

## 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 dH<sub>2</sub>O.



#### Post-Fixation

Incubate 5 min in 1% formaldehyde solution. Wash in TBS buffer, then in dH<sub>2</sub>O.



#### Heat Pretreatment

Incubate 15 min @ 98°C in Heat Pretreatment Solution EDTA, then wash in dH<sub>2</sub>O.



#### Proteolysis

Apply Pepsin Solution for approx. 15 min @ 37°C, then wash in dH<sub>2</sub>O.



#### 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 dH<sub>2</sub>O. 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 carcinoma<sup>1</sup>. 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) specimens<sup>2</sup>. 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 carcinoma<sup>3</sup> while the absence of such an infection indicates benign reactive conditions<sup>4</sup>.

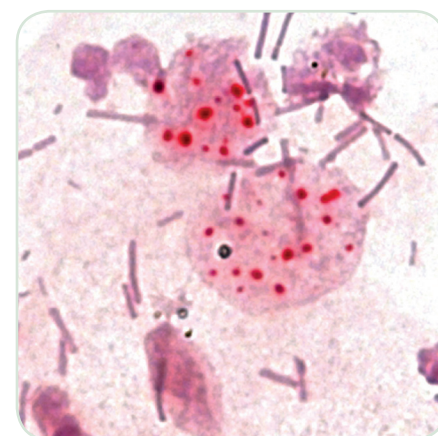
#### HPV-CISH

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.

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

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

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

<sup>4</sup> 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 <sub>2</sub> , 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.

For a detailed description of all new products, please visit : <http://zytovision.com/productinfo.html>

# Distributors

## AFRICA

**Algeria, Burkina, Cameroun, Central African Republic, Côte d'Ivoire, Gabun, Guinea, Morocco, Niger, Senegal, Chad, Tunisia**  
CliniSciences s.a.s  
Fon: +33 1 42 53 14 53  
Fax: +33 1 46 56 97 33  
Email: info@clinisciences.com  
www.clinisciences.com

**Egypt**  
Advanced Diagnostic Services (ADS) Ltd.  
Fon: +44 7783 124383  
Fax: +44 1553661700  
Email: adserviceuk@aol.com

**Saudi Arabia**  
The Gene East  
Fon/Fax: +966 1 4778977  
Email: sales@thegenes.net  
www.thegenes.net

## AMERICA

**Brazil**  
INOPAT IMP. E EXP. LTDA ME  
Fon: +55 (11) 3865-6623  
Fax: +55 (11) 3865-6623  
Email: inopat@inopat.com.br  
www.inopat.com.br

**Canada**  
Gene Diagnostic Inc.  
Fon: +86-571-8604 2915  
Fax: +86-571-8603 1967  
Email: info@genediagnostic.com  
www.genediagnostic.com

**USA**  
Axxora LLC  
Fon: +1 800 900 0065  
Fax: +1 800 550 8825  
Email: axxora-usa@axxora.com  
www.axxora.com

BIO SB, Inc.  
Fon: +1 805 692-2768  
Fax: +1 805 692-2769  
Email: info@biosb.com  
www.biosb.com

**Uruguay**  
Tanirel SA  
Fon: +598 (2) 4804895  
www.tanirel.com.uy

## ASIA

**China**  
Beijing Hualisentai Bio-scientific Co., LTD  
Fon: +86 10 83587896  
Fax: +86 10 83560538  
Email: hualisentai@163.com  
www.hualisentai.com

Gene Diagnostic Inc.  
Fon: +86-571-8604 2915  
Fax: +86-571-8603 1967  
Email: info@genediagnostic.com  
www.genediagnostic.com

**India**  
Vision Diagnostic (India) Pvt. Ltd.  
Fon: +91 11 2279 3914  
Fax: +91 11 2279 4914  
Email: info@vision-groups.com  
www.vision-groups.com

**Indonesia**  
Pt. Biozatif Indonesia  
Fon: +62 21 4788-5303  
Fax: +62 21 4788-5304  
Email: sales@biozatif.net  
www.biozatif.net

