Instructions for use

Imegen® Quimera Screening Multiplex Plus

Ref. IMG-116-26

CE IVD

Manufactured by::
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healthincode

Health in Code guarantees that its products are free of defects, in both materials and workmanship. This guarantee remains in force until the expiration date, as long as the conservation practices described in this manual are adhered to.

Our products are intended for *in vitro diagnostic* use. Health in Code provides no guarantee, whether explicit or implicit, that extends beyond the proper functioning of the components of this kit. Health in Code's sole obligation, in relation to the aforementioned guarantees, shall be to either replace the product or reimburse the cost of it, per the client's preference, provided that materials or workmanship prove to be defective. Health in Code is not liable for any cost or expense, direct or indirect, or damage or harm incurred by the customer or user as a result of use of the product by the buyer or user.

All Health in Code products undergo strict quality control. The **Imegen® Quimera Screening Multiplex Plus** dPCR kit has passed all internal validation tests, thus guaranteeing the reliability and reproducibility of each assay.

If you have any questions about the use of this product or its protocols, feel free to contact our Technical Department:

Telephone: +34 963 212 340
E-mail: tech.support@healthincode.com

Modifications to the Instructions for Use (IFU)

Version 06	Format edition
Version 07	In section 3, specifying the necessary amount of DNA needed for the analysis.
Version 08	Update of section Troubleshooting
Version 09	Change of manufacturer's identification from Imegen to Health in Code.

contents

01
General information

The analysis of molecular chimerism resulting from allogenic transplantation has become an established method to follow transplant progression, since it offers accurate and valuable information that allows targeting post-transplant treatments or interventions with the purpose of anticipating any potential risk of relapse, rejection, or graft-versus-host disease. This approach is highly useful not only to determine the risk of relapse, rejection, or graft-versus-host disease, but also to assess the response to different treatment modalities.

The whole **Imegen® Quimera** kit family has been developed in collaboration with the Carlos Haya Regional Hospital (Malaga, Spain), included in the Andalusian regional public healthcare service (Servicio Andaluz de Salud, SAS). As a result of this agreement, Health in Code holds the **exclusive worldwide license** on the *know-how* of the products for their commercial manufacturing and exploitation.

References

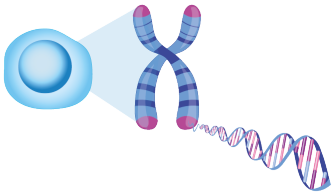
- *Jiménez-Velasco A, Barrios M, Román-Gómez J, Navarro G, Buño I, Castillejo J, et al. Reliable quantification of hematopoietic chimerism after allogeneic transplantation for acute leukemia using amplification by real-time PCR of null alleles and insertion/deletion polymorphisms. Leukemia. 2005; 1-8.*
- *Stahl T, Böhmeb M, Krögera N, Fehse B. Digital PCR to assess hematopoietic chimerism after allogeneic stem cell transplantation. Experimental Hematology. 2015; 43:462-468*

> Procedure for hematopoietic chimerism analysis:

1. EXTRACTION OF GENOMIC DNA

1h

Genomic DNA extracted from peripheral blood or bone marrow samples.



2. SCREENING FOR INFORMATIVE POLYMORPHISMS

2h30'

A genotyping assay allows identifying an informative polymorphism suitable for patient follow-up.

3. MARKER SELECTION FOR PATIENT FOLLOW-UP

10'

In hematopoietic stem cell transplant cases, a polymorphism is considered informative when detected in the recipient and not in the donor

MARKER		RECIPIENT	DONOR	INFORMATIVE
Q116-6I	[FAM]	+ −	+ −	✗
Q116-3I	[VIC]	+ −	+ −	✗
Q116-7I	[FAM]	+ −	+ −	✓
Q116-12D	[VIC]	+ −	+ −	✗

4. QUANTIFICATION OF FOLLOW-UP MARKER

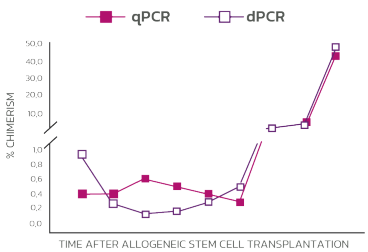
dPCR 4h
qPCR 2h30'

Molecular chimerism is quantified based on the number of copies of the informative marker relative to the number of copies of the reference gene (β-globin).

