VIASURE
by certest

Real Time PCR Detection Kit

Mycoplasma genitalium with Macrolide Resistance Assay for BD MAX™ System

Instructions for use

CE IVD
2797

These instructions for use apply to the following references:

PRODUCT	REFERENCE
VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System	444224

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

EN To download IFUs in other languages, please visit **certest.es/viasure/labeling**. Once there, please follow the instructions to access the language required. If you need additional information, please contact: viasure@certest.es.

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CS Chcete-li si stáhnout návody k použití (IFU) v jiných jazycích, přejděte na stránku **certest.es/viasure/labeling**. Jakmile se tam dostanete, postupujte podle pokynů pro přístup k požadovanému jazyku. Pokud potřebujete další informace, kontaktujte prosím: viasure@certest.es.

DA Hvis du vil downloade brugsanvisninger på andre sprog, kan du gå til **certest.es/viasure/labeling**. Når du er der, bedes du følge instruktionerne for at få adgang til det sprog, du har brug for. Hvis du har brug for yderligere oplysninger, kan du kontakte: viasure@certest.es.

DE Um die IFUs in anderen Sprachen herunterzuladen, gehen Sie bitte zu **certest.es/viasure/labeling**. Wenn Sie dort sind, folgen Sie den Anweisungen, um auf die gewünschte Sprache zuzugreifen. Wenn Sie weitere Informationen benötigen, wenden Sie sich an: viasure@certest.es.

EL Για λήψη των Οδηγίες χρήσης (IFUs) σε άλλες γλώσσες, μεταβείτε στη διεύθυνση **certest.es/viasure/labeling**. Μόλις φτάσετε εκεί, ακολουθήστε τις οδηγίες για να αποκτήσετε πρόσβαση στη γλώσσα που χρειάζεστε. Εάν χρειάζεστε πρόσθετες πληροφορίες, επικοινωνήστε με τη διεύθυνση: viasure@certest.es.

ES Para descargar las Instrucciones de Uso (IFU) en otros idiomas, por favor, entre en **certest.es/viasure/labeling.** Una vez esté allí, siga las instrucciones para acceder al idioma que necesite. Si necesita información adicional, contacte: viasure@certest.es.

FR Pour télécharger la notice d'utilisation (IFU) dans d'autres langues, consultez **certest.es/viasure/labeling.** Une fois sur le site, suivez les instructions pour accéder à la langue de votre choix. Si vous avez besoin d'informations supplémentaires, contactez: viasure@certest.es.

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IT Per scaricare le istruzioni per l'uso (IFU) in altre lingue, vai su **certest.es/viasure/labeling**. Una volta che sei lì, segui le indicazioni per accedere alla lingua di cui hai bisogno. Se hai bisogno di ulteriori informazioni, contatta: viasure@certest.es.

LT Norėdami atsisiųsti naudojimo instrukciją kitomis kalbomis, apsilankykite adresu **certest.es/viasure/labeling**. Atidarę šį tinklalapį vykdykite rodomas instrukcijas, kol rasite reikiamą kalbą. Jei reikia daugiau informacijos, kreipkitės adresu: viasure@certest.es.

LV Lai lejupielādētu lietošanas pamācību citās valodās, lūdzu, apmeklējiet vietni **certest.es/viasure/labeling**. Pēc vietnes lapas atvēršanas izpildiet norādījumus, lai piekļūtu vajadzīgajai valodai. Ja nepieciešama papildu informācija, lūdzu, sazinieties ar: viasure@certest.es.

NB Hvis du vil laste ned en bruksanvisning (IFU) på et annet språk, kan du gå inn på **certest.es/viasure/labeling**. Følg instruksjonene på nettsiden for å få tilgang til det språket du ønsker. Hvis du trenger ytterligere informasjon, kan du sende en e-post til: viasure@certest.es.

PT Para transferir instruções de utilização (IFU) noutros idiomas europeus, aceda a **certest.es/viasure/labeling**. Uma vez lá, siga as instruções para acessar ao idioma que pretende. Se necessitar de informações adicionais, entre em contacto connosco através do: viasure@certest.es.

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SV För att ladda ner bruksanvisningen på andra språk, gå in på **certest.es/viasure/labeling** och följ instruktionerna. Om du behöver ytterligare information, kontakta: viasure@certest.es.

SK Ak si chcete stiahnuť návody na použitie v iných jazykoch, prejdite na stránku **certest.es/viasure/labeling**. Po otvorení stránky postupujte podľa pokynov na prístup k požadovanému jazyku. Ak potrebujete ďalšie informácie, obráťte sa na: viasure@certest.es.

FI Lataa suomenkielinen turvallisuusopas osoitteesta **certest.es/viasure/labeling**. Seuraa annettuja ohjeita. Mikäli tarvitset lisätietoja, ota yhteyttä: viasure@certest.es.

Consult **certest.es/viasure/labeling** if your language is not on the list. Contact viasure@certest.es if your language is not on the website.

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

ENGLISH

1. Intended purpose

The VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System is an automated qPCR test designed for the qualitative detection of DNA from *Mycoplasma genitalium* and specific point mutations (conferred by base substitutions in *23S rRNA*) implicated in macrolide resistance in vaginal swabs and male and female urine specimens from patients suspected of *M. genitalium* infection by their healthcare professional (HCP). This test is intended to be used as an aid in the diagnosis of infection with *M. genitalium* and the detection of potential resistance to macrolides 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' NA 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 *M. genitalium* and mutations at the *23S rRNA* gene associated to macrolide resistance.

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

Sexually transmitted infections (STIs) are a major public health problem worldwide, affecting quality of life and causing serious morbidity and mortality.

Mycoplasma genitalium (MG) is a common cause of non-gonococcal urethritis (NGU) and non-chlamydial urethritis in men and cervicitis in women, and is reported to be associated with pelvic inflammatory disease, infertility, and preterm birth (Baumann et al., 2018; Jensen et al., 2022; van der Schalk et al., 2020). MG is a flask-shaped organism with a slightly curved terminal organelle, capable of cause inflammation in the urogenital tract by adhesion to host epithelial cells, eliciting acute inflammatory signals via highly expressed innated immune sensors (Gnanadurai & Fifer, 2020). This microorganism is a slow growing organism known to be the smallest prokaryote capable of independent replication, which has emerged over the last few decades as a sexually transmitted pathogen due to its capacity of colonizing the reproductive tract in men and women (Gnanadurai & Fifer, 2020; Jensen et al., 2022). MG infection during pregnancy has been linked to preterm birth and may play a role in early pregnancy loss and neonatal infections (Heavey, 2017). It can

coexist with *Chlamydia trachomatis* and other sexually transmitted infections, making it challenging to determine its independent effects (Heavey, 2017).

Macrolides are a class of drugs used in the management and treatment of various bacterial infections, such as pneumonia, sinusitis, pharyngitis, tonsillitis, uncomplicated skin infections and otitis media or *Helicobacter pylori* infection, but are also commonly used to treat sexually transmitted infections such as gonococcal and chlamydial infections (Patel & Hashmi, 2023). The mechanism of action consists of binding to the bacterial 50S ribosomal subunit (close to the peptidyl transferase site (V region)) or to the A2058 and A2059 (*Escherichia coli* numbering) residues of 23S rRNA, causing the cessation of bacterial protein synthesis (van der Schalk et al., 2020).

The growing issue of macrolide resistance is a serious concern, with global resistance rates ranging between 30% and 100% (Gnanadurai & Fifer, 2020). Bacteria primarily develop resistance to macrolides through two mechanisms: single nucleotide polymorphisms (SNPs) and rRNA methylation. However, since MG lacks the necessary enzymes for methylation, it can only develop resistance via target modification through SNPs (van der Schalk et al., 2020). Resistance often results from a single base mutation at position A2058 or A2059 (based on *Escherichia coli* numbering) in the 23S rRNA, with minimal impact on bacterial fitness, enabling continued transmission (Gnanadurai & Fifer, 2020). Azithromycin is the most commonly used macrolide for treating MG infections, although josamycin and pristinamycin are now also being recommended (Gnanadurai & Fifer, 2020; Jensen et al., 2022; van der Schalk et al., 2020).

Culturing MG is difficult, as it requires weeks to months for growth, making standard susceptibility testing impractical. While testing for antibiotic susceptibility using MG strains grown in Vero cell cultures has produced results similar to traditional broth dilution methods, this approach is not feasible for primary diagnostic or most reference laboratories (Gnanadurai & Fifer, 2020).

Due to the absence of a cell wall, MG is not visible on Gram-stained genital secretions. Additionally, antibody-based serology tests are unreliable due to cross-reactivity with other mycoplasmas, including *Mycoplasma pneumoniae* (Gnanadurai & Fifer, 2020). However, nucleic acid amplification tests (NAATs), such as polymerase chain reaction and transcription-mediated amplification, provide accurate detection of MG (Baumann et al., 2018; Heavey, 2017; Jensen et al., 2022). NAATs may be performed on several types of genital samples, but vaginal swabs for women and first-void urine samples for men appear to provide the best results when testing for MG (Heavey, 2017). Because of the high rate of antimicrobial resistance, it is recommended to conduct simultaneous testing for genotypic resistance to guide appropriate treatment (Gnanadurai & Fifer, 2020).

