VIASURE
by certest

Real Time PCR Detection Kit

HSV-1, HSV-2 & Treponema pallidum Assay for BD MAX™ System

Instructions for use

CE 2797 IVD

These instructions for use apply to the following references:

PRODUCT	REFERENCE
VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System	444222

Table A 1. Reference for product to be used with the BD MAX™ System.

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Note: The user should notify the manufacturer and the competent authority of the Member State in which he is established as a user and/or patient of any serious incident related to the product.

Content

1. Intended purpose 6
2. Summary and Explanation 6
3. Principle of the procedure 7
4. Reagents provided 8
5. Reagents and equipment to be supplied by the user 8
6. Transport, storage and use conditions 9
7. Precautions for users 9
8. Test procedure 11
8.1. Sample collection, transport and storage 11
8.2. Sample preparation and DNA extraction 12
8.3. PCR protocol 13
8.3.1. Creating PCR test program for VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System 13
8.3.2. BD MAX™ Rack set up 16
8.3.3. BD MAX™ Instrument set up 17
8.3.4. BD MAX™ results report 18
9. Result interpretation 18
10. Limitations of the test 20
11. Quality control 22
12. Analytical performance characteristics 22
12.1. Analytical linearity 22
12.2. Analytical sensitivity 23
12.2.1. Limit of Blank (LoB) 23
12.2.2. Limit of Detection (LoD) 24
12.3. Measuring range 24
12.4. Accuracy 24
12.4.1. Trueness (Veracity) 24
12.4.2. Precision 26

12.4.3. Accuracy 28
12.5. Analytical specificity and reactivity 29
12.5.1. Analytical Specificity 29
12.5.2. Analytical reactivity 34
12.6. Metrological traceability 36
13. Clinical performance characteristics 36
Bibliography 37
Symbols for IVD components and reagents 37
Trademarks 38

ENGLISH

1. Intended purpose

The VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System is an automated qPCR test designed for the simultaneous qualitative detection of DNA from Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), and *Treponema pallidum* (Syphilis) in anogenital and oral skin lesion swab specimens from patients suspected of HSV-1, HSV-2 and *T. pallidum* infection by their healthcare professional (HCP). This test is intended to be used as an aid in the diagnosis of infection with the above-mentioned microorganism in combination with patient's clinical signs and symptoms and/or epidemiological risk factors. Positive results are indicative of the nucleic acid (NA) target's presence but do not preclude the presence of other pathogens not detected by the test. Negative results do not preclude the presence of the NA targets and should not be used as the sole basis for treatment, or other patient management decisions. The assay uses the BD MAX™ System for automated extraction of DNA and subsequent qPCR employing the reagents provided combined with universal reagents and disposables for the BD MAX™ System. DNA is extracted from samples, amplified using qPCR and detected using specific primers and fluorescent reporter dye probes for HSV-1, HSV-2 and *T. pallidum*.

The product is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures (including training on the Real Time PCR instrument (thermocycler) and nucleic acid extraction system).

2. Summary and Explanation

Human Herpes Simplex Virus (HSV) types 1 and 2 are two of the most prevalent human viruses worldwide, which are commonly associated to diseases such as cold sores, genital herpes, herpes stromal keratitis, meningitis, and encephalitis (Zhu & Viejo-Borbolla, 2021). HSV primarily affects the skin and mucus membranes, causing persistent infections consisting of periods of quiescence (latency) and recurrent disease (reactivation) (Cole, 2020; Minaya et al., 2017). Transmission of both HSV-1 and HSV-2 occurs through oral contact, while HSV-2 infections occur later, normally through sexual transmission, and the clinical manifestations of these viruses are highly variable: asymptomatic, mild, or life-threatening (Cole, 2020; WHO, 2023; Zhu & Viejo-Borbolla, 2021). In most immunocompetent individuals, HSV causes mild and self-resolving disease (Zhu & Viejo-Borbolla, 2021). However, HSV infection is also associated with high morbidity and mortality in certain individuals, like immunocompromised patients who suffer recurrent HSV infection (Cole, 2020; Zhu & Viejo-Borbolla, 2021). It has been noted that an infection with one type of HSV typically generates immunity that prevents reinfection with the same serotype, but not with the other (Zhu & Viejo-Borbolla, 2021).

The spirochete *Treponema pallidum* is recognized as the causative agent of venereal syphilis, yaws, endemic syphilis, and pinta, all of which are multistage infections that can only be distinguished based on clinical, epidemiological, and geographic criteria (Mercuri et al., 2022; Peeling et al., 2023; Radolf et al., 2016; Tudor et al., 2022). *T. p. pertenue* is associated with yaws, *T. carateum* with pinta, and *T. p. endemicum* with endemic syphilis, but *T. pallidum* subspecies *pallidum* is the only subspecies linked to venereal syphilis (Mercuri et al., 2022; Tudor et al., 2022).

Syphilis progresses through multiple stages, each presenting distinct signs and symptoms, such as chancres in primary syphilis and rashes or ulcers in secondary syphilis (CDC, 2023; Mercuri et al., 2022; Peeling et al., 2023). In fact, due to the variety of clinical manifestations, syphilis is nicknamed as “the great imitator” (Peeling et al., 2023; Tudor et al., 2022). This disease has affected various at-risk populations throughout history, and although it was nearly eradicated in 1998, its incidence has been rising since 2000 (Mercuri et al., 2022).

Testing to differentiate HSV-1 from HSV-2 is always advised, as it influences both prognosis and management. The CDC recommends confirming the clinical diagnosis with type-specific virology and serology tests (Cole, 2020). A reliable laboratory method for diagnosing acute genital HSV infections is the detection of HSV-1 and HSV-2 DNA through polymerase chain reaction (PCR) (Cole, 2020). Relating to *T. pallidum*, direct detection and serologic testing have been used for laboratory diagnosis since this pathogen cannot be cultured (Forrestel et al., 2020). However, serologic tests are unable to distinguish syphilis from other treponemal infections due to the high genetic similarity among pathogenic treponemes (Forrestel et al., 2020). Nucleic acid amplification techniques, such as PCR, enable the detection of bacterial DNA in clinical samples during the early stages of the disease, offering a potential strategy for controlling the syphilis epidemic due to their high sensitivity (Peeling et al., 2023).

3. Principle of the procedure

VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System is designed for the simultaneous qualitative detection of DNA from Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), and *Treponema pallidum* (Syphilis) in anogenital and oral skin lesion swab specimens. After DNA isolation, the identification of HSV-1, HSV-2 and *T. pallidum* is performed by the amplification of a conserved region of the *glycoprotein L UL1* gene of HSV-1, *glycoprotein G US4* gene of HSV-2 and *16S rRNA* gene of *T. pallidum*, using specific primers and fluorescent-labelled probes.

VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System is based on the 5’exonuclease activity of DNA polymerase. During DNA amplification, this enzyme cleaves the probe bound to the complementary DNA sequence, separating the quencher dye from the reporter. This reaction generates an increase in the fluorescent signal which is proportional to the quantity of target template. This fluorescence is measured on the BD MAX™ System.

VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System contains in each tube all the necessary components for real-time PCR assay (specific primers/probes, dNTPs, buffer, polymerase) in a stabilized[1] format, as well as an endogenous internal control (EIC) (*human RNAse P* gene) to follow-up the integrity of the sample, to monitor the extraction process and/or discard the inhibition of the polymerase activity. Human housekeeping genes are involved in basic cell maintenance and, therefore, are expected to be present in all nucleated human cells and maintain relatively constant expression levels.

Target	Channel	Gene
HSV-1	475/520	*Glycoprotein L UL1* gene
HSV-2	530/565	*Glycoprotein G US4* gene
T. pallidum	630/665	*16S rRNA* gene
Endogenous Internal control (EIC)	585/630	*RNAse P*

Table 1. Target, channel and genes.

4. Reagents provided

VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System includes the following materials and reagents detailed in Table 2:

Reagent/Material	Description	Concentration Range	Code	Amount
HSV-1, HSV-2 & Treponema pallidum reaction tube	Lyoprotectors and Stabilizers	±6 g/100 mL*	1E foil	2 pouches of 12 transparent tubes
	Nucleotide triphosphate (dNTPs)	±1 mM*		
	Primers and Probes	0.2-1 nMol/µL*		
	Enzymes	10-100 U/rxn*		
Rehydration Buffer tube	Saline Solution Mixture	±13 mM	11 foil	1 pouch of 24 transparent tubes
	Buffer (TRIS, pH)	±67 mM		

Table 2. Reagents and materials provided in VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System with Cat. N°. 444222.
* For component in stabilized format, the concentration range means after rehydration.

5. Reagents and equipment to be supplied by the user

The following list includes the materials that are required for use but not included in the VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System.

- Real-time PCR instrument: BD MAX™ System (Ref: 441916).
- BD MAX™ ExK™ TNA-3 (Ref:442827 or 442828).
- BD MAX™ PCR Cartridges (Ref: 437519).
- Vortex.
- Micropipettes (accurate between 2 and 1000 µL).

[1] Please note that both "stabilized", and "lyophilized" terms are used indistinguishable and as synonyms throughout the entire document.

- Nuclease-free water.
- Filter tips.
- Powder-free disposable gloves.

