DIACARTA

QuantiVirus™ HPV E6/E7 mRNA Test

Comparable Sensitivity and Better Specificity for HPV Screening and Triage Test

IMPORTANT FACTS ABOUT HPV

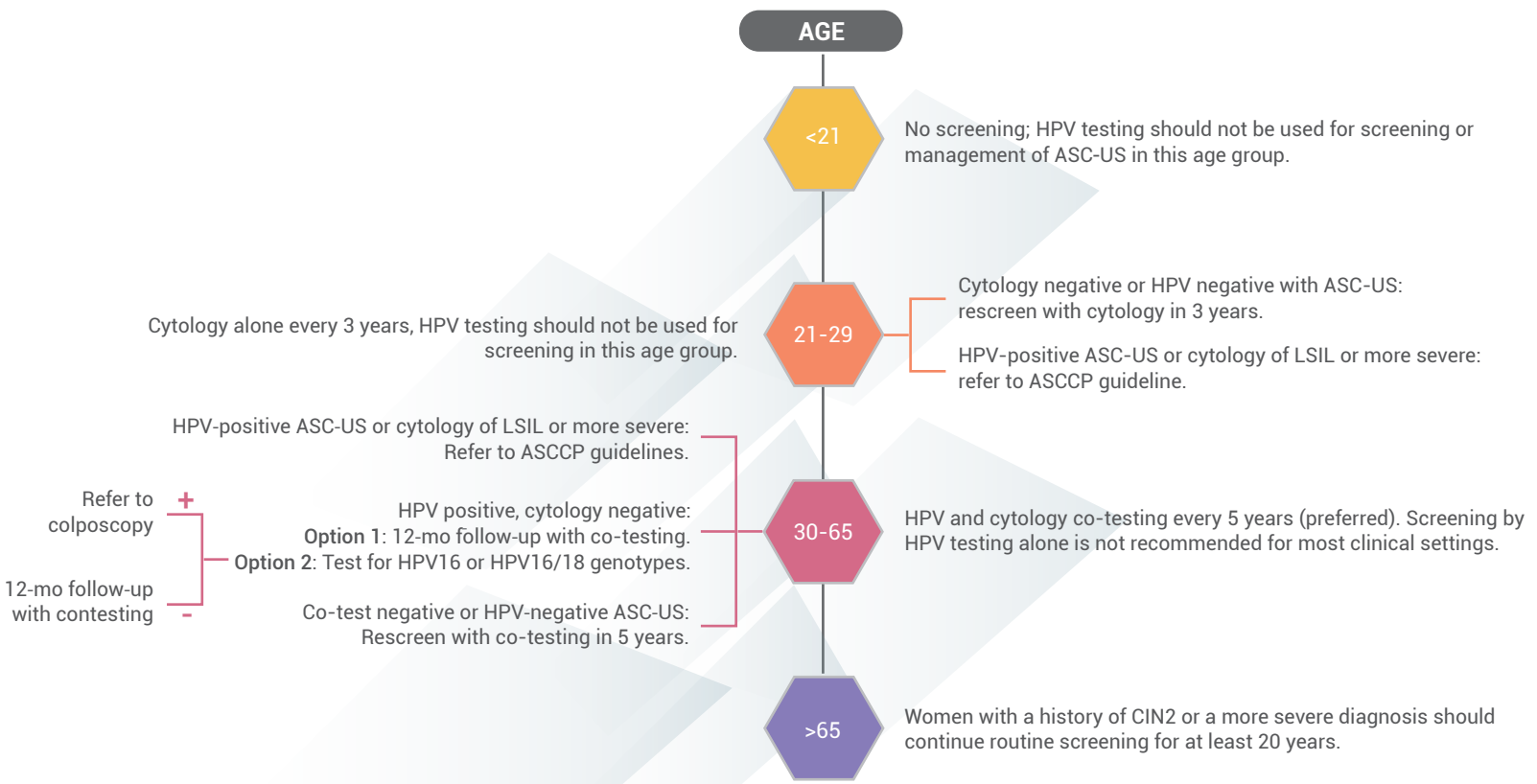
Human papillomavirus (HPV) is mostly spread through sexually transmitted infection. Although most of the infections will clear within two years (transient infection), some will stay in cells for the life time without a cure. With more than 150 different types, the HPV viruses are classified as low-risk and high-risk types. Luckily, persistent infection with high-risk HPV is necessary, but not sufficient, to develop cancer. The challenge is to screen for the high-risk type infected individuals with progression towards cancer. The most common cancer types caused by HPV are cervical cancer in women and neck/head cancer in men.