5. PATIENT FOLLOW-UP FOR HEMATOPOIETIC CHIMERISM

10'

During follow-up, hematopoietic chimerism values are plotted in a graph to study the transplant patient's progression over time.



02
Intended use

The **Imegen® Quimera Screening Multiplex Plus** kit allows selecting informative markers for the follow-up of hematopoietic stem cell transplant patients by simultaneous analysis of 16 insertion/deletion polymorphisms (INDELs) in 8 independent multiplexed real-time PCR reactions.

To determine the informativity of polymorphisms, the kits **Imegen® Quimera Screening Multiplex Plus** (IMG-116-26) and **Imegen® Quimera Screening Multiplex Plus II** (IMG-116-25) have been developed. In cases of hematopoietic stem cell transplant, a polymorphism is considered informative when detected in the transplant recipient but not in the donor, while in solid organ studies, a marker is considered informative when detected in the donor but not in the recipient.

No molecular analysis is necessary to determine the informativity of the SRY and RhD markers. In cases of hematopoietic stem cell transplant, the SRY marker is considered informative when the recipient is a male and the donor is a female, while the RhD marker is considered informative when the recipient's blood type is Rh+ and the donor's is Rh-.

The **Imegen® Quimera Screening Multiplex Plus** kit can only be used for in vitro diagnosis and is aimed at professionals in molecular biology.

03

Technical characteristics

The **Imegen® Quimera Screening Multiplex Plus** kit consists of a 16-biomarker genotyping assay that includes null alleles and INDELs (insertions/deletions), which allows identifying informative markers for hematopoietic chimerism analysis. Along with Imegen® Quimera Screening Multiplex Plus II and with the SRY marker, located in Chr Y, it includes 33 markers.

It uses a combination of specific oligonucleotides and fluorescent hydrolysis probes to detect the presence or absence of 16 multiplexed polymorphic markers.

33 markers in 19 chromosomes

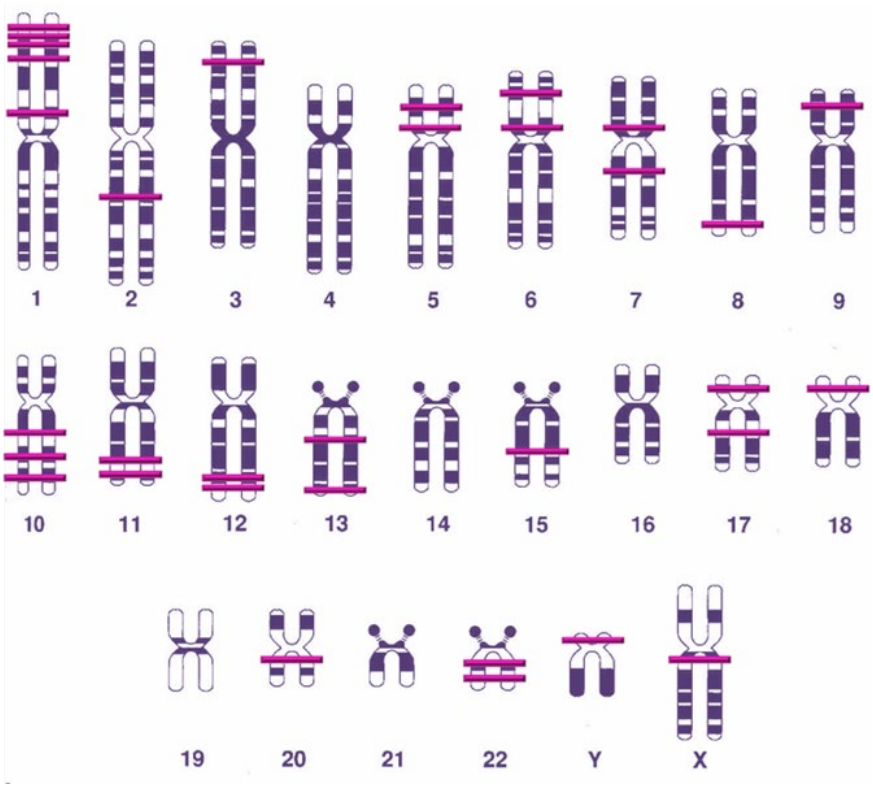


Figure 1. Chromosomic representation of the biomarkers included in genotyping assays and follow-up for chimerisms..

