# RIDA®GENE CAP Bac

REF PG2705

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## 1. Intended use

For *in vitro* diagnostic use. RIDA®GENE CAP Bac is a multiplex real-time PCR for the direct qualitative detection and differentiation of *Chlamydophila pneumoniae (Chlamydia pneumoniae)*, *Legionella pneumophila*, and *Mycoplasma pneumoniae* in human bronchoalveolar lavage (BAL).

The RIDA®GENE CAP Bac multiplex real-time PCR is intended to use as an aid in diagnosis for community-acquired pneumonia (CAP) caused by *Chlamydophila pneumoniae, Legionella pneumophila*, or *Mycoplasma pneumoniae*.

## 2. Summary and explanation of the test

Community-acquired pneumonia (CAP) is the most frequently recorded infectious disease worldwide and is the infectious disease which most frequently leads to death in Western nations. In Germany, there are up to 600,000 cases of CAP annually, and the mortality varies between 0.6 % and 14 % depending on whether CAP is outpatient or inpatient.[1] Bacteria are the most frequent pathogens of CAP, and a distinction is drawn between typical and atypical pathogens. Atypical bacteria cannot be cultivated in a regular culture of sputum or blood and also cannot be visualized by gram staining. This makes it difficult to detect atypical CAP bacteria since standard methods generally make identification impossible. The most frequent atypical CAP bacteria include *Mycoplasma pneumoniae (M. pneumoniae), Legionella* spp., and *Chlamydophila pneumoniae (C. pneumoniae)*.[2]
Up to 20 % of outpatient pneumonias are triggered by *M. pneumoniae*.[3]
*M. pneumoniae* is a highly infectious bacterium without a cell wall that is primarily transmitted in the air through droplets or by direct or indirect contact through smear infections. *M. pneumoniae* does not belong to the normal flora of humans and is generally detected in children and young adults. Pneumonia develops in 5 % to 25 % of cases of infection with *M. pneumoniae* and generally requires further treatment with antibiotics.[4]
*Chlamydophila pneumoniae* is a gram-negative bacteria and is generally transmitted through the air.[5] An infection with *C. pneumoniae* is generally difficult to overcome, but mild.[5,6] It is estimated that *C. pneumoniae* has a prevalence rate of 50 % to 70 %, and 60 % of the population will have experienced a *C. pneumoniae* infection by the age of 20. A severe case can lead to atypical pneumonia. In Germany, it is estimated that up to 5 % of CAP pneumonias are caused by *C. pneumoniae*.[1,5] The bacteria can dwell in the upper respiratory tract for many years so the danger of infection may exist for a long time. Classic antigen detection methods such as ELISA have only a narrow specificity and can be used only after several weeks of an acute infection. PCR can detect the pathogen reliably from respiratory samples or tissues.[5]
The Legionella strain belongs to the *Legionellaceae* family and is divided into 40 species with more than 70 serogroups. Legionella bacteria are facultative intracellular gram-negative bacteria whose peak infection rate occurs in summer and early fall. With Legionnaires' disease, a differentiation is made between community

acquired, travel-associated, and nosocomial infections. In the USA, the mortality rate of nosocomial infections is between 15 % and 20 %. In Europe, 12 % of all Legionella infections are fatal.[7] All Legionella species are to be considered potentially pathogenic to humans. In Europe, however, most community-acquired illnesses are caused by pathogens of the species *Legionella pneumophila* serogroup 1. An infection with *Legionella pneumophila* primarily leads to Legionnaires' disease (also termed legionellosis).[8]

## 3. Test principle

RIDA®GENE CAP Bac is a multiplex real-time PCR for the direct qualitative detection of *Chlamydophila pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae* in human bronchoalveolar lavage (BAL).
Following DNA isolation, the specific gene fragments (if present) of *Chlamydophila pneumoniae* (16S-rRNA), *Legionella pneumophila* (16S-rRNA), and *Mycoplasma pneumoniae* (IGS) are amplified. The amplified target sequences are detected using hydrolysis probes that are attached to a quencher at one end and a fluorescent reporter dye (fluorophore) at the other end. The probes hybridize with the amplicons in the presence of a target sequence. During extension, the Taq-Polymerase separates the reporter from the quencher. The reporter emits a fluorescence signal that is detected by the optical unit of a real-time PCR device. The fluorescence signal increases with the quantity of formed amplicons. The RIDA®GENE CAP Bac test contains an Internal Control DNA (ICD) to be able to control the specimen preparation and/or potential PCR inhibition.

## 4. Reagents provided

**Table 1**: Reagents provided (The reagents provided in the kit are sufficient for 100 determinations.)

| Kit code | Reagents | Amount | | Lid color |
|---|---|---|---|---|
| **1** | Reaction Mix | 2x | 1050 µl | Yellow |
| **2** | Taq-Polymerase | 1x | 80 µl | Red |
| **D** | Internal Control DNA | 2x | 1700 µl | Orange |
| **N** | No Template Control | 1x | 450 µl | White |
| **P** | Positive Control | 1x | 200 µl | Blue |

## 5. Storage instructions

- All reagents must be stored away from light at -20 °C and, if unopened, can be used until the expiration date printed on the label. After the expiration date, the quality guarantee is no longer valid.
- All reagents should be carefully thawed prior to use (e.g., in a refrigerator at 2 – 8 °C).
- Repeated freezing/thawing of up to 20 times does not have an impact on the test property (if necessary, create aliquots after the first thaw and refreeze reagents immediately).
- Cool all reagents during PCR preparation (2 – 8 °C).

