Instructions for Use

In-vitro-Diagnostic

IVD

The res1plex *direct* PCR is a real-time qPCR test for simultaneous *in vitro* detection and differentiation of DNA from ***Mycoplasma pneumoniae, Chlamydia pneumoniae*** and ***Legionella pneumophila*****.**

Ref.No.:
FBC119

The res1plex *direct* PCR was validated with the Roche LightCycler® 480 II and with the BioRad CFX Opus 96™. In general, Lab *direct* qPCR tests are compatible with other qPCR cyclers (e.g. AJ qTOWER or LightCycler® 480 I).

Content

1. Name of the Device

res1plex *direct* PCR

2. Intended Purpose

The res1plex *direct* PCR is an assay for *in vitro* examination of bacterial nucleic acids in nasal/nasopharyngeal and oral/oropharyngeal swabs to provide information to aid to diagnose symptomatic or asymptomatic patients under suspicion of respiratory tract infections with *Mycoplasma pneumoniae*, *Chlamydia (Chlamydophila) pneumoniae* and/or *Legionella pneumophila*.

The IVD medical device detects the DNA of the aforementioned pathogens by qualitative measurements based on qPCR and is intended for use in medical laboratories or health institutions by laboratory personnel specifically trained in qPCR and *in vitro* diagnostic techniques. It has to be used in combination with conventional nucleic acid extraction systems for DNA extraction and qPCR cyclers for detection and analysis.

3. Pathogen Information

Mycoplasma pneumoniae, a leading agent of community-acquired pneumonia, is a very small-cell bacteria that lacks a cell wall. *M. pneumoniae* has a small genome of approximately 800 kilobases that has remained largely unchanged over time and across geographic regions [1]. The widespread administration of macrolide antibiotics has contributed to the rise and dissemination of macrolide-resistant strains in several countries, including Taiwan [2,3]. Approximately 8% of the *M. pneumoniae* genome consists of repetitive elements—RepMP1, RepMP2/3, RepMP4, and RepMP5—which are involved in recombination processes that promote surface antigen variability, thereby aiding the bacterium's adaptation and survival [4]. Epidemiological observations suggest that *M. pneumoniae* epidemics occur in cycles every 3 to 7 years, as reflected in outbreaks documented in Japan and Europe during 2011–2012, 2014–2015, and 2015–2016 [5, 6, 7].

Chlamydophila pneumoniae belongs to the phylum Chlamydiae, a group of obligate intracellular bacteria that parasitize a wide variety of eukaryotic organisms, ranging from amoebae and insects to vertebrates, including humans [8]. *C. pneumoniae* is the most prevalent intracellular bacterium associated primarily with respiratory tract infections, although it can occasionally cause extrapulmonary conditions. Most people are exposed to this pathogen at some point during their lives. Antibody prevalence reaches about 50% by age 20 and increases to approximately 80% by ages 60 to 70, indicating that infections are often asymptomatic and individuals can experience multiple infections over their lifetime [9, 10].

Legionella pneumophila is a Gram-negative, aerobic bacterium classified within the *Legionellaceae* family and genus *Legionella*. To date, more than 60 species have been identified, encompassing at least 79 distinct serogroups. These bacteria are facultative intracellular parasites capable of replicating within host cells, notably amoebae in aquatic environments as well as human macrophages. Although all *Legionella* species possess pathogenic potential, community-acquired infections in Europe are predominantly attributed to *Legionella pneumophila* serogroup 1. Within this serogroup, monoclonal subtypes can be further differentiated, with strains reacting to the monoclonal antibody 3-1 regarded as particularly virulent [11].

Legionella pneumophila is transmitted to humans mainly via inhalation of contaminated aerosols originating from water systems such as cooling towers, showers, and fountains, where the bacteria can proliferate. Person-to-person transmission does not occur. Once inhaled, the bacteria can infect the lungs, leading to illnesses such as Legionnaires' disease or Pontiac fever. Environmental factors like warm water temperatures and stagnant conditions favor bacterial growth and facilitate transmission [11]. During the summer and autumn months, cases of legionellosis tend to rise consistently. This seasonal pattern may be linked to increased travel during holiday periods, which involves potential exposure risks such as staying in hotels or using whirlpools, as well as

water stagnation in domestic plumbing systems during extended absences. Additionally, elevated temperatures in these seasons can encourage the proliferation of *Legionella pneumophila* in cold water systems and cooling plants, thereby heightening the likelihood of infection [11].

