick the fingertip with a sterile lancet.
3. Wipe the first drop of blood. Massage the hand again from palm to fingertip to get a sufficient amount of blood.
4. Collect sample with one of the pipettes included in the test kit.
5. Follow the chapter “TEST PROCEDURE”.
6. Use the sample immediately to avoid clotting, following the test procedure. Do not freeze whole blood samples.

Vein puncture:

1. Collect whole blood sample from vein in accordance with your local guidelines. Please use one of the pipettes included in the kit to collect a drop of whole blood from the tube.
2. Follow the chapter “TEST PROCEDURE”.
3. The test procedure should start immediately after sample collection to avoid blood clotting . Do not freeze whole blood samples.

Serum:

Use 25 µl serum for the test procedure.

TEST PROCEDURE

Bring the test, all reagents and/or external controls to room temperature (15-30°C) prior to the test procedure.

1) Take one tube with HPV-reagent and add 1 drop (30µl) of whole blood sample (alternative 25µl serum).
2) Mix the specimen with the HPV-reagent by pipetting up and down and/or shaking the closed tube several times.
3) Ensure that all material is mixed together. Pay particular attention to material that may be retained in the tube lid.

Abviris Deutschland GmbH
Alter Teichweg 22a
22949 Ammersbek
Deutschland

Tel.: +49 (0)4102 – 677 56 14
Fax: +49 (0)4102 – 677 56 15
E-mail: info@abviris.de
www.abviris.de

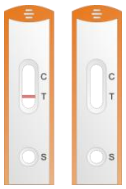
4) Put the tube in the tube holder and incubate the solution 10 (+/-1) minutes. Discard the first pipette.
5) Remove the test device from the pouch and put the cassette on a flat surface. Use the test cassette as soon as possible after removal of the pouch, but within one hour.
6) Take a fresh pipette and put 4 drops of the solution into the specimen well (S) of the cassette. Avoid bubbles while dropping. Start the timer.
7) Read the result after 10 minutes. Do not read the result later than 15 minutes.

INTERPRETATION OF THE RESULTS

The interpretation of test results is performed visually as follows.

NEGATIVE:
Two lines appear. One line in the control region (C) and one line in the test region (T) appeared. A negative test result indicates that no HPV16 antibodies are present in the specimen or that the amount of HPV16 antibodies is below the cut off level of the rapid test. The colour intensity of the lines (T-line and C-line) may be different.

POSITIVE:
One line appears in the control (C) region. No line is visible in the test (T) region. If no line in the test region appears, it is indicated that HPV 16 L1 antibodies, above the cut off level, are detected in the specimen.



INVALID:
If the control line does not appear, then the test result is inconclusive and must be regarded as invalid. This indicates a possible error in performing the test or a failure of the test materials. Repeat the assay with a new cassette device. Pay special attention to the instructions (see TEST PROCEDURE). If the problem persists, contact your supplier.

QUALITY CONTROL

Internal quality control

The test includes an internal procedure control. A red coloured line appearing in the control region (C) represents an internal control. It indicates that enough sample specimen is used, the migration of the specimen on the membrane works properly and the test procedure is correct.

External quality control

Control reagents are not provided with the test kit. Good Laboratory Practice should be applied to assure quality of test performance. It is recommended to perform one test of each kit with a negative control.

OUTCOME AND FOLLOW-UP

In case of a positive test result, please refer to your local guidelines for screening and/or management of HPV induced tumors. If no specific guidelines are in place, it is recommended to proceed as follows:

1) An immediate visual in-depth inspection of the oral cavity including the oropharynx should be performed; suspicious cells should be checked by a pathologist
2) Referral of the patient to an oral surgeon and/or head and neck specialist for further investigation of the oropharyngeal region;
3) A regular visual inspection of the oral cavity including the oropharynx every 3 to 6 months for a period of 2 years should be performed.

Please note: HPV16 potentially infects other cells (e.g. anus or genitals). In case no suspect findings in the oral cavity are observed, it is strongly recommended to let these areas be checked as well from a specialist.

