DOCUMENT Nº2.1:

DEVICE DESCRIPTION

HPV2

IRAN REGISTRATION

CONFIDENTIAL

EDICIÓN	FECHA	MODIFICACIONES
1	29.07.2017	INITIAL VERSION

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1. INTRODUCTION

The main goal of the new version of CLART® HPV2is to improve the sensitivity and the specificity of the product in order to provide a more accurate tool for diagnosis of human HPV.

As a result, GENOMICA has designed a product with a sensitivity and specificity reaching 99% and 100% respectively.

Current knowledge has established that infection with Human Papillomavirus (HPV) is the main cause of invasive cervical cancer and cervical intraepithelial neoplasia (Bosch et al. 2002; Walboomers et al. 1999).

Interestingly, although approximately 100 types of HPV have been described to date, only 50 have been isolated from the anogenital mucosa. These anogenital types are sexually transmitted and have been classified in two epidemiological groups according to their association with cervical cancer (Dunne et al., 2007):

- **Low oncogenic risk type HPVs - types** 6, 11, 32, 40, 42, 44, 54, 55, 61, 62, 64, 71, 72, 74, 81, 83, 84, 87, 89 and 91.
- **High oncogenic risk HPVs - types**: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82 and 85.

This classification particularly highlights the relevance of identifying the HPV types causing the infection, which will determine future patient care.

2. PRODUCT DESCRIPTION

CLARTHuman Papillomavirus 2 Kit detects up to 35 (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 70, 71, 72, 73, 81, 82, 83, 84, 85 y 89) of the most clinically relevant HPV types (see Fig. 1) in a wide range of samples.

Detection of the different HPV genotypes is achieved by PCR amplification of a 450 bp fragment within the highly conserved L1 region of the virus. This highly conserved sequence presents slight variations among each individual HPV type that allows its genomic identification by recognition of the viral DNA by specific probes. This slight variation guaranties the detection specificity.

This approach presents a number of advantages:

- Its high sensitivity allows detection of minimal quantities of viral DNA.
- Its high specificity allows detection of specific HPV genotypes by recognising a highly conserved sequence of the viral genome.
- The test can be easily performed in hospital laboratories.
- It can quickly detect the presence of up to 35 of the most clinically relevant HPV types within hours.

CLART® HPV2kit has been designed and validated for use on swabs, cell suspensions, tissues fixed in formol or paraffin wax.

The sensitivity obtained with the CLART® HPV2kit, combining the amplification and its visualization in the array, is so high that it is not necessary to perform double amplifications (nested-PCR), thus avoiding any contamination risk involved.

One of the main drawbacks of genomic amplification is the utilization of poor quality DNA samples (too short DNA, degradation of the DNA, or loss of DNA during extraction) or the presence of DNA polymerase inhibitors (e.g., hemoglobin, remains of paraffin wax, salts etc.) in the samples to be analyzed, thus interfering with the genomic amplification and resulting in false negatives. However, the CLART® HPV2eliminates false negatives using internal controls within the same tube where the sample is analyzed, and that are amplified at the same time as the viral DNA.

Every reaction (amplification) tube of the kit contains the following primers:

- A pair of primers that amplify a fragment of the human gene CFTR (genomic DNA or DNA from the patient). It is used as genomic DNA control.
- A pair of primers that amplify a modified plasmid that is included in the tube and which is used as a amplification reaction control.
- HPV primers.

The reaction tube has been designed in order to favour the amplification of the HPV against the other two controls. Among these two controls, the genomic DNA will amplify preferentially compared to the amplification reaction control.

The reason for this design is:

Genomic DNA control would only be essential for confirming a negative result, since it reports that DNA from the patient was present in the sample even if no HPVs were found.

PCR control would only be essential if no amplification in the tube is found, because it will help to distinguish between an inhibited PCR and a sample where no DNA has been found.

