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Point-of-need nucleic acid test for
Staphylococcus aureus enterotoxin genes in
raw ruminants' milk

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Background and Objectives

Systematic review (Shalaby et. al., 2023)

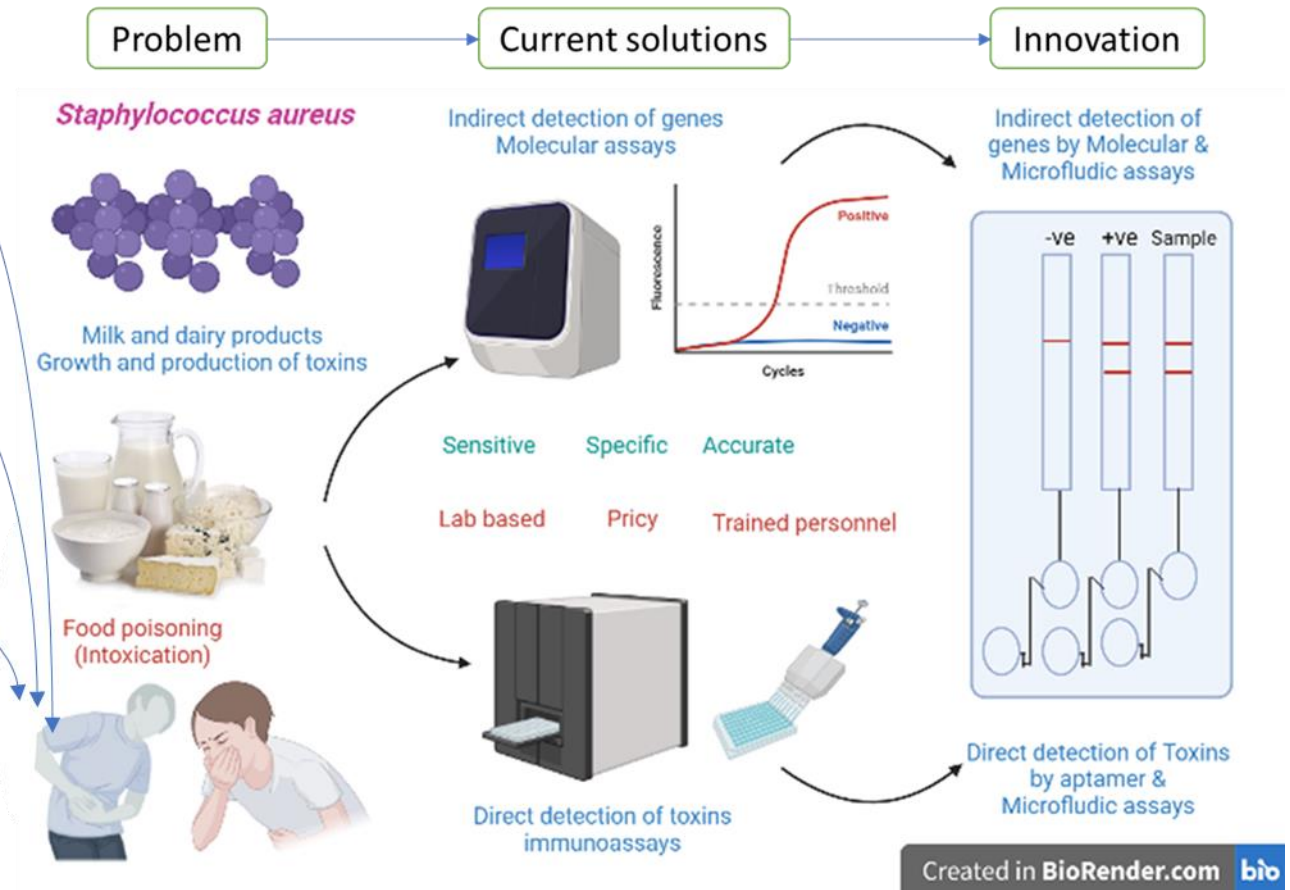
Reports available only from some subregions¹