## 6. Additional necessary reagents and necessary equipment

The RIDA®GENE CAP Bac multiplex real-time PCR test can be used with the following extraction platforms and real-time PCR devices:

**Table 2**: Necessary equipment

| **Extraction platforms** | |
|---|---|
| R-Biopharm | RIDA® Xtract |
| Promega | Maxwell® RSC |
| **Real-time PCR devices** | |
| R-Biopharm | RIDA®CYCLER |
| Roche | LightCycler® 480II, LightCycler® 480 z |
| Agilent Technologies | Mx3005P |
| Applied Biosystems | ABI 7500 |
| Bio-Rad | CFX96™ |
| QIAGEN | Rotor-Gene Q |

**Note: When using Rotor-Gene Q (QIAGEN), use only 0.1 ml reaction vials.**

Should you have to use other extraction procedures or real-time PCR devices, please contact R-Biopharm to check the compatibility at mdx@r-biopharm.de.

- RIDA®GENE Color Compensation Kit IV (PG0004) when using LightCycler® 480II and LightCycler® 480 z
- Real-time PCR consumables (plates, reaction vials, films)
- Centrifuge with rotor for reaction vials or plates
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Pipette tips with filters
- Powder-free disposable gloves
- PCR water (nuclease-free)

## 7. Precautions for users

For *in vitro* diagnostic use only.

### 7.1 General warnings and precautions for the users

This test must be carried out only by trained laboratory personnel. The guidelines for working in medical laboratories must be followed. Read through the instructions for use carefully prior to testing and strictly adhere to the instructions.

Biological specimens must be viewed as potentially infectious and must be handled and disposed of appropriately, like all reagents and materials that come into contact with potentially infectious specimens. Avoid direct contact with biological specimens. Avoid squirting or spraying. Materials that come into contact with biological specimens must be disposed of at least in accordance with applicable regulations. Flammable disposable materials must be burned. Liquid waste containing acids or bases must be neutralized prior to disposal.

Users are responsible for proper disposal of all reagents and materials after use in accordance with appropriate safety standards. For disposal, please adhere to national regulations.

For further details, see the safety data sheets (SDS) at www.r-biopharm.com.

Wear personal protective equipment (appropriate gloves, lab coat, safety glasses, face mask) when handling reagents and specimens, and wash hands meticulously after completing the test. Do not pipette samples or reagents using your mouth. Do not smoke, eat, drink, or use cosmetic products in work areas. Avoid contact with broken skin and mucous membranes.

Dispose of test kit once the expiration date has lapsed. Use only the reagents provided with the product and recommended by the manufacturer. Do not use reagents from other lots. Do not use reagents from other manufacturers.

### 7.2 Molecular biological warnings and precautions for the users

Molecular biological procedures such as nucleic acid extraction, amplification, and detection require trained and qualified laboratory personnel to avoid the risk of erroneous results, especially resulting from the degradation of the nucleic acid in the specimens or contamination of the specimens with amplification products.
For manual processing, ensure that sample preparation/extraction, PCR preparation, and PCR are carried out in different rooms in order to avoid cross-contamination. Never bring amplification products into areas where sample preparation/extraction and PCR preparation are taking place. Each area (sample preparation/extraction, PCR preparation, and PCR) requires separate lab coats, gloves, and implements designated exclusively for that area. Lab coats, gloves, and implements from the

PCR area should never be used in the sample preparation/extraction or PCR preparation area.
The specimens should be used only for this type of analysis and must be processed under the biosafety cabinet. Never open vials of different specimens at the same time. Pipettes used for sample preparation must be used for this purpose only.
The required reagents must be processed under a laminar flow workbench. The reagents needed for amplification must be prepared for use in a test procedure. Pipettes used for reagents must be used for this purpose only. Only positive displacement pipettes or pipettes with filter tips may be used. The pipette tips must be sterile and free of DNAse and RNAse as well as DNA and RNA.
To avoid contamination, handle amplification products such that their spread into the environment is avoided as much as possible. Pipettes used for amplification products must be used only for this purpose.

## 8. Collection and storage of specimens

### 8.1 DNA preparation from bronchoalveolar lavage (BAL)

For DNA isolation from BAL, specimens must be used that were stored according to laboratory guidelines, but not stored or transported for longer than 24 hours after being taken. Specimens that were transported and/or stored for more than 24 hours should be stored for up to 72 hours at +2 °C to +8 °C. Specimens stored for longer than 72 hours must be kept at -70 °C.[9]
It is recommended to produce aliquots of the specimens to avoid repeated thawing and freezing. Frozen specimens should be thawed immediately prior to extraction to prevent degradation of the nucleic acid.

A commercially obtainable nucleic acid extraction kit (such as RIDA® Xtract (R-Biopharm)) or nucleic acid extraction system (such as Maxwell® RSC (Promega)) is recommended for DNA preparation from bronchoalveolar lavage (BAL). The manufacturer's instructions must be observed.

The RIDA®GENE CAP Bac test contains an Internal Control DNA which indicates potential PCR inhibition, checks the integrity of the reagents, and confirms successful nucleic acid extraction. The Internal Control DNA can be used either only as inhibition control or as extraction control for specimen preparation and as inhibition control.

If the Internal Control DNA is used only as inhibition control, 1 µl of the Internal Control DNA must be added to the master mix (see Table 4).

If the Internal Control DNA is used as extraction control for specimen preparation **and** as inhibition control, 20 µl of the Internal Control DNA must be used during extraction. The Internal Control DNA should be added to the specimen-lysis buffer mix and should **not** be added directly to the specimen material. We recommend pipetting 1 µl per reaction of the

Internal Control DNA to both the PCR mix for the negative control and the positive control.

## 9. Test procedure

### 9.1 Master-Mix preparation

The total number of the reactions needed for PCR (samples and control reactions) must be calculated. One positive and one negative control must be included in each test run.

Adding an additional 10 % volume to the master mix is recommended in order to balance out the pipette loss (see Tab. 3, Tab. 4). Prior to use, thaw, mix, and briefly centrifuge the Reaction Mix, the Taq-Polymerase, the Positive Control, the No Template Control, and the Internal Control DNA. Always cool all reagents during work steps (2 – 8 °C).

