

HPV Detection on Cytology Specimens

Protocol Overview

This is a condensed protocol and should not replace the manual included in each product.



Remove Coverslip

Soak slides in xylene until coverslips can be removed easily.



Destaining

After rehydration, destain for 5-15 min in 0.5% HCl-ethanol, then wash in dH₂O.



Post-Fixation

Incubate 5 min in 1% formaldehyde solution. Wash in TBS buffer, then in dH₂O.



Heatpretreatment

Incubate 15 min @ 98°C in Heat Pretreatment Solution EDTA, then wash in dH₂O.



Proteolysis

Apply Pepsin Solution for approx. 15 min @ 37°C, then wash in dH₂O.



Hybridization

Apply Probe, cover with coverslip, seal with rubber cement, and co-denature for 5 min @ 75°C. Hybridize 1 h @ 37°C.



Stringency Wash

Remove coverslip by soaking in TBS buffer, incubate for 5 min in TBS @ 55°C, then wash in TBS buffer.



Detection

Apply primary antibody, incubate 30 min @ 37°C, then wash in TBS buffer. Apply secondary antibody, incubate 30 min @ 37°C, then wash in TBS buffer.



Stain & Cover

Apply chromogen solution, incubate, then wash in dH₂O. Counterstain with hematoxylin solution and mount slide with a permanent mounting solution.



Evaluation

Evaluate slides using a light microscope.

ZytoLight® Cytology Implementation Kit

Introduction

Human papillomavirus (HPV) infections are associated consistently with histologically defined preinvasive lesions and are accepted as a necessary cause of invasive cervical carcinoma¹. Thus, testing for HPV high-risk types (HPV-HR) is the recommended method of triage for patients with a diagnosis of Atypical Squamous Cells of Undetermined Significance (ASCUS) and Low grade Squamous Intraepithelial Lesion (LSIL) on Papanicolaou (Pap) specimens². Detection of HPV-HR in case of Pap smear interpretations of ASCUS is a sensitive method for identifying women who harbor underlying High grade Squamous Intraepithelial Lesions (HSIL) or squamous carcinoma³ while the absence of such an infection indicates benign reactive conditions⁴.

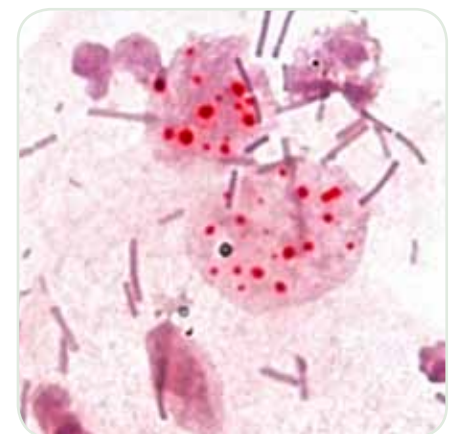
HPV-CISH

While in case of liquid-based specimens testing for HPV is well established and can be easily used, conventional Pap smears often lack this possibility as additional cells for further molecular tests are not available. In this case, HPV detection using chromogenic *in situ* hybridization (CISH) is the method of choice as this test is performed directly on Pap smears omitting the need

for further cells. Additionally, as CISH results are interpreted using light microscopy, HPV-CISH on Pap slides, either conventional smears or liquid-based, will detect HPV infections in the context of cell morphology improving the diagnostic relevance and significance.

ZytoFast® PLUS CISH Solutions

The ZytoFast® PLUS CISH Cytology Implementation Kit in combination with any Digoxigenin-labeled ZytoFast® HPV Probe is an outstandingly convenient solution for HPV detection on Pap slides using CISH, providing you with a detailed protocol as well as with all necessary reagents and controls for performing successful CISH.



HPV detection (red signals) on destained cells of a Pap smear, counterstained with hematoxylin, using the ZytoFast® PLUS CISH Cytology Implementation Kit.

¹ Evans MF, et al. (2006) Cancer 103: 1054-64.

² Qureshi MN, et al. (2005) Acta Cytol 49: 120-6.

³ Solomon D, et al. (2001) J Natl Cancer Inst 93: 293-9.

⁴ Cox JT (2001) Best Pract Res Clin Obstet Gynaecol 15: 715-41.

ZytoFast® Products for CISH analysis

Prod. No.	Product	Label	Tests* (Volume)
Coming soon	ZytoFast HPV type 16/18/31/33/35/39/45/51/52/56/58/59/66/68/82 Probe C€ IVD	Digoxigenin	40 (400 µl)
Coming soon	ZytoFast PLUS CISH Cytology Implementation Kit C€ IVD Incl. Pepsin Solution, Wash Buffers, 10x MgCl ₂ , 10x PBS, Antibodies, Chromogenic Substrate, Counterstain Solution, Mounting Solution		40

* Using 10 µl probe solution per test. C€ IVD only available in certain countries. All other countries research use only! Please contact your local dealer for more information.

ZytoFast® CISH probes are tagged using the unique ZytoFast® HighTag System providing improved signal intensity.

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