Findings Related to HPV Infection

- Nearly 80 million Americans, or one in every four, are infected with HPV
- 14 million people including teens are infected with HPV annually
- 45% of Americans from age 18 to 59 carry HPV
- Up to 70% of initial infections clear the virus within 2 years
- Among the women with HPV, significant portion of them develops low-grade cervical lesions
- Most of low-grade cervical lesions regress spontaneously, but 15% progress to high-grade cervical lesions
- One-third of the high-grade lesions develop cervical cancer within 10 years
- Cervical cancer more frequently develops in mid-age women (35 to 45), rarely under 20
- 15% of cervical cancer develops in women over 65
- In the U.S., 13,240 people are newly diagnosed with cervical cancer and 4,170 people died of cervical cancer in 2018

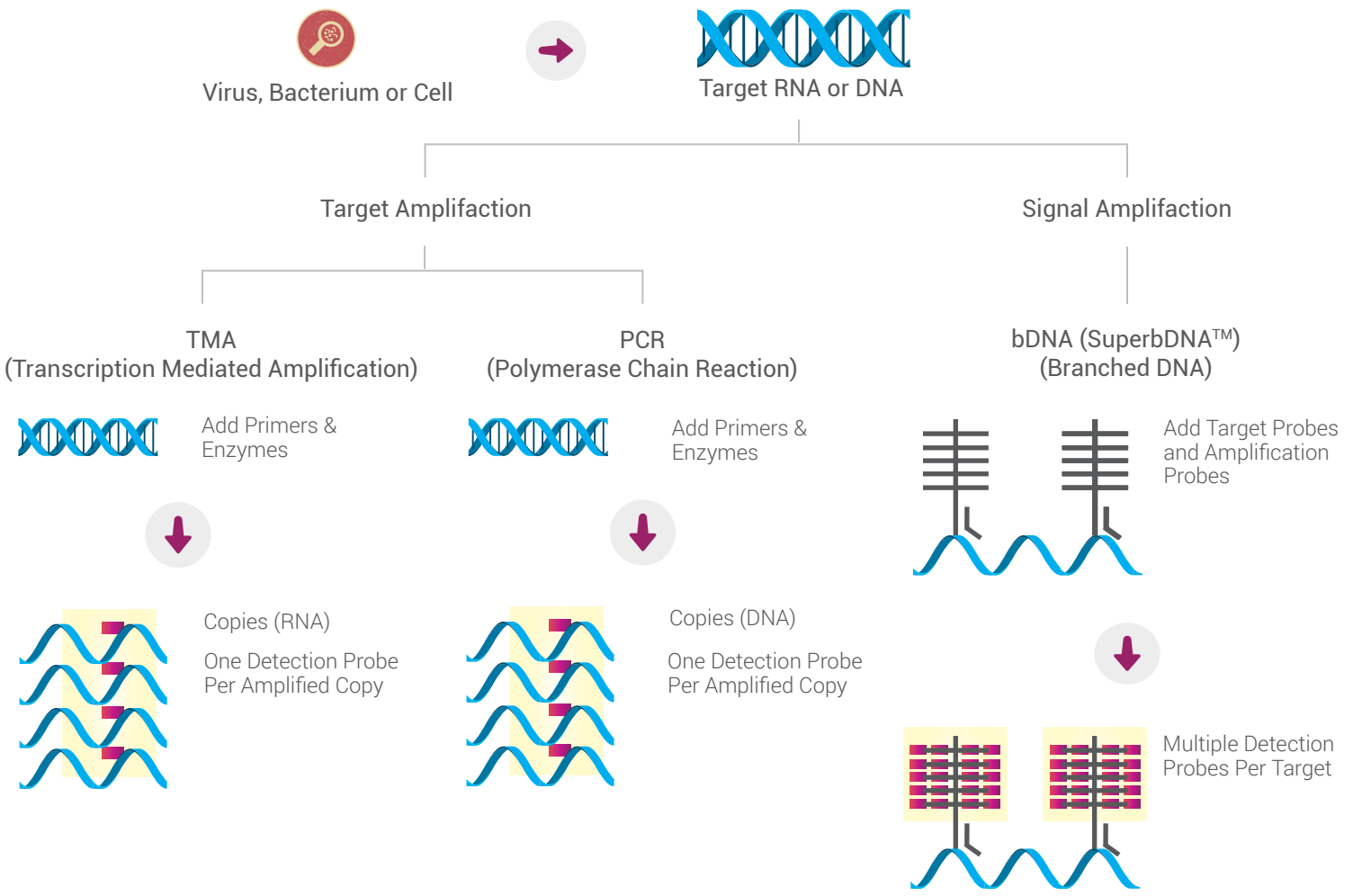
GUIDLINES FOR HPV TESTING

Finding persistent infection and the high-risk type HPV infection help monitoring progression of the virus leading to cervical cancer. The 2012 cervical cancer screening guidelines from American Cancer Society provides screening strategies, including cytology examination, high-risk hpv testing options and follow-up detection.



