BACTERIAL MENINGITIS REALTIME PCR KIT

REF RTPCR014-LPD-R Σ 48

RUO For research use only

INTENDED PURPOSE

Real Time PCR kit for detection of nucleic acids from *Streptococcus pneumoniae* (SPN), *Listeria monocytogenes* (LMO), *Streptococcus agalactiae* (SAG), *Haemophilus influenzae* (HIN), *Neisseria meningitidis* (NME) and *Escherichia coli* K1 (ECOK1) in human cerebrospinal fluid (CSF) samples.

INTRODUCTION

Streptococcus pneumoniae or pneumococcus is an α-haemolytic capsulated streptococcus that is found in the normal flora of the human oropharynx. It can cause pneumonia, sinusitis, otitis media, meningitis or endocarditis.

Listeria monocytogenes is a Gram-positive, facultative anaerobic, asporogenous, motile, short-bacillary bacterium. It has been isolated from diverse animals, but soil and vegetable matter are their primary habitats. Transmitted through contaminated food, it shows tropism for the central nervous system and is able to cross the placenta and infect the fetus.

Streptococcus agalactiae or Lancefield group B streptococcus is a ß-haemolytic streptococcus that is found in the normal gastrointestinal flora and can colonize the urogenital tract. It is the main cause of neonatal sepsis in newborns infected intrapartum through their mothers.

Haemophilus influenzae is a Gram-negative, facultative anaerobic, coccobacillary bacterium that requires growth factors V and X. It is an obligate parasite found in the normal flora of healthy children. Capsulated strains of serotype b are frequent cause of meningitis in non-vaccinated children.

Neisseria meningitidis or meningococcus is a Gram-negative, oxidase-positive, aerobic, coccal bacterium that appears microscopically under diplococcal arrangement. Strains are serogrouped on the basis of their capsular polysaccharides. The meningococcus usually inhabits the human nasopharynx without causing detectable disease although it may cause meningococcal meningitis. Six serogroups (A, B, C, W, X and Y) are responsible for virtually all cases of meningitis.

Escherichia coli is the principal Gram-negative rod responsible for meningitis and sepsis in newborn and premature infants. Most of the *E. coli* strains isolated from the cerebrospinal fluid (78–92 %) possess the K1 capsular antigen as an essential virulence determinant. *E. coli* K1 remains a major cause of high neonatal mortality and morbidity. It is therefore important to identify the K1-encapsulated *E. coli* rapidly and reliably to start the appropriate treatment as soon as possible.

TEST PRINCIPLE

It is based on the amplification of specific nucleic acid fragments of *Streptococcus pneumoniae* (SPN), *Listeria monocytogenes* (LMO), *Streptococcus agalactiae* (SAG), *Haemophilus influenzae* (HIN), *Neisseria meningitidis* (NME), and *Escherichia coli* K1 (ECOK1) by real time PCR in human cerebrospinal fluid (CSF) samples.

Two master mix (VIRCELL BME RT-PCR MIX A and VIRCELL BME RT-PCR MIX B) are provided for screening and confirmation using one independent target for each microorganism.

VIRCELL BME RT-PCR MIX A targets a specific fragment of the *lytA* gene for SPN, *hly* gene for LMO and *cfb* gene for SAG, while in VIRCELL BME RT-PCR MIX B targets a specific fragment of *bexB* and *siaT* genes for HIN, *ctrA* and *sodC* genes for NME and *neuB* gene for ECOK1.

An amplification control is included to check the absence of carry-over of amplification inhibitors and the correct amplification set-up. This control consists of exogenous nucleic acids and specific oligo pair/probe for its amplification.

The technique is divided into 2 main steps: DNA extraction and amplification/detection with specific oligo pairs and probes. In VIRCELL BME RT-PCR MIX A, SPN DNA is detected in FAM channel, LMO DNA is detected in HEX/VIC channel and SAG DNA is detected in Texas/ROX channel, while in VIRCELL BME RT-PCR MIX B, HIN DNA is detected in FAM channel, NME DNA is detected in HEX/VIC channel and ECOK1 DNA is detected in Texas/ROX channel. The internal control is detected in Cy5 channel.

KIT FEATURES

VIRCELL RT-PCR MIX and VIRCELL POSITIVE CONTROL are lyophilized. It is necessary to reconstitute them before use (see "Preparatory treatment of the device" section). The rest of the reagents are ready to use.

This kit is based on amplification and detection using real time PCR.

MATERIALS PROVIDED

[1] VIRCELL BME RT-PCR MIX LPD: 1 plate with 96 tubes divisible into 12 strips with 8 tubes. Each strip has 4 tubes with BME RT-PCR MIX A containing Taq polymerase, buffer, specific primers/probe for SPN (*lytA* gene), LMO (*hly* gene) and SAG (*cfb* gene) and, as internal control, primers/probe and 4 tubes with BME RT-PCR MIX B containing Taq polymerase, buffer, specific primers/probe for HIN (*bexB* and *siaT* genes), NME (*ctrA* and *sodC* genes) and ECOK1 (*neuB* gene). Also, primers/probe for internal control. 1 reaction per tube. Lyophilized.

See Figure 1 for the distribution of BME RT-PCR MIX A and BME RT-PCR MIX B on the strip.

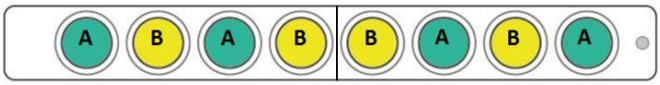


Figure 1: Distribution of BME RT-PCR MIX A and BME RT-PCR MIX B on the strip.
Legend: A= BME RT-PCR MIX A and B= BME RT-PCR MIX B

[3] VIRCELL BME POSITIVE CONTROL: 1 vial containing a mixture of lyophilized non-infectious nucleic acids to be used as positive control. Red cap.

[4] VIRCELL NEGATIVE CONTROL: 200 µl of deionized water to be used as negative control. Green cap.

[5] VIRCELL PCR MIX RECONSTITUTION SOLUTION: 2 x 1 ml of aqueous solution to reconstitute the PCR mix. Yellow cap.

[6] VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION: 500 µl of aqueous solution to reconstitute the positive control. Brown cap.

[7] VIRCELL RT-PCR MIX CAPS: 12 strips of 8 caps RT-PCR compatible.

Special materials required but not provided:

-Microbiological safety cabinet.
-DNA/RNA extraction kit (see recommendations in "Assay procedure").
-Real Time PCR thermocycler.
-Precision micropipettes.
-Sterile tips with aerosol barrier.
-Microcentrifuge.
-PCR cabinet (recommended).
-Vortexer.

STORAGE AND HANDLING CONDITIONS

Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

IN-USE STABILITY

VIRCELL POSITIVE CONTROL reconstituted: store it between -25°C and -15°C and use until expiration date. Avoid more than 10 freeze-thaw cycles during this time period.

VIRCELL RT-PCR MIX reconstituted: store it between 2ºC and 8ºC and use before 60 minutes.

Rest of reagents: Refer to package label for expiration date (at 2-8°C).

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

WARNINGS AND PRECAUTIONS

1. For research use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
4. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
5. Wear personal protective equipment when handling samples and reagents. Wash hands properly after handling the samples and reagents. All procedures must be carried out in accordance with the approved safety standards.
6. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
7. Never pipette by mouth.

8. Do not use in the event of damage to the package.
9. Do not use the kit after expiration date.
10. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
11. Keep containers for samples and reagents closed while they are not being handled.
12. Avoid using samples subjected to repeated freeze-thaw cycles.
13. Handle in aseptic conditions to avoid microbial contaminations.
14. Reagents in this kit could include nucleic acids. Observe the local regulations for waste disposal.
15. Dispose of unused reagents and waste in accordance with all applicable regulations.
16. Use kit components only. Do not mix components from different kits or manufacturers. Only VIRCELL NEGATIVE CONTROL, VIRCELL PCR MIX RECONSTITUTION SOLUTION and VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION are compatible with the equivalents in other RTPCR VIRCELL references and lots.
17. Specimens should be handled as in the case of infectious samples using safety laboratory procedures. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.
18. Testing of all the samples at the earliest interval following collection will help ensure the most accurate test results. Variation in storage times during specimen shipment has not been assessed.
19. It is recommended to have two different areas to perform the test: Pre-Amplification and Amplification areas.
20. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of kit reagents or amplification mixtures. All reagents should be closely monitored to purity.
21. It is recommended to use conventional DNA/RNA purification kits.

CONDITIONS FOR COLLECTION, HANDLING AND PREPARATION OF THE SPECIMEN

The kit can be used with human cerebrospinal fluid samples.
Do not delay transport and laboratory investigations. Specimens could be stored at 2-8°C for up to 4 hours after collection, if delay is expected storage between -90 °C and -70 °C is recommended.

PREPARATORY TREATMENT OF THE DEVICE

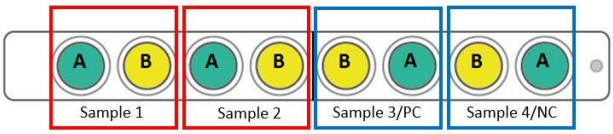
All reagents supplied are ready to use, except for the VIRCELL RT-PCR MIX [1] and the VIRCELL POSITIVE CONTROL [3].
[1] VIRCELL RT-PCR MIX. For reconstitution add 15 µl of VIRCELL PCR MIX RECONSTITUTION SOLUTION [5] per tube.

⚠The reconstituted VIRCELL RT-PCR MIX must be used within 60 minutes of adding the reconstitution solution stored at 2-8ºC if the start of the test is delayed. In this case, a freeze rack is recommended.
[3] VIRCELL POSITIVE CONTROL. Follow the next steps to reconstitute it:
- Centrifuge the corresponding tube for 5 seconds at 5000 g.
- Add 100 µl of VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION [6]
- Mix with vortex for 1-2 seconds.
- Centrifuge the tube for 5 seconds at 5000 g.

After reconstitution, the VIRCELL POSITIVE CONTROL [3] can be frozen at temperature between -25°C and -15°C to be used in subsequent reactions.

ASSAY PROCEDURE

1. DNA extraction (performed in the Pre-Amplification area):
   1.1. It is recommended to use a commercial extraction kit for DNA/RNA extraction. In order to use commercial extraction kits, follow the manufacturer instructions. Consult with Customer Service.
2. Amplification using RT-PCR (performed in the Amplification area):
   2.1. Preparation of the VIRCELL RT-PCR MIX tubes: the plate with 96 tubes provided could be easily torn off into one or more individual 8-tube strips depending of the samples to be tested. Please note each 8-tube strip will be used for 4 samples. Add 15 µl of VIRCELL PCR MIX RECONSTITUTION SOLUTION [5] per tube. Maintain cold after reconstitution/thawing.
   2.2. Addition of the sample: Add 5 µl of each extracted DNA/RNA sample to one tube containing RT-PCR MIX A and one tube containing RT-PCR-MIX B. Add 5 µl of VIRCELL POSITIVE CONTROL [3] and VIRCELL NEGATIVE CONTROL [4] to the corresponding tubes containing RT-PCR MIX A and RT-PCR MIX B. The negative control is water.



   2.3. Secure caps VIRCELL RT-PCR MIX CAPS [7] on the tubes.
   2.4. It is recommended to briefly centrifuge the plate/strips of tubes with the purpose of ensuring vial content is at the bottom of the tube.
   2.5. RT-PCR program: Insert the PCR tubes in the real time thermocycler and run the following program*:

1 cycle	95 ºC	3 minutes
45 cycles	95 ºC	15 seconds
	58 ºC	45 seconds*

* Fluorescence data (FAM, HEX/VIC, Texas/ROX and Cy5) should be collected.

INTERNAL QUALITY CONTROL

Each batch is subjected to internal quality control testing before releasing, complying with strict specifications.

VALIDATION PROTOCOL FOR USERS

It is recommended to include one negative control in each run performed. The negative control will monitor reagent or environmental contamination.
The positive control is recommended to be included on each run. The positive control monitors for reagent failures and for correct operation of essential procedure.
The thermocycler software is likely to automatically calculate the baseline fluorescence value (threshold) based on the amplification curve for each target (fluorescence detection). Nevertheless, it is recommended to set the thresholds for the different detection channels individually. In order to set a threshold for each target, it is recommended to use as a reference the amplification curves of the positive and negative controls. The threshold should be fixed at the beginning of the exponential reading of fluorescence and above the background signal.
The controls result interpretation is as follows:

CONTROL	SPN (FAM)	LMO (HEX/VIC)	SAG (Texas/ROX)	HIN (FAM)	NME (HEX/VIC)	ECOK1 (Texas/ROX)	IC (Cy5)	Interpretation
VIRCELL BME POSITIVE CONTROL	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Correct
	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Invalid
VIRCELL NEGATIVE CONTROL	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40)	Correct
	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	No amplification or Ct >40	Invalid

INTERPRETATION OF RESULTS

The result interpretation is described in the tables below:

Table 1: RT-PCR MIX A

RESULT	SPN (FAM)	LMO (HEX/VIC)	SAG (Texas/ROX)	IC[1] (Cy5)	Interpretation
1	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Invalid (sample/kit/ setup related)
2	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40)	Negative
3	Amplification (Ct < 40)	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40) or no amplification	SPN
4	No amplification or Ct >40	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 40) or no amplification	LMO
5	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	SAG
6	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	SPN + SAG
7	Amplification (Ct < 40)	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 40) or no amplification	SPN + LMO
8	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	LMO + SAG
9	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	SPN + LMO + SAG

Table 2: RT-PCR MIX B

RESULT	HIN (FAM)	NME (HEX/VIC)	ECOK1 (Texas/ROX)	IC[1] (Cy5)	Interpretation
1	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Invalid (sample/kit/ setup related)
2	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40)	Negative
3	Amplification (Ct < 40)	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40) or no amplification	HIN
4	No amplification or Ct >40	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 40) or no amplification	NME
5	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	ECOK1
6	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	HIN + ECOK1
7	Amplification (Ct < 40)	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 40) or no amplification	HIN + NME
8	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	NME + ECOK1
9	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	HIN +NME + ECOK1

[1] In case of a high copy number of the target nucleic acid, the amplification of the internal control (IC) in results 3 to 9 (Table 1) and 3 to 9 (Table 2) may be affected. The late amplification or absence of IC amplification does not change the interpretation of the result.
In case of invalid or inconclusive result, it is recommended to re-extract DNA/RNA from original specimen and re-test it. In the case of failure of amplification of internal control, improper extraction of nucleic acids or inhibition of amplification could be assumed. Testing a new sample is recommended.

SYMBOLS USED IN LABELS

For research use only


Use-by (expiry date)

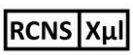
Store at x-y °C

Contains sufficient for <n> test

Batch code

Catalogue number


Consult instructions for use



Reconstitute in <X> µl


Manufacturer

Current version Nr.: L-RTPCR014-LPD-R-EN-01
Date: 2024/06/26
Updates: New reference