3. Principle of the procedure

VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System is designed for the simultaneous qualitative detection and differentiation of DNA from *Mycoplasma genitalium* and specific genetic markers associated with macrolide resistance and sensitivity in vaginal swabs and male and female urine specimens. After DNA isolation, the identification of *M. genitalium* and macrolide resistance and sensitivity genetic markers is performed by the amplification of a specific region of the *MgPa adhesin* gene of *M. genitalium* and the *23S rRNA* gene, whose specific point mutations are implicated in macrolide resistance and sensitivity, using specific primers and fluorescent-labelled probes. The intended population of the test includes individuals with signs or suspicion of STI, sexual partners of individuals diagnosed with *M. genitalium* infection, and high-risk populations such as men who have sex with men (MSM), individuals with HIV, and patients attending sexual health clinics.

VIASURE *Mycoplasma genitalium with Macrolide Resistance* 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 *Mycoplasma genitalium with Macrolide Resistance* 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
Macrolide-resistance	475/520 (FAM)	*23S rRNA*
Macrolide-sensitivity	530/565 (HEX)	*23S rRNA*
Mycoplasma genitalium	585/630 (ROX)	*MgPa adhesin*
Endogenous Internal Control (EIC)	680/715 (Cy5.5)	*RNAse P*

Table 1. Target, channel and genes.

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

4. Reagents provided

VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System includes the following materials and reagents detailed in Table 2:

Reagent/Material	Description	Concentration Range	Code	Amount
Mycoplasma genitalium with Macrolide Resistance reaction tube	Lyoprotectors and Stabilizers	±6 g/100 mL*	1F 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 *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System with Cat. N°. 444224.
* 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 *Mycoplasma genitalium with Macrolide Resistance* 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).
- 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 for 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 *Mycoplasma genitalium with Macrolide Resistance* 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.*
Mycoplasma genitalium with Macrolide Resistance reaction tube (1F 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 *Mycoplasma genitalium with Macrolide Resistance* 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 *Mycoplasma genitalium with Macrolide Resistance* 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 *Mycoplasma genitalium with Macrolide Resistance* 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. Avoid contamination and contact with skin, eyes and clothes.
- 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 *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System has been tested in clinician-collected vaginal swab specimens using Copan eSwab® (Copan's Liquid Amies Elution Swab) and self-collected first-void male and female urine specimens in a sterile, preservative-free specimen collection cup. 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. Specimens submitted for molecular testing must be stored in controlled conditions so that nucleic acids do not degrade during storage. Repeated freeze-thaw cycles should be avoided in order to prevent degradation of the sample and nucleic acids.

The clinical specimens must be collected, transported and stored according to appropriate laboratory guidelines and/or laboratory policy manuals.

An internal specimen stability study was conducted with VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System using negative vaginal matrix collected in Copan eSwab®, female and male urine matrix spiked with the reference macrolide-sensitive strain (Amplirun® Total Macrolide Resistant MGE Control) at 3xLoD concentration. The stability was analysed by means of two different assays: primary stability (25°C: 4 hours and 2 days; 4°C: 1 and 2 days; -20°C: 12 months), and nested stability. For the nested stability assay, samples incubated at 25°C for 4 hours and samples incubated at 4°C for 1 day were analysed 3 days after addition to the sample buffer tube (SBT); samples incubated at 25°C for 2 days and samples incubated at 4°C for 2 days were analysed 7 days after addition to the SBT. Besides samples were analysed after going through five freezing (at -80°C) and thawing (at 25°C) cycles. Results showed a good performance of samples stored at all conditions tested, meeting the acceptance criteria initially stated.

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 400 µL of the vaginal swab or 750 µL of the urine specimen 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.

   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 *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System

Note: If you have already created the test for the VIASURE *Mycoplasma genitalium with Macrolide Resistance* 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 MGM.

   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	500 (µL) (urine specimens' protocol) 425 (µL) (vaginal specimens' protocol)
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. N/A = Not applicable.

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: 1F (concerning *Mycoplasma genitalium with Macrolide Resistance* 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)	Res	40	200	0	40
530/565 (HEX)	Sen	40	200	0	40
585/630 (ROX)	Mg	40	200	0	40
630/665 (Cy5)	-	-	-	-	-
680/715 (Cy5.5)	EIC	60	200	0	35/40*

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.

*Due to the variability in the number of human cells contained in urine specimens, the Cut-off value for endogenous internal control (EIC) target is set in 35 for vaginal swab specimens and 40 for female and male urine specimens to ensure a proper sample collection.

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	-	3.0	0.0	0.0	0.0
	530/565	3.0	-	0.0	0.0	0.0
	585/630	0.0	0.0	-	-	0.0
	630/665	0.0	0.0	0.0	0.0	-
	680/715	0.0	0.0	0.0	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	95°C	-
Denaturation and Annealing/Extension (Data collection)	Annealing/Extension	2-Temperature	45	10	95°C	-
				58	60°C	✓

Table 7. PCR protocol.

In the "Result Logic" tab:

12) In the "Target" field name your target: i.e. Res (up to seven alphanumeric characters). Repeat steps 12-15 for each target (i.e. Sen or Mg) 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	Res	PCR	✓
680/715	EIC	PCR	✓

Table 8. PCR channels selection in the "Result logic" tab for Res (Macrolide-resistant) target.

Wavelength	Alias	Type	Analyze
530/565	Sen	PCR	✓
680/715	EIC	PCR	✓

Table 9. PCR channels selection in the "Result logic" tab for Sen (Macrolide-sensitivity) target.

Wavelength	Alias	Type	Analyze
585/630	Mg	PCR	✓
680/715	EIC	PCR	✓

Table 10. PCR channels selection in the "Result logic" tab for Mg (*Mycoplasma genitalium)* 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 for vaginal specimens and tables 14-16 for urine samples.

Vaginal swabs specimens

Result	Res (475/520)	EIC (680/715)
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 Res (Macrolide resistance) target in vaginal specimen protocol. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Result	Sen (530/565)	EIC (680/715)
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 Sen (Macrolide sensitivity) target in vaginal specimen protocol. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Result	Mg (585/630)	EIC (680/715)
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 Mg *(Mycoplasma genitalium)* target in vaginal specimen protocol. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Urine specimens

Result	Res (475/520)	EIC (680/715)
POS	Valid	Valid
POS	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 14. List of the combination of result types and Result logic for Res (Macrolide resistance) target in urine specimen protocol. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Result	Sen (530/565)	EIC (680/715)
POS	Valid	Valid
POS	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 15. List of the combination of result types and Result logic for Sen (Macrolide sensitivity) target in urine specimen protocol. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Result	Mg (585/630)	EIC (680/715)
POS	Valid	Valid
POS	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 16. List of the combination of result types and Result logic for Mg *(Mycoplasma genitalium)* target in urine specimen protocol. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Note: According to the Ct Max previously defined (table 5):

i. The result type for Res (475/520), Sen (530/565) or Mg (585/630) channels is considered "Valid" when the Ct value obtained is ≤40; and "Invalid" when the Ct value obtained is >40.

ii. The result type for EIC (680/715) channel is considered "Valid" when the Ct value obtained is ≤35 for vaginal swabs specimens and ≤40 for urine specimens; and "Invalid" when the Ct value obtained is >35 and >40, respectively.

* Although the Cut-off for EIC target in urine specimens is 40, it is possible not to observe amplification curve in the EIC in the case of positive signal in both Res target or Sen target and Mg target. In this case, the Result logic is POS (Positive). See Section 9. Result interpretation.

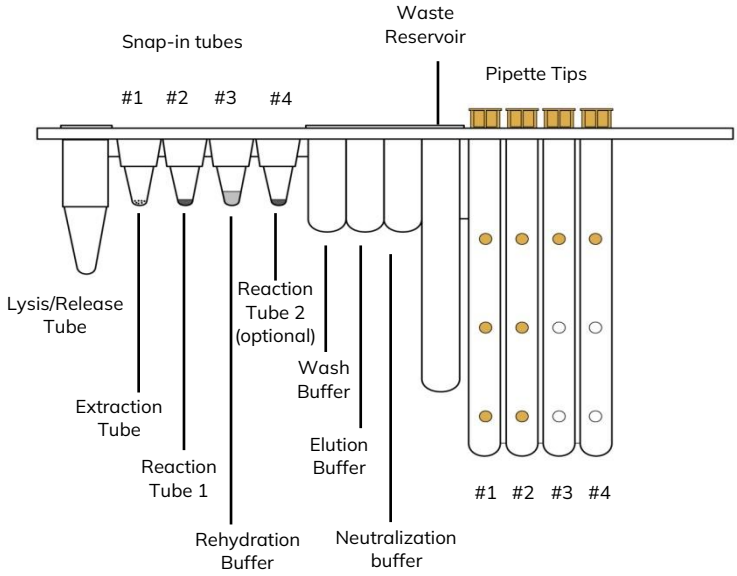
16) Click the "Save" button to save the test.

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 *Mycoplasma genitalium with Macrolide Resistance* reaction tubes (1F 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 MGM (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 Tables 17-18.

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. Please note that according to the result logic defined, it is possible not to observe amplification curve in the EIC in urine samples in the case of positive signal in both Mg target and Res target or Sen target. Read carefully the interpretation for patient’s individual urine samples (Table 18).