**Table 3**: Example of the calculation and production of the master mix for 10 reactions (ICD extraction and inhibition control)

| Kit code | Components of the master mix | Quantity per reaction | 10 reactions (plus 10 %) |
|---|---|---|---|
| 1 | Reaction Mix | 19.3 µl | 212.3 µl |
| 2 | Taq-Polymerase | 0.7 µl | 7.7 µl |
| | **Overall** | **20 µl** | **220 µl** |

Mix the master mix and then centrifuge for short time.

**Table 4**: Example of the calculation and production of the master mix for 10 reactions (ICD only as inhibition control)

| Kit code | Components of the master mix | Quantity per reaction | 10 reactions (plus 10 %) |
|---|---|---|---|
| 1 | Reaction Mix | 19.3 µl | 212.3 µl |
| 2 | Taq-Polymerase | 0.7 µl | 7.7 µl |
| D | Internal Control DNA | 1.0 µl | 11 µl |
| | **Overall** | **21.0 µl** | **231.0 µl** |

Mix the master mix and then centrifuge for short time.

## 9.2 Preparation of the PCR-Mix

Pipette 20 µl of the master mix into each reaction vial (vial/plate).

**Negative control:** Pipette 5 µl of the No Template Control to the pre-pipetted master mix.

**Note: We recommend pipetting 1 µl of the Internal Control DNA into the PCR mix for the negative control when using the Internal Control DNA as extraction control for specimen preparation and as inhibition control.**

**Specimens:** Pipette 5 µl eluate to the pre-pipetted master mix.

**Positive control:** Pipette 5 µl of the Positive Control to the pre-pipetted master mix.

**Note: We recommend pipetting 1 µl of the Internal Control DNA into the PCR mix for the positive control when using the Internal Control DNA as extraction control for specimen preparation and as inhibition control.**

Seal the reaction vials or plates, briefly centrifuge at slow speed, and transfer into the real-time PCR device. Start PCR according to device settings (see Table 5, Table 6, Table 7, Table 8).

## 9.3 PCR instrument set-up

### 9.3.1 DNA real-time PCR profile

**Table 5**: DNA real-time PCR profile for LightCycler® series Rotor-Gene Q, RIDA®CYCLER

| | |
|---|---|
| Initial denaturation | 1 min, 95 °C |
| Cycles | 45 cycles |
| PCR Denaturation<br>Annealing/Extension | 10 sec, 95 °C<br>15 sec, 60 °C |
| Temperature Transition Rate/<br>Ramp Rate | Maximum |

**Note: Annealing and extension take place in the same step.**

**Table 6**: DNA real-time PCR profile for Mx3005P, ABI7500, and CFX96™

| | |
|---|---|
| Initial denaturation | 1 min, 95 °C |
| Cycles | 45 cycles |
| PCR Denaturation<br>Annealing/Extension | 15 sec, 95 °C<br>30 sec, 60 °C |
| Temperature Transition Rate/ Ramp Rate | Maximum |

**Note: Annealing and extension take place in the same step.**

### 9.3.2 Universal real-time PCR profile

**Note: The universal real-time PCR profile for DNA tests should be used only if RIDA®GENE DNA and RIDA®GENE RNA real-time PCR tests are combined in one run.**

**Table 7**: Universal real-time PCR Profile for LightCycler® series and RIDA®CYCLER

| | |
|---|---|
| Reverse transcription | 10 min, 58 °C |
| Initial denaturation | 1 min, 95 °C |
| Cycles | 45 cycles |
| PCR Denaturation<br>Annealing/Extension | 10 sec, 95 °C<br>15 sec, 60 °C |
| Temperature Transition Rate/ Ramp Rate | Maximum |

**Note: Annealing and extension take place in the same step.**

**Table 8**: Universal real-time PCR profile for Mx3005P, ABI7500, Rotor-Gene Q, and CFX96™

| | |
|---|---|
| Reverse transcription | 10 min, 58 °C |
| Initial denaturation | 1 min, 95 °C |
| Cycles | 45 cycles |
| PCR Denaturation<br>Annealing/Extension | 15 sec, 95 °C<br>30 sec, 60 °C |
| Temperature Transition Rate/ Ramp Rate | Maximum |

**Note: Annealing and extension take place in the same step.**

## 9.4 Detection channel setting

**Table 9**: Selection of appropriate detection channels

| Real-time PCR device | Detection | Detection channel | Comment |
|---|---|---|---|
| **R-Biopharm RIDA®CYCLER** | *Chlamydophila pneumoniae* | Green | **-** |
| | ICD | Yellow | |
| | *Legionella pneumophila* | Orange | |
| | *Mycoplasma pneumoniae* | Red | |
| **Roche LightCycler® 480II** | *Chlamydophila pneumoniae* | 465/510 | **RIDA®GENE Color Compensation Kit IV (PG0004) is required.** |
| | ICD | 533/580 | |
| | *Legionella pneumophila* | 533/610 | |
| | *Mycoplasma pneumoniae* | 618/660 | |
| **Roche LightCycler® 480 z** | *Chlamydophila pneumoniae* | 465/510 | **RIDA®GENE Color Compensation Kit IV (PG0004) is required.** |
| | ICD | 540/580 | |
| | *Legionella pneumophila* | 540/610 | |
| | *Mycoplasma pneumoniae* | 610/670 | |
| **Agilent Technologies Mx3005P** | *Chlamydophila pneumoniae* | FAM | **Set the reference dye to none.** |
| | ICD | HEX | |
| | *Legionella pneumophila* | ROX | |
| | *Mycoplasma pneumoniae* | Cy5 | |
| **ABI 7500** | *Chlamydophila pneumoniae* | FAM | **Set the passive reference dye ROX to none.** |
| | ICD | VIC | |
| | *Legionella pneumophila* | ROX | |
| | *Mycoplasma pneumoniae* | Cy5 | |
| **Bio-Rad CFX96™** | *Chlamydophila pneumoniae* | FAM | **-** |
| | ICD | VIC | |
| | *Legionella pneumophila* | ROX | |
| | *Mycoplasma pneumoniae* | Cy5 | |
| **Qiagen Rotor-Gene Q** | *Chlamydophila pneumoniae* | Green | **The gain settings must be set to 5 (default) for all channels.** |
| | ICD | Yellow | |
| | *Legionella pneumophila* | Orange | |
| | *Mycoplasma pneumoniae* | Red | |

