Candida albicans Real-time PCR Kit, IVD

REF MD04891, 96 reactions

For professional* in vitro *diagnostic use only

CE IVD

EN

Instructions for Use (IFU)

IM-008en

VERSION 02/2022, December 2022

NZYTech genes & enzymes
Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal
Tel.:+351.213643514 Fax: +351.217151168
www.nzytech.com

Contents

1. Introduction

Candida albicans is a diploid, unicellular fungus that naturally colonizes the human intestinal tract of ± 80% of the population. *C. albicans* is, however, an opportunistic yeast that can cause oral and genital infections. Infection is accompanied by a morphologic change from a unicellular organism to a pathogenic multicellular filamentous form. In the commensal form, the fungus lives in the gastrointestinal tract and its growth is regulated by other microorganisms as well as the host's immune system. The switch between commensal and pathogenic forms, known as phenotypic switching, can be regulated by differential gene expression coordinated by several transcription factors. Ultimately, the phenotypic change to the multicellular form allows penetration of the mucous membrane from which infection is initiated. *C. albicans* infection causes candidiasis, which can be superficial or systemic when it becomes a life-threatening disease. Superficial infections cause symptoms such as itching and irritation, which can usually be eliminated with antifungal medications. Nevertheless, individuals with a weakened immune system or with diabetes have an increased risk of developing infection. When overgrowth occurs, it can lead to urinary tract infections, genital yeast infections, oral thrush and mucocutaneous thrush. In most severe cases, when *C. albicans* enters the bloodstream or organs, it can lead to loss of vision, blood and bone infections, endocarditis, meningitis, or inflammation of the intra-abdominal lining. Real-time PCR is the fastest and most reliable method to perform an accurate diagnosis of *C. albicans* infection.

2. Intended Use

NZYTech's Candida albicans Real-time PCR Kit, IVD, is a molecular test based on real-time PCR technology, intended for the rapid detection and qualitative diagnosis of defined pathogenic nucleic acids in human biological samples. The use of the kit is indicated in patients with inflammatory symptoms of the vaginal tract, for diagnosis and control of infection caused by *C. albicans*. The application of the kit does not depend on population and demographic aspects. There are no contraindications to use the *C. albicans* real-time PCR detection kit. However, this test serves as an adjunct to the diagnosis and its result should be combined with the clinical signs and symptoms of fungal infection. Thus, a positive result indicates the presence of *C. albicans* DNA, but clinical correlation from the history and other diagnostic information is necessary to determine the patient's infection status. A negative result does not exclude the existence of *C. albicans* and should not be used as the sole instrument for the patient's treatment decision. Testing must be performed by specialized and qualified laboratory technicians, especially in real-time PCR technique and molecular *in vitro* diagnostic. Suitable clinical samples include vaginal swabs of epithelial cells. The kit should only be used as indicated in this user manual.

3. Principles of the Assay

NZYTech's Candida albicans Real-time PCR Kit, IVD, provides the set of reagents, enzymes, and oligonucleotides (primers and probes) for the qualitative detection of the *C. albicans* genome, using the real-time PCR technique (see equipment specification requirements in Section 6). The kit detects the RPR1 gene which was previously identified as a good genetic marker for *C. albicans*. NZYTech's Candida albicans Real-time PCR Kit, IVD, was designed to have a broad detection profile, while remaining specific to the *C. albicans* genome. The kit's primers and probes have 100% homology to 100% of the *C. albicans* sequences available in the GenBank database (November, 2022). In addition, the set of oligonucleotides was specifically designed for the detection of this organism and do not show significant homology with other genomes, which reflects the high specificity and detection sensitivity of the test. As such, the kit was designed to be specific to *C. albicans* genome and to avoid detection of other organisms causing vaginal infections. The internal control, included in kit, validates the efficacy of the extraction process as well as the absence of PCR inhibitors potentially present in the human biological samples. Periodically, NZYTech revisits *C. albicans* target gene sequence and, if necessary, will release a new version of this kit. Additionally, the kit includes two external controls (a positive control and a negative control) as described below. The positive control consists of nucleic acid fragments that contains the two target sequences detected by the kit (RPR1 fungal gene and the human β-actin gene).