Results should be read and analyzed using the following tables:

Interpretation of results for vaginal swabs specimens

Macrolide-resistant (name target: Res)	Macrolide-sensitivity (name target: Sen)	*M. genitalium* (name target: Mg)	Interpretation for patient's individual samples
NEG	POS	POS	Macrolide-sensitivity and *M. genitalium* DNA detected, Macrolide-resistant DNA not detected
POS	NEG	POS	Macrolide-resistant and *M. genitalium* DNA detected, Macrolide-sensitivity DNA not detected
POS	POS	POS	Macrolide-resistant, Macrolide-sensitivity and *M. genitalium* DNA Detected INCONCLUSIVE RESULT[1]
POS	POS	NEG	Macrolide-resistant and Macrolide-sensitivity DNA Detected, *M. genitalium* DNA not detected. INCONCLUSIVE RESULT[1]
POS	NEG	NEG	Macrolide-resistant DNA Detected, Macrolide-sensitivity and *M. genitalium* DNA not Detected. INCONCLUSIVE RESULT[1]
NEG	POS	NEG	Macrolide-sensitivity DNA Detected, Macrolide-resistant and *M. genitalium* DNA not Detected. INCONCLUSIVE RESULT[1]
NEG	NEG	POS	*M. genitalium* DNA Detected, Macrolide-resistant and Macrolide-sensitivity DNA not Detected. INCONCLUSIVE RESULT[1,2]
NEG	NEG	NEG	Macrolide-resistant, Macrolide-sensitivity and *M. genitalium* DNA not detected
UNR	UNR	UNR	Unresolved (UNR) Result obtained in the presence of inhibitors in the PCR reaction, samples below the limit of detection or when a general problem (not reported by an error code) with the sample processing and/or amplification steps occurs.[3]
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.[4]
INC	INC	INC	Incomplete assay result (INC). Due to BD MAX™ System failure. Assay result displayed in case of failure to complete run.[4]

Table 17. Sample interpretation for vaginal swab samples.

1 Retesting is required. It is recommended to repeat the assay from the same Sample Buffer Tube (SBT) or from the primary sample preparing a new SBT. In case of a continued inconclusive result, obtain a new specimen (more concentrated if possible) and retest.

NOTE: Vaginal swab specimens can be kept without transfer to the SBT for up to 2 days if stored at 25°C or 4°C. 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 vaginal swab specimens can be kept in the SBT for a maximum of 7 days at 25ºC or 4ºC (if previously stored at 25ºC or 4ºC for a maximum of 2 days).

2 The kit detects the following mutations associated with macrolide resistance: *23S rRNA* gene (A2058T, A2058C, A2058G, A2059C, A2059G). In case of the presence of another mutation, the kit is not validated for its detection, therefore, amplification will be observed in the ROX channel (detection of *M. genitalium*) and not in the FAM and HEX channels (detection of macrolide resistance and macrolide sensitivity, respectively).

3 The Endogenous Internal Control (EIC) must show an amplification signal with Ct value ≤ 35 to be considered. If there is an absence of signal for EIC or Ct value > 35, the result is considered as Unresolved (UNR) and retesting is required. Check the result report and Ct values of the selected targets and carry out appropriate action considering the following:

I. When targeted genes results are invalid (Ct > 40), it is required to repeat the assay from the same SBT or from the primary sample preparing a new SBT. If an UNR result is obtained again, they must be considered two scenarios. On the one hand, the concentration of the sample may be below the LoD, therefore it would be recommended to obtain a more concentrated sample. On the other hand, the potential presence of inhibitors in the PCR reaction should be considered and it would be recommended to dilute these samples 1/10. Follow the laboratory guidelines and/or microbiology laboratory policy manuals.

II. When Macrolide-resistant, Macrolide-sensitivity and/or *M. genitalium* targeted genes results are valid (Ct ≤ 40), it is possible to see no amplification or amplification from EIC with a Ct value >35 when testing highly concentrated samples, due to a preferential amplification of target-specific nucleic acids. If considered necessary, dilute these samples 1/10, prepare the Sample Buffer Tube (SBT) again and repeat testing. Follow the laboratory guidelines and/or microbiology laboratory policy manuals.

4 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.

Interpretation of results for urine samples

Macrolide-resistant (name target: Res)	Macrolide-sensitivity (name target: Sen)	*M. genitalium* (name target: Mg)	Interpretation for patient's individual samples
NEG	POS	POS	Macrolide-sensitivity and *M. genitalium* DNA detected, Macrolide-resistant DNA not detected
POS	NEG	POS	Macrolide-resistant and *M. genitalium* DNA detected, Macrolide-sensitivity DNA not detected
POS	POS	POS	Macrolide-resistant, Macrolide-sensitivity and *M. genitalium* DNA Detected INCONCLUSIVE RESULT[1]
POS	POS	NEG	Macrolide-resistant and Macrolide-sensitivity DNA Detected, *M. genitalium* DNA not detected. INCONCLUSIVE RESULT[1]
POS	NEG	NEG	Macrolide-resistant DNA Detected, Macrolide-sensitivity and *M. genitalium* DNA not Detected. INCONCLUSIVE RESULT[1]

NEG	POS	NEG	Macrolide-sensitivity DNA Detected, Macrolide-resistant and *M. genitalium* DNA not Detected. INCONCLUSIVE RESULT[1]
NEG	NEG	POS	*M. genitalium* DNA Detected, Macrolide-resistant and Macrolide-sensitivity DNA not Detected. INCONCLUSIVE RESULT[1,2]
NEG	NEG	NEG	Macrolide-resistant, Macrolide-sensitivity and *M. genitalium* DNA not detected
UNR	POS	POS	Macrolide-sensitivity and *M. genitalium* DNA detected, Macrolide-resistant DNA not detected [3]
POS	UNR	POS	Macrolide-resistant and *M. genitalium* DNA detected, Macrolide-sensitivity DNA not detected [3]
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.[4]
POS	POS	UNR	Unresolved (UNR) – Repeat Testing [1]
POS	UNR	UNR	Unresolved (UNR) – Repeat Testing [1]
UNR	POS	UNR	Unresolved (UNR) – Repeat Testing [1]
UNR	UNR	POS	Unresolved (UNR) – Repeat Testing [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.[5]
INC	INC	INC	Incomplete assay result (INC). Due to BD MAX™ System failure. Assay result displayed in case of failure to complete run. [5]

Table 18. Sample interpretation for urine samples.

1 Retesting is required. It is recommended to repeat the assay from the same Sample Buffer Tube (SBT) or from the primary sample preparing a new SBT. In case of a continued inconclusive result, obtain a new specimen (more concentrated if possible) and retest.

NOTE: Urine specimens can be kept without transfer to the SBT for up to 4 hours if stored at 25°C or 1 day if stored at 4°C. 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 urine samples can be kept in the SBT for up to 3 days at 25°C (if previously stored at 25°C for a maximum of 4 hours) or for up to 3 days at 4ªC (if previously stored or at 4°C for a maximum of 1 day).

2 The kit detects the following mutations associated with macrolide resistance: *23S rRNA* gene (A2058T, A2058C, A2058G, A2059C, A2059G). In case of the presence of another mutation, the kit is not validated for its detection, therefore, amplification will be observed in the ROX channel (detection of *M. genitalium*) and not in the FAM and HEX channels (detection of macrolide-resistant and macrolide-sensitivity, respectively).

3 Although the Cut-off for Endogenous Internal Control (EIC) target in urine specimens is set in a value of 40, due to the low number of human cells in the urine it is allowed not to observe amplification curve in the EIC in the case of positive signal in both macrolide resistance (FAM channel) or sensitivity (HEX channel) target and *Mycoplasma genitalium* (ROX channel) target. In this

case, there is no risk of reporting a false-positive result because the amplification should be observed simultaneously in two different channels.

4 The 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 SBT or from the primary sample preparing a new SBT, or to obtain a more concentrated sample. 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. Follow the laboratory guidelines and/or microbiology laboratory policy manuals.

5 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). 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.
- VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System has been validated only with vaginal swabs and male and female urine 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 macrolide-resistant *M. genitalium* and/or macrolide-sensitivity *M. genitalium* samples containing high concentrations of target DNA or contamination due to PCR products from previous reactions.
- 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 *M. genitalium* strains or Macrolide-resistant and Macrolide-sensitivity genetic markers.
    - A bacterial 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 this 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.
- The kit detects the following mutations associated with macrolide resistance: *23S rRNA* gene (A2058T, A2058C, A2058G, A2059C, A2059G). Macrolide-resistant *M. genitalium* strain that contains the A2059T mutation, which is less prevalent, cannot be detected by the device. Just in this case, it is possible to observe amplification in ROX channel (detection of *M. genitalium*) and not in FAM and HEX channels (detection of macrolide resistance and macrolide sensitivity, respectively).
- 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 macrolide-resistant *M. genitalium* and macrolide-sensitivity *M. genitalium* targets sequences.
- Negative results do not preclude the presence of macrolide-resistant and/or macrolide-sensitivity *M. genitalium* 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

infection caused by *M. genitalium* 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) and/or antimicrobial resistance are negative and clinical observations, patient history and epidemiological information suggest that *M. genitalium* 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 *Mycoplasma genitalium with Macrolide Resistance* 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 *Mycoplasma genitalium with Macrolide Resistance* 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 *Mycoplasma genitalium with Macrolide Resistance* 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 vaginal matrix, male and female urine specimens containing a known concentration of specific and synthetic DNA belonging to macrolide-sensitive or macrolide-resistant *M. genitalium* (ranging from 2E+07 to 2E+00 copies/µL). The arithmetic mean, standard deviation and coefficient of variation of Ct values, as well as the efficiency and the regression coefficient of the PCR reaction were calculated, and examples of the amplification plot resulting from an assay in one of the matrixes evaluated are included below.

Figure 2. Dilution series of macrolide-sensitive *M. genitalium* (*23S rRNA* gene (wild type) + *MgPA adhesin* gene) synthetic DNA (2E+07 to 2E+00 copies/µL) template run on the BD MAX™ System (530/565 (HEX) channel).