Optional:

- External control materials can be run as part of the quality control procedure of the assay performance. Commercially available control material and/or samples previously characterized as positive or negative can be used as external positive control (EPC) or external negative control (ENC), respectively. The selection and validation of the EPC and ENC must be done according to applicable local, state, and/or federal regulations and the laboratory's standard Quality Control procedures. Additionally, when using commercially available control material the user must follow the respective instructions for use.

6. Transport, storage and use conditions

- The kits can be shipped and stored at 2-30°C until the expiration date which is stated on the kit label.
- Avoid vibrations during transport to prevent liquid leakage.
- After opening the aluminum pouches which contain the reaction tubes, the product can be used up to 28 days at 2-30°C. Keep the vial away from light.

The following table summarises the conditions for transport, storage and use of the overall kit and each component:

Component	Transport Conditions	Storage Conditions	In-use conditions
Entire VIASURE *HSV-1, HSV-2 & Treponema pallidum Assay* for BD MAX™ System	2-30°C during the shelf life stated in the kit label.	**Before use:** 2-30°C during the shelf life stated in the kit label.	** See in-use conditions of each component.*
HSV-1, HSV-2 & Treponema pallidum reaction tube (1E foil)		**Before use:** 2-30°C during the shelf life stated in the kit label. **Once pouch is opened with the silica gel**: 2-30°C for up to 28 days.	Room temperature.
Rehydration Buffer tube		**Before use:** 2-30°C during the shelf life stated in the kit label. **Once pouch is opened with the silica gel**: 2-30°C for up to 28 days.	Room temperature.

Table 3. Summary of the conditions for transport, storage and use of the VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System and each component.

7. Precautions for users

- The product is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- For *in vitro* diagnostic use.
- Instructions for use of the VIASURE product and the BD MAX™ System User's Manual must be read carefully before using the VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System. Do not perform the assay until the information about procedures, safety precautions and limitations described therein have been understood.

- Do not use expired reagents and/or materials.
- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective box is open or broken upon arrival.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Do not use reagents if desiccant is not present or broken inside reagent pouches.
- Do not remove desiccant from reagent pouches.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Close protective pouches of reagents promptly with the zip seal after each use to protect the master mix from sunlight. Remove any excess air in the pouches prior to sealing.
- Protect reagents against humidity. Prolonged exposure to humidity may affect product performance.
- To avoid label deterioration, do not use the product near solvents.
- An appearance of the reaction mixture in stabilized format, normally found at the bottom of the tube, different from the usual one (without conical shape, inhomogeneous, smaller/larger in size and/or color different from whitish) does not alter the functionality of the test.
- Make sure reaction tube and rehydration buffer tube are snapped into place securely during the BD MAX™ rack set up.
- In cases where other PCR tests are conducted in the same general area of the laboratory, care must be taken to ensure that the VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System, BD MAX™ ExK™ TNA-3 extraction kit, or any additional reagents required for testing and the BD MAX™ System are not contaminated. Always avoid microbial and ribonuclease (RNase)/deoxyribonuclease (DNase) contamination of reagents. The use of sterile RNase/DNase-free disposable aerosol resistant or positive displacement pipette tips is recommended. Use a new tip for each specimen. Gloves must be changed before manipulating reagents and cartridges (BD MAX™ PCR Cartridge).
- To avoid contamination of the environment by amplicons, do not break apart the BD MAX™ PCR Cartridge after use. The seals of the BD MAX™ PCR Cartridge are designed to prevent contamination.
- Design a unidirectional workflow. It should begin in the Extraction Area and then move to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area in which the previous step was performed.
- Follow Good Laboratory Practices. Wear protective clothing, use disposable gloves, goggles, and mask. Do not eat, drink, smoke or apply cosmetic products in the working area. Wash your hands after finishing the test.
- Samples must be treated as potentially infectious and/or biohazardous, as well as all the reagents and materials that have been exposed to the samples and they must be handled according to the national safety regulations. Take necessary precautions during the collection, transport, storage, handling, and disposal of samples.

- Samples and reagents must be handled in a biological safety cabinet. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples. Dispose of waste in compliance with local and state regulations.
- Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces.
- In accordance with Regulation (EC) No 1907/2006 (REACH), VIASURE Assays for BD MAX™ System do not require Material Safety Data Sheets on account of their classification as non-hazardous to health and the environment, because they do not contain substances and/or mixtures which meet the hazard classification criteria available in Regulation (EC) No 1272/2008 (CLP), or which are in concentrations higher than the value established in the mentioned regulation for their declaration. A statement declaring no requirement of Material Safety Data Sheet could be requested to Certest Biotec S.L.
- Make sure that the definition of the PCR test program on the BD MAX™ System is done following the instructions in the section 'PCR protocol' (Sample extraction parameters, custom barcodes, PCR settings, etc.).
- Consult the BD MAX™ System User's Manual for additional warnings, precautions and procedures.
- The certificate of analysis is not included with the device; however, it could be downloaded from Certest Biotec S.L. website (www.certest.es) in case of need.

8. Test procedure

8.1. Sample collection, transport and storage

The VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System has been tested in anogenital and oral skin lesion swab specimens collected using Copan ESwab® (Copan's Liquid Amies Elution Swab) (hereinafter natural skin lesion samples). Other types of samples must be validated by the user.

Collection, storage, and transport of specimens should be maintained per the conditions validated by the user. Overall, clinical samples should be collected and labelled appropriately in clean containers with or without transport media (depending on sample type). After collection, specimens should be placed in a biohazard bag and should be transported and processed as soon as possible to guarantee the quality of the test. The specimens should be transported at Room Temperature (RT) or at 2±2°C for up to 48 hours, following the local and national regulations for the transport of pathogen material. For long term transport, we recommend shipping at -20°C or lower[2]. Specimens submitted for molecular testing must be stored in controlled conditions so that nucleic acids do not degrade during storage. It is recommended to use fresh

[2] IDSA guideline (Miller, J. M., Binnicker, M. J., Campbell, S., ... & Pritt, B. S. (2018). A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. *Clinical Infectious Diseases*, 67(6), e1-e94))

specimens for the test, but in the event that this is not possible or in the case of a retrospective study, the samples should be stored preferentially at -70 or -80 °C and, as a second option, at -20 °C[3].

The clinical specimens must be collected, transported and stored according to appropriate laboratory guidelines and/or laboratory policy manuals. As examples, refer to IDSA guideline (Miller, J. M., Binnicker, M. J., Campbell, S., ... & Pritt, B. S. (2018). A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. *Clinical Infectious Diseases*, 67(6), e1-e94) or Sánchez-Romero, M. I., García-Lechuz Moya, J. M., González López, J. J. & Orta Mira, N. Collection, transport and general processing of clinical specimens in Microbiology laboratory. Enfermedades Infecc. y Microbiol. Clin. (English ed.) 37, 127–134 (2019).

Please note: Specimen collection, transport, and storage conditions indicated above are suggested based on the recommendations for genitourinary tract samples intended to be used for nucleic acid detection, as appear in the referenced guides previously mentioned. Nevertheless, we recommend following laboratory guidelines, and/or laboratory policy manual for proper transport and preservation of samples.

An internal specimen stability study was conducted with VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System using natural skin lesion negative matrix spiked with Human herpesvirus 1, Strain KOS (ATCC® VR-1493 Ref. FR-310 (IRR)), Herpes Simplex Virus Type 2, Strain MS (Ref. 0810006CF, Zeptometrix) and *Treponema pallidum*, strain SS14 (Washington University) at 3xLoD concentration. The stability was analysed by means of three different assays: nested primary specimen stability with stability in the SBT at 2±2°C and 25±2°C, primary specimen stability at -20±2°C and freeze (-80°C) - thaw cycles (25°C). Results showed a good performance of samples stored at all conditions tested. In conclusion, regarding primary specimen stability, natural skin lesion samples are stable after 48 hours at 2±2°C and 25±2°C. Regarding stability in the SBT, samples are stable after 7 days in the SBT at 2±2°C and 25±2°C (having spent up to 48h at 2±2°C and 25±2°C before addition to SBT). Besides, samples are stable after 6 months of storage at -20±2°C and for at least 5 freeze-thaw cycles.

8.2. Sample preparation and DNA extraction

Perform the sample preparation according to the recommendations in the instructions for use of the extraction kit used, BD MAX™ ExK™ TNA-3.

1. Pipette 500 µL of sample into a BD MAX™ ExK™ TNA-3 Sample Buffer Tube and close the tube with a septum cap. Ensure complete mixing by vortexing the sample at high speed for 1 minute. Proceed to BD MAX™ System Operation.

[3] Sánchez-Romero, M. I., García-Lechuz Moya, J. M., González López, J. J. & Orta Mira, N. Collection, transport and general processing of clinical specimens in Microbiology laboratory. Enfermedades Infecc. y Microbiol. Clin. (English ed.) 37, 127–134 (2019).

Note: Ensure that vortexing is performed a few minutes before starting the run. If the same BD MAX™ ExK™ TNA-3 Sample Buffer Tube is used for retesting, it is recommended to manually shake the tube a few minutes before starting the test to ensure proper homogenization of the sample.

Note that application-specific extraction preparation procedures should be developed and validated by the user and that some other samples may require pre-processing.

