Simplexa™ Mycoplasma pneumoniae (English)

Product Code MOL0400
Rev. EU-Trial

A real time polymerase chain reaction (PCR) assay intended for the qualitative detection of the presence or absence of *Mycoplasma pneumoniae* in extracted nucleic acid

Product regulatory status within the United States:
For Research Use Only

Outside of the United States:
For Research Use Only

INTENDED USE

Focus Diagnostics' Simplexa™ Mycoplasma pneumoniae assay is a real-time polymerase chain reaction (PCR) test intended for the qualitative detection of the presence or absence of *Mycoplasma pneumoniae* in extracted nucleic acid. The assay is indicated for testing humans for presumptive diagnosis of *Mycoplasma pneumoniae* infection. The predictive value of a positive or negative result depends on the population's prevalence and the pretest likelihood of a suspected *Mycoplasma pneumoniae* case.

SUMMARY AND EXPLANATION

Pneumonia is a lower respiratory tract infection characterized by the inflammation of the lung tissue. It is commonly classified into two groups: Community-acquired pneumonia (CAP) and nosocomial pneumonia. Community-acquired pneumonia is subdivided into typical and Atypical Pneumonia, based on clinical manifestations and etiological agents[1]. According to Centers for Disease Control and Prevention (CDC), Community-acquired pneumonia affects approximately 4 million people in United States each year, and about 20% of those require hospitalization[2].

Mycoplasma pneumoniae infection occurs world-wide and is the most common cause of community-acquired pneumonia in the 5 to 20 year-old age group[3]. Pneumonia caused by *Mycoplasma pneumoniae* is categorized as an atypical pneumonia since treatment and diagnosis are different from historically recognized causes of Community-acquired pneumonia and typical pneumonia such as *Streptococcus pneumoniae*. Atypical pneumonia is usually presented with a gradual onset of pneumonia associated with headache, fever, muscle aches, sore throat, initial dry cough which may persist for weeks, and abnormal or diffuse chest X-ray. However, it is practically impossible to distinguish the different types of pneumonia based on clinical symptoms alone[2].

Mycoplasma pneumoniae is difficult to culture. Mycoplasma cultures from clinical samples may take 2-3 weeks, and the success rate depends on proper sample collection, prompt sample processing, and the expertise of the microbiology laboratory personnel. It is conventionally diagnosed by serological methods, which often requires paired sera collected in a proper window of infectious stage yet still lack either specificity or sensitivity[4].

TEST PRINCIPLES

The Focus Diagnostics' Simplexa™ Mycoplasma pneumoniae assay is a nucleic acid amplification test that uses real-time PCR amplification to enable the detection of *Mycoplasma pneumoniae*. The test combines fluorescently labeled probe technology with a real-time PCR amplification and detection system. Each probe molecule contains a fluorophore and a quencher. The target sequence is amplified by the primers, and binding of the probe element to the amplified DNA fragment results in separation of the fluorophore from the quencher and generation of fluorescent signal.

MATERIALS PROVIDED

Focus Diagnostics' Simplexa™ Chlamydophila pneumoniae assay contains sufficient materials for 48 reactions. Upon receipt, store all reagents at -10°C to -30°C (Do not use a frost-free freezer). After first use, store thawed reagents at 2°C to 8°C up to 30 days. Appropriately stored reagents are stable through the end of the expiration month as indicated on the kit packaging.

Table 1. Kit Component Table
Simplexa™ Mycoplasma pneumoniae assay: Part # MOL0400

Kit Components	Acronym	Part Number	Number in Kit	Color Code
Atypical Pneumonia Primer Mix	AP MM	MOL0401	2	Brown
Master Mix	MM	MOL0001	2	Green
DNA Internal Control	IC-DNA	MOL0011	2	Blue
Atypical Pneumonia Positive Control	AP PC	MOL0421	2	Red
No Template Control	NTC	MOL0002	2	Clear
Diluent	DL	MOL0003	5	Clear
Nuclease-free water	H_2O	MOL0004	1	Clear

Atypical Pneumonia Primer Mix 2 vials, (63 µl per vial) | REF | MOL0401 | REAG | A

The Primer Mix contains fluorescently dye-labeled primers and probes specific for detection of *Mycoplasma pneumoniae* and for the Internal Control template. See Table 2 below for dye specifications.

Table 2. Fluorescent Dye-Labeled Probe/Primers included in the Simplexa™ Mycoplasma pneumoniae assay.