The necessary material for this study is genomic DNA, mainly from peripheral blood. The necessary amount of DNA is 450 ng of the recipient's pre-transplant sample and 450 ng of the donor's sample.

IMG-116-26 Screening Multiplex Plus

Name of biomarker	Chromosomal Position
3I	20q11.22
6I	10q26.2
12D	5p13.2
7I	Xq28
11I	1p13.3
5I	10q21.2
4I	17p13.2
10I	22q13.32
23I	13q34
28I	12q24.23
32I	3p25.3
31I	6p21.2
29D	17q21.31
30D	7q21.3
27D	18p11.22
24I	1p34.1

IMG-116-25 Screening Multiplex Plus II

Name of biomarker	Chromosomal Position
33I	1p36.13
37I	5p15.32
38I	6p12.3
44I	13q14.11
43I	12q24.21
49I	2q21.2
39I	7p12.3
50I	1p36.11
45I	15q21.3
9I	22q11.22
41I	10q23.33
20I	8q24.22
46I	9p23
47I	11q23.2
42I	11q22.3
RhD	1p36.11

Table 1. Chromosomal position of biomarkers. The SRY marker, located in Chr Y, is also available.

The clinical performance of this kit has been validated using genomic DNA from peripheral blood or bone marrow from human samples. The limit of detection has been determined to be 0.01%.

The cumulative informativity of this panel, together with the SRY and RhD markers, is 99.1%. If the **Imegen® Quimera Screening Multiplex Plus II** marker panel is also analyzed, the cumulative informativity is 99.98%.

Health in Code is certified against the norm **UNE-EN ISO 13485:2018 Medical devices: Quality management systems - Requirements for regulatory purposes** by the SPANISH AGENCY OF MEDICINES AND MEDICAL DEVICES for the design, development, and production of medical devices for in vitro diagnostic use:

- Genetic testing kits
- Software for the bioinformatics analysis of genetic data

04

Safety warnings and precautions

1. It is recommended to strictly follow the instructions in this manual, especially regarding the handling and storage conditions of the reagents.

2. Do not mouth-pipette.

3. Do not smoke, eat, drink, or apply cosmetics in areas where kits and samples are handled.

4. Any cuts, abrasions, and other skin injuries must be properly protected.

5. Do not pour the remains of reagents down the drain. It is recommended to use waste containers established by the legal norm and manage their treatment through an authorized waste management facility.

6. In the event of an accidental spill of any of the reagents, avoid contact with the skin, eyes, and mucous membranes and rinse with a large amount of water.

7. Safety data-sheets (MSDS) of all dangerous substances contained in this kit are available on request.

8. This product requires the manipulation of samples and materials of human origin. It is recommended to consider all materials of human origin as potentially infectious and manipulate them according to level 2 of the OSHA norm on biosafety and bloodborne pathogens or other practices related to biosafety of materials that contain or are suspected to contain infectious agents.

9. The reagents included in this kit are not toxic, explosive, infectious, radioactive, magnetic, corrosive, or environmental biological pollutants.

10. This kit has been validated with specific equipment and under specific conditions that may vary widely among laboratories. Therefore, it is recommended that each laboratory conduct an internal validation when the kit is to be used for the first time.

11. The manufacturer assumes no responsibility for any damage or failure of the assay caused by substituting reagents included in the kit for ones not provided by Health in Code.

12. The manufacturer does not guarantee the assay's reproducibility when the user uses reagents that have not been validated by Health in Code but are considered by the user equivalent to those provided in the kit.

05

Content and storage conditions of the kit

This kit contains sufficient reagents to analyze 10 different genomic DNA samples or 5 recipient/donor cases.

The kit consists of one 8-tube strip, each tube containing a Screening Master mix. Each Master is composed of two oligonucleotide pairs and two TaqMan®-MGB probes with different labels (FAM™ or VIC®) for the simultaneous analysis of two different polymorphisms.

