WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: *care*HPV™ Test
WHO reference number: PQDx 0085-028-00

***care*HPV™ Test** with product code **614015**, manufactured by **QIAGEN GmBH**, **CE-mark regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 13 July 2018.

Summary of WHO prequalification assessment for *care*HPV™ Test

	Date	Outcome
PQ listing	13 July 2018	listed
Dossier assessment	1 August 2017	MR
Site inspection(s) of quality management system	September 2015 December 2015	MR
Product performance evaluation	13 December 2017	MR

MR: Meets requirements

Intended use:
The *care*HPV™ Test technology is an in vitro nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of 14 high-risk types of HPV DNA in cervical specimens. The HPV types detected by the test are the high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

The use of this test is indicated as a primary screening test in women 30 years and older to detect high-risk HPV infection, which is a risk factor for developing high-grade cervical intraepithelial neoplasia (CIN 2/3+).

This test is “For Professional Use Only” by trained and validated laboratory personnel. The instructions for use must be read carefully before using the test.

The *care*HPV™ Test is not intended for use in screening women under the age of 30 or women who are pregnant. The use of this test has not been evaluated for the management of women with the following conditions:

- prior cytologic or histologic abnormalities
- have undergone a hysterectomy procedure
- are postmenopausal
- are HIV+ with additional risk factors

- are immunocompromised
- have been exposed to Diethylstilbestrol
- have a history of sexually transmitted diseases

Assay description:
The *care*HPV™ Test is a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescent detection. When specimens containing high-risk HPV DNA are present, the HPV DNA hybridizes to complementary RNA from the probe mix. The magnetic microparticle solid support displays anti-DNA–RNA hybrid antibodies that capture the DNA–RNA hybrids, allowing separation and removal of unbound non-specific material. Next, alkaline phosphatase-linked anti-hybrid antibodies are added to bind and detect the captured hybrids. Further washing removes unbound alkaline phosphatase conjugate, leaving alkaline phosphatase that is bound in proportion to the amount of hybridized HPV DNA. Finally, a chemiluminescent substrate is added that is hydrolyzed by the bound alkaline phosphatase to produce light in direct proportion to the amount of alkaline phosphatase present, which correlates with the amount of hybridized HPV DNA present.

The signal produced by the hydrolyzed substrate is measured to give a result in relative light units (RLU) quantified by a luminometer. A RLU value equal to or greater than the cutoff value (CO) means that the specimen contains sufficient amount of high-risk HPV DNA to be considered clinically positive. A RLU value below the CO means that the specimen contains insufficient or no high-risk HPV DNA and is considered clinically negative.

Test kit contents:

Component	**96 tests (product code 614015)**
Assay microplate	1
Negative Calibrator	1 (0.5 ml)
Positive Calibrator	1 (0.5 ml)
Reagent 1 (purple label)	1 (3 ml)
Indicator Dye	1 (0.35 ml)
Stabilized biologics • Reagent 2 (yellow label) • Reagent 3 (brown label) • Reagent 4 (red label) • Reagent 6 (green label)	4
Reconstitution diluents • Reagent 2 diluent • Reagent 3 diluent • Reagent 4 diluent • Reagent 6 diluent	4 4.5 ml 3 ml 5 ml 5 ml

Reagent 5 (blue label)	250 ml
Reagent 5 nozzle	1
Assay Data Recording Sheet	1
Handbook	1

Items required but not provided:

Item	
Consumables: Specimen collection material: *care*HPV Collection Medium (CCM) and *care*Brushes Foam specimen tube rack Fixed volume pipette Repeat pipette Repeat-pipette tips appropriate for dispensing 20 µl, 25 µl, and 40 µl Plate sealers Powder-free gloves Paper towels Bleach 70% Isopropyl alcohol Personal protection equipment	
Equipment:	**Catalog number**
*care*HPV Test System (cat. no. 9001772), including:	
*care*HPV Test Controller	9001775
*care*HPV Test Luminometer	9001773
*care*HPV Test Shaker	9001774
*care*HPV Test Magnetic Plate Holder	9019960

Storage:
The test kit should be stored between 4°C and 25°C.

Shelf-life upon manufacture:
12 months.

Warnings/limitations:
Please refer most current version of instructions for use.

Prioritization for prequalification

Based on the established criteria, *care*HPV™ Test was given priority for WHO prequalification.

Dossier assessment

QIAGEN GmBH submitted a product dossier for *care*HPV™ Test as per the "*Instructions for compilation of a product dossier*" (PQDx_018 v1). The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

Commitments for prequalification:

1. Further reproducibility studies must be undertaken. The final report must be submitted no later than 01 December 2018.
2. As a commitment to prequalification, the manufacturer has agreed to change the specimen storage claims in the revision of the IFU required for prequalification. In addition, new studies are required to investigate the findings of false positives and false negatives over time using more than one lot of specimen collection media. The results of these studies and any consequent amendments to the relevant IFU must be submitted by no later than 01 December 2018.
3. As a commitment to prequalification, the manufacturer has agreed to add the symbol for "keep away from sunlight" in the next revision of the IFU.

WHO will follow-up on implementation of these commitments at the next re-inspection.

Based on the product dossier screening and assessment findings, the product dossier for *care*HPV™ Test meets WHO prequalification requirements.

Manufacturing site inspection

A comprehensive inspection was performed at the sites of manufacture QIAGEN Shenzhen Co. Ltd, 6&7/F, R3-B, High-tech Industrial Park, Shenzhen China, and QIAGEN Sciences, 19300 Germantown Road, Germantown, MD, 20874, United States of *care*HPV™ Test in September and December 2015, respectively, as per the "*Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics*" (PQDx_014 v1). The inspection found that the manufacturer had an acceptable quality management system and manufacturing practices in place that ensured the consistent manufacture of a product of good quality.

The manufacturer's responses to the nonconformities found at the time of the inspection were accepted on 10 April 2018. The effectiveness of the implementation of corrective actions initiated in response to these nonconformities will be followed up at the next inspection of the QIAGEN Germantown facility scheduled for the second half of 2018.

Commitments for prequalification:
N/A
Based on the site inspection and corrective action plan review, the quality management system for *care*HPV™ Test meets WHO prequalification requirements.

Product performance evaluation

*care*HPV™ Test was evaluated by the Scottish Human Papilloma Virus Reference Laboratory Edinburgh, NHS Lothian, Scotland from 14 March to 10 May 2017.

*care*HPV™ Test technology is an in vitro nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of 14 high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, ,59, 66 and 68. A volume of 50µl of specimen is needed to perform the assay. This type of assay requires additional laboratory equipment and can be performed in laboratories with limited facilities.

Analytical specimens:
The limit of detection was not verified. In this evaluation, the LOD for HPV either genotype could not be estimated by probit analysis as the only dilution detected was 1×10^6 IU/ml.

Clinical specimens:
In this limited performance evaluation, we found, an initial positive percent agreement (95% CI) of 70.93% (60.14-80.22) and an initial negative percent agreement (95% CI) of 96.68% (94.40-98.22) compared to the benchmark results.

The final positive percent agreement (95% CI) was 74.42% (63.87-83.22), and the negative percent agreement (95% CI) was 97.45% (95.36-98.77).

In this study, the error rate was 0%.

Protocol limitations:

- While WHO standards can provide insight into analytical sensitivity of the assay, it is noted that these are not bio-specimens validated for formal calibration purposes, nor do they represent or resemble the biological matrix of clinical specimens for which the assay under evaluation was validated. These aspects have implications for the comparison between the LOD reported in the instructions for use and that observed in this study and should be taken into account if there is disagreement.

- This laboratory evaluation protocol was designed to verify the ability for the assays under evaluation to detect HPV DNA (in line with the manufacturer's stated intended use). Pathology data were not available given the setting/infrastructure where patient sampling occurred. Consequently, clinical performance relative to

CIN2+ could not be assessed nor could the level of *clinically relevant* discordance between the test under evaluation and the benchmark test.

