



Chlamydia Direct

Molecular test system for the detection of Chlamydia trachomatis

For Research Use Only. Not for use in Diagnostic Procedures

Catalog Number: 12-MGCD 1
Size: 50Tests
Version: G-2003-03-31 – ALPCO 06/24/04

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ASSAY INFORMATION

Technical Data

Sample material:	synovial fluid, urine
Required sample size:	up to 1,000 μ L sample per single determination
Total processing time:	approx. 4 h
Sensitivity:	1 genome equivalent per reaction
Storage:	see materials supplied
Order code:	012-MGCD 1
Package size:	50 tests
Controls:	amplification control (control DNA)

Materials Supplied, Storage and Stability

Components	Cat.-No.	Content	Preparation	Storage at	Shelf life
Buffered wash solution (white cap)	MGWA	3 vials à 20 mL	ready to use	2 – 8 °C	Until the expiration date; 3 months after opening
Lysis reagent, violett (white cap)	MGLR	1 vial 5 mL	ready to use	2 – 8 °C	Until the expiration date; 3 months after opening
Neutralization buffer (red cap)	MGNB	1 vial 5 mL	ready to use	2 – 8 °C	Until the expiration date; 3 months after opening
Amplification mix (blue cap)	MGAC	2 vials. à 500 μ L	ready to use	-20 °C	Until the expiration date
Positive control (red cap)	MGCTC1	1 vial 150 μ L	ready to use	-20 °C	Until the expiration date
Probe (green cap)	MGFC	1 vial 250 μ L	ready to use	-20 °C	Until the expiration date
Dipsticks	MGDS	2 vials à 25 strips	ready to use	2 – 8 °C	Until the expiration date
Assay buffer (blue cap)	MGCB	1 vial 10 mL	ready to use	2 – 8 °C	Until the expiration date; 3 months after opening
Evaluation sheet	MGCDES	2			

Material safety data sheets are available on request.

Materials Required

- Thermocycler with a block for 0.2 mL vials; preferably with lid heater
- Microcentrifuge with minimal 20,000 g (e. g. Eppendorf 5415C or compatible instrument)
- Waterbath or thermomixer for 95 °C
- Vortex
- Rack made of plastic or aluminium for PCR-reaction tubes
- Ice bath
- Adjustable pipettes for 10 μ L, 100 μ L and 1,000 μ L (three separate sets for extraction, amplification and detection)
- Pipet tips with filter
- 0.2 mL PCR-sample vials compatible with the thermocycler (e.g. individual vials, 8-well-strips or 96-well microtiter plates)
- 1.5 mL reaction tubes (tubes with a screw cap or, exceptionally, Eppendorf Safe-lock tubes)
- microtiter plates with 8 well strips

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Reagents Required

- Standard Taq DNA-Polymerase without 3'-5'-Exonuclease activity, (e. g. from Qiagen, Eppendorf or Peqlab)

Important Note: *This kit uses the PCR process which is covered by patents owned by Hoffmann-La Roche. No authorization, implicit or explicit license to practice PCR or any method using PCR is covered by purchasing this product. Information on licenses may be obtained by contacting Hoffmann-La Roche AG, CH-4002 Basel/Switzerland.*

- Enzyme specific reaction buffer with MgCl₂

Specimen Collection and Preparation

The GenLine Chlamydia Direct assay has been developed for the analysis of synovial fluid and urine. A sample volume of 1,000 µL is required for a single determination. If a prolonged storage is intended, samples should be stored at -20 °C. Repeated freeze-thaw cycles of samples or reagents should be avoided.

Warnings and Precautions

All reagents of this test kit are strictly intended for *in vitro* diagnostic use only. Use by staff, who is specially trained in methods applied in *in vitro* diagnostics (EN 375).

Please adhere strictly to the sequence of pipetting steps provided in this protocol.

