Genvinset®

HLA Behçet v5

INSTRUCTIONS FOR USE

*Kit for the HLA-B*51/52 alleles group detection*

For In Vitro Diagnostic use

Rev.04/2022-10-10

CE 2797

Camino del Pilón 86, Casa 7, Local
50011 – Zaragoza (Spain)

www.bdrdiagnostics.com

Product code:	UDI-DI:	Store
GVS-B505-24 (24 tests)	8437016942659	from -30°C to -18°C
GVS-B505-48 (48 tests)	8437016942666	

Genvinset®

HLA Behçet v5

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1- Information for safety

Please, read completely these instructions for use and follow them during the use of the current IVD kit.

The IVD kit shall be used by experts with strong experience in DNA analysis and interpretation of genetic results.

Consult the manufacturer if there are doubts involving the description of the assay method. Contact by phone +34 976 094 603 or email address *customersupport@bdrdiagnostics.com*.

The IVD kit has a limited shelf life. Make sure that the shelf life is not expired before using the kit. Reagents from a kit beyond its expiry date might be degraded, which could impair results. Discard the expired reagents following applicable regulations.

The contamination of the sample or the reagent can render incorrect results. Be careful during the DNA extraction and sample and reagents manipulation.

This kit can be damaged during transport or storage. Do not use the kit in case of suspected damage during transport. Follow carefully the storage conditions described in the label and the IFU.

Ensure that the waste is managed according to local regulations. Incorrect waste management can result in environmental contamination.

The toxicological properties of the kit have not been studied in-depth, so it is recommended to avoid contact with skin and mucous membranes. Do not ingest. Manual Safety Data Sheets (MSDS) are available for the customer by request.

Ensure that this kit is adequate for the requested analysis by the clinician.

2- Intended Use

Genvinset® HLA Behçet v5 is a semi-automated *in vitro* diagnostic kit for the qualitative detection of the HLA-B*51/52 group of alleles in genomic DNA extracted from whole blood, associated with Behçet's disease predisposition, by Real-Time PCR using TaqMan® probes technology.

Patients who can benefit from this determination are those referred by a specialist. The results of this test should not be the only ones on which the therapeutic decision is based and should be used as an aid in the diagnosis together with results of other markers of the disease.

The intended user of the kit is technical personnel trained to carry out the protocol and the interpretation of results described in the instructions for use.

3- Summary and explanation

Behçet's Disease (BD) is a form of vasculitis that manifests with mouth ulcers, orogenital ulcers, uveitis, skin inflammation, enterocolitis and inflammation in other organs[1,2]. Despite being a worldwide-distributed illness, BD is more frequent in the area that extends from East Asia to the Mediterranean basin (silk road), with the highest prevalence in Turkey[1,3]. It is also relatively common in countries such as Japan and Iran[1,2].

According to various scientific studies, there are several genetic factors that partially determine the susceptibility to develop the illness[1,3,4], More specifically, it is known that BD is

associated with the HLA-B*5 antigen, belonging to the Major Histocompatibility Complex (MHC), encoded by the HLA-B*51/52 group of alleles[4]. Therefore, the HLA-B*5 antigen is a risk factor strongly associated with BD[4,5].

The nature of the clinical manifestations of BD established the question of whether the HLA-B*5 antigen may have a modulating effect on the expression of the disease. Results from different studies suggest that HLA-B*5 positive patients develop more frequently symptoms that affect the central nervous system or organs such as the eye[4,6], whereas HLA-B*5 negative patients more often develop thrombophlebitis[4].

4- Procedure principles

The test is based on real-time PCR technology with TaqMan® probes. Each sample is analysed with:

- A pair of primers for the amplification of HLA-B*51/52 alleles and a pair of primers for β-globin (*HBB*) gene amplification that serves as internal positive control (IPC).
- A hydrolysis probe specific for the HLA-B*51/52 alleles labelled at the 5` end with FAM fluorophore and a hydrolysis probe specific for the IPC (β-globin; *HBB* gene) labelled at the 5' end with HEX fluorophore. Both probes are labelled at the 3´ end with a quencher that inhibits fluorescence emission when the probe is intact.