Performance characteristics		
Analytical performance		
Limit of Detection	LOD for HPV either genotype could not be estimated by probit analysis. Last dilution detected was 1 x 10^6 IU/ml.	
Clinical performance		
	Initial (95% CI)	**Final (95% CI)**
Positive percent agreement %	70.93% (60.14-80.22)	74.42% (63.87-83.22)
Negative percent agreement %	96.68% (94.40-98.22)	97.45% (95.36-98.77)
Invalid rate	0%	

Key operational characteristics	
Validated specimen types	Cervical specimens collected in *care*HPV Collection Medium™ (CCM™) using the *care*HPV Cervical Brush (*care*Brush)
Number of steps	52
Time to result	Approximately 3h30 min
In-use stability of reagents	Prepared reagents must be stored between 15–30 °C for no longer than 8 hours.

Labelling

1. **Labels**
2. **Instructions for use**

1. Labels

*care*HPV™ Test Kit（96）
Label（CE）

文件控制信息 （Document Control Information）

编制者	审核者	批准者	所有部门
李芳	徐嫚嫚、吴湄湄、王嘉	王嘉	生产

发放部门	生产、QA

修订历史（Revision History）

版次	生效日期	更改申请表编号	内容描述
1	2012-05-01	N/A	初次发布（Initial release）
2	2012-08-01	N/A	删去 NC,PC 标签中的危险品标识（包括试剂标签及盒面标签）增加 5 种语言的风险提示标签。
3	2015-08-04	Production-201507008	删去说明书，删去产品标签上的橙色色块，标签上的部分打印内容更改，将 5 种语言的风险提示标签替换成印有 13 种语言的风险提示语的 A4 纸并添加透明信封袋。
4	2017-11-20	Production-201709010	将 Negative Calibrator 和 Positive Calibrator 的包装材料由 10ml Polypropylene tube、 Natural Push Cap 变更为 Carclo RCS VIAL 、Carclo Push Cap。
5	2018-06-01	P-Production-201804001	1. 在所有半成品标签名称前添加“*care*HPV”字样。 2 . 盒面标签添加避免阳光直射标识。

careHPV™ Test Kit（96）
Label（CE）

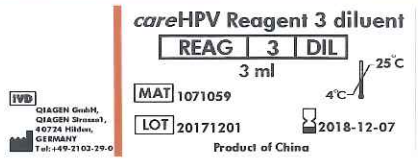
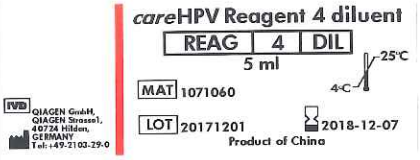
1 careHPV Test Kit

1.1 包装盒样板见附件 1

1.2 标签样板及标签对应物料

序号	标签		标签对应物料			
	编号	样板	SAP 编码	名称	规格数量	包装材料
1	L0417-1121	careHPV Negative Calibrator CAL - 0.5ml MAT 1071050 LOT 20171201 2018-12-05 Product of China IVD QIAGEN GmbH, QIAGEN Strasse1, 40724 Hilden, GERMANY Tel:+49-2103-29-0	1071050	careHPV Negative Calibrator	0.5ml×1	Carclo RCS VIAL Carclo Push Cap
2	L0418-1122	careHPV Positive Calibrator CAL + 0.5ml MAT 1070960 LOT 20171201 2018-12-07 Product of China IVD QIAGEN GmbH, QIAGEN Strasse1, 40724 Hilden, GERMANY Tel:+49-2103-29-0	1070960	careHPV Positive Calibrator	0.5ml×1	Carclo RCS VIAL Carclo Push Cap
3	L0161-1123	careHPV Indicator Dye MAT 1071052 LOT 20171201 INDIC 0.35ml 2018-12-04 IVD QIAGEN GmbH, QIAGEN Strasse1, 40724 Hilden, GERMANY Tel:+49-2103-29-0	1071052	careHPV Indicator Dye	0.35ml×1	3ml tube 3ml tube' cap 3ml tube' insert
4	L0419-1124	careHPV Reagent 1 REAG 1 3 ml MAT 1071051 LOT 20171201 2018-12-04 Product of China contains sodium hydroxide IVD QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY Tel:+49-2103-29-0	1071051	careHPV Reagent 1	3ml×1	15ml 旋盖窄口离心管
5	L0420-1125	careHPV Reagent 2 REAG 2 MAT 1071053 LOT 20171201 2018-12-07 Product of China IVD QIAGEN GmbH, QIAGEN Strasse1, 40724 Hilden, GERMANY Tel:+49-2103-29-0	1071053	careHPV Reagent 2	1 瓶	15ml 旋盖窄口离心管
6	L0421-1126	careHPV Reagent 3 REAG 3 MAT 1071054 LOT 20171201 2018-12-13 Product of China IVD QIAGEN GmbH, QIAGEN Strasse1, 40724 Hilden, GERMANY Tel:+49-2103-29-0	1071054	careHPV Reagent 3	1 瓶	15ml 旋盖窄口离心管
7	L0422-1127	careHPV Reagent 4 REAG 4 MAT 1071055 LOT 20171201 2018-12-03 Product of China IVD QIAGEN GmbH, QIAGEN Strasse1, 40724 Hilden, GERMANY Tel:+49-2103-29-0	1071055	careHPV Reagent 4	1 瓶	15ml 旋盖窄口离心管
8	L0423-1128	careHPV Reagent 6 REAG 6 MAT 1071057 LOT 20171201 2018-12-21 Product of China IVD QIAGEN GmbH, QIAGEN Strasse1, 40724 Hilden, GERMANY Tel:+49-2103-29-0	1071057	careHPV Reagent 6	1 瓶	15ml 旋盖窄口离心管
9	L0420-1129	careHPV Reagent 2 diluent REAG 2 DIL 4.5 ml MAT 1071058 LOT 20171201 2018-12-05 Product of China contains 2-[bis(2-hydroxyethyl) amino]ethanesulphonic acid; Polyacrylic acid; sodium hydroxide IVD QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY Tel:+49-2103-29-0	1071058	careHPV Reagent 2 diluent	4.5ml×1	15ml 旋盖窄口离心管

careHPV™ Test Kit（96）
Label（CE）

10	L0421-1130		1071059	careHPV Reagent 3 diluent	3ml×1	15ml 旋盖窄口离心管
11	L0422-1131		1071060	careHPV Reagent 4 diluent	5ml×1	15ml 旋盖窄口离心管
12	L0423-1132		1071061	careHPV Reagent 6 diluent	5ml×1	15ml 旋盖窄口离心管
13	L0303-1133	见背面	N/A	careHPV Reagent 2 careHPV Reagent 3 careHPV Reagent 4 careHPV Reagent 6	N/A	铝箔袋
14	L0424-1134	见背面	1071062	careHPV Reagent 5 nozzle	1 个	热封袋
15	L0425-1135	见背面	1070930	careHPV Assay Microplate	1 块	封口密实袋 17×10cm
16	L0426-1136	见背面	1071056	careHPV Reagent 5	250ml×1	250ml 窄口塑料瓶
17	N/A	见附件 2	N/A	13 种语言风险提示	N/A	N/A
18	N/A	见附件 2	1081204	透明信封袋	N/A	N/A
19	L0210-1137	见包装盒面	614015	包装盒	15*13*14.8 x1	包装盒

careHPV Reagent 2	REAG 2 LOT 20171201	2018-12-07	4°C 25°C
careHPV Reagent 3	REAG 3 LOT 20171201	2018-12-13	
careHPV Reagent 4	REAG 4 LOT 20171201	2018-12-03	
careHPV Reagent 6	REAG 6 LOT 20171201	2018-12-21	Product of China