Tube	Reactions	Markers	Conservation	Rehydration
1	10 Reactions	Q116-6I Q116-3I	4°C	33 µL of water/vial*
2	10 Reactions	Q116-7I Q116-12D	4°C	33 µL of water/vial*
3	10 Reactions	Q116-11I Q116-5I	4°C	33 µL of water/vial*
4	10 Reactions	Q116-4I Q116-10I	4°C	33 µL of water/vial*
5	10 Reactions	Q116-23I Q116-28I	4°C	33 µL of water/vial*
6	10 Reactions	Q116-32I Q116-31I	4°C	33 µL of water/vial*
7	10 Reactions	Q116-30D Q116-29D	4°C	33 µL of water/vial*
8	10 Reactions	Q116-27D Q116-24I	4°C	33 µL of water/vial*

Table 2. Components of the Imegen® Quimera Screening Multiplex Plus kit and their storage temperatures

* Once rehydrated, the reagents must be stored at -20 °C.

06

Necessary equipment, reagents, and material not included in the kit

Equipment:

- Real-time PCR thermal cycler
- Micropipettes (10 µL, 20 µL, and 200 µL)
- Vortex mixer

Reagents:

- Nuclease-free water
- Master Mix 2X (HotStart DNA polymerase)

Materials:

- Optical 96-well plates or 0.2 ml optical tubes
- Optical film for 96-well plates or optical lids for 0.2 ml tubes
- Filter pipette tips (10 µL, 20 µL, and 200 µL)
- Sterile 1.5 ml tubes
- Powder-free latex gloves

Complementary kits

As a complementary kit, in case **Imegen® Quimera Screening Multiplex Plus** does not identify any informative marker, the use of **Imegen® Quimera Screening Multiplex Plus II** is recommended; this kit offers 16 new markers **(REF: IMG-116-25)**.

Once a polymorphism has been identified as informative, we recommend acquiring the corresponding **Imegen® Quimera dPCR** kit from out catalog to perform patient follow-up and, therefore, analysing the transplanted organ and assessing the risk of relapse. The **Imegen® Quimera** kits allow quantifying the amount of informative marker (chimerism), either absolutely or relative to the total genomic DNA amount using a reference gene (β-globina). The reference gene is analyzed simultaneously with the informative marker in a multiplexed reaction; in addition, it is used as qualitative and quantitative control of the tested DNA sample.

Kit name	Reference
Imegen® Quimera SRY dPCR	IMG-116-27
Imegen® Quimera RhD dPCR	IMG-116-28
Imegen® Quimera Q116-3I dPCR	IMG-116-29
Imegen® Quimera Q116-4I dPCR	IMG-116-30
Imegen® Quimera Q116-5I dPCR	IMG-116-31
Imegen® Quimera Q116-6I dPCR	IMG-116-32
Imegen® Quimera Q116-7I dPCR	IMG-116-33
Imegen® Quimera Q116-11I dPCR	IMG-116-34
Imegen® Quimera Q116-10I dPCR	IMG-116-35
Imegen® Quimera Q116-12D dPCR	IMG-116-36
Imegen® Quimera Q116-23I dPCR	IMG-116-37
Imegen® Quimera Q116-24I dPCR	IMG-116-38
Imegen® Quimera Q116-20I dPCR	IMG-116-40
Imegen® Quimera Q116-27D dPCR	IMG-116-41
Imegen® Quimera Q116-28I dPCR	IMG-116-42
Imegen® Quimera Q116-29D dPCR	IMG-116-43
Imegen® Quimera Q116-30D dPCR	IMG-116-44
Imegen® Quimera Q116-31I dPCR	IMG-116-45
Imegen® Quimera Q116-32I dPCR	IMG-116-46
Imegen® Quimera Q116-33I dPCR	IMG-116-47
Imegen® Quimera Q116-9I dPCR	IMG-116-48
Imegen® Quimera Q116-37I dPCR	IMG-116-49
Imegen® Quimera Q116-38I dPCR	IMG-116-50
Imegen® Quimera Q116-39I dPCR	IMG-116-51
Imegen® Quimera Q116-41I dPCR	IMG-116-52
Imegen® Quimera Q116-42I dPCR	IMG-116-53
Imegen® Quimera Q116-43I dPCR	IMG-116-54
Imegen® Quimera Q116-44I dPCR	IMG-116-55
Imegen® Quimera Q116-45I dPCR	IMG-116-56
Imegen® Quimera Q116-47II dPCR	IMG-116-57
Imegen® Quimera Q116-49I dPCR	IMG-116-58
Imegen® Quimera Q116-50I dPCR	IMG-116-59
Imegen® Quimera Q116-46II dPCR	IMG-116-60