**Iran**  
LifeTebGen Co.  
Fon: +98 21 55491015  
Fax: +98 21 88334906  
Email: info@lifetebgen.com

**Israel**  
Rhenium Co. LTD  
Fon +972 (2) 5335599  
Fax +972 (2) 5335590  
Email: info@rhenium.co.il

**Jordan**  
Paramount International  
Fon: +96-2-79-597-0957  
Email: zeid.taher@gmail.com

**Japan**  
Cosmo Bio Co., Ltd  
Fon: +81 3 5632 9617  
Fax: +81 3 5632 9618  
Email: export@cosmobio.co.jp  
www.cosmobio.co.jp

Wako Pure Chemical Ind., Ltd.  
Free Fon: 0120-052-099  
Free Fax: 0120-052-806  
Email: labchem-tec@wako-chem.co.jp  
www.wako-chem.co.jp

**Malaysia**  
Nano Life Quest Sdn. Bhd.  
Fon: +603 8062-4678  
Fax: +603 8062-4678  
Email: info@nanolifequest.com  
www.nanolifequest.com

**Russia**  
OOO Microtests b.m.v.  
Fon: +7 495 9699826  
Fax: +7 495 981 13 51  
Email: info@microtesty.ru  
www.microtesty.ru

**Singapore**  
Precision Technologies Pte Ltd  
Fon: +65-6273 4573  
Fax: +65-6273 8898  
Email: scitech@pretech.com.sg  
www.pretech.com.sg

**South Korea**  
Essence Medical Inc.  
Fon: +82-31-707-1544  
Fax: +82-31-707-1999  
Email: info@essencemedical.com  
www.essencemedical.com

**Taiwan**  
Wonderwork Enterprise Co.  
Fon: +886 (2) 2736-3138  
Fax: +886 (2) 2736-6771  
Email: wonderwork.tw@gmail.com

**Thailand**  
Medicare Supply Ltd. Part.  
Fon: +662 553 6013  
Fax: +662 5783076  
Email: medicareupply@yahoo.com

**Turkey**  
SitoGen Biomedikal ve Laboratuvar Sist. San. Tic. Ltd. Sti.  
Fon: +90 216 - 489 3344  
Fax: +90 216 - 489 0220  
Email: sitogen@sitogen.com.tr  
www.sitogen.com.tr

**United Arab Emirates**  
Advanced Diagnostic Services (ADS) Ltd.  
Fon: +44 7783 124383  
Fax: +44 1553661700  
Email: adserviceuk@aol.com

## EUROPE

**Austria**  
Sanova Pharma GesmbH  
Fon: +43 1 801 04 2560  
Fax: +43 1 801 04 2570  
Email: medicalsystems@sanova.at  
www.medicalsystems.sanova.at

**Belgium**  
Klinipath BVBA  
Fon: +32 14 7021-00  
Fax: +32 14 7021-01  
Email: info@klinipath.be  
www.klinipath.be

**Bulgaria**  
Labsystems Ltd.  
Fon: +359 2 9657917  
Fax: +359 2 9657930  
Email: labsystems@labsystems.bg  
www.labsystems.bg

**Croatia**  
Sanova Pharma GesmbH  
Fon: +43 1 801 04 2560  
Fax: +43 1 801 04 2570  
Email: medicalsystems@sanova.at  
www.medicalsystems.sanova.at

**Czech Republic**  
Pragostem s.r.o.  
Fon/Fax: +420466414176  
Email: info@pragostem.cz  
www.pragostem.cz

**Denmark**  
Nordic BioSite ApS  
Fon: +46 85444 3340  
Fax: +46 (0)8 756 94 90  
Email: info@nordicbiosite.dk  
www.nordicbiosite.dk

**Estonia**  
Nordic BioSite AB  
Fon: +46 85444 3340  
Fax: +46 8 756 94 90  
Email: info@biosite.se  
www.biosite.se

**Finland**  
Immuno Diagnostic Oy  
Fon: +358 3 6153 70  
Fax: +358 3 682 2039  
Email: info@immunodiagnostic.fi  
www.immunodiagnostic.com

**France**  
CliniSciences s.a.s  
Fon: +33 1 42 53 14 53  
Fax: +33 1 46 56 97 33  
Email: info@clinisciences.com  
www.clinisciences.com

**Germany**  
ZYTOMED Systems GmbH  
Fon: +49 30-804 984 990  
Fax: +49 30-804 984 999  
Email: info@zytomed-systems.de  
www.zytomed-systems.de