8.3. PCR protocol

Note: Please, refer to the BD MAX™ System User's Manual for detailed instructions.

8.3.1. Creating PCR test program for VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System

Note: If you have already created the test for the VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System, you can skip step 8.3.1 and go directly to 8.3.2.

1) On the "Run" screen of the BD MAX™ System, select the "Test Editor" tab.
2) Click the "Create" button.

In the "Basic Information" tab:

3) Within the "Test Name" field, name your test: i.e. VIASURE HHT.

   Note: The test name must be unique and must have a maximum of twenty characters.

4) In the "Extraction Type" drop down menu, select "ExK TNA-3".
5) In the "Master Mix Format" drop down menu, choose "Type 5: Concentrated Lyophilized MM with Rehydration Buffer".
6) In the "Sample Extraction Parameters" field select "User Defined" and adjust the following parameters values (table 4).

Sample Extraction Parameters	Value (units)
Lysis Heat Time	15 (min)
Lysis Temperature	55 (°C)
Sample Tip Height	1600 (steps)
Sample Volume	475 (µL)
Wash Volume	500 (µL)
Neutralization Volume	N/A
DNase Heat Time	N/A

Table 4. Parameters of sample extraction performed with BD MAX™ ExK™ TNA-3.

7) In the "Ct Calculation" field select "Call Ct at Threshold Crossing" (selected by default).

8) If running software version 5.00 or higher and have barcoded foil snap-in tubes, in the "Custom Barcodes" field select the following configuration:

   a. Snap-In 2 Barcode: 1E (concerning *HSV-1, HSV-2 & Treponema pallidum* reaction tube).

   b. Snap-In 3 Barcode: 11 (concerning Rehydration Buffer tube).

In the "PCR Settings" tab:

9) In "PCR Settings" field enter the following parameters described in Table 5: "Alias" (up to seven alphanumeric characters), "PCR Gain", "Threshold", "Ct Min" and "Ct Max".

Channel	Alias	PCR Gain	Threshold	Ct Min	Ct Max
475/520 (FAM)	HSV1	40	200	0	40
530/565 (HEX)	HSV2	60	200	0	40
585/630 (ROX)	EIC	40	200	0	40
630/665 (Cy5)	TRE	60	200	0	40
680/715 (Cy5.5)	-	0	0	0	0

Table 5. PCR settings.

Note: It is recommended to set the minimum threshold values listed above for each channel as a starting point, but the final settings must be determined by the end-user during the result interpretation, in order to ensure that thresholds fall within the exponential phase of the fluorescence curves and above any background signal. The threshold value for different instruments may vary due to different signal intensities.

10) In the "Color compensation" field enter the following parameters (Table 6).

		False Receiving Channel				
	Channel	475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520	-	4	0	0	0
	530/565	0	-	0	0	0
	585/630	0	0	-	4	0
	630/665	0	0	0	-	0
	680/715	0	0	0	0	-

Table 6. "Color compensation" parameters.

In the "Melt Settings" tab no action is needed, it is not applicable to this product.

In the "Test Steps" tab:

11) Enter the step name (up to twenty characters) and set the following parameters to define each step of the PCR protocol: "Profile Type", "Cycles", "Time" and "Temperature" and select the "Detect" field to define the detection step (Table 7). Click the "Add" button to add a new step and repeat until all the steps needed are defined.

    Note: The "Type" field must be empty.

Step	Step name	Profile Type	Cycles	Time (s)	Temperature	Detect
Initial denaturation	IN-denaturation	Hold	1	120	98°C	-
Denaturation and Annealing/Extension (Data collection)	Annealing/Extension	2-Temperature	45	10	95°C	-
				41	63°C	✓

Table 7. PCR protocol.

In the "Result Logic" tab:

12) In the "Target" field name your target: i.e. HSV1 (up to seven alphanumeric characters). Repeat steps 12-15 for each target (i.e. HSV2 or TRE) following the tables specific for the target being defined.

13) Click the "Analyze" checkbox to include the desired wavelengths (PCR channels) in the target result analysis (Tables 8-10).

Wavelength	Alias	Type	Analyze
475/520	HSV1	PCR	✓
585/630	EIC	PCR	✓

Table 8. PCR channels selection in the "Result logic" tab for HSV1 (Herpes simplex virus 1) target.

Wavelength	Alias	Type	Analyze
530/565	HSV2	PCR	✓
585/630	EIC	PCR	✓

Table 9. PCR channels selection in the "Result logic" tab for HSV2 (Herpes simplex virus 2) target.

Wavelength	Alias	Type	Analyze
585/630	EIC	PCR	✓
630/665	TRE	PCR	✓

Table 10. PCR channels selection in the "Result logic" tab for TRE (*Treponema pallidum)* target.

14) Click the "Edit Logic" button.

15) In the "Edit Logic" window all the combinations of result types are listed. For each row, in the "Result" drop down menu select the result that is called when the conditions in that row are met, following tables 11-13.

Result	HSV1 (475/520)	EIC (585/630)
POS	Valid	Valid
UNR	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 11. List of the combination of result types and Result logic for HSV1 (Herpes simplex virus 1) target. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Result	HSV2 (530/565)	EIC (585/630)
POS	Valid	Valid
UNR	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 12. List of the combination of result types and Result logic for HSV2 (Herpes simplex virus 2) target. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Result	TRE (630/665)	EIC (585/630)
POS	Valid	Valid
UNR	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 13. List of the combination of result types and Result logic for TRE (*Treponema pallidum*) target. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Note: According to the Ct Max previously defined (table 5), the result type for HSV1 (475/520), HSV2 (530/565), EIC (585/630) and TRE (630/665) channels is considered “Valid” when the Ct value obtained is ≤40; and “Invalid” when the Ct value obtained is >40.

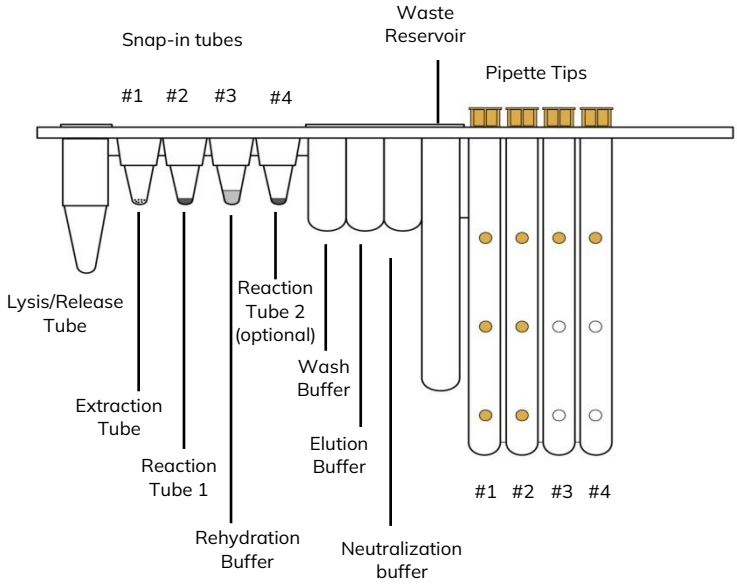
16) Click the “Save” button to save the test.

8.3.2. BD MAX™ Rack set up

1) For each sample to be tested, remove one Unitized Reagent Strips from the BD MAX™ ExK™ TNA-3 kit. Gently tap each strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes and load on the BD MAX™ System sample racks.
2) Remove the required number of BD MAX™ ExK™ TNA Extraction Tubes (B4) (white foil) from their protective pouch. Snap the Extraction Tube(s) (white foil) into its corresponding positions in the TNA strip (Snap position 1, white color coding on the rack. See Figure 1). Remove excess air, and close pouch with the zip seal.
3) Determine and separate the appropriate number of *HSV-1, HSV-2 & Treponema pallidum* reaction tubes (1E foil) and snap into their corresponding positions in the strip (Snap position 2, green color coding on the rack. See Figure 1).
    a. Remove excess air, and close aluminum pouches with the zip seal.
    b. In order to carry out a correct rehydration, please make sure that the lyophilized product is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.
4) Remove the required number of Rehydration Buffer tubes (11 foil) and snap into their corresponding positions in the strip (Snap position 3, non-color coding on the rack. See Figure 1).
    a. Remove excess air, and close the pouch with the zip seal.

b. In order to ensure a correct transfer, please make sure that the liquid is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the buffer is at the bottom of the tube.

Figure 1. BD MAX™ TNA Reagent Strip (TNA) from the BD MAX™ ExK™ TNA-3 kit.



8.3.3. BD MAX™ Instrument set up

1) Select the “Worklist” tab on the “Run” screen of the BD MAX™ System software v4.50A or higher.
2) In the “Test” drop down menu, select the desired test: i.e. VIASURE HHT (if not already created see Section 8.3.1).
3) In the “Kit Lot Number” drop down menu, select the appropriate lot number for the kit (found on the outer box of extraction kit used) (optional).

