OncoE6™ Cervical Test

Instructions for Use

IVD

In Vitro Diagnostic

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Intended Use

- The Onco***E6***™ Cervical Test is a qualitative test that detects elevated levels of E6 oncoprotein expressed from cells infected by the Human Papillomavirus types 16 and / or 18.
- E6 oncoprotein is necessary for oncogenic transformation of cervical epithelial cells. Detection of elevated levels of E6 oncoprotein in cells from cervico-vaginal specimens indicates an existing precancerous or cancerous lesion, or elevated risk of future precancer or cancer.
- The Onco***E6***™ Cervical Test is an aid to further assess the likelihood that malignancy is present when used in conjunction with independent clinical evaluations. The test is not intended as a screening or stand-alone diagnostic assay.

Principles of the Procedure

The Onco***E6***™ Cervical Test uses cell lysates generated from cervical (tipped polyester) swab specimens or from specimens collected in PreservCyt® solution (cervical PreservCyt® specimen). The lysate is incubated with alkaline phosphatase (AP) conjugated high-affinity monoclonal antibodies (mAbs) to E6 oncoprotein of HPV subtypes 16 and 18 (E6 16/18). A nitrocellulose test strip, with two capture lines (consisting of immobilized mAbs to E6 16/18), is placed into the specimen lysate/mAb-AP mix. This mix migrates through the test strip membrane by capillary action. A ternary complex (capture mAb-E6-detector mAb) may form if the E6 oncoprotein of HPV types 16 and/or 18 is present in the mix. Upon addition of an enzyme substrate, the ternary complex becomes visible as a purple line at the respective locations (E6 of HPV subtype 16 or 18) on the strip.

The result is positive if the test is valid and a purple test line of any intensity can be visualized; the result is negative if no test line is present on a valid test.

Warnings and Precautions

- Biohazard: biological samples such as tissues, body fluids, and blood have the potential to transmit infectious diseases; for handling of these substances, follow all applicable local, state/provincial, and/or national regulations.
- Use routine laboratory precautions. Do not eat, drink, or smoke in designated work areas.
- Use of this test is limited to personnel who has completed Arbor Vita Corporation's approved training program.
- Do not use kit component that appears damaged or irregular.
- Lysis Solution A is an irritant. Avoid eye and skin contact. In case of exposure, immediately use shower, eyewash fountain, hand/face spray unit, and other emergency equipment to flush affected area(s) with water. Seek medical attention if necessary.

Specimens

Onco***E6***™ Cervical Test is for use with cervical swab specimens or cervical PreservCyt® specimens.

Storage

Appropriate storage conditions and associated stability data of the Onco***E6***™ Cervical Test Kit components are summarized below.

Table 1. Kit Storage Conditions

Onco***E6***™ Cervical Test Kit	Temperature	Duration
Specimen Collection Kit Part #2001000	No temperature restriction	See product label for actual expiration date
Liquid Specimen Preparation Kit Part #2002000	2°C to 25°C	
Specimen Processing Kit Part #2000000	2°C to 25°C	
Starter Kit Part #2000031	No temperature restriction	No expiration date

Table 2. Stability of Specimen

	Temperature	Duration
Cervical Swab Specimen	-85°C to -65°C	At least 12 weeks
	-25°C to -15°C	12 weeks
	2°C to 8°C	14 days
	15°C to 25°C	72 hours
Cervical PreservCyt® Specimen	-25°C to -15°C	For long-term storage
	2°C to 8°C	14 days
	15°C to 25°C	72 hours

Materials Provided

Table 3. Onco***E6***™ Cervical Test Kit Contents (each of the Specimen Collection Kit, Liquid Specimen Preparation kit and Specimen Processing kit contains reagents to process **24 tests**)

