Development of a multiple cross displacement amplification combined with nanoparticles-based biosensor assay to detection of a variety of pathogens

Journal Club

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Rapid, ultrasensitive, and highly specific identification of *Brucella abortus* utilizing multiple cross displacement amplification combined with a gold nanoparticles-based lateral flow biosensor

Jiang *et al. BMC Microbiology* (2022) 22:20
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BMC Microbiology

RESEARCH Open Access

Multiple Cross Displacement Amplification Coupled with Lateral Flow Biosensor (MCDA-LFB) for rapid detection of *Legionella pneumophila*

Luxi Jiang[1†], Rumeng Gu[1,2†], Xiaomeng Li[3], Meijun Song[1], Xiaojun Huang[1] and Deguang Mu[4*]

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Development of a multiple cross displacement amplification combined with nanoparticles-based biosensor assay to detect *Neisseria meningitidis*

Shijun Li, Chunting Liu, Ying Liu, Qing Ma, Yue Wang & Yi Wang

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BMC Microbiology

RESEARCH Open Access

Reliable detection of *Burkholderia pseudomallei* using multiple cross displacement amplification label-based biosensor

Xiaoxia Wang[†], Licheng Wang[†], Huaxiong Zhu, Chongzhen Wang and Xiong Zhu[*]

Traditional diagnosis

Bacterial culture

- **Advantages:**

Reliability

Intuitiveness

- **Disadvantages:**

Time-consuming

Complicated workflow of detection

Risk of infection for laboratory personnel

Overuse of antibiotics reduces the utility of culture-based methods

Traditional diagnosis

- **Serological assays**
- Specificity is indeed the major limitation
- Low diagnostic sensitivity
- Cross reaction

PCR based methods

- Expensive
- Special requirements(thermal cycler)
- Sensitivity still needs to be further improved in individuals with low bacterial load
- Complicated to perform in resource poor laboratories in many developing countries

The ideal method

- Simple
- Rapid
- Sensitive
- Reliable
- Readily available

Isothermal amplification techniques

- Loop-mediated isothermal amplification (LAMP)
- Multiple cross displacement amplification (MCDA)

Isothermal amplification techniques

- Greater specificity and sensitivity
- Requires simpler equipment or machines

Limit of detection and time for MCDA method targeting L. monocytogenes, compared to LAMP

Assays	Regions recognized	LoD (no./reaction)*	Fastest time (min)	Time for LoD (min)
MCDA	10	62.5 fg	15	40
LAMP	8	1 pg	25	52

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SCIENTIFIC REPORTS

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Rapid and Sensitive Isothermal Detection of Nucleic-acid Sequence by Multiple Cross Displacement Amplification

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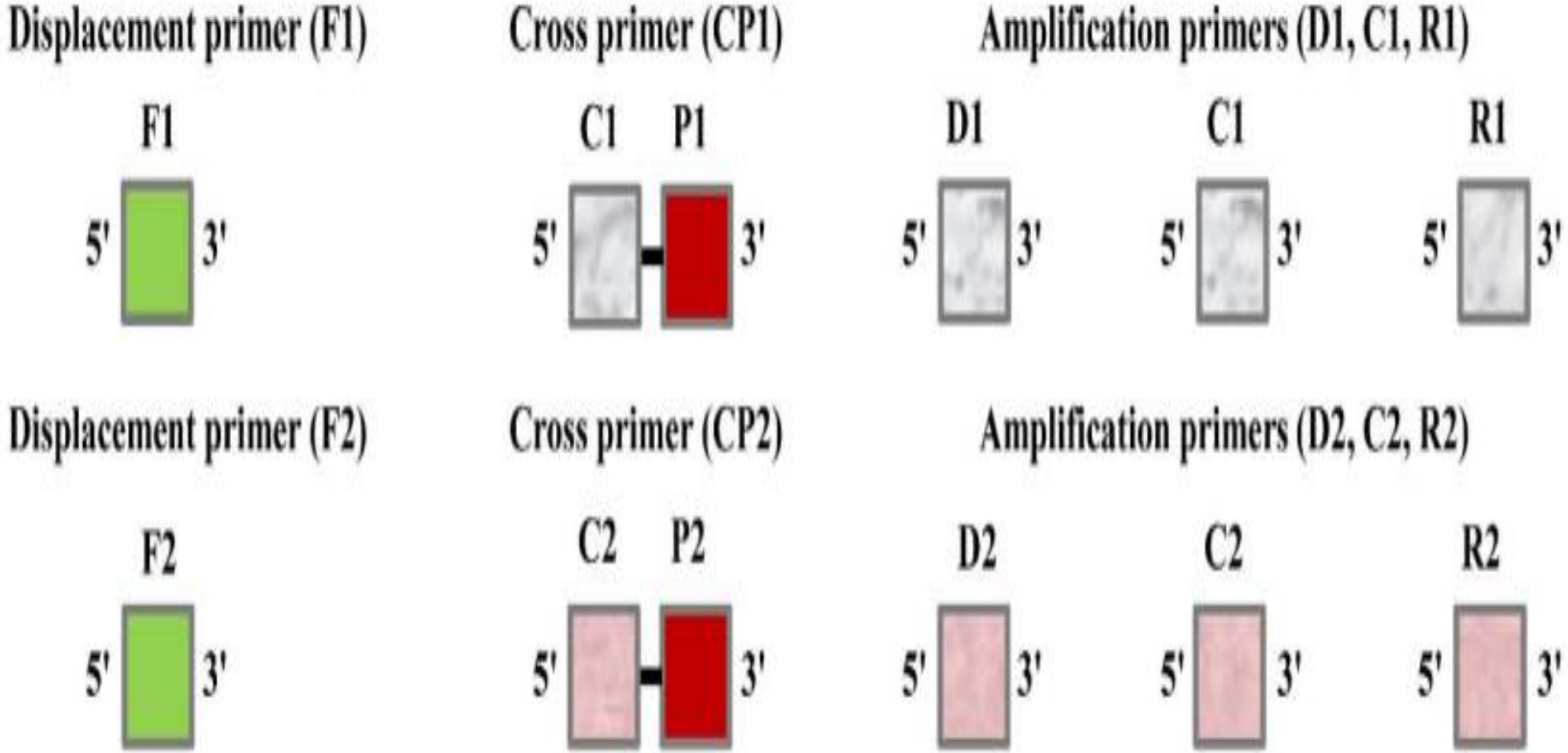
Yi Wang[1,*], Yan Wang[1,*], Ai-Jing Ma[1], Dong-Xun Li[2], Li-Juan Luo[1], Dong-Xin Liu[3], Dong Jin[1], Kai Liu[1] & Chang-Yun Ye[1]