   Note: Lot numbers must be defined in the “Inventory” display before they can be selected here.
4) Enter the Sample Buffer Tube identification number into the “Sample tube” field, either by scanning the barcode with the scanner or by manual entry.
5) Fill the “Patient ID” and/or “Accession” field and click the Tab or enter key. Continue until all Sample Buffer Tubes barcodes are entered. Ensure that the Specimen/Patient ID and the Sample Buffer Tubes are accurately matched.
6) Place the prepared Sample Buffer Tube into the BD MAX™ Rack(s).
7) Load the rack(s) into the BD MAX™ System (Rack A is positioned on the left side of the BD MAX™ System and Rack B on the right side).
8) Place the required number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System.
9) Close the BD MAX™ System door.
10) Click “Start” to begin the procedure.

8.3.4. BD MAX™ results report

1) From the menu bar, click the “Results” button.
2) Either double click on your run in the list or press the “view” button.
3) The “Print” and "Export" buttons at the bottom of the screen will be enabled.

To print the results:

1. Click on “Print” button.
2. In the “Print” preview window of the run report select: “Run Details”, “Test Details” and “Plots”.
3. Click on “Print” to print the report or click “Export” to export a PDF of the report to a USB.

To export the results:

1. Click on “export” button to transfer the report (PDF and CSV file) to a USB.
2. When the export is complete, the success/fail icon appears in the “Results Export” window.

9. Result interpretation

For a detailed description on how to analyze data, refer to the BD MAX™ System User’s manual.

The analysis of the data is done by the BD MAX™ software according to the manufacturer´s instructions. The BD MAX™ software reports Ct values and amplification curves for each detector channel of each sample tested in the following way:

- Ct value of 0 indicates that there was no Ct value calculated by the software with the specified Threshold (see Table 5). Amplification curve of the sample showing a “0” Ct value must be checked manually.

- Ct value of -1 indicates that no amplification process has occurred, that there was no Ct value calculated by the software or the Ct value calculated is below the specified threshold or above the stablished Ct Max (Cut-off).

- Any other Ct value should be interpreted in correlation with the amplification curve and according to the result logic defined, following the interpretation guidelines outlined in Table 14.

Check Endogenous Internal Control (EIC) signal to verify the correct functioning of the amplification mix. In addition, check that there is no report of BD MAX™ System failure.

Results should be read and analyzed using the following table:

HSV-1 (name target: HSV1)	HSV-2 (name target: HSV2)	*T. pallidum* (name target: TRE)	Interpretation for patient's individual samples
POS	POS	POS	HSV-1, HSV-2 and *Treponema pallidum* DNA detected.
POS	POS	NEG	HSV-1 and HSV-2 DNA detected. *Treponema pallidum* DNA not detected.
POS	NEG	POS	HSV-1 and *Treponema pallidum* DNA detected. HSV-2 DNA not detected.
NEG	POS	POS	HSV-2 and *Treponema pallidum* DNA detected. HSV-1 DNA not detected.
POS	NEG	NEG	HSV-1 DNA detected. HSV-2 and *Treponema pallidum* DNA not detected.
NEG	POS	NEG	HSV-2 DNA detected. HSV-1 and *Treponema pallidum* DNA not detected.
NEG	NEG	POS	*Treponema pallidum* DNA detected. HSV-1 and HSV-2 DNA not detected.
NEG	NEG	NEG	HSV-1, HSV-2 and *Treponema pallidum* DNA not detected.
UNR	UNR	UNR	Unresolved (UNR) Result obtained in the presence of inhibitors in the PCR reaction or when a general problem (not reported by an error code) with the sample processing and/or amplification steps occurs.[1]
IND	IND	IND	Indeterminate assay result (IND). Due to BD MAX™ System failure. Assay result displayed in case of an instrument failure linked to an error code.[2]
INC	INC	INC	Incomplete assay result (INC). Due to BD MAX™ System failure. Assay result displayed in case of failure to complete run. [2]

Table 14. Sample interpretation.

1 The Endogenous Internal Control (EIC) must show an amplification signal with Ct value ≤ 40 to be considered. If there is an absence of signal for EIC or Ct value > 40, the result is considered as Unresolved (UNR) and retesting is required. It is recommended to repeat the assay from the same Sample Buffer Tube (SBT), from the same specimen preparing a new SBT or from a new specimen (more concentrated if possible). It may also occur that the Unresolved (UNR) result is due to the presence of inhibitors in the PCR reaction. In these cases, it is recommended to dilute these samples 1/10.

NOTE: natural skin lesion samples can be kept without transfer to the SBT for up to 48 hours if stored at 2±2°C - 25±2°C (expressed in ESwab® from Copan). In case of retesting from the same SBT, it is recommended to manually shake the SBT to ensure proper homogenization of the sample. Please note that natural skin lesion samples can be kept in the SBT for up to 7 days at 2±2°C - 25±2°C.

2 Indeterminate (IND) or Incomplete (INC) results may be obtained due to a system failure and retesting is required. Refer to the BD MAX™ System's User Manual for interpretation of warning and error codes.

Note: When using external controls, they should yield the following expected results; negative for ENC and positive for EPC (known positive specimens are expected to be positive only for the microorganism(s) present in the specimen). An ENC that yields a positive test result is indicative of a contamination event or specimen handling failure. An EPC that yields a negative result is indicative of a specimen handling/preparation

problem. Review the specimen handling/preparation technique. When an external control failure occurs, retesting is required.

In case of a continued ambiguous result, it is recommended to review the instructions for use, the extraction process used by the user; to verify the correct performance of each PCR steps and review the parameters; and to check the sigmoid shape of the curve and the intensity of fluorescence.

The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms, and other diagnostic tests.

10. Limitations of the test

- The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms, and other diagnostic tests.
- Although this assay can be used with other types of samples, it has been validated only with anogenital and oral skin lesion swab specimens.
- For good test performance, the lyophilized product should be at the bottom of the tube and not adhered to the top area of the tube or the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.
- Possible crosstalk might be observed in empty channels of the BD MAX™ System if there is no target to be detected, so it is required to select only the channels where these are amplified when interpretation of results is performed. Please contact to viasuresupport@certest.es for any queries.
- The quality of the test depends on the quality of the sample; nucleic acid must be properly extracted from clinical samples.
- This test is a qualitative test and does not provide quantitative values or indicate the number of organisms present. It is not possible to correlate the Ct values obtained by PCR with the concentration of the sample as they depend on the thermal cycler used and the run itself.
- Extremely low levels of target below the limit of detection might be detected, but results may not be reproducible.
- Please note the intended measurement range of the assay, as samples with concentrations above or below this range may give erroneous results.
- There is a possibility of false positive results due to cross-contamination by HSV-1, HSV-2 and/or *T. pallidum* samples containing high concentrations of target DNA or contamination due to PCR products from previous reactions.
- The specific primer and probe combinations for detection of the *glycoprotein L UL1* gene of HSV-1, *glycoprotein G US4* gene of HSV-2 and *16S rRNA* gene of *T. pallidum*, used in VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System, do not show significant combined homologies with

the human genome, human microflora, or other microorganisms, which might result in predictable false positive.

- False Negative results may arise from several factors and their combinations, including:
    - Improper specimens' collection, transport, storage, and/or handling methods.
    - Improper processing procedures (including DNA extraction).
    - Degradation of the DNA during sample shipping/storage and/or processing.
    - Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown HSV-1, HSV-2 and/or *T. pallidum* strains.
    - A microbial load in the specimen below the limit of detection for the assay.
    - The presence of qPCR inhibitors or other types of interfering substances. The impact of vaccines, some antiviral therapeutics, antibiotics, chemotherapeutics, immunosuppressant drugs or antifungals used to prevent the infection or used during the treatment of the infection has not been evaluated.
    - The effect of interfering substances has only been evaluated for those indicated in section 12.5.1 (study of interfering substances) of these instructions for use. Please, see this section to check the most common endogenous and exogenous substances that induce a total or partial interference of qPCR reaction. Other substances not indicated in this part could lead to erroneous results.
    - Failure to follow instructions for use and the assay procedure.
- A positive test result does not necessarily indicate the presence of viable microorganisms and does not imply that these microorganisms are infectious or are the causative agents for clinical symptoms. However, a positive result is indicative of the presence of HSV-1, HSV-2 and/or *T. pallidum* targets sequences.
- Negative results do not preclude the presence of HSV-1, HSV-2 and/or *T. pallidum* DNA in a clinical specimen and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak microbial levels during infections caused by HSV-1, HSV-2 and/or *T. pallidum* have not been determined. The collection of multiple specimens (types and time points) from the same patient may be necessary to detect the pathogen.
- If diagnostic tests for other Sexually Transmitted Diseases (STD) are negative and clinical observations, patient history and epidemiological information suggest that HSV-1, HSV-2 and/or *T. pallidum* infection is possible, then a false negative result should be considered, and a re-testing of the patient should be discussed.
- Fluorescence values may vary due to multiple factors such as: PCR equipment (even being the same model), extraction system, type of sample, previous treatment of the sample, etc... among others.
- Positive and negative predictive values are highly dependent on prevalence in all *in vitro* diagnostic tests. VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System performance may vary depending on the prevalence and population tested.

- In the case of obtaining Unresolved, Indeterminate or Incomplete results using VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System, retesting will be required. Unresolved results may be due to the presence of inhibitors in the sample or an incorrect rehydration of lyophilized reaction mix tube. If there is an instrument failure, Indeterminate or Incomplete results will be obtained.

