IVD REF CER-020

PAPILLODIA

Instruction for Use – English

Please read this user manual carefully before using the test

INTENDED USE

PapilloDia is a rapid immunochromatographic test intended to be used for the qualitative detection of HPV 16/18 E6&E7 oncoproteins in female cervical swab samples. This test is for professional use only and is intended to be used as an aid in the diagnosis of Cervical Pre-cancer and Cancer

SUMMARY

Cervical cancer is a type of cancer that occurs in the cells of the cervix, the lower part of the uterus that connects to the vagina. The major risk factor for cervical cancer is the infection with Human Papilloma Virus (HPV), particularly from high-risk virus types, such as 16 and 18. Cancer of the cervix uteri is the seventh most common in women in the WHO European Region, accounting for about 3.8% of the total. The current vaccines are highly effective in preventing infections with HPV types 16 and 18. Effective primary (HPV vaccination) and secondary prevention approaches (screening for, and treating precancerous lesions) will prevent most cervical cancer cases. The viral E6 and E7 oncoproteins are necessary for malignant conversion. The detection of these proteins in a rapid and easy test can be a useful tool for the diagnostic of cervical pre-cancer and cancer.

DETECTION PRINCIPLE

PapilloDia is a sandwich immunochromatographic assay with one individual test strip containing a conjugate pad impregnated with colloidal gold coupled with monoclonal antibodies specific to HPV 16 & 18 oncoproteins E6 and. E7. The T line is coated with monoclonal antibodies specific to E6 and E7 oncoproteins and the C line is coated with antibodies specific to gold nanoparticles. If the sample is positive, the antigens in the sample react with the nanoparticles and form a complex (Antigen – monoclonal antibody – gold nanoparticles). The mixture then moves upward on the membrane by capillary action. As the sample flows through the nitrocellulose membrane, the specific antibodies present on the membrane (T line) capture the colored conjugate complex and form a red line. If the sample is negative, there is no antigens present or the antigens may be present in a concentration lower than the detection limit. The antibodies present on the membrane (Test line) will not capture the antigen-red-colored conjugate complex (not formed), and the red line will not appear. Whether the sample is positive or not, the nanoparticle complex continues to move through the membrane to the immobilized specific antibodies placed in the control line (C line). The antibodies present on the membrane will react with the nanoparticles and capture the complex to form a red line. The presence of this red line serves as: (1) verification that sufficient volume is added, (2) that proper flow is obtained and (3) an internal control for the reagents. The control line must always appear.

KIT COMPONENTS

Components	Quantity per kit
PapilloDia test in pouch with desiccant	20
Extraction buffer A (0.2 M NaOH) – yellow cap	1 x 10 ml
Extraction buffer B (0.2 M HCl) – white cap	1 x 10 ml
Extraction Tube	20
Tube support	1
Instructions for use	1

Note: The components of kits cannot be exchanged

Materials Required but Not Supplied

1. Cervical swab (eg. #25–806 1PD, Puritan Medical Products)
2. Timer
3. Protective equipment

STORAGE CONDITIONS AND SHELF LIFE

1. The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch and external packaging.
2. The test must remain in the sealed pouch until use and the test must be use within 1 hour after opening the aluminum foil pouch.
3. Do not freeze.
4. Cares should be taken to protect components in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

SAMPLE PREPARATION

Sample type: Cervical swab

The quality of specimen obtained is of extreme importance. As much as cervical epithelial cell should be collected by the swab.

- Use only Dacron or Rayon tipped sterile swabs with plastic shafts. Swabs with cotton tips or wooden shafts are not recommended. The use of Cytobrush was not evaluated with this test.
- Before specimen collection, remove excess of mucus from the endocervical area with a separate swab or sterile gauze and discard. Insert the swab into the cervix until only the bottommost fibers are exposed. Gently rotate the swab for 15-20 seconds in one direction. Pull the swab out carefully!
- Do not place the swab in any transport device containing medium since transport medium interferes with the assay. The test is detecting antigen (proteins) and the viability of the organisms is not required for the assay. Put the swab to the extraction tube if the test may be run immediately. If immediate testing is not possible, the patient samples should be placed in a dry transport tube for storage or transport. The swabs may be stored for 24 hours at room temperature (15-30°C), 1 week at 4°C or no more than 6 months at -20°C. All specimens should be allowed to reach a room temperature (15-30°C) before testing.