However, when HPVs are present in the sample, there is always a preference to amplify genotypes instead of amplifying the controls. Hence, under certain conditions (i.e. high copy numbers of a particular HPV genotype or several HPV genotypes present in one sample) internal controls may not appear.

3. DESCRIPTION OF THE PRINCIPLE OF THE ASSAY METHOD

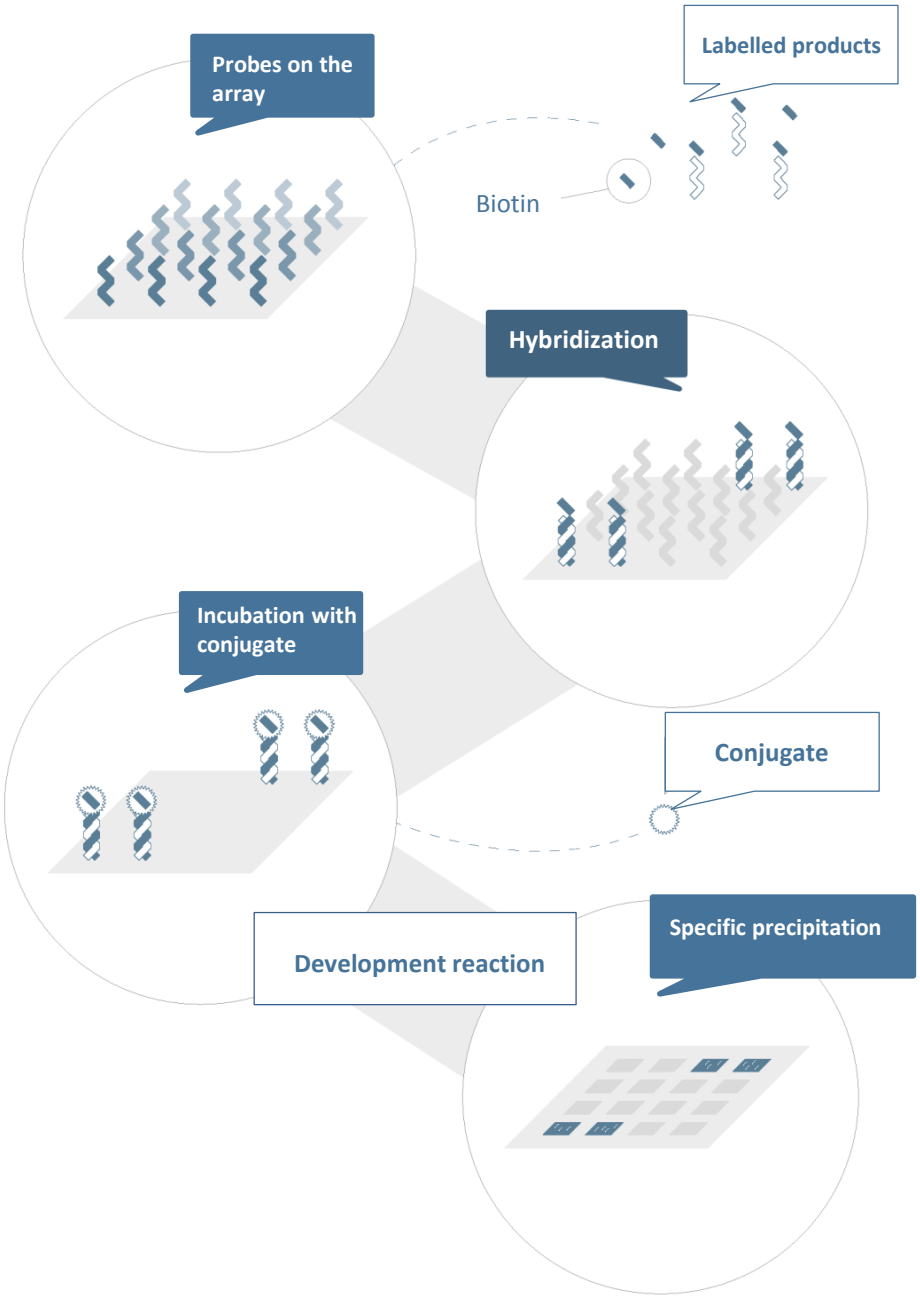
The product kit **CLART® HPV2** is a platform for IVD based in DNA microarray. Both amplification phase via PCR and visualization and interpretation are automatic. Final visualization of results is performed with CLART STRIPS (CS).