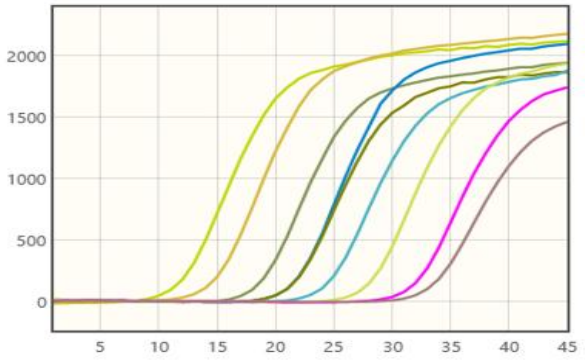


Figure 3. Dilution series of macrolide-resistant *M. genitalium* (*23S rRNA* gene (A2058C mutation) + *MgPA adhesin* gene) synthetic DNA (2E+07 to 2E+00 copies/µL) template run on the BD MAX™ System (475/520 (FAM) channel).

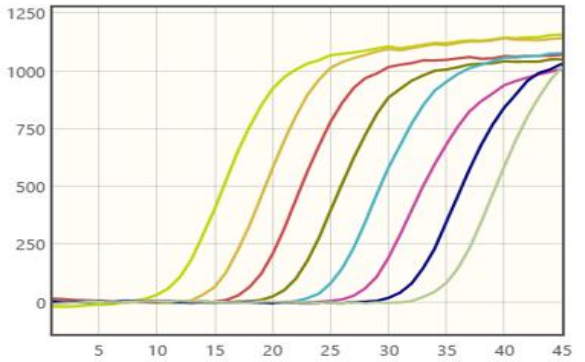


Figure 4. Dilution series of macrolide-resistant *M. genitalium* (*23S rRNA* gene (A2058G mutation) + *MgPA adhesin* gene) synthetic DNA (2E+07 to 2E+00 copies/µL) template run on the BD MAX™ System (475/520 (FAM) channel).

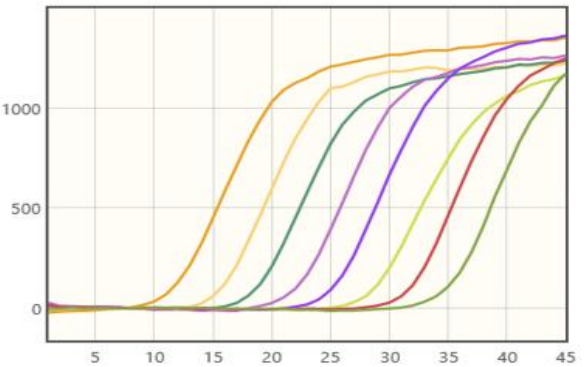


Figure 5. Dilution series of macrolide-resistant *M. genitalium* (*23S rRNA* gene (A2058T mutation) + *MgPA adhesin* gene) synthetic DNA (2E+07 to 2E+00 copies/µL) template run on the BD MAX™ System (475/520 (FAM) channel).

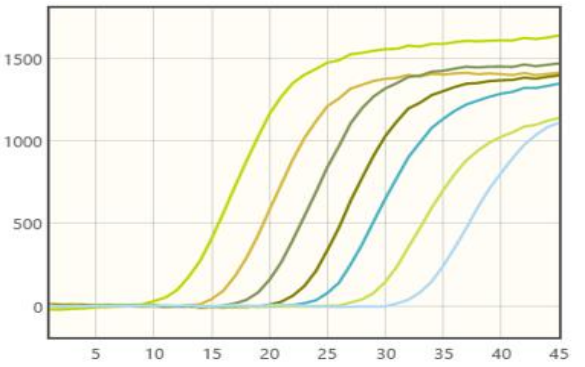


Figure 6. Dilution series of macrolide-resistant *M. genitalium* (*23S rRNA* gene (A2059C mutation) + *MgPA adhesin* gene) synthetic DNA (2E+07 to 2E+00 copies/µL) template run on the BD MAX™ System (475/520 (FAM) channel).

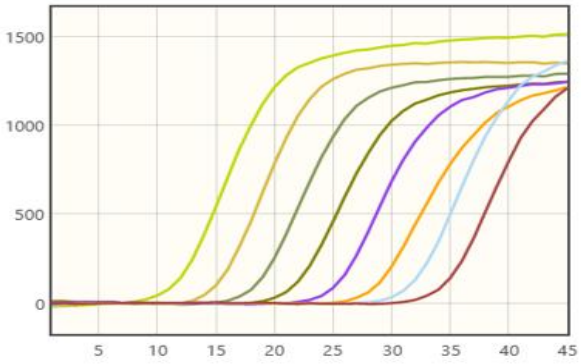
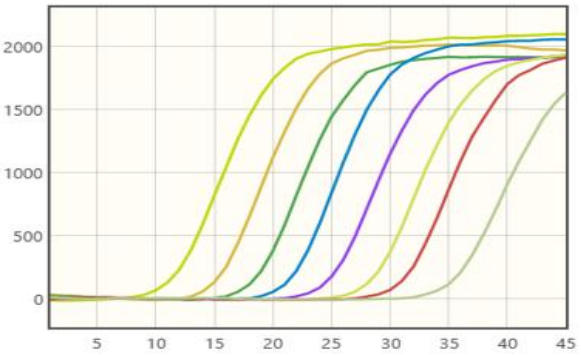


Figure 7. Dilution series of macrolide-resistant *M. genitalium* (*23S rRNA* gene (A2059G mutation) + *MgPA adhesin* gene) synthetic DNA (2E+07 to 2E+00 copies/µL) template run on the BD MAX™ System (475/520 (FAM) channel).



12.2. Analytical sensitivity. Limit of Detection (LoD)

Analytical sensitivity or limit of detection (LoD) of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System was analysed with three lots using vaginal matrix, male and female urine specimens. The reference strains or synthetic DNA (in case no strain available), used are detailed in the following table:

Target		Strain/Synthetic DNA	External reference
Macrolide-sensitivity + *Mycoplasma genitalium*	*23S rRNA* gene (wild type) + *MgPA adhesin* gene	*Mycoplasma genitalium* strain M30	49895™
Macrolide-resistant + *Mycoplasma genitalium*	*23S rRNA* gene (A2058C mutation) + *MgPA adhesin* gene	Synthetic DNA (MGRXPC)	N/A
	23S rRNA gene (A2058G mutation) + *MgPA adhesin* gene	AMPLIRUN® TOTAL MACROLIDE RESISTANT MGE CONTROL	MBTC029
	23S rRNA gene (A2058T mutation) + *MgPA adhesin* gene	Synthetic DNA (MGRXPC)	N/A
	23S rRNA gene (A2059C mutation) + *MgPA adhesin* gene	Synthetic DNA (MGRXPC)	N/A
	23S rRNA gene (A2059G mutation) + *MgPA adhesin* gene	AMPLIRUN® TOTAL MACROLIDE RESISTANT MGE CONTROL	MBTC029

Table 19. Reference strains and synthetic DNA used for Limit of Detection assay.

VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System showed the detection limit indicated in the following table, with a positive rate of ≥ 95%.

Limit of Detection (LoD) (copies/µl)						
Matrix	Macrolide-sensitive *M. genitalium*	Macrolide-resistant *M. genitalium* (A2059G)	Macrolide-resistant *M. genitalium* (A2058G)	Macrolide-resistant *M. genitalium* (A2058C)	Macrolide-resistant *M. genitalium* (A2058T)	Macrolide-resistant *M. genitalium* (A2059C)
Female urine	3.33E-02	3.00E+00	9.99E-01	6.00E+00	6.00E+00	2.00E+00
Male urine	1.00E-01	9.00E-01	3.00E-01	2.00E+00	2.00E+00	2.00E+00
Vaginal	1.00E-01	2.70E+00	3.33E-01	2.00E+00	6.00E+00	2.00E+00

Table 20. Limit of detection of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System.

Therefore, the results obtained show that the VIASURE device's sensitivity is consistent and reliable across production batches.

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 and synthetic DNA belonging to macrolide-sensitive *M. genitalium* and macrolide-resistant *M. genitalium*. Results allowed to confirm the correct target detection from of the targets at the measuring range indicated in the following table:

	Measuring range (copies/µl)					
Target	Female urine matrix		Male urine matrix		Vaginal matrix	
Macrolide-sensitive *M. genitalium*	2E+07	2E+01	2E+07	2E+00	2E+07	2E+00
Macrolide-resistant *M. genitalium* (A2059G)	2E+07	2E+00	2E+07	2E+00	2E+07	2E+00
Macrolide-resistant *M. genitalium* (A2058G)	2E+07	2E+01	2E+07	2E+01	2E+07	2E+00
Macrolide-resistant *M. genitalium* (A2058C)	2E+07	2E+01	2E+07	2E+00	2E+07	2E+00
Macrolide-resistant *M. genitalium* (A2058T)	2E+07	2E+00	2E+07	2E+01	2E+07	2E+01
Macrolide-resistant *M. genitalium* (A2059C)	2E+07	2E+01	2E+07	2E+01	2E+07	2E+00

Table 21. Measuring range of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System.

In conclusion, measuring range of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System was successfully determined according to the acceptance validation criteria initially stated, ensuring reliable, accurate and reproducible results across a wide spectrum of bacterial loads, affirming its utility in various clinical diagnostic scenarios.

12.4. Accuracy

12.4.1. Trueness (Veracity)

The veracity of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System was evaluated by testing reference material listed below.