HPV DIAGNOSTICS/TESTING METHODS

Although multiple methods can be used to infer the presence of HPV, the molecular diagnostic methods are the most sensitive and commonly used methods. Three types of methods are developed: non-amplifcation DNA hybridization methods (southern blot, dot blot), nucleic acid amplification methods (PCR and qPCR), and signal amplification methods (branched DNA or SuperbDNA™).



HPV mRNA Testing V.S. HPV DNA Testing

Although HPV DNA test was first developed for HPV test, the assay can't distinguish the difference between transient and pertinent (transforming) infection. The population of patients with transient infection that eventually clears the virus from the system would also test positive, but will be negative later. Due to the false-positive results from the HPV DNA test, the assay specificity is compromised.

On the other hand, the mRNA test uses biomarker E6/E7 mRNA, which is expressed only in pertinent infection and is necessary, although not sufficient, for cervical cancer development.

The biomarker only detects the pertinent infection and therefore provides better specificity than HPV DNA test.

Although some physicians do not think lower specificity is an issue because they think it would be a small price to pay for these false-positive patients than the price for treating cancer if left out by mistake. But what they fail to realize is the follow-up cost, the potential damage to the cervix and unnecessary emotional pain all add up to offset the inconvenience of switching to a more specific and accurate HPV mRNA test.

INTRODUCING QUANTIVIRUS™ HPV E6/E7 mRNA TEST

SUPERBDNA™ TECHNOLOGY | CE/IVD CERTIFIED

*QuantiVirus™ is NOT a U.S. FDA approved product

QuantiVirus™ HPV E6/E7 mRNA Test is a CE/IVD-certified, highly sensitive and specific signal amplification nucleic acid probe assay. Targeting high-risk HPV mRNA from the E6/E7 oncogenes, which are essential for development of cervical cancer, DiaCarta's QuantiVirus™ HPV mRNA Test detects HPV oncogenes E6/E7 mRNA from 14 high-risk type (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and genotypes HPV 16 and HPV 18 directly from cervical samples (PreservCyt® and SurePath® preservation fluid) for cervical cancer screening and directly from saliva for head & neck cancer screening in men. Unlike HPV DNA tests, the test focuses on persistent infections, rather than transient ones, thus proving a better predictor of CIN2+ lesions. It may be used as triage of HPV DNA+ samples as an alternative to cytology, that needs extensive experience and is operator-dependent.The assay not only detects the E6/E7 mRNA from the 14 most common types of high-risk HPV. If the test is positive, the samples are further tested if they are type 16 or 18.

The QuantiVirus™ HPV E6/E7 mRNA Assay is a sandwich nucleic acid hybridization procedure to detect HPV E6/E7 mRNA in cervical samples without RNA purification or RT-PCR. After HPV mRNA is released from the cells by lysis, the RNA is captured onto a microwell by a set of target-specific, synthetic oligonucleotide capture extenders.

Another set of target-specific, synthetic oligonucleotides called label extender hybridizes to both the viral mRNA from the HPV genome and the synthetic pre-amplifier probes. The pre-amplifier probe subsequently hybridizes to an amplifier probe forming a branched DNA (bDNA) complex. Multiple copies of an alkaline phosphatase (AP) labeled probe are then hybridized to the immobilized amplifier probe. Detection is achieved by incubating the AP-bound complex with a chemiluminescent substrate. Light emission is directly related to the amount of HPV RNA present in each sample, and results are recorded as relative light units (RLUs) by the DiaCarta QuantiVirus™ Benchtop Luminometer.