careHPV Reagent 5 nozzle

REAG 5 NOZZLE

MAT 1071062 4°C 25°C

LOT 20171201 2019-01-01

Product of China

IVD

QIAGEN GmbH,
QIAGEN Strasse1,
40724 Hilden,GERMANY
Tel:+49-2103-29-0

careHPV Assay Microplate

PLATE

MAT 1070930 4°C 25°C

LOT 20171201 2019-01-01

Product of China

IVD

QIAGEN GmbH,
QIAGEN Strasse1,
40724 Hilden,
GERMANY
Tel:+49-2103-29-0

careHPV Reagent 5

REAG 5

250 ml

MAT 1071056 4°C 25°C

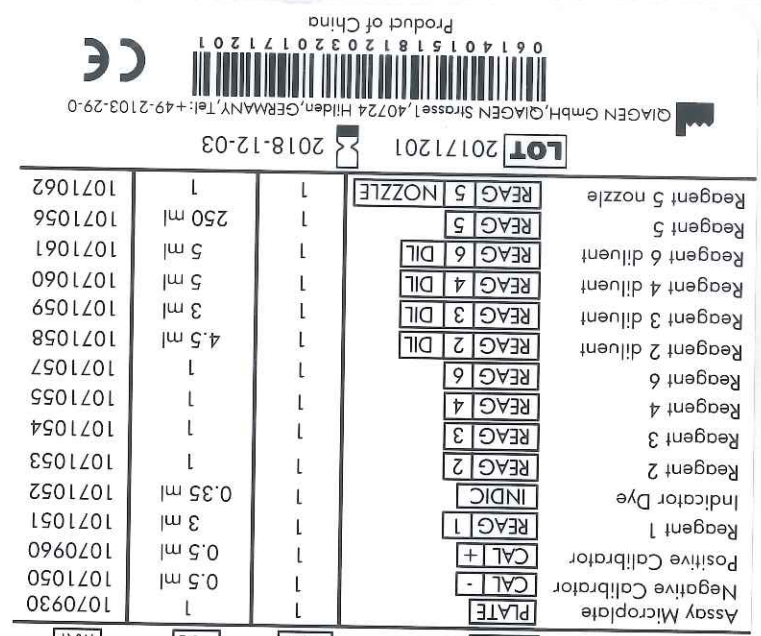
LOT 20180404 2019-04-10

Product of China

IVD

QIAGEN GmbH,
QIAGEN Strasse1,
40724 Hilden,
GERMANY
Tel:+49-2103-29-0

附件1


careHPV™ Test Kit
Σ 96
REF 614015
COMP
NUM
VOL
MAT
Assay Microplate PLATE 1 1 107093O
Negative Calibrator CAL - 1 0.5 ml 1071050
Positive Calibrator CAL + 1 0.5 ml 1070960
Reagent 1 REAG 1 1 3 ml 1071051
Indicator Dye INDIC 1 0.35 ml 1071052
Reagent 2 REAG 2 1 1 1071053
Reagent 3 REAG 3 1 1 1071054
Reagent 4 REAG 4 1 1 1071055
Reagent 6 REAG 6 1 1 1071057
Reagent 2 diluent REAG 2 DIL 1 4.5 ml 1071058
Reagent 3 diluent REAG 3 DIL 1 3 ml 1071059
Reagent 4 diluent REAG 4 DIL 1 5 ml 1071060
Reagent 6 diluent REAG 6 DIL 1 5 ml 1071061
Reagent 5 REAG 5 1 250 ml 1071056
Reagent 5 nozzle REAG 5 NOZZLE 1 1 1071062
LOT 20171201
2018-12-03
QIAGEN GmbH,QIAGEN Strasse1,40724 Hilden,GERMANY,Tel:+49-2103-29-0
Product of China
careHPV™ Test Kit
Σ 96
REF 614015
IVD
4°C
25°C
CE
+EQIA614015O/

2. Instructions for use

July 2018

*care*HPV® Test Kit Handbook

Σ 96 (cat. no. 614015)

IVD

For detection of 14 high-risk human papillomavirus (HPV) genotypes by nucleic acid hybridization

For use with:

- *care*HPV Test System
- *care*Brush
- *care*HPV Collection Medium

CE

REF 614015

QIAGEN GmbH
QIAGEN Strasse 1
40724 Hilden
GERMANY

R6 MAT 1058802EN

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QIAGEN Sample and Assay Technologies

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- microRNA research and RNAi
- Automation of sample and assay technologies

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Contents

Intended Use

The *care*HPV Test technology is an in vitro nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of 14 high-risk types of HPV DNA in cervical specimens. The HPV types detected by the test are the high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

The use of this test is indicated as a primary screening test in women 30 years and older to detect high-risk HPV infection, which is a risk factor for developing high-grade cervical intraepithelial neoplasia (CIN 2/3+).

This test is "For Professional Use Only" by trained and validated laboratory personnel. Read these instructions for use carefully before using the test.

The *care*HPV Test is not intended for use in screening women under the age of 30 or women who are pregnant. The use of this test has not been evaluated for the management of women with the following conditions:

- prior cytologic or histologic abnormalities
- have undergone a hysterectomy procedure
- are postmenopausal
- are HIV+ with additional risk factors
- are immunocompromised
- have been exposed to Diethylstilbestrol
- have a history of sexually transmitted diseases

Summary and Explanation

The presence of certain HPV types in the female genital tract is associated with a number of diseases, including condyloma, Bowenoid papulosis, cervical, vaginal, and vulvar intraepithelial neoplasia, and cancer (1, 2). More than 100 types of HPV have been identified and are generally classified as high-risk or low-risk depending on their known association or lack of association with cancer and its precursor lesion, high-grade cervical intraepithelial neoplasia (CIN 2/3+). It is generally accepted that these viruses are predominantly sexually transmitted and that high-risk HPV types are a major recognized risk factor for development of cervical cancer (2–6). Infection of the cervix with high-risk HPV types can be associated with cytological and histological changes that are detected by Pap screening, colposcopy, or biopsy.

Human papillomaviruses are composed of an icosahedral viral particle (virion) containing an 8000 base pair double-stranded circular DNA molecule surrounded by a protein capsid. Following infection of epithelial cells, the viral DNA becomes established throughout the entire thickness of the epithelium, but intact virions are found only in the upper layers of the tissue. Thus, viral DNA can be found either in virions or as episomal or integrated HPV sequences, depending upon the type and grade of lesion.

Historically, HPV types 16 and 18 have been regarded as high-risk cancer-associated types (2, 7, 8) and HPV types 31, 33, and 35 have been demonstrated to have an intermediate association with cancer (2, 9). This intermediate association is due to the fact that these types are more frequently detected in CIN 2/3+ rather than in cancers. Therefore, cancers associated with the presence of these types are less common than cancers that are associated with high-risk HPV types 16 and 18 (2, 10). These 5 HPV types combined together account for about 80 percent of cervical cancers (2, 11, 12). Additional high- and intermediate-risk HPV types, including types 39, 45, 51, 52, 56, 58, 59, and 68, have been identified as the principal HPV types detectable in the remaining cancers (2, 12–18). HPV type 66 has been classified as a probable high-risk type (19), and due to the increased specificity of the *care*HPV Test, HPV type 66 was added to the probe mix.

The absolute risk of developing an incident cytologic abnormality following an HPV infection with types detected by the *care*HPV Test has not been adequately described and is known to vary in different populations (6).

Although current scientific literature suggests that persistent infection with high-risk HPV is the main risk factor for development of high-grade cervical neoplasia and cancer (2, 4, 5, 8, 20–26), apparent persistence may represent continuous infection with a single HPV type, with multiple HPV types, or reinfection. Nonetheless, women who are repeatedly Pap negative and high-risk HPV negative appear to be at low risk for having or developing cervical precancerous lesions (5, 20, 27, 28).

Principle of the Procedure

The *care*HPV Test utilizes the same Hybrid Capture® 2 technology developed for QIAGEN's *digene*® HC2 High-Risk HPV DNA Test (HC2 Test). The *care*HPV Test is a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescent detection. When specimens containing high-risk HPV DNA are present, the HPV DNA hybridizes to complementary RNA from the probe mix. The magnetic microparticle solid support displays anti-DNA–RNA hybrid antibodies that capture the DNA–RNA hybrids, allowing separation and removal of unbound non-specific material. Next, alkaline phosphatase-linked anti-hybrid antibodies are added to bind and detect the captured hybrids. Further washing removes unbound alkaline phosphatase conjugate, leaving alkaline phosphatase that is bound in proportion to the amount of hybridized HPV DNA. Finally, a chemiluminescent substrate is added that is hydrolyzed by the bound alkaline phosphatase to produce light in direct proportion to the amount of alkaline phosphatase present, which correlates with the amount of hybridized HPV DNA present.