TEST PROCEDURE

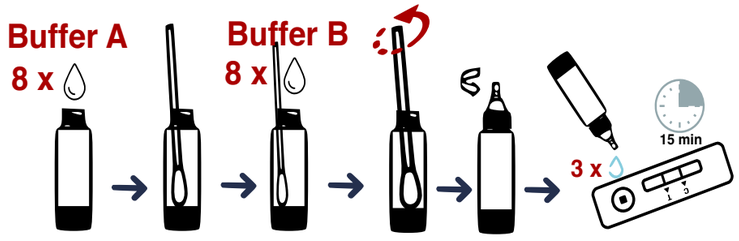
Bring all reagent to room temperature (15-30°C) before testing. Open the aluminum foil pouch and take out the test cassette just before testing.

1. **Prepare swab samples**

- Place a clean extraction tube in the workstation. Add 8 drops of Extraction Buffer A in the extraction tube.
- Immerse the patient swab into the extraction tube and wait 2 minutes. While waiting, use a circular motion to roll the swab against the side of the extraction tube so that the liquid is expressed from the swab and can reabsorb.
- At the end of the extraction time, add 8 drops of Extraction Buffer B to the tube and extract for another 1 minute in the same way. Then squeeze the swab firmly against the tube to extract as much liquid as possible from the swab. Discard the swab following guidelines for handling infectious agents.
- The extracted specimen can remain at room temperature for 60 minutes without affecting the test result.

2. **Testing of samples**

- Remove the test from its sealed pouch, and place it on a clean, horizontal surface. Label the device with patient or control identification. The assay should be performed within one hour.
- Add 3 drops (approximately 100 µl) of extracted sample from the Extraction Tube to the sample well on the test cassette. Avoid trapping air bubbles in the specimen well (S), and do not drop any solution in the test membrane.
- Read the result **after 15 minutes.** Do not interpret the results after 20 minutes.



RESULTS INTERPRETATION

+ The presence of one red line(s) in test area (T), regardless of the intensity of the test line, indicates a positive result

- A single control line (C) indicates a negative result.

● If the control line (C) does not appear, the result is invalid.

1. The color intensity of the test lines cannot be used as quantitative indications. Any line in the Test (T) area is considered positive.
2. If the test result is invalid, repeat the test. If it happens again, stop using this lot and contact your supplier.

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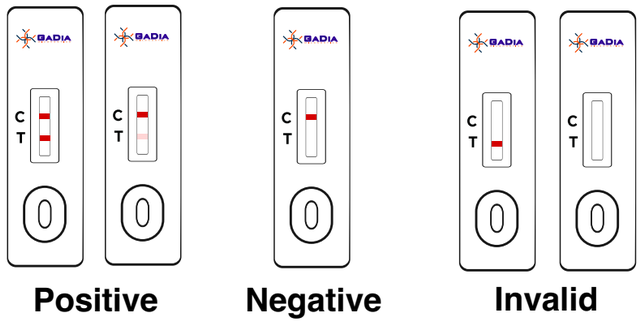

LIMITATIONS

1. The test procedure, precautions and interpretation of results for this test must be followed strictly when testing
2. The test is for professional use only and not for self-testing.
3. This test is a qualitative assay and will not give a quantitative result.
4. Detection of HPV 16/18 E6&E7 Oncoproteins is dependent on the quantity of proteins present in the specimen. This may be affected by specimen collection methods and patient factors such as age, history of STD, presence of symptoms, etc. The minimum detection level of this test may vary according to serovar.
5. The expression of E6&E7 oncoproteins is only indicative of the risk of cervical cancer and pre-cancer occurs, the positive results do not confirm the cancer or pre-cancer stage and the negative results cannot exclude the happening of cervical cancer and pre-cancer.
6. The test results of this kit should be used as an aid for the rapid detection of E6&E7 oncoproteins. The results must be confirmed with alternative or complementary diagnostic procedures. The clinical management of patients should be comprehensively considered in conjunction with their symptoms, medical history, other laboratory tests and treatment responses.

PERFORMANCES

1. Limit of detection (LOD)

The Limit of Detections (LODs) were determined based on antigen concentration at 10 ng/ml

2. Hook effect

No Hook effect has been observed with concentration up to 100 ng/mL of antigens.