**Greece**  
BioSURE R&T Cell Co.  
Fon: +30 210-92.23.246,  
Fax: +30 210-92.23.252  
Email: info@biosure.gr  
www.biosure.gr

MENARINI Diagnostics SA  
Fon: +30 210 9944950  
Fax: +30 210 9945029  
Email: mendiagr@otenet.gr  
www.menarinidiagnostics.gr

**Hungary**  
Izinta Trading Co. Ltd.  
Fon: +36 (1) 392 265458  
Fax: +36 (1) 392 265153  
Email: info@izinta.hu  
www.izinta.hu

**Italy**  
Bio-Optica Milano S.p.A.  
Fon: +39 (02) 2127131  
Fax: +39 (02) 2154155  
Email: info@bio-optica.it  
www.bio-optica.it

**Latvia**  
Nordic BioSite AB  
Fon: +46 85444 3340  
Fax: +46 8 756 94 90  
Email: info@biosite.se  
www.biosite.se

**Lithuania**  
Nordic BioSite AB  
Fon: +46 85444 3340  
Fax: +46 8 756 94 90  
Email: info@biosite.se  
www.biosite.se

**Republic of Moldova**  
AP Bioresearch SRL (ICS)  
Fon: +37 322-238166  
Fax: +37 322-224050  
Email: apbioresearch@company.md

**Netherlands**  
Immunologic B.V.  
Fon: +31 316-250309  
Fax: +31 316-280809  
Email: info@immunologic.nl  
www.immunologic.nl

**Norway**  
Nordic BioSite AS  
Fon: +46 85444 3340  
Fax: +46 (0)8 756 94 90  
Email: info@biosite.no  
www.nordicbiosite.no

**Poland**  
Immuniq  
Fon: +48 32 60710-15  
Fax: +48 32 60710-14  
Email: info@immuniq.pl  
www.immuniq.pl

**Portugal**  
ACL - Alfredo Cavalheiro, Lda  
Fon: +351 214387487  
Fax: +351 214387489  
Email: acl@confor.pt

**Romania**  
SC GenomicALL Solutions SRL  
Fon: +40 7 52081534  
Fax: +40 7 49710190  
Email: office@genomicall.ro  
www.genomicall.ro

**Russia**  
OOO Microtests b.m.v.  
Fon: +7 495 9699826  
Fax: +7 495 981 13 51  
Email: info@microtesty.ru  
www.microtesty.ru

**Slovakia**  
Pragostem s.r.o.  
Fon/Fax: +420466414176  
Email: info@pragostem.cz  
www.pragostem.cz

**Slovenia**  
Sanova Pharma GesmbH  
Fon: +43 1 801 04 2560  
Fax: +43 1 801 04 2570  
Email: medicalsystems@sanova.at  
www.medicalsystems.sanova.at

**Spain**  
Anacrom Diagnostics  
Fon: +34 954155282  
Fax: +34 954154734  
Email: anacrom@anacrom.com  
www.anacrom.com

Menarini Diagnostics S.A.  
Fon: +34 935071000  
Fax: +34 932781801  
Email: info@menarini.es  
www.menarinidiag.es

**Sweden**  
Nordic BioSite AB  
Fon: +46 85444 3340  
Fax: +46 8 7569490  
Email: info@biosite.se  
www.biosite.se

**Switzerland**  
LabForce AG  
Fon: +41 61 795 96 20  
Fax: +41 61 795 96 21  
Email: info@labforce.ch  
www.labforce.ch

**Turkey**  
SitoGen Biomedikal ve Laboratuvar Sist. San. Tic. Ltd. Sti.  
Fon: +90 216 - 489 3344  
Fax: +90 216 - 489 0220  
Email: sitogen@sitogen.com.tr  
www.sitogen.com.tr

**United Kingdom/Eire**  
Source BioScience LifeSciences  
Fon: +44 115 973 9018  
Fax: +44 115 973 9021  
Email: lifesciences.customerservice@sourcebioscience.com  
www.lifesciences.sourcebioscience.com

## REST OF THE WORLD

ZytoVision GmbH  
Fon: +49 471 4832 300  
Fax: +49 471 4832 509  
Email: export@zytovision.com  
www.zytovision.com



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# ZYTOVISION



ZytoVision GmbH · Fischkai 1

D - 27572 Bremerhaven · Germany · www.zytovision.com