Probe/Primer	Fluorophore	Excitation	Emission	Targeted Gene
Mycoplasma pneumoniae	FAM	495 nm	520 nm	P1
Internal Control	Quasar 670	647 nm	667 nm	NA

Master Mix, 2 vials, (315 µl per vial) | REF | MOL0001 | REAG | B

The **Simplexa™** Master Mix contains enzyme, buffer, stabilizing additives, dNTPs and $MgCl_2$: See Table 3 below for specifications.

DNA Internal Control, 2 vials, (125 µl per vial) | REF | MOL0011 | CONTROL | IC

The Internal Control is a 250 bp DNA fragment of non-coding sequence. See Table 3 below for specifications.

Atypical Pneumonia Positive Control, 2 vials, (50 µl per vial) | REF | MOL0421 | CONTROL | +

The Positive Control is a 483 bp DNA fragment containing target regions of *M.pneumoniae*. See Table 3 below for specifications.

No Template Control, 2 vials, (50 µl per vial) | REF | MOL0002 | CONTROL | -

The No Template Control is nuclease-free water. See Table 3 below for specifications.

Diluent, 5 vials, (1.0 ml per vial) | REF | MOL0003 | DIL | SPE

The Diluent is for sample dilution and for preparation of Positive and No Template Controls for extraction. See Table 3 below for specifications.

Nuclease-Free Water 1 vial, (500 µl) | REF | MOL0004 | H_2O | SQ

Purified water that does not contain DNase or RNase.

Table 3. Summary of reagents provided.

Reagent	Reactions per Kit	Vials per Kit	Reactions per Vial	Volume per Reaction, µL
Atypical Pneumonia Primer Mix	48	2	24	2.5
Master Mix	48	2	24	12.5
DNA Internal Control	48	2	24	5
Atypical Pneumonia Positive Control	20	2	10	5
No Template Control	20	2	10	5
Diluent	20	5	4	250
Nuclease-free Water	48	1	48	5

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Real-time PCR instrument
2. Single or multi-channel micropipette(s) with an accuracy range between 1-10 µL, 10-100 µL and 100-1000 µL. NOTE: Dedicated micropipettes are required for extraction, as well as for the pre- and post-PCR areas.
3. -10°C to -30°C freezer, manual defrost (Do not use a frost-free freezer)
4. Laminar flow hood for extractions
5. Bench top centrifuge for low speed centrifugation of 96-well plates or other reaction vessels.
6. Microcentrifuge
7. Vortex mixer
8. Nucleic Acid Extraction Reagents or Kit
9. Sterile, RNase/ DNase-free disposable aerosol-barrier micropipettor tips
10. RNase/DNase-free 1.5 ml polypropylene microcentrifuge tubes and racks
11. RNase/DNase-free 96 well thermocycler plate rack or appropriate tube rack.
12. 96-well optical reaction plate or other reaction vessels.
13. Optical adhesive cover
14. Disposable powder-free gloves

MATERIALS REQUIRED AND SUPPLIED AS AN ACCESSORY

1. Simplexa™ Software

SHELF LIFE AND HANDLING

1. Store reagents at -10°C to -30°C (Do not use a frost-free freezer).
2. Do not use kits or reagents beyond their expiration dates.
3. Allow reagents to warm to room temperature (approximate range 18°C to 25°C) before use.
4. Once thawed, store the unused reagent at 2°C to 8°C for no more than 30 days.
5. Do not refreeze.
6. Store materials in the dark and protect from light.
7. When properly stored, kit reagents are stable through the end of the month indicated on the expiration date on the package.

WARNINGS AND PRECAUTIONS

1. Global Product regulatory status is for research use only.
2. Follow universal precautions. All patient specimens should be considered potentially infectious and handled accordingly.
3. Wear personal protective equipment, such as (but not limited to) gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the test.
4. Do not pipette by mouth.
5. Do not smoke, drink, eat, handle contact lenses or apply make-up in areas where kit reagents and/or human specimens are being used.
6. Dispose of unused kit reagents and human specimens according to local, state and federal regulations.