LIMITATIONS

- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated. A negative test result does not rule out an HPV-infection.
- The Prevo-Check® rapid test is an IVD medical device. It detects antibodies against HPV16 L1 only. A detection of antibodies with the Prevo-Check® rapid test does not reveal anything about the presence of antibodies against the L1 protein of other HPV types.
- Patients who are vaccinated against HPV16 are supposed to show high antibody titers. The test should not be performed with patients who were immunized less than 6 years ago, since a positive result is expected, even in absence of an infection with HPV16. A negative test result does not exclude an infection with HPV.
- The Prevo-Check® specifically detects antibodies against HPV16 L1. The Prevo-Check® does not indicate the presence of antibodies from other HPV types.

EXPECTED VALUES

Currently, more than 25% of all oral squamous cell carcinoma are associated with HPV16. The rate increased constantly during recent years. Especially cancers of tonsils (>90%) and oropharynx (>66%) are associated with HPV16 very often. Oral cancer represents the 6th most common cancer type with >500,000 cases annually worldwide. Men are afflicted three to four times more often than women.

DIAGNOSTIC SENSITIVITY AND SPECIFITY

913 samples of persons with known state of health have been analysed with Prevo-Check®. In the group were healthy persons in the age from 30 years on without symptoms indicating no tumor or infection (CRP < 1.0 mg/L) and patients with confirmed HPV16 induced head-neck tumor and positive HPV16 DNA and p16 test results. Specificity of 99.3% and sensitivity of 94.4% have been evaluated. The accuracy with Prevo-Check compared to the clinical diagnosis was 99.2%.

ANALYTICAL SENSITIVITY AND SPECIFITY

The analytical sensitivity of Prevo-Check® results has been determined with the DRH1 standard.
No Hook effect could be observed up to a concentration of 100µg/ml of the HPV16 L1 specific antibody DRH1. Due to the competitive test principle no Hook effect in higher concentrations than 100µg/mL is expected.
There was no cross reactivity observed with HPV type 8,11,18,35 and 39 up to a concentration of 16 IU/ml (type 18) respectively 10 µg/mL.
No interferences could be observed for:

Total protein in serum (100 g/L), albumin (55 g/L), immunglobulin (35 g/L), hemoglobin (1 g/L); lipids (15 g/L), cholesterin (6 g/L), triglycerides (4.5 g/L); acetaminophen (20 mg/dl); acetylsalicylic acid (20 mg/dl); Ampicillin (4 mg/dl); ascorbic acid (20 mg/dl); bilirubin (10 mg/dl); coffein (20 mg/dL); creatinin (200 mg/dl); ethanol (0.4 %); glucose (2000 mg/dl); Ibuprofen (20 mg/dl); rheumatoid factors(200 IU/ml).

INTER- AND INTRA LOT VARIATION/REPRODUCIBILITY

The test results obtained with the same or different lots and reproducibility between different users show same results.

LITERATURE

1) Frazer, Ian, 2007. Correlating Immunity with protection for HPV infection, International Journal of Infectious Diseases 11 (Supplement 2), S10-S16.
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3) Applebaum, Katie M., et al., 2007, Lack of Association of Alcohol and Tobacco with HPV16-Associated Head and Neck Cancer, Journal of the National Cancer Institute, Oxford Journals, 2007;99:1801-10
4) D'Souza G et al., Case-Control Study of Human Papillomavirus and Oropharyngeal Cancer, in The New England Journal of Medicine, 356/2007, S.1944–56.

SYMBOLS

IVD	In-vitro diagnostic medical device		For single use only
Σ	Content		Expiration date
LOT	Lot number		Storage temperature
	Manufacturer		Read instructions for use

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Abviris Deutschland GmbH
Alter Teichweg 22a
22949 Ammersbek
Deutschland

Tel.: +49 (0)4102 – 677 56 14
Fax: +49 (0)4102 – 677 56 15
E-mail: info@abviris.de
www.abviris.de