

**Supplementary Information for**

**A shelf-stable fluorogenic isothermal amplification assay for detection of *Burkholderia pseudomallei***

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## **Calculation of the shelf-life of the shelf-stable fluorogenic LAMP reagent by using Q<sub>10</sub> method**

The LAMP reagents stored at 56 °C and maintain its reactivity for 14 days.

Age of lyophilized fluorogenic LAMP reagent = 14 days at 56 °C

Ambient temperature = 25°C

$$Q_{10} = 1.8$$

Acceleration factor (based on 31°C temperature difference):

$$(1.8)^{3.1} = 6.19$$

Length of time at elevated temperature = 14 days

### **Estimation of shelf life**

Accelerated age = age × acceleration factor

$$14 \text{ days} \times 6.19 = 86.59 \text{ days}$$

Shelf life = accelerated age + actual age

$$86.59 \text{ days} + 14 \text{ days} = 100.6 \text{ days}$$

**Table S1.** Oligonucleotide sequences for LAMP reaction

<b>Primers</b>	<b>Sequences</b>
<b>FIP</b>	5'- TTG CTG TTC TCG GCA TGC CGA ATT CCG GCG CTT CAA TCT GCT -3'
<b>BIP</b>	5'- TCG ATT GCG CGA TCG CCA TTT TTG CGC GTC ATC TGT TGC TAG -3'
<b>F3</b>	5'- TCC CAC ACC GGT TGC T -3'
<b>B3</b>	5'- TCG ATG AGG CGT GAG GT -3'
<b>LB</b>	5'- ACG CAC TGC CTG ACA ATC CG -3'

**Table S2.** Composition of the aqueous buffer for lyophilized fluorogenic LAMP reagents mix

<b>Components</b>	<b>Final composition</b>	
	Final concentration	Final Volume
<b>PCR grade water</b>	-	7.0 $\mu$ l
<b>Betaine (5 M)</b>	0.8 M	4.0 $\mu$ l
<b>Thermopol Buffer solution (10X)</b>	1X	2.5 $\mu$ l
<b>MgSO<sub>4</sub> (100 mM)</b>	6.0 mM	1.5 $\mu$ l
<b>Total Volume</b>		15.0 $\mu$ l

**Table S3.** Analytical specificity test of ambient temperature stable fluorogenic-LAMP assay

Bacteria strains	Fluorogenic LAMP detection	
	Colour change	Interpretation
<b><i>Burkholderia pseudomallei</i></b>		
BUPS00814 (GenBank accession no. KR869096)	+	Positive
BUPS00114 (GenBank accession no. KR869097)	+	Positive
BUPS00214 (GenBank accession no. KR869098)	+	Positive
BUPS00314 (GenBank accession no. KR869099)	+	Positive
BUPS00414 (GenBank accession no. KR869100)	+	Positive
BUPS00514 (GenBank accession no. KR869101)	+	Positive
BUPS00614 (GenBank accession no. KR869102)	+	Positive
BUPS00714 (GenBank accession no. KR869103)	+	Positive
BUPS2314	+	Positive
BUPS2414	+	Positive
BUPS2614	+	Positive
BUPS2714	+	Positive
BUPS2814	+	Positive
BUPS2914	+	Positive
BUPS3014	+	Positive
BUPS3114	+	Positive
BUPS3214	+	Positive
BUPS3314	+	Positive
BUPS3414	+	Positive
BUPS3514	+	Positive
BUPS3614	+	Positive
BUPS3714	+	Positive
BUPS3814	+	Positive
BUPS3914	+	Positive
BUPS5513	+	Positive
<b>Other <i>Burkholderia</i> species</b>		
<i>Burkholderia thailandensis</i> BUTH00114 (GenBank accession no. KR869105)	-	Negative
<i>Burkholderia cepacia</i> BUCE00114 (GenBank accession no. KR869104)	-	Negative
<b><i>Gram positive bacteria</i></b>		
Group A <i>Streptococcus</i>	-	Negative
Group B <i>Streptococcus</i>	-	Negative
Group F <i>Streptococcus</i>	-	Negative
Group G <i>Streptococcus</i>	-	Negative
<i>Listeria monocytogenes</i>	-	Negative
<i>Bacillus subtilis</i>	-	Negative
<i>Enterococcus gallinarum</i>	-	Negative
<i>Enterococcus faecium</i>	-	Negative
<i>Enterococcus faecalis</i>	-	Negative
<i>Staphylococcus aureus</i>	-	Negative