As the PCR reaction proceeds, the 5'→3'exonuclease activity of Taq polymerase cleaves the probes hybridized to their complementary sequence, separating the fluorophore from the quencher and producing a fluorescent signal (in real-time), which is proportional to the amount of PCR product generated and monitored in a real-time PCR instrument. Thus:

- In the case of samples with one or two copies of the HLA-B*51/52 alleles (HLA-B*51/52 positive), the FAM-labelled probe binds to its complementary DNA sequence at the HLA-B*51/52 alleles, the HEX-labelled probe binds to its complementary DNA sequence at the IPC and the following is observed:
    - Fluorescent signal in the FAM channel (λmax 518 nm) and
    - Fluorescent signal in the HEX channel (λmax 556 nm).
- In the presence of samples with no copies of HLA-B*51/52 alleles (HLA-B*51/52 negative), the HEX-labelled probe binds to its complementary DNA sequence at the IPC and the following is observed:
    - No signal or weak signal in the FAM channel and
    - Fluorescent signal in the HEX channel.

5- Kit contents

➔ GVS-B05-24 (24 tests)

- GVS-B5v5-PM: 1 vial x 192 µL Primer Mix (PM) - Blue cap
- GVS-B5v5-MM: 1 vial x 240 µL Master Mix (MM) - Red cap
- GVS-B5v5-C+: 1 vial x 15 µL Positive Control (C+) - Green cap
- GVS-RB: 1 vial x 100 µL Reaction Blank (RB) - Natural cap

➔ GVS-B505-48 (48 tests)

- GVS-B5v5-PM: 2 vials x 192 µL Primer Mix (PM) - Blue cap
- GVS-B5v5-MM: 2 vials x 240 µL Master Mix (MM) - Red cap

- GVS-B5v5-C+: 1 vial x 15 µL Positive Control (C+) - Green cap
- GVS-RB: 1 vial x 100 µL Reaction Blank (RB) - Natural cap

6- Kit storage

All the components of the kit must be stored between -30°C and -18°C upon receipt. Under these conditions, they are stable up to their expiry date indicated on the label.

Do not perform more than 3 freeze/thaw cycles on the kit vials as this may reduce the sensitivity of the assay and affect the results. If assays are to be performed with few samples, it is recommended to use aliquots of the reagents to reduce freeze/thaw cycles.

Due to the photosensitive nature of the Primer Mix, avoid continuous exposure to light.

7- Materials required but not supplied

General

- Disposable gloves
- Lab coat

Consumables

- Filter tips (P200, P20 and P10)
- 1.5 mL autoclaved tubes
- Compatible consumables for each real-time PCR instrument

Equipment

- Vortex mixer
- Centrifuge
- Micropipettes (P200, P20 and P10)
- Real-time PCR instrument, with FAM and HEX/VIC detection channels. The following devices have been validated:
    - 7500, and QuantStudio 5 Dx Real-Time PCR Systems, Applied Biosystems™.
    - LightCycler® 96 and LightCycler® 480 Systems, Roche.
    - Rotor-Gene® Q, Qiagen®.
    - DT Lite Real-Time PCR System, DNA-Technology.
    - qTOWER³G, Analytik Jena.
    - CFX Opus 96 Real-Time PCR, BioRad.
    - Mic qPCR, Molecular Systems.

8- Sample collection and preparation

Samples must be collected in line with the instructions for use of the collection device (not included with the kit) and under national and international guidelines.

The present test should only be performed with DNA extracted from whole blood samples preserved with anticoagulant agents such as EDTA or citrate. Heparin can interfere with the PCR process and should be avoided.

The technique is compatible with several DNA extraction methods. Before delivering results with a diagnostic purpose, a validation assay with such extraction method should be done.

CAUTION!

All biological and blood samples should be treated as potentially infectious. When manipulating them, take the corresponding basic and universal precautions.

9- Usage procedures

➔ PCR setup

CAUTION!

- Define pre- and post-PCR work areas that should be kept separated to reduce the risk of contamination. Prepare the PCR in the pre-PCR area. Use a lab coat and disposable gloves.
- Work on ice or over a cool block. Minimize the time between preparation and the start of the assay.
- For each assay, it is recommended to test the contamination control (Reaction Blank) and the Positive Control (C+) included in the kit.