Detection of the different HPV genotypes is achieved by **PCR amplification of a 450 bp fragment within the highly conserved L1 region of the virus**. This highly conserved sequence

presents slight variations among each individual HPV type that allows its genomic identification by recognition of the viral DNA by specific probes. This slight variation guaranties the detection specificity). The platform is based on a low density microarray fixed in the bottom of an 8-well strip (CLART® Strip-CS) (Fig. 2), thus rendering a very efficient and easy to use system. This technology allows the simultaneous detection of multiple molecular markers for diagnostic use while providing the controls needed to ensure the reliability of the results. Moreover, this system considerably simplifies the processes of hybridization and visualization when compared with classic microarray systems.

Figure 1. **CLART Strip®-CS** *platform in the form of an 8-well strip.*

CLART® HPV2 detection system is based on the precipitation of an insoluble product at those sites of the microarray where the hybridization of the amplified products by specific probes takes place. Hybridization of the amplified PCR product is detected by generation of an insoluble precipitate at the sites of the microarray where the amplified products have been captured by the probes. This is achieved by marking the amplified products with biotin during the PCR procedure. Biotinilated products hybridize to their specific probes attached to the microarray surface and become immobilised. These immobilised biotinilated products are recognised by the streptavidin of a streptavidin-peroxidase conjugate, thus providing with peroxidase activity to the hybridized products. Peroxidase activity will then metabolise o-Dianisidine and produce an insoluble product which will precipitate in those places where hybridization occurred.


Probes on the array
Labelled products
Biotin
Hybridization
Incubation with conjugate
Conjugate
Development reaction
Specific precipitation

4. COMPONENTS OF THE ASSAY

CLART® HPV2 kit contains enough reagents for the analysis of 16, 48 or 96 clinical samples. Components of the kit are provided at their optimal storage temperatures, and remain stable until the expiration date is reached, upon observance of recommended storage conditions.

Kit components are displayed herein:

3.1. Extraction and purification reagents

Shipped and stored at 4ºC (Format for analysis of 16 samples), or Room Temperature (Formats for the analysis of 48 or 96 samples).

Components:

Purification columns adapted to 2 ml tubes

2 ml tubes

T1 Buffer

B3 Buffer

B5 Buffer

BE Buffer

BW Buffer

Proteinase K, lyophilized (keep at -20ºC when resuspended)

PB Buffer

Note: In the format for analysis of 16 samples, Proteinase K already resuspended is sent at -20ºC, together with the amplification reagents.

4.2. Amplification reagents

There are two formats under which amplification reagents are shipped and stored, depending on whether the tubes are lyophilized or not:

Non-lyophilized tubes: Shipped and stored at -20ºC. They are provided ready-to-use. Only the exact number of required tubes should be thawed on ice. Remaining ones should be kept at -20ºC.

Note: Boxes containing amplification tubes include a self-adhesive and irreversible temperature indicator; Red color displayed on the visualization window of the indicator means that the package has exceeded at some time the storage temperature of –20ºC and reagents should be discarded.

Lyophilized tubes: Sent at 4ºC or at Room Temperature, and stored at 4ºC. Lyophilized tubes are provided in the form of strips of 8 tubes within a thermosealed aluminium bag.

Once the bag has been opened, it is recommended that the strips that are not to be used immediately, be stored together with the desiccant bag within the original aluminium bag, sealed with tape. Under these conditions the strips may be stored up to a maximum of 30 days.