3. Interfering substances and cross-reactions

PapilloDia rapid test has been shown to detect HPV type 16 and 18 oncoproteins. Cross reactivity with other organisms has been studied. The following organisms were not detected using the test.

Microorganisms	Concentration	Results
Acinetobacter calcoaceticus	10^7 cfu/ml	NEG
Salmonella typhi	10^7 cfu/ml	NEG
Staphylococcus aureus	10^7 cfu/ml	NEG
Neisseria catarrhalis	10^7 cfu/ml	NEG
Neisseria meningitidis	10^7 cfu/ml	NEG
Escherichia coli	10^7 cfu/ml	NEG
Streptococcus faecalis	10^7 cfu/ml	NEG
Pseudomonas aeruginosa	10^7 cfu/ml	NEG
Ureaplasma Urealyticum	10^7 cfu/ml	NEG
Proteus vulgaris	10^7 cfu/ml	NEG
Acinetobacter spp.	10^7 cfu/ml	NEG
Candida albicans	10^7 cfu/ml	NEG
Neisseria gonorrhoea	10^7 cfu/ml	NEG
Neiiseria lactamica	10^7 cfu/ml	NEG
Gardnerella vaginalis	10^7 cfu/ml	NEG
Streptococcus faecium	10^7 cfu/ml	NEG
Trichomonas vaginalis	10^7 cfu/ml	NEG
Mycoplasma hominis	10^7 cfu/ml	NEG

4. Repeatability and reproducibility

Reproducibility and reproducibility of the test have been evaluated internally with three different lots and a coefficient of variation (CV) of less than 10% was observed.

5. Clinical performances

A retrospective performance evaluation study was conducted on vaginal swab samples. PapilloDia rapid test was compared to immunohistochemical assay (IHC) [6]. The performance of the test is strongly dependent of the stage of the cervical cancer

		IHC	
		+	-
PapilloDia	+	58	8
	-	13	63
Sensitivity	**81,7%**	(CI95%: 70.4-89.5%)	
Specificity	**88,7%**	(CI95%: 78.5-94.7%)	
PPV	**87,9%**	(CI95%: 77.0-94.3%)	
NPV	**82,9%**	(CI95%: 72.2-90.2%)	

WARNING AND PRECAUTIONS

1. Read the instruction for use carefully before using the test.
2. This product is for *in vitro* diagnostic and professional use only.
3. Do not reuse the test or kit components
4. Do not use the test after expiry date and do not freeze the test.
5. Read the test results within the specific time to avoid wrong interpretation.
6. Do not use the components from different batches or different types of reagents.
7. Properly dispose the specimen and used materials following the local biohazardous disposal regulation.
8. Use protective equipment when using the test and handling samples as they may contain infectious agents, human or animal components
9. Humidity and temperature can adversely affect results, use the test in standard laboratory humidity and temperature conditions.
10. The Extraction buffers (A & B) contain acid and alkaline solutions. Avoid eyes and skin contact. In case of contact with eyes, rinse immediately with plenty of water.
11. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

REFERENCES

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2. Kong et al., Analysis of the role of the human papillomavirus 16/18 E7 protein assay in screening for cervical intraepithelial neoplasia: a case control study. BMC Cancer 20, 999 (2020)
3. Schweizer et al., Feasibility study of a human papillomavirus E6 oncoprotein test for diagnosis of cervical precancer and cancer. J Clin Microbiol. 2010 Dec;48(12):4646-8
4. Krings et al., Performance of OncoE6 cervical test with collection methods enabling self-sampling. BMC Women's Health (2018) 18:68
5. Bhattarakosol et al., Immunogold-agglutination assay for direct detection of HPV-16 E6 and L1 proteins from clinical specimens. J Virol Methods. 2018 May; 255:60-65.
6. Stiasny et al., Immunohistochemical Evaluation of E6/E7 HPV Oncoproteins Staining in Cervical Cancer. Anticancer Res. 2016 Jun;36(6):3195-8.

SYMBOLS

	Manufacturer		Expiry Date
	Do not reuse	LOT	Lot Number
	Manufacturing date	EC REP	European Authorized Representative
	Consult instructions for use	IVD	In vitro diagnostic medical device
	Temperature limitation	REF	Catalog number
	Sufficient for <n> Test	CE	CE Marking
	Not for near patient testing (IVDR only)		Not for self-testing (IVDR only)

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SWISS QUALITY

CE IVD

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