**Gram negative bacteria**

<i>Enterohaemorrhagic Escherichia coli (EHEC)</i>	-	Negative
<i>Enteroinvasive Escherichia coli (EIEC)</i>	-	Negative
<i>Enterotoxigenic Escherichia coli (ETEC)</i>	-	Negative
<i>Enteropathogenic Escherichia coli (EPEC)</i>	-	Negative
<i>Shigella flexneri</i>	-	Negative
<i>Klebsiella pneumonia</i>	-	Negative
<i>Yersinia enterocolitica</i>	-	Negative
<i>Acinetobacter baumannii</i>	-	Negative
<b><i>Aeromonas hydrophila</i></b>	-	Negative

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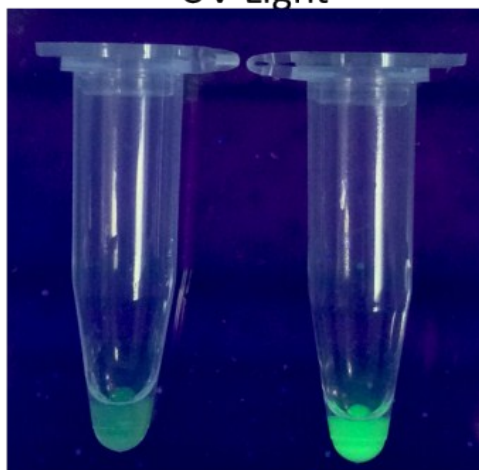
Normal Lighting



-

+

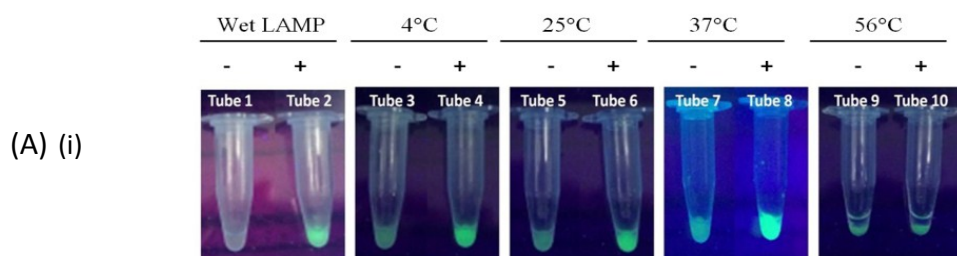
UV Light

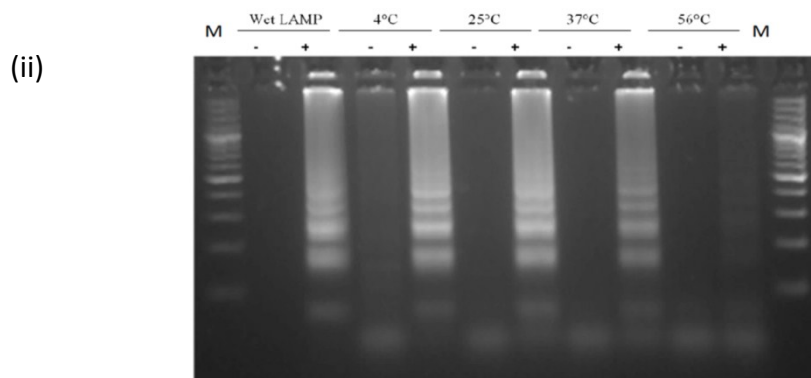


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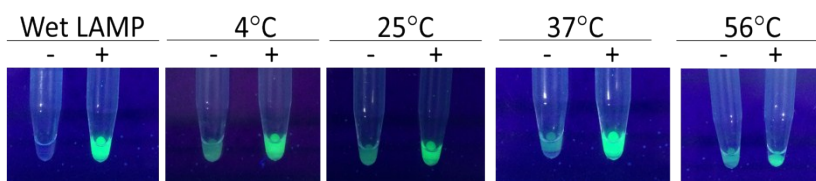
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**Fig. S1.** The fluorescence of positive (+) and negative (-) samples under different lighting.

