

In the name of God

 frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 22 July 2022
DOI 10.3389/fcimb.2022.949514

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OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to

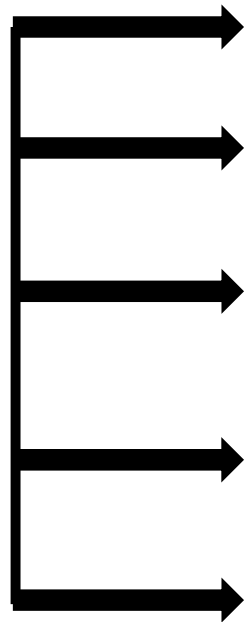
Sensitive and visual
identification of *Chlamydia
trachomatis* using multiple cross
displacement amplification
integrated with a gold
nanoparticle-based lateral flow
biosensor for point-of-care use

Presenter: Z.Fooladfar

Supervisor: Dr.M.Motamedifar



Outline



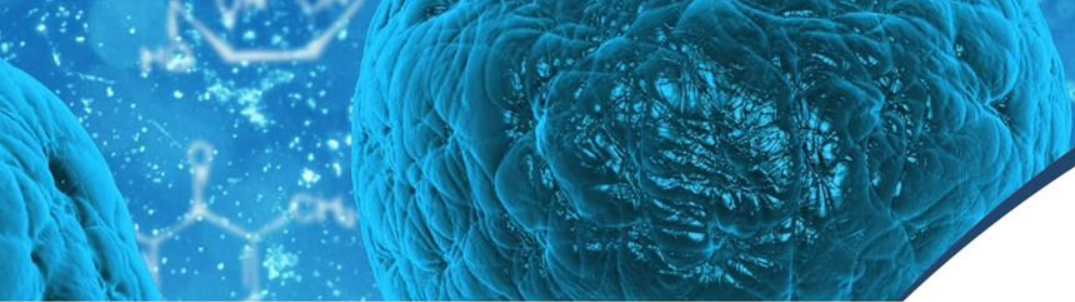
Introduction

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Discussion

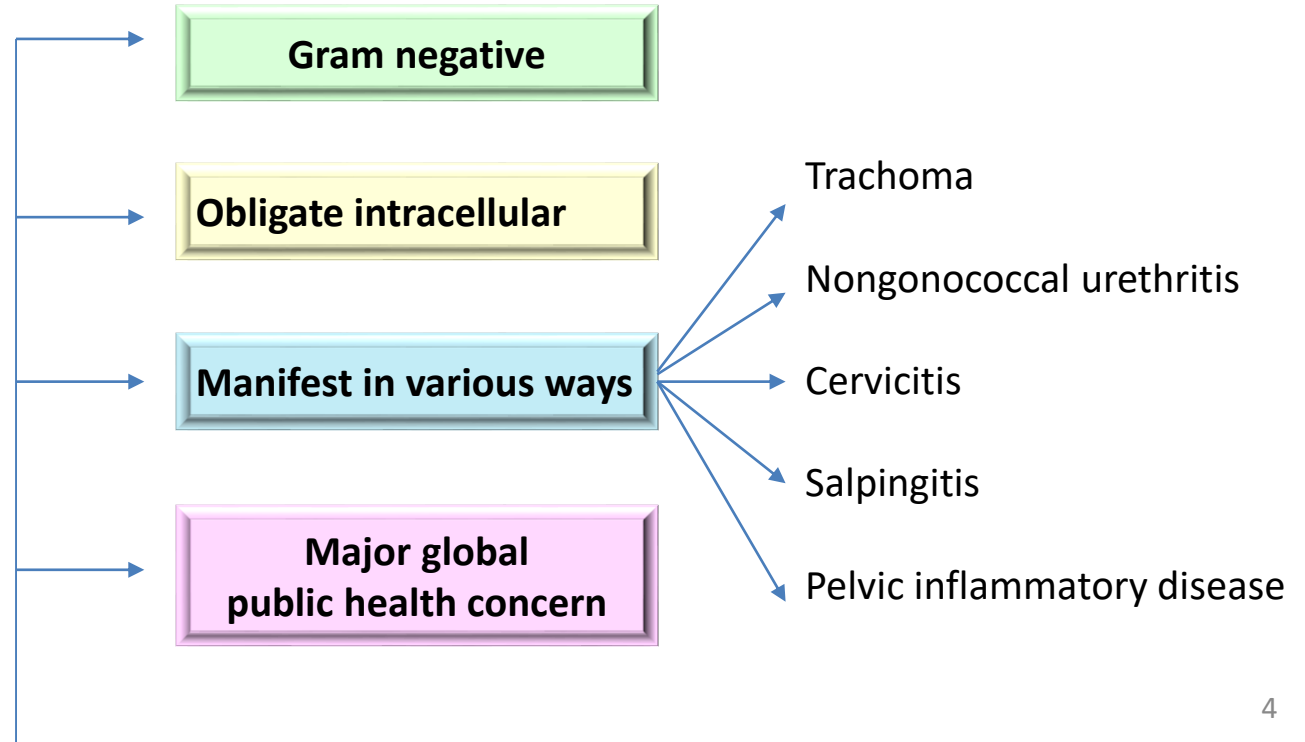
Conclusions

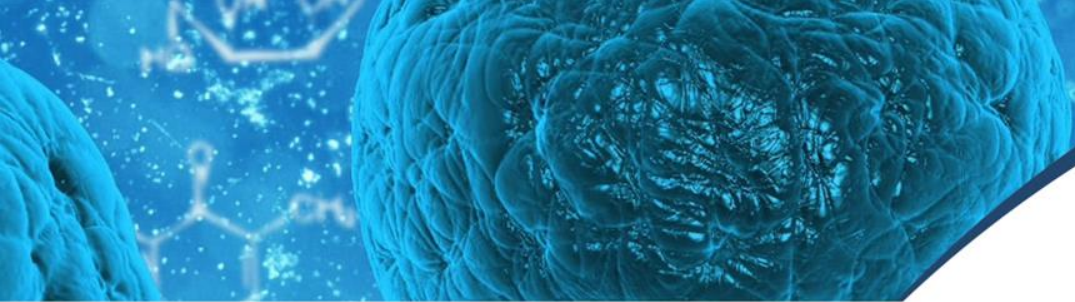



introduction

1.introduction

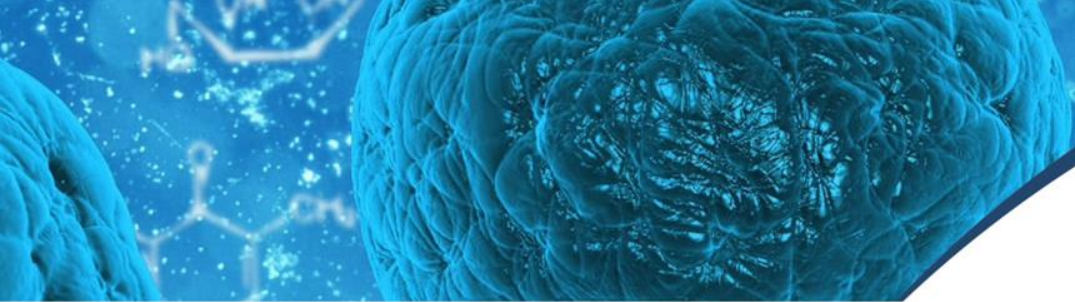
Chlamydia trachomatis



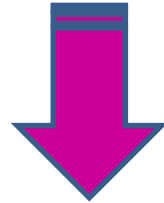


- Asymptomatic infections are common in both female and male **If untreated**  **cause severe complications**
- Maternal infection is associated with serious adverse pregnancy outcomes, including **miscarriage, stillbirth, low birth weight, preterm birth, or direct fetal infection**

Also *C. trachomatis* is a cofactor in human immunodeficiency virus transmission and human papillomavirus related-cervical cancer



Establishing **a specific, sensitive, rapid, inexpensive, and easy-to-interpret point-of-care (POC) testing system** for *C. trachomatis* would be important

