Primerdesign™ Ltd

R01006

genesig™PLEX Joint Infection Real-Time PCR Multiplex Kit

Kit version: 1

Staphylococcus aureus (PRK14902 gene region)

Cutibacterium (rpsD & purB genes)

genesig™PLEX kit

100 tests

Specificity of primers and probes last reviewed on:
March 2023

For general laboratory and research use only

Introduction

Staphylococcus aureus

Staphylococcus aureus is a spherical, Gram-positive bacterium of the *Staphylococcaceae* family. The genome of this species is around 2.8Mb in length. Virulence factors for *S. aureus* are encoded by phages, pathogenicity islands, staphylococcus cassette chromosome and plasmids, which contain genes for antibiotic resistance such as those found in Methicillin Resistant *Staphylococcus aureus* (MRSA).

S. aureus can be found on the skin and mucosal membranes of healthy individuals, but any abrasion to these barriers allows the bacterium to enter the wound, colonise and cause infection. As this bacterium lives on the skin, it can be transmitted easily between individuals and is commonly seen in hospital acquired infections.

S. aureus can cause a variety of different infections ranging from mild skin infections such as impetigo to invasive diseases and toxic mediated diseases, and is a common cause of bone and joint infections.

Cutibacterium

Cutibacterium (formerly known as Propionibacterium) are Gram-positive, slow growing cutaneous anaerobic commensals that have been reported in prosthetic joint infections. Their genomes are approximately 2.5 Mb in length and there are five species within the genus: *Cutibacterium acnes*, *Cutibacterium granulosum*, *Cutibacterium avidum*, *Cutibacterium modestum* and *Cutibacterium namnetense*. The key species responsible for infections in joints are the former four, with *C. acnes* responsible in most cases.

Diagnosing *Cutibacterium* for treatment has proved challenging as typical signs of infection are absent, often with the only symptom being pain. Due to the slow growth rate microbial cultivation diagnoses are often inconclusive, as they are usually only several days in duration, whereas *Cutibacterium* typically requires at least a 14-day cultivation time. Molecular diagnostics are therefore the preferred methodology as they provide a more rapid diagnosis.

Specificity

The genesig™PLEX kit is designed for the in vitro detection of *Staphylococcus aureus*, *Cutibacterium acnes*, *Cutibacterium avidum*, *Cutibacterium modestum* and *Cutibacterium granulosum*.

The assays within this kit are predicted to detect over 95% of sequences available on the NCBI database at the time of design.

The dynamics of genetic variation means that new sequence information may become available after the initial design. Primerdesign periodically reviews the detection profiles of our kits and when required releases new versions.

If you require further information or have a specific question about the detection profile of this kit, then please send an email to techsupport@primerdesign.co.uk and our team will answer your question.

Kit contents

- **Multiplex primer/probe mix (2x 50 reactions BROWN)**
  FAM, VIC, and Cy5 labelled (see table below)

Target	Fluorophore
Cutibacterium	FAM
S.aureus	VIC
Internal extraction control	Cy5

- **Multiplex positive control template (RED)**
- **Internal extraction control DNA (BLUE)**
- **2x Lyophilised oasig™ Master Mix (SILVER)**
- **2x oasig™ resuspension buffer (BLUE)**
- **Template preparation buffer (YELLOW)**
  For resuspension of the positive control template
- **RNase/DNase free water (WHITE)**
  For resuspension of the primer/probe mix

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Extraction kit

This kit is recommended for use with genesig EASY DNA/RNA Extraction kit or exsig®Mag however, it is designed to work well with all processes that yield high quality RNA and DNA with minimal PCR inhibitors.

Pipettors and filter tips

Vortex and centrifuge

1.5ml tubes

qPCR plates or tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20ºC on arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20ºC for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances. Primerdesign does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and DNA integrity. Always run at least one negative control with the samples. To prepare a negative control, replace the template DNA sample with RNase/DNase free water.

Dynamic range of test

Under optimal PCR conditions genesig kits have very high priming efficiencies of >90% and can detect between $1X10^6$and $1X10^2$ copies of target template.

Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primerdesign genesig detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applera Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

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Principles of the test

Real-time PCR

Individual primer and probes designed for each pathogen have been combined into a single reaction and these can be detected through the different fluorescent channels as described in the kit contents.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target DNA. Fluorogenic probes are included in the same reaction mixture which consists of a DNA probe labelled with a 5`-dye and a 3`-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control

For a positive control, the kit contains a single positive control that contains templates for the 2 targets in the test. The kit positive control will give a *Cutibacterium* signal through the FAM channel and an *S. aureus* signal through the VIC channel. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting each target are working properly in that particular run. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false positive results. This can be achieved by handling these components in a post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control

To confirm the absence of contamination, a negative control, or No Template Control (NTC) reaction should be included every time the kit is used. For this reaction, the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run.

Internal DNA Extraction Control

When performing DNA extraction, it is often advantageous to have an exogenous source of DNA template that is spiked into the lysis buffer. This control DNA is then co-purified with the sample DNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR for the control DNA also indicates that PCR inhibitors are not present at a high concentration. Within the Multiplex primer/probe mix are primers and probes to detect the exogenous DNA using qPCR. The primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control DNA does not interfere with detection of the target DNA even when present at low copy number. The Internal control is detected through the Cy5 channel and gives a Cq value of 28+/-3.