4. Testing Principle

The res1plex *direct* PCR is a multiplex qPCR test for the detection and differentiation of the following pathogens:

- *Mycoplasma pneumoniae*
- *Chlamydia (Chlamydophila) pneumoniae*
- *Legionella pneumophila*

A separate Internal Control (IC) is added to each sample prior to nucleic acid extraction and serves as control for nucleic acid isolation from the biological specimen as well as for amplification.
The nucleic acid eluate is added to the ready-to-use reaction solution that contains all reagents necessary for qPCR. The analysis can be performed on various qPCR cyclers.

The res1plex *direct* PCR contains primers and probes specific for the targets listed in Table 1 as well as the Internal Control. The probes are each labelled with fluorescent reporter dyes and a second dye that serves as a quencher and suppresses the fluorescence signals of intact probes.

The analysis is performed by determining Ct (cycle threshold) values. The Ct value describes the cycle in which the signal rises above a certain threshold for the first time. The more target copies are present in the sample, the lower the Ct value.

Table 1: Analytes and target genes of res1plex direct PCR

ANALYTE	GENE	REGION (NUCLEOTIDES)
Mycoplasma pneumoniae	*CARDS Mp180*	444509 - 444589
Chlamydia pneumoniae	*argR*	232755 - 232855
Legionella pneumophila	*xcpW*	1479962 - 1479857

5. Package Content

Each kit contains the following vials which are sufficient for 96 reactions (see Table 2).

Table 2: Package content of res1plex direct PCR

MATERIAL	LID COLOUR	#VIALS; VOLUME	#RXNS.	COMMENT	ACTIVE INGREDIENT(S)
Solution A	green	1x; 1050 µL	96	Reaction mix	Polymerase (approx. 0.2 U/µl); Primers and probes (approx. 150-200 nM)
Internal Control	blue	1x; 400 µL	96	Artificial nucleic acid target	Mix of RNA (approx. 10^7 copies/µl) and plasmid DNA (approx. 1 pg/µl)
Positive Control	red	1x; 50 µL	4	DNA of target analytes	DNA plasmids with target regions of *M.pneumoniae, C.pneumoniae* and *L.pneumopila* (each approx. 0.01 pg/µl)

6. Configuration

The res1plex *direct* PCR is available in one configuration, which is suitable for use on a qPCR instrument with at least 4 detection channels (FAM/HEX/Red610/Cy5).

7. Additional Equipment and Reagents (not provided)

- qPCR cycler (with at least 4 detection channels)
- Disposable protective gloves, powder-free
- PCR reaction tubes/microtiter plate plus lids/adhesive optical film
- Pipettes
- Sterile filter-tips for PCR testing (DNA/RNA-free)
- Table centrifuge
- DNA extraction kit (QIAamp DNA Mini Kit Qiagen N.V. or similar devices)
- Negative Control (no-template control, molecular grade water, or any other negative control according to the laboratory´s standard procedure)

8. Transport, Storage and Stability

The res1plex *direct* PCR is shipped on dry ice. All components must be stored at -25 °C to -18 °C in the dark immediately after receipt. All reagents must be kept at +2 °C to +8 °C during procedure steps. Exposure to light should be avoided. You may thaw and refreeze the kit components up to 4 times. The package bears an expiry date, after which no quality guarantee can be given.

9. Warnings, Safety Precautions and Additional Information Access

The res1plex *direct* PCR is intended for *in vitro* diagnostic use only. The test should only be performed by personnel trained in molecular diagnostic techniques. If the user makes changes to the product or the application instructions, results may not correlate with the intended purpose.

All serious incidents relating to the kit must be notified to the manufacturer and the national competent authority of the EU Member State where the laboratory and/or patient is located.

- Before performing the test, read the entire instructions for use and follow them carefully. Deviations from the given test protocols can lead to invalid results.
- All patient samples must be treated as potentially infectious material.
- Discard sample and assay waste that was in contact with patient material according to your local, regional, or national safety regulations.
- Do not use the test beyond the expiration date.
- Do not use the test with opened or damaged packaging.
- Protect reagents from heat, moisture, and light.
- Do not replace or mix the reagents with reagents from other batches or other chemicals.
- Avoid contamination of the test by microorganisms and nucleases (DNases).
- Any carry-over of samples during handling and processing of the test may result in false positive test results.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner.
- Always wear disposable gloves in each area and change them before entering a different area.
- The test kits are intended for single use and must not be reused after performing qPCR.

- If contamination of the qPCR cycler is suspected, cleaning and maintenance must be carried out according to the system´s manual.
- In the event of failure of the controls (see chapter 14/Results), the test result cannot be evaluated and a repeat run should be considered.
- Safety Data Sheets (SDS) are available for download via https://frizbiochem.de/downloads/
- Pending EUDAMED entry, the Summary of Safety and Performance (SSP) can be downloaded via https://frizbiochem.de/downloads/

10. Specimen Collection, Handling, Transport, and Storage

Proper sample collection, storage and transport are critical for PCR analyses in general. Inadequate sample collection, improper sample handling and/or transport can lead to false negative results. Therefore, ensure that:

- The instructions of the medical laboratory and/or the manufacturer of the sample collection system for sample collection, transport and storage are followed.
- The sample collector is instructed to collect the sample in accordance with applicable national laws, regulatory requirements and the instructions for use of the sampling tool.

11. Important Points before Starting

This test is intended for use with samples derived from nasal/nasopharyngeal and oral/oropharyngeal swabs. Other specimen types may cause invalid results.

In every qPCR run one Positive Control and one Negative Control should be included. The Positive Control consists of nucleic acids of respective target pathogens. The Negative Control (e.g. molecular grade water) must be provided by the user. A failed Positive or Negative Control will invalidate the qPCR run and the results must not be reported.

Appropriate nucleic acid extraction methods must be conducted prior to using this assay. Nucleic acid extraction reagents are not part of the res1plex *direct* PCR. Performance evaluation studies have been conducted using the QIAamp DNA Mini Kit (Qiagen N.V.) for nucleic acid extraction.

12. Test Procedure

All components must be stored at -25 °C to -18 °C in the dark. All reagents must be kept at +2 °C to +8 °C during procedure steps.

Table 3: Test procedure of res1plex direct PCR

TEST PROCEDURE	
Sample preparation	
1	Thaw Internal Control only.
2	Add Internal Control to the nucleic acid extraction in accordance with the laboratory´s standard procedure (e.g., add 4µL/sample to lysis buffer; add one additional water sample and use as Negative Control).
3	Put the Internal Control back in the kit box in the deep freezer.
4	Perform nucleic acid extraction according to your laboratory's standard procedure.
qPCR	
5	Thaw Solution A and Positive Control only.
6	Pipette 10 µL/well of Solution A into the PCR microtiter plate/reaction tubes. (If IC is not added to sample during extraction, 0.1 µl IC/sample could be added to Solution A).
7	Add 10 µL/well of eluate from nucleic acid extraction; add 10 µL Positive Control per run; add 10 µL Negative Control per run.
8	Put Solution A and the Positive Control back in the kit box in the deep freezer.
9	Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided.
10	Briefly centrifuge the microtiter plate or reaction tubes.
11	Place the filled plate/reaction tubes in the qPCR cycler.
12	Start program.

13. Instrument Settings

The following thermal profile is to be used (Table 4). Channel settings depend on the qPCR cycler used and should be checked before starting the analysis (Table 5). Make sure to activate the detection mode of your qPCR cycler before starting the analysis.

*Table 4: Instrument settings of res1plex direct PCR**

STEPS	TEMPERATURE [°C]	TIME	ACQUISITION	#CYCLES
Initial step (optional)[#]	55	5 min	-	1
Denaturation	95	3 min	-	1
Amplification	95	5 sec	-	45
	60	15 sec	on	
	72	15 sec	-	

**If required, the amplification step can be adapted to a two-step cycling protocol by combining the two 60°C/15sec and 72°C/15sec steps into a single 60°C/30sec step.*

[#]only for mixed RNA-/DNA runs

Table 5: Channel settings of res1plex direct PCR (Ref. No.: FBC119)

	Mycoplasma pneumoniae	***Chlamydia pneumoniae***	**Internal Control**	***Legionella pneumophila***
	CARDS Mp180	*argR*	artificial NA	*xcpW*
Reporter dye	FAM	HEX	Red 610	Cy5
Colour	green	yellow-green	orange	red
Emission [nm]	520	560	610	670
Quencher	Black Hole Quencher			

The res1plex *direct* PCR was validated with the BioRad CFX Opus 96™ and Roche LightCycler® 480 II. In general, FRIZ Lab *direct* qPCR tests are compatible with many qPCR cyclers. FBC119 is suitable for qPCR cyclers with at least the four channels: FAM, HEX, Red 610, and Cy5.

14.Results

Positive samples show a qPCR typical amplification curve that crosses a certain threshold generating the Ct value (see Figure 1).

A positive signal is characterized by a sigmoidal curve exhibiting both a baseline phase and an exponential phase. Signals that do not exhibit this curve pattern, despite having a Ct value, are considered negative. The threshold shall be set within the exponential phase. It should be noted that in the case of low positive samples, the plateau phase might not be visible due to the cut off after 45 cycles. Thus, the presence of a plateau phase is not an essential requirement for defining a positive signal in such cases.

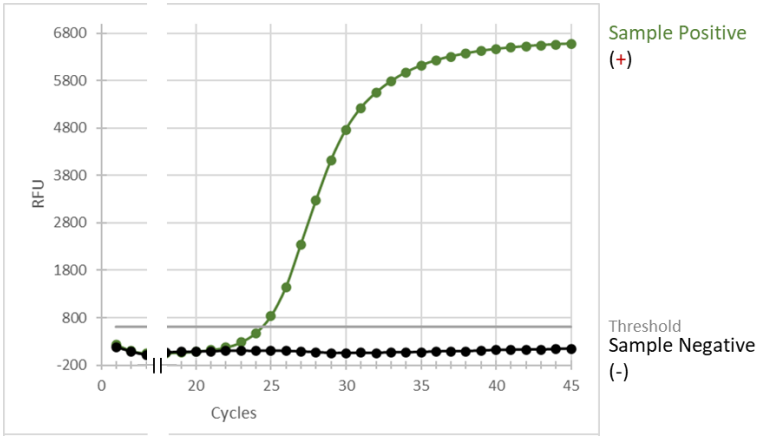

Figure 1: Exemplary amplification curves

- The Negative Control must not show amplification curves in all channels except for the IC channel.
- The Positive Control must show amplification curves in all target channels. If the IC is directly included in Solution A, an amplification curve must also be observed in the IC channel of the Positive Control. The Ct value of the Positive Control must be < 36. A Positive Control with a higher Ct value indicates procedural problems.
- The IC should show a positive amplification curve in both positive and negative samples.

For distribution of results within the channels see Table 6.

Table 6: Results of res1plex direct PCR (Ref. No.: FBC119)

Mycoplasma pneumoniae	***Chlamydia pneumoniae***	**Internal Control**	***Legionella pneumophila***		
FAM	**HEX**	**Red 610**	**Cy5**	**Result**	**Interpretation**
+	–	+	–	Valid	*Mycoplasma pneumoniae* detected.
–	+	+	–	Valid	*Chlamydia pneumoniae* detected.
–	–	+	+	Valid	*Legionella pneumophila* detected.
–	–	+	–	Valid	No *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* or *Legionella pneumophila* detected.
+/–	+/–	–	+/–	Invalid	The test result cannot be evaluated.

During the analysis of a res1plex *direct* PCR test with BioRad CFX Opus 96™, activation of ´´Fluorescence Drift Correction´´ is recommended. ´´Fluorescence Drift Correction´´ could be activated through the following steps:

Settings →Baseline Setting → Apply Fluorescence Drift Correction

15. Limitations of the Method

The results support the differential diagnosis of infections with *Mycoplasma pneumoniae, Chlamydia pneumoniae* and *Legionella pneumophila.*

Positive results indicate the presence of the respective pathogen, but do not exclude a co-infection with other pathogens.

A negative result does not exclude the presence of *Mycoplasma pneumoniae, Chlamydia pneumoniae* and/ or *Legionella pneumophila* as results depend on correct sampling, the absence of inhibitors and sufficient DNA to be detected. Invalid results may be obtained if the sample contains inhibitors that prevent lysis, extraction, amplification or detection of the target nucleic acids.

Test results should always be seen in the context of the clinical findings and should not be used as the only basis for diagnosis, treatment, or other patient management decisions. Therapeutic consequences of the diagnostic results must be drawn in relation to the clinical findings by an appropriate healthcare professional.

The detection of analyte target does not mean that they are the causative agents of clinical symptoms.