In this kit, the qualitative detection of DNA is based on the real-time PCR technology, which is a reference methodology in laboratory diagnosis. It is a methodology of high sensitivity and specificity to accurately detect the presence of this organism. NZYTech Candida albicans Real-time PCR Kit, IVD, is based on the principle of researching the presence of Candida fungus DNA, isolated and purified with an extraction system. The extracted DNA is subjected to a PCR amplification, in a single reaction, using two highly specific primer/probe sets exploiting the TaqMan® principle. In the presence of *C. albicans* DNA, the TaqMan® probe specifically binds to conserved regions of the RPR1 gene which are flanked by two specific primers pairs. A second primers/probe set acts as an internal control, detecting the human β-actin gene (ACTB), which allows confirming the efficiency of the process of capturing the biological material collected from the patient. In addition, this internal control demonstrates that no reaction inhibition has occurred by PCR inhibitors potentially present in the clinical/environmental samples. To allow identification of the amplification of the two specific targets in a single reaction, probes specific for *C. albicans* and for human β-actin are labelled with FAM™ and HEX™ reporter dyes, respectively. Thus, this kit consists of a duplex assay where the target specific to *C. albicans* is detected in the FAM optical channel and the human target gene is detected in the HEX optical channel. These oligonucleotides/probe sets are provided in optimized concentrations to ensure that human DNA, even when present at extremely high concentrations, does not limit the efficiency of the *C. albicans* primers/probe sets.

4. Kit Composition

NZYTech's Candida albicans Real-time PCR Kit, IVD, provides a comprehensive set of reagents sufficient to perform 96 qPCR reactions in a single step.

Kit Component		Volume (per vial)	Number of tubes
C. albicans MMix	NZYSupreme qPCR Probe Master Mix (2x)	1050 µL	1
C. albicans PPMix	*C. albicans*/ACTB primers/probe Mix	205 µL	1
C. albicans POS	*C. albicans*/ACTB Positive Control	105 µL	1
NTC	No-Template Control	105 µL	1

5. Storage, Stability and Handling Conditions

NZYTech's Candida albicans Real-time PCR Kit, IVD, is shipped refrigerated. Upon receipt of the kit, all components should be immediately stored at -85°C to -15°C. When in use, kit components should be promptly placed in the freezer after use to minimize the exposure time to room temperature. In addition:

- Minimise the number of freeze-thaw cycles by storing working aliquots. If appropriate, kit components may be aliquoted in smaller volumes after thawing.
- The C. albicans PPMix component should be stored protected from light. Particularly, do not expose NZYSupreme qPCR Probe Master Mix (2x) to direct sun light after combining with primers/probe mix.
- If the package that protects the kit arrived damaged, please contact NZYTech.
- Beware to the expiry date indicated on the packaging. NZYTech does not recommend using the kit after the expiry date. On this date, the kit must be discarded following the disposal instructions in **Section 8.2**.

6. Materials and Instrumentation Required but Not Provided

- Real-time PCR Instrument that detects FAM and HEX/JOE/VIC (at emission wavelengths of 520 and 556 nm, respectively). See in **Section 11** the instrument models for which the kit was validated.
- Equipment and consumables for isolating DNA of biological/clinical samples.
- RNase/DNase free qPCR plasticware: PCR tubes of 1,5 or 2 mL, strips, caps, 96-well plates, adhesive films.
- Pipettors and filter tips (RNase/DNase free).
- Disposable gloves.
- Vortex and centrifuge.

7. Sample Collection and Preparation

The kit is designed to detect DNA extracted from vaginal swabs. Different factors, such as the biological sample collection procedure, transport, storage, and sample processing time, are critical to ensuring sample integrity and achieving optimal results. Collected samples should be tested as soon as possible. Inappropriate sample collection, handling and/or transport of specimens may result in a false result. Extracted nucleic acids constitute the starting material for the assay with NZYTech's Candida albicans Real-time PCR Kit, IVD. NZYTech recommends the use of NZY Plant/Fungi gDNA Isolation kit (MB177, NZYTech) for nucleic acids extraction, as this kit has been validated for the extraction of *C. albicans* samples. Please ensure that DNA samples are suitable in terms of purity, concentration, and nucleic acid integrity. Since ethanol is a strong inhibitor of real-time PCR, it is necessary to eliminate this component before the elution of nucleic acids during the extraction process. NZYTech's kit contains an internal control that targets human DNA co-purified with *C. albicans* DNA. Human DNA is amplified with the set of oligonucleotides (primers and probe) from the human β-actin gene. The introduction of the internal control is useful in assessing the efficiency of DNA extraction and isolation and/or in detecting the presence of potential inhibitors during sample processing.