In addition to the amplification tubes, SD solution (Diluting Solution) is provided. Store at Room Temperature (20-25 ºC).

4.3. Visualization components

Visualization components are divided into two groups, according to optimal shipping and storage temperatures:

Shipped at 4ºC and stored at Room Temperature:

- **CLART-Strip® (CS)**, each well including all specific probes for detection of all HPV types to be detected.

Note: Required **CS** units are shipped in a sealed pouch. Each unit should be kept until use, in the unopened pouch, at room temperature (i.e. 25ºC maximum) and protected from direct light and high temperatures.

Shipped and stored at 4ºC:

DC (Conjugate Diluent).

SH (Hybridization Solution).

CJ (Conjugate Solution).

RE (Development Solution). Keep away from light.

TL (Wash Buffer).

Microtiter plate adaptor and plastic lid.

4.4. Other components

GENOMICA's **CAR®** or CLINICAL ARRAY READER (Figure 3).

CAR® grants automatic reading, analysis and interpretation of up to 12 **CS** units (i.e., to a maximum of 96 samples) *per* run. It displays a user-friendly and intuitive graphical interface (CLEIS), and includes updates of GENOMICA's proprietary image processing software SAICLART® as well as kit-specific Software.

Note: CAR® is to be used exclusively with GENOMICA's diagnostic kits.

FIGURE 3. CAR® (CLINICAL ARRAY READER)

GENOMICA's **autoclart®**.

autoclart® allows automatic processing of up to 12 CSs strips (96 samples) during the visualization step.

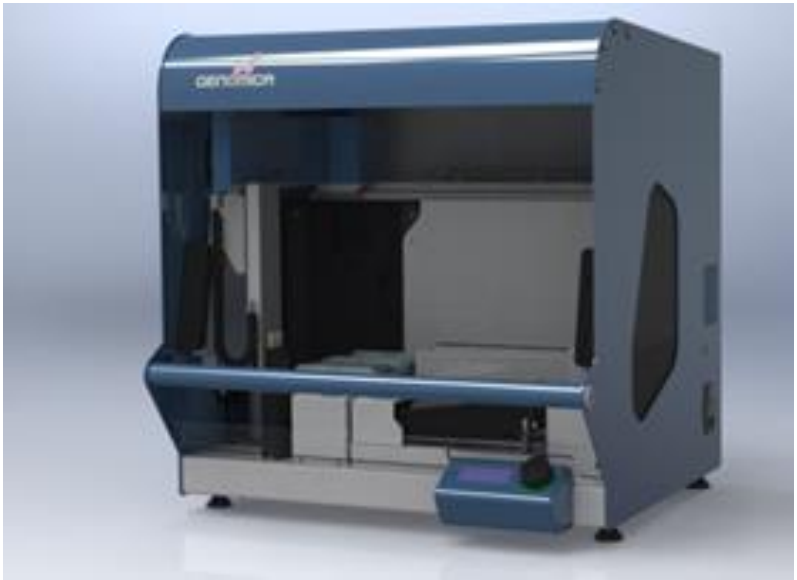


FIGURE 4. AUTOCLART®

GENOMICA's **autoclart® plus**.

autoclart® plus is a fully automated electromedical device capable of processing up to 96 samples per run, starting from the denatured amplification product, and ending with issuance of the corresponding diagnostic report.