Item	Quantity
Onco***E6***™ Cervical Test Specimen Collection Kit Part #2001000	
Swabs, polyester tipped, sterile, specimen collection device	24 swabs
Tube, swab specimen storage container	24 tubes
Onco***E6***™ Cervical Test Liquid Specimen Preparation Kit Part #2002000	
Specimen Rinse Solution	1 bottle
Onco***E6***™ Cervical Test Specimen Processing Kit Part #2000000	
Lysis Solution **A**	1 bottle
Conditioning Solution v1.1 **B**	1 bottle
Enhanced Detector v1.1 **C** (3 vials / pouch)	8 pouches
Wash Solution v1.1 **D**	1 bottle
Developing Solution **E**	1 bottle
Test Unit (3 test strips / pouch)	8 pouches
Positive Control v1.1 **(+)** (1 vial / pouch)	1 pouch
Lysis tube	24 tubes
Amber Wash vial	24 vials
Development tube	24 tubes
Onco***E6***™ Cervical Test Starter Kit Part #2000031	
Solution Stand v1.1	1 unit
Test Platform	2 units
Reading Guide	2 units
Quick Guide	1 sheet

Equipment Required

- Microcentrifuge for 1.5 to 2 mL tubes, 12,000 x g ± 4,000 xg
- 2 Micropipettes (max 200 µL; max 1000 µL; adjustable)
- Tube rotator (end-over-end rotation), 8 ± 2 RPM
- Thermometer (calibrated)
- Timer

Procedure

- Do not run test below 15°C or above 30°C.
- Open only one swab specimen storage container, or one cervical PreservCyt® specimen vial, and one lysis tube at a time to avoid negligent mistakes.
- Pipet all solutions slowly, and avoid formation of bubbles for accuracy. Slow pipetting is especially important for the Lysis Solution A which is **viscous**.
- Change pipette tip between all pipetting steps.
- **Make sure all the reagents are equilibrated to room temperature before use.**

1. SPECIMEN PREPARATION

For Cervical swab specimen: go to **step 1.1**

For Cervical PreservCyt® specimen: go to **step 1.2**

1.1 CERVICAL SWAB SPECIMEN PREPARATION

a) Label lysis tube.

b) Open swab specimen storage tube.

c) Grasp the swab by its handle and carefully remove it from storage container. You may use a piece of lab tissue to touch the swab handle.

d) Insert swab head into the correspondingly labeled lysis tube.

e) Grasp the swab handle just above swab head.

f) Position swab such that the upper border of swab head aligns approximately with the rim of the tube .

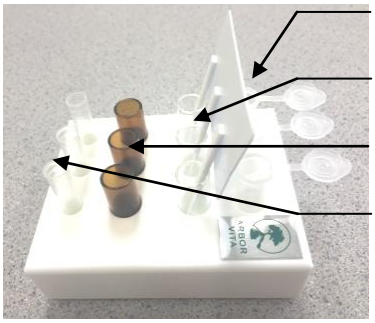
g) Firmly press on lysis tube lid and quickly bend swab handle downwards until it breaks and swab head drops into lysis tube (Picture 1).

Note: when breaking the swab handle, grasp it as close as possible to the tube, to facilitate breaking.

Picture 1. *Breaking swab handle*

h) Place the lysis tube into the first row of the Test Platform (Picture 2).

i) Add 930 µL of Lysis Solution A to the lysis tube containing the swab head.



Picture 2. *Vial/Tube allocation in the Test Platform*

1.2 CERVICAL PRESERVCYT® SPECIMEN PREPARATION

a) Obtain cervical PreservCyt® specimen vial and label a lysis tube accordingly. If the specimen vial was stored at cold temperature, equilibrate at room temperature for 30 minutes. Just prior to use, invert 10 times to suspend cell solution.

b) Slowly aspirate 2 mL of cell solution from cervical PreservCyt® specimen vial. Transfer into the correspondingly labeled lysis tube.

Note: a quantity of at least 10% of biological material (cells) present in the sample after initial collection from the patient is required for a valid test outcome .

c) Spin lysis tube, at 10,000 x g for 5 minutes.

Note: place lysis tube with the cap hinge facing outward into the centrifuge, facilitating location of the pellet (hinge side).

