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New closed tube loop mediated isothermal amplification assay for prevention of product cross-contamination

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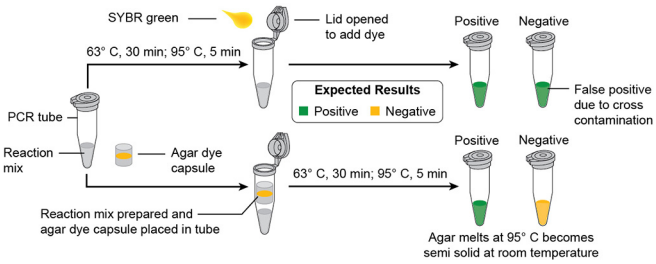
GRAPHICAL ABSTRACT



ABSTRACT

Loop mediated isothermal amplification (LAMP) assay, a promising diagnostic test, has been developed for detection of different pathogens of human as well as animals. Various positive points support its use as a field level test but the major problem is product cross contamination leading to false positive results. Different methods were adopted by various researchers to control this false positive amplification due to cross contamination but all have their own advantages and disadvantages. A new closed tube LAMP assay based on agar dye capsule was developed in the present study and this technique has some advantages over the other closed tube technique.

- Agar at the concentration of 1.5% was used to sandwich SYBR green dye I with the aid of intradermal syringe. This agar dye capsule was placed over the LAMP reaction mixture before it was amplified.
- To eliminate the hazardous nature of Ultra Violet (UV) light during result visualization of LAMP products, the present study demonstrates the use of Light Emitting Diode (LED) lights for result visualization.

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- LAMP was carried out for *Brucella* species detection using this modified techniques yielding good results without any cross contamination and LED showed similar fluorescence compared to UV.

ARTICLE INFO
Method: Closed Tube LAMP technique
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Method details

The present work was designed to develop a closed tube LAMP assay using agar dye capsule using SYBR green dye I, having good sensitivity and also the use of Light Emitting Diode (LED) bulbs to fluoresce dye instead of UV light. LAMP assay for detection of *Brucella* spp. was designed using this modified closed tube method combined with LED technique for result visualization.

*Optimization of LAMP assay for **Brucella** spp.*

Three *Brucella* species (*B. abortus* S99, *B. abortus* S19, *B. melitensis*, *B. suis*) and 11 non-*Brucella* species were used in the present study for optimization and also to assess specificity of LAMP assay (Table 1).

LAMP primers were designed using primer explorer online software version 4 for the detection of *Brucella* spp. targeting *Omp*25 gene. F3 and FIP primers were modified from the earlier report of Lin et al. [1] so as to accommodate loop primers. Loop primers were designed using same software in order to see the effect of loop primers on reaction time. Sequences of the primers used are presented in the Table 2. LAMP reaction conditions were adopted as per Lin et al. [1] and optimization for time, temperature and for different chemicals were carried out. PCR targeting *bcsp*31 gene of *Brucella* was used alongside to compare the results with LAMP [2].

The 25 μl final volume of LAMP mixture contained 5 pM of each outer primer (F3 and B3), 40 pM of each inner primer (FIP and BIP) and 20 pM of each loop primer (LF and LB). Thermopol buffer 2.5 μl [1× buffer comprised of 25 mM Tris–HCl pH 8.8, 12.5 mM KCl, 12 mM $(NH_4)_2SO_4$ and 0.125% Tween 20 (New England Biolabs, USA)], 8 mM $MgSO_4$, 1.2 mM dNTPs, 1 M Betaine were the other components in the reaction along with nuclease free water (NFW) to make the volume to 22 μl. To the mixture 2 μl of

Table 1
Bacterial isolates used.

S. no.	Bacterial isolates	Source
1	*Brucella abortus* S19 (vaccine strain)	Division of Biological Standardization
2	*Brucella abortus* S99 (diagnostic reference strain)	Division of Biological Standardization
3	*Brucella melitensis* 16M	Division of Veterinary Public Health
4	*Brucella melitensis* field isolate	General Bacteriology Lab
5	*Brucella suis*	Division of Veterinary Public Health
6	*Escherichia coli*	Division of Veterinary Public Health
7	*Salmonella* Typhimurium	Division of Veterinary Public Health
8	*Yersinia enterocolitica*	Division of Veterinary Public Health
9	*Pasteurella multocida* B:2	Division of Bacteriology and Mycology
10	*Clostridium chauvoei*	Division of Bacteriology and Mycology
11	*Clostridium perfringens*	Division of Bacteriology and Mycology
12	*Staphylococcus aureus* field isolate	General Bacteriology Lab
13	*Campylobacter jejuni* field isolate 1	Division of Veterinary Public Health
14	*Campylobacter jejuni* field isolate 2	Division of Veterinary Public Health
15	*Listeria monocytogenes*	Division of Veterinary Public Health
16	*Shigella flexneri*	Division of Veterinary Public Health