7. Workflow in the laboratory should proceed in a uni-directional manner, beginning in the Pre-Amplification Area(s) and moving to the Amplification/Detection Area. Reagent preparation should be performed in Pre-Amplification Area I. Specimen extraction should be performed in a separate Pre-Amplification Area dedicated for specimen extraction. Plate set up should be performed in an additional separate pre-amplification area Pre-Amplification Area II. Supplies and equipment used for reagent preparation should not be used for specimen preparation activities or for pipetting or processing amplified DNA or other sources of target nucleic acid. Post-amplification supplies and equipment should remain in the Amplification/Detection Area at all times. Disposable gloves must be worn in each area and must be changed before leaving that area.
8. Contamination of patient specimens or reagents can produce erroneous results. Use aseptic techniques. Pipette and handle reagents carefully to avoid mixing of samples from adjacent wells.
9. Pipette and handle reagents carefully to avoid mixing of samples from adjacent wells.
10. Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
11. Do not substitute or mix reagent from different kit lots or from other manufacturers.
12. Do not interchange the reagent tube caps. This may cause contamination and compromise the test results.
13. Use only protocols described in this insert. Deviations from the protocol or the use of times or temperatures other than those specified may give erroneous results. Assays performed outside of the protocol specifications should be repeated.
14. Assay set up should be performed at room temperature (approximate range 18ºC to 25ºC).
15. Do not re-use wells that have already been exposed to patient samples or reagents.
16. Dispose of amplified samples without opening reaction vessel.
17. If different lots of the same Simplexa™ kit are set up on the same plate, positive and negative controls from each lot need to be tested.
18. If different Simplexa™ kits are set up on the same plate, positive and negative controls from each kit need to be tested.

INSTRUCTIONS FOR USE

I. SPECIMEN PREPARATION AND HANDLING

Performed in area dedicated for specimen extraction

1. Specimen should be equilibrated to room temperature (approximate range 18ºC to 25ºC) immediately before the sample preparation.
2. Specimen should not be diluted
3. Add 5 µl internal control (IC) to each clinical sample prior to extraction.
4. Aliquot 250 µl each of Diluent to two tubes, one for No Template Control (NTC) and one for Positive Control (PC).
5. Add 5 µl IC and 5 µl NTC to the tube labeled as 'NTC'.
6. Add 5 µl IC and 5 µl PC to the tube labeled as 'PC'.
7. Extract nucleic acids from the samples and controls using a validated procedure.
   (Note: Prepare No Template Control (NTC) and Positive Control (PC) for each sample batch.).
8. If the extracted sample is not used immediately, it should be stored at -10ºC to -30ºC.

II. REAGENT PREPARATION

1. Launch the Simplexa™ software, Create a New Plate or Worklist, select a Kit and Lot number (add a new lot if required)
2. Enter or Import sample IDs, and add the desired number of extra reactions
3. Save Plate or Worklist
4. Print Plate or Worklist Setup information. The volumes of reagents required are based on the single reaction volumes listed in Table 4.

Performed in Pre-Amplification area I

Use RNase/DNase-free tubes only.

1. Remove the appropriate number of tubes of Master Mix, Primer Mix and Nuclease- free Water from -10°C to -30ºC storage, and allow to equilibrate to room temperature (approximate range 18ºC to 25ºC). Each vial contains sufficient reagent for 24 reactions.
2. Assemble the total amount of Reaction Mix required by pipetting the components in the following order into a 1.5 ml polypropylene microcentrifuge tube: a) Nuclease-free Water, b) Master Mix, and c) Primer Mix.
3. Mix by vortexing with two 1 second pulses.
4. Centrifuge for 5 seconds to collect the contents to the bottom of the tube.
5. Proceed to Plate Set Up.

Table 4. Reagent volumes used to prepare the Reaction Mix.

Reagent	1 reaction (volume)
Nuclease-free Water	5.0 µl
Master Mix	12.5 µl
Primer Mix	2.5 µl

III. PLATE SET UP

Performed in Pre-Amplification area II; use RNase/DNase-free plates only

1. **Simplexa™** software will create a plate map of sample placement in the 96-well optical plate or appropriate reaction vessel.
   - The first sample of the plate is the Positive Control (POS).
   - The last sample of the plate is the No Template Control (NTC).

Example of a 96-well plate map:

	1	2	3	4	5	6	7	8	9	10	11	12
A	POS	S	S	S	S	S	S	S	S	S	S	S
B	S	S	S	S	S	S	S	S	S	S	S	S
C	S	S	S	S	S	S	S	S	S	S	S	S
D	S	S	S	S	S	S	S	S	S	S	S	NTC
E												
F												
G												
H												

POS: Positive Control; first sample added to the plate.

S: Patient Sample
NTC: No Template Control; last sample added to the plate.