1. **Synthetic cDNA fragments**
   - Synthetic cDNA fragment for *23S rRNA* (wild type) and *MgPA adhesin* genes of macrolide-sensitive *M. genitalium*: MGRXPC, HEX and ROX channels.
   - Synthetic cDNA fragment for *23S rRNA* (A2058C mutation) and *MgPA adhesin* genes of macrolide-resistant *M. genitalium*: MGRXPC, FAM and ROX channels.
   - Synthetic cDNA fragment for *23S rRNA* (A2058G mutation) and *MgPA adhesin* genes of *M. genitalium* macrolides resistant: MGRXPC, FAM and ROX channels.
   - Synthetic cDNA fragment for *23S rRNA* (A2058T mutation) and *MgPA adhesin* genes of *M. genitalium* macrolides resistant: MGRXPC, FAM and ROX channels.
   - Synthetic cDNA fragment for *23S rRNA* (A2059C mutation) and *MgPA adhesin* genes of *M. genitalium* macrolides resistant: MGRXPC, FAM and ROX channels.
   - Synthetic cDNA fragment for *23S rRNA* (A2059G mutation) and *MgPA adhesin* genes of M. *genitalium* macrolides resistant: MGRXPC, FAM and ROX channels.

2. **The American Type Culture Collection (ATCC®)**

External Reference	Microorganism	Product name	Variety
49895	*Mycoplasma genitalium*	*Mycoplasma genitalium* Tully et al.	Strain M30
33530	*Mycoplasma genitalium*	*Mycoplasma genitalium* Tully et al.	Strain G37
49898	*Mycoplasma genitalium*	*Mycoplasma genitalium* Tully et al.	Strain TW48-5G
49123	*Mycoplasma genitalium*	*Mycoplasma genitalium* Tully et al.	Strain TW10-5G
49899	*Mycoplasma genitalium*	*Mycoplasma genitalium* Tully et al.	Strain UMTB-10G
49896	*Mycoplasma genitalium*	*Mycoplasma genitalium* Tully et al.	Strain TW10-6G
49897	*Mycoplasma genitalium*	*Mycoplasma genitalium* Tully et al.	Strain R32G [R32]

Table 22. Reference material from American Type Culture Collection (ATCC).

3. **Controls**

External Reference	Microorganism	Product name	Variety
MBTC029	*Mycoplasma genitalium*	AMPLIRUN® TOTAL MACROLIDE RESISTANT MGE CONTROL	- Sensitive type strain -A2059G mutation in *23 rRNA* gene - A2058G mutation in *23 rRNA* gene

Table 23. Control material from Vircell S.L.

4. External Quality Assessment (EQA) programs

External Reference	Provenance	Microorganism	Product name	Variety
MG23S-03	QCMD	*Mycoplasma genitalium*	*Mycoplasma genitalium* G37 (WT)	Strain G37
MG23S-06	QCMD	*Mycoplasma genitalium*	*Mycoplasma genitalium* M6303	*23S rDNA* mutation A2059G, macrolide resistant
MG23S-07	QCMD	*Mycoplasma genitalium*	*Mycoplasma genitalium* M6593	*23S rDNA* mutation A2059G, macrolide resistant
MG101S-04	QCMD	*Mycoplasma genitalium*	*Mycoplasma genitalium* M6303	Macrolide resistant
MG101S-06	QCMD	*Mycoplasma genitalium*	*Mycoplasma genitalium* M6593	Macrolide resistant

Table 24. Reference material from External Quality Assessment (EQA) programs.

12.4.2. Precision

To determine the precision of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System, intra- assay (repeatability), inter-assay, inter-batch and inter-equipment assays (reproducibility) were performed using vaginal matrix, female and male urine matrix spiked with a known concentration of the reference strains: *Mycoplasma genitalium* strain M30 (Ref: 49895™) for macrolide-sensitive *M. genitalium* and AMPLIRUN® TOTAL MACROLIDE RESISTANT MGE CONTROL (Ref: MBTC029) for macrolide-resistant *M. genitalium*.

Intra-assay

The intra-assay was tested by analysing six replicates of all samples in the same run using VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System. A summary of results is shown in the table below.

Macrolide-sensitive *M. genitalium* strain						
Matrix	**Target**	**Sample**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
Male urine	Macrolide-sensitive	3xLoD	530/565 (HEX)	32.02	0.27	0.85
		5xLoD	530/565 (HEX)	30.98	0.32	1.03
		Negative control	530/565 (HEX)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	31.57	0.34	1.09
		5xLoD	585/630 (ROX)	30.72	0.26	0.86
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	34.38	0.80	2.32
		5xLoD	680/715 (CY5.5)	34.05	0.53	1.55
		Negative control	680/715 (CY5.5)	35.23	1.20	3.41
Female urine	Macrolide-sensitive	3xLoD	530/565 (HEX)	34.02	0.83	2.44
		5xLoD	530/565 (HEX)	33.50	0.67	2.01
		Negative control	530/565 (HEX)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	33.65	0.28	0.84
		5xLoD	585/630 (ROX)	33.05	0.54	1.64
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.

	EIC	3xLoD	680/715 (CY5.5)	32.75	0.50	1.53
		5xLoD	680/715 (CY5.5)	33.22	0.37	1.12
		Negative control	680/715 (CY5.5)	32.97	0.23	0.71
Vaginal	Macrolide-sensitive	3xLoD	530/565 (HEX)	32.48	0.29	0.88
		5xLoD	530/565 (HEX)	30.90	0.71	2.30
		Negative control	530/565 (HEX)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	31.32	0.44	1.41
		5xLoD	585/630 (ROX)	30.10	0.66	2.19
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	31.12	0.47	1.50
		5xLoD	680/715 (CY5.5)	31.20	0.61	1.97
		Negative control	680/715 (CY5.5)	31.23	0.25	0.80

Table 25. Intra-assay results of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System with macrolide-sensitive *M. genitalium* strain. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, Neg = negative, n.a. = not applicable.

Macrolide-resistant *M. genitalium* strain						
Matrix	**Target**	**Sample**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
Male urine	Macrolide-resistant	3xLoD	475/520 (FAM)	36.17	0.62	1.70
		5xLoD	475/520 (FAM)	35.05	0.63	1.81
		Negative control	475/520 (FAM)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	35.00	0.99	2.84
		5xLoD	585/630 (ROX)	34.08	0.50	1.48
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	32.58	1.24	3.80
		5xLoD	680/715 (CY5.5)	32.12	0.39	1.22
		Negative control	680/715 (CY5.5)	33.28	0.66	1.99
Female urine	Macrolide-resistant	3xLoD	475/520 (FAM)	33.72	0.51	1.52
		5xLoD	475/520 (FAM)	33.30	0.37	1.12
		Negative control	475/520 (FAM)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	32.67	0.48	1.48
		5xLoD	585/630 (ROX)	31.62	0.27	0.86
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	31.45	0.29	0.92
		5xLoD	680/715 (CY5.5)	30.73	0.56	1.83
		Negative control	680/715 (CY5.5)	34.45	0.44	1.27
Vaginal	Macrolide-resistant	3xLoD	475/520 (FAM)	34.33	1.34	3.90
		5xLoD	475/520 (FAM)	33.75	0.27	0.79
		Negative control	475/520 (FAM)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	32.55	1.15	3.52
		5xLoD	585/630 (ROX)	32.02	0.34	1.07
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	32.13	0.73	2.28
		5xLoD	680/715 (CY5.5)	31.20	0.72	2.29
		Negative control	680/715 (CY5.5)	34.15	0.50	1.46

Table 26. Intra-assay results of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System with macrolide-resistant *M. genitalium* strain. (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 *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System. A summary of results is shown in the table below.

Macrolide-sensitive *M. genitalium* strain						
Matrix	**Target**	**Sample**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
Male urine	**Macrolide-sensitive**	3xLoD	530/565 (HEX)	34.48	0.85	2.46
		5xLoD	530/565 (HEX)	33.66	0.75	2.22
		Negative control	530/565 (HEX)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	34.05	0.60	1.75
		5xLoD	585/630 (ROX)	33.38	0.56	1.68
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	34.06	1.65	4.85
		5xLoD	680/715 (CY5.5)	34.04	1.85	5.44
		Negative control	680/715 (CY5.5)	34.10	1.74	5.09
Female urine	**Macrolide-sensitive**	3xLoD	530/565 (HEX)	34.36	1.80	5.24
		5xLoD	530/565 (HEX)	34.01	1.84	5.42
		Negative control	530/565 (HEX)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	34.56	1.84	5.33
		5xLoD	585/630 (ROX)	33.78	1.33	3.94
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	33.81	1.75	5.18
		5xLoD	680/715 (CY5.5)	33.29	0.98	2.94
		Negative control	680/715 (CY5.5)	34.04	1.39	4.10
Vaginal	**Macrolide-sensitive**	3xLoD	530/565 (HEX)	33.06	0.90	2.73
		5xLoD	530/565 (HEX)	31.90	0.68	2.13
		Negative control	530/565 (HEX)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	32.04	0.89	2.77
		5xLoD	585/630 (ROX)	30.98	0.69	2.24
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	31.21	0.51	1.62
		5xLoD	680/715 (CY5.5)	31.03	0.55	1.76
		Negative control	680/715 (CY5.5)	30.84	0.77	2.49

Table 27. Inter-assay results of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System with macrolide-sensitive *M. genitalium* strain. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, Neg = negative, n.a. = not applicable.