1. Thaw all the kit components before starting the assay. Vortex vigorously the Primer Mix vial and carefully mix the Master Mix vial. Centrifuge briefly to collect the volume at the bottom of the tubes.
2. Prepare the reaction mix for n+1 samples, using the quantities indicated in the following table:

	Vol. per sample (µL)
Master Mix	10
Primer Mix	8

   Gently mix and centrifuge to ensure that all volume settles to the bottom of the tube.
3. Pipette 18 µL of this mix into the PCR plate/tubes.
4. Add into each well 2 µL of DNA (recommended concentration between 10 and 200 ng/µL), Reaction Blank, or Positive Control (C+).
5. Seal the plate/tubes using the appropriate sealer and centrifuge briefly to remove any bubbles. Ensure that all the volume settles to the bottom of the well.
6. Place the plate/tubes in the thermal cycler and set up the thermal cycler amplification program as described in the following section.

➔ Thermal cycler configuration

1. Set up the following readout channels:
   - FAM channel for FAM-labelled probe detection.
   - HEX/VIC channel for HEX-labelled probe detection.
2. Set up the appropriate amplification profile*, and start the run:

	Cycles	Temperature (°C)	Time (mm:ss)	Analysis
Denaturation	1	95	05:00	X
Cycles	40	95	00:10	X
		66	**00:30 (*)**	Single
Cooling	1	15	∞	X

(*) For both Rotor-Gene® (Qiagen) and DT-Lite (DNA-Technology) real-time PCR thermal cyclers, please set up the following amplification program:

	Cycles	Temperature (°C)	Time (mm:ss)	Analysis
Denaturation	1	95	05:00	X
Cycles	40	95	00:10	X
		66	**00:25**	Single
Cooling	1	15	∞	X

➔ Disposal

Waste products shall be managed according to local regulations.

10- Results

➔ Results visualization

The analysis of the results is performed with the specific software of the real-time PCR instrument being used, and according to the manufacturer's instructions for use.

Once the run is finished, select the linear scale to visualize the amplification curves. It is recommended to check the correct behaviour of the obtained amplification curves:

- An amplification signal is considered positive if a quick and regular increase of fluorescence values (exponential) is observed (sigmoidal amplification) with Ct<35.
- A weak fluorescent, background or exponential signal with Ct>35 should not be considered a positive amplification. This assay allows the detection of some of the alleles included in the group of highly polymorphic HLA alleles, among which little differences in their sequences have been described. Therefore, weak non-specific signals from other similar in sequence but not-detected HLA alleles can be observed in the FAM channel. The appearance of these signals does not invalidate the assay.

A sample is considered positive if it produces an exponential amplification with Ct<35. A sample is considered negative if it produces a non-exponential amplification with low intensity or an exponential amplification with a Ct value >35.

CAUTION!

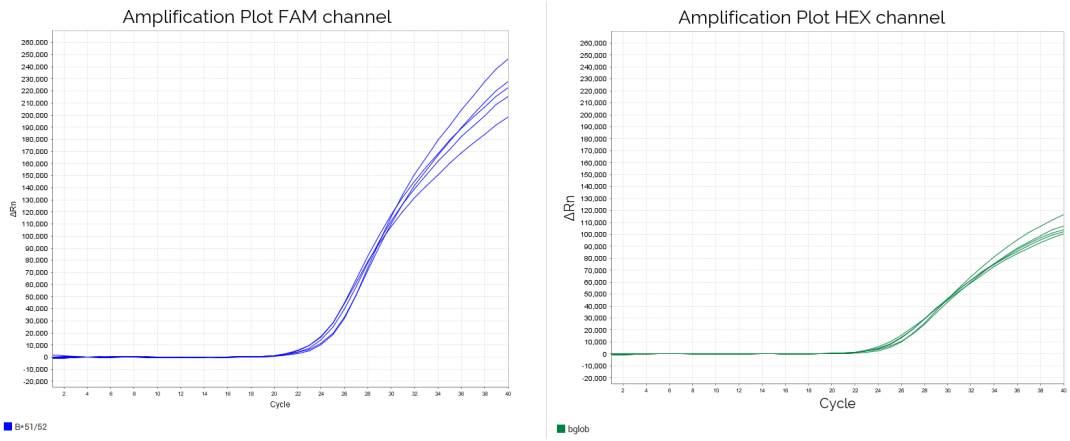
To determine the Ct value in each channel, adjust the threshold line as follows:

Select linear scale view and choose the region where the fluorescence signal is stable before exponential amplification. Place the threshold line above this background signal, so that it crosses close to the inflection point of the amplification curve. This line should slightly exceed the value of the highest fluorescence obtained with negative samples for the allele detected in this channel.