Store all reagents in original vials at the temperatures indicated on the respective labels! Do not interchange kit components from different lots and assays. The expiration dates stated on the labels of the shipping container and all vials have to be observed. The reagents should be used within 30 days after opening the containers. Do not use kit components beyond their expiration dates.

Patient's samples have to be treated as potentially infectious. The samples should be handled in a lamina air flow bench

The assay reagents contain preservation substances against microbial growth, so avoid contact with skin and/or mucous membranes.

Run the assay in the laboratory only in one direction: A) Sample preparation / DNA-isolation; B) DNA-amplification; C) detection. These three steps must be performed in three separate areas of the laboratory. If possible three different rooms, individually equipped with instrumentation, pipets and the package insert for the test modules should be used. Use the reagents and instruments only for this activity and not for other purposes. Do not transfer pipets or other equipment from one area to the other.

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Assay Procedure

Attention:

- Do not interchange components of different lots.
- Bring all required reagents to room temperature (18 – 28 °C)
- Process all steps for the DNA isolation under a laminar flow.
- Use only pipet tips with contamination protection (filter tips).
- Open and close reaction tubes individually.
- If gloves are contaminated, change them immediately.
- Do not use more than 500 µL synovial fluid from samples which contain blood or are taken from severely inflamed joints. Higher volumes of these samples may inhibit the amplification reaction and can therefore lead to false negative results. The other steps and reagent volumes of the DNA isolation procedure remain unchanged.
- Separate the area of amplification from the area where the detection is performed.
- Do not open vials containing amplified samples in the area of amplification.
- Amplified samples, controls and denaturated PCR products (single stranded DNA) are a significant potential source of contaminations and must be pipetted and discarded with utmost care.
- Use an extra set of pipets for the preparation of the PCR reactions and use exclusively pipet tips with contamination protection!
- Open reaction tubes containing DNA carefully in order to avoid the formation of aerosols. If possible, centrifuge for a few seconds.

A. DNA-Isolation

1. Bring samples to room temperature and mix them before processing. Prepare and label the appropriate number of 1.5 mL reaction vials (vials with screw caps). Label one additional vial (extraction control), and process it with the other samples beginning with step A5.
2. Transfer **1,000 µL** of the sample to the labeled, sterile reaction tubes. Open only one tube at a time and close it immediately after the addition of the fluid.
3. Centrifuge samples for **20 minutes** at **3,500 g**.
4. Discard **950 µL** of the supernatant by using a filter tip. Do not touch or whirl the pellet with the pipet tip. It does not affect the extraction if some excess fluid remains in the reaction tube!
5. Open a reaction tube, cover the pellet with **1,000 µL** buffered wash solution and close the tube immediately. Continue to process the other samples in the same way. For every sample use a fresh filter tip. Mix the reaction tubes on the vortex.
6. Centrifuge the samples for **10 minutes** at **20,000g**.
7. Discard the supernatant carefully.
8. Add **75 µL** lysis reagent to the pellet and mix the reaction tubes on a vortex.
9. Incubate the reaction tubes for **15 minutes** at **95 °C** either in a water bath or in a thermomixer.
10. Centrifuge the reaction tubes for **30 seconds** at **5,000g**.
11. Add **75 µL** of neutralization buffer and mix.
12. Centrifuge reaction tubes for **30 seconds** at **5,000g**.
13. The supernatant is used for the amplification.

Important:

If there is a demand to stop sample processing, it can be done at this step. Until further processing samples can be stored in the refrigerator for up to 24 hours or at -20 °C for prolonged times.

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B. PCR-amplification:

1. Label the appropriate number of sterile 0.2 mL PCR reaction tubes. Process all pipetting steps immediately on ice. Include a positive control with control DNA and at least one negative control in each run.
2. Preparation of the PCR mastermix:

The amplification reaction is performed with **15 µL** DNA extract in a reaction volume of **100 µL**.