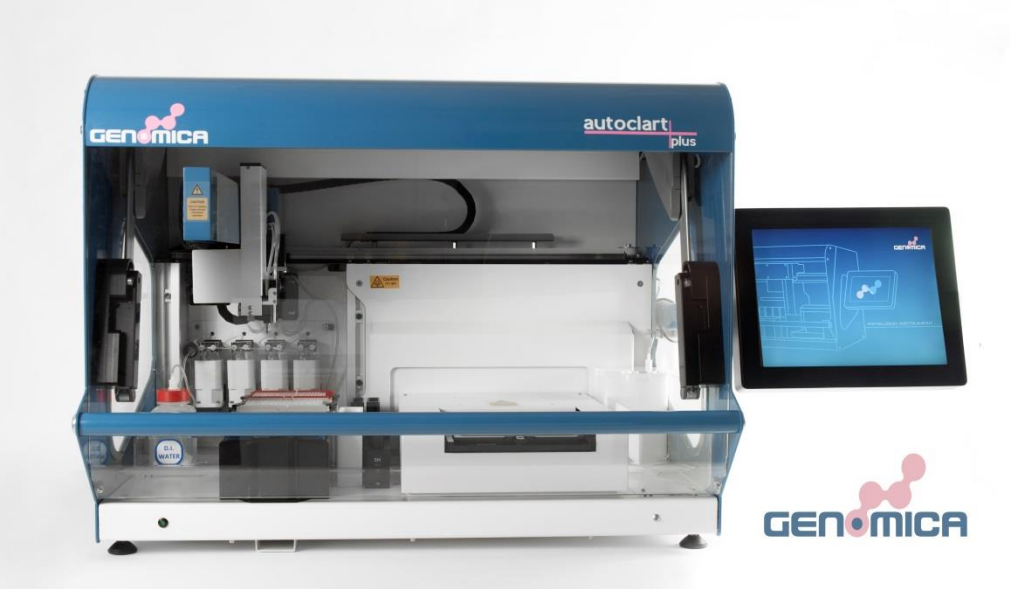


FIGURE 5. AUTOCLART® PLUS

ITEMS REQUIRED BUT NOT PROVIDED

A list of all items required but not provided is displayed below:

4.5. Reagents and materials

Distilled water.

96% Ethanol.

Disposable gloves.

Filter tips or positive displacement pipettes.

Crushed ice container or cool tube-holder.

1.5 mL autoclaved Eppendorf tubes.

1.5 mL tube grids.

0.5 mL/0.2 mL tube holder.

Saline solution (0.9% NaCl).

4.6. Equipment

Microcentrifuge.

Thermal cycler.

Biosafety cabinet.

Three adjustable micropipettes ranging from 1-20 µL, 20-200 µL and 200-1000 µL for the pre-PCR area.

Three adjustable micropipettes ranging from 1-20 µL, 20-200 µL, and 200-1000 µL for the post-PCR area.

Termobloque (Thermomixer) compatible with 96-well skirted plates and adjustable shaking at 25°C, 30°C and 65ºC.

Vortex.

Vacuum pump (optional).

5. PAKAGING

SECUNDARY PACKAGING:

The kit package is made of cardboard.

The secondary labeling is fixed on the packaging. This packaging has imprinted the name and the address of the manufacturer.

Front of the packaging	***Back of the packaging***
	
In this packaging is printed the name and the adress of the manufacturer	
	

Please note: The amplification reagent package includes a self-adhesive and irreversible temperature indicator; the appearance of a reddish color on the visualization window indicates that, at a certain moment, products have exceeded storage temperature of -20 ^{o}C and they should not be used.

6. STORAGE

Reagents included in the kit have been grouped into various packages, depending on the temperature at which they should be stored.

Extraction and Purification reagents : They should be stored at room temperature.

Amplification reagents : They should be stored at -20ºC.

Visualization reagents :. This visualization kit should be stored at 2ºC to 8ºC

Components	Storage Temperature
Hybridization Solution (SH)	**STORE AT 2ºC TO 8ºC**
Conjugate (CJ)	**STORE AT 2ºC TO 8ºC**
Conjugate Diluent (DC)	**STORE AT 2ºC TO 8ºC**
Development Solution (RE)	**STORE AT 2ºC TO 8ºC**
Wash Buffer (TL)	**STORE AT 2ºC TO 8ºC**
CS Strips (Arrays)	**STORE AT 20ºC TO 22Cº (ROOM TEMPERATURE)**

7. EXPIRATION DATE

Stability experiments are intended confirm that functionality of the reagents was not affected by their shelf-life, always within the expiration date limits recommended by the manufacturer.