8. Precautions and Warnings

As in any analytical testing procedure, good laboratory practices are essential. Carefully follow the procedures and guidelines provided in this handbook to ensure that the test is performed correctly. Any deviation from them may result in assay failure or cause erroneous results. Due to high sensitivity of the kit, special care must be taken to keep reagents and PCR amplification mixes free from contamination.

8.1 Safety Information

Before using the kit, please consult the Safety Data Sheet (SDS) that is available at NZYTech website (www.nzytech.com). This kit detection should be performed only by staff trained in the relevant technical and safety procedures in appropriately equipped laboratories. International and national guidelines on laboratory biosafety should be followed in all circumstances.

8.2 Handling and Procedural Requirements

- Only for professional *in vitro* diagnostic use.
- Do not use this kit after expiration date.
- Do not use the test components, if kit sealing is damaged.
- Do not interchange reagents of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- DNase/RNase-free disposable plasticware and pipettes should be used in all procedures.
- Sample preparation, reaction set up and amplification should be performed in different working areas. The order of tasks in the lab should be unidirectional. Always wear disposable gloves in each area and change them before entering a different area. If possible, change your coat.
- Select specific materials and equipment for each individual work area and do not transfer them from one area to another.
- Biological samples must be handled as if they are infectious following proper biosafety precautions.

- Positive control contains high copy number templates; it should be opened and processed away from test samples and kit components to avoid cross-contamination.
- Always use the NTC provided in the kit.
- Handle post-amplification plates with care and dispose them immediately after the end of the testing; plates should always be discarded into a proper biohazard container after use.
- At the end of each test, clean/disinfect the surfaces of work areas and equipment with an appropriate disinfectant solution to remove any traces of nucleic acids.
- Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations.
- All results should be interpreted by a health-care professional in the context of patient medical history and clinical symptoms.
- This test cannot exclude diseases caused by other pathogens.
- A negative result for any PCR test does not conclusively rule out the possibility of infection.
- Follow good laboratory practices, wear protective clothing, permanently wear disposable powder-free gloves, use goggles and mask. Do not eat, drink, or smoke in the working area.

9. Testing Procedure

Please read the instructions for use carefully before performing the assay. Beware that pipetting steps and plate set-up should be performed on benchtop coolers or ice. After the plate is poured start immediately the real-time PCR protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artefacts that reduce the sensitivity of detection. Prior to the experiment, gently mix the reaction tubes provided, centrifuge for 5 seconds to collect contents at the bottom of the tube and place tubes on ice. **We strongly recommend pipetting C. albicans POS, the kit Positive Control, last to avoid cross contaminations.**

9.1 Reaction set-up

1. Prepare a qPCR mix sufficient for the number of tests to be performed with a 5% additional volume for pipetting losses. Proceed according to the table below that specify the volumes for 1 and *n* tests (where *n* corresponds to the total number of reactions):

Component	**1 test volume (µL)**	***n* tests (*) volume + 5% (µL)**
C. albicans MMix	10	*n* x 10.5
C. albicans PPMix	2	*n* x 2.1
Final Volume	**12**	***n* x 12.6**

(*) To calculate the total number of reactions needed for each assay, count the number of samples and add two more for the Negative and the Positive control, respectively.

2. Pipette 12 µL of the qPCR mix into individual wells according to your real-time PCR experimental plate set-up.

3. For the negative control, add 8 µL of NTC instead of DNA template into the negative control well. The final volume should be 20 µL.

4. For the biological samples, add 8 µL of each DNA sample into the sample wells, according to your experimental plate set-up. The final volume in each well should be 20 µL.

5. For the positive control, add 8 µL of C. albicans POS instead of DNA template into the positive control wells. The final volume should be 20 µL.