The signal produced by the hydrolyzed substrate is measured to give a result in relative light units (RLU) quantified by a luminometer. A RLU value equal to or greater than the cutoff value (CO) means that the specimen contains sufficient amount of high-risk HPV DNA to be considered clinically positive. A RLU value below the CO means that the specimen contains insufficient or no high-risk HPV DNA and is considered clinically negative.

Materials Provided

Kit contents

***care*HPV Test Kit**		**(96)**
Catalog no.		**614015**
Number of tests*		**96**
Assay Microplate	**PLATE**	1
Negative Calibrator	**CAL** **–**	0.5 ml
Positive Calibrator	**CAL** **+**	0.5 ml
Reagent 1 (purple cap sticker)	**REAG** **1**	3 ml
Indicator Dye	**INDIC**	0.35 ml
Stabilized biologics (4)		
Reagent 2 (yellow cap sticker)	**REAG** **2**	1
Reagent 3 (brown cap sticker)	**REAG** **3**	1
Reagent 4 (red cap sticker)	**REAG** **4**	1
Reagent 6 (green cap sticker)	**REAG** **6**	1
Reconstitution diluents (4)		
Reagent 2 Diluent (yellow cap sticker)	**REAG** **2** **DIL**	4.5 ml
Reagent 3 Diluent (brown cap sticker)	**REAG** **3** **DIL**	3 ml
Reagent 4 Diluent (red cap sticker)	**REAG** **4** **DIL**	5 ml
Reagent 6 Diluent (green cap sticker)	**REAG** **6** **DIL**	5 ml
Reagent 5 (blue cap sticker)	**REAG** **5**	250 ml
Reagent 5 Nozzle	**REAG** **5** **NOZZLE**	1

* Note that the calibrators required for assay calibration verification must be included with each performance of the test. See "Quality Control," page 28 for further information.

Materials Required but Not Provided

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- *care*HPV Test System (cat. no. 9001772), including:
  - *care*HPV Test Controller
  - *care*HPV Test Luminometer
  - *care*HPV Test Shaker
  - *care*HPV Test Magnetic Plate Holder
- Foam specimen tube rack (for example, VWR® Foam Tube Racks for 15 mm or 16 mm diameter tubes, catalog no. 60828-91)*
- 50 µl fixed-volume pipet†
- Repeat pipet capable of dispensing 20 µl, 25 µl, and 40 µl†
- Repeat-pipet tips appropriate for dispensing 20 µl, 25 µl, and 40 µl
- Disposable 200 µl extra-long aerosol-barrier pipet tips
- Plate sealers
- Powder-free gloves
- Paper towels

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

† Make sure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Warnings and Precautions

Warnings

For in vitro diagnostic use.

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN® kit and kit component.

Handle all specimens and disposed materials as if capable of transmitting infectious agents. Clinical specimens should be handled at the biosafety level (BSL) 2 level as recommended for any potentially infectious human serum or blood specimen (29, 30).

Clean and disinfect all spills of specimens using a suitable disinfectant in accordance with national and local regulations. Refer also to the disinfection and sterilization chapter in the World Health Organization's Laboratory Biosafety Manual (31).

Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials in accordance with national and local regulations.

Safety and risk statements for components

The following hazard and precautionary statements apply to components of the *care*HPV Test kit in either dried or reconstituted form.

Negative Calibrator

Contains: 1% ethoxylated nonylphenol. Warning! Causes mild skin irritation. If skin irritation occurs: Get medical advice/attention.

Positive Calibrator

Contains: 1% ethoxylated nonylphenol. Warning! Causes mild skin irritation. If skin irritation occurs: Get medical advice/attention.

Reagent 1

Contains: sodium hydroxide. Danger! Causes severe skin burns and eye damage. May be corrosive to metals. Dispose of contents/container to an approved waste disposal plant. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF ON SKIN (or hair): Remove/take off immediately all contaminated clothing. Rinse skin with water/shower. Immediately call a POISON CENTER or doctor/physician. Store locked up. Wear protective gloves/protective clothing/ eye protection/face protection.

Reagent 2 diluent

Contains: 2.2 M 2-[bis(2-hydroxyethyl)amino]ethanesulphonic acid; 2.6% polyacrylic acid; 0.7 M sodium hydroxide. Danger! Causes severe skin burns and eye damage. May cause respiratory irritation. Dispose of contents/container to an approved waste disposal plant. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF ON SKIN (or hair): Remove/take off immediately all contaminated clothing. Rinse skin with water/ shower. Immediately call a POISON CENTER or doctor/ physician. Store in a well-ventilated place. Keep container tightly closed. Wear protective gloves/protective clothing/eye protection/face protection.

Reagent 3

Contains: 0.4% ethoxylated nonylphenol; 0.04% sodium azide. Warning! May be harmful if swallowed. Causes mild skin irritation. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Dispose of contents/container to an approved waste disposal plant.

Reagent 4

Contains: 0.04% sodium azide. Warning! May be harmful if swallowed. Call a POISON CENTER or doctor/physician if you feel unwell.

Reagent 6

Contains: 0.1 M 2-amino-2-methyl-1-propanol. Warning! Causes mild skin irritation. If skin irritation occurs: Get medical advice/attention.

Precautions

The user must always adhere to the following precautions when performing the *care*HPV Test:

- The components in this test kit have been tested as a unit and must not be interchanged with components from other sources or from different test kits.
- Nucleic acids are very sensitive to environmental nuclease degradation. Nucleases are present on human skin and on surfaces or materials handled by humans. Work surfaces must be clean and covered with disposable pads; technicians must wear powder-free gloves when performing all test steps.
- Prevent contamination of the Assay Microplate and Reagent 6 (green cap sticker) with exogenous alkaline phosphatase. Substances that may contain alkaline phosphatase include Reagent 4 (red cap sticker), bacteria, saliva, hair, and oils from the skin. Covering the microplate after Reagent 5 addition and during incubation with Reagent 6 is especially important because exogenous alkaline phosphatase may react with Reagent 6, producing false-positive results.
- Reagents 1, 2, 3, 4, and 6 must be prepared prior to starting the test and used within 8 hours of preparation. Failure to do so may cause an invalid assay. If the assay is invalid, the test must be repeated using a new kit.
- Indicated reagent volumes must be accurately dispensed. Failure to do so could result in erroneous test results. Ensuring that the noted color changes occur will help confirm that the required volumes have been dispensed.
- When using the repeat pipet, the user should first dispense several times into a waste reservoir to flush the pipet tip of any air bubbles and ensure accurate delivery.
- The Test Data Recording Sheet (see "Appendix: Test Data Recording Sheet," page 41) indicates the required microplate well locations for the Negative Calibrator (microplate wells A1, B1, C1), Positive Calibrator (microplate wells D1, E1, F1), and clinical specimens (microplate wells G1 and all subsequent microplate wells).
- When performing the *care*HPV Test, refer to the appropriate *care*HPV Test System user manuals for instrument instructions and troubleshooting.

Reagent Storage and Handling

Upon receipt, store the *care*HPV Test kit between 4 °C and 25 °C. Do not use the *care*HPV Test Kit beyond the expiration date on the kit label.

Store prepared reagents between 15 °C and 30 °C for no longer than 8 hours. Discard the kit and all prepared reagents if not used for testing within 8 hours of reagent preparation.

Specimen Handling and Storage

Use only cervical specimens collected in *care*HPV Collection Medium with a *care*Brush. Refer to the *care*Brush Instructions For Use (IFU) for additional specimen collection details.

Store clinical specimens in *care*HPV Collection Medium between 15 °C and 30 °C for 7 days or between 2 °C and 8 °C for 21 days.