- a) Place the Taq-DNA-Polymerase directly into an ice bath.
- b) Bring all other reagents required for the preparation of the PCR mastermix to room temperature and intermix on a vortex before putting them into an ice bath.
- c) Place the reaction tube rack in the ice bath and pipet all reagents on ice.

Remark: For the preparation of the PCR mastermix a Taq-DNA-Polymerase and an enzyme-specific reaction buffer (concentrate) is required which are not supplied with the kit. The buffer for the Taq-Polymerase is usually concentrated 10-fold and therefore 10 µL are needed for a 100 µL reaction (please verify!).

Important: Use the 10x reaction buffer **with MgCl₂**!

Table 1: Preparation of the PCR mastermix

Reagent	Volume per reaction	Volume multiplied by the number of samples
Sterile water	54.5 µL *	
10x reaction buffer with MgCl₂	10 µL	
Amplification mix (blue cap)	20 µL	
Taq-DNA-Polymerase (5 units/µL)	0.5 µL (equivalent with 2.5 units)	
Final volume	85 µL *	

* Adjust water volume to get a final volume of **85 µL**.

The PCR mastermix is stable for 1 hour at 2 - 8 °C; do not freeze.

3. Prepare the PCR mastermix according to the example given in Table 1 in a 1.5 mL reaction vial. Dispense **85 µL** in each of the PCR reaction tubes and close the tubes.
4. Add **15 µL** of DNA extract of the patient's sample, the positive control (red cap) and sterile water (negative control) resp., to the PCR mastermix aliquots. Open the PCR reaction tubes individually and close them immediately after pipetting. Handle DNA extracts with great care in order to avoid contaminations with other samples.
5. If a thermocycler without lid heating is used, the samples must be overlaid with mineral oil (30 µL).
6. Transfer the samples into the thermocycler and start the amplification program (see Table 2). For thermocyclers with lid heating adjust the temperature to 110 °C.

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Table 2: Programming of the thermocycler**Adjust lid heating to 110 °C**

1 Cycle	Denaturation*	5 min*	at 95 °C
40 Cycles	Denaturation	30 sec	at 94 °C
	Annealing	30 sec	at 55 °C
	Elongation	60 sec	at 72 °C
1 Cycle	End-Elongation	5 min	at 72 °C

* If a so-called „Hot Start“-polymerase is used, the first denaturation step has to be extended to 10 to 15 minutes. **Please refer to the manufacturers instruction!**

- The run of the whole program needs approx. 2 hours.
- The thermocycler should be adjusted to the maximum heating and cooling power.

C. Product detection

First of all the amplification products are hybridized with the specific probe. Afterwards the so formed complex is detected with the lateral flow strips.

C1. Hybridization**Important Notes:**

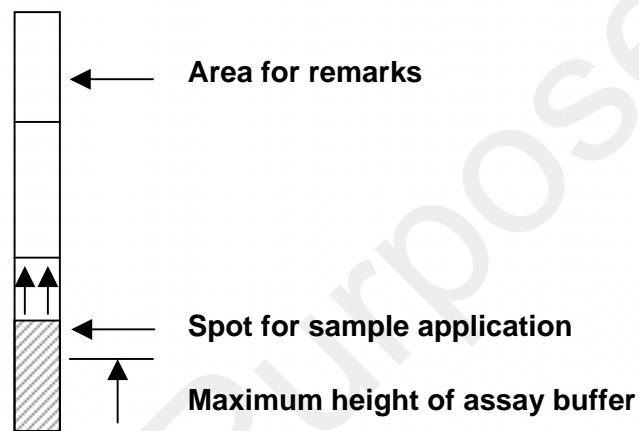
- no cooling below 56 °C should occur between the steps 2 and 3!
 - it is recommended to write a short hybridization program for the thermocycler in order to allow the precise control of temperature and time.
 - alternatively the incubations for the hybridization can be made in a water bath or in a thermomixer, but only if the temperatures can be adjusted with a precision of ± 1 °C.
1. Dispense **4 μ L** probe (green cap) in each reaction tube and add **10 μ L** of each PCR product. Close the tubes carefully!
 2. Incubate the hybridization mix for **5 minutes at 95 °C** (denaturation).
 3. Incubate the mix for **15 minutes at 56 °C** (hybridization).