11. Quality control

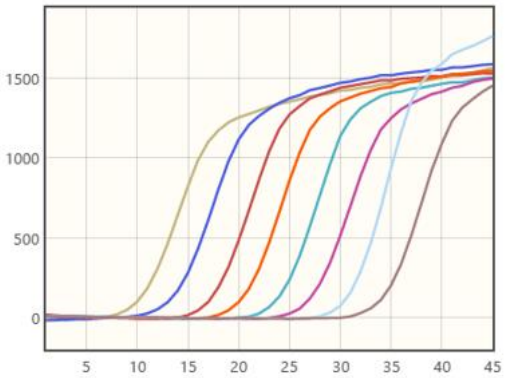
VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System contains an Endogenous Internal Control (EIC) in each reaction tube which confirms the correct performance of the technique. Besides, the use of external controls (EPC and ENC) allows to confirm the assay performance. External controls are not used by the BD MAX™ System for the purpose of result interpretation but considered as a sample. External Positive Control (EPC) is intended to monitor a potential failure of the assay reagents, while External Negative Control (ENC) is intended to detect environmental or reagent contamination by target nucleic acids.

12. Analytical performance characteristics

12.1. Analytical linearity

The linearity of the assay was determined and confirmed by testing a series of ten-fold dilutions of artificial skin lesion matrix[4] (Artificial Matrix for Cutaneous Lesion, Biochemazone BZ394) containing a known concentration (ranging from 2E+07 to 2E+01 copies/µl) of specific and synthetic DNA belonging to HSV-1, HSV-2 and *T. pallidum*. Example of the amplification plot resulting from an assay is included below:

Figure 2. Dilution series of HSV-1 (2E+07 to 2E+01 copies per µl) template run on the BD MAX™ System (475/520 (FAM) channel).



[4] Due to the limited availability of anogenital and oral skin lesion swab specimens, a natural matrix versus artificial matrix equivalence study was performed to confirm that the artificial matrix could be used in validation assays. Therefore, both natural and artificial matrices were tested and confirmed to be equivalent.

Figure 3. Dilution series of HSV-2 (2E+07 to 2E+01 copies per µl) template run on the BD MAX™ System (530/565 (HEX) channel).

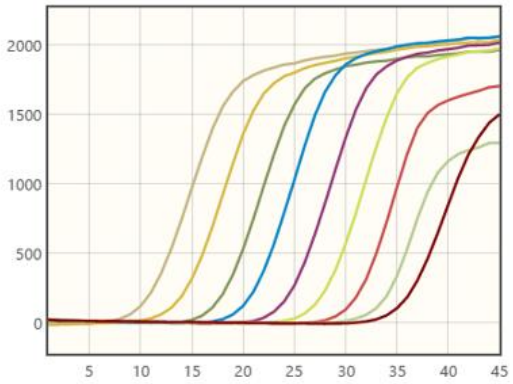
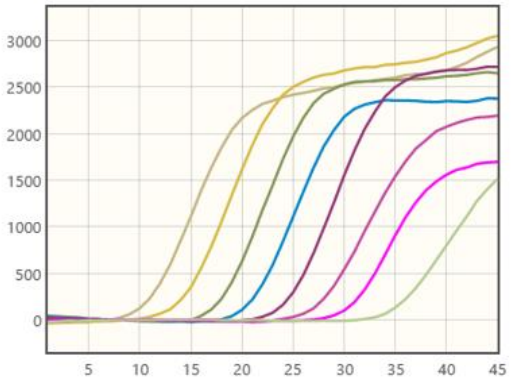


Figure 4. Dilution series of *T. pallidum* (2E+07 to 2E+01 copies per µl) template run on the BD MAX™ System (630/665 (Cy5) channel).



12.2. Analytical sensitivity

Analytical sensitivity is often referred to as the limit of detection (LoD). In practice, LoD and Limit of Blank (LoB) parameters are determined as part of analytical sensitivity evaluation.

12.2.1. Limit of Blank (LoB)

To determine the “limit of blank” (LoB), a non-template control assay was performed using three lots of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System, and 24 replicates of three types of samples: natural skin lesion negative samples, artificial skin lesion matrix and RNase/DNase free water.

The absence of signal in the FAM, HEX and Cy5 channels was verified, as well as the presence of signal for the Endogenous Internal Control (EIC) in the ROX channel in the case of negative matrix (natural or simulated skin lesion matrix). In conclusion, the results obtained confirmed that the product does not generate non-specific amplifications in the different matrixes tested.

12.2.2. Limit of Detection (LoD)

The limit of detection (LoD) of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System was analysed with three lots using natural skin lesion samples. The reference strains used were Human herpesvirus 1, Strain KOS (ATCC® VR-1493, Ref FR-310 (IRR)), Herpes Simplex Virus Type 2, Strain MS (Ref, 0810006CF (Zeptometrix)) and *Treponema pallidum*, strain SS14 (Washington University). In conclusion, VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System showed a LoD of 2.52E+02 TCID50/ml for HSV-1, 6.30E+00 TCID50/ml for HSV-2 and 6.60E+03 cells/ml for *T. pallidum*, with a positive rate of ≥95%, in natural skin lesion samples.

12.3. Measuring range

The measuring range of the assay was determined by testing a series of ten-fold dilutions containing a known concentration of specific DNA belonging to HSV-1, HSV-2 and *T. pallidum*. Results allowed to confirm the correct target detection from 2E+07 to 2E+00 copies/µl for HSV-1 and *T. pallidum* targets, and from 2E+07 to 2E-01 copies/µl for HSV-2 target.

In conclusion, measuring range of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System was successfully determined, ensuring reliable, accurate and reproducible results across a wide spectrum of viral/bacterial loads, affirming its utility in various clinical diagnostic scenarios.

12.4. Accuracy

12.4.1. Trueness (Veracity)

The veracity of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System was evaluated by testing reference material listed below spiked in skin lesion matrix.

1. **Synthetic DNA fragments**
    - Synthetic DNA fragment for *glycoprotein L UL1* gene of HSV-1: HSV1.XPC, FAM channel.
    - Synthetic DNA fragment for *glycoprotein G US4* gene of HSV-2: HSV2.XPC, HEX channel.
    - Synthetic DNA fragment for *16S rRNA* gene of *T. pallidum*: TREXPC, Cy5 channel.

All the synthetic DNA fragments were detected correctly in the appropriate channel.

2. The American Type Culture Collection (ATCC®)

External Reference	Microorganism	Product Name	Variety	Result
VR-1493	Herpes simplex virus 1	Human herpesvirus 1	Strain KOS	Detected
VR-260	Herpes simplex virus 1	Human Herpesvirus 1	Strain HF	Detected
VR-539	Herpes simplex virus 1	Human Herpesvirus 1	Strain MacIntyre	Detected
VR-733	Herpes simplex virus 1	Human Herpesvirus 1	Strain F	Detected
VR-3393	Herpes simplex virus 2	Human Herpesvirus 2	Strain G	Detected
VR-1779	Herpes simplex virus 2	Human Herpesvirus 2	Strain ATCC-2011-2	Detected
BAA-2642SD	*Treponema pallidum*	Quantitative Synthetic *Treponema pallidum* DNA	N/A	Not Detected

Table 15. Reference material from the American Type Culture Collection (ATCC®).

All the strains from the ATCC were correctly detected in the appropriate channel and the EIC showed amplification with a Ct value ≤40, except for the Quantitative Synthetic *Treponema pallidum* DNA (ATCC code BAA-2642SD). This synthetic DNA includes fragments of the *polA* gene, *23S* gene, *16S* gene, *flaA, 47kDa* protein gene and *bmp,* however, the absence of detection at very high concentration (up to 4.70E+04 copies/µl) using the VIASURE Assay suggests that its target region within *16S* gene is not included in the synthetic DNA from ATCC. Therefore, it cannot be used as reference material.

3. National Institute for Biological Standards and Control (NIBSC)

External Reference	Microorganism	Product Name	Variety	Result
16/368	Herpes simplex virus 1	1st WHO IS for Herpes Simplex Virus type-1 (HSV-1)	N/A	Detected
17/122	Herpes simplex virus 2	1st WHO IS for Herpes Simplex Virus type-2 (HSV-2)	N/A	Detected

Table 16. Reference material from National Institute for Biological Standards and Control (NIBSC).

All the strains from the NIBSC were correctly detected in the appropriate channel and the EIC showed amplification with a Ct value ≤40.

4. Control material

External Reference	Provenance	Microorganism	Product Name	Variety	Result
0810006CF	ZeptoMetrix	Herpes simplex virus 2	Herpes Simplex Virus Type 2 (HSV-2)	Strain MS	Detected
MBC109	Vircell S.L.	*Treponema pallidum*	AMPLIRUN® TREPONEMA PALLIDUM DNA CONTROL	N/A	Detected
N/A	University of Washington	*Treponema pallidum*	Heat-killed *Treponema pallidum*	Nichols strain	Detected
N/A	University of Washington	*Treponema pallidum*	Heat-killed *Treponema pallidum*	SS14 strain	Detected

Table 17. Control material for HSV-2 and *T. pallidum* from Vircell S.L, ZeptoMetrix and University of Washington.

All the strains and reference material used were correctly detected in the appropriate channel and the EIC showed amplification with a Ct value ≤40.