➔ Interpretation of results

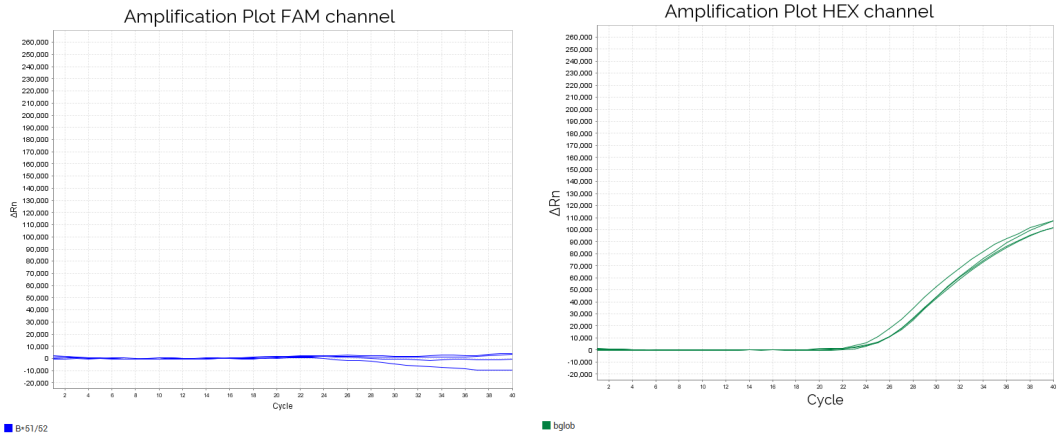
Results obtained with this kit must be interpreted by visualization of the amplification curves in both FAM and HEX channels. Select "linear scale" and determine the absence/presence of sigmoidal amplification in each channel.

HLA-B*51/52 positive samples



Exponential amplification with Ct<35 in both FAM and HEX channel

HLA-B*51/52 negative samples



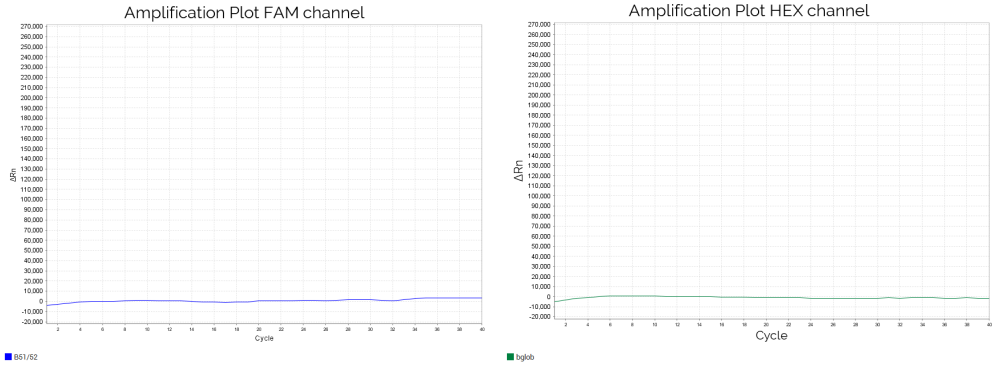
No signal or low intensity signal in FAM channel and exponential amplification in HEX channel (Ct<35)

11- Quality control

This kit includes a Reaction Blank and a Positive Control (C+) that should be included in each assay. An adequate behaviour of the control samples is a guarantee of the proper performance of the kit.