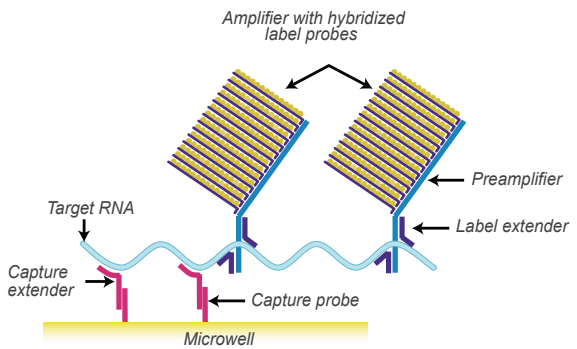


Figure: SuperbDNA™ technology overview

QuantiVirus™ HPV E6/E7 mRNA Test Streamlined Workflow

Step 1: Collect Samples | Step 2: Capture E6/E7 | Step 3: Amplifier | Step 4: Label Probe - AP | Step 5: Substrates | Step 6: Read and Report

DiaCarta Offers QuantiReader™ Benchtop Luminometer for Reading and Reporting QuantiVirus™ Results

The QuantiReader™ Microplate Luminometer is designed for the modern diagnostic laboratory delivering high levels of sensitivity and precision from a compact and easy-to-use platform.

- Affordable 96-well microplate luminometer delivers exceptional sensitivity and precision making it the ideal solution for routine laboratory use.
- User-friendly-Simple wizard-driven software allows for rapid experimental design and set-up. Integrated computer reduces installation time and space requirements while providing greater flexibility in assay format development and use. The result is an instrument with superior performance that is easy to use, is affordable, and can be customized to your laboratory's needs.

CLINICAL DATA FOR QUANTIVIRUS™ HPV E6/E7 mRNA TEST

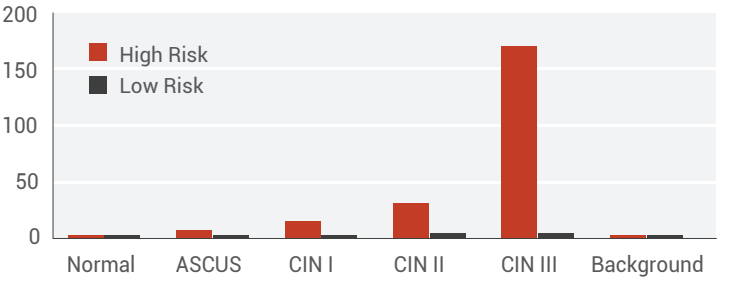
QuantiVirus™ HPV E6/E7 mRNA test and Hybrid capture 2 (HC2) DNA test from Qiagen (DiGene) are compared in analysis of 262 cervical smears collected in a screening population in China. DiaCarta's QuantiVirus™ assay has compatible sensitivity with HC2.

		DiaCarta's QuantiVirus™		Qiagen's HC2 Test	
		HPV E6/E7 mRNA		HPV DNA	
Group	# of Patients	Positive	Percentage	Positive	Percentage
Normal	87	10	11.5%	20	**20.3%**
CIN 1	63	23	36.5%	39	**61.9%**
CIN 2	46	23	50.0%	34	**73.9%**
CIN 4	55	37	67.3%	43	**78.2%**
Cervical	11	11	100.0%	11	**100.0%**
Cancer	262	104	39.7%	147	**56.1%**

When the test results are compared for prediction of CIN2 and worse, QuantiVirus™ HPV E6/E7 mRNA test has better specificity and accuracy than HC2.

	DiaCarta's QuantiVirus™		Qiagen's HC2 Test	
	bDNA Method		HC2 Method	
	Percentage	95% CI	Percentage	95% CI
Sensitivity	63.4% (71/112)	54.5%-72.3%	78.6% (88/112)	71.0%-86.2%
Specificity	78.0% (117/150)	71.4%-81.6%	60.7% (91/150)	52.8%-68.5%
Accuracy	71.8% (118/262)	66.3%-77.2%	68.3% (179/262)	62.7%-74.0%

An efficient and accurate assay should not have cross-reactivity between high-risk and low-risk HPV types. When cross-reactivity is tested for QuantiVirus™ HPV E6/E7 mRNA assay, the samples directly from the Pap smear is used for testing both high risk and low risk samples, no cross-activity is found.



QuantiVirus™ HPV E6/E7 mRNA test is based on SuperbDNA™ technology and uses signal amplification to detect the presence of E6 and E7 mRNA directly from the sample without mRNA purification or amplification. The test shows comparable sensitivity but better specificity compared with the HC2 test. The assay does not show cross-activity between the high-risk and low-risk type HPV testing.