The stability of the extraction and Amplification part is determined by the stability of the amplification tubes. This stability has been assessed and is for one year. The results are presented below.

The stability of the Visualization part is determined by the stability of its most critical component: the Develoment Solution.

For further details see DOCUMENT STABILITY DATA

8. INTENTED USE

FUNTION

CLART® HPV2 is intended for following use: genotyping of human papillomavirus via genomic identification for *IN VITRO* DIAGNOSIS.

USER

CLART® Human Papillomavirus 2 is intended to be used by professional people with adequate qualification because it requires the interpretation of the results obtained from the assay.

USED SAMPLES

- Swabs.
- Cell suspensions.
- . Formalin, ethanol and paraffin wax-embedded samples.

ENVIROMENTAL CONDITIOND OF USE

This assay should be performed in two physically separated areas, in order to avoid sample contamination with the previously amplified product. Separate working materials should be available in each area (pipettes, tips, tubes, grids, gloves, etc.) which should never be used outside these areas.

1. Pre-PCR area: DNA extraction, sample preparation and addition of the extracted material to the amplification tubes are performed in this area. Sample manipulation must be carried out within a biosafety cabinet (BSC).

2. Post-PCR area: Amplification and visualization of the amplified product are carried out in this area. The material of this area should never come into contact with the material of the extraction area. Avoid entering the pre-PCR area after having worked in the visualization area.

Always use gloves. It is recommended to change gloves quite frequently, and it is mandatory to change gloves before start working in each of the aforementioned areas. New gloves must always be used when DNA is added to the amplification tubes.

Clean working areas (laboratory cabinets, hoods, grids, pipettes) thoroughly with a 10% diluted bleach solution **after every sample batch processing;** it is mandatory to disinfect all working areas in case of contamination. For thermocyclers and thermomixers, it is advised to clean them before and after used, in these same conditions.

Always use filter tips and positive displacement pipettes to avoid contamination due to micropipettes. Different sets of pipettes should be used in each area.

5. Use disposable and autoclaved laboratory material.

6. Never mix reagents from two different vials, even if they belong to the same lot.

7. Close reagent tubes immediately after use in order to avoid contamination.

8. Discard the micropipette tip after pipetting.

9. GENOMICA is not responsible for the results obtained with the kit if other samples different to the ones indicated are used.

CONDITION BEING TREATED AND GROUP OF PATIENTS.

Detection and typing of human papillomavirus

automatization

GENOMICA's "Clinical Array Technology (CLART®)" is our own proprietary microarray-based technology for *in vitro* diagnostics.

CLART® technology is based on detection of microorganisms or genotypes in biological samples by multiplex-PCR and visualization of the amplicons by means of low density microarrays (specific capture and labeling) printed on the flat-bottom surface of microtiter-plate wells (Fig 1).

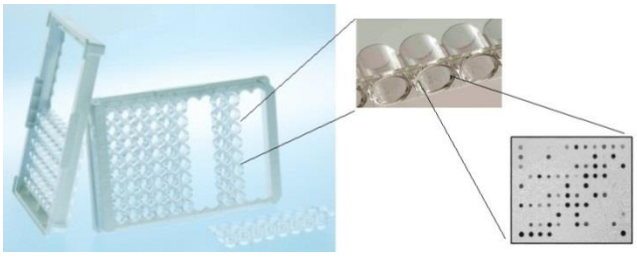


Fig. 1

CLART® microarrays are imaged in a colorimetric array readers called: Clinical Array Reader (CAR). This reader provides a fully automated analysis and interpretation of microarray tests, avoiding user intervention in the process of producing the result of the test (Fig 2).