Table 3. Imegen® Quimera dPCR kits for digital PCR follow-up

07
Assay protocol

7.1 Reagents preparation

All the reagents included in this kit are freeze-dried. Before using any of our kits, the first step is to rehydrate the reagents by adding 33 µL of nuclease-free water per vial*. To enable resuspension of each component, it is recommended to shake and spin the tubes containing the reagents and store them at 4 °C for one hour before their use.

**If these reagents are not to be used immediately after rehydration, storage at -20 °C is recommended.*

7.2 Preparation of amplification reactions

The assay must include the following reactions:

- Reactions with the recipient's sample
- Reactions with the donor's sample

The simultaneous analysis of the 16 markers using the **Imegen® Quimera Screening Multiplex Plus** kit requires the preparation of eight different PCR mixes. Each PCR mix must contain:

- Screening Multiplex Master Mix
- PCR Master Mix 2x (not included in the kit)

The following protocol is recommended to prepare the amplification reactions:

1. Thaw the 8-tube strip containing the Screening Masters and the DNA from both the recipient and the donor. Vortex each reagent and keep cold.
2. Add 45 µL of PCR Master Mix 2X and 18 µL of recipient DNA to 25 ng/µL in a 1.5 mL tube.
3. Add 45 µL of PCR Master Mix 2X and 18 µL of donor DNA to 25 ng/µLin a 1.5 mL tube.
4. Vortex and pipette 7 µL of Master Mix with recipient DNA in 8 wells and 7 µL of Master Mix with donor DNA in other 8 wells.
5. Add 3 µL of each Screening Master Mix both to the wells containing recipient DNA and to those containing donor DNA.

7.3 Settings for the real-time PCR program

Depending on the equipment used to perform real-time PCR, the instructions below must be followed to set up the amplification program:

Tube	Markers	Insertion (Allele +)	Deletion (Allele -)	Marking	Quencher
1	Q116-6I	X		FAM™	MGB
	Q116-3I	X		VIC®	
2	Q116-7I	X		FAM™	
	Q116-12D		X	VIC®	
3	Q116-11I	X		FAM™	
	Q116-5I	X		VIC®	
4	Q116-4I	X		FAM™	
	Q116-10I	X		VIC®	
5	Q116-23I	X		FAM™	
	Q116-28I	X		VIC®	
6	Q116-32I	X		FAM™	
	Q116-31I	X		VIC®	
7	Q116-30D		X	FAM™	
	Q116-29D		X	VIC®	
8	Q116-27D		X	FAM™	
	Q116-24I	X		VIC®	

Table 4. Information about the probes included in the Imegen® Quimera Screening Multiplex Plus kit

7500 Fast or StepOne Plus Real-Time PCR system (Thermo Scientific)

- Type of experiment: Quantitation —Standard curve
- Ramp rate: standard
- Reaction volume: 10 µL
- ROX™ baseline reference: included
- Fluorophores of TaqMan® probes:
- Optimal program:

Fields	Phase 1 Enzymatic activation	Phase 2 PCR	
No. of cycles	1 initial cycle	50 cycles	
		Denaturation	Primer binding/extension
Temperature	95°C	95°C	60°C
Time	10 minutes	15 seconds	1 minute*

Table 5. Optimal PCR program for the 7500 FAST or StepOne Plus PCR systems

**Fluorescence detection*

Lightcycler 480 (Roche)

› Optimal program:

Fields	Phase 1 Enzymatic activation	Phase 2 PCR			Phase 3
No. of cycles	1 initial cycle	50 cycles			1 final cycle
		Denaturation	Primer binding	Extension	
Temperature	95°C	95°C	60°C	72°C	40°C
Time	10 minutes	5 seconds	10 seconds	15 seconds*	20 seconds

Table 6. Optimal PCR program for Lightcycler 480

**Fluorescence detection*

› Analysis: Fit points for all samples

08
Analysis of results

The analysis of results is based on the detection of an informative polymorphism, i.e. one detected in the recipient but not in the donor.