European Union²

25 outbreaks (2007 - 2011)
117 outbreaks (2019 - 2020)

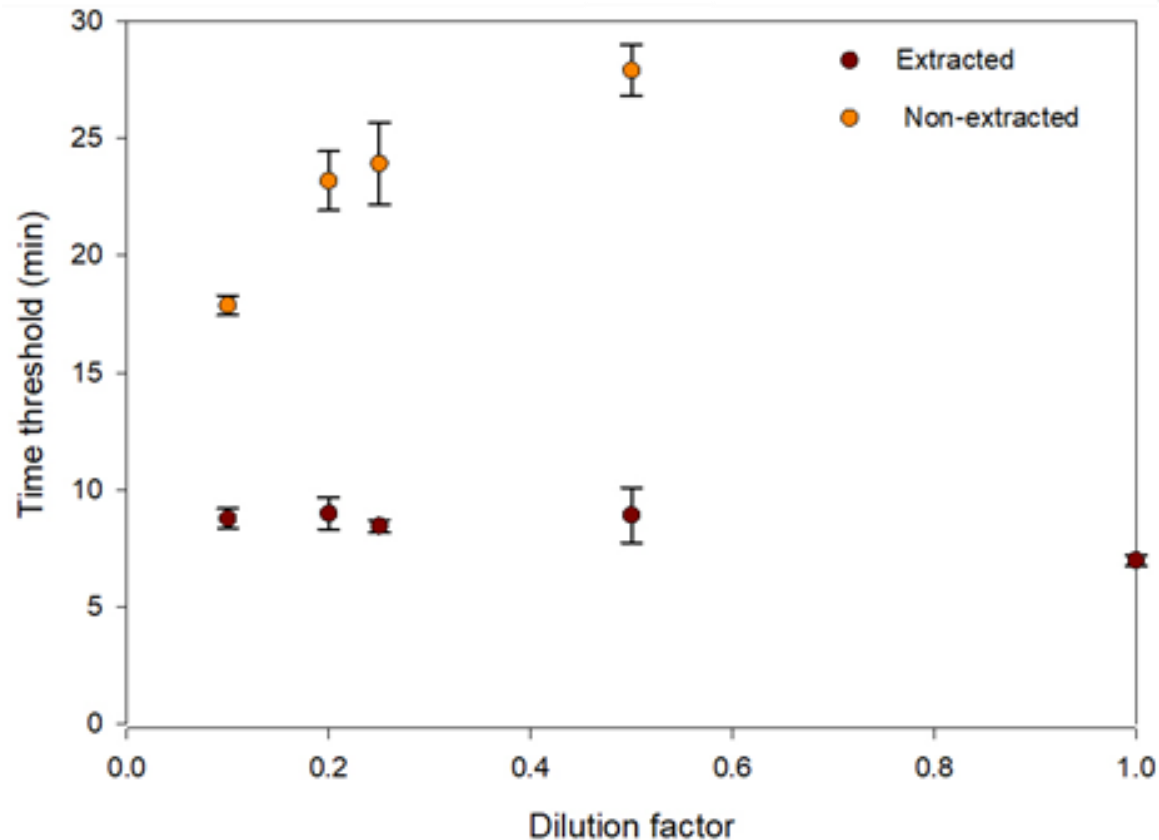
United States³

42 outbreaks (1993 - 1997)
75 outbreaks (2009 - 2015)



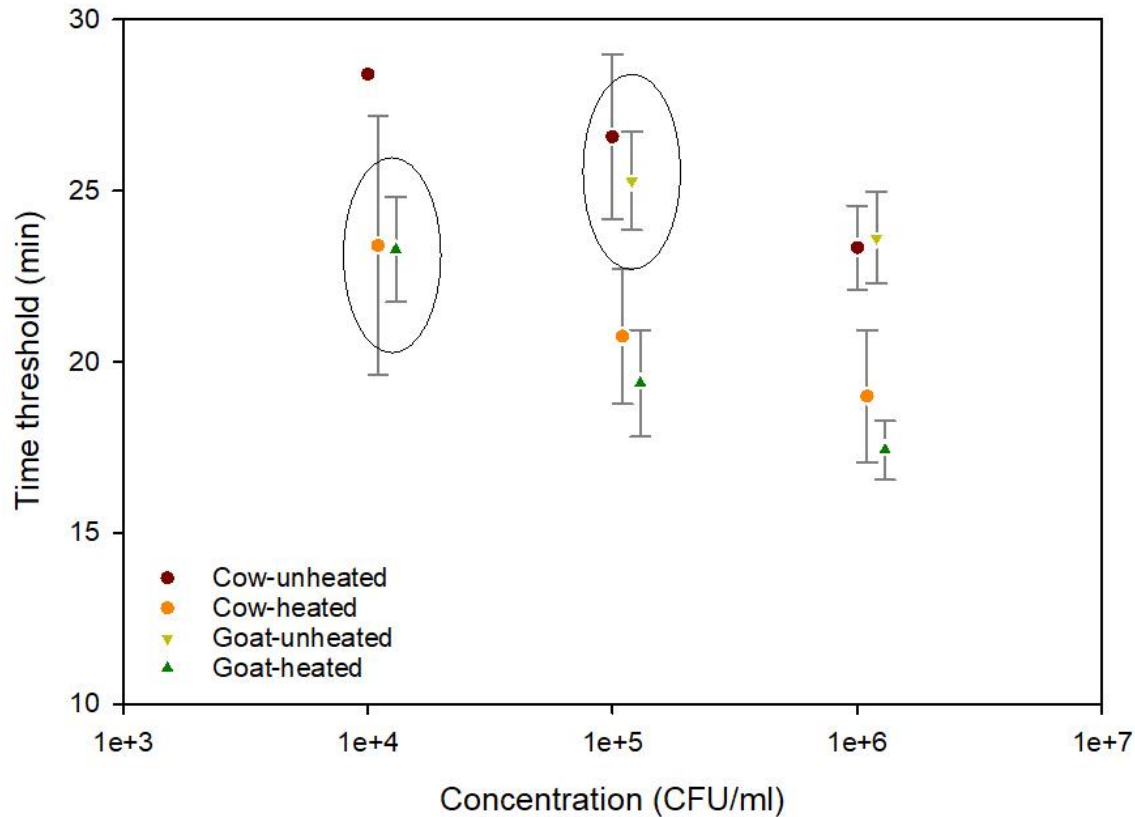
¹ WHO 2015, ² EFSA 2021, ³ Oslen et. al., 2000 and Dewey-Mattia et. al., 2018

Optimization of sample preparation for LAMP amplification (*Staphylococcus aureus* enterotoxin B gene)



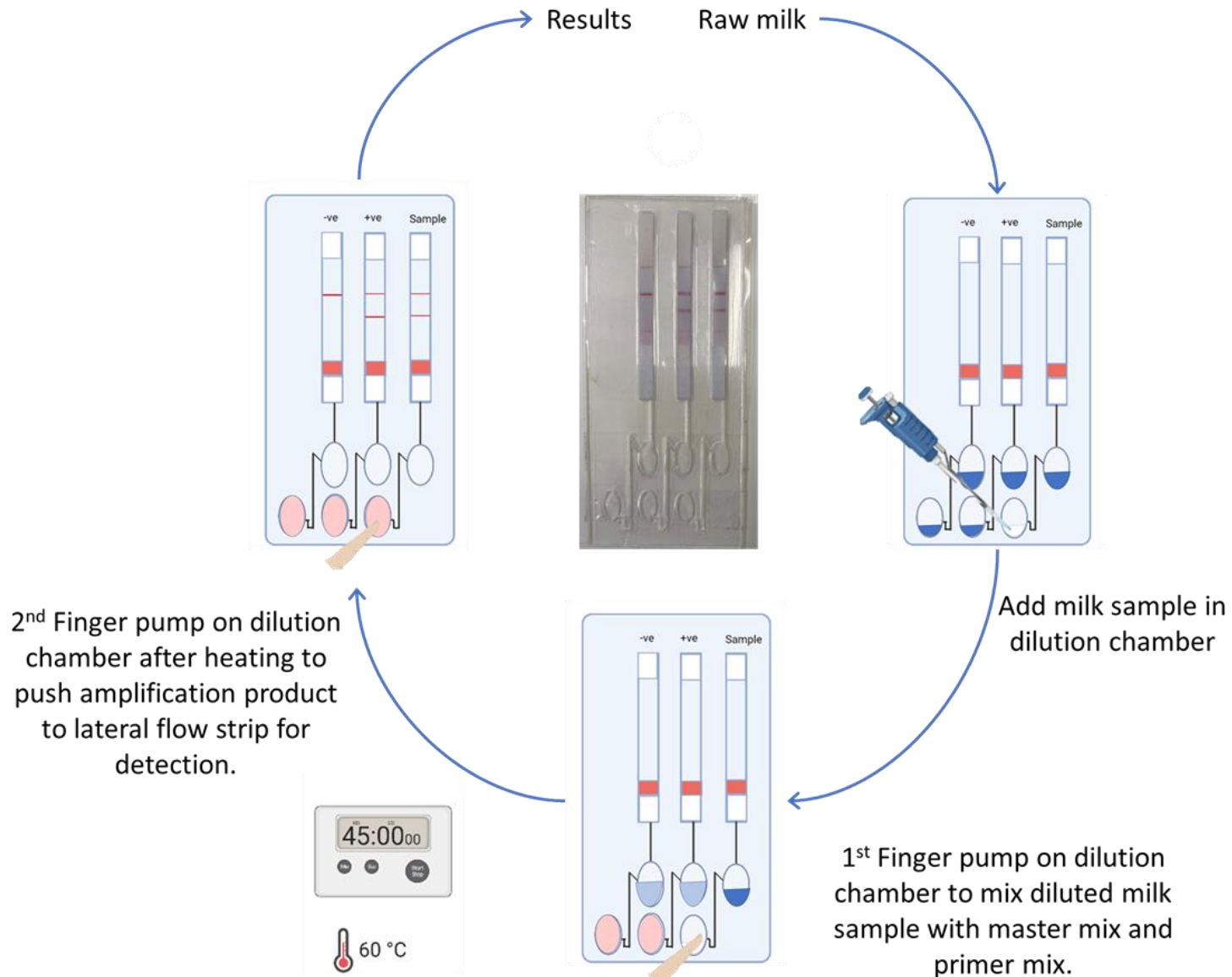
- Dilution as a preparation step
- Spiked diluted cow milk samples
- 1:2, 1:4, 1:5 and 1:10 dilution factor
- **Extracted** and **non-extracted** samples
- Amplification time

Limit of detection (LOD) of multiplex LAMP assay (*Staphylococcus aureus* enterotoxin A and B genes)



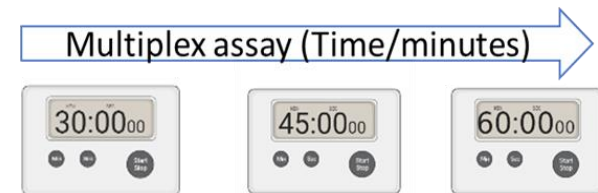
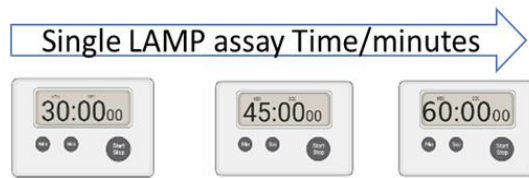
- Spiked 1:10 diluted cow (●) and goat (▼) milk samples
- With and without heating at 95 °C for 10 min
- LOD: 10⁴ and 10⁵ CFU/ml