Pellet must be visible and ≥ 1.5 mm at its widest diameter, otherwise the specimen is considered as invalid (Quantity Not Sufficient - QNS).

d) After centrifugation, remove as much supernatant as possible, while avoiding to disturb or to remove the pellet.

e) Add 930 µL of Specimen Rinse Solution to the Lysis tube.

f) Shake lysis tube vigorously for 3 seconds to resuspend the pellet.

g) Spin lysis tube at 10,000 x g for 5 minutes.

Note: place lysis tube with the cap hinge facing outward into the centrifuge, facilitating location of the pellet (hinge side).

h) Retrieve lysis tube from microcentrifuge, without disturbing the pellet, and place the lysis tube into the first row of the Test Platform (Picture 2).

i) Remove as much supernatant as possible, while avoiding to disturb / or to remove the pellet; discard supernatant.

j) Add 372 µL of Lysis Solution A to the lysis tube.

2. E6 Oncoprotein Extraction

a) Shake lysis tube vigorously for 3 seconds.

b) Rotate lysis tube end-over-end at 8 **± 2** RPM for 15 minutes.

c) Spin lysis tube at 10,000 x g for 15 seconds.

3. Conditioning and Clarification

a) Add 87 µL (for cervical swab specimens) or 35 µL (for cervical PreservCyt® specimen) of Conditioning Solution v1.1 B to the lysis tube.

b) Shake lysis tube vigorously for 3 seconds.

c) Rotate lysis tube end-over-end, at 8 **± 2** RPM for 15 minutes.

d) Spin lysis tube at 10,000 x g for 10 minutes.

4. Lysate Application to Enhanced Detector v1.1 C

a) During step 3d, obtain as many Enhanced Detector v1.1 C pouch(es) as needed (one pouch contains 3 Enhanced Detector v1.1 C vials).

b) Tear open each pouch and remove the Enhanced Detector v1.1 C vials: one vial for each sample.

c) Place Enhanced Detector v1.1 C vials into the second row of the Test Platform (Picture 2).

d) Upon completion of Step 3.d, retrieve lysis tube from microcentrifuge and place into the first row of the Test Platform.

e) Transfer 200 µL of supernatant from the lysis tube into the appropriate Enhanced Detector v1.1 C vial.

f) Place Enhanced Detector v1.1 C vial on a flat surface and swirl vial in a circular motion for 5 to 10 seconds; place back into the Test Platform.

Note: handle one Enhanced Detector v1.1 C vial at a time to prevent sample mix-ups.

g) Let sit for 5 minutes.

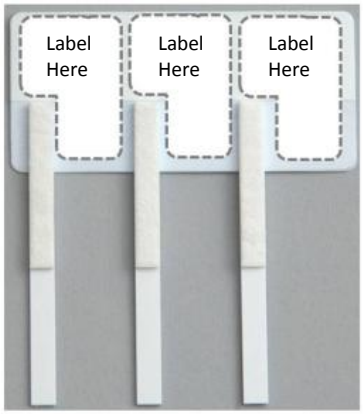
h) Swirl each Enhanced Detector vial v1.1 C once more for 5-10 seconds, as described above.

5. Test Unit Preparation

- Do not use Test Unit if damaged (bent bridge, bent or scratched strip).
- Do not modify Test Unit (remove a strip from the adhesive bridge, cut the adhesive bridge between strips, etc).
- Grab Test Unit pouch at the edges; grab Test Unit only at rigid bridge; do not touch the strips.

a) Tear open the Test Unit pouch and carefully remove Test Unit.

b) Place Test Unit on a clean surface (for example the empty pouch) and label Test Unit in area shown in Picture 3.



Picture 3. *Labeling area on the bridge of the Test Unit*

6. STRIP TEST RUN

For correct positioning and support of the Test Unit, always place the appropriate tubes in all 3 wells per row on the Test Platform. If running fewer than 3 specimens, use empty amber wash vial(s) and development tube(s) to fill the corresponding wells on the Test Platform.

a) Place Test Unit into the Enhanced Detector v1.1 C vials such that it is slanted backwards (Picture 2).

b) Let sit for 55 minutes (referred as "run").