Resuspension protocol

To minimise the risk of contamination with foreign DNA, we recommend that all pipetting is performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer and probe mix is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the primer/probe mix in the RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component – resuspend in water	Volume
Pre-PCR pack	
Multiplex primer/probe mix (**BROWN**)	55µl

3. **Resuspend the internal control template and positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component – resuspend in template preparation buffer	Volume
Pre-PCR heat-sealed foil	
Internal extraction control DNA (**BLUE**)	500µl
Post-PCR heat-sealed foil	
Positive control template (**RED**)*	500µl

*** This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.**

4. **Resuspend the lyophilised Master Mix in oasig resuspension buffer, according to the table below:**

Component – resuspend in oasig resuspension buffer	Volume
Lyophilised Master Mix (SILVER)	525µl

DNA extraction

The internal extraction control DNA can be added to either lysis/extraction buffer or to the sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control DNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. Add 4µl of the Internal extraction control DNA (BLUE) to each sample in DNA lysis/extraction buffer per sample.
2. Complete DNA extraction according to the manufacturer's recommended protocols.

qPCR detection protocol

For optimum performance and sensitivity
All pipetting steps and experimental plate set up should be performed on ice. After the plate is prepared, proceed immediately to the amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. **For each DNA sample prepare a reaction mix according to the table below:**
   Include sufficient reactions for positive and negative controls.

Component	Volume
oasig lyophilised Master Mix (SILVER)	10µl
Multiplex primer/probe mix (**BROWN**)	1µl
RNase/DNase free water (**WHITE**)	4µl
Final volume	**15µl**

2. **Pipette 15µl of this mix into each well according to your qPCR experimental plate set up.**

3. **Pipette 5µl of DNA sample into each well according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.

4. **Pipette 5µl of positive control template into each well according to your plate set up.**
   The positive control contains templates for *Cutibacterium* and *S. aureus*. The final volume in each well is 20µl.

qPCR amplification protocol

Amplification conditions using oasig Master Mix

	Step	Time	Temp
Cycling x 50	Enzyme activation	2 mins	95°C
	Denaturation	10 secs	95°C
	DATA COLLECTION*	60 secs	60°C

* Fluorogenic data should be collected during this step through the FAM, VIC, and Cy5 channels.

Interpretation of results

Positive control

The positive control well should give an amplification plot through the FAM channel (*Cutibacterium*) and the VIC channel (*S. aureus*). There is no Internal control template within the positive control so the Cy5 channel should give no signal (flat amplification plot). The positive control signals indicate that the kit is working correctly to detect each target.

No template control (NTC)

The NTC should give a flat line (flat amplification plots) through all channels. Signals in the NTC indicate cross contamination during plate set up.

Internal DNA extraction control

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quality and quantity of DNA added to the PCR reaction and the individual machine settings. Cq values of 28±3 are within the normal range. When amplifying a target sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Sample data

Presence of the targets are detected in the channels indicated in the kit contents section. Positive signals indicate positive tests for those targets. It may be possible for samples to contain multiple targets, therefore positive results in the FAM and VIC channels may be present.

Cutibacterium DNA is known to be highly prevalent within the air and environment generally and the negative control may therefore give a late positive signal due to environmental contamination. The section below gives guidance on how to interpret results where environmental contamination is evident.

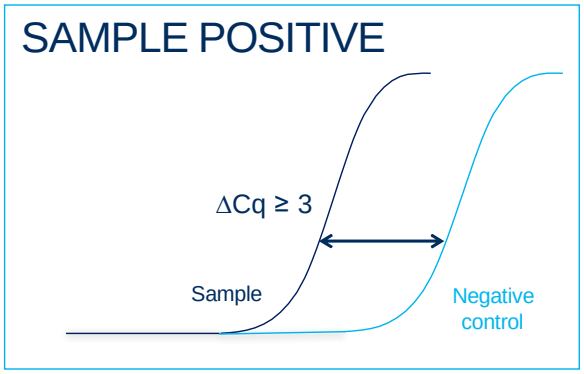
Summary of data interpretation

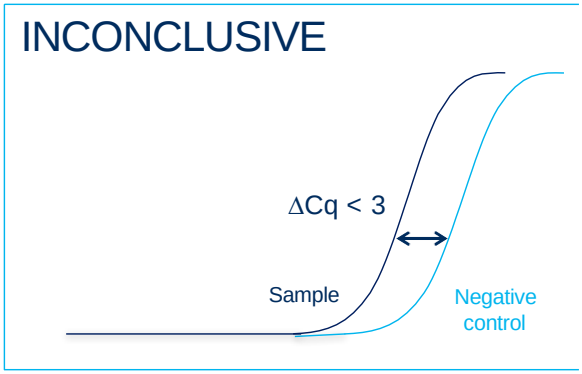
Target (FAM/VIC)	Internal extraction control (Cy5)	Positive Control	Negative Control	Interpretation
FAM +	**+ / -**	**+**	**-**	***CUTIBACTERIUM* POSITIVE RESULT**
VIC +	**+ / -**	**+**	**-**	***S. AUREUS* POSITIVE RESULT**
-	**+**	**+**	**-**	**NEGATIVE RESULT**

+ / -	**+ / -**	**+**	**≤30**	**EXPERIMENT FAILED** Due to test contamination
+ / -	**+ / -**	**+**	**>30**	*****
-	**-**	**+**	**-**	**SAMPLE PREPARATION FAILED**
+ / -	**+ / -**	**-**	**+ / -**	**EXPERIMENT FAILED**

Positive control template (RED) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

* Where the test sample is positive, and the negative control is positive with a Cq >30, the sample must be reinterpreted based on the relative signal strength of the two results:





If the sample amplifies ≥ 3 Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.

If the sample amplifies < 3 Cq earlier than the negative control, then the positive sample result is invalidated, and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.