The table below shows the possible results:

Reagents	Results		Informativity	
	Recipient	Donor	Bone marrow	Solid organ
Polymorphism	+	+	Non-informative	Non-informative
Polymorphism	+	-	Informative	Non-informative
Polymorphism	-	-	Non-informative	Non-informative
Polymorphism	-	+	Non-informative	Informative

Table 7. Information about the possible results obtained using the Imegen® Quimera Screening Multiplex Plus kit

In the event that no informative markers have been detected, please contact our technical support team (tech.support@healthincode.com).

The figure below is an example of the result of two multiplexed markers. The VIC-labeled marker would be informative in the case of bone marrow transplant, but not in the case of solid organ transplantation, as noted in Table 7.

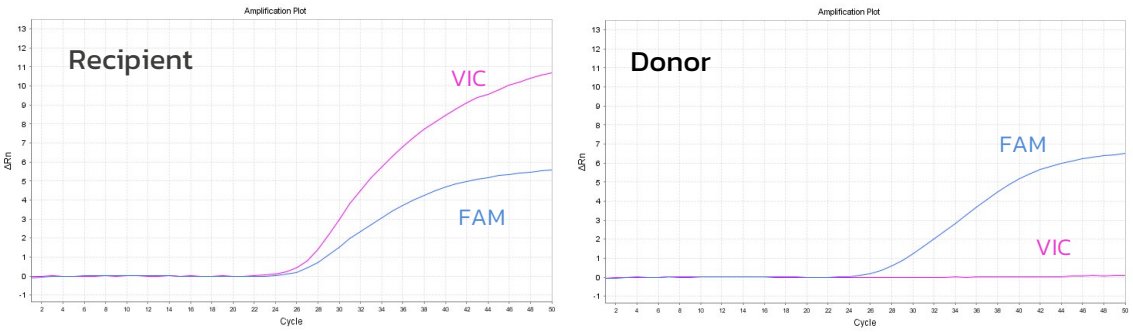


Figure 2. Results obtained using the 7500 FAST Real-time PCR System for recipient and donor samples. Two genetic markers are multiplexed in each PCR reaction. The blue amplification curves represent the amplification signal in the FAM channel, while the pink amplification curves represent the amplification signal in the VIC channel..

Imegen® Quimera Software (Patient follow-up appplication)

Health in Code has designed and developed a user-friendly application that allows creating a patient database, as well as recording screening results for informative polymorphisms, their quantification in the different follow-up samples from a patient, and the medical actions taken in regard to said patient during follow-up. Moreover, the user can plot all the medical actions and the patient's progression and can export the results.

A video tutorial about how to use our **Imegen® Quimera** application is available at the following link: *https://youtu.be/K38cV3hacm8*