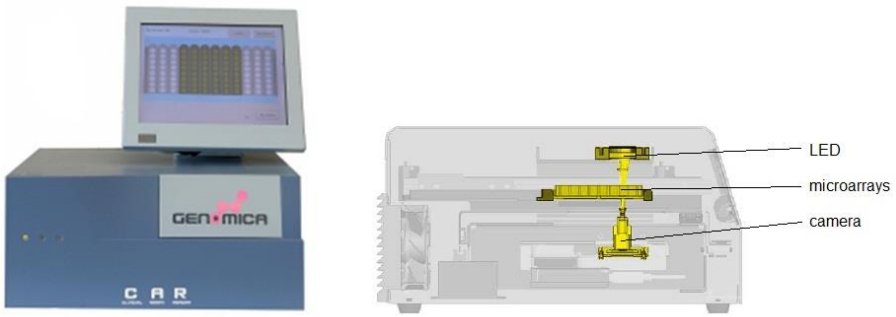


Fig. 2

The reader is able to scan a full microtiter plate (96 arrays). Image analysis and interpretation of these microarrays is driven by specific software for each kit that is designed and implemented by GENOMICA as part of our development process. One of the key characteristics of this analysis is that it is fully automated. That is, GENOMICA's software is able to recognize the microarray in the image, identify its signals, and get to the results without the need of user intervention of any kind.

In 2013, GENOMICA launched a semi-automated solution for the processing of CLART® microarrays under the brand name of autoclart (Fig. 3).

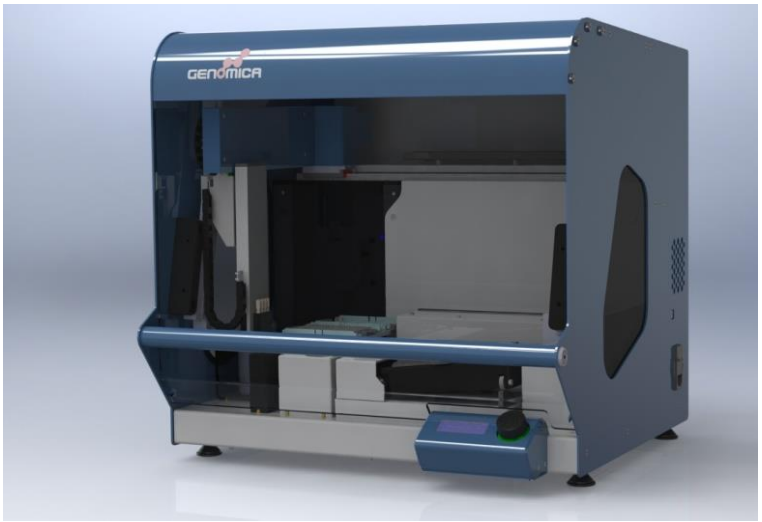


Fig. 3

This instrument automates prewashing, hybridization, conjugation and development of up to 96 microarrays in multiples of 4. Samples are added to the microplate wells manually by the user (3-10 µl of previously denatured PCR product). Upon protocol completion, the microarrays are ready to be imaged and automatically-interpreted at the CAR reader.

Liquid handling is based on an X/Y/Z mechanism for addition and aspiration of reagents. For addition it incorporates a four-channel head with a disposable-tip picking and ejection system. Precise volume control is achieved using a peristaltic pump. Incorporated into the tip picking system it has four aspirating needles, for the removal of waste reagent. In order for the aspiration to be efficient and avoid physical contact with the microarrays, it is able to tilt the microtiter plate and drive the needles to side of the well. Heating and cooling is based on a Peltier unit that gives accurate and precise temperature control above and below room temperature.