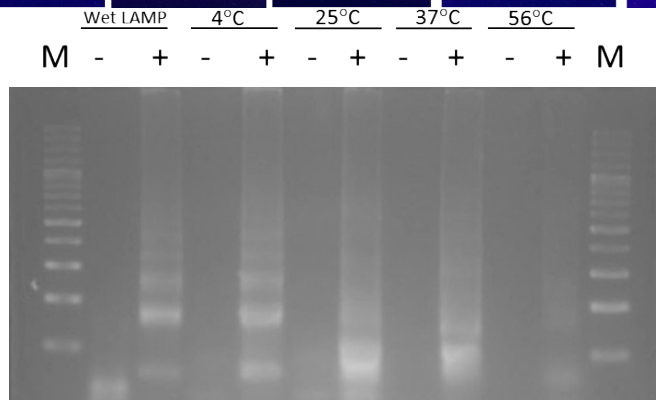




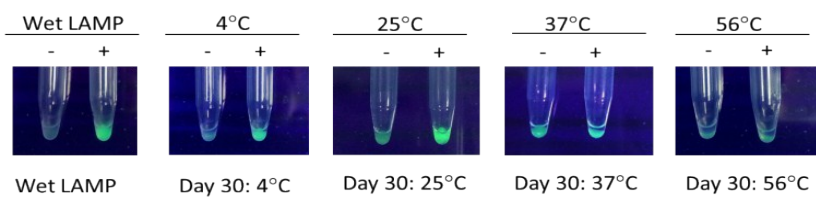
(B) (i)



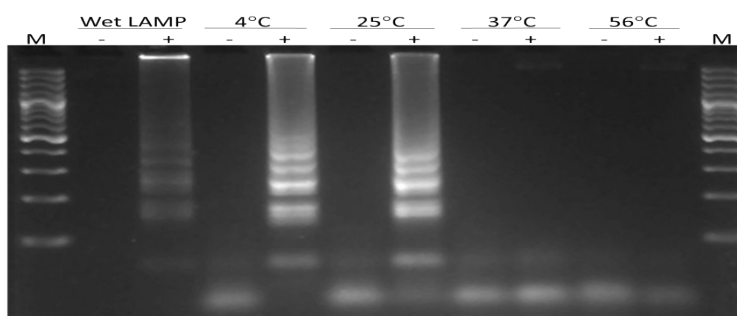
(ii)



(C) (i)

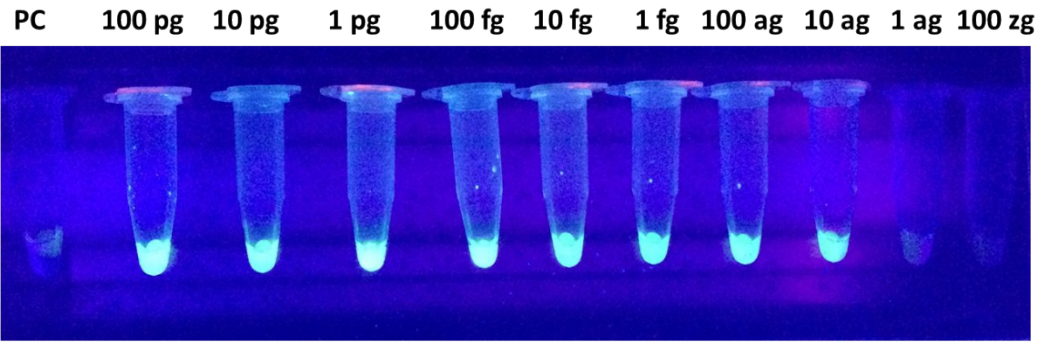


(ii)