## 10. Quality control

Specimens are evaluated using the analysis software of the respective real-time PCR device according to the manufacturer’s instructions. The negative control and positive control must show the correct results (see Table 10, Fig. 1, Fig. 2, Fig. 3).

The Positive Control comes at a concentration of $10^3$ copies/µl. It is used in a total quantity of 5 x $10^3$ copies in every PCR run.

**Table 10**: A valid PCR run must meet the following conditions:

| Sample | Result | ICD Ct | Target gene Ct |
|---|---|---|---|
| Positive control | Positive | N/A *[1] | See Quality Assurance Certificate |
| Negative control | Negative | Ct > 20 | 0 |

**[1] A Ct value for the ICD is not essential to achieve a positive result for the positive control.*

If the positive control is not in the specified Ct range but the negative control is valid, all reactions need to be reanalyzed, including the controls.

If the negative control is not negative but the positive control is valid, all reactions need to be reanalyzed, including the controls.

If the specified values are not met, check the following things before repeating the test:

- Expiration date of the reagents used
- Functionality of the devices used
- Correct test procedure

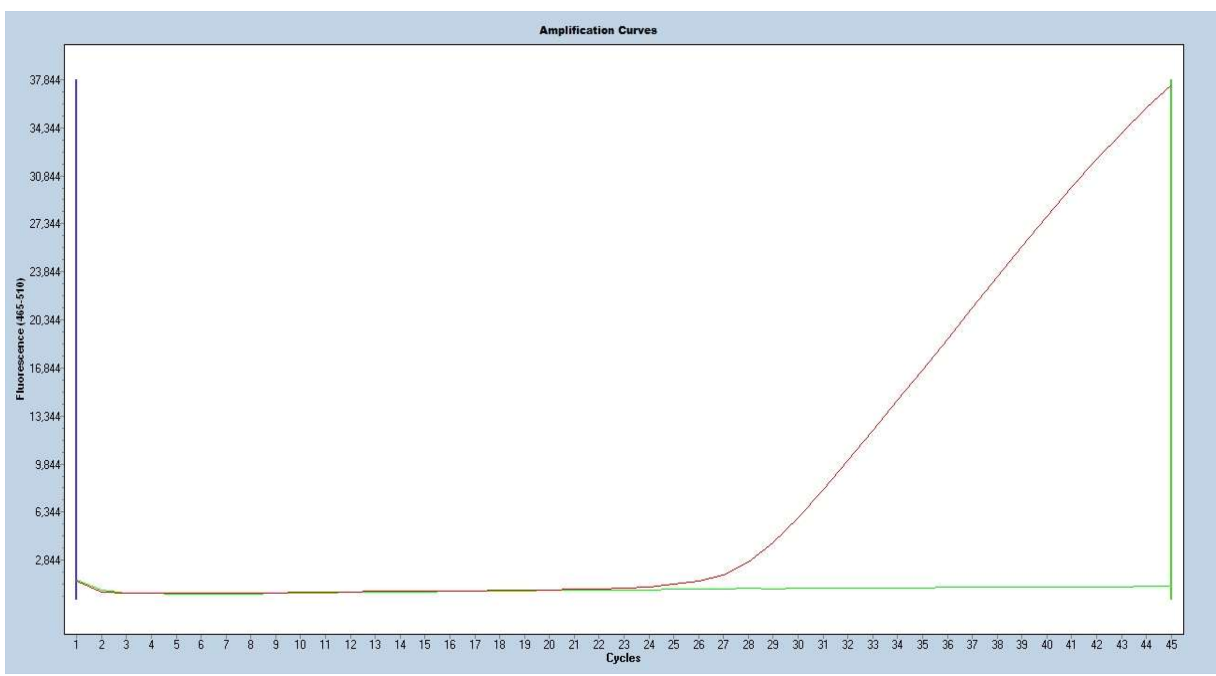


**Fig. 1**: Correct run of the positive control (red) and negative control (green) (*Chlamydophila pneumoniae*) on LightCycler® 480II

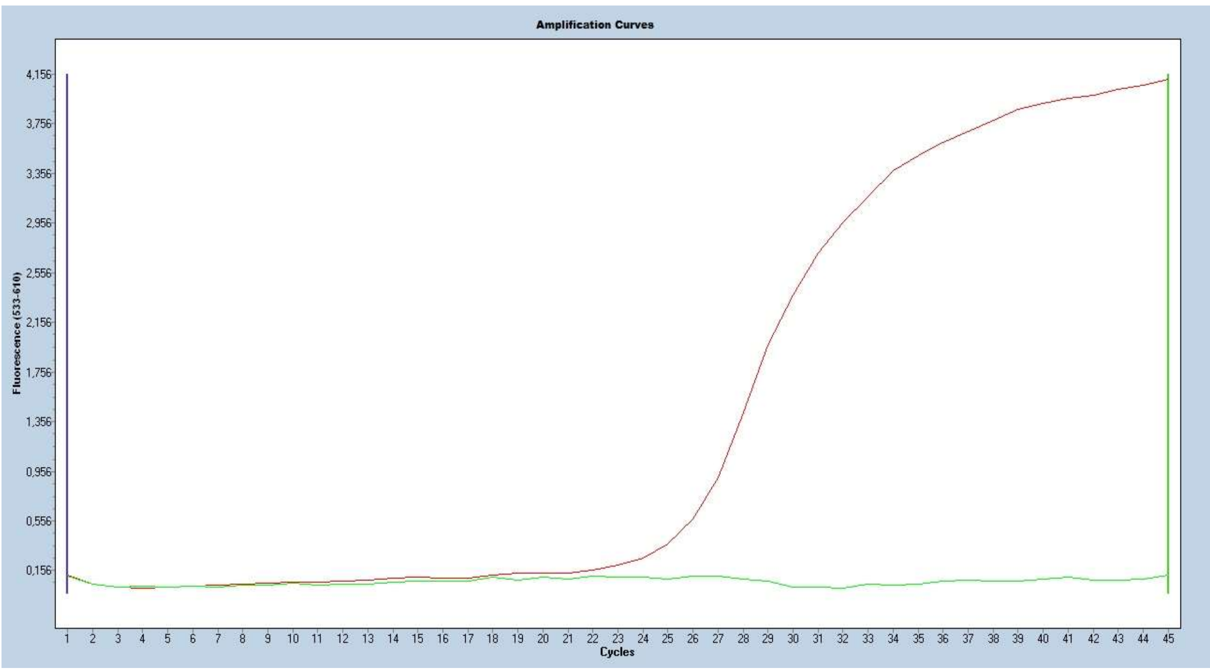


**Fig. 2**: Correct run of the positive control (red) and negative control (green) (*Legionella pneumophila*) on LightCycler® 480II

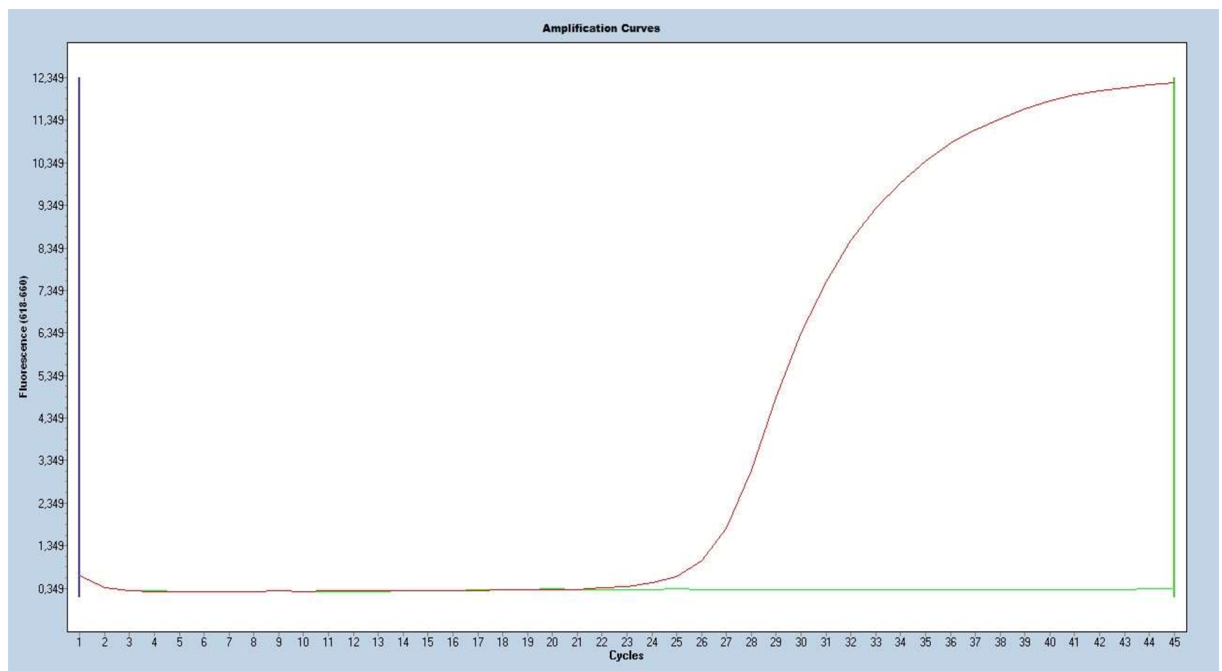


**Fig. 3**: Correct run of the positive control (red) and negative control (green) (*Mycoplasma pneumoniae*) on LightCycler® 480II

## 11. Result interpretation

The result interpretation is done according to Table 11.

**Table 11**: Sample interpretation

| Detection of | | | | |
|---|---|---|---|---|
| ***Chlamydophila pneumoniae*** | ***Legionella pneumophila*** | ***Mycoplasma pneumoniae*** | **ICD** | **Result** |
| **positive** | negative | negative | **positive / negative** | ***C. pneumoniae* detectable** |
| negative | **positive** | negative | **positive / negative** | ***L. pneumophila* detectable** |
| negative | negative | **positive** | **positive / negative** | ***M. pneumoniae* detectable** |
| **positive** | **positive** | negative | **positive / negative** | ***C. pneumoniae* and *L. pneumophila* detectable** |
| **positive** | negative | **positive** | **positive / negative** | ***C. pneumoniae* and *M. pneumoniae* detectable** |
| negative | **positive** | **positive** | **positive / negative** | ***L. pneumophila* and *M. pneumoniae* detectable** |
| **positive** | **positive** | **positive** | **positive / negative** | ***C. pneumoniae, L. pneumophila*, and *M. pneumoniae* detectable** |
| negative | negative | negative | **positive** | **Target genes not detectable** |
| negative | negative | negative | **negative** | **Invalid** |

A specimen is rated positive if the specimen DNA and the Internal Control DNA show an amplification signal in the detection system.

A specimen is also rated positive if the specimen DNA displays an amplification signal but no amplification signal can be found in the detection system for the Internal Control DNA. Detecting the Internal Control DNA is not necessary in this case because high amplicon concentrations can cause a weak or absent signal of the Internal Control DNA.

A specimen is rated negative if the specimen DNA does not show an amplification signal, but an amplification signal for the Internal Control DNA can be found in the detection system. An inhibition of the PCR reaction can be ruled out by the detection of the Internal Control DNA.

A specimen is invalid if the specimen DNA and the Internal Control DNA do not show an amplification signal in the detection system. There are PCR inhibitors in the sample, or an error occurred during the extraction process. The extracted specimen should be diluted 1:10 with PCR water and re-amplified, or the isolation and purification of the specimen should be improved

## 12. Limitations of the method

1. The diagnosis should not be based on the result of the molecular biological test alone, but should always take the patient's medical history and symptoms into account.
2. This test is only valid for bronchoalveolar lavage (BAL).
3. The presence of PCR inhibitors can lead to non-evaluable results.
4. The results obtained with this product depend on adequate specimen identification, extraction, transport, storage, and handling. It is therefore critical to perform these steps carefully and follow the instructions provided by the manufacturer of the nucleic acid extraction products. Improper specimen sampling, transport, storage, and handling or a pathogen load below the test's analytical sensitivity can lead to false negative results.
5. Use of this product requires work clothing and work areas that are suitable for handling potentially infectious biological specimens and chemical preparations classified as hazardous in order to prevent accidents involving possible serious consequences for the user and other individuals.
6. This product may be used only by qualified personnel trained in performing molecular biological procedures such as extraction, amplification, and detection of nucleic acids. This requirement should prevent false results.
7. For manual processing, ensure that sample preparation/extraction, PCR preparation, and PCR are carried out in different rooms in order to prevent false-positive results.
8. For manual processing, separate clothing and instruments are required for sample preparation/extraction, PCR preparation, and PCR in order to prevent false-positive results during the use of this product.
9. As with all PCR-based in vitro diagnostic tests, extremely low concentrations of the target sequences under the limit of detection (LoD) can be detected. The results obtained are not always reproducible.
10. Mutations or polymorphisms in the primer or probe binding sites can interfere with the detection of new or unknown variants and can lead to false-negative results using RIDA®GENE CAP Bac.
11. As with all PCR-based *in vitro* diagnostic tests, extremely low concentrations of the target sequences under the limit of detection (LoD) can be detected. The results obtained are not always reproducible.
12. A positive test result does not necessarily indicate the presence of viable organisms. A positive result indicates that the target genes (*Chlamydophila pneumoniae* (16S-rRNA), *Legionella pneumophila* (16S-rRNA), and *Mycoplasma pneumoniae* (IGS)) are present.
13. The substances paracodeine, ciprofloxacin, and guaifenesin/dextromethorphan can manifest interfering properties even in small amounts.

## 13. Performance characteristics

### 13.1 Clinical performance

The RIDA®GENE CAP Bac multiplex real-time PCR was compared in an external laboratory with the pathogen-specific real-time PCR test method of the laboratory (analogous to PCR test methods accredited according to DIN EN ISO 15189:2007) using LightCycler® 480 II and LightCycler® 2.0. Overall, 282 retention samples of nucleic acid extracts from respiratory clinical test material (BAL) were tested. An invalid result was detectable in 17 samples (6 %) due to the negative detection of the internal control DNA, which indicates a sensitive detection of inhibitor results on the part of the RIDA®GENE CAP Bac assay. These specimens were not included in the evaluation.

The results of the individual pathogens are shown in Tables 12 to 14:

**Table 12**: Detection of *Chlamydia pneumoniae* (CP)

| | | **RIDA®GENE CAP Bac – *C. pneumoniae*** | | |
|---|---|---|---|---|
| | | Positive | Negative | Overall |
| **In-house CP** | Positive | 30 | 0 | 30 |
| | Negative | 0 | 235 | 235 |
| | Overall | 30 | 235 | 265 |

| | |
|---|---|
| Sensitivity | 100.0 % |
| Specificity | 100.0 % |
| PPV | 100.0 % |
| NPV | 100.0 % |

**Table 13**: Detection of *Mycoplasma pneumoniae* (MP)

| | | RIDA®GENE CAP Bac – *M. pneumoniae* | | |
|---|---|---|---|---|
| | | Positive | Negative | Overall |
| **In-house MP** | Positive | 50 | 0 | 50 |
| | Negative | 0 | 215 | 215 |
| | Overall | 50 | 215 | 265 |

| | |
|---|---|
| Sensitivity | 100.0 % |
| Specificity | 100.0 % |
| PPV | 100.0 % |
| NPV | 100.0 % |

**Table 14**: Detection of *Legionella pneumophila* (LP)

| | | RIDA®GENE CAP Bac – *L. pneumophila* | | |
|---|---|---|---|---|
| | | Positive | Negative | Overall |
| **In-house LP** | Positive | 50 | 0 | 50 |
| | Negative | 5 | 210 | 215 |
| | Overall | 55 | 210 | 265 |

| | |
|---|---|
| Sensitivity | 100.0 % |
| Specificity | 97.7 % |
| PPV | 90.9 % |
| NPV | 100.0 % |

### 13.2 Analytical sensitivity

The RIDA®GENE CAP Bac real-time multiplex PCR has a detection limit of ≥ 50 DNA copies per reaction for *Chlamydophila pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae*.

Figures 4, 5, and 6 below show dilution series of *Chlamydophila pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae* (each 5 x $10^5$ to $10^1$ DNA copies/reaction) on LightCycler® 480II.

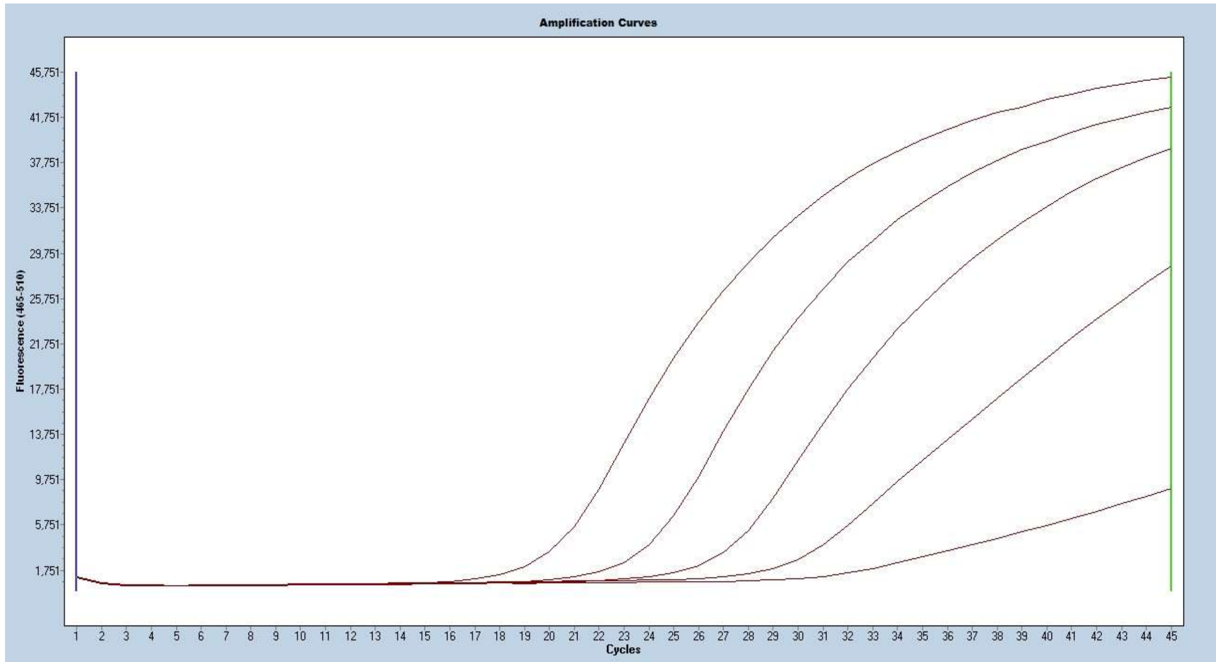


**Fig. 4:** Dilution series of *Chlamydophila pneumoniae* (5 x $10^5$ to $10^1$ DNA copies/reaction) on LightCycler® 480II

