



An evaluation of alternative buffers for the transport and detection of *Moraxella bovis* and *M. bovoculi* on cotton wool swabs

Louis H. Maartens^{a,b,c,*}, Bruce Gummow^{a,b}, John D. Grewar^a, Peter N. Thompson^a,
Jacqueline A. Picard^b

^a Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa.

^b Discipline of Veterinary Science, James Cook University, Townsville, Australia.

^c Research and Development Section, Deltamune Pty Ltd, Pretoria, South Africa.

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ABSTRACT

Bovine keratoconjunctivitis is a globally important inflammatory condition affecting the eyes of cattle. The reliable detection of *Moraxella bovis* and *M. bovoculi* on conjunctival specimens is crucial for observational studies aiming to unravel the complex epidemiology of this disease. The stability of *Moraxella* DNA was evaluated in three experiments using cotