Evaluation of methods of DNA extraction from *Staphylococcus aureus* in milk for use in real-time PCR

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**ABSTRACT.** The aim of this study was to evaluate the repeatability and performance of 4 methods of extracting DNA from *Staphylococcus aureus* (SAU) and the gene encoding bovine mitochondrial cytochrome B (BMCB) in milk samples from cows with subclinical mastitis for use in amplification by real-time polymerase chain reaction. Two milk samples were obtained from cows naturally infected with *S. aureus* and subjected to the following extraction methods: Qiagen DNA extraction kit; Axyprep DNA extraction kit; in silica column boil and in silica column method. After extraction in duplicate, eluates were subjected to purification and precipitation to determine purity (A\textsubscript{260}/A\textsubscript{280} ratio) and concentration (μg/μL) by spectrophotometry and amplification by real-time polymerase chain reaction of target genes (SAU and BMCB). There was no effect of
the DNA extraction method on DNA concentration and threshold cycle for *BMCB* and *SAU*. The purity ratio ($A_{260}/A_{280}$) was higher when using Qiagen DNA extraction (1.76 ± 0.136) compared to the other methods tested. Our results indicate that the DNA extraction kit from Qiagen produces samples of the highest purity ratio compared to other methods.

**Key words:** DNA; Extraction; Mastitis; *Staphylococcus aureus*; Real-Time Polymerase Chain Reaction