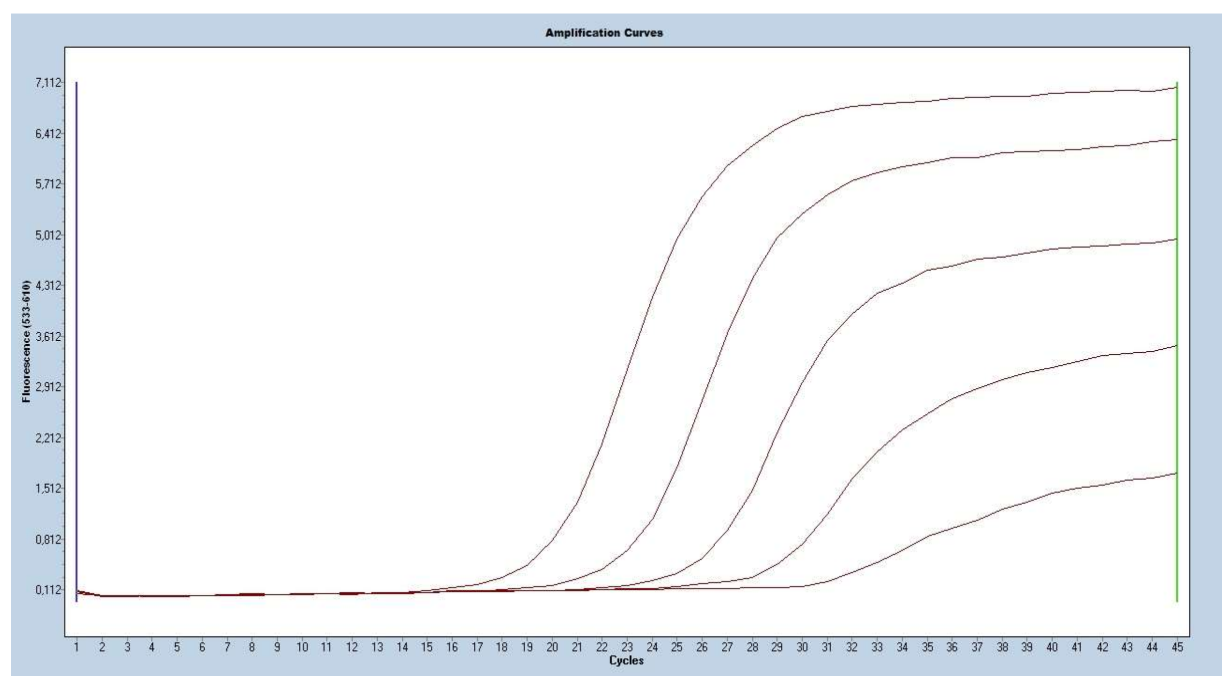


**Fig. 5:** Dilution series of *Legionella pneumophila* (5 x $10^5$ to $10^1$ DNA copies/reaction) on LightCycler® 480II

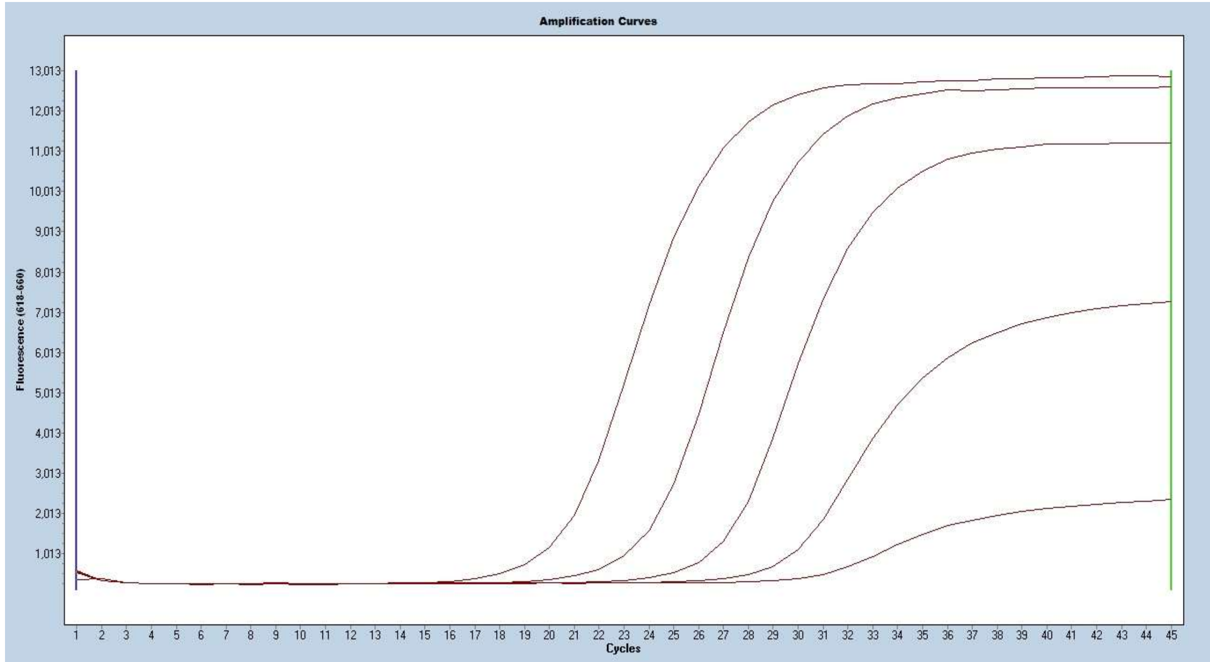


**Fig. 6:** Dilution series of *Mycoplasma pneumoniae* (5 x $10^5$ to $10^1$ DNA copies/reaction) on LightCycler® 480II

The limit of detection of the overall process depends on the specimen matrix, the DNA extraction, and the DNA content.

### 13.3 Analytical specificity

The RIDA®GENE CAP Bac multiplex real-time PCR is specific to *Chlamydophila pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae*. No cross-reactivities with the following species were detected (see Table 12):

**Table 15**: Cross-reactivity testing

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Adenovirus 1, human, strain Adenoid 71 | - | Echovirus 11 | - | *Klebsiella pneumoniae* | - | Parainfluenza virus serotype 3 | - |
| Adenovirus 7, human, strain Gomen | - | Enterovirus type 71 | - | *Legionella bozemanii* | - | Parainfluenza virus 4b, human, strain CH19503 | - |
| *Bordetella parapertussis* | - | *Haemophilus influenzae* Rd | - | *Legionella longbeachae* | - | Respiratory syncytial virus, human, strain 9320 | - |
| *Bordetella pertussis* | - | Herpes simplex virus 1, strain McIntyre | - | *Mycoplasma fermentans* | - | Respiratory syncytial virus, human, strain Long | - |
| *Chlamydia abortus* | - | Herpes simplex virus 2, strain MS | - | *Mycoplasma hominis* | - | Rhinovirus, genogroup A, human | - |
| *Chlamydia psittaci* | - | Human metapneumovirus | - | *Neisseria meningitidis* | - | *Staphylococcus haemolyticus* | - |
| Coronavirus 229E, human | - | Influenza virus, infectious A/PR/8/34 | - | Parainfluenza virus 1, human strain C35 | - | *Staphylococcus hominis* subsp. *novobiosepticus R22* | - |
| Coxsackie B4, human | - | Influenza B (B/Lee/40) | - | Parainfluenza virus 2, human, strain Greer | - | *Streptococcus pneumoniae* | - |

### 13.4 Analytical reactivity

The reactivity of the RIDA®GENE CAP Bac multiplex real-time PCR test was investigated using *Legionella pneumophila* serogroups (see Table 13). The tested *Legionella pneumophila* serogroups were detected using the RIDA®GENE CAP Bac multiplex real-time PCR test.

**Table 16**: Analytical reactivity testing

| ***Legionella pneumophila*** | | | | | |
|---|---|---|---|---|---|
| Serogroup 1 | + | Serogroup 6 | + | Serogroup 11 | + |
| Serogroup 2 | + | Serogroup 7 | + | Serogroup 12 | + |
| Serogroup 3 | + | Serogroup 8 | + | Serogroup 13 | + |
| Serogroup 4 | + | Serogroup 9 | + | Serogroup 14 | + |
| Serogroup 5 | + | Serogroup 10 | | | |

## 14. Version history

| Version number | Chapter and designation |
|---|---|
| 2019-09-10 | Release version |

## 15. Explanation of symbols

General symbols

| Symbol | Meaning |
|---|---|
| IVD | For in vitro diagnostic use |
| | Consult instructions for use |
| LOT | Lot number |
| | Expiry |
| | Store at |
| REF | Article number |
| Σ | Number of tests |
| | Date of manufacture |
| | Manufacturer |

Test-specific symbols

Reaction Mix

Taq-Polymerase

Internal Control DNA

No Template Control

Positive Control

## 16. Literature


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