Development of internal control for coupled detection of *Staphylococcus aureus* in milk and meatproducts by single-step, dual loop-mediated isothermal amplification combined with hydroxynaphthol blue (HNB) and nanogold particle probe (AuNP)

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Abstract: Food poisoning by*Staphylococcus aureus*enterotoxin A (SEA) is one of the leading major illnesses worldwide. In 2015, World Health Organization (WHO) reported approximately600 million people were sick by SEA globally, and more than 100,000 patients were in Thailand yearly. This illness leads to 420,000 deaths per year, which one-third are children under the age of 5. The conventional method of detecting food and drinks contaminated with SEA is by *S. aureus* cultivation which takes more than 18 h, not specific to the *S. aureus* strains that have SEA gene, and hence low specificity comparing to the DNA detection polymerase chain reaction (PCR) serves as a standard DNA detection method, yet the cost is high due to the requirement of thermalcycler (80,000-100,000 bahts) and the DNA extraction step from milk and meat samples. These reasons lead to necessity for specific and sensitive, simple and easy to use, rapid,inexpensive, and appropriate for local detection and point-of-care diagnostic. Researchers therefore, chose loop-mediated isothermal amplification (LAMP),which detects specific DNA by primers and special enzyme to allow reaction in 20 – 40 minutesat 60-65^{o}C without a thermal cycler required. This research continues from our previous research that developed *S. aureus* SEA gene LAMP detection for cow milk and ground pork products, by adding internal control for milk and pork (dual LAMP), respectively. The essential of internal control is to indicatereliability the method for non-contaminated food sample, when the reaction for *S. aureus* is absent but internal control is present. Moreover, this research reducesDNA extraction step and stop-reaction step into a single-step LAMP (ssLAMP) (63 ^{o}C 25 minutes incubation). We also combinedual, ssLAMP with hydroxynaphthol blue (HNB) and nanogold particle probe (AuNP) so the results can be read by naked eyes. The specificity ofthe developed internal controlLAMP isvalidated using positive (eg. Pasteurized, UHT, and sterile cow milk) and negative (soy, goat, and human breast milk)controls. The detection limit is up to 10-100 copies, or equivalent to 0.01 fgof the DNA target. In conclusion, the developed internal controlLAMP is promising for the field uses and in coupled with *S. aureus* SEA gene LAMP (dual, ssLAMP) for the test kit development because of its high specificity and sensitivity (detection limit10-100 copies) simple, rapid, andlow cost (less than 100 bahts per test) compared with the standard conventional and PCR method (400-1,000 bahts per test).

Keywords: *Staphylococcus aureus*, LAMP, internal control

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