Macrolide-resistant *M. genitalium* strain						
Matrix	**Target**	**Sample**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
Male urine	**Macrolide-resistant**	3xLoD	475/520 (FAM)	35.98	1.00	2.78
		5xLoD	475/520 (FAM)	35.31	0.90	2.56
		Negative control	475/520 (FAM)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	34.61	1.08	3.12
		5xLoD	585/630 (ROX)	33.52	0.59	1.76
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	33.10	0.76	2.30
		5xLoD	680/715 (CY5.5)	32.54	0.74	2.27
		Negative control	680/715 (CY5.5)	34.65	1.30	3.74
Female urine	**Macrolide-resistant**	3xLoD	475/520 (FAM)	33.08	1.28	3.87
		5xLoD	475/520 (FAM)	31.98	0.79	2.48
		Negative control	475/520 (FAM)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	31.92	1.01	3.15
		5xLoD	585/630 (ROX)	30.91	0.70	2.27
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	31.15	0.43	1.40
		5xLoD	680/715 (CY5.5)	30.14	0.54	1.78
		Negative control	680/715 (CY5.5)	33.54	1.09	3.25
Vaginal	**Macrolide-resistant**	3xLoD	475/520 (FAM)	34.72	1.34	3.87
		5xLoD	475/520 (FAM)	34.22	1.27	3.71

		Negative control	475/520 (FAM)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	32.52	1.16	3.57
		5xLoD	585/630 (ROX)	32.36	1.33	4.11
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	30.61	0.44	1.42
		5xLoD	680/715 (CY5.5)	30.59	0.64	2.08
		Negative control	680/715 (CY5.5)	30.98	0.55	1.78

Table 28. Inter-assay results of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System with macrolide-resistant *M. genitalium* strain. (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 *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System. A summary of results is shown in the table below.

Macrolide-sensitive *M. genitalium* strain						
Matrix	**Target**	**Sample**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
Male urine	Macrolide-sensitive	3xLoD	530/565 (HEX)	32.77	0.89	2.71
		5xLoD	530/565 (HEX)	31.46	0.57	1.81
		Negative control	530/565 (HEX)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	31.70	0.47	1.48
		5xLoD	585/630 (ROX)	30.52	0.50	1.64
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	34.74	0.67	1.92
		5xLoD	680/715 (CY5.5)	34.96	1.48	4.22
		Negative control	680/715 (CY5.5)	35.44	1.01	2.85
Female urine	Macrolide-sensitive	3xLoD	530/565 (HEX)	34.72	0.81	2.34
		5xLoD	530/565 (HEX)	33.39	0.77	2.39
		Negative control	530/565 (HEX)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	34.24	1.19	3.46
		5xLoD	585/630 (ROX)	32.96	0.77	2.32
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	35.46	1.79	5.08
		5xLoD	680/715 (CY5.5)	34.58	1.15	3.32
		Negative control	680/715 (CY5.5)	33.53	0.58	1.74
Vaginal	Macrolide-sensitive	3xLoD	530/565 (HEX)	31.62	0.98	3.11
		5xLoD	530/565 (HEX)	30.88	0.83	2.67
		Negative control	530/565 (HEX)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	31.02	0.94	3.03
		5xLoD	585/630 (ROX)	30.37	0.73	2.40
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	31.29	0.46	1.47
		5xLoD	680/715 (CY5.5)	31.26	0.38	1.23
		Negative control	680/715 (CY5.5)	31.06	0.39	1.27

Table 29. Inter-batch results of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System with macrolide-sensitive *M. genitalium* strain. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, Neg = negative, n.a. = not applicable.

Macrolide-resistant *M. genitalium* strain						
Matrix	**Target**	**Sample**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
Male urine	**Macrolide-resistant**	3xLoD	475/520 (FAM)	36.46	1	2.73
		5xLoD	475/520 (FAM)	35.47	0.76	2.14
		Negative control	475/520 (FAM)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	34.68	0.76	2.20
		5xLoD	585/630 (ROX)	33.85	0.63	1.86
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	33.57	1.25	3.73
		5xLoD	680/715 (CY5.5)	32.55	0.61	1.88
		Negative control	680/715 (CY5.5)	32.49	0.73	2.24
Female urine	**Macrolide-resistant**	3xLoD	475/520 (FAM)	32.81	0.96	2.93
		5xLoD	475/520 (FAM)	32.38	0.84	2.59
		Negative control	475/520 (FAM)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	31.27	1.15	3.68
		5xLoD	585/630 (ROX)	30.57	0.92	3.02
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	30.25	0.91	3.02
		5xLoD	680/715 (CY5.5)	30.13	0.58	1.92
		Negative control	680/715 (CY5.5)	33.51	1.02	3.06
Vaginal	**Macrolide-resistant**	3xLoD	475/520 (FAM)	34.34	1.39	4.05
		5xLoD	475/520 (FAM)	33.96	0.62	1.82
		Negative control	475/520 (FAM)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	32.66	1.26	3.85
		5xLoD	585/630 (ROX)	32.23	0.51	1.60
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	31.43	0.75	2.40
		5xLoD	680/715 (CY5.5)	30.76	0.58	1.87
		Negative control	680/715 (CY5.5)	32.24	1.44	4.46

Table 30. Inter-batch results of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System with macrolide-resistant *M. genitalium* strain. (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 six replicates of the same samples used for intra-assay, inter-assay and inter-batch, using the VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System. These assays were run at three laboratory sites with three different BD MAX™ System. A summary of results is shown in the table below.

Macrolide-sensitive *M. genitalium* strain						
Matrix	**Target**	**Sample**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
Male urine	**Macrolide-sensitive**	3xLoD	530/565 (HEX)	31.96	0.43	1.35
		5xLoD	530/565 (HEX)	31.00	0.40	1.30
		Negative control	530/565 (HEX)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	31.78	0.56	1.75
		5xLoD	585/630 (ROX)	30.88	0.9	0.94
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	34.48	0.87	2.53
		5xLoD	680/715 (CY5.5)	34.51	0.87	2.51
		Negative control	680/715 (CY5.5)	32.46	0.62	1.92
Female urine	**Macrolide-sensitive**	3xLoD	530/565 (HEX)	34.31	0.97	2.81
		5xLoD	530/565 (HEX)	33.72	0.52	1.54

		Negative control	530/565 (HEX)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	33.85	0.80	2.35
		5xLoD	585/630 (ROX)	33.38	0.63	1.88
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	33.16	0.56	1.69
		5xLoD	680/715 (CY5.5)	33.06	0.44	1.32
		Negative control	680/715 (CY5.5)	33.08	0.62	1.87
Vaginal	Macrolide-sensitive	3xLoD	530/565 (HEX)	32.55	0.81	2.48
		5xLoD	530/565 (HEX)	32.57	0.52	1.60
		Negative control	530/565 (HEX)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	31.42	0.88	2.81
		5xLoD	585/630 (ROX)	31.54	0.44	1.40
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	31.12	0.33	1.07
		5xLoD	680/715 (CY5.5)	33.58	0.56	1.68
		Negative control	680/715 (CY5.5)	30.84	0.50	1.61

Table 31. Inter-equipment results of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System with macrolide-sensitive *M. genitalium* strain. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, Neg = negative, n.a. = not applicable.

Macrolide-resistant *M. genitalium* strain						
Matrix	**Target**	**Sample**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
Male urine	Macrolide-resistant	3xLoD	475/520 (FAM)	36.58	1.31	3.58
		5xLoD	475/520 (FAM)	35.13	0.53	1.51
		Negative control	475/520 (FAM)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	34.81	0.86	2.46
		5xLoD	585/630 (ROX)	34.00	0.44	1.30
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	32.29	0.90	2.79
		5xLoD	680/715 (CY5.5)	31.88	0.67	2.11
		Negative control	680/715 (CY5.5)	32.89	0.77	2.34
Female urine	Macrolide-resistant	3xLoD	475/520 (FAM)	33.67	0.43	1.28
		5xLoD	475/520 (FAM)	32.85	0.64	1.96
		Negative control	475/520 (FAM)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	32.46	0.44	1.35
		5xLoD	585/630 (ROX)	31.44	0.42	1.34
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	31.40	0.26	0.82
		5xLoD	680/715 (CY5.5)	30.66	0.43	1.41
		Negative control	680/715 (CY5.5)	34.59	0.65	1.87
Vaginal	Macrolide-resistant	3xLoD	475/520 (FAM)	34.47	0.89	2.59
		5xLoD	475/520 (FAM)	33.46	0.61	1.84
		Negative control	475/520 (FAM)	Neg	n.a.	n.a.
	M. genitalium	3xLoD	585/630 (ROX)	32.75	0.77	2.35
		5xLoD	585/630 (ROX)	31.77	0.63	1.97
		Negative control	585/630 (ROX)	Neg	n.a.	n.a.
	EIC	3xLoD	680/715 (CY5.5)	31.87	0.57	1.78
		5xLoD	680/715 (CY5.5)	31.27	0.59	1.88
		Negative control	680/715 (CY5.5)	33.72	0.54	1.61

Table 32. Inter-equipment results of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System with macrolide-resistant *M. genitalium* strain. (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 across all tested matrixes, complying with the acceptance validation criteria initially stated.

12.5. Analytical specificity and reactivity

The analytical specificity and analytical reactivity were evaluated for the VIASURE *Mycoplasma genitalium with Macrolide Resistance* 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 two components to be considered for analytical specificity: Cross-reactivity and Interference. Cross-reactivity may occur when genetically related sequences are present in a patient specimen, while interference may happen if the presence of specific substances potentially present in the sample matrixes 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/or search and/or alignment tools as BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and an in-house bioinformatic analysis software. BLAST analysis over each primer and probe over the NCBI Genbank Nucleotide Database and an in-house bioinformatic analysis were performed.