QUANTIVIRUS™ ASSAY PRODUCT SPECIFICATION

PRODUCT SPECIFICATION			
Product Name	QuantiVirus™ HPV E6/E7 mRNA Test	Instruments Validated	QuantiReader™ Benchtop Luminometer; Molecular Device SpectraMax L Microplate Reader
Intended Use	For In Vitro Diagnostic Use (CE/IVD) or For Research Use	Detection Chemistry	Chemiluminescence
Sample Type	Cervical Samples, Saliva, FFPE and Head-Neck Samples	Turnaround Time	8 hours
Pack Size	96 Reactions	Stability	Stable for 12 Months at Recommended Conditions

ORDERING INFORMATION

Product Name	Pack Size	Catalog Number (CE/IVD)	Catalog Number (Research-Use-Only)
QuantiVirus™ HPV E6/E7 mRNA Test for Cervical Cancer	96 Reactions	DC-01-0001	DC-01-0001R
QuantiVirus™ HPV E6/E7 mRNA Test for Head and Neck Cacner	96 Reactions	DC-01-0002	DC-01-0002R

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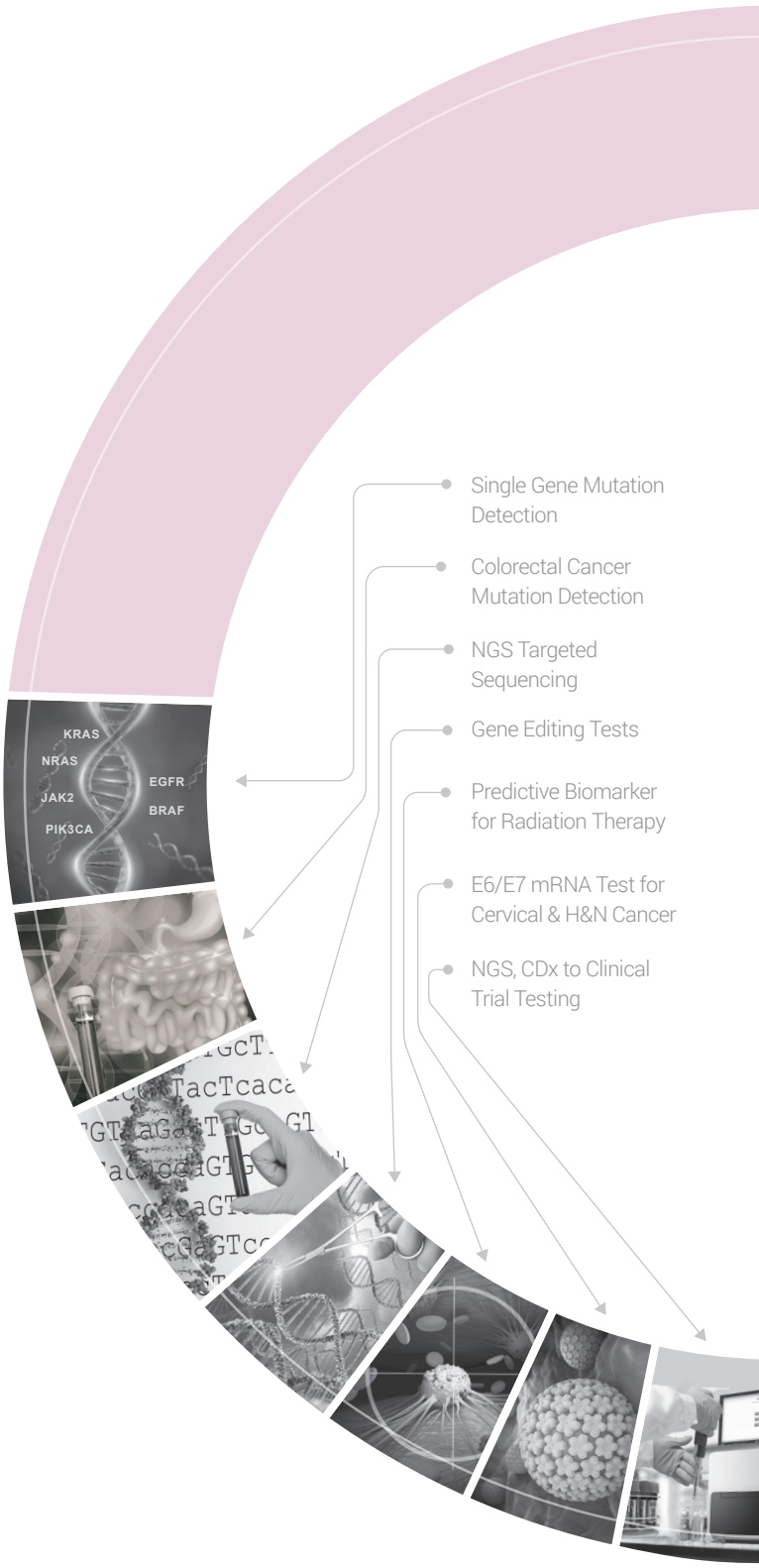
Redefining Precision Molecular Diagnostics through Cancer Gene Mutation Detection



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