During the last 5-10 minutes of the run

c) Place three empty amber wash vials into the third row of the Test Platform (Picture 2).

d) Add 200 µL of Wash Solution v1.1 D into each amber wash vial.

e) Place three empty development tubes into the fourth row of the Test Platform (Picture 2).

f) Add 750 µL of Developing Solution E to each development tube.

7. WASH STEP

a) At completion of the 55-minute run (Step 6.b), transfer Test Unit (from Enhanced Detector v1.1 C vials) into the amber wash vials, ensure that Test Unit is slanted backwards (Picture 2).

b) Let sit for 12 minutes.

Note: do not "drop" Test Unit into amber wash vials. Ensure that all three strips sit onto the bottoms of the amber wash vials.

Important: during the wash step, do not move Test Platform or touch Test Unit.

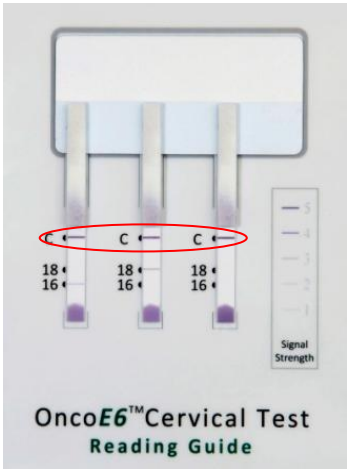
8. DEVELOPING STEP

a) Record the room temperature at the location the test is being performed, and determine the appropriate test development time using the table below.

Room Temperature (°C)	15.0 – 20.9	21.0 – 25.0	25.1– 30.0
Development Time	25 min	20 min	15 min

b) Transfer Test Unit from amber wash vials into the set of development tubes. Let sit for the time determined in Step 8.a.

c) At completion of development, immediately place Test Unit onto Reading Guide (aligning each internal positive control lines (line C) with the "C" marks on Reading Guide as shown in Picture 4); and proceed to step 9. "Test Results Interpretation".



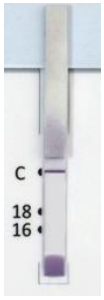
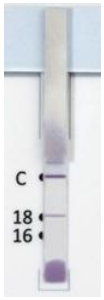
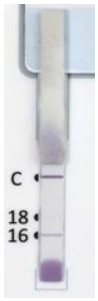
Picture 4. *Test Unit positioning on Reading Guide*

9. TEST RESULTS INTERPRETATION

Results should be read and recorded within 3 minutes after completion of the development step.

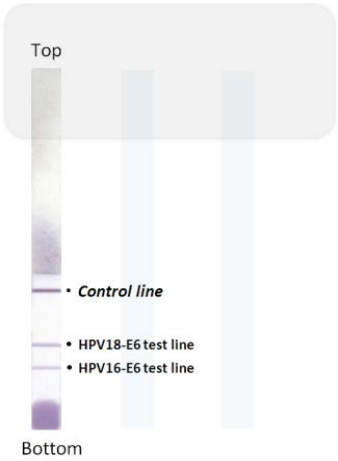
Inspect test area of each strip and interpret result following Table 4 below.

Table 4. Test Result Interpretation

Test is **Negative**
Only the internal positive control line (line C) is visible. 
Test is **Positive for HPV18 E6 oncoprotein**
The internal positive control line (line C) and the 18 line are visible. 
Test is **Positive for HPV16 E6 oncoprotein**
The internal positive control line (line C) and the 16 line are visible. 
Test is **Invalid**
-There is no internal positive control line (line C); -Control or Test lines appear as broken lines, or do not cover full width of strip; -There is an overall strong dark purple background; -There are streaks or dots obscuring the lines.

10. RUNNING THE ONCO*E6*™ CERVICAL TEST POSITIVE CONTROL V1.1 (+)

Use of Onco***E6***™ Cervical Test Positive Control v1.1 (+) allows to generate positive HPV16/18 E6 test lines in the absence of E6 oncoprotein positive specimens. The Positive Control v1.1 contains a mixture of types HPV16 and HPV18 E6 oncoproteins (Picture 5).