Integration of developed LAMP assay in a point-of-need device

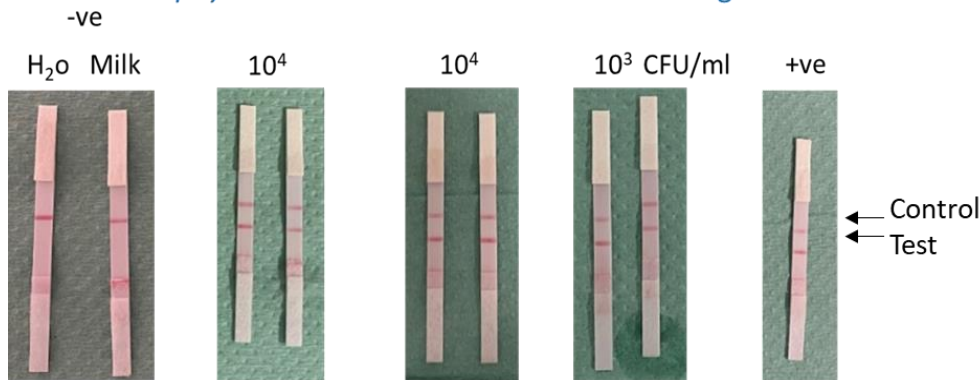


Evaluation of increasing amplification time of developed LAMP and multiplex LAMP assay in a point-of-need device

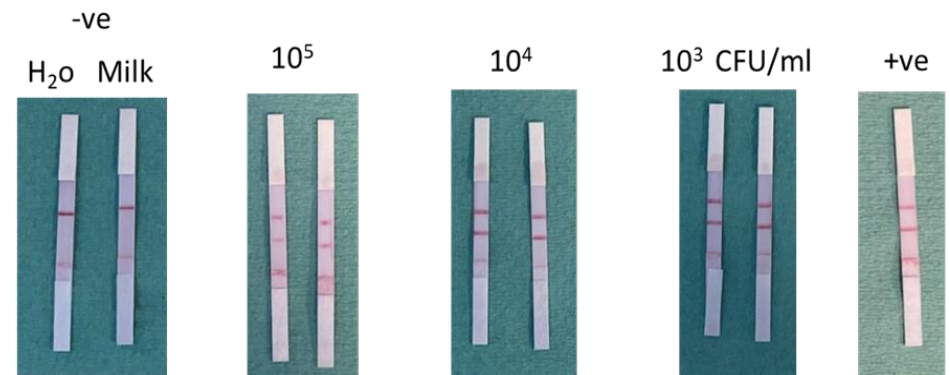
- Single LAMP and multiplex LAMP assays
- Different amplification times 30, 45 and 60 minutes
- Different enterotoxigenic *S. aureus* strains
- Diluted 1:10 spiked goat milk samples



Staphylococcus aureus enterotoxin A and B genes

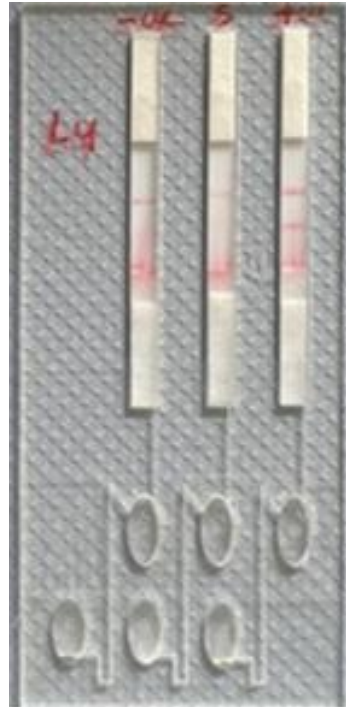


Staphylococcus aureus DSMZ 19041/ 19044 reference strains



Field testing of developed point-of-need device

- Multiplexed LAMP assay with LOD below recommended value for food safety applications
- Low-cost device enables detection directly from milk for onsite use
- Field testing in Turkey – July 2023
- No false positive or false negatives with controls
- None of the samples were positive
- Device handling and preparation was easy
- No leakage or contamination



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- OHRBID Lab members.
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