6. Cover and seal the plate with an appropriate optical adhesive film or caps before proceeding with the qPCR and detection steps.

7. Place the reaction plate in the real-time PCR instrument and run the qPCR protocol according to the section below.

9.2 Programming the real-time PCR instrument

The table below displays a standard protocol optimized on a few platforms. However, these conditions may be adapted and validated to suit different machine-specific protocols.

Cycles	Temperature	Time	Step
1	95 °C	3 min	Polymerase activation
40	95 °C	5 s	Denaturation
	60 °C	30 s	Annealing/Extension*

** Depending on the qPCR instrument select suitable detection channels. Fluorogenic data should be collected during this step through channels FAM and HEX/JOE/VIC.*

The fluorescent dyes and detection channels used for this kit are:

Fluorescent Dyes & Detection Channels

Targets	Fluorescent dye	Detection Channels
C. albicans (RPR1) specific gene	FAM™	FAM
Human β-actin (ACTB) gene	HEX™	HEX/JOE/VIC
C. albicans POS	FAM™ & HEX™	FAM & HEX/JOE/VIC

NZYTech's Candida albicans Real-time PCR Kit, IVD, was validated for the following Real Time PCR Systems: Applied Biosystem® 7500 FAST, Applied Biosystem® QuantStudio 5, Roche Life Science LightCycler® 480 II and Bio-Rad® CFX96. If other equipment is used, the kit should be validated by the user by using previously characterised samples (both positive and negative).

10. Data Analysis

10.1 Run Validation Criteria

Before analysing samples results, we recommend verifying if the real-time PCR test is valid. Thus, for each plate, please confirm if the results for Positive and Negative controls performed as expected, according to the following criteria:

- **Positive control:** the amplification of FAM (*C. albicans*) and HEX (ACTB) curves are positive. Positive control is expected to amplify at 20<Ct<25, in both channels FAM and HEX. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

- **Negative control (no template reaction):** no amplification is detected. If the negative control has amplification curves (FAM e HEX) with a sigmoidal shape, sample contamination may have occurred. Repeat the test following good qPCR practices.

If the controls are according with expected, the test is **valid**. Please proceed with interpretation of results for the tested samples.
If any of the controls do not exhibit the expected performance, the assay was compromised or executed improperly and should be considered **invalid**. **Please, repeat the test.** If the problem persists, contact the manufacturer

10.2 Test Results Interpretation

***C. albicans* is detected** if the FAM amplification curve displays a sigmoidal shape with a Ct≤36, regardless of what result is obtained for the ACTB (HEX) assay.

***C. albicans* is not detected** if FAM curve does not amplify (Ct>36), while the ACTB (HEX) assay displays a positive sigmoidal curve with Ct≤40.

The **test is invalid** if the *C. albicans* e ACTB assays are negative. The test should be repeated with nucleic acid re-purified from the sample.

The following table summarises the interpretation of principal results (evaluate the overall shape of the amplification curves; **only sigmoidal amplification curves are indicative of true amplification**).

C. albicans (FAM)	**ACTB** (HEX)	**Results interpretation**
+ (Ct≤36)	+ (Ct≤40)	*C. albicans* → **POSITIVE**
+ (Ct≤36)	- (Ct>40)*	*C. albicans* → **POSITIVE**
- (Ct>36)	+ (Ct≤40)	*C. albicans* not detected → **NEGATIVE**
- (Ct>36)	- (Ct>40)	Invalid test, repeat extraction and qPCR run

* *Detection of the Internal Control on the HEX detection channel is not required for positive results on the FAM detection channel. High amounts of target DNA in the sample may cause the Internal Control signal to be strongly reduced or absent.*

Note: Interpretation of results must account for the possibility of false negative and false positive results.

• False negative results may be caused by:

- Unsuitable collection, handling and/or storage of samples.
- Sample degradation.
- Presence of qPCR inhibitors.
- Mutations in the genome of the pathogenic organism.
- Failure to follow procedures in this handbook.
- Use of unauthorised extraction kit or real-time PCR platform.

• False positive results may be caused by:

- Unsuitable handling of samples containing high concentration of DNA of *C. albicans*.
- Unsuitable handling of the positive control C. albicans POS.
- Unsuitable handling of amplified product (post-amplification plate).