Procedure

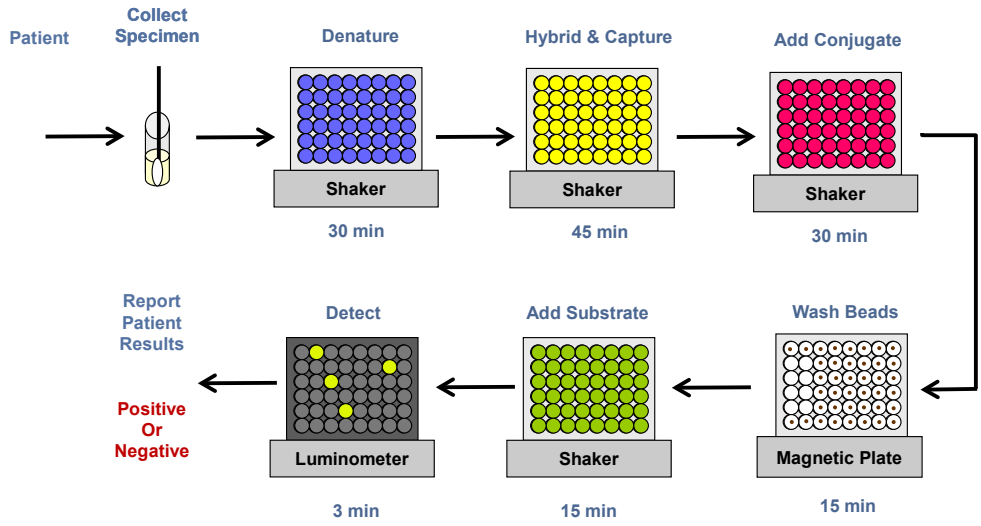

Figure 1. General workflow for *care*HPV Test System.

Preparing specimens

1. **Record the following information on the Test Data Recording Sheet (see "Appendix: Test Data Recording Sheet," page 41):**
   - Testing site
   - Testing date
   - Operator ID
   - Room temperature
   - *care*HPV Test Kit lot number
2. **Place the clinical specimen tubes into the foam specimen tube rack(s).**

   On the Test Data Recording Sheet, complete the plate map by recording in the applicable microplate well locations the IDs of all specimens to be tested (microplate well G1 and all subsequent microplate wells).

   Note: The calibrators are not placed in the specimen tube rack.
3. **Make sure that the specimen tube caps are tightly closed.**

4. **Mix the specimens as follows:**

   Invert the specimen tube rack 180 degrees and give a hard shake once, quickly, in the inverted position.

   Promptly return the rack to the upright position and give a hard shake once, quickly, in the upright position.

   Repeat this mixing step continuously for 5 minutes.

Starting the *care*HPV Test System

The *care*HPV Test System requires approximately 15 seconds after receiving power to display the "Startup" screen.

1. **Touch the "*care*HPV" icon on the *care*HPV Test Controller to begin the *care*HPV Test.**

   The *care*HPV Test Controller displays the 7 steps of the *care*HPV Test that will be performed.

2. **Record the microplate run number on the Test Data Recording Sheet.**
3. **Allow the *care*HPV Test Shaker to warm to the required temperature for the performance of the test.**

   The *care*HPV Test System requires approximately 13–15 minutes to reach the required temperature.

Preparing reagents

Reconstitute the *care*HPV Test reagents as described below. Use prepared reagents within 8 hours of preparation. Failure to do so may cause an invalid assay.

Important points before starting

- Indicator Dye does not have a number on the bottle; it is paired with Reagent 1.
- The containers of the stabilized biologics and the diluents are color-coded for ease of use.

Things to do before starting

- To reduce possible errors, line up the stabilized biologics by order with the diluent bottles that have the same numbers.
- Tap the stabilized biologics bottles on the bench before opening.

1. **Add 1 drop of Indicator Dye to the Reagent 1 bottle (purple cap sticker). Replace the cap of the Reagent 1 bottle and invert 10 times to thoroughly mix the reagent.**

   The color of the reagent changes from clear to purple.

2. **Add the contents of the Reagent 2 diluent bottle (yellow cap sticker) to the Reagent 2 bottle (yellow cap sticker). Replace the cap of the Reagent 2 bottle and invert 10 times to thoroughly mix the reagent.**

   Note: Mix gently to avoid foam.

3. **Add the contents of the Reagent 3 diluent bottle (brown cap sticker) to the Reagent 3 bottle (brown cap sticker). Replace the cap of the Reagent 3 bottle and invert 10 times to thoroughly resuspend the reagent.**
4. **Add the contents of the Reagent 4 diluent bottle (red cap sticker) to the Reagent 4 bottle (red cap sticker). Replace the cap of the Reagent 4 bottle and invert 10 times to thoroughly resuspend the reagent.**
5. **Add the contents of the Reagent 6 diluent bottle (green cap sticker) to the Reagent 6 bottle (green cap sticker). Replace the cap of the Reagent 6 bottle and invert 10 times to thoroughly resuspend the reagent.**

   Note: Reagent 6 is light sensitive. Reagent 6 is in a brown-colored bottle to protect it from direct sunlight.

6. **Remove the cap from the Reagent 5 bottle (blue cap sticker).**
7. **Cut open the package holding the Reagent 5 nozzle.**
8. **Remove the Reagent 5 nozzle from the packaging and attach to the Reagent 5 bottle.**

   Do not place the Reagent 5 nozzle on the bench; remove it directly from the sealed bag and attach it to the bottle.

Protocol 1: Microplate preparation and 30–minute incubation

Things to do before starting

- Clean and cover the work surface with disposable pads, and wear powder-free gloves when performing all test steps.
- Complete the Test Data Recording Sheet ("Appendix: Test Data Recording Sheet," page 41) by recording the IDs of the calibrators to be pipetted into the applicable microplate well locations; observe the required placement for the Negative Calibrator (microplate wells A1, B1, C1) and Positive Calibrator (microplate wells D1, E1, F1).
- Confirm the testing site, testing date, operator ID, room temperature, *care*HPV Test Kit lot number, microplate run number, and microplate well locations of all IDs of clinical specimens to be pipetted were recorded as described in "Preparing specimens," page 15.

1. **Using the repeat pipet and a new tip, add 25 µl of Reagent 1 (purple cap sticker) to each microplate well.**
2. **Using the fixed-volume pipet and a new, clean pipet tip for each calibrator or specimen, add the indicated volumes to the specified microplate wells, as follows:**
   - Dispense 50 µl of Negative Calibrator into microplate wells A1, B1, and C1.
   - Dispense 50 µl of Positive Calibrator into microplate wells D1, E1, and F1.
   - According to the Test Data Recording Sheet, dispense 50 µl of each specimen into the bottom of the remaining microplate wells, beginning with microplate well G1. Record on the Test Data Recording Sheet any specimens that appear dark in color.

   Important: False-positive test results could occur due to contamination of the *care*HPV Test with non-specific RNA–DNA hybrids endogenous to cervical specimens. It is important during transfer of the specimen to the microplate well that the specimen is delivered directly to the bottom of the microplate well without the pipet tip touching the sides of the microplate well.

Important: Specimens containing blood or other biological materials appearing dark in color may not affect the results of the test, but may not give the proper color change following Reagent 2 addition. Make a note of the sample number for any that are dark in color on the Test Data Recording Sheet.

3. **Apply a new plate sealer and securely cover the microplate according to the following procedure:**
   a. Remove the paper from the plate sealer.
   b. Place the plate sealer over the microplate, being sure to cover all microplate wells.
   c. Press the plate sealer over the microplate and tear off the tab on each end of the plate sealer.
4. **Confirm the *care*HPV Test Shaker is at the proper temperature to start the test.**
5. **At the prompt, open the *care*HPV Test Shaker lid and place the microplate into the *care*HPV Test Shaker with the A1 microplate well oriented in the top left corner. Close the lid.**
6. **Touch the "1" icon on the *care*HPV Test Controller to begin the 30–minute incubation.**
7. **Proceed to "Protocol 2: Reagent 2 addition and 15–minute incubation," starting on page 20.**

Protocol 2: Reagent 2 addition and 15–minute incubation

1. **When prompted by the *care*HPV Test Controller, remove the microplate from the *care*HPV Test Shaker and place the microplate on the bench top.**
2. **Carefully remove the plate sealer to prevent splashing and cross-contamination between microplate wells; discard the plate sealer.**
3. **Promptly insert the microplate back into the *care*HPV Test Shaker.**
4. **Swirl the Reagent 2 bottle (yellow cap sticker) to mix and, using the repeat pipet and a new tip, add 40 µl of Reagent 2 to each microplate well.**
5. **Apply a new plate sealer and securely cover the microplate, as previously described on page 19, while the microplate is in the *care*HPV Test Shaker.**
6. **Close the *care*HPV Test Shaker lid.**
7. **Touch the "2" icon on the *care*HPV Test Controller to begin a 15–minute incubation.**
8. **Proceed to "Protocol 3: Reagent 3 addition and 30–minute incubation," starting on page 21.**

Protocol 3: Reagent 3 addition and 30–minute incubation

1. **When prompted by the *care*HPV Test Controller, remove the microplate from the *care*HPV Test Shaker and place the microplate on the bench top. Leave the *care*HPV Test Shaker lid open.**
2. **Make sure that the color of each sample has changed from purple to yellow. Carefully note on the Test Data Recording Sheet any samples that have not changed color.**

   Note: Specimens that contain blood or other biological materials may not give the proper color change; these specimens were recorded as dark in color on the Test Data Recording Sheet in Protocol 1. This dark color will not affect the results of the test and the user should proceed with testing these specimens.