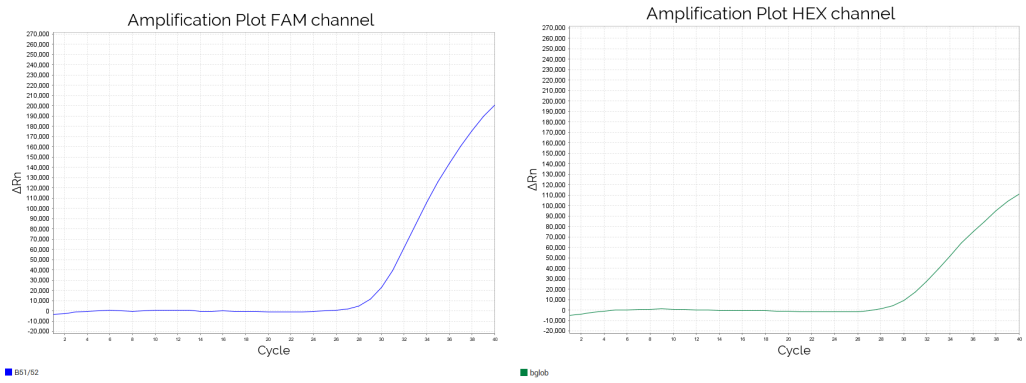
The results are considered valid if the following amplification pattern is obtained:

Reaction Blank



No signal in both FAM and HEX channels or amplification with Ct>35

Positive Control (C+)



Exponential amplification with a Ct<35 value in both FAM and HEX channels.

The IPC is detected in the HEX channel and used as an internal positive control for each analysed sample. Therefore, the result of a sample is considered valid if there is, at least, sigmoidal amplification with Ct<35 in the HEX channel.

If adequate behaviour is observed in the above-mentioned controls, proceed with the interpretation of the samples as indicated in the previous section.

The result is considered invalid and should be repeated if:

- An amplification curve with Ct<35 is observed in FAM and/or HEX channel in the Reaction Blank.
- Non-exponential amplification signal is observed or an amplification signal with Ct>35 appears in the Positive Control.
- DNA samples with amplification curves with Ct>35 in FAM and/or HEX channels must be considered doubtful and should be retested performing a new DNA extraction.

12- Specific operation data

➔ Analytical specificity

Cross-reactivity has been assessed in three independent Genvinset® HLA Behçet v5 kit validation studies, as stated in the "Diagnostic sensitivity and specificity" section.

Due to the highly polymorphic nature of the HLA system, the *in silico* alignment of primers and probes in the most common HLA database (IMGT-HLA) gave a list of detected, no

detected and no tested with possible low-intensity signal alleles that can be found at *www.bdrdiagnostics.com*. It would be possible that intronic regions of some non-frequent alleles have not yet been sequenced, so the *in silico* alignment of the kit primers and probes in those areas is unknown. No cross-reaction phenomena with other DNA regions have been reported.

Interference has been studied by means of a bibliographic search. Heparin can inhibit Taq polymerase activity and compete with target nucleic acid, so the collected blood must be treated with other anticoagulants, as stated in the "Sample collection and preparation" section. Some substances found in blood are known as PCR inhibitors: haemoglobin, hemin, bilirubin, bile salts, lactoferrin, and immunoglobulin G. The polymerase included in the Genvinset® HLA Behçet v5 kit has demonstrated high resistance to inhibition, and Master Mix composition is designed to deal with interference substances. Nevertheless, the presence of potentially inhibitory substances must be eliminated during DNA extraction and purification protocols. Before delivering results with a diagnostic purpose, a validation assay with such extraction method should be done.

➔ Analytical sensitivity

LoD: A dilution assay was performed using two DNA samples (HLA-B*51/52 positive and HLA-B*51/52 negative). Input DNA levels tested ranged from 40 ng to 0.016 ng (**) in a three-fold dilution series. Each level was tested in triplicate. The following data were obtained:

- Detection Limit = 0.49 ng (Ct<35)

Upper limit: 2 samples with different genotypes (HLA-B*51/52 positive and negative) were assayed in a two-fold dilution series ranging from 800 ng to 6.25 ng (**). Assay performance remained acceptable at all input levels: suitable sigmoidal amplification curves and genotyping calls were accurately made at all levels (with Ct values<35).

(**) DNA concentration was measured using Nanodrop 1000 Spectrophotometer (Thermo Scientific).