Aligned sequences with a percentage of alignment less than 80% of homology were considered unlikely to be detected. Results obtained were as follows:

***Mycoplasma genitalium* (*MgPA adhesin* gene)**

All analysed sequences were below 80% of homology with *Mycoplasma genitalium* (*MgPA adhesin* gene) primers and probe set.

Therefore, the VIASURE *Mycoplasma genitalium* target designs should not cause false positives in detecting *Mycoplasma genitalium* when other organisms are present.

***Mycoplasma genitalium* (*23S rRNA* gene)**

BLAST analysis filtered by the *23S rRNA* gene of *M. genitalium* (excluding *M. genitalium*) shows high homology between the primers and probes and several "*Uncultured Mycoplasma sp.* (taxonomy ID: 167967)" sequences and one *"Synthetic Mycoplasma genitalium JCVI-1.0* (taxonomy ID: 488339)" sequence.

"Uncultured Mycoplasma sp. (taxonomy ID: 167967)" sequences are detected with the primers and probes of macrolide-resistant and macrolide-sensitive *M. genitalium*, therefore, there is no risk of cross-reactivity in

the product, since to give a sample as positive, it is necessary the detection in the channel of the microorganism (*M. genitalium*) and in the channel of the macrolide-resistant or macrolide-sensitivity.

"Synthetic Mycoplasma genitalium JCVI-1.0 (taxonomy ID: 488339)" is detected with the macrolide-sensitive primers and probe. It is a sequence corresponding to the target microorganism and must be detected. This sequence appears in the cross-reactivity as it has a different taxonomy ID than the one used in the exclusion.

Therefore, none of the sequences analysed, including those showing a homology higher than 80%, could affect the correct detection of *Mycoplasma genitalium* (*23S rRNA* gene).

Analytical specificity wet testing

Cross-reactivity wet testing

The cross-reactivity of VIASURE *Mycoplasma genitalium with Macrolide Resistance* 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 and 1E+04 – 1E+05 pfu (plaque-forming unit)/ml for viruses). No cross-reactivity was detected between any of the following microorganisms tested, except for the targeted microorganisms.

Cross-reactivity testing					
Acinetobacter baumannii	-	*Haemophilus ducreyi*	-	*Mycoplasma genitalium* strain TW48-5G	-/+
Aspergillus fumigatus	-	Haemophilus influenzae	-	*Mycoplasma genitalium* strain UMTB-10G	-/+
Atopobium vaginae	-	Herpes simplex virus 2	-	*Mycoplasma genitalium* Tully et al. strain TW10-5G	-/+
Bacteroides fragilis	-	Human Herpesvirus 1, Strain HF	-	*Mycoplasma hominis*	-
Candida albicans	-	Human papillomavirus 16	-	*Neisseria gonorhoeae*	-
Candida dubliniensis	-	Human papillomavirus 18	-	*Neisseria meningitidis*	-
Candida glabrata	-	*Klebsiella oxytoca*	-	*Proteous mirabilis*	-
Candida krusei	-	*Klebsiella pneumoniae*	-	*Pseudomonas aeruginosa*	-
Candida parapsilosis	-	*Listeria innocua*	-	*Serratia marcescens*	-
Candida tropicalis	-	*Listeria ivanovii* subsp. ivanovii	-	*Staphylococccus aureus*	-
Chlamydia trachomatis Serovars Panel	-	*Listeria monocytogenes*	-	*Stenotrophomonas maltophilia*	-
Enterobacter cloacae	-	*Listeria monocytogenes*	-	*Streptococcus pneumoniae*	-
Enterococcus faecalis	-	*Mycoplasma genitalium* strain G37	-/+	*Treponema pallidum*	-
Enterococcus faecium	-	*Mycoplasma genitalium* strain R32G [R32]	-/+	*Trichomonas vaginalis*	-
Escherichia coli	-	*Mycoplasma genitalium* strain TW10-6G	-/+	*Ureaplasma urealyticum*	-
Gardnerella vaginalis	-				

Table 33. Reference 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 met the acceptance validation criteria and indicate high specificity of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System for the detection of the targeted macrolide-resistant *M. genitalium* and macrolide-sensitive *M. genitalium*, 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 interfering microbial agents

A study of interfering microbial agents was performed to analyse the potential interfering microbial agents for VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System. A panel of different clinically, environmentally, and phylogenetically relevant microorganisms was tested in presence of the reference strains: *Mycoplasma genitalium* strain M30 (Ref: 49895™) for macrolide-sensitive *M. genitalium* and AMPLIRUN® TOTAL MACROLIDE RESISTANT MGE CONTROL (Ref: MBTC029) for macrolide-resistant *M. genitalium*. 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 and 1E+04 – 1E+05 pfu (plaque-forming unit)/ml for viruses). Each point analysis was conducted once per specimen.

Positive Matrix Control and Negative Matrix Control (PMC and NMC, respectively) are included as controls of the test. PMC corresponds to the vaginal matrix, female and male urine matrix spiked with the specific macrolide-sensitive and macrolide-resistant *M. genitalium* strains without any interfering microbial agent, whereas NMC corresponds to the negative matrixes without any interfering microbial agent.

Microorganism Name	Concentration tested	Result
PMC	-	n.a.
NMC	-	n.a.
Acinetobacter baumannii	8.10E+05 cfu/mL	N.I
Gardnerella vaginalis	4.40E+01 cfu/ul	N.I
Haemophilus influenzae	5.20E+02cfu/ul	N.I
Herpes simplex virus 1	1.60E+05 TCID50/mL	N.I
Listeria monocytogenes	5.80E+06 ufc/ml	N.I
Mycoplasma hominis	4.70E+06 cfu/ml	N.I
Pseudomonas aeruginosa	4.09E+05 cfu/ml	N.I
Staphylococccus aureus	4.60E+05 cfu/ml	N.I
Streptococcus pneumoniae	1.80E+01 cfu/ul	N.I
Treponema pallidum	3.40E+04 cells/ml	N.I
Klebsiella oxytoca	7.60E+03 cop/ul	N.I
Escherichia coli	n.a.	N.I
Aspergillus fumigatus	n.a.	N.I

Atopobium vaginae	4.52E+03 cfu/ul	N.I
Candida albicans	4.18E+04 cfu/ul	N.I
Candida glabrata	2.46E+03 cfu/ul	N.I
Candida tropicalis	2.88E+03 cfu/ul	N.I
Chlamydia trachomatis Serovars E	6.40E+05 IFU/ml	N.I
Enterobacter cloacae	1.28E+03 cfu/ul	N.I
Enterococcus faecalis	5.00E+04 cfu/ml	N.I
Enterococcus faecium	3.50E+04 cfu/ml	N.I
Herpes simplex virus 2	7.24E+03 TCID50/mL	N.I
Human papillomavirus 16	1.00E+02 IU/ul	N.I
Human papillomavirus 18	1.00E+02 IU/ul	N.I
Klebsiella pneumoniae	3.65E+03 cfu/ul	N.I
Listeria monocytogenes	n.a.	N.I
Neisseria gonorhoeae	6.20E+03 cfu/ul	N.I
Neisseria meningitidis	5.70E+04 cfu/ul	N.I
Proteous mirabilis	2.55E+03 cfu/ul	N.I
Serratia marcescens	n.a.	N.I
Stenotrophomonas maltophilia	9.20E+03 cfu/ul	N.I
Ureaplasma urealyticum	2.00E+04 cfu/ul	N.I

Table 34. Interfering microbial agents assay. N.I. = No interference., n.a. = not applicable.

In conclusion, no interference was observed in the detection of macrolide-sensitive and/or macrolide-resistant *M. genitalium* RNA in vaginal matrix, female and male urine 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 *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System. A total of 17, 25 and 28 potentially interfering substances were added to the negative vaginal swab, male urine and female urine matrix, respectively, enriched with the reference strains: *Mycoplasma genitalium* strain M30 (Ref: 49895™) for macrolide-sensitive *M. genitalium* and AMPLIRUN® TOTAL MACROLIDE RESISTANT MGE CONTROL (Ref: MBTC029) for macrolide-resistant *M. genitalium*. 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 vaginal matrix collected in Copan eSwab®, female and male urine matrix spiked with the specific macrolide-sensitive and macrolide-resistant *M. genitalium* strains without interfering substance, whereas NMC corresponds to the negative matrixes without interfering substance nor added microorganisms/reference material. The following results were obtained:

Vaginal matrix		
Substance name	Concentration tested	Result
PMC	-	n.a.
NMC	-	n.a.
Acyclovir	6.60E-02 mg/ml	N.I.
Metronidazole	1.23E-01 mg/ml	N.I.
Clotrimazole	2.50E+00 mg/ml	N.I.
Miconazole Nitrate	2.50E+00 mg/ml	N.I.
Conceptrol Contraceptive Gel (Nonoxynol-9)	2.50E+00 mg/ml	N.I.
Tioconazole	2.50E+00 mg/ml	N.I.
Leukocytes/monocytes	2.00E+06 cell/ml	N.I.
Premeno Duo (Hyaluronic acid and lactic acid)	2.50E+00 mg/ml	N.I.
Femenine Deodorant Spray	5.00E+01 µl/ml	N.I.
Vaginal Lubricant Liquid – water-based Durex Frescor	5.00E+01 µl/ml	N.I.
Vaginal Lubricant – oil based SOIVRE Intim Oil	5.00E+01 µl/ml	N.I.
DermoVagisil Vaginal cream	2.50E+00 mg/ml	N.I.
Preparation H Hemorrhoid Gel HEMOAL	2.50E+00 mg/ml	N.I.
Estradiol	7.50E-03 ng/ml	N.I.
Progesterone	6.00E+00 ng/ml	N.I.
Semen	5%	N.I.
Whole Blood	1%	N.I.