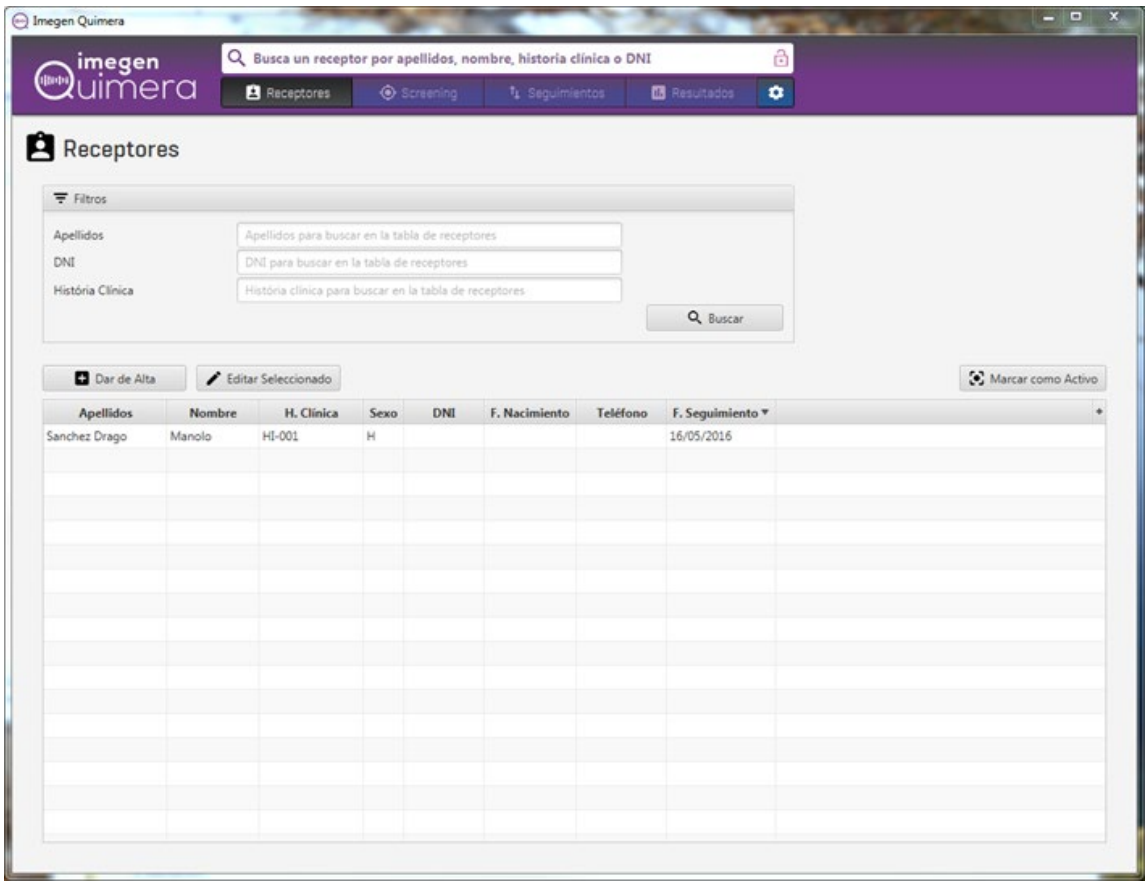


Figure 3. View of the patient follow-up application developed by Health in Code.

09
Troubleshooting

The table below graphically summarizes the possible test results that can be obtained for the different positive and negative controls and from one DNA sample in one run, along with their interpretation and the most likely reasons for each possible result:

Control	C_T Polymorphism	Result	Cause
Tested sample	Detected < 30	+	Expected result
	Detected < 30	+	PCR contamination with human DNA[1] or sample concentration below the value specified in the protocol[2]
	Not detected	-	Expected result
Negative PCR control	Not detected	-	Expected result
	Detected	+	PCR contamination with human DNA[1]

Table 8. Possible results for controls and samples

[1] **PCR contamination by human DNA:** PCR contamination may be due to mishandling of the sample, the use of contaminated reagents, or environmental contamination. Thoroughly clean the laboratory and equipment where the PCR process was performed. If necessary, use fresh aliquots of the reagents used for the PCR and repeat the test.

[2] **Inadequate sample concentration:** late marker amplification may be due to test DNA concentrations lower than the value specified in the protocol. In this case, it is recommended to quantify the sample again by absorbance or fluorescence. If necessary, use fresh aliquots of the reagents used for the PCR and repeat the test.

10
Limitations

10.1. Equipment

Imegen® Quimera Screening Multiplex Plus has been validated for use with the following real-time PCR platforms:

- 7500 FAST Real-Time PCR System (ThermoFisher Scientific)
- StepOne Plus Real-Time PCR System (ThermoFisher Scientific)
- LightCycler 480 (Roche)

If a different brand or model of thermal cycler is used, the amplification program may need to be adjusted. Should you need further information or advice, please contact our technical support service.

10.2. Reagents

Imegen® Quimera Screening Multiplex Plus has been validated using the reagents included in the kit. We recommend using the PCR reagents indicated by the manufacturer of the thermal cycler to be used for real-time PCR assays, as mentioned in section 6 (Necessary equipment, reagents and materials not included in the kit). Should you have any questions, please contact our technical support team.

10.3. Product stability

Optimal performance of this product is achieved provided that the specified recommended storage conditions are applied, within the optimal product expiration date associated with each production batch.