12.4.2. Precision

To determine the precision of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System, intra- assay (repeatability), inter-assay, inter-batch and inter-equipment assays (reproducibility) were performed with artificial skin lesion matrix spiked with the reference strains Human herpesvirus 1, Strain KOS (ATCC® VR-1493, Ref FR-310 (IRR)), Herpes Simplex Virus Type 2, Strain MS (Ref, 0810006CF (Zeptometrix)) and *Treponema pallidum*, strain SS14 (Washington University).

Intra-assay

The intra-assay was tested by analysing six replicates of all samples in the same run using VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System. A summary of results is shown in the table below.

Target	Channel	Sample type	Ct ($\bar{x}$)	σ	CV%
HSV-1	475/520 (FAM)	3xLoD	33.32	0.49	1.48
		5xLoD	32.93	0.12	0.37
		Negative control	NEG	N/A	N/A
HSV-2	530/565 (HEX)	3xLoD	31.08	0.40	1.28
		5xLoD	32.00	0.24	0.74
		Negative control	NEG	N/A	N/A
EIC	585/630 (ROX)	3xLoD	30.42	0.15	0.48
		5xLoD	29.90	0.48	1.60
		Negative control	NEG	N/A	N/A
Treponema pallidum	630/665 (Cy5)	3xLoD	34.17	0.55	1.61
		5xLoD	31.95	0.43	1.35
		Negative control	NEG	N/A	N/A

Table 18. Intra-assay results of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, NEG = negative, N/A = not applicable.

Inter-assay

The inter-assay was tested by testing four replicates of the different samples on three different days by three different operators, using the VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System. A summary of results is shown in the table below.

Target	Channel	Sample type	Ct ($\bar{x}$)	σ	CV%
HSV-1	475/520 (FAM)	3xLoD	33.21	0.47	1.41
		5xLoD	31.85	0.78	2.43
		Negative control	NEG	N/A	N/A
HSV-2	530/565 (HEX)	3xLoD	33.83	0.56	1.66
		5xLoD	32.68	0.48	1.48
		Negative control	NEG	N/A	N/A
EIC	585/630 (ROX)	3xLoD	29.82	0.43	1.43
		5xLoD	29.94	0.40	1.33
		Negative control	29.78	0.46	1.54
Treponema pallidum	630/665 (Cy5)	3xLoD	32.61	1.07	3.29
		5xLoD	31.86	1.24	3.88
		Negative control	NEG	N/A	N/A

Table 19. Inter-assay results of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, NEG = negative, N/A = not applicable.

Inter-batch

The inter-batch values were determined with six replicates of the different samples by using three batches of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System. A summary of results is shown in the table below.

Target	Channel	Sample type	Ct ($\bar{x}$)	σ	CV%
HSV-1	475/520 (FAM)	3xLoD	33.26	0.46	1.38
		5xLoD	33.29	0.33	1.00
		Negative control	NEG	N/A	N/A
HSV-2	530/565 (HEX)	3xLoD	30.98	0.86	2.78
		5xLoD	32.70	0.58	1.79
		Negative control	NEG	N/A	N/A
EIC	585/630 (ROX)	3xLoD	30.04	0.57	1.89
		5xLoD	30.26	0.56	1.86
		Negative control	29.97	0.69	2.31
Treponema pallidum	630/665 (Cy5)	3xLoD	33.53	0.76	2.26
		5xLoD	32.24	0.55	1.69
		Negative control	NEG	N/A	N/A

Table 20. Inter-batch results of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, NEG = negative, N/A = not applicable.

Inter-equipment

The inter-equipment values were determined with four replicates of the same samples used for intra-assay, inter-assay and inter-batch, using the VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System and three different BD MAX™ System. A summary of results is shown in the table below.

Target	Channel	Sample type	Ct ($\bar{x}$)	σ	CV%
HSV-1	475/520 (FAM)	3xLoD	33.71	0.52	1.54
		5xLoD	32.68	0.59	1.79
		Negative control	NEG	N/A	N/A
HSV-2	530/565 (HEX)	3xLoD	33.56	0.80	2.39
		5xLoD	32.65	0.42	1.28
		Negative control	NEG	N/A	N/A
EIC	585/630 (ROX)	3xLoD	29.51	0.77	2.59
		5xLoD	29.23	0.39	1.33
		Negative control	29.98	0.52	1.75
Treponema pallidum	630/665 (Cy5)	3xLoD	32.28	0.62	1.93
		5xLoD	31.19	0.40	1.29
		Negative control	NEG	N/A	N/A

Table 21. Inter-equipment results of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, NEG = negative, N/A = not applicable.

In conclusion, the precision study confirmed the reliable performance and consistency of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System.

12.4.3. Accuracy

The accuracy experiment was conducted through the analysis of panels from External Quality Assessment (EQA) Programmes. Samples containing HSV-1, HSV-2 or *T. pallidum*, among other non-target microorganisms, were analysed. All test results, shown in the following table, were compared with the final reports provided by the EQA Programmes organizers.

Target	ORA	TP	TN	FP	FN	PPA	NPA
HSV-1	1 (0.51-1)	4	30	0	0	1 (0.40-1)	1 (0.88-1)
HSV-2	1 (0.90-1)	12	22	0	0	1 (0.74-1)	1 (0.85-1)
Treponema pallidum	0.97 (0.85-0.99)	7	26	0	1	0.88 (0.47-0.99)	1 (0.87-1)

Table 22. Accuracy results using VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System. (ORA) = overall agreement, (TP) = true positive, (TN) = true negative, (FP) = false positive, (FN) = false negative, (PPA) = Positive Precent Agreement, (NPA) = Negative Percent Agreement.

In conclusion, the VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System provides high accuracy in the detection of the DNA of HSV-1, HSV-2 and *T. pallidum*, with excellent NPA and PPA values across all tested strains.

12.5. Analytical specificity and reactivity

The analytical specificity and analytical reactivity were evaluated for the VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System *in silico* and experimentally, using different starting material such as certified reference strains, certified reference RNA/DNAs and material from the EQAs programmes.

12.5.1. Analytical Specificity

Analytical specificity is the assay's ability to detect the intended target. There are four components to be considered for analytical specificity: cross-reactivity, co-infection, microbial agents interference and substances interference. Cross-reactivity may occur when genetically related microorganisms are present in a patient specimen, while microbial interference may happen if those genetically related microorganisms alter the detection of the target microorganisms when they are present. Similarly, co-infection study aims to assess whether target microorganisms at different concentrations in the same sample could alter the detection between them. Finally, substances interference may happen if the presence of specific substances potentially present in the sample matrix affects the performance of the qPCR.

Cross-reactivity *in silico* analysis

The Cross-reactivity was assessed by using reference sequences of the pathogens from NCBI Genbank (https://www.ncbi.nlm.nih.gov/genbank/) and with alignments tools such as BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) an in-house bioinformatic analysis software.

The results to be shown only present sequences with an homology percentage higher than 80%. Aligned sequences with an alignment percentage of less than 80% of homology were considered unlikely to be detected.

Treponema pallidum

All analysed sequences were below 80% of homology with *T. pallidum* primers and probe set.

In silico cross-reactivity analysis revealed 100% homology with *Treponema paraluiscuniculi* (Taxid ID: 53435 and 545766). However, according to a NCBI search these sequences were found to be heterotypic or taxonomically synonyms and also correspond to *Treponema pallidum*.

Therefore, none of the sequences analyzed, including those showing a homology higher than 80%, could affect the correct detection of *Treponema pallidum*.

Human alphaherpesvirus 1

All analysed sequences were below 80% of homology with HSV-1 primers and probe set.

In silico cross-reactivity analysis revealed 98.33% homology with Chimpanzee alpha-1 herpesvirus strain 105640 (Taxid ID: 332937). The host of this strain of alphaherpesvirus is the chimpanzee (*Pan troglodytes*). Herpesviruses are highly host specific and share a long synchronous evolution with their hosts. This virus has not been identified in humans and is not considered zoonotic virus at the moment, so it does not interfere in the detection of HSV-1.

Therefore, none of the sequences analyzed, including those showing a homology higher than 80%, could affect the correct detection of HSV-1.

Human alphaherpesvirus 2

All analysed sequences were below 80% of homology with HSV-2 primers and probe set.

In conclusion, the HSV-1, HSV-2 and *T. pallidum* target designs of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System should not cause false positives in detecting *Treponema pallidum*, human alphaherpesvirus 1 and human alphaherpesvirus 2 when other organisms are present.

Analytical specificity wet testing

Cross-reactivity wet testing

The cross-reactivity of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System was confirmed by testing a panel of different microorganisms associated with sexually transmitted infection symptoms or environmentally, and phylogenetically relevant microorganisms. When possible and the concentration data available, the microorganisms interfering were evaluated at medically relevant levels (usually 1E+05 – 1E+06 cfu (colony-forming unit)/ml for bacteria/fungi and 1E+04 – 1E+05 pfu (plaque-forming unit)/ml for viruses). No cross-reactivity was detected between any of the following microorganisms tested, excepted for the target microorganisms.