Table 35. Potential interference substances in vaginal matrix. N.I: No reportable interfere / I: Interfere, n.a. = not applicable.

Male urine		
Substance name	Concentration tested	Result
PMC	-	n.a.
NMC	-	n.a.
Ibuprofen	2.19E-01 mg/ml	N.I.
Naproxen	3.60E-01 mg/ml	N.I.
Amoxicilin	5.40E-02 mg/ml	N.I.
Azythromycin	1.10E-02 mg/ml	N.I.
Ceftriaxone	8.40E+01 mg/dl	N.I.
Erythromycin	1.38E-01 mg/ml	N.I.
Metronidazole	1.23E-01 mg/ml	N.I.
Sulfamethoxazole	1.12E+00 mg/ml	N.I.
Tetracycline Hydrochloride	2.40E-02 µg/ml	N.I.
Trimethoprim	4.20E-02 µg/ml	N.I.
Albumin	1.00E+01 mg/ml	N.I.
Bilirubin	4.00E-01 mg/ml	N.I.

Glucose (Dextrose)	1.00E+01 mg/ml	N.I.
Leukocytes/monocytes	2.00E+06 cell/ml	N.I.
Low pH (HCl)	N/A	N.I.
High pH (NaOH)	N/A	N.I.
Semen	5%	N.I.
Urea	1.20E+00 mg/ml	N.I.
Uric Acid	2.35E-01 mg/ml	N.I.
Whole Blood	1%	I.*
	0.25%	N.I.
	0.125%	N.I.
Antocianine (cranberry vaccinium macrocarpon)	1.09E-01 mg/ml	N.I.
Talquistina	2.64E+00 ml Talq/ml dvte	N.I.
4-Acetamidophenol	1.56E-01 mg/ml	N.I.
Phenazopyridine Hydrochloride	1.60E-01 mg/ml	N.I.
Salic Acid	1.50E-01 mg/ml	N.I.

Table 36. Potential interference substances in male urine matrix. N.I: No reportable interfere / I: Interfere, n.a. = not applicable.

* Inhibition observed only for Macrolide-resistant *Mycoplasma genitalium* target.

Female urine matrix		
Substance name	Concentration tested	Result
PMC	-	n.a.
NMC	-	n.a.
Ibuprofen	2.19E-01 mg/ml	N.I.
Naproxen	3.60E-01 mg/ml	N.I.
Amoxicilin	5.40E-02 mg/ml	N.I.
Azythromycin	1.10E-02 mg/ml	N.I.
Ceftriaxone	8.40E+01 mg/dl	N.I.
Erythromycin	1.38E-01 mg/ml	N.I.
Metronidazole	1.23E-01 mg/ml	N.I.
Sulfamethoxazole	1.12E+00 mg/ml	N.I.
Tetracycline Hydrochloride	2.40E-02 µg/ml	N.I.
Trimethoprim	4.20E-02 µg/ml	N.I.
Albumin	1.00E+01 mg/ml	N.I.
Bilirubin	4.00E-01 mg/ml	N.I.
Glucose (Dextrose)	1.00E+01 mg/ml	N.I.
Leukocytes/monocytes	2.00E+06 cell/ml	N.I.
Low pH (HCl) 4	N/A	I.*
Low pH (HCl) 5	N/A	I.*
Low pH (HCl) 6	N/A	N.I.
High pH (NaOH)	N/A	N.I.

Semen	5%	N.I.
Urea	1.20E+00 mg/ml	N.I.
Uric Acid	2.35E-01 mg/ml	N.I.
Whole Blood	1%	N.I.
Antocianine (cranberry vaccinium macrocarpon)	1.09E-01 mg/ml	N.I.
Talquistina	2.64E+00 ml Talq/ml dvte	N.I.
4-Acetamidophenol	1.56E-01 mg/ml	I.*
	3.90E-02 mg/ml	N.I.
	9.75E-03 mg/ml	N.I.
Phenazopyridine Hydrochloride	1.60E-01 mg/ml	N.I.
Salic Acid	1.50E-01 mg/ml	N.I.
17-α Ethinylestradiol	7.50E-03 ng/ml	N.I.
Norethindrone	1.60E+01 ng/ml	N.I.
Femenine Deodoran Spray	5.00E+01 µl/ml	N.I.

Table 37. Potential interference substances in female urine matrix. N.I: No reportable interfere / I: Interfere, n.a. = not applicable.

* Inhibition observed only for Macrolide-sensitive *Mycoplasma genitalium* target.

In conclusion, different potentially interfering substances, both endogenous and exogenous, were tested on VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System. The results obtained lead to the conclusion that, at the concentrations tested, no interference of any of 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 *Mycoplasma genitalium with Macrolide Resistance* 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 2,399 analysed sequences (sequences downloaded from the database with duplicates removed). The results obtained are shown in the following table:

Mycoplasma genitalium					
Gene	Aligned sequences: 486				
	Without mismatches	With mismatches	Sequences with confirmed detection	Sequences with no detection	Sequences with unknown detection
MgPA adhesin	74.07%	25.93%	74.07%	0%	25.93%*
Mycoplasma genitalium					
Gene	Aligned sequences: 17				
	Without mismatches	With mismatches	Sequences with confirmed detection	Sequences with no detection	Sequences with unknown detection
23S RNA	82.35%	17.65%	100%	0%	0%

Table 38. 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.

*It should be noted that 20.78% of the total of aligned sequences (101/486) presented non-critical mismatches which are considered to be detected.

To sum up, the inclusivity analysis shown a correct detection of *MgPA adhesin* and *23S rRNA* genes *of M. genitalium* with the VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System.

Analytical reactivity wet testing

The analytical reactivity of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System for *Mycoplasma genitalium* was evaluated against the DNA from the following strains, showing positive results:

- *Mycoplasma genitalium* strain G37 (ATCC code: 33530)
- *Mycoplasma genitalium* strain TW48-5G (ATCC code: 49898)
- *Mycoplasma genitalium* strain TW10-5G (ATCC code: 49123)
- *Mycoplasma genitalium* strain UMTB-10G (ATCC code: 49899)
- *Mycoplasma genitalium* strain TW10-6G (ATCC code: 49896)
- *Mycoplasma genitalium* strain R32G [R32] (ATCC code: 49897)

12.6. Metrological traceability

This assay is not designed for measuring purposes.

13. Clinical performance characteristics

The clinical performance of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System was tested using vaginal and urine samples. The results were as follows:

	Site	Sample type	Workflow	Target
1	Certest Biotec S.L. in collaboration with Hospital Universitario Miguel Servet (Zaragoza, Spain)	Vaginal swabs	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	Macrolide-sensitive *Mycoplasma genitalium*
				Macrolide-resistant *Mycoplasma genitalium*
2	Certest Biotec S.L. (Zaragoza, Spain) using samples from Cerba Xpert	Urine	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	Macrolide-sensitive *Mycoplasma genitalium*
				Macrolide-resistant *Mycoplasma genitalium*

Table 39. 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 *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System were calculated in relation to each comparator assay as shown in the following table:

Site	Comparator assay	Target	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV	LR+	LR-
1	Allplex™ MG & AziR Assay (Seegene)	MG macrolides resistant	43	96	0	2	0.96 (0.85-0.99)	1.00 (0.96-1.00)	1.00 (0.91-1.00)	0.98 (0.93-1.00)	183.46 (11.55-2914)	0.055 (0.016-0.182)
		MG macrolide sensitive	50	86	3	2	0.96 (0.87-0.99)	0.97 (0.91-0.99)	0.94 (0.85-0.99)	0.98 (0.92-1.00)	28.53 (9.37-86.88)	0.040 (0.010-0.155)
2	Allplex™ MG & AziR Assay (Seegene)	MG macrolides resistant	44	107	0	0	1.00 (0.92-1)	1.00 (0.97-1)	1.00 (0.92-1)	1.00 (0.97-1)	213.6 (13.4-3394)	0.011 (0.001-0.176)
		MG macrolide sensitive	54	95	0	2	0.96 (0.88-0.99)	1.00 (0.96-1)	1.00 (0.93-1)	0.98 (0.93-0.99)	183.6 (11.6-2916)	0.044 (0.013-0.148)

Table 40. 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 *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System.

In conclusion, results show high agreement to detect macrolide-sensitive and macrolide-resistant *Mycoplasma genitalium* using VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System.

14. Summary of Safety and Performance

The Summary of Safety and Performance of VIASURE *Mycoplasma genitalium with Macrolide Resistance* Assay for BD MAX™ System can be downloaded from the webpage: **certest.es/viasure/labeling**. This summary will also be found on the EUDAMED website (https://ec.europa.eu/tools/eudamed).

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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	Contains sufficient for <n> test	UDI Unique Device Identification	REF Catalogue number
CE marking	Keep away from sunlight			

Trademarks

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Change Control		
Version No.	**Changes**	**Date**
00	Original version	18/02/2026

Table A 2. Control change table.

Revision: 18th February 2026

VIASURE

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F-566 rev.03