➔ Diagnostic sensitivity and specificity

153 different samples were analysed in different studies at different laboratories. These samples were previously typed by a genotyping methodology different from Genvinset®. The following results were obtained:

		Genvinset® HLA Behçet v5 kit	
	HLA-B*51/52	HLA-B*51/52 Positive	HLA-B*51/52 Negative
Previous method	Positive	45	0
	Negative	0	108

There is a 100% match in the results obtained with the Genvinset® HLA Behçet v5 kit and the genotypes previously obtained by a different typing method.

➔ Accuracy

Repeatability studies consists in the measurement of within-run variability, through the analysis of replicas of every kind of sample that can be measured by the kit (HLA-B*51/52 positive and negative samples). Each sample was analysed in duplicate.

Genvinset® HLA Behçet v5 kit showed 100% repeatability.

A study to determine the reproducibility of the reagent was performed. It allows to estimate the variability between runs, lots, real-time thermal cyclers and operators. Samples used represent the full range of expected analyte that can be measured with Genvinset® HLA Behçet v5 kit, that is, HLA-B*51/52 positive and negative samples. Three different lots in three real-time thermal cyclers were assayed by different operators.

Genvinset® HLA Behçet v5 kit showed 100% reproducibility.

➔ Trueness

The trueness of the analytical procedure of Genvinset® HLA Behçet v5 kit is assessed by comparison with a reference method. The study was developed as an internal validation of the reagent, in which trueness has been demonstrated with a 100% value. See the "Diagnostic Sensitivity and Specificity" section.

13- Procedure limitations

- The kit detects the HLA B*51/52 alleles included in the "HLA alleles detected_GVS-B5v5" at *www.bdrdiagnostics.com*. Due to the highly polymorphic nature of the HLA alleles, weak signals from other alleles similar in sequence could appear.
- Mutations or polymorphisms at the annealing primer/probe sites may result in the lack of allele definition. Other technologies could be necessary to resolve the typing.
- All the recommendations mentioned in this document should be carefully followed. Any performances that do not meet such indications, can lead to poor results.
- Do not use the kit if there are any suspicions of possible loss of reactivity, contamination, external box deterioration or any other incidence that might affect the kit's performance.
- All Genvinset® reagent manipulations must be done according to good laboratory practice, being adapted to local regulations.
- Do not mix components from other kits or lot numbers.
- Do not use the kit after its expiration date. Discard expired reagents according to applicable regulations.
- The real-time PCR thermal cycler must be calibrated according to the manufacturer´s recommendations and should be used accordingly to the manufacturer's instructions.
- Due to the complexity of HLA typing, data and result interpretation must be revised by qualified personnel.
- This serves as an auxiliary tool for the diagnosis of patients with suspected HLA-B*51/52-associated disorders. Use these results in conjunction with clinical data and results of other tests performed on the patient.

14- Troubleshooting guide

➔ No amplification signal is detected in any sample (neither in positive controls) or the intensity is very low

- The real-time PCR instrument is not correctly programmed. The thermal profile is not correct/the reading channels are not correctly configured/the selected fluorophores are not suitable.
    - Check that the instrument has been programmed correctly.
- The positions of the samples and controls indicated during the preparation of the assay do not match the positions in which they have been placed in the device.
    - Correctly assign the position of the samples.

- The reagent does not work properly.
    - Ensure that the kit is stored at an appropriate temperature (between -30°C and -18°C) and protected from light. Avoid unnecessary freeze/thaw cycles. Do not use it beyond the expiry date.
- The indicated amounts of each of the reagents have not been added to the reaction mix.
    - Check the volume of each component added to the mix.
- The consumables used are not compatible with the equipment being used.
    - Make sure that the right consumables have been used (compatible with the PCR instrument used).

➔ No signal is detected in clinical samples (signal appears in positive control)

- Poor quality of the DNA used.
    - Check the Absorbance ratio 260/280 and discard poor-quality samples. Avoid the presence of inhibitors (heparin, haemoglobin, hemin, bilirubin, bile salts, lactoferrin, immunoglobulin G). Repeat sampling and DNA extraction.
- Inadequate DNA concentration.
    - Adjust the DNA concentration to the recommended concentration range.
    - Check that the DNA concentration is above the limit of detection.
- Inhibition of amplification.
    - Collect whole blood in EDTA or citrate tubes.
- No sample was added.
    - Repeat the assay making sure that samples have been added.