Negative results do not preclude infection and the test result should not be used as the sole basis for treatment or other patient management decisions. In addition, this test cannot rule out diseases caused by other bacterial or viral pathogens.

11. Performance Evaluation

The performance of NZYTech's Candida albicans Real-time PCR Kit, IVD, was validated for the instruments: Applied Biosystems® 7500 FAST, Applied Biosystems® QuantStudio 5 e Bio-Rad® CFX96™. If other equipment is used, the kit should be validated by the user by using previous characterised samples (both positive and negative).

11.1 Expected Results

A typical amplification plot observed for a clinical *C. albicans* positive sample is present in Figure 1. In situations where the sample contains high amounts of *C. albicans* DNA, the HEX/JOE/VIC channel curve, corresponding to the human β-actin gene, may be absent or exhibit an atypical shape.

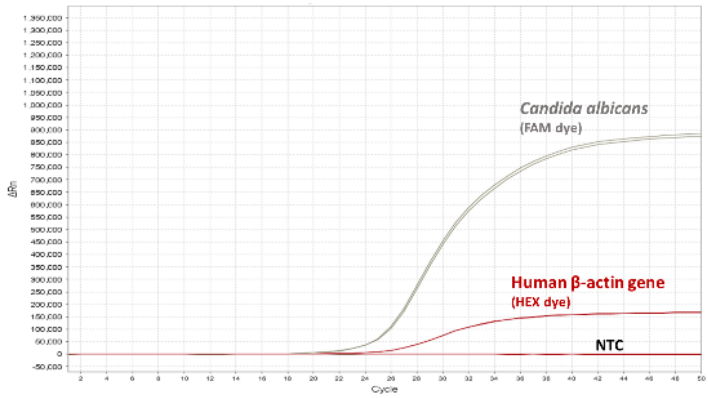

Figure 1. Detection of *C. albicans* and human β-actin (ACTB) genes in a clinical *C. albicans* positive sample. Gray curve: detection of the *C. albicans* target sequence (RPR1 gene) through the FAM channel. Red curve: detection of the human β-actin sequence (ACTB) through the HEX channel. NTC negative control.

11.2 Limit of Detection (LoD) - Analytical Sensitivity

The analytical sensitivity was defined as the lowest concentration of analyte that could be reliably detected with 95% confidence. This was assessed by testing *C. albicans* nucleic acids at different copy numbers, spiked into DNA extracted from negative vaginal samples, using 3 different kit batches following typical testing reaction conditions. Tests were repeated over 4 days, producing 48 replicates for each concentration tested. Together, the data revealed that NZYTech´s Candida albicans Real-time PCR Kit, IVD detects 5 copies/reaction or 0,250 copies/µL of *C. albicans* DNA with a confidence ≥95%. Thus, the tentative Limit of Detection (LoD) was determined to be 250 copies/mL for *C. albicans.*

The different LoDs were confirmed by two different operators using three kit batches in an experiment with a total of 96 replicates, thus ensuring that analytical sensitivity is maintained under different testing conditions. The LoD study established the lowest concentrations of *C. albicans* (copy number/mL) that can be detected by the Candida albicans Real-time PCR Kit, IVD in at least 96% of cases. All assays were performed using the Applied Biosystem® QuantStudio™ 5 Real Time PCR instrument and the analysis was performed using the instrument's software.

Finally, the capacity of NZYTech's Candida albicans Real-time PCR Kit, IVD, to detect the pathogen at different load range was determined by testing different nucleic acid loads under standard testing conditions. Figure 2 displays the amplification curves for the different nucleic acid quantities.