Displacement primer (F1)
F1
5'
3'
Cross primer (CP1)
C1
P1
5'
3'
Amplification primers (D1, C1, R1)
D1
C1
R1
5'
3'
Displacement primer (F2)
F2
5'
3'
Cross primer (CP2)
C2
P2
5'
3'
Amplification primers (D2, C2, R2)
D2
C2
R2
5'
3'

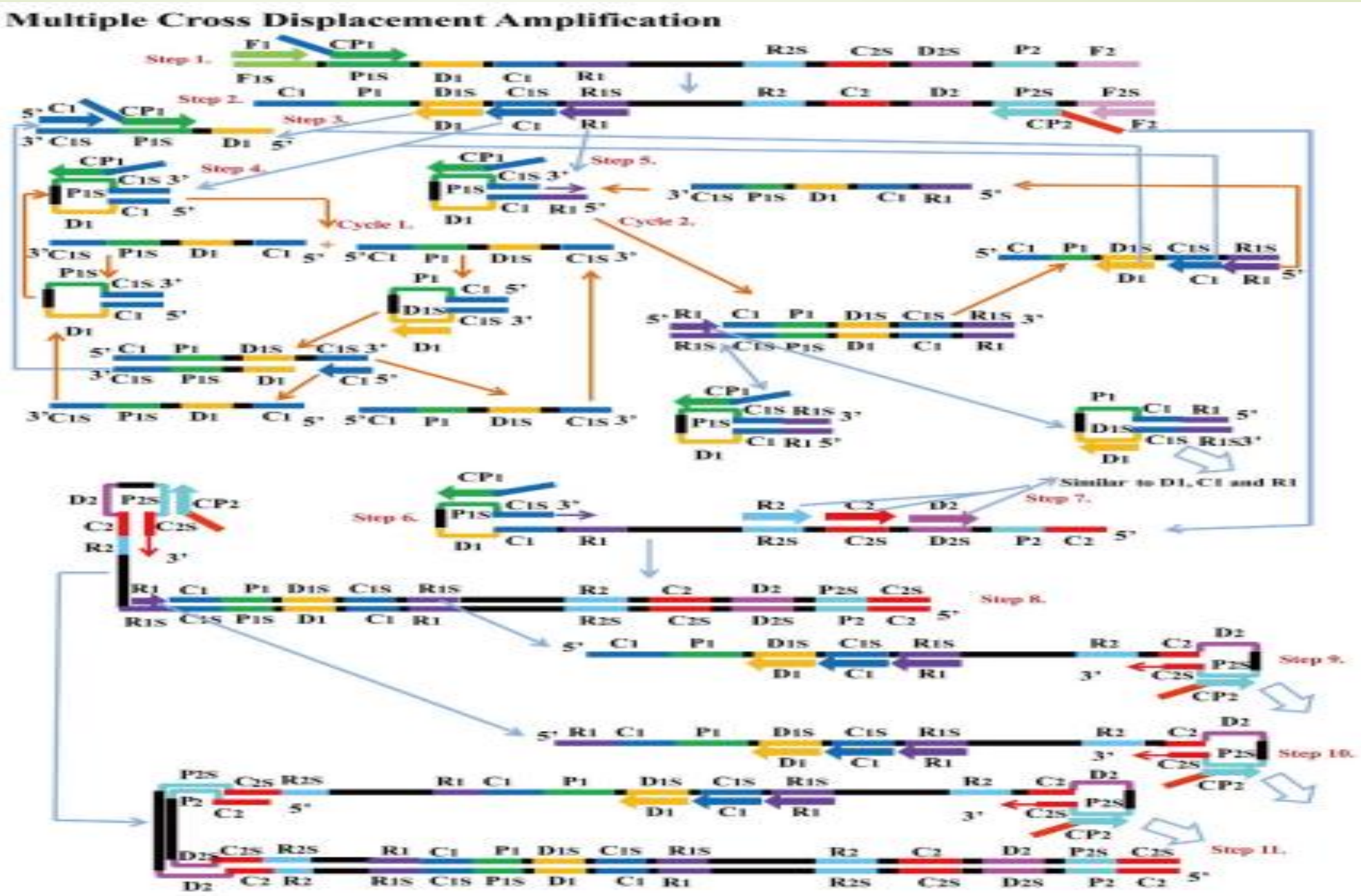


Figure 1. The principle of multiple cross displacement amplification. The schematic showing the mechanism of the novel MCDA assay.

Two monitoring techniques

- Colorimetric indicator
- Gel electrophoresis analysis

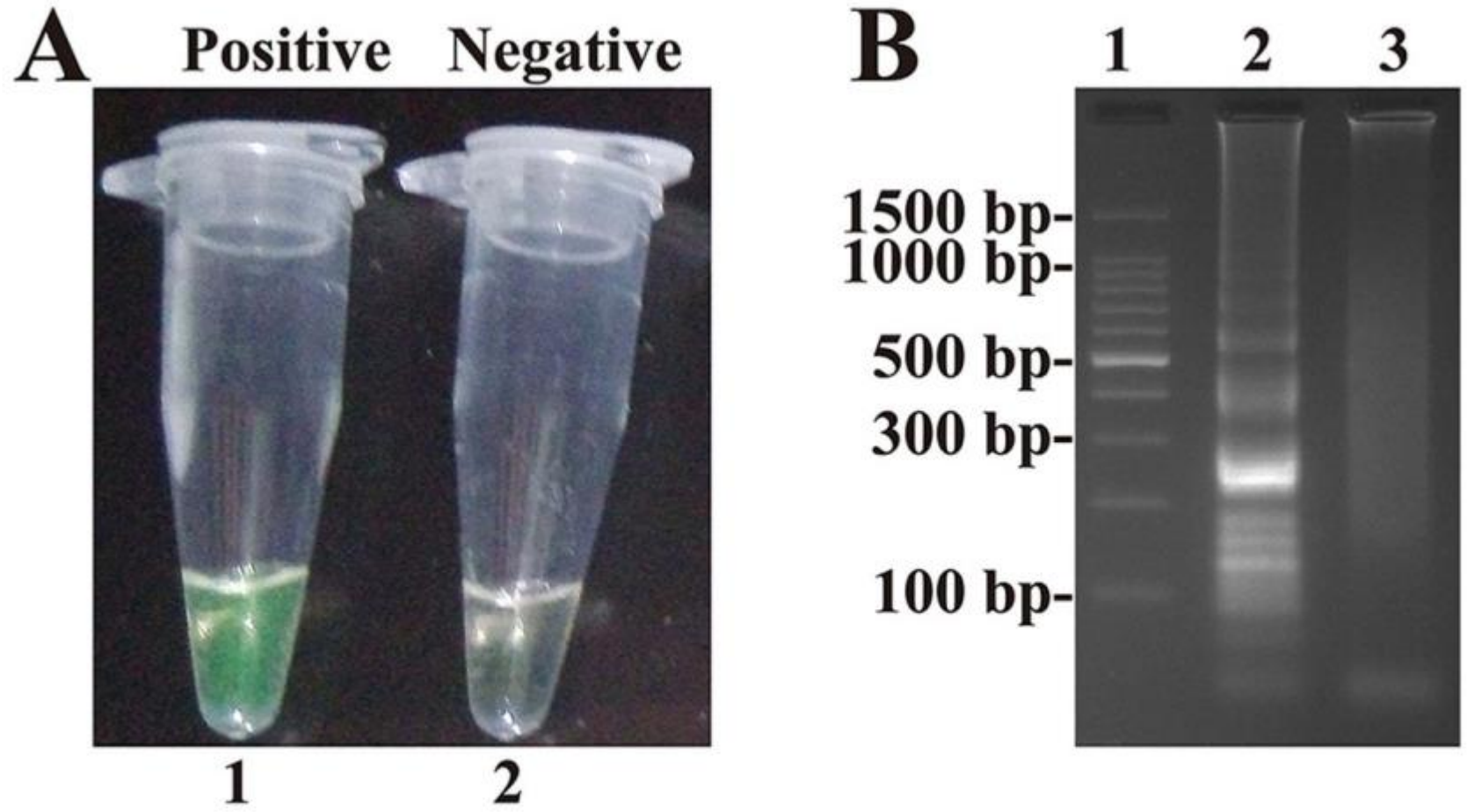
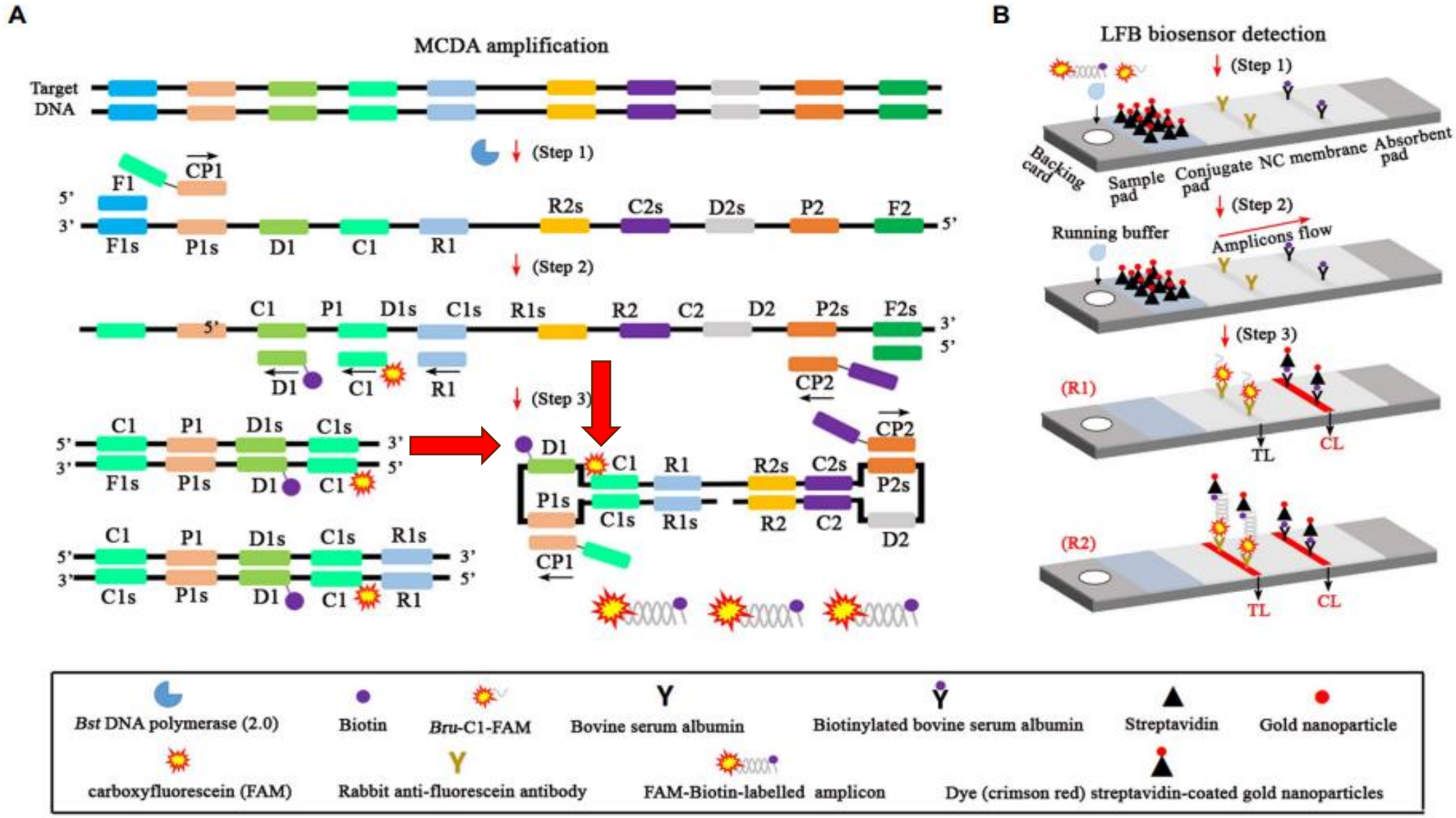
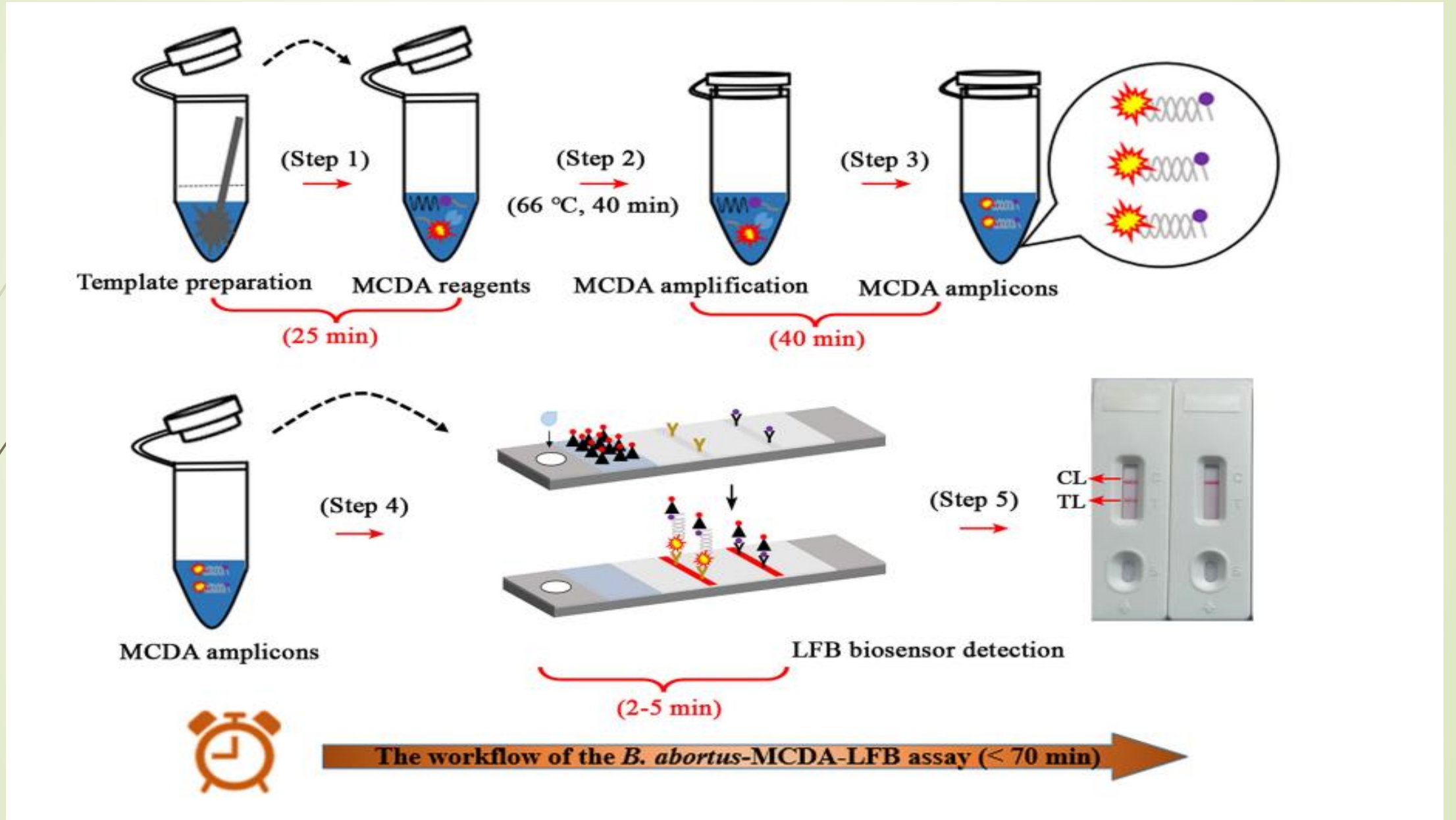


Figure 3. Confirmation and detection of MCDA products. (**A**) Color change of MCDA tubes; tube 1, positive amplification; tube 2, negative amplification. (**B**) 2.5% agarose gel electrophoresis applied to MCDA products; lane 1, DL 100-bp DNA marker; lane 2, positive MCDA products; lane 3, negative control (no DNA).

Difficult to distinguish specific from non-specific amplification accurately

LFB detection




(Step 1)
(Step 2)
(66 ℃, 40 min)
(Step 3)
Template preparation
MCDA reagents
MCDA amplification
MCDA amplicons
(25 min)
(40 min)
(Step 4)
(Step 5)
CL
TL
MCDA amplicons
LFB biosensor detection
(2-5 min)
The workflow of the B. abortus-MCDA-LFB assay (< 70 min)

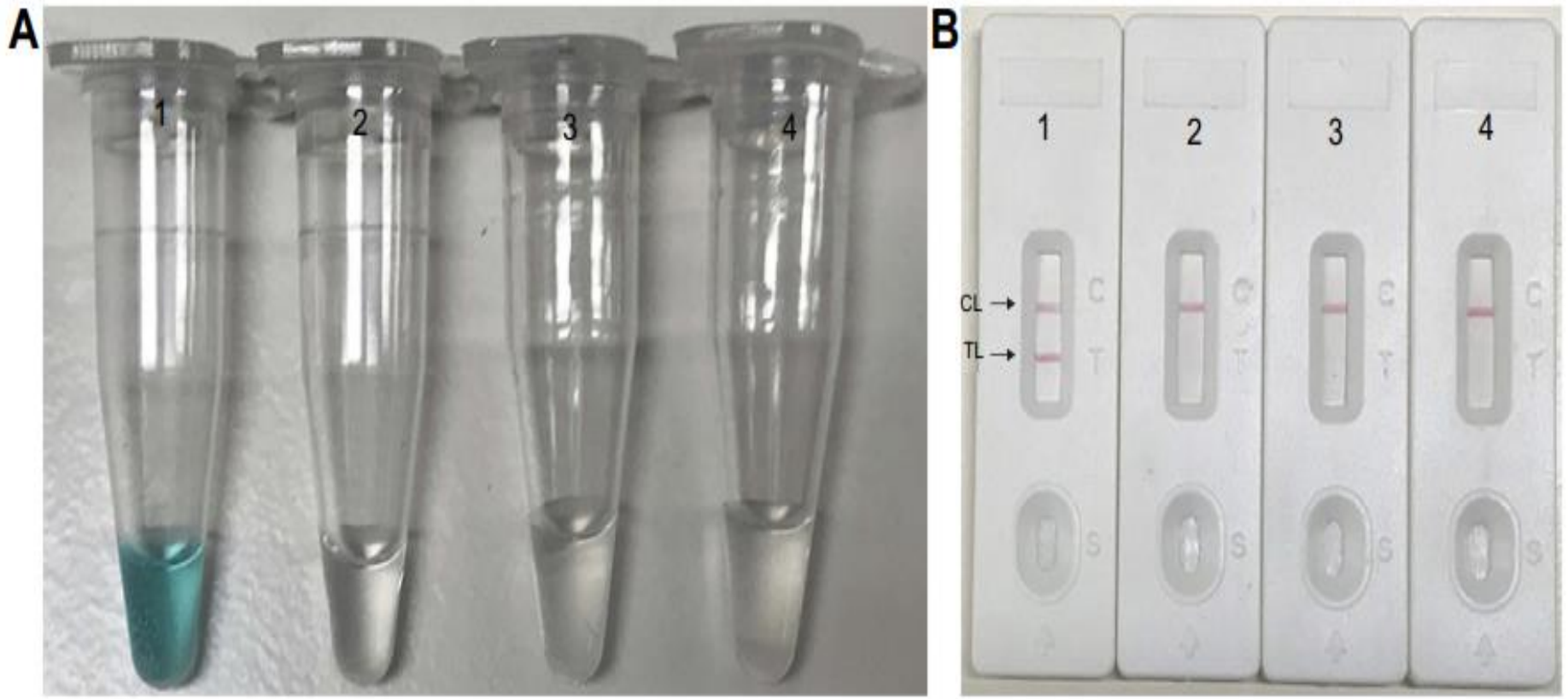


Figure 2 Confirmation and detection of Neisseria *meningitidis*-MCDA products. **(A)** By the MG method, amplification products of the *N. meningitidis*-MCDA assay were visually analyzed by observation of the color change. **(B)** A lateral flow biosensor was applied for visual detection of *N. meningitidis*-MCDA products. Tube 1/Biosensor 1: positive amplification of *N. meningitidis* strain 13007 Tube 2/Biosensor 2: negative control of *Staphylococcusaureus* (GZCDC isolate); Tube 3/Biosensor 3: negative control of Streptococcus*pneumoniae* (GZCDC isolate); Tube 4/Biosensor 4: blank control (DW).

Abbreviations: TL, test line; CL, control line; MCDA, multiple cross displacement amplification; MG, malachite green; GZCDC, Guizhou Provincial Center for Disease Control and Prevention; DW, double-distilled water.

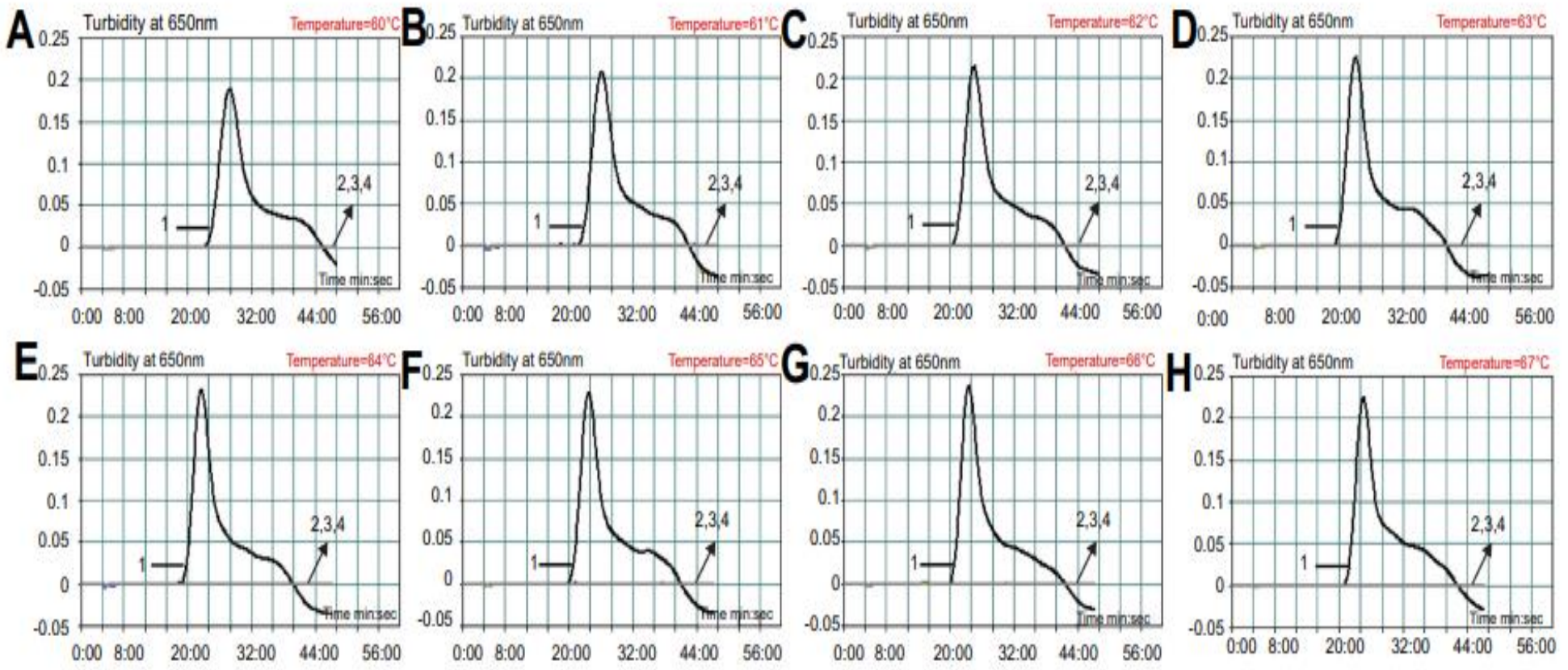


Figure 3 Reaction temperature optimization for *N. meningitidis*-MCDA primers. The standard MCDA reactions for detection of *N. meningitidis* were monitored by the determination of real-time turbidity, and the DNA concentrations were displayed with corresponding curves marked in the figures. The threshold value was 0.1 and a turbidity >0.1 was set as positive. A total of 8 kinetic graphs (**A**–**H**) were produced at different temperatures points (60–67°C, 1°C intervals) with target DNA at the level of 10 pg per reaction. The graphs from (**B–H)** show strong amplification.

Abbreviation: MCDA, multiple cross displacement amplification.

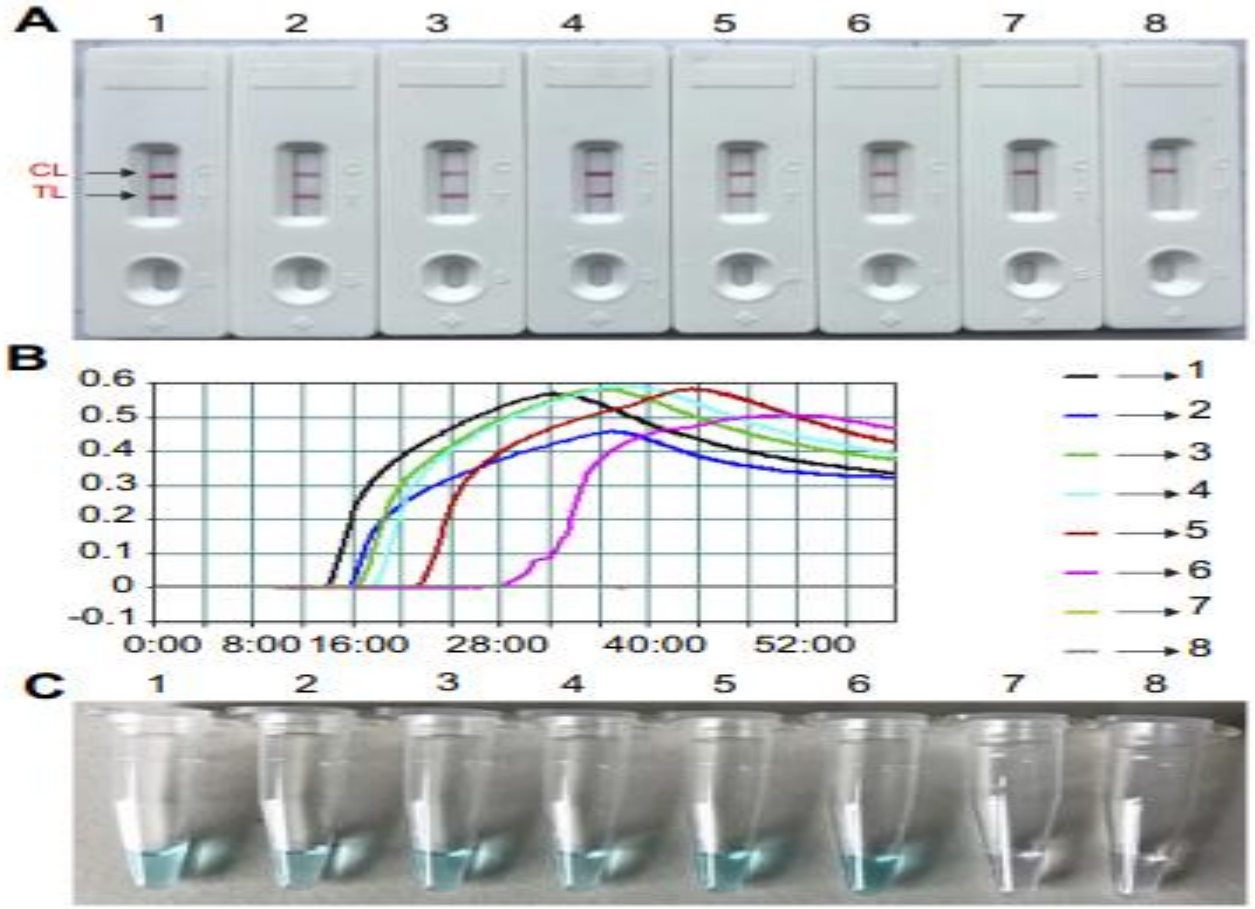


Figure 4 Sensitivity analysis of the MCDA-LFB assay using serial dilutions of genomic DNA extracted from *Neisseriameningitidis* strain 13007. A total of three monitoring techniques, including the lateral flow biosensor **(A)**, real-time turbidity **(B)** and colorimetric indicator (MG; **C**) methods, were applied to analyze the amplification products. Serial dilutions of target templates were subjected to standard MCDA reactions. Biosensors **(A)**/Tubes **(B)**/Turbidity signals **(C)** 1–8 represent the DNA levels of 1 ng per reaction, 100 pg per reaction, 10 pg per reaction, 1 pg per reaction, 100 fg per reaction, 10 fg per reaction, 1 fg per reaction of target templates and the blank control (DW), respectively. The genomic DNA levels of 1 ng per reaction, 100 pg per reaction, 10 pg per reaction, 1 pg per reaction, 100 fg per reaction, 10 fg per reaction produced positive reactions.

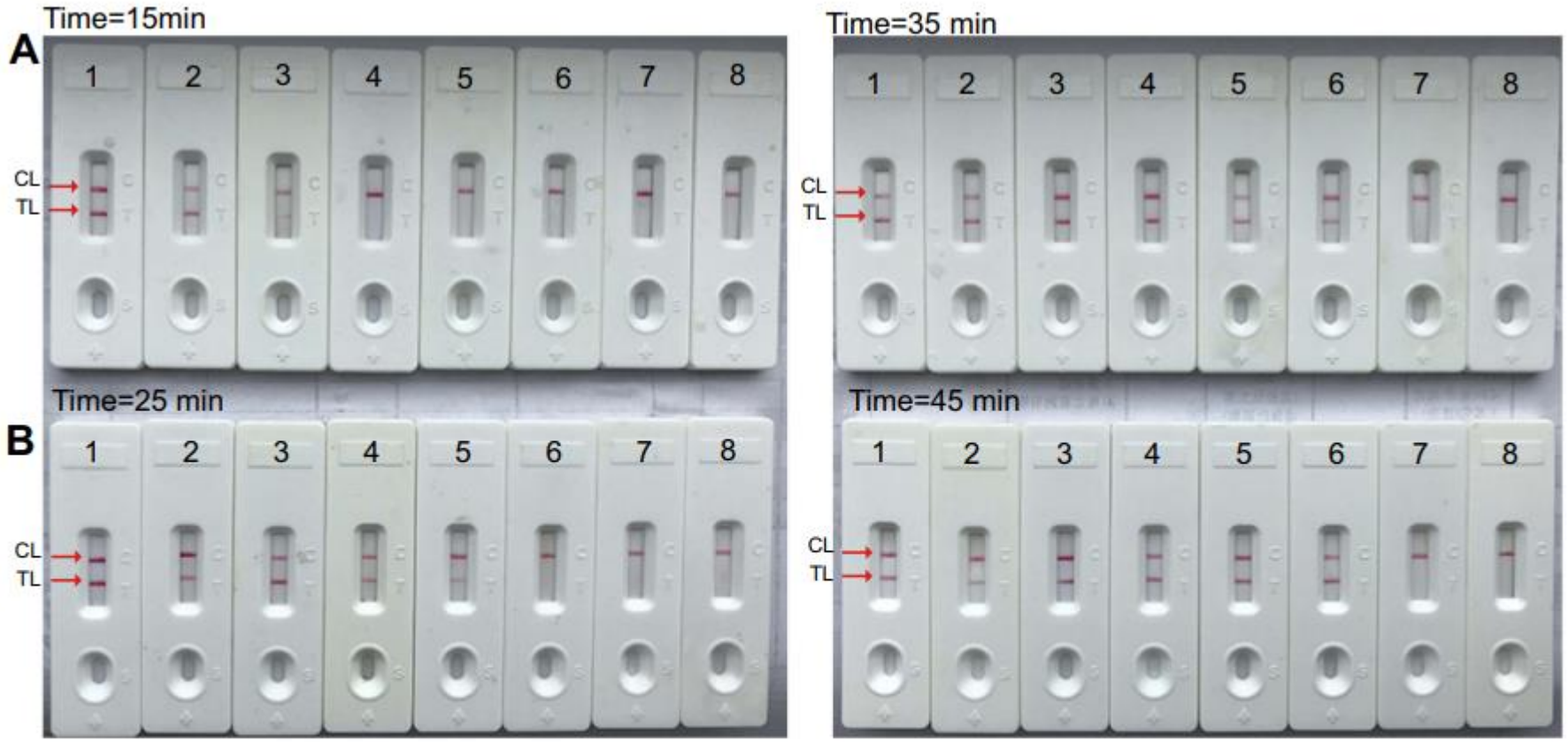


Figure 5 The optimal duration of time required for Neisseria *meningitidis*-MCDA-LFB method. Four distinct reaction times (**A**, 15 mins; **B**, 25 mins; **C**, 35 mins; **D**, 45 mins) were examined and compared at 64°C. Biosensors 1, 2, 3, 4, 5, 6, 7 and 8 represent DNA levels of 1 ng of templates, 100 pg of *N. meningitidis* templates, 10 pg of *N. meningitidis* templates, 1 pg of *N. meningitidis* templates, 100 fg *N. meningitidis* templates, 10 fg *N. meningitidis* template, 1 fg *N. meningitidis* template and blank control (DW), respectively. The best sensitivity was observed when the amplification lasted for 35 mins (C).

Abbreviations: TL, test line; CL, control line; MCDA-LFB, multiple cross displacement amplification with lateral flow biosensor; DW, double-distilled water.

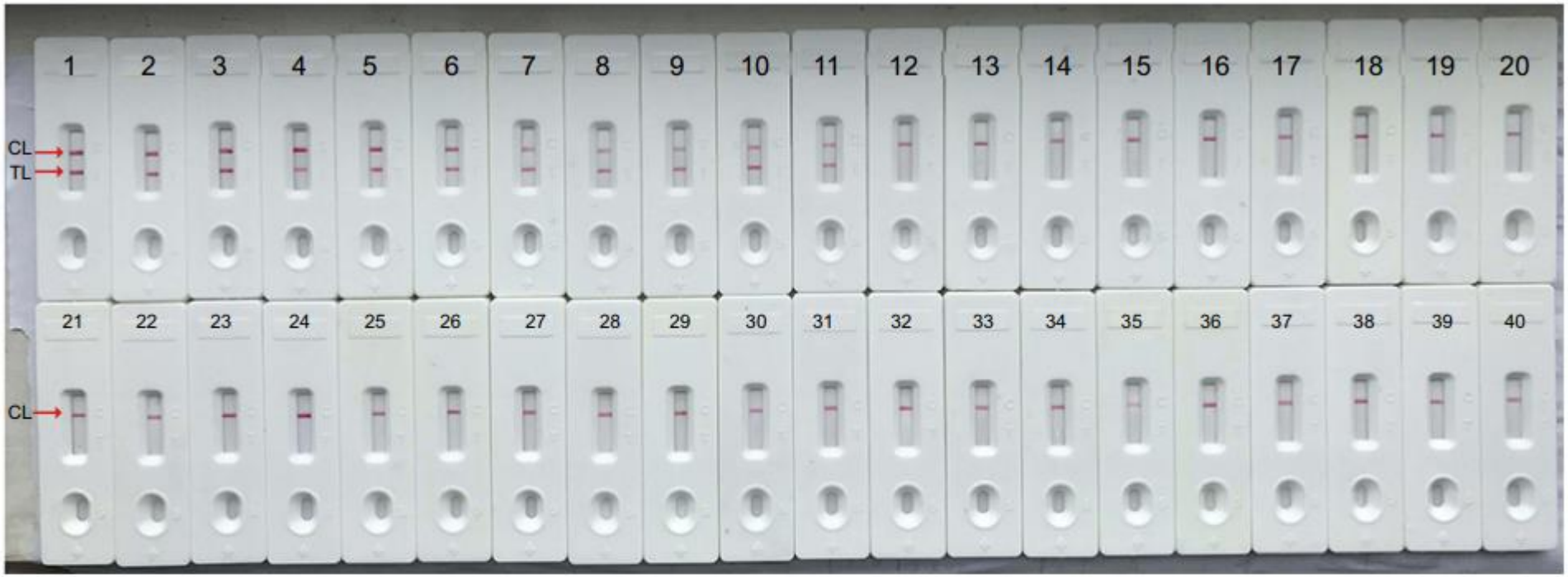


Figure 6 Specificity analysis of the *Neisseriameningitidis*-MCDA-LFB assay for different bacterial strains. MCDA reactions were carried out using different genomic DNA templates and were analyzed visually. Biosensors 1, 2, 3, 4, 5, 6, 7, 8, 9 used *N. meningitidis* serogroup A, *N. meningitidis* serogroup B, *N. meningitidis* serogroup C, *N. meningitidis* serogroup D, *N. meningitidis* serogroup W-135, *N. meningitidis* serogroup 29-E, *N. meningitidis* serogroup X, *N. meningitidis* serogroup Y, *N. meningitidis* serogroup Z; 10–11, Biosensors 10–11 used *N. meningitidis* isolate GZCDC001, *N. meningitidis isolate* GZCDC002, respectively; Biosensors 12–39 used *Bordetella pertussis*, *Bordetella parapertussis*, *Hemophilus parainfluenza*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Mycoplasma pneumoniae*, *Legionellae bacillus*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Salmonella*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, invasive *E.coli*, enterohemorrhagic *E. coli*, enteroaggregative *E. coli*, *Streptococcus suis*, *Vibrio cholerae*, *Vibrio parahemolyticus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Bacillus cereus*, *Bacillus proteus*, *Enterobacter cloacae; Listeria monocytogenes*, *Shigella flexneri*, *Shigella boydii*, respectively; Biosensor 40 used the blank control (DW).

Abbreviations: MCDA-LFB, multiple cross displacement amplification with lateral flow biosensor; DW, double-distilled water; GZCDC, Guizhou Provincial Center for Disease Control and Prevention.

TABLE 3 Comparison of four methods for the detection of 56 whole blood samples.

Methods[a]	Culture	
	Positive	Negative
B. abortus-PCR		
Positive	7	0
Negative	2	47
B. abortus-LAMP-LFIA		
Positive	9	0
Negative	0	47
B. abortus-MCDA-LFB		
Positive	9	0
Negative	0	47

[a]PCR, polymerase chain reaction; LAMP, loop-mediated isothermal amplification; LFIA, lateral flow immunoassay biosensor; MCDA, multiple cross displacement amplification; AuNPs-LFB, gold nanoparticles-based lateral flow biosensor.

Table 3 Comparison of conventional PCR, culture-biotechnical and MCDA-LFB for the detection of *N. meningitidis* in clinical samples

Detection method	Samples (n=56)	
	Positive	Negative
PCR	16	40
Culture	19	37
MCDA-LFB	19	37

Table 3 Comparison of PCR, culture-biotechnical, and MCDA-LFB assays for the detection of *L. pneumophila* in sputum samples

Detection methods	Sputum samples ($n = 88$)	
	Positive	**Negative**
MCDA-LFB	5	83
Culture	5	83
PCR	4	84

Table 3 Comparison of culture-biotechnical, MCDA-LFB, conventional PCR, for the detection of *Listeria monocytogenes* in raw meat samples

Detection methods	Pork samples (n=61)	
	Positive	Negative
Culture	13	48
MCDA-LFB	13	48
PCR	10	51

Abbreviations: LFB, lateral flow biosensor; MCDA, multiple cross displacement amplification; PCR, polymerase chain reaction.

Conclusion

- MCDA-LFB has strength for the **rapid detection** with advantages in **specificity**, **sensitivity**, **time-saving** and **detection cost**

- it possesses limitation when compared with methods which can be used for serogroup identification and antimicrobial resistance detection

The End

thank you for your attention!