Cross-reactivity testing					
Acinetobacter baumannii 5377 [NCDC KC 755]	-	Haemophilus influenzae Strain L-378	-	*Listeria monocytogenes* Serovar 4b	-
Aspergillus fumigatus Fresenius 1863 CECT 2071	-	Hepatitis A HM175/18f	-	Monkeypox virus	-
Atopobium vaginae	-	Herpes Simplex Virus type-1 1st WHO IS for HSV-1	+	*Mycoplasma genitalium* Strain M30	-
Bacteroides fragilis	-	Herpes Simplex Virus type-2 1st WHO IS for HSV-2	+	*Mycoplasma hominis* Strain LBD-4	-
Candida albicans	-	Herpes Virus 2 Strain MS	+	*Neisseria gonorhoeae* Strain NCTC 8375 [B 5025]	-
Candida glabrata	-	Human Herpesvirus 1 HF strain	+	*Neisseria meningitidis* Strain M2091	-

Cross-reactivity testing					
Candida krusei Issatchenkia orientalis Kudryavtsev 1960 CECT 1433	-	Human Herpesvirus 1 Strain F	+	*Proteous mirabilis* NCDC 2059-70	-
Candida parapsilosis	-	Human Herpesvirus 1 Strain KOS (ATCC-VR-1493)	+	*Pseudomonas aeruginosa* Strain RH 815	-
Candida tropicalis (Castellani) Berkhout 1923 CECT 1005	-	Human Herpesvirus 1 Strain MacIntyre	+	*Serratia marcescens* Subsp. marcescens	-
Chlamydia trachomatis Serovar E	-	Human Herpesvirus 2 Strain ATCC-2011-2	+	*Staphylococccus aureus* Subsp. Aureus, strain Seattle 1945	-
Citrobacter freundii	-	Human Herpesvirus 2 Strain G	+	*Stenotrophomonas maltophilia* Strain 810-2	-
Enterobacter cloacae subsp. cloacae	-	Human papillomavirus 16	-	*Treponema pallidum* DNA Control, Vircell	+
Enterococcus faecalis Type strain CECT 8120	-	Human papillomavirus 18	-	*Treponema pallidum* Strain Nichols	+
Enterococcus faecium vanA, strain CECT 5253	-	*Klebsiella oxytoca* Strain MIT 10-5243	-	*Treponema pallidum* Strain SS14	+
Epstein-Barr Virus 1st WHO IS for Epstein-Barr Virus EBV	-	*Klebsiella pneumoniae* Strain UHKPC57	-	*Trichomonas vaginalis*, QCMD TV18S-01	-
Escherichia coli Type strain	-	*Listeria innocua*	-	*Ureaplasma urealyticum* T-strain 960 (CX8) [960, CIP 103755, NCTC 10177]	-
Gardnerella vaginalis 594 [NCTC 10287]	-	*Listeria ivanovii* Subsp. londoniensis	-	Varicella Zoster Virus Strain Ellen	-
Haemophilus ducreyi Class I	-	*Listeria monocytogenes* Serovar 1/2	-		

Table 23. Reference pathogenic microorganisms included in the cross-reactivity assay. The +/- result refers to the positive or negative result obtained in the different channels depending on the target detected. In case a microorganism tested is one of the targets detected by the device, a positive result is obtained in their corresponding channel, but a negative result is obtained in the other channels.

In conclusion, the results from cross-reactivity assays indicate high specificity of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System, thereby minimizing the risk of false-positive results. Since no non-specific amplifications were observed with other related microorganisms, this suggests that the device is capable of accurately distinguishing the targets.

Study of co-infection

A study of co-infection was conducted using Human herpesvirus 1, Strain KOS (ATCC® VR-1493, Ref FR-310 (IRR)), Herpes Simplex Virus Type 2, Strain MS (Ref, 0810006CF (Zeptometrix)) and *Treponema pallidum*, strain SS14 (Washington University) at different concentrations, to confirm that the presence of any of them, independently of the concentration, does not alter the detection between them. Three natural skin lesion negative matrix samples spiked with the reference material, one target at low concentration (3xLoD) and the other targets at a very high concentration, usually 1E+04 – 1E+05 units/ml, if possible, were analysed.

The results confirm that the detection of the microorganisms targeted is not altered when analysed with VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System in coinfection at different concentrations.

Study of interfering microbial agents

A study of microbial interference was performed to analyse the potential interfering microbial agents for VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System. A panel of different clinically, environmentally, and phylogenetically relevant microorganisms was tested in presence of HSV-1, HSV-2 and *T. pallidum* targets using natural skin lesion negative matrix spiked with Human herpesvirus 1, Strain KOS (ATCC® VR-1493, Ref FR-310 (IRR)), Herpes Simplex Virus Type 2, Strain MS (Ref, 0810006CF (Zeptometrix)) and *Treponema pallidum*, strain SS14 (Washington University). When possible and the concentration data available, the virus, bacteria and/or fungi interfering were evaluated at medically relevant levels (usually 1E+05 – 1E+06 cfu/ml for bacteria/fungi and 1E+04 – 1E+05 pfu/ml for viruses).

Positive Matrix Control and Negative Matrix Control (PMC and NMC, respectively) are included as controls of the test. PMC corresponds to the negative matrix spiked with specific target strains without any interfering microbial agent, whereas NMC corresponds to the negative matrix without any interfering microbial agent.

Microorganism Name	Concentration tested	Result
PMC	N/A	N.I
NMC	N/A	N.I
Aspergillus fumigatus	Not available	N.I
Atopobium vaginae	4.52E+03 cfu/µl	N.I
Candida albicans	4.18E+03 cfu/µl	N.I
Candida glabrata	2.46E+03 cfu/µl	N.I
Candida parapsilosis	Not available	N.I
Candida tropicalis	3.04E+04 cp/µl	N.I
Chlamydia trachomatis Serovar E	3.20E+06 IFU/ml	N.I
Enterobacter cloacae	1.28E+03 cfu/µl	N.I
1st WHO IS for Epstein-Barr Virus EBV	5.00E+04 IU/mL	N.I
Gardnerella vaginalis 594 [NCTC 10287]	4.40E+05 cfu/ml	N.I
Haemophilus ducreyi Class I	1.40E+02 cp/µl	N.I
Haemophilus influenzae	1.20E+03 cfu/µl	N.I
Hepatitis A	2.80E+03 TCID50/µl	N.I
Klebsiella oxytoca	Not available	N.I
Klebsiella pneumoniae	Not available	N.I
Listeria innocua	Not available	N.I
Listeria ivanovii subsp. londoniensis	Not available	N.I
Listeria monocytogenes (CECT 4031)	2.90E+03 cfu/µl	N.I
Listeria monocytogenes (CECT 935)	2.70E+03 cfu/µl	N.I
Mycoplasma hominis	4.40E+03 cfu/µl	N.I
Mycoplasma genitalium	3.80E+03 cfu/µl	N.I
Neisseria gonorhoeae	6.20E+03 cfu/µl	N.I
Neisseria meningitidis	5.70E+04 cfu/µl	N.I
Pseudomonas aeruginosa	4.90E+04 cfu/µl	N.I

Staphylococccus aureus	4.60E+04 cfu/µl	N.I
Stenotrophomonas maltophilia	9.20E+03 cfu/µl	N.I
Ureaplasma urealyticum	2.00E+04 cfu/µl	N.I
Acinetobacter baumannii 5377 [NCDC KC 755]	8.10E+05 cfu/ml	N.I
Bacteroides fragilis	3.40E+02 cp/µl	N.I
Enterococcus faecalis Type strain	5.00E+05 cfu/ml	N.I
Enterococcus faecium vanA	3.50E+05 cfu/ml	N.I
Human papillomavirus 16	1.00E+03 IU/µl	N.I
Human papillomavirus 18	1.00E+03 IU/µl	N.I
Serratia marcescens	2.30E+05 cfu/ml	N.I
Varicella-Zoster Virus	7.26E+04 cfu/ml	N.I
Citrobacter freundii Type strain	6.20E+02 cfu/µl	N.I
Escherichia coli Type strain	5.20E+02 cfu/µl	N.I
Proteous mirabilis	2.55E+01 cfu/µl	N.I
Monkeypox virus	1.50E+03 cp/ml	N.I

Table 24. Interfering microbial agents assay. N.I. = No interference, (PMC) = positive matrix control, (NMC) = negative matrix control, (TCID50): Tissue Culture Infectious Dose 50%, (IU): International Units, (cfu): colony-forming units, (cp): copies, (IFU): infection unit.

In conclusion, no interference was observed in the detection the nucleic acid targeted with any of the microorganisms tested.

Study of interfering substances

A study of interfering substances was performed to test the potential interfering effect of endogenous and exogenous substances on VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System. A total of 22 potentially interfering substances were added to the natural skin lesion negative matrix enriched with the reference strains: Human herpesvirus 1, Strain KOS (ATCC® VR-1493, Ref FR-310 (IRR)), Herpes Simplex Virus Type 2, Strain MS (Ref, 0810006CF (Zeptometrix)) and *Treponema pallidum*, strain SS14 (Washington University); and evaluated with six replicates.