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C2. Detection:

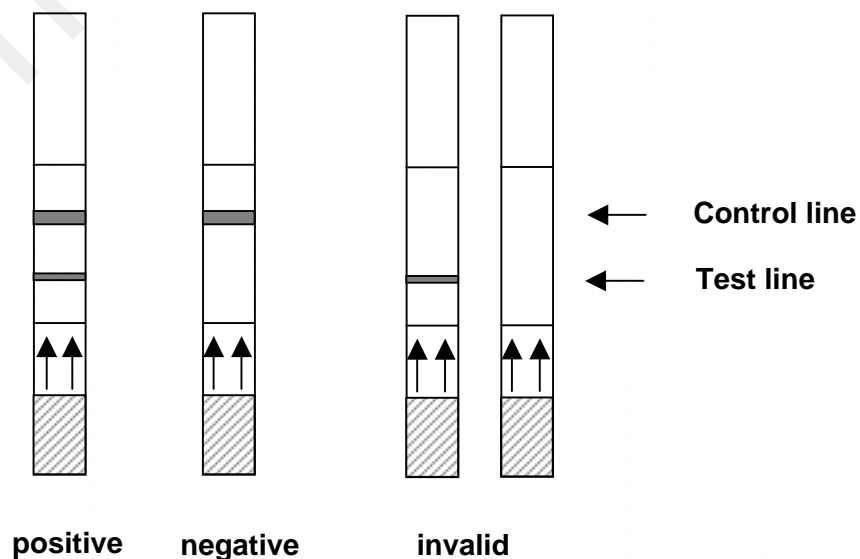
Important notes:

- Only touch and mark the covered areas of the strips
 - Keep the vial containing the dipsticks closed (protection of moisture)
1. Take the required number of strips from the vial and label them.
 2. Pipet **150 μL** assay buffer for every sample in individual reaction tubes or in wells of a microtiterplate.
 3. Pipet **7 μL** of the hybridization-mix close to the edge of the foil onto the free membrane part of the strip and incubate for **1 minute**.
 4. Immerse the strips with the membrane into the assay buffer of the tubes/wells of the microtiterplate and allow an incubation of **10 minutes**. The control line must be visible if results should be read.



Interpretation of Results

The test run is only valid, if the control line is clearly visible!



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Positive and negative controls of a run must show correct results to report any diagnostic results, i.e. positive control must show a clearly visible test line and negative control must be completely negative.

If the **PCR-negative control** shows a visible test line, all results of the test run must be rejected and the run must be repeated.

Case 1	<p>Two lines are visible: Test line and control line.</p> <p>Notes:</p> <p>(i) even a weak staining of the test line has to be interpreted as positive. If needed the test must be repeated for confirmation.</p> <p>(ii) depending on the concentration positive results may become visible even before the entire incubation time is over.</p> <p>(iii) with high concentration of the hybridization product intensity of the control line is affected</p>	The detection of DNA of <i>Chlamydia trachomatis</i> is positive .
Case 2	<p>One line is visible at the location of the control line.</p> <p>Do not interpret results before the whole incubation time is over.</p>	The detection of DNA of <i>Chlamydia trachomatis</i> is negative .

Trouble Shooting

Problem	Possible reason	Recommendation
No control line visible.	a) Assay buffer is wrong or does not work b) Test strips expired c) Wrong storage of test strips	Use new assay buffer. Use new test strips. Store at 2 – 8 °C.
Test strip shows a negative result, in contrast to this an agarose gel shows a band.	a) Band on the gel is a result of an unspecific PCR product. b) The hybridization was not successful c) No probe added	Check specificity of the PCR product (e.g. hybridization with a specific probe, restriction analysis or sequencing) Check hybridization conditions Repeat hybridization
Mineral oil	Mineral oil on the test strip may slow down or even inhibit the reaction.	Pipet the PCR product carefully from the bottom of the vial.