➔ Signal detected in the negative control

- Pipetting error.
    - Change the pipette tip each time DNA is added to a well. Check that the sample added to the well corresponds to what is written on the worksheet.
- Contamination of the Primer Mix/Master Mix/Reaction Blank vial.
    - Repeat the assay with fresh aliquots.
- The PCR preparation area is contaminated.
    - Clean surfaces, instruments, lab coats, and change consumables and reagents. Repeat the assay.

➔ Fluorescence intensity varies between samples or abnormal amplification curves

- Dirtiness outside the reaction tube interferes with the detection of fluorescence.
    - Clean the equipment thoroughly. Check that the outside of the tubes/plate is clean. Handle the plate/tube with gloves.
- The volume is not at the bottom of the well or there are bubbles.
    - Centrifuge the tubes/plate before placing them in the thermal cycler.
    - Check if there are any bubbles. If so, perform a brief spin to remove them.
- The plate/tubes have not been closed properly.
    - Repeat the assay checking that the tubes/plates have been sealed correctly.
- DNAs with different concentrations have been used or the sample used contains a reaction inhibitor.
    - Repeat the sampling and DNA extraction.
- Presence of polymorphisms or mutations at the probe/primer binding sites.

- Contact our Technical Support department through *customersupport@bdrdiagnostics.com*

15- References

1) Nair, J. R. & Moots, R. J. Behcet's disease. *Clinical Medicine* **17**, 71 (2017).
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3) Yurdakul, S. Epidemiology of Behçet Syndrome and regional differences in disease expression. *Behçet Syndrome* 21–35 (2019) doi:10.1007/978-3-030-24131-5_3.
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6) Shenavandeh, S., Jahanshahi, K. A., Aflaki, E. & Tavassoli, A. Frequency of HLA-B5, HLA-B51 and HLA-B27 in patients with idiopathic uveitis and Behçet's disease: a case-control study. *Reumatologia/Rheumatology* **56**, 67–72 (2018).

16- Notice to purchaser

- This product has been developed for *in vitro* diagnostic purposes.
- When the kit is used within the territory of any Member State of the European Union, should there be any serious incident regarding the use of the kit, the user must report it to the manufacturer (regulatory@bdrdiagnostics.com) and the Competent Authority of their country and/or the patient´s country. Serious incident reporting for all other countries must be conducted according to local requirements for each country.
- The Summary of Safety and Performance (SSP) is uploaded into the EUDAMED database and it is accessible to the users of the kit (https://ec.europa.eu/tools/eudamed/#/screen/home).
- Blackhills Diagnostic Resources, S.L.U. products should not be resold, modified for reselling, or used to manufacture other commercial products without the written consent of Blackhills Diagnostic Resources, S.L.U.
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- TaqMan® is a registered trademark of Roche Molecular Systems, Inc.
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17- Changes control

Version	Description of the modification
Rev. 00	First version of the document.
Rev. 01	Expression and ortographic corrections
Rev. 02	Updating of validated qPCR thermal-cyclers
Rev. 03	Addition of "CE" logo and "For in vitro diagnostic use" mention.
Rev. 04	Thermal cycling program modification. Change in Positive Control, Primer Mix and Master Mix volumes indicated in labels. Change in composition of Primer and Master mixes. Change in the list of validated real-time PCR thermal-cyclers. Update of the samples analyzed in the validation of the kit. New sections: *Accuracy* and *Trueness*. Information regarding the intended user, intended patient and interferences has been added. Correction of typos and translation mistakes. Inclusion of information about the SSP in the EUDAMED database and its accessiblility to all users. Insertion of UDI-DI codes

18- Explanation of symbols used on the labels

IVD	*In vitro* diagnostic medical device		Expiration date
REF	Catalogue number	Σ	Contents sufficient for <n> tests
LOT	Lot number		Manufacturer
	Temperature limit		Keep away from sunlight
CONTROL +	Positive control		Consult electronic Instructions for Use document
CE	This product fulfils the requirements of Directive 98/79/EC on *in vitro* diagnostic medical device		