Positive Matrix Control and Negative Matrix Control (PMC and NMC, respectively) are included as controls of the test. PMC corresponds to the negative matrix spiked with specific target strains without any interfering substance, whereas NMC corresponds to the negative matrix without interfering substance nor added microorganisms/reference material. The following results were obtained:

Substance name	Concentration tested	Result
PMC	N/A	N.I.
NMC	N/A	N.I.
Acyclovir	6.60E-02 mg/ml	N.I.
Albumin	1.00E+01 mg/ml	N.I.
Whole Blood	1.00E+00% (v/v)	N.I.

Leukocytes/ monocytes	1.00E+06 cell/ml	N.I.
Mucin	2.50E+00 mg/ml	N.I.
Clamiox (hidrocortisone)	2.50E+00 mg/ml	N.I.
Neosayomol (difenhidramina)	3.87E-02 mg/ml	N.I.
Letibalm (lip balm)	2.50E+00 mg/ml	N.I.
Casein	7.00E+00 mg/ml	N.I.
Hemoal (benzocaina)	2.50E+00 mg/ml	N.I.
Durex Frescor	5.00E+01 µl/ml	N.I.
SOIVRE Intim Oil	5.00E+01 µl/ml	I
	1.25E+01 µl/ml	N.I.
Liade	7.20E+00 mg/ml	N.I.
Female urine	1.00E+01% (v/v)	N.I.
Male urine	1.00E+01% (v/v)	N.I.
Feces	2.20E-01 % (v/v)	N.I.
Seminal fluid	5.00E+00% (v/v)	N.I.
Halibut	2.50E+00 mg/ml	N.I.
Vagisil Cream	7.50E-01 mg/ml	N.I.
Cornstarch	2.50E+00 mg/ml	N.I.
Talquistina	2.64E+00% (v/v)	I
	6.60E-01% (v/v)	I
	3.30E-01% (v/v)	I
	1.70E-01% (v/v)	N.I.
Tioconazole	2.50E+00 mg/ml	N.I.

Table 25. Potential interference substances. N.I: No reportable interfere / I: Interfere.

Different potentially interfering substances, both endogenous and exogenous, were tested on VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System. Interference was observed when testing SOIVRE Intim Oil at 50 µl/ml and Talquistina at 2.64% (v/v). Dilutions were performed until reaching the concentration at which these interference effects were no longer observed: 12.5 µl/ml for SOIVRE Intim Oil and 0.17% (v/v) for Talquistina. The results obtained lead to conclude that, at the final concentrations tested, no interference of any the evaluated substances is observed.

12.5.2. Analytical reactivity

Analytical reactivity can be defined as the percentage of target microbial strains or DNA/RNA samples that give the correct positive result. Analytical reactivity was studied in silico and by wet analyses.

Analytical reactivity *in silico* analysis

The Analytical Reactivity of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System was assessed by using publicly available nucleotide sequence database as NCBI GenBank

(https://www.ncbi.nlm.nih.gov/genbank/), and an in-house bioinformatic analysis software, in order to demonstrate that the target genes can be correctly detected by the device under study. *In silico* analysis of the primers and probe design was performed through alignment against a total of 4,941 analysed sequences (sequences downloaded from the database with duplicates removed) for *T. pallidum*, 9,779 for HSV-1 and 8,689 for HSV-2. The results obtained are shown in the following table:

Treponema pallidum					
Gene	Aligned sequences: 607				
	Without mismatches	With mismatches	Sequences with confirmed detection	Sequences with no detection	Sequences with unknown detection
16S rRNA	99.84%	0.16%	99.84%	0%	0.16%
Human alphaherpesvirus 1					
Gene	Aligned sequences: 513				
	Without mismatches	With mismatches	Sequences with confirmed detection	Sequences with no detection	Sequences with unknown detection
UL1	87.33%	12.67%	97.66%	0%	2.34%
Human alphaherpesvirus 2					
Gene	Aligned sequences: 549				
	Without mismatches	With mismatches	Sequences with confirmed detection	Sequences with no detection	Sequences with unknown detection
US4	93.44%	6.56%	93.44%	0%	6.56%

Table 26. Analytical reactivity *in silico* assay. "Aligned sequences" = number of sequences aligned without or with mismatches from the total of analysed sequences, "Sequences with confirmed detection" = sequences without mismatches or wet-analysed whose detection is guaranteed, "Sequences with no detection" = sequences previously in silico-analysed whose experimental detection cannot be guaranteed due to previous negative experimental evidence, "Sequences with unknown detection" = sequences previously in silico-analysed whose experimental detection cannot be guarantee due to lack of experimental evidence.

To sum up, the inclusivity analysis shown a correct detection of *16S rRNA, UL1* and *US4* genes of T. pallidum, HSV-1 and HSV-2, respectively, with the VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System.

Analytical reactivity wet testing

The analytical reactivity of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System for HSV-1 was evaluated against the DNA from Human Herpesvirus 1, HF strain (ATCC code VR-260), Human Herpesvirus 1, strain MacIntyre (ATCC code VR-539), Human Herpesvirus 1, strain F (ATCC code VR-733) and 1st WHO IS for Herpes Simplex Virus type-1 (HSV-1) (NIBSC 16/368), showing positive results.

The analytical reactivity of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System for HSV-2 was evaluated against the DNA from Human Herpesvirus 2, strain G (ATCC code VR-3393), Human Herpesvirus 2, strain ATCC-2011-2 (ATCC code VR-1779) and 1st WHO IS for Herpes Simplex Virus type-2 (HSV-2) (NIBSC 17/122), showing positive results.

The analytical reactivity of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System for *T. pallidum* was evaluated against the DNA from *Treponema pallidum*, Nichols strain (University of Washington) and *Treponema pallidum* DNA Control (Vircell MBC109), showing positive results.

12.6. Metrological traceability

This assay is not designed for measuring purposes.

13. Clinical performance characteristics

The clinical performance of VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System was tested using anogenital and oral skin lesion swab specimens collected by nursing staff using the Copan eSwab®. For the collection, the sterile swab (FLOQSwab) was placed into the supplied vial containing 1 mL Copan's Liquid Amies Elution Swab. The results were as follows:

	Site	Sample type	Workflow	Target
1	Certest Biotec S.L. (Zaragoza, Spain) in collaboration with Hospital Universitario Miguel Servet (Zaragoza, Spain) using samples from the Biobanco del Sistema de Salud de Aragón (BSSA)	Anogenital and oral skin lesion swab (Retrospective study)	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	HSV-1
				HSV-2
				T. pallidum
2	Certest Biotec S.L. (Zaragoza, Spain) using samples from Cerba Xpert	Anogenital and oral skin lesion swab (Retrospective study)	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	HSV-1
				HSV-2
				T. pallidum

Table 27. Site, sample type, workflow, and target.

True positive and negative values, false positive and negative values, sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV) and the likelihood ratios (LR) for VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System were calculated. The results and analysis of the different studies were compiled as a single reference value due to both studies used the same reference method, type of samples and inclusion/exclusion criteria. The results are shown in the following table:

Site	Comparator assay	Target	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV	LR+	LR-
1+2	Allplex™ Genital Ulcer Assay (Seegene)	HSV-1	50	96	0	1	0.98 (0.90-1)	1 (0.96-1)	1 (0.93-1)	0.99 (0.94-0.99)	188.4 (11.86-2992)	0.029 (0.006-0.14)
		HSV-2	49	97	0	1	0.98 (0.89-0.99)	1 (0.96-1)	1 (0.93-1)	0.99 (0.94-0.99)	190.2 (11.98-3021)	0.03 (0.006-0.14)
		T. pallidum	40	106	0	1	0.98 (0.87-0.99)	1 (0.97-1)	1 (0.91-1)	0.99 (0.96-0.99)	206.36 (12.98-3280)	0.036 (0.007-0.173)

Table 28. True positive (TP) and negative values (TN), false positive (FP) and false negative (FN) values, sensitivity, specificity, Positive Predictive Values (PPV), Negative Predictive Values (NPV) and the likelihood ratios (LR) for VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System.

In conclusion, results show high agreement to detect HSV-1, HSV-2 and *T. pallidum* using VIASURE *HSV-1, HSV-2 & Treponema pallidum* Assay for BD MAX™ System.

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Symbols for IVD components and reagents

IVD	*In vitro* diagnostic device		Keep dry		Use by		Manufacturer	LOT	Batch code
	Consult instructions for use		Temperature limitation	Σ	Contains sufficient for <n> test	UDI	Unique Device Identification	REF	Catalogue number
CE	CE marking		Keep away from sunlight						

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Certest Biotec, S.L.
Pol. Industrial Río Gállego II, Calle J, Nº 1, 50840, San Mateo de Gállego, Zaragoza (Spain)
Tel. (+34) 976 520 354 | viasure@certest.es | www.certest.es

Australian sponsor information: Becton Dickinson Pty Ltd., 66 Waterloo Road.
Macquarie Park NSW 2113, Australia

New Zealand sponsor information: Becton Dickinson Limited, 14B George Bourke Drive.
Mt. Wellington Auckland 1060, New Zealand

Change Control		
Version No.	**Changes**	**Date**
00	Original version	27/07/2025

Table A 2. Control change table.

Revision: 00

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Certest Biotec, S.L.
Pol. Industrial Río Gállego II · Calle J, Nº1 50840,
San Mateo de Gállego, Zaragoza (Spain)

(+34) 976 520 354

viasure@certest.es

www.certest.es

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