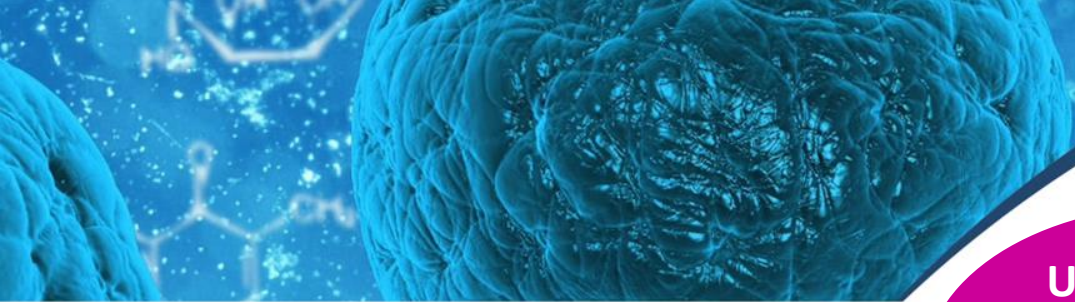


Multiple cross displacement amplification integrated with a gold nanoparticle-based lateral flow biosensor (MCDA-AuNps-LFB)



Multiple cross displacement amplification (MCDA)

1. Novel isothermal amplification approach
2. Attractive alternative to traditional nucleic acid amplification procedures such as PCR
3. MCDA is highly **specific, sensitive, robust, cost-effective, easy-to-operate**, and **does not require costly thermocycling facilities**
4. The strategy was previously used for the rapid detection of various pathogens, including SARS-CoV-2, *Neisseria gonorrhoeae*, and *Candida tropicalis*



Ten specially designed primers spanning ten distinct regions of target sequence

MCDA

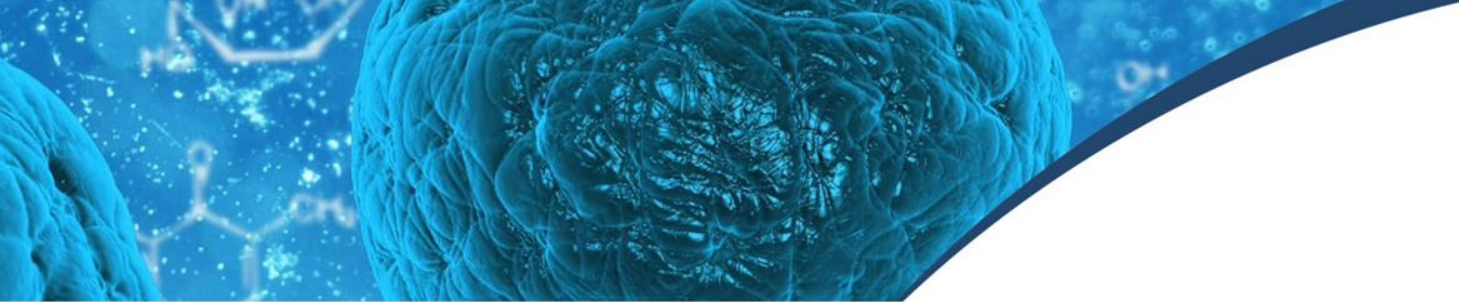
Utilizes only a polymerase with strand displace activity

At a constant temperature

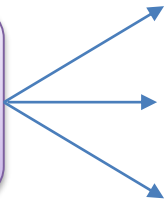


The gold nanoparticle-based lateral flow biosensor (AuNPs-LFB)

1. Paper-based platform
2. Highly attractive for POC diagnostics
3. It is **easy to manufacture, inexpensive, sensitive, and specific, and robustly and rapidly detects targets**



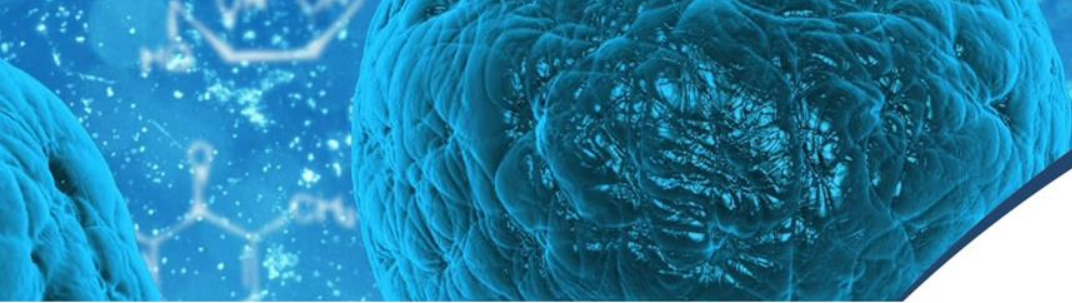
AuNPs



The **most common** nanomaterials used as **optical labels** in LFB

Easily synthesized, biocompatible, size-tunable, stable over time

Display a **strong red signal visible** to the naked eye

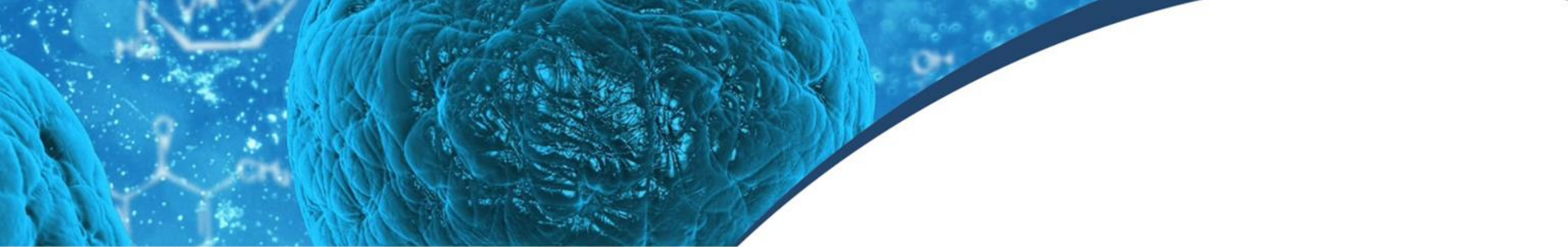


In this study:

MCDA-AuNPs-LFB was devised for the visual and rapid detection of *C. trachomatis* by targeting the **ompA** gene from several serovars (A–K, L1, L2, and L3)

The complete diagnostic process is accomplished within **40 min**

Using **clinical genital secretion samples** from patients



Material and method



Reagents

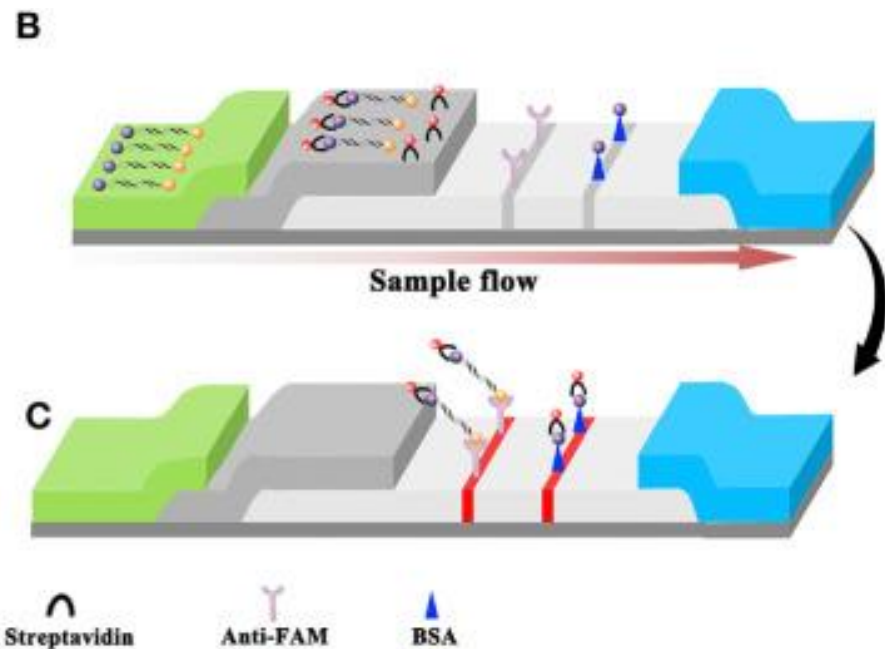
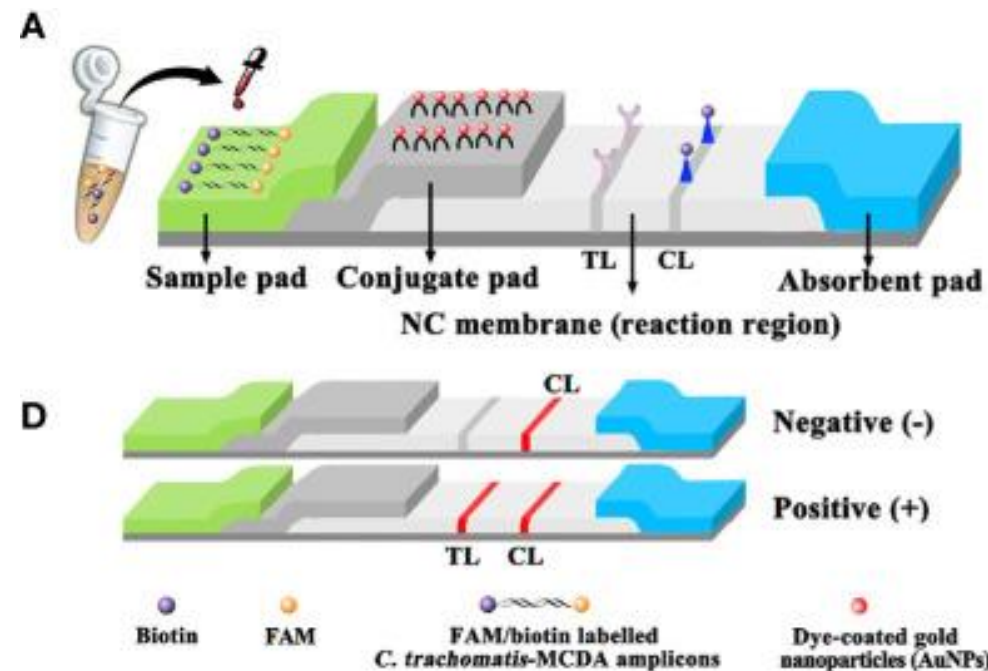
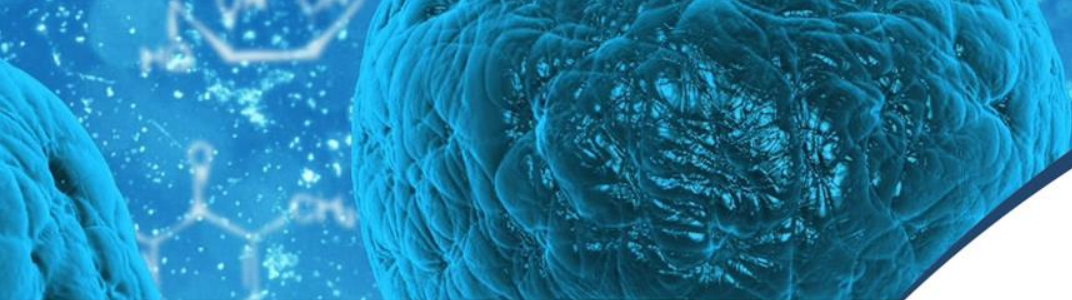
✓ AuNP-based LFB materials

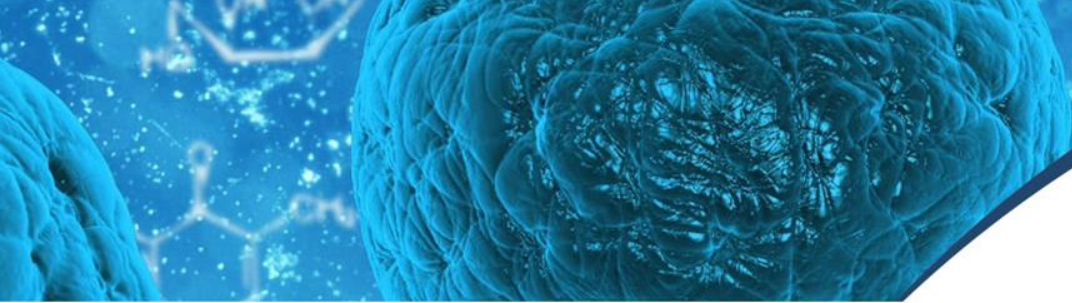
- Crimson red dye streptavidin-coated AuNPs
- Biotinylated bovine serum albumin (biotin-BSA)
- rabbit antifuorescein antibody (anti-FAM)

✓ LFB sections

- Nitrocellulose Membranes
- Sample pad
- Conjugate pad
- Adsorption pad

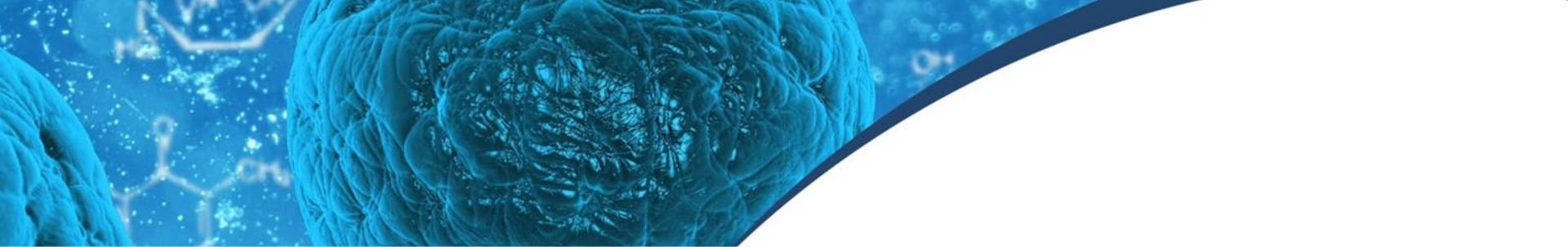
laminated on plastic
adhesive backing





Amplification reagent

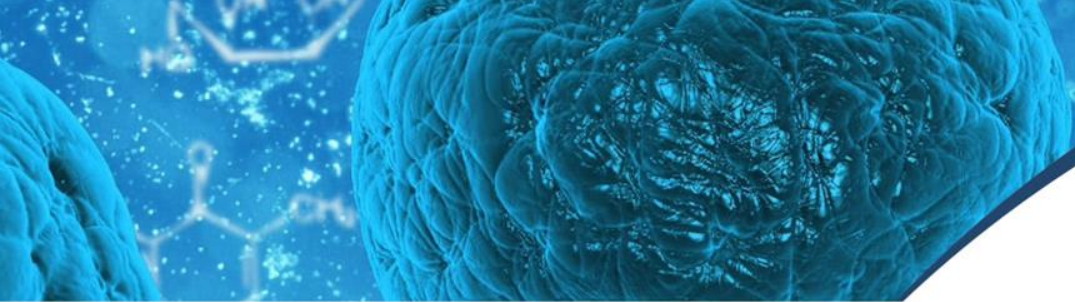
- Nucleic acid releasers
- Isothermal amplification kits
- Colorimetric indicator malachite green (MG)
- Commercial PCR diagnostic kits for *C. trachomatis*



Preparing target DNA and clinical samples:

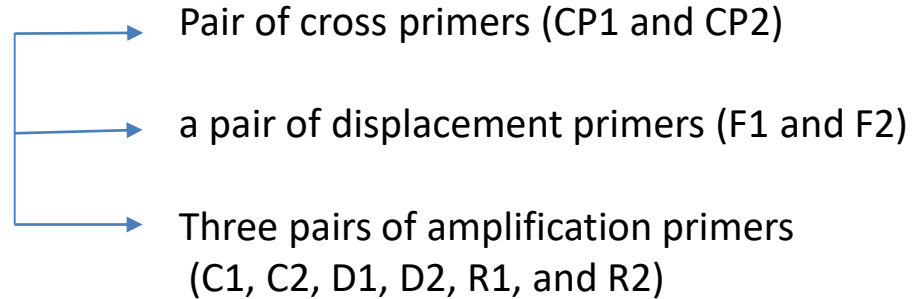
- ❖ Collected suspected *C. trachomatis*-infected genital secretion samples
- ❖ Crude genomic DNA was extracted using Nucleic Acid Releasing Agents

Briefly, a genital secretion sample was mixed with 100 μ l of Nucleic Acid Releasing Agent for 5 min cell lysis, and the supernatant was used as a template for *C. trachomatis*-MCDA assay.



MCDA primer design:

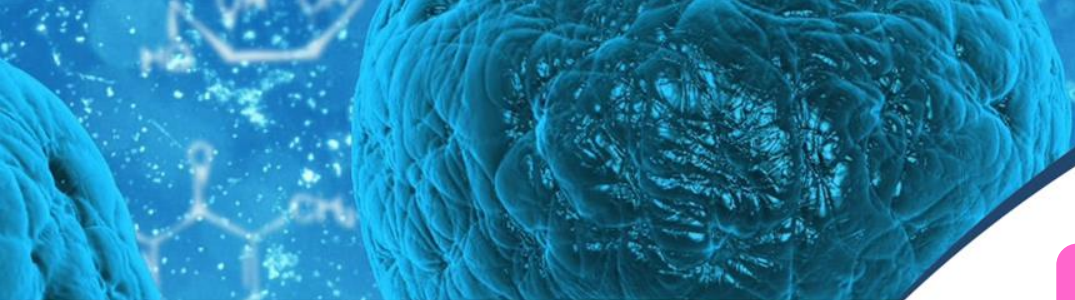
- Suite of 10 MCDA primers was designed to amplify 10 different sections of *C. trachomatis* ompA



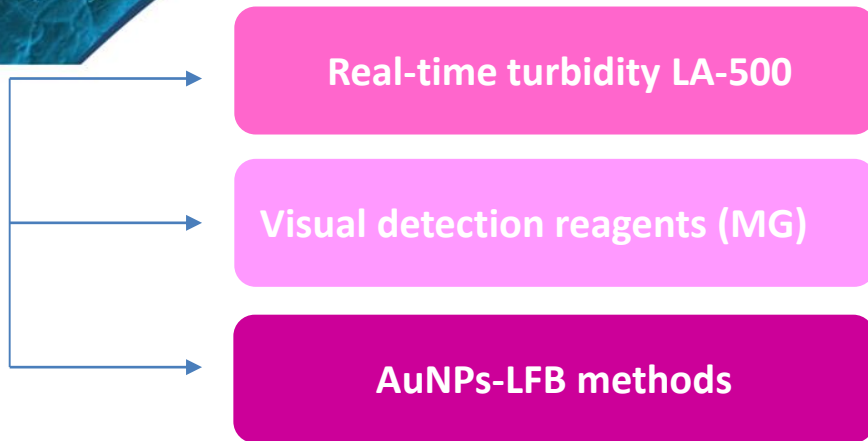
- **OmpA genes** from 14 *C. trachomatis* serological variants (serovar A, B, C, D, E, F, G, H, I, J, K, L1, L2, and L3) were aligned

conserved sequences selected for MCDA primer design.

□ **Bst DNA polymerase** was used in this study instead of *Thermus aquaticus* DNA polymerase.

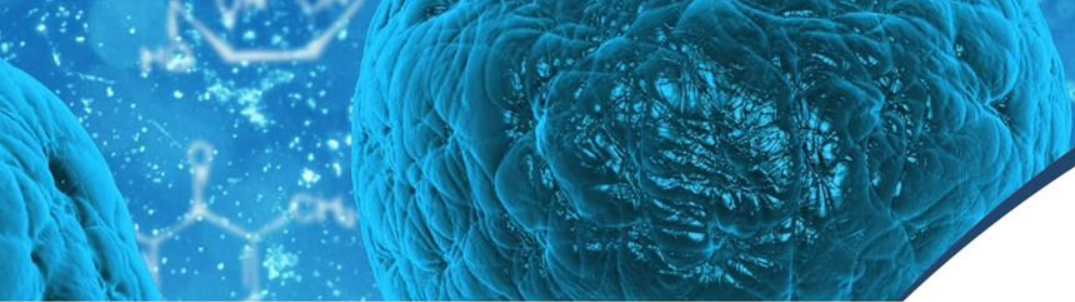


Amplification products were examined



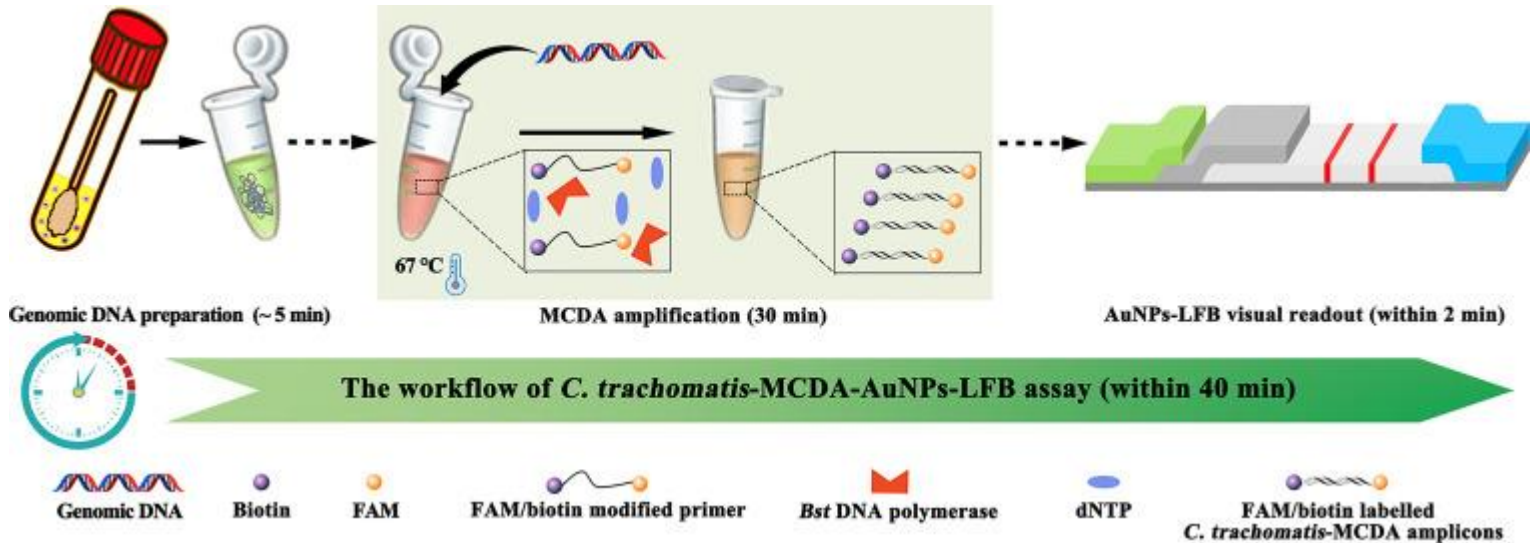
For positive result

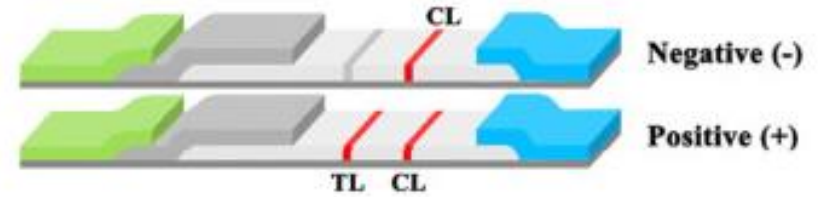
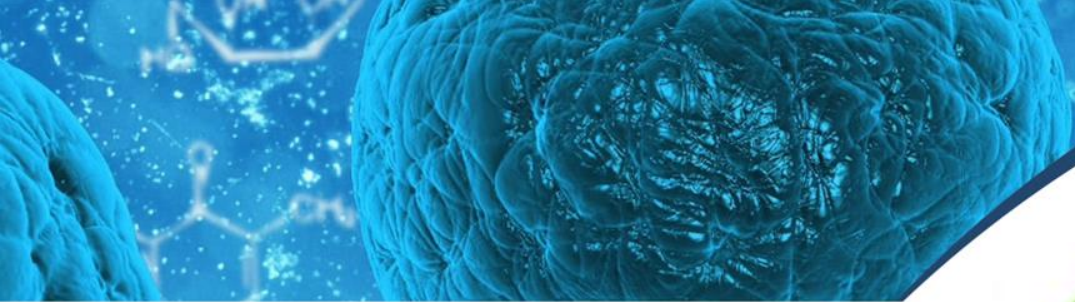
- AuNPs-LFB detection, both **Control Line** and **Test Line** simultaneously appeared on the biosensor
- Visual MG analysis, reaction mixtures changed to **light green**
- A real-time turbidity **value >0.1**



Result

Assay system overview



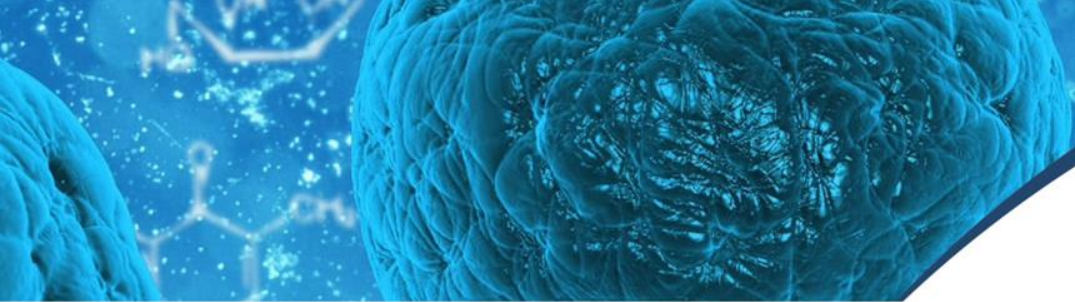


For positive result

- FAM/biotin labeled ompA-MCDA amplicons were specifically captured by anti-FAM at the TL
- SA-AuNPs were captured by biotin-BSA at the CL

For negative result

- Only SA-AuNPs were captured by biotin-BSA at the CL



Confirming the *C. trachomatis* MCDA assay:

To validate the reaction system

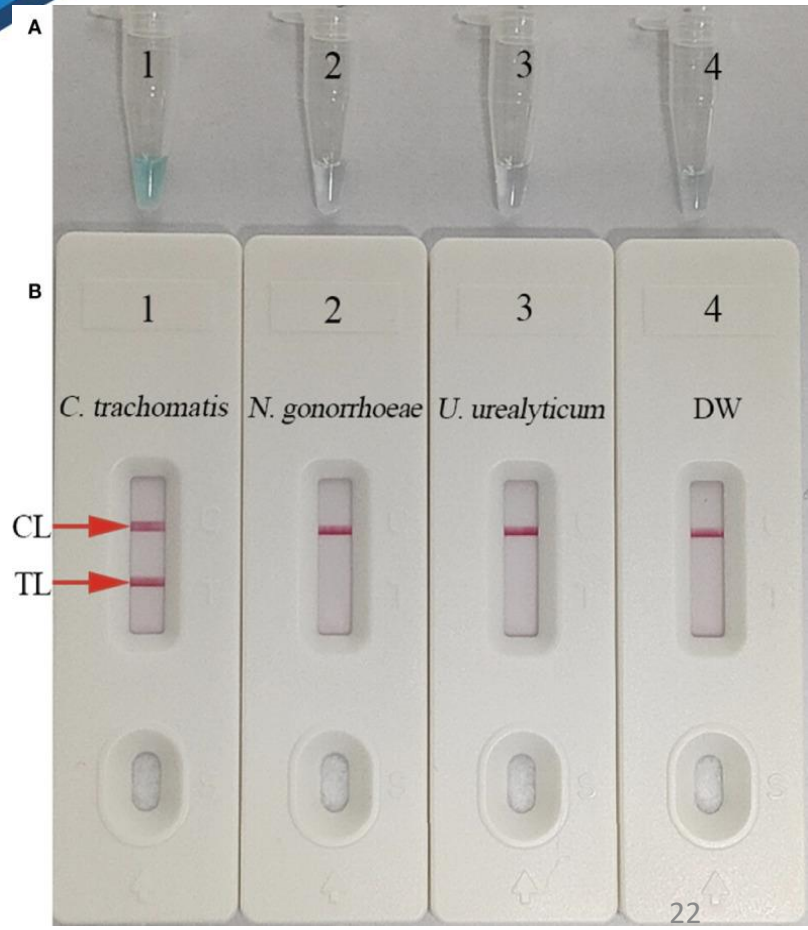


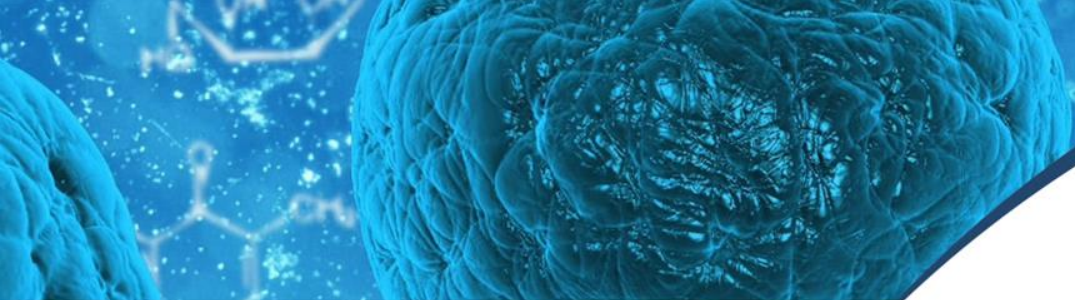
MCDA amplification mixture for



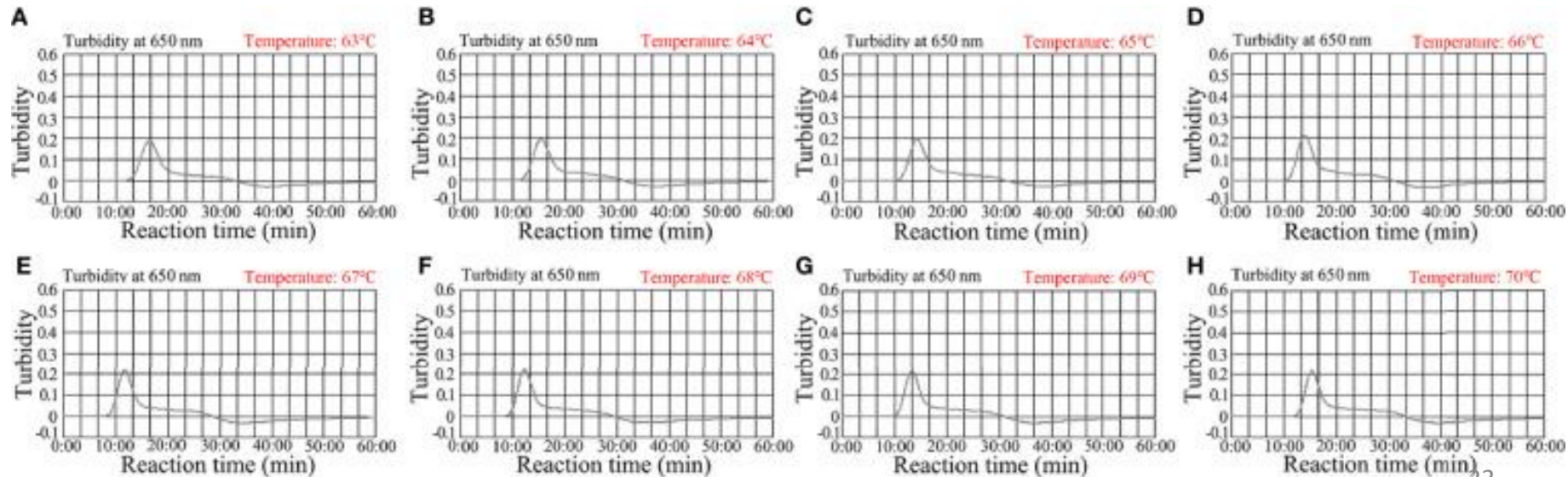
MG AuNP-LFB

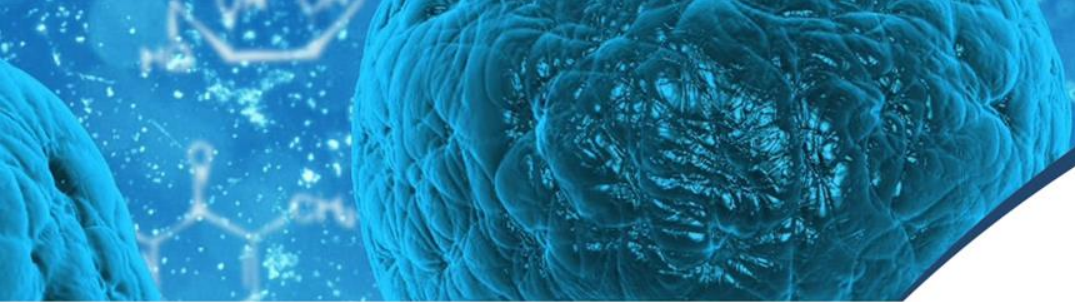
- *C. trachomatis* ompA plasmid
- *Neisseria gonorrhoeae*
- *Ureaplasma urealyticum*





Determining an optimal reaction assay temperature:

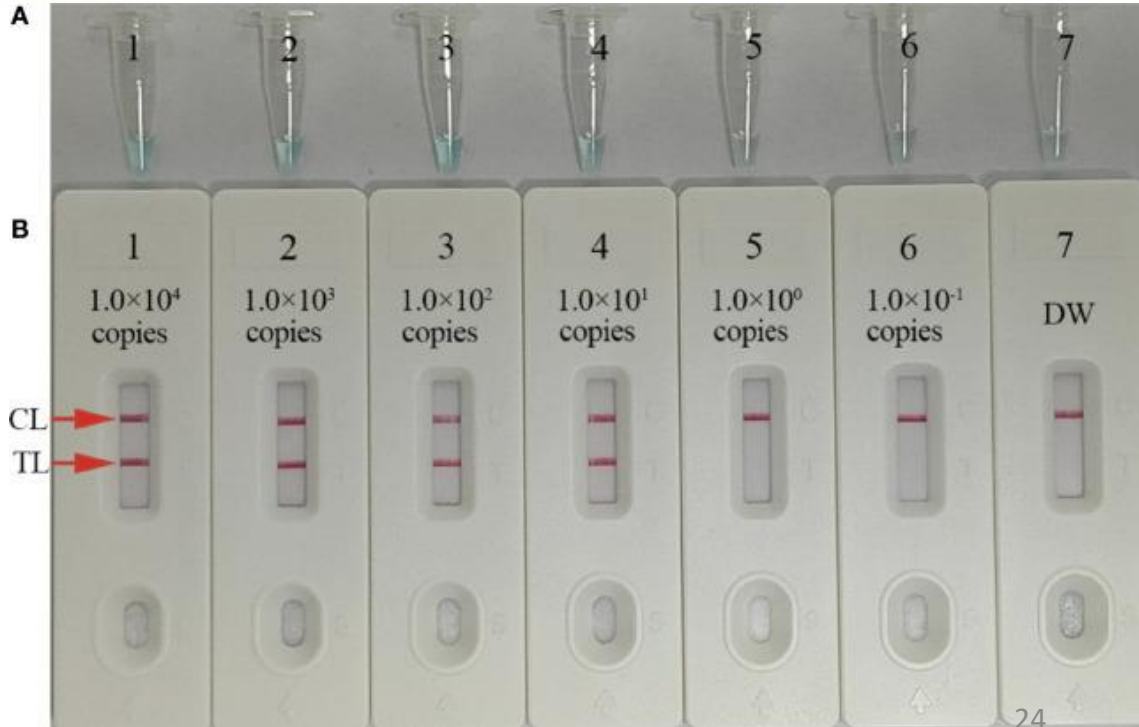


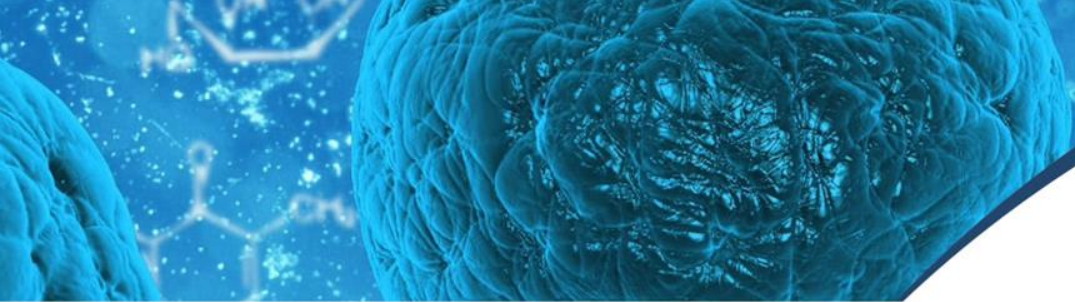


Assay sensitivity:

- 1) Prepared 10-fold serial dilutions of *C. trachomatis* ompA standard plasmids
- 2) MCDA reactions were conducted
- 3) Results were visualized by MG and AuNPs-LFB

LoD : 10 copies/test

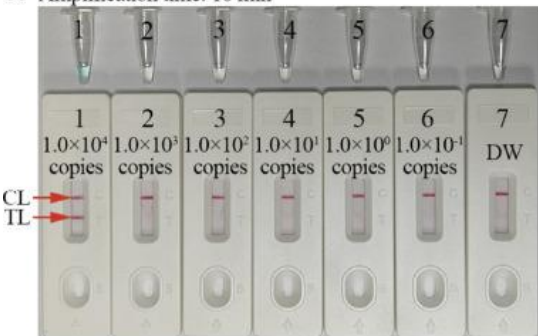




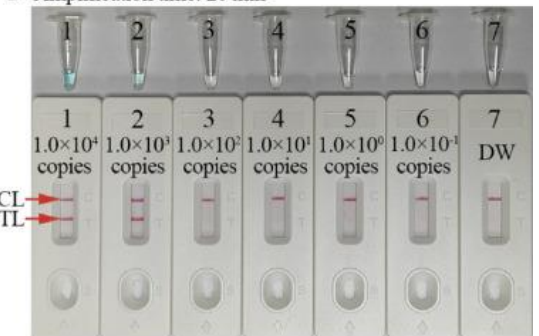
Optimizing the assay reaction time:

- prepared 10-fold serial dilutions of *C. trachomatis* ompA standard plasmids
- during isothermal amplification stages(67°C)
- different reaction times (20, 30, 40, and 50 min)
- MCDA reactions were conducted
- results were visualized by MG and AuNPs-LFB

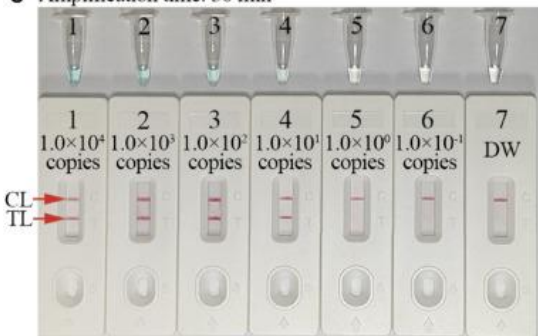
A Amplification time: 10 min



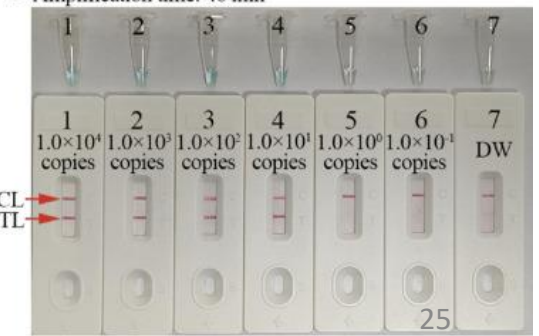
B Amplification time: 20 min

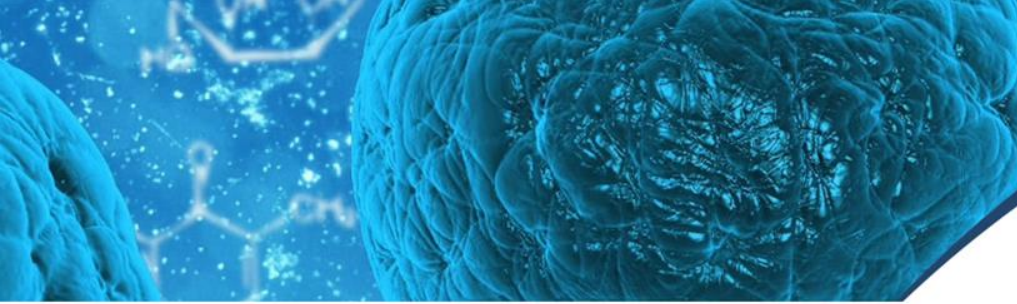


C Amplification time: 30 min



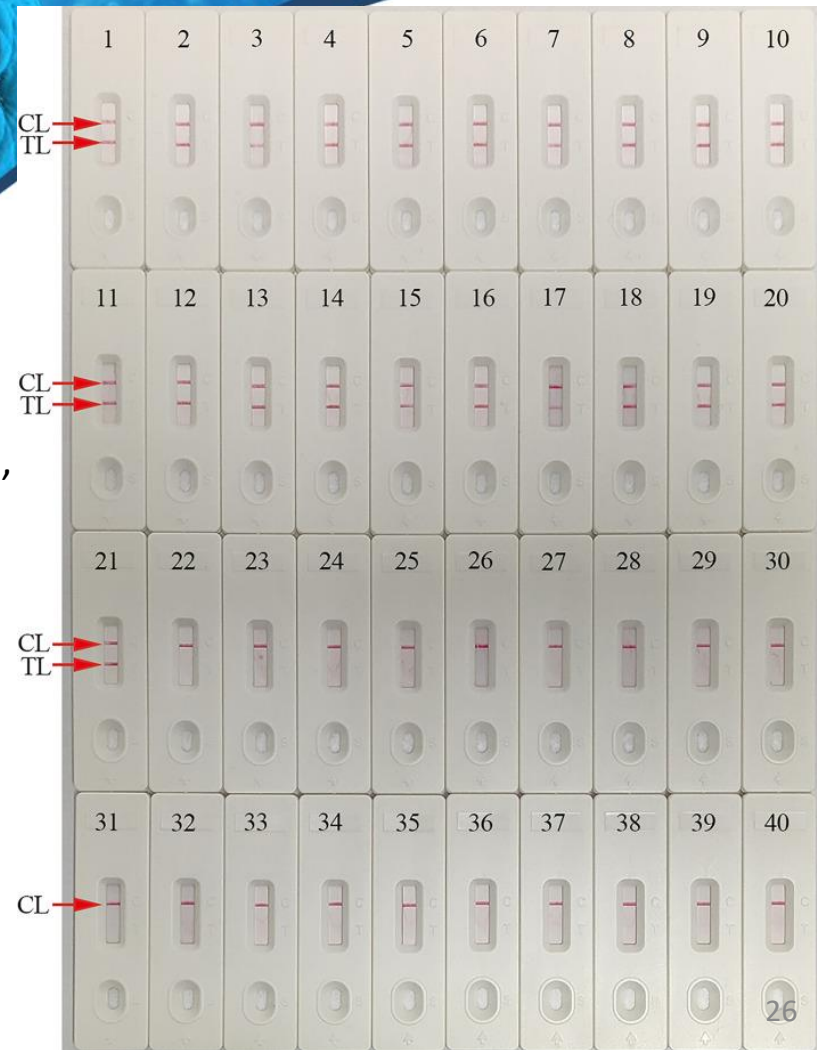
D Amplification time: 40 min

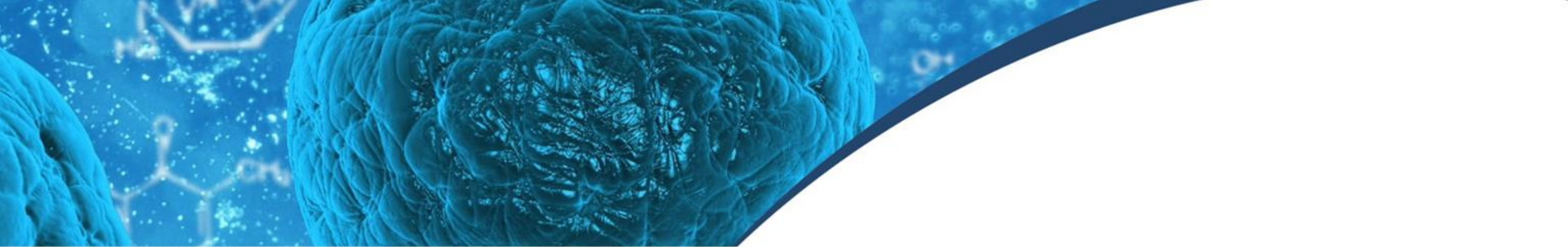




Assay specificity:

- Biosensors 1–14, *C. trachomatis* serovars A, B, C, D, E, F, G, H, I, J, K, L1, L2, and L3 ompA-plasmids.
- Biosensors 15–21, *C. trachomatis* (clinical samples)
- Biosensors 22-39 non-*C. trachomatis* strains
- Biosensors 40 negative control (distilled water, DW)





Assay evaluation using clinical samples:

Table 3

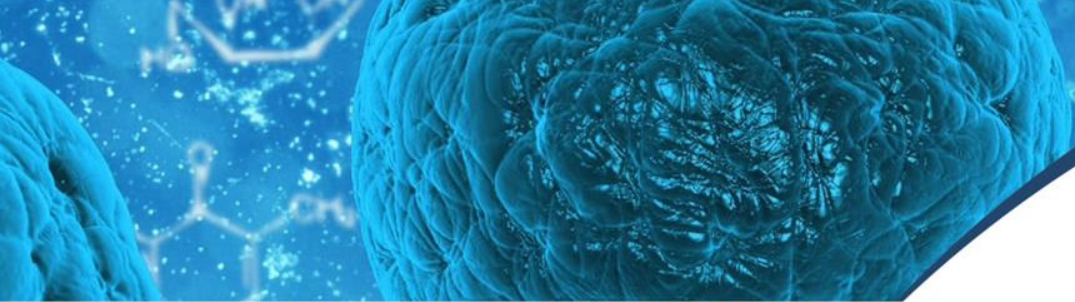
Comparing *C. trachomatis* levels in clinical samples using our MCDA-AuNPs-LFB assay with a qPCR method.

<i>C. trachomatis</i> - MCDA-AuNPs-LFB	<i>C. trachomatis</i> real-time TaqMan PCR(reference method)			Sensitivity (%)	Specificity (%)	PPV ^a (%)	NPV ^b (%)
	Positive	Negative	Total				
Positive	56	3	59	100	96.20	94.92	100
Negative	0	76	76				
Total	56	79	135				

[Open in a separate window](#)

^aPPV, positive predictive value;

^bNPV, negative predictive value.