9. RISK CLASS OF THE PRODUCT: CLASSIFICATION

CLASSIFICATION IN EUROPE

The Classification of an IVD Medical Device is based on the following criteria:

- The intended use and indications for use as specified by the manufacturer (specific disorder, condition or risk factor for which the test is intended)
- The technical/scientific/medical expertise of the intended user (lay person or professional)
- The importance of the information to the diagnosis (sole determinant or one of several), taking into consideration the natural history of the disease or disorder including presenting signs and symptoms which may guide a physician
- The impact of the result (true or false) to the individual and/or to public health

The device is subject to special national rules that apply within a particular jurisdiction.

In Europe, the In Vitro Diagnostic Medical Devices Directive 98/79/CE describes, for the purposes of the conformity assessment procedures, the groups IVDs into four categories:

• General IVDs, i.e. all IVDs other than those covered by Annex II and IVDs for self-testing;

• IVDs for self-testing (a device intended by the manufacturer to be able to be used by lay persons in a home environment) excluding self-test devices covered in Annex II;

• IVDs in Annex II List B of the Directive: Which, amongst others, includes reagents products for rubella, toxoplasmosis and phenylketonuria, cytomegalovirus, Chlamydia as well as devices for self testing for blood sugar.

• IVDs in Annex II List A of the Directive: Which includes reagents and products for HIV I and II, Hepatitis B, C and D, and reagent products for determining ABO systems and anti-kell.

The device concerned is intended to be used to detect the presence of, or exposure to, a transmissible agent that causes a life-threatening often incurable disease with a high risk of propagation.

CLART® Human Papillomavirus 2 2 meets the provisions of the EC Council Directive 98/79/EC on in vitro diagnostic medical devices, transposed to the Spanish legislation by the Royal Decree 1662/2000 of 29th September 2000. MDD 98/79/EC.

It is intended to be used by professional people with adequate qualification but it is not included in Annex II of 98/79/CE Directive. This kit only requires Declaration of Conformity from GENOMICA (Annex III, 98/79/CE Directive).

CLASSIFICATION IN BRAZIL

In brazil according to the **RDC 36 (2015/08/26) / IN 03/2015 (2015/08/26) / RDC 40/2015** ***published on 2015/08/27 , CLART® Human Papillomavirus 2 is classified as Class III.***

TECHNICAL STANDARDS USED

For further details see DECLARATION OF CONFORMITY

10. MARKETING

GENOMICA has noT adressed any procedure in order to have authorized the publicity of its products in any country

11. BACKGROUND

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Ejegod DM., Rebolj M., Bonde J.: "*Comparison of analytical and clinical performance of CLART HPV2 genotyping assay to Linear Array and Hybrid Capture 2: a split-sample study*". DOI 10.1186/s12885-015-1223-z

12. ONCOGENIC RISK OF THE HPV TYPES DETECTABLE WITH CLART® HPV2

TYPE	ONCOGENIC RISK *
PVH 6	Low Risk
PVH 11	Low Risk
PVH 16	High Risk
PVH 18	High Risk
PVH 26	Problable High Risk
PVH 31	High Risk
PVH 33	High Risk
PVH 35	High Risk
PVH 39	High Risk
PVH 40	Low Risk
PVH 42	Low Risk
PVH 43	Low Risk
PVH 44	Low Risk
PVH 45	High Risk
PVH 51	High Risk
PVH 52	High Risk
PVH 53	Probable High Risk
PVH 54	Low Risk

TYPE	ONCOGENIC RISK *
PVH 56	High Risk
PVH 58	High Risk
PVH 59	High Risk
PVH 61	Low Risk
PVH 62	Low Risk
PVH 66	High Risk
PVH 68	High Risk
PVH 70	Low Risk
PVH 71	Low Risk
PVH 72	Low Risk
PVH 73	Probable High Risk
PVH 81	Low Risk
PVH 82	Probable High Risk
PVH 83	Low Risk
PVH 84	Low Risk
PVH 85	Low Risk
PVH 89	Low Risk

*According to: *Bouvar d V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F et al.*

A review of human carcinogens -Part B: biological agents. Lancet Oncol