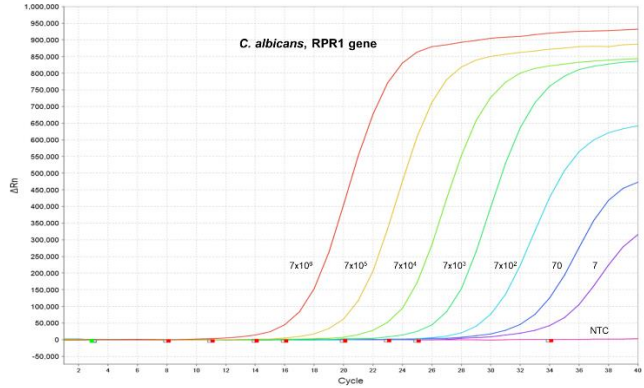

Figure 2. Sensitivity of the NZYTech's Candida albicans Real-time PCR Kit, IVD. Amplification plot (cycle number versus fluorescence - ΔRn) of 1:10 serial dilutions of the *C. albicans* DNA, from 7 x 10^6 copies to 7 copies per reaction through the FAM channel. NTC, No Template Control (negative control).

11.3 Analytical Reactivity (Inclusivity) and Analytical Specificity

Inclusivity and cross-reactivity were evaluated by *in silico* analysis of oligonucleotide probes and primers against pathogens related to *C. albicans* and normal pathogens that cause infection with similar symptoms, respectively. Upon *in silico* analysis the assay design was found to detect *C. albicans* and exhibited no reactivity with non-related species.

In vitro cross-reactivity assays (exclusivity) were performed to confirm that the Candida albicans Real-time PCR Kit, IVD kit does not react with other colonizing and pathogenic microorganisms commonly found in human clinical specimens of the vaginal tract. This study was performed using a commercial panel of vaginal pathogens marketed by ZeptoMetrix®, namely the NATtrol™ Vaginal Panel® (#NATVP-BD). This panel includes representative samples of true clinical specimens of bacterial and fungal origin, including *Atopobium vaginae* Z242, *Candida albicans* Z006, *Gardnerella vaginalis* Z247, *Lactobacillus crispatus* Z246, *Trichomonas vaginalis* Z070 and BVAB2 Recombinant. Data obtained using three different batches of the kit confirmed that, except for *C. albicans* which as expected was identified by the kit, none of the microorganisms tested interfered with the performance of the kit or generated a detectable amplification signal.

In addition, the kit was tested in the amplification of nucleic acids of common vaginal tract microbes, including *Actinomyces naeslundii, Candida glabrata, Candida tropicalis, Chlamydia trachomatis, Gardnerella vaginalis, Lactobacillus acidophilus, Mesomycoplasma lagogenitalium, Mycoplasma hominis, Neisseria gonorrhoeae, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus sp.* and *Veillonella parvula* (Leibniz-Institut DSMZ). Data obtained using three different kit batches confirmed that none of the microorganisms tested interfered with the performance of the kit or generated a detectable amplification signal. Thus, considering the tested organisms the kit has an analytical specificity of 100%.

Interference by substances putatively found in vaginal samples on the sensitivity of detection by the Candida albicans Real-time PCR Kit, IVD kit, was evaluated in an assay using 33 potential

interfering substances (see table below). In this study, artificial samples were mixed with vaginal samples contrived with *C. albicans* DNA. Artificial samples were prepared by adding *C. albicans* DNA at 3x LoD concentration to a negative clinical matrix. Control samples with no *C. albicans* DNA were also prepared. Potentially interfering substances were tested at concentrations that represent the highest levels expected in vaginal samples based on a literature review. A negative control using water as the added substance was also included. The data revealed that none of the substances tested interfere with the sensitivity of detection of *C. albicans* by the Candida albicans Real-time PCR Kit, IVD. All experiments were performed on the Applied Biosystems® QuantStudio™ 5 real-time PCR instrument.