2. Plate set up
   a) Add 20.0 µl Reaction Mix to each reaction tube or well.
   b) Add 5.0 µl of extracted patient samples to S wells.
   c) Add 5.0 µl of the extracted Positive Control to the POS well.
   d) Add 5.0 µl of the extracted No Template Control to the NTC well.
   e) Tightly seal the reaction tubes or wells.
   f) Centrifuge the plate at 2000 x g for 2 minutes.
   g) Proceed immediately to Real Time PCR Instrument Set Up.

IV. REAL-TIME PCR INSTRUMENT SET UP

Simplexa™ Mycoplasma pneumoniae assay is run using a real-time detection thermocycler capable of detecting the following fluorescent dyes: FAM (excitation max 495 nm, emission max 520 nm) and Quasar 670 (excitation max 647 nm, emission max 667 nm).
The instrumentation must have consumables that accommodate a 25 µl reaction volume and it must also be programmable to the following conditions:
1. Temp – 4 °C to 99 °C in 1 °C intervals
2. Time – 1 second to ∞ in 1 second intervals
3. Set detection/read intervals

The following cycling program should be used to run the Simplexa™ Mycoplasma pneumoniae assay:
Stage 1: 95°C for 10min, 1 cycle
Stage 2: Step 1 - 95°C for 15 sec
Step 2 - 60 °C for 35 sec*, 45 cycles
*Set data collection at this step

V. DATA ANALYSIS

AUTOMATED Simplexa™ INTERPRETATION OF TEST RESULTS

1) Import Instrument Data for plates or worklists that were initially set up using Simplexa™ software
2) Generate, Review, and Print Sample Reports with interpretations

Run validity Criteria

1) The NTC well should be:
FAM or equivalent: Not detected
Quasar 670 or equivalent: Detected (Ct <40)
If the results match the above continue with step 2. If results do not match the above, the run is Invalid.

2) The Positive Control well should be:
FAM or equivalent: Detected (Ct <39)
Quasar 670 or equivalent: Detected (Ct <40)
If the results match the above, then go on to interpret patient sample results. If not, the entire run is invalid.

Table 5. Interpretation of Results

Assay Result Reported		Interpretation of Result
FAM or equivalent *Mycoplasma pneumoniae*	**Quasar 670 or equivalent Internal Control**	
Detected	NA*	*M.pneumoniae* DNA detected
Not Detected	Detected	No target DNA detected
Not Detected	Not Detected	Invalid Test

NA = not applicable, * Detection of the Internal Control is not required for a positive result.

LIMITATIONS

1. All results from this and other tests must be correlated with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
2. The prevalence of infection will affect the test's predictive value.
3. As with other tests, negative results do not rule out *Mycoplasma pneumoniae* infections.
4. False negative results may occur when the infecting organism has genomic mutations, insertions, deletions, or rearrangements.
5. As with other tests, false positive results may occur. False-positive PCR test results are more likely in persons with nonspecific clinical symptoms such as runny nose, sneezing, and sore throat. Repeat testing or testing with a different device may be indicated in some settings.
6. This test is a qualitative test and does not provide the total amount of detected organism present.
7. This test cannot distinguish the different strains of *Mycoplasma pneumoniae*.
8. The performance of this test has not been established for patients without the symptoms of *Mycoplasma pneumoniae* infection.
9. The performance of this test has not been established for monitoring treatment of *Mycoplasma pneumoniae* infection.
10. This test cannot rule out diseases caused by other bacterial or viral respiratory pathogens.

EXPECTED VALUES

To be established.

PERFORMANCE CHARACTERISTICS

To be established.

REFERENCES

1. McDonough EA, Barrozo CP, Russell KL, Metzgar D. A multiplex PCR for detection of Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila, and Bordetella pertussis in clinical specimens. Mol Cell Probes. 2005 Oct;19(5):314-22.
2. Bernstein JM. Treatment of Community-Acquired Pneumonia -- IDSA guidelines. *Chest.* 1999;115.
3. Cosentini R, Tarsia P, Blasi F, Roma E, Allegra L. Community-acquired pneumonia: role of atypical organisms. Monaldi Arch Chest Dis. 2001 Dec; 56(6):527-34.
4. Carroll KC. Laboratory diagnosis of lower respiratory tract infections: controversy and conundrums. J Clin Microbiol. 2002 Sep;40(9):3115-20.

This package insert is **not yet available** in French, German, Italian, and Spanish at www.focusdx.com, and is **not yet available** in other languages from your local distributor.

AUTHORIZED REPRESENTATIVE
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TECHNICAL ASSISTANCE

If questions arise concerning the kit or its reagents, please contact Focus Diagnostics' Technical Services personnel.

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ORDERING INFORMATION

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