The res1plex *direct* PCR could detect the presence of the respective nucleic acid of the target pathogen although the clinical symptoms are not observed anymore or the patient is recovered from the diseases state of the target pathogen.

High concentrations of blood in the specimen (> 1% (v/v)) may inhibit the qPCR and a false-negative result could occur.

Mutations or polymorphisms in primer and probe binding regions can interfere with the detection of new variants that may result in false negative results.

16. Analytical Performance

16.1 Analytical Sensitivity

The limit of detection (LoD) was determined with serial dilutions of genomic DNA. The analytes were tested in 12 replicates per concentration on a BioRad CFX Opus 96™. The 95% confidence interval (95 CI) was determined using Logistic Regression (Logit) with the GraphPad Prism 10.0.2 software (see Table 7).

Table 7: Limit of Detection of res1plex direct PCR

ANALYTES	LOD (COPIES/REACTION)	UPPER LIMIT	LOWER LIMIT
Mycoplasma pneumoniae	22.8	34.5	14.0
Chlamydophila pneumoniae	8.2	12.4	5.1
Legionella pneumophila	5.8	6.4	5.2

16.2 Other Analytical Performance Parameters

Other analytical performance data, such as analytical specificity including cross-reactivity, endogenous and exogenous interfering substances, inclusivity and competitive interference as well as precision (repeatability and reproducibility) were performed and demonstrated no impact on assay performance, with all test acceptance criteria met.

17. Diagnostic Performance

In total, 285 nasal/nasopharyngeal and oral/oropharyngeal samples were tested. These samples were extracted using the QIAamp DNA Mini Kit (Qiagen N.V.). Eluates were used for parallel analysis with the res1plex *direct* PCR and a CE certified test from another manufacturer in direct comparison on a Bio-Rad CFX Opus 96™. In the event of a discrepancy between the results, another CE certified test from another manufacturer was used to resolve the discrepancy.

Diagnostic sensitivity and specificity are summarized in the following:

***Mycoplasma pneumoniae* samples**

		positive	negative	
res1plex	positive	50	0	Sensitivity: 98.0% (89.5 - 99.9)
	negative	1	234	Specificity: 100.0% (98.4 - 100.0)

***Chlamydophila pneumoniae* samples**

		positive	negative	
res1plex	positive	8	0	Sensitivity: 100.0% (63.1 - 100.0)
	negative	0	277	Specificity: 100.0% (98.7 - 100.0)

***Legionella pneumophila* samples**

		positive	negative	
res1plex	positive	11	0	Sensitivity: 100.0% (71.5 - 100.0)
	negative	0	274	Specificity: 100.0% (98.7 - 100.0)

The diagnostic sensitivity of the res1plex *direct* PCR is dependent on the DNA extraction method used to isolate DNA from biological specimens. It is the responsibility of the user to qualify the extraction methods used.

CE 0123 This product complies with the requirements of the Regulation (EU) *2017*/746 (*IVDR*) for *in vitro* diagnostic medical devices.

18. Symbols

Please note following symbol descriptions according to EN ISO 15223-1.

Graphic	Title	Description
	In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.
	Manufacturer	Indicate the medical device manufacturer.
	Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed.
	Use-by date	Indicates the date after which the medical device is not to be used.
	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.
	CE marking European Conformity	--
	Catalogue number (Reference number)	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	Content sufficient for <n> tests	Indicates the total number of tests that can be performed with the medical device.
	Do not reuse	Indicates a medical device that is intended for one single use only.
	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	Keep away from sunlight	Indicates a medical device that needs protection from light sources.
	Unique device identifier	Indicates a carrier that contains unique device identifier information.

Document Version:

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Version 1.0 Date: 2026-07-29	First version

19.Technical Assistance and Contact Information

For technical assistance, product support, or questions related to the performance or interpretation of this test, users may contact the manufacturer directly.

Manufacturer:	FRIZ Biochem GmbH
Address:	Floriansbogen 2-4 / 82061 Neuried / Germany
Telephone:	+49 (0) 89 72 44 09 0
Email (Technical Assistance):	support@frizbiochem.de
Email (General Enquiry):	info@frizbiochem.de
Website:	www.frizbiochem.de

Technical support is available during regular business hours, and all inquiries will be handled by qualified personnel. Users are encouraged to contact the manufacturer immediately in the event of suspected malfunction, unexpected results, or any issue requiring expert clarification.

20.References

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