Potential Interferent	Interferent name	Active Ingredient	Final concentration in sample	Interference Yes (Y) or No (N)
Anti-Fungal	Anidulafungin	Anidulafungin	5 mg/mL	N
Anti-Fungal	Flucitosine	Flucytosine	5 mg/mL	N
Anti-Fungal	Voriconazole	Voriconazole	5 mg/mL	N
Anti-Fungal	Anfotericin B	Amphotericin B (20 µg/mL)	10% v/v	N
Antimicrobials	Gino-Canesten	Clotrimazole (10 mg/g)	10% w/v	N
Antimicrobials	Lomexin	Fenticonazole nitrate	10% w/v	N
Medicine	Progeffik	Progesterone	5 mg/mL	N
Washing	Betadine 100 mg/mL	Povidone iodine (100 mg/ml)	10% v/v	N
Washing	Cien Gel Para Higiene Íntima	-	10% v/v	N
Washing	Saugella Homme	Lactic acid	10% v/v	N
Washing	Sabão azul e branco	-	10 mg/mL	N
Washing	D'aveia Ginecológico	-	10% w/v	N
Washing	Palmolive Glicerina Natural	Sodium Palmate and Sodium Oleate	10 mg/mL	N
Washing	Palmolive Indulging Delight	-	10 mg/mL	N
Washing	Continente Gel Íntimo	-	10% v/v	N
Lubricant	Warm Up Cherry	-	10% v/v	N
Lubricant	Control - Thai Passion	-	10% v/v	N
Topical Products	Lauroderme	Zinc oxide (23mg/g) + Salicylic acid (2mg/g)	10 mg/mL	N
Topical Products	Bepanthene Pomada	Dexpantenol (50 mg/g)	10% w/v	N

Potential Interferent	Interferent name	Active Ingredient	Final concentration in sample	Interference Yes (Y) or No (N)
Topical Products	Halibut	Zinc oxide (150mg/g)	10% w/v	N
Topical Products	L-Mesitran Soft	40% medical grade honey	10% w/v	N
Topical Products	Climacare - Gel Vaginal	Hialuronic acid & Lactic acid	10% v/v	N
Topical Products	Elixir de Argan Oil & Go	Paraffinum Liquidum	10% v/v	N
Topical Products	WOMAN ISDIN Hidratante Vaginal	Glycerin (11%)	10% v/v	N
Natural inhibitors	Sexual fluids	-	Scraping in transport medium	N
Natural inhibitors	Seminal fluid	-	5% v/v	N
Natural inhibitors	Saliva	-	10% v/v	N
Natural inhibitors	Urine	-	10% v/v	N
Natural inhibitors	Whole blood	Glucose, Hormones, Enzymes, Ions, Iron, etc	10 % v/v	N
Natural inhibitors	Mucus	Immunoglobulin, Lysozyme, Polymers	Scraping in transport medium	N
Natural inhibitors	Menstruation	-	Scraping in transport medium	N
Natural inhibitors	Plasma	-	10 % v/v	N
Absolute ethanol	Ethanol	Alcohol	5% v/v	N

11.4 Precision

Assay precision for the NZYTech's Candida albicans Real-time PCR Kit, IVD was determined by the repeated testing of positive samples representing two pathogen load levels, 3x LoD and 30x LoD copies per reaction, spiked into DNA extracted from negative vaginal samples, using 3 different kit batches, and following typical testing reaction conditions. Precision was evaluated by measuring Cq average, Cq coefficient of variation and % of replicate detection, as described below for each case. The data is resumed in table displayed below.

11.4.1. Repeatability

Repeatability was assessed by one operator by analysing 12 replicates of each sample (3x LoD and 30x LoD copies per reaction), accounting for a final number of 24 tests performed per target.

11.4.2. Daily Reproducibility

Daily reproducibility was assessed by one operator by analysing 48 replicates of each sample (3x LoD and 30x LoD copies per reaction), for 4 days, with 12 replicates of each concentration per day (a total of 96 assays per target were performed).

11.4.3. Lot-to-lot Reproducibility

Reproducibility between lots was assessed by one operator through the analysis of 72 replicates of each sample (3x LoD and 30x LoD copies per reaction) using 3 different kit batches with 24 replicates per batch.

11.4.4. Operator Reproducibility

Operator reproducibility was assessed by testing 36 replicates of each sample (3x LoD and 30x LoD copies per reaction), by three different operators with 12 replicates per operator.

11.4.5. Inter-instrument Reproducibility

Inter-instrument reproducibility was measured by one operator through the testing 24 replicates of each sample (3x LoD and 30x LoD copies per reaction), in five different qPCR instruments: Applied Biosystem® QuantStudio™ 5, Applied Biosystem® 7500, Applied Biosystem® StepOnePlus, Roche® LightCycler 96™ and Bio-Rad® CFX96™, in a total of 60 tests per sample.

Precision of NZYTech's Candida albicans Real-time PCR Kit, IVD.