Discussion



In this study

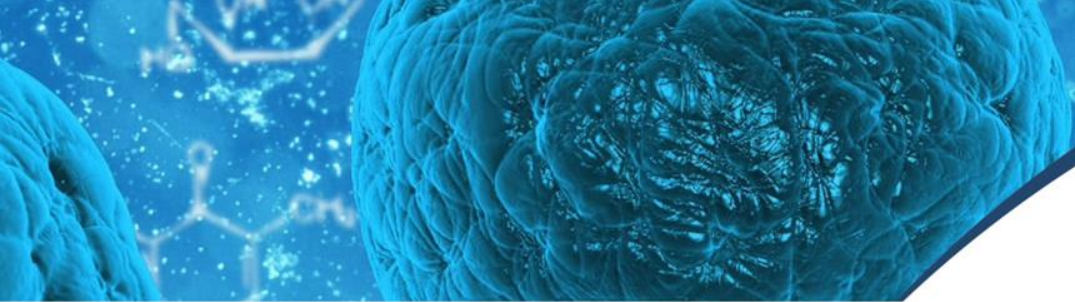
- ✓ Established an **accurate, rapid, easy-to-interpret, inexpensive, specific, and sensitive POC** *C. trachomatis* testing system, using **MCDA** for ompA amplification, followed by an **AuNPs-LFB** visual specific readout.
- ✓ Sample : suspected *C. trachomatis* infection genital secretion
- ✓ Compare with *C. trachomatis* real-time TaqMan PCR kit :

NAATs method:

Unaffordable and inaccessible in less developed regions as they require **robust laboratory infrastructures, expensive instruments, and trained personnel**

But: MCDA system required **simple devices** → a water bath that maintained 67°C for 30 min

All-in cost for each test was approximately \$5.5 USD



The whole process takes 40 min

- ✓ Nucleic acid extraction (approximately 5 min)
- ✓ MCDA (30 min)
- ✓ Visual results readout (approximately 2 min)

✓ Other Isothermal amplification technologies, including **loop mediated isothermal amplification (LAMP)** and **cross-priming amplification (CPA)** have also been used to identify *C. trachomatis*.

BUT MCDA is as a **more sensitive** than traditional PCR and other Isothermal amplification methods.

LAMP

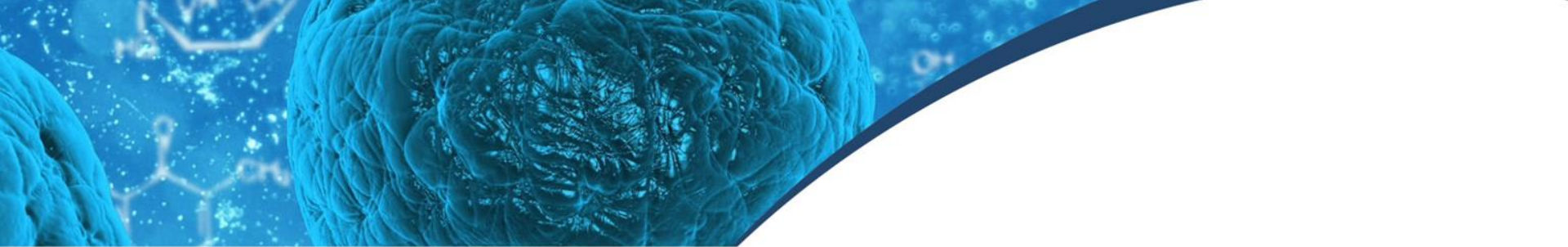
**LoD: 11.25 copy
In 1 hour**

CPA

**LoD: 45 copy
In 1.5 hour**

MCDA

**LoD: 10 copy
In 30 min**

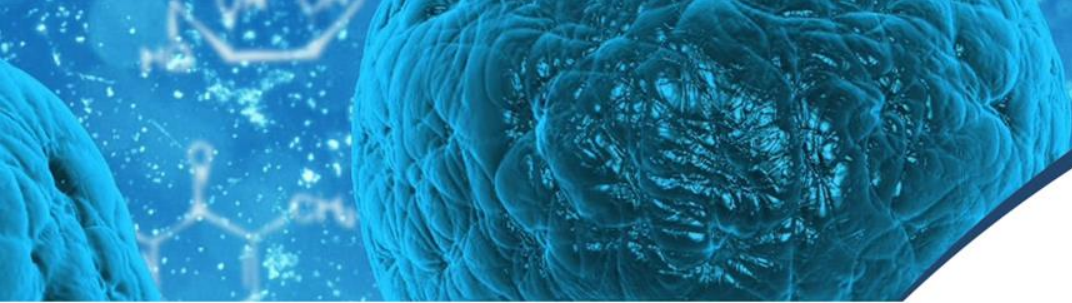


To rapidly and visually analyze *C. trachomatis*-MCDA amplification products:

AuNPs-LFB was used

- **Robust**
- **Inexpensive**
- **User-friendly**
- **Sensitive and specific**



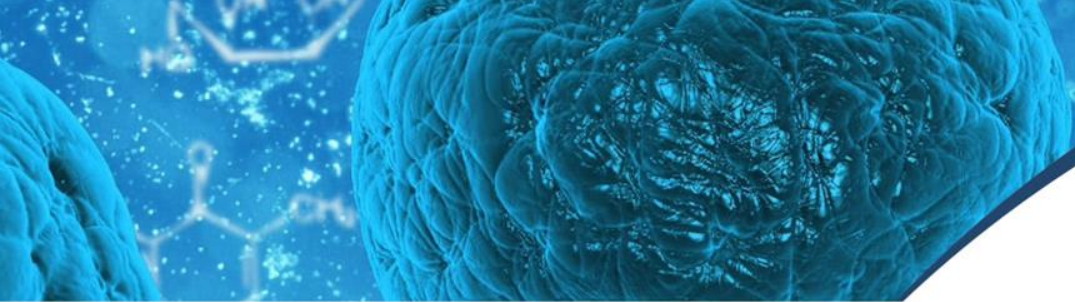


 Drawbacks of this study:

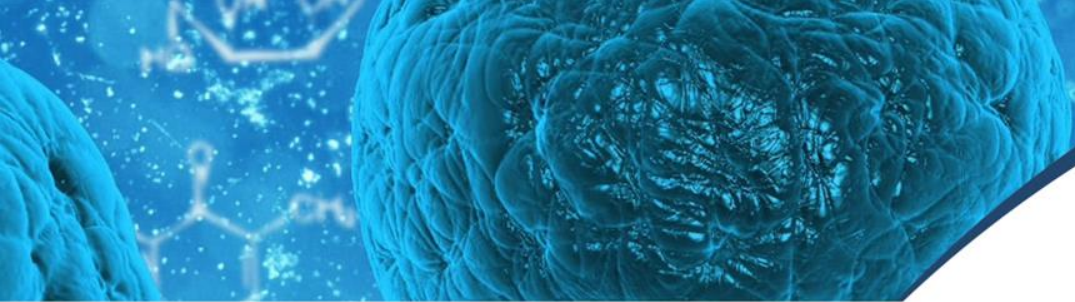
For further evaluation of this assay, it needs to be compared with a highly sensitive method as reference, including **more samples** with **low copy numbers**.

This assay can be **used for qualitative detection** of *C. trachomatis*, but **not for measurement of the concentrations** of *C. trachomatis* in sample

C. trachomatis-MCDA reaction tubes must be taken off for AuNP-LFB detection. Thus, there is a **risk of carry-over contamination**.



Conclusions



Novel method was designed:

For rapid, highly specific, sensitive, user-friendly, and visual identification of *C. trachomatis*

LoD was 10 copies/reaction

The assay showed no cross-reactions with non-*C. trachomatis* microbes

The detection procedure was completed within 40 min

Did not require expensive instrumentation

This novel assay has great potential for the POC testing and identification of *C. trachomatis* in clinical settings, particularly in low-income regions.