   Any microplate wells that were not noted as dark specimens but have not turned yellow will produce invalid results and must be eliminated from result interpretation. Repeat testing for these specimens. Make note of the specimens to be retested and record them on the Test Data Recording Sheet.
3. **Carefully remove and discard the plate sealer.**
4. **Promptly insert the microplate back into the *care*HPV Test Shaker. Swirl the Reagent 3 bottle (brown cap sticker) to mix and, using the repeat pipet, add 20 µl of Reagent 3 to each microplate well.**
5. **Apply a new plate sealer and securely cover the microplate, as previously described on page 19. Close the *care*HPV Test Shaker lid.**
6. **Touch the "3" icon on the *care*HPV Test Controller to begin a 30–minute incubation.**
7. **When prompted by the *care*HPV Test Controller, remove the microplate from the *care*HPV Test Shaker. Leave the *care*HPV Test Shaker lid open.**

   Keep the microplate horizontal and steady to avoid splashing across microplate wells.
8. **Carefully secure the microplate on the *care*HPV Test Magnetic Plate Holder.**
9. **Leave the *care*HPV Test Magnetic Plate Holder containing the microplate on the bench top. Carefully remove and discard the plate sealer.**

10. **Touch the "3" icon on the *care*HPV Test Controller to begin a 3–minute incubation.**

    Note: This incubation occurs on the bench top and the *care*HPV Test Controller counts down the incubation time.

11. **Proceed to "Protocol 4: Reagent 4 addition and incubation," starting on page 23.**

Protocol 4: Reagent 4 addition and incubation

Important: Make sure the 3–minute incubation from Protocol 3 has completed before starting this procedure.

1. **Decant and blot the microplate as follows:**
   a. Firmly grip the bottom of the *care*HPV Test Magnetic Plate Holder and sides of the microplate in one hand (microplate faces up).
   b. Invert the *care*HPV Test Magnetic Plate Holder upside down (180 degrees) over a waste collector and decant the liquid from the microplate one time with force.
   c. While holding the *care*HPV Test Magnetic Plate Holder in this inverted position (microplate facing down), place it onto a clean blotting paper towel and blot the microplate.
   d. Return the *care*HPV Test Magnetic Plate Holder to the bench top with the microplate facing up.
2. **Swirl the Reagent 4 bottle (red cap sticker) to mix. Using the repeat pipet and a new tip, add 40 µl to each microplate well.**
   The microplate remains on the *care*HPV Test Magnetic Plate Holder.
3. **Apply a new plate sealer and securely cover the microplate, as previously described on page 19.**
4. **Carefully remove the microplate from the *care*HPV Test Magnetic Plate Holder to prevent splashing and place the microplate on the bench top.**
5. **Touch the "4" icon on the *care*HPV Test Controller to begin the timer for the bench top incubation.**
   Note: This incubation starts with the microplate on the bench top to allow the *care*HPV Test Shaker to cool down. The *care*HPV Shaker lid should remain open to cool. The remainder of the incubation is performed with the microplate in the *care*HPV Test Shaker.
6. **When prompted by the *care*HPV Test Controller, place the microplate in the *care*HPV Test Shaker and close the lid for the remainder of the incubation.**
7. **When prompted by the *care*HPV Test Controller, remove the microplate from the *care*HPV Test Shaker and secure the microplate on the *care*HPV Test Magnetic Plate Holder.**

8. **Carefully remove and discard the plate sealer.**
9. **Touch the "4" icon on the *care*HPV Test Controller to begin a 3–minute incubation.**

   Note: This incubation occurs on the bench top.
10. **Proceed to "Protocol 5: Reagent 5 addition and microplate wash," starting on page 25.**

Protocol 5: Reagent 5 addition and microplate wash

Important point before starting

- To avoid bubbles and cross-contamination during washing, dispense Reagent 5 bubbles into a waste reservoir, and then move directly to filling the microplate without stopping the Reagent 5 flow.
- When washing the microplate, fill each microplate well to the top without overflowing

1. **When prompted by the *care*HPV Test Controller, decant and blot the microplate, as previously described on page 23.**
2. **Return the *care*HPV Test Magnetic Plate Holder to the bench top with the microplate facing up.**
3. **Wash the microplate by gently filling each microplate well with Reagent 5 (blue cap sticker).**
4. **Touch the "5" icon on the *care*HPV Test Controller to begin a 3–minute incubation.**

   Notes:

   - The "5" icon will have a flashing blue halo until the "5" icon is touched to start the 3–minute incubation. The *care*HPV Test Controller will count down to the completion of the incubation.
   - At the touch of the "5" icon, a blue-filled droplet with a black number inside appears on the *care*HPV Test Controller display.
5. **At the end of the incubation, decant and blot the microplate, as previously described on page 23.**

   Note: The "5" icon will have a flashing blue halo at the end of the incubation.
6. **The *care*HPV Test Controller will prompt 4 more times. Each time the *care*HPV Test Controller prompts, repeat the wash of the microplate (steps 3–5 of this protocol), for a total of 5 washes.**

   Note: Touching the "5" icon starts the 3–minute incubation; make sure to add Reagent 5 to the microplate wells before touching the "5" icon.
7. **Leave the microplate in the *care*HPV Test Magnetic Plate Holder.**
8. **Proceed to "Protocol 6: Reagent 6 addition and incubation," starting on page 26.**

Protocol 6: Reagent 6 addition and incubation

1. **When prompted by the *care*HPV Test Controller, swirl the Reagent 6 bottle (green cap sticker) to mix and, using the repeat pipet, add 40 µl of Reagent 6 to each microplate well.**
2. **Apply a new plate sealer and securely cover the microplate, as previously described on page 19.**
3. **Carefully remove the microplate from the *care*HPV Test Magnetic Plate Holder; place the covered microplate on the *care*HPV Test Shaker and close the lid.**
4. **Touch the "6" icon on the *care*HPV Test Controller to begin a 15–minute incubation.**
5. **When prompted by the *care*HPV Test Controller (after 2 minutes), remove the microplate from the *care*HPV Test Shaker.**
6. **Carefully remove and discard the plate sealer.**
7. **At the prompt, open the *care*HPV Test Luminometer lid and lift the microplate cover.**
8. **Place the microplate into the *care*HPV Test Luminometer with the microplate oriented with the A1 microplate well in the upper right corner.**
9. **Close the lid to finish the incubation.**

   Notes:

   - The incubation will continue with the incubation time counting down and displaying an active "6" icon.
   - At the end of the incubation, the *care*HPV Test System proceeds immediately to Protocol 7 of the test without user intervention. The *care*HPV Test Luminometer initiates microplate measurement. The screen will display an active "7" icon while the microplate is being measured.
   - The duration of the microplate measurement is approximately 3 minutes. After the microplate is measured, the "Results" screen will display.

10. **Proceed to "Interpretation of Results," page 27.**

Interpretation of Results

Specimen results are interpreted automatically by the *care*HPV Test System. Specimens with a RLU to CO ratio (RLU/CO) ≥1.0 are considered positive and specimens with a RLU/CO <1.0 are considered negative or not detected. The results are displayed graphically on the *care*HPV Test Controller screen.

When the *care*HPV Test Controller displays the "Results" screen with test results, transcribe the result shown for each microplate well onto the Test Data Recording Sheet.