Variable		*C. albicans* (copies/reaction)	
		3x LoD	30x LoD
Repeatability	n	12	12
	Mean Cq	32,90	29,65
	Coefficient of Variation (%)	1,18	0,48
	% Replicate Detection	100	100
Daily Reproducibility	n	48	48
	Mean Cq	32,85	29,65
	Coefficient of Variation (%)	1,41	0,56
	% Replicate Detection	100	100
Lot-to-lot Reproducibility	n	72	72
	Mean Cq	32,91	29,66
	Coefficient of Variation (%)	1,45	0,56
	% Replicate Detection	100	100
Operator Reproducibility	n	36	36
	Mean Cq	33,00	29,66
	Coefficient of Variation (%)	1,4	0,53
	% Replicate Detection	100	100
Inter-instrument Reproducibility	n	60	60
	Mean Cq	32,47	29,04
	Coefficient of Variation (%)	2,06	1,23
	% Replicate Detection	100	100

11.5 Clinical evaluation

The clinical performance of NZYTech's Candida albicans Real-time PCR Kit, IVD, was evaluated using vaginal specimens, in an independent molecular diagnostic laboratory. The comparator was a routine microbiologic culture method. In total, 20 clinical samples were tested, namely 10

negative samples and 10 positive clinical samples for *Candida albicans*. The data revealed a 100% agreement for all positive and negative samples tested.

12. Quality Control

All components of NZYTech's Candida albicans Real-time PCR Kit, IVD, are tested following the protocols described above. The duplex qPCR system allows the detection of targets described for the identification of *C. albicans* DNA as well as human DNA (β-actin gene, ACTB). Positive amplifications are observed for target genes, positive control and internal controls through FAM and HEX/JOE/VIC channels, according to respective primers/probe set reporter dyes.

13. Technical Support

For Technical support, please contact our dedicated technical support team by Phone: +351 (0) 21 364 35 14 or Email: info@nzytech.com.

14. Trademarks and Disclaimers

All trademarks that appear in this manual are the property of their respective owners.

15. Explanation of Symbols

IVD	*in vitro* diagnostic medical device		Consult instructions for use
REF	Catalogue number		Manufacturer
LOT	Batch code		Use by
	Temperature limitation		Sufficient for
CONTROL +	Positive control		Keep away from the sun light (primer/probe mix)
CONTROL -	Negative control		

16. Conformity Declaration

Product Name: Candida albicans Real-time PCR Kit, IVD

Catalogue Number: MD04891

Intended use: *Candida albicans* qualitative detection

Manufacturer: NZYTech - Genes & Enzymes,

Estrada do Paço do Lumiar, Campus do Lumiar

Edifício E, R/C,

1649-038, Lisboa

Portugal

We, NZYTech, Lda – Genes & Enzymes, hereby declare that this product, to which this declaration of conformity relates, is in conformity with the following standard and other normative documents ISO 9001:2015, following the provisions of the 98/79/EC Directive and of the Regulation (EU) 2017/746 on *in vitro* diagnostic medical devices as transposed into the national laws of the Member States of the European Union.

The product technical file is maintained at NZYTech, Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal.

Joana Brás, PhD

Technical Director

17. References

Silva Dantas, A., Lee, K. K., Raziunaite, I., Schaefer, K., Wagener, J., Yadav, B., & Gow, N. A. (2016). Cell biology of *Candida albicans*–host interactions. Current opinion in microbiology, 34, 111-118.

Dadar, M., Tiwari, R., Karthik, K., Chakraborty, S., Shahali, Y., & Dhama, K. (2018). *Candida albicans*-Biology, molecular characterization, pathogenicity, and advances in diagnosis and control–An update. Microbial pathogenesis, 117, 128-138.

Calderone, R. A., & Fonzi, W. A. (2001). Virulence factors of *Candida albicans*. Trends in microbiology, 9(7), 327-335.

https://www.cdc.gov/fungal/diseases/candidiasis/. Access date: 13/01/2022.

Kozel, T. R., & Wickes, B. (2014). Fungal diagnostics. Cold Spring Harbor perspectives in medicine, 4(4), a019299.

nzytech
genes & enzymes

Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal
Tel.:+351.213643514 Fax: +351.217151168
www.nzytech.com