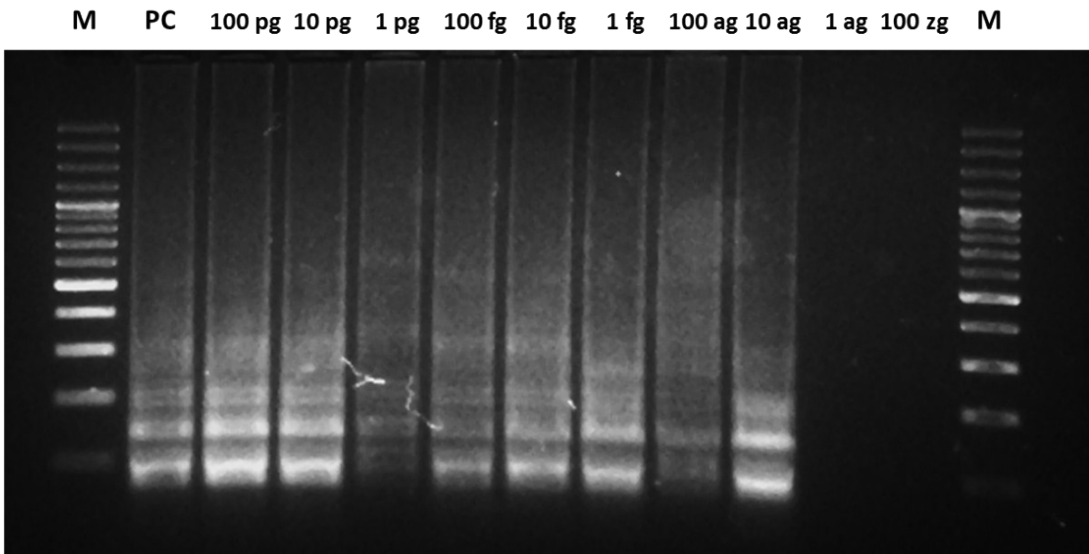


**Fig. S2.** Shelf-life of formulated LAMP reagents and its buffer stored at various temperature for 7 (A), 14 (B) and 30 (C) days were determined by visualized on UV illuminator (i) and agarose gel stained with RedSafe (ii). Lane M, 100bp PLUS DNA Ladder as DNA size marker, LAMP reagent tested with target DNA (+); LAMP reagent tested without target DNA (-);

(A)

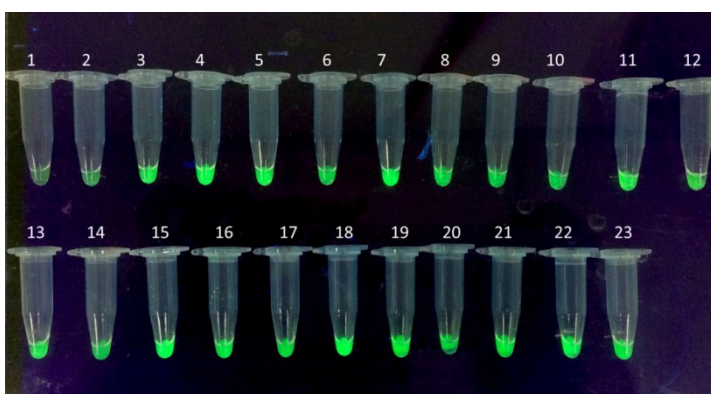


(B)

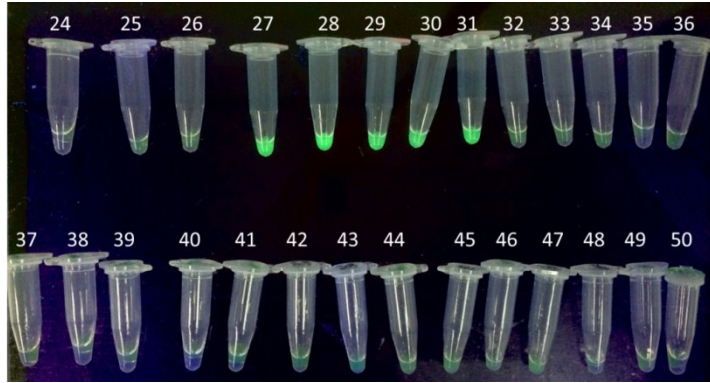


**Fig. S3.** Analytical sensitivity assessment of ambient temperature stable fluorogenic-LAMP assay performed with 10-fold serially diluted genomic DNA and visualized on UV illuminator (A) or by visualization on agarose gel stained with RedSafe (B). Lane M, 100 bp PLUS DNA Ladder as DNA size marker; Lane PC, Positive control wet LAMP without fluorescence detection reagent.

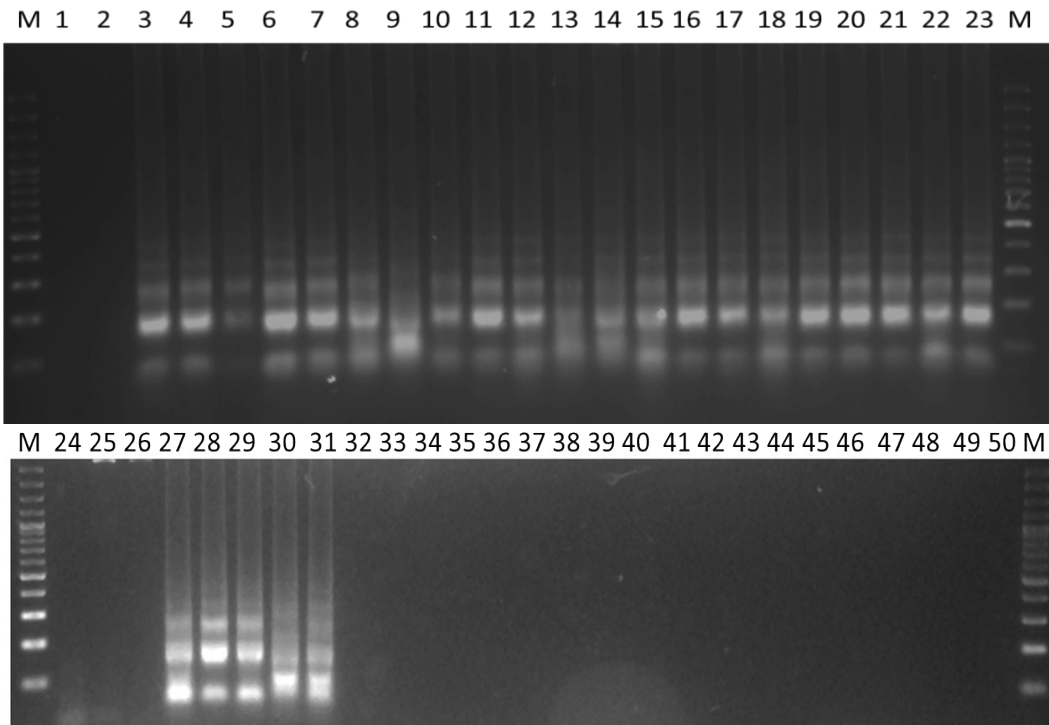
(A)







(B)



**Fig. S4.** Analytical specificity assessment of ambient temperature stable fluorogenic-LAMP assay performed visualization on UV illuminator (A) and agarose gel stained with RedSafe (B). Lane M, 100bp PLUS DNA ladder; Lane 1 & 24, Non template control; Lane 2 & 26, *B. thailandensis* BUTH00114 (GenBank accession no. KR869105); Lane 3 & 27, positive control *B. pseudomallei* strain BUPS00814 (GenBank accession no. KR869096); Lane 4, *B. pseudomallei* strain (GenBank accession no. KR869101); Lane 5, *B. pseudomallei* strain (GenBank accession no. KR869102); Lane 6, *B. pseudomallei* strain (GenBank accession no. KR869103); Lane 7, *B. pseudomallei* strain BUPS2314; Lane 8, *B. pseudomallei* strain BUPS2414; Lane 9, *B. pseudomallei* strain BUPS2614; Lane 10, *B. pseudomallei* strain BUPS2714; Lane 11, *B. pseudomallei* strain BUPS2814; Lane 12, *B. pseudomallei* strain BUPS2914; Lane 13, *B. pseudomallei* strain BUPS3014; Lane 14, *B. pseudomallei* strain BUPS3114; Lane 15, *B. pseudomallei* strain BUPS3214; Lane 16, *B. pseudomallei* strain BUPS3314; Lane 17, *B. pseudomallei* strain BUPS3414; Lane 18, *B. pseudomallei* strain BUPS3514; Lane 19, *B. pseudomallei* strain BUPS3614; Lane 20, *B. pseudomallei* strain BUPS3714; Lane 21, *B. pseudomallei* strain BUPS3814; Lane 22, *B. pseudomallei* strain BUPS3914; Lane 23, *B. pseudomallei* strain

BUPS5513; Lane 24, Non template control; Lane 25, *B. cepacia* BUCE00114 (GenBank accession no. KR869104); Lane 28, *B. pseudomallei* strain (GenBank accession no. KR869097); Lane 29, *B. pseudomallei* strain (GenBank accession no. KR869098); Lane 30, *B. pseudomallei* strain (GenBank accession no. KR869099); Lane 31, *B. pseudomallei* strain (GenBank accession no. KR869100); Lane 32, Group A *Streptococcus*; Lane 33, Group B *Streptococcus*; Lane 34, Group F *Streptococcus*; Lane 35, Group G *Streptococcus*; Lane 36, *L. monocytogenes*; Lane 37, *B. subtilis*; Lane 38, *E. gallinarum*; Lane 39, *E. faecium*; Lane 40, *E. faecalis*; Lane 41, *S. aureus*; Lane 42, EHEC; Lane 43, EIEC; Lane 44, ETEC; Lane 45, EPEC; Lane 46, *S. flexneri*; Lane 47, *K. pneumonia*; Lane 48, *Y. enterocolitica*; Lane 49, *A. baumannii*; Lane 50, *A. hydrophila*.