Picture 5. *Strip appearance upon application of the Positive Control v1.1*

a) Label a lysis tube as “Mix A+B”.

b) Add 930 µL of Lysis Solution A to “Mix A+B” tube.

c) Add 87 µL of Conditioning Solution v1.1 B to “Mix A+B” lysis tube.

d) Shake “Mix A+B” lysis tube vigorously for 3 seconds.

e) Tear open the Positive Control v1.1 (+) pouch and remove the Positive Control v1.1 (+) vial.

f) Add 200 µL of “Mix A+B” to Positive Control v1.1 (+) vial.

g) On a flat surface, swirl vial in a circular motion for 5 to 10 seconds.

h) Let sit for 5 minutes and repeat the swirling motion.

i) Obtain an Enhanced Detector v1.1 C vial and place it into the second row of the Test Platform (Picture 2).

j) Pipette the entire contents into the Enhanced Detector v1.1 C vial.

k) Place Enhanced Detector v1.1 C vial on a flat surface and swirl vial in a circular motion for 5 to 10 seconds and place back into the Test Platform.

l) Let sit for 5 minutes and repeat the swirling motion.

m) Continue by following Step 5 - 9 of the above procedure.

Note: it is convenient to prepare the Positive Control during the centrifugation Step 3.d. Take care not to confuse the Positive Control v1.1 vial (marked as "+") with the Enhanced Detector v1.1 vial; do not place the test strip assigned for the Positive Control into the Positive Control v1.1 vial, but place it into the Enhanced Detector v1.1 vial containing the Positive Control solution.

Troubleshooting

Problem	Possible Cause and Solution
Background shows vertical lines or streaks	-Test Unit not properly positioned in wash vials or was disturbed during wash step. Repeat sample run starting at Step 3.d. -Excess bubbles during wash step. Repeat sample run starting at Step 3.d, pipet Wash Solution v1.1 D more slowly to wash vial. -Test was run outside of specified temperature range. Repeat sample run starting at Step 3.d.
Internal positive control line (line C) has weak signal	-Cell debris pellet was transferred. Repeat sample run starting at Step 3.d. -Sample contains interfering substance. Obtain new sample and rerun. - Too much Conditioning Solution v1.1 B. Obtain new test components and sample, and rerun. -Developing Solution was too cold. Repeat sample run starting at Step 3.d, and make sure Developing Solution is equilibrated to room temperature before use. -Test components stored incorrectly. Obtain new test components and sample and rerun; or, repeat sample run starting at Step 3.d with new test components.
Test area of test strip is dark purple	-Strip not adequately washed. Repeat sample run starting at Step 3.d.
Test area of the Test strip is white (no signal at the test lines including the internal positive control line (line C))	-Conditioning Solution v1.1 B was not used. Repeat sample run starting at Step 3. adding 68.3 µL of Conditioning Solution v1.1 B to the lysate if processing a cervical swab specimen. If processing a cervical PreservCyt® specimen, obtain a new specimen and rerun. -Developing Solution E was not used. Repeat sample run starting at Step 3.d. -Enhanced Detector v1.1 C was not used. Repeat sample run starting at Step 3.d.

Limitations of the Procedure

- Not a screening test.
- Not a stand-alone diagnostic test.
- Use in conjunction with other clinical patient evaluations.

Bibliography

- Doorbar, J., Clin Sci **110**, 525-541 (2006)
- McCredie, M.R.E et al., Lancet Oncol **9**, 425-434 (2008)
- Yim, E.K. & J.S. Park, Biomarker Insights **2**, 215-225 (2006)
- Blatt, AJ. et al. Cancer (Cancer Cytopathol) 2015;123:282-8.
- Schweizer, J. et al., J Clin Microbiol **48**(12), 4646-4648; doi: 10.1128/JCM.01315-10 (2010)
- Zhao, FH. et al.(2013) Canc Prev Res, 6 p. 938-948
- Qiao, Y. et al. (2013) Int J Canc, 134 (12), p. 2891-2901
- Valdez, M. et al. (2016) Int J Canc, 138 (6), p1453-1461
- Yu, LL. Et al. (2016) Canc Epi Biom Prev, 25 (7) 1167-1174
- Mariano VS, (2016) PLoS One, 11 (10)