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ADDITIONAL INFORMATION

Method

The Chlamydia Direct test is a molecular system for the isolation, amplification and qualitative detection of *Chlamydia trachomatis* specific DNA in synovial fluid and urine. The test consist of three modules:

1. Sample preparation: isolation of DNA from synovial fluid and urine
2. PCR amplification of the target-DNA of *Chlamydia trachomatis*
3. Detection of the PCR product by hybridization with a specific probe and detection of the hybridization products applying an immunochromatographic method

1. Sample preparation

Extraction and purification of DNA from 1,000 µL sample material.

2. PCR-amplification

The isolated DNA of *Chlamydia trachomatis* is used for a specific amplification. During amplification the product is labeled for subsequent detection.

3. Detection

For the detection of the amplified product a lateral flow test device is used. This test identifies the DNA-sequence of the amplified product after hybridization with a sequence specific probe. Only amplification products that bind to the probe result in the formation of a red band at the location of the test line on the test strip (positive result).

The immunochromatographic detection on the lateral flow test device is based on the interaction of various antibodies directed against the respective labels of the DNA. The complex of the amplification product and the probe binds in a first step to antibodies conjugated to gold particles (gold conjugate). The immune complexes diffuse through the analytical membrane. At the location of the test line a molecule is coated which binds the complex described above and a band becomes visible by time in the case of positive results. Excess gold conjugat continues to migrate until it is bound by a second antibody which is coated on the membrane of the test device. This line is formed in every run and can be used to show the correct performance of the test strip.

Test Characteristics

Sensitivity

The analytical sensitivity of the GenLine Chlamydia Direct assay has been evaluated by serial dilutions of *Chlamydia trachomatis* cells in synovial fluid and urine. Dilution series of 10^4 to 10^{-2} *Chlamydia trachomatis* bacteria per mL patients sample were prepared. From each concentration 1 mL has been processed according to this protocol. The results have shown that the GenLine Chlamydia Direct test could detect up to 1×10^1 cells per mL. This is equivalent to the amount of 1 genome equivalent per amplification reaction.

The sensitivity of detecting DNA is comparable to an agarose gel which is stained with ethidiumbromid (sensitivity approx. 10 ng DNA).

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Short Instruction: Milenia[®] GenLine Chlamydia Direct

(all volumes in μL)

A. DNA Isolation

Steps	Vial	Solution	Sample
Pipet		Sample	1,000
Centrifuge 20 min at 3,500 g Discard 950 μL of supernatant			
Pipet		Buffered Wash Solution	1,000
Centrifuge 10 min at 20,000 g Discard supernatant			
Pipet		Lysis Reagent	75
Mix and incubate 15 min at 95 °C Centrifuge 30 s at 5,000 g			
Pipet		Neutralizing Buffer	75
Mix and centrifuge 30 s at 5,000 g			
continue the test or store supernatant (-20°C)			

B. PCR Amplification

Steps	Vial	Solution	Sample	Positive Control	Negative Control
Pipet		PCR mastermix	85	85	85
Pipet		DNA extract	15	-	-
Pipet		DNA control	-	15	-
Pipet		dest. water	-	-	15
start the thermocycler program (p. 7, tab. 2)					

C. Product Detection

Steps	Vial	Solution	Sample	Positive Control	Negative Control
Pipet		Probe (green cap)	4	4	4
Pipet		PCR Product	10	10	10
Incubate 5 min at 95 °C Incubate 15 min at 56 °C					
Pipet (into extra vials)		Assay Buffer	150	150	150
Pipet (onto the strip)		Hybridization mix	7	7	7
Incubate 1 min Dip strips into the assay buffer Incubate 10 min					
Interpret results					

For details see pages 4-7 of the assay procedure.

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