Test results are indicated, as follows:

- **Green** microplate wells indicate specimens with a negative test result (that is, high-risk HPV DNA not detected).

  Note: **Green** microplate wells also indicate acceptable results for the negative and positive calibrators.

- **Yellow** microplate wells (displaying a "+") indicate specimens with a positive test result (that is, high-risk HPV DNA detected).

- **Gray** microplate wells with a large red circle with a slash over the middle of the plate indicate an invalid assay (for example, due to failed calibrators).

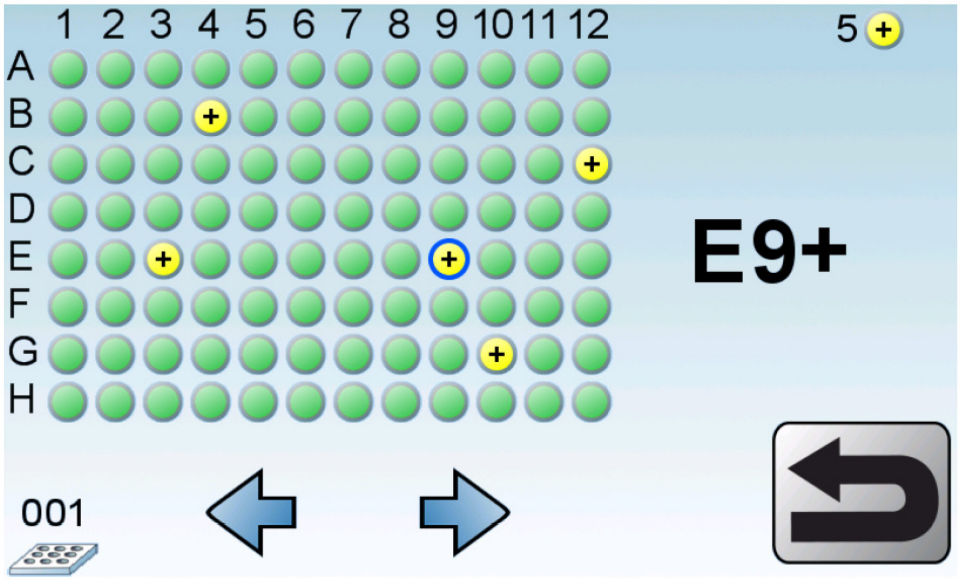

Figure 2. Example of sample results displayed on the *care*HPV Test Controller.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the *care*HPV Test Kit is tested against predetermined specifications to ensure consistent product quality. Acceptable ranges have been established only for the *care*HPV Test System.

The *care*HPV Test Controller performs assay calibration verification to ensure that the reagents and furnished calibrator materials are functioning properly, permitting accurate determination of the test result. The assay calibration verification consists of the following:

- The Negative Calibrator is tested in triplicate with each test. The Negative Calibrator mean ($NC\bar{x}$) must be ≥10 and ≤750 RLU, and the resultant coefficient of variation (CV) must be ≤25% in order for the assay to be valid.
- The Positive Calibrator is tested in triplicate with each test. The resultant CV must be ≤25% in order for the assay to be valid.
- The Positive Calibrator mean ($PC\bar{x}$) and $NC\bar{x}$ results are used to calculate the $PC\bar{x}$ /$NC\bar{x}$ ratio. The ratio must be ≥2.0 and ≤15.0 for the assay to be valid.

The *care*HPV Test System will complete the calculation of the above 3 quality control standards. When the above standards are met, the test results are valid and the *care*HPV Test Controller displays the "Results" screen. When the above standards are not met, the test results are invalid and the *care*HPV Test Controller displays an "Invalid" screen.

Limitations

- Refer to the *care*HPV Test System user manual for additional limitations specific to the use of that system.
- Detection of HPV using the *care*HPV Test does not differentiate HPV types or infection with more than one type and cannot evaluate persistence of any one type.
- The analytical sensitivity for HPV 45 and HPV 52 is lower in comparison to the other genotypes tested in the *care*HPV Test.

- Infection with HPV is not an indicator of cytological changes or underlying CIN 2/3+, nor does it imply that CIN 2/3+ or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN 2/3+ or cancer.
- The *care*HPV Test does not detect HPV low-risk types (6, 11, 42, 43, 44, and many other low-risk types).
- A small amount of cross-hybridization between HPV types 6 and 42 (low-risk HPV types) and the *care*HPV Test exists. Specimens with high levels (≥2 ng/ml) of HPV 6 or HPV 42 DNA may be positive.
- It has been reported in the literature that a complex probe mix, similar to that used in this test, may cause false-positive results due to cross-hybridization with HPV types 11, 53, 54, 55, 66, MM4, MM7, MM8, or MM9 (32). Although several of these HPV types are rare or novel types not often encountered with high-grade disease, specimens containing high levels of these HPV DNA types may incorrectly be reported as positive with the *care*HPV Test (10, 33).
- Cross-reactivity between the *care*HPV Test and the plasmid pBR322 is possible. The presence of pBR322 homologous sequences has been reported in human genital specimens, and false positive results could occur in the presence of high levels of bacterial plasmid.
- A negative result does not exclude the possibility of HPV infection. HPV infection may exist below the limit of detection for the test, or sampling error during specimen collection may cause a false-negative test result.
- A negative high-risk HPV result does not exclude the possibility of future cytological abnormalities or underlying CIN 2/3+ or cancer. A small proportion of high-grade lesions occur in women who are high-risk HPV negative by existing technologies (6).
- If antifungal cream is present at the time a specimen is collected for HPV testing, there is a likelihood of obtaining a false-positive result.
- If high concentrations of blood, contraceptive jelly, or douche are present at the time a specimen is collected for HPV testing, there is a likelihood of obtaining a false-negative result should this specimen contain HPV DNA concentration near the CO.

Performance Characteristics

Clinical performance for the use of *care*HPV Test in screening for cervical cancer and precancerous lesions

A multi-center clinical study using the *care*HPV Test was conducted at the Cancer Institute and Hospital, Chinese Academy of Medical Sciences (CICAMS), Sun Yat-Sen University Cancer Center, and Nanjing Drum Tower Hospital in China. Cervical specimens were collected using the *care*Brush and *care*HPV Collection Medium from women (30–59 years) in a general screening population and outpatients of cervical clinics. A total of 1279 women were enrolled in this study, representing a relatively equal distribution across the 3 hospitals; 1241 participants completed the study. The 3 study sites collected specimens from approximately 147 patients diagnosed with cervical cancer or precancerous lesions (CIN 2/3+), 162 patients with benign lesions (inflammation/mild cervical intraepithelial neoplasia, CIN 1), and 932 cases of normal control.

Acetic acid staining was also performed for visual examination (VIA). Liquid-based cytology was performed at each hospital, and the results recorded using the Bethesda Classification. The *care*HPV Test, the HC2 Test, and PCR testing were performed for each patient specimen. All *care*HPV testing was performed at room temperature (15–30°C)*. Test results were compared to the disease status of each patient. Disease status was based on the results of histologic evaluation. Women with a positive HC2 Test result or VIA were returned for colposcopy and biopsy. Test results were compared to disease status to assess the test's clinical sensitivity, clinical specificity, as well as negative and positive predictive values for detecting high-grade cervical neoplasia (see Table 1, below).

* Additional clinical data for *care*HPV testing performed in Hyderabad, India shows valid assay performance at temperatures up to 36.6°C with a maximum relative humidity of 75% for temperatures up to 31°C, decreasing linearly to 27% at 36.6°C.

Table 1. Performance characteristics of the *care*HPV Test in a general screening population

	Pathological diagnosis		
***care*HPV Test**	**Positive (CIN 2/3+)**	**Negative (<CIN 2)**	**Total**
Positive	129	160	289
Negative	18	934	952
Total	**147**	**1094**	**1241**

Where:

- Sensitivity [TP/(TP+FN)] = 87.76% (129/147); 95%CI = 81.69–92.34%
- Specificity [TN/(TN+FP)] = 85.37% (934/1094); 95%CI = 83.19–87.38%
- Positive predictive value = 44.64% (129/289)
- Negative predictive value = 98.11% (934/952)

The prevalence of HPV infection in a population may affect positive predictive, as values decrease when testing populations with low prevalence or individuals with no risk of infection.

The positivity rate of the *care*HPV Test and the HC2 Test was 23.29% (289/1241) and 25.06% (311/1241), respectively. The HC2 Test and the *care*HPV Test detect the same 13 HPV types with the *care*HPV Test additionally detecting HPV type 66. This difference would not be expected to result in significantly different performance profiles for the 2 tests. The concordance between the *care*HPV Test and HC2 Test was 93.71%, as shown in Table 2.

Table 2. Comparison of the *care*HPV Test versus the *digene* HC2 High-Risk HPV DNA Test

	***digene* HC2 High-Risk HPV DNA Test**		
***care*HPV Test**	**Positive**	**Negative**	**Total**
Positive	261	28	289
Negative	50	902	952
Total	**311**	**930**	**1241**

Kappa = 0.829 (P<0.0001)

Consistent rate = 1163/1241 = 93.71% (95% CI = 92.26%–94.97%)

The concordance between the *care*HPV Test versus PCR-based HPV detection was 90.89%, as shown in Table 3, below. HPV nucleic acid was amplified using a PCR-based fluorescent detection kit [Ganglong Biotechnology (Shenzhen) Co., Ltd].

Table 3. Comparison of *care*HPV Test versus PCR-based HPV detection

	PCR-based HPV detection		
***care*HPV Test**	**Positive**	**Negative**	**Total**
Positive	263	26	289
Negative	87	865	952
Total	**350**	**891**	**1241**

Kappa = 0.763 (P<0.0001)

Consistent rate = 1128/1241 = 90.89% (95% CI = 89.20%–92.40%)

Analytical performance testing conditions

Studies for analytical sensitivity, cross-reactivity, and interfering substances were performed at room temperature (15–30 °C) in a controlled laboratory environment. Additional analytical testing was performed in an environmental chamber, showing valid test performance at 15–40 °C and 15–75% relative humidity (noncondensing); maximum 75% relative humidity for temperatures up to 31 °C decreasing linearly to 50% relative humidity at 40 °C.

Analytical sensitivity

To demonstrate the analytical sensitivity of the *care*HPV Test, a panel of HPV plasmid DNA targets was tested to verify that each of the 14 high-risk HPV types is detected with a PC$\bar{x}$ /NC$\bar{x}$ ratio ≥2.0. Each of the 14 HPV DNA types was prepared at a HPV target concentration of 1.0 pg/ml (5000 copies/assay) in Negative Calibrator. The concentration prepared replicates the target plasmid concentration of the Positive Calibrator.

Each HPV type was tested in replicates of 8. The mean signal, the CV, and the signal-to-noise ratio for each HPV type were calculated. The results are shown in Table 4, on the next page.

Table 4. Summary of the *care*HPV Test analytical sensitivity for each HPV DNA type at 1 pg/ml

HPV type	Mean signal (RLU)	Coefficient of variation	Signal-to-noise ratio
16	672	15%	5.3
18	611	14%	4.9
31	623	12%	4.9
33	564	8%	4.5
35	678	10%	5.4
39	611	7%	4.4
45	321	9%	2.5
51	676	12%	5.4
52	370	8%	2.7
56	739	10%	5.3
58	558	10%	4.4
59	686	8%	5.4
66	636	12%	4.6
68	534	11%	3.8

Cross-reactivity

Cross-reactivity with micro-organisms

Studies indicate that the *care*HPV Test does not cross-react with the following micro-organisms (see Table 5, below) at the following concentrations:

- *Chlamydia trachomatis* (3.5 x 10^2 to 2.0 x 10^3 IFU/ml)
- *Trichomonas vaginalis* (8 x 10^5 cells/ml)
- Pathogens listed in Table 5 (1.5 x 10^4 to 9.8 x 10^9 CFU/ml)

Table 5. Potentially cross-reactive pathogens

Pathogen	Pathogen
Acinetobacter sp.	*Mycoplasma hominis*
Acinetobacter lwoffi	*Neisseria gonorrhoeae*
Bacteroides fragilis	*Neisseria lactamica*
Candida albicans	*Neisseria sicca*
Chlamydia trachomatis	*Peptostreptococcus anaerobius*
Enterobacter cloacae	*Prevotella melaninogenica*
Enterococcus faecalis (Streptococcus)	*Proteus vulgaris*
*Escherichia coli (HB101)**	*Serratia marcescens*
Escherichia coli	*Staphylococcus aureus*
Gardnerella vaginalis	*Staphylococcus epidermidis*
Haemophilus ducreyi	*Streptococcus pyogenes*
Klebsiella pneumoniae	*Treponema phagedenis*
Lactobacillus acidophilus	*Trichomonas vaginalis*
Mobiluncus curtisii	*Ureaplasma urealyticum*
Mobiluncus mulieris	

* Both the *E. coli* strain used to grow plasmids (HB101) and a clinical isolate of *E. coli* were tested.

Cross-reactivity with viral or plasmid DNA

The following DNA types were tested for cross-reactivity at the following concentrations:

- Herpes simplex II (1 x 10^6 PFU/ml)
- pBR322 (4 ng/ml)

The Herpes simplex II showed no cross-reactivity.

The pBR322 plasmid showed cross-reactivity in the *care*HPV Test, which is not unexpected. The pBR322 is used as the vector for the HPV plasmid and it is

difficult to remove the entire vector pBR322 DNA when isolating the HPV insert. The presence of pBR322 homologous sequences has been reported in human genital specimens, and false-positive results could occur in the presence of high levels of pBR322 DNA.

Cross-reactivity with human genomic DNA

Studies indicate that the *care*HPV Test does not cross-react with human genomic DNA at 250 ng/ml.

Cross-reactivity determined by blast method

Sequence analyses (blast method) were completed for the following to make sure there were no overlapping, cross-reactive sequences:

- HIV, HBV, EBV, CMV
- Adenovirus 2
- *Neisseria meningitides*

Interfering substances

The effect of substances that may be found in cervical specimens (whole blood, douche, antifungal cream, contraceptive jelly, and vaginal lubricant) was evaluated in the *care*HPV Test. The substances were added in 2 different amounts (50 µl and 100 µl) to Negative Calibrator, Positive Calibrator, and 5 pg/ml HPV 16 in Negative Calibrator. The Negative Calibrator, Positive Calibrator, and 5 pg/ml HPV 16 in Negative Calibrator were also tested without substances.

False-positive results were observed with the antifungal cream at both concentrations, but no false-positive results were observed with any of the other substances at any concentration tested.

A false-negative result may be reported in a clinical specimen with a HPV DNA concentration close to that of the CO (1 pg/ml) if high levels of blood, contraceptive jelly, or douche are present at the time a specimen is collected.

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Symbols

The following symbols may appear on the packaging and labeling:

Symbol	Symbol definition
	Contains sufficient for <N> tests
	Use by
	In vitro diagnostics
	Catalog number
	Lot number
	Material number
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	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Consult instructions for use

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Appendix: Test Data Recording Sheet

Testing site: _____ Testing date: _____

Operator ID: _____ Room temperature: ___ °C careHPV Test Kit lot number: _____ Microplate run number: _____

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC											
B	NC											
C	NC											
D	PC											
E	PC											
F	PC											
G												
H												

Ordering Information

Product	Contents	Cat. no.
*care*HPV Test System	This includes the following items: ■ *care*HPV Test Controller ■ careHPV Test Luminometer ■ careHPV Test Shaker ■ careHPV Test Magnetic Plate Holder	9001772
*care*HPV Test Luminometer	Microplate chemiluminescent detection instrument for use with the *care*HPV Test System	9002140
*care*HPV Test Shaker	Heater/shaker for use with the *care*HPV Test System	9002141
*care*HPV Test Controller	Touch-screen device with application software for use with the *care*HPV Test System	9002142
*care*Brush	Package of 50 pre-scored cervical brush collection devices	619024
*care*HPV Collection Medium	Package of 50 tubes, each containing 1 ml of *care*HPV Collection Medium	619025
*care*HPV Test Magnetic Plate Holder	Magnetic plate holder for *care*HPV Test	9019960
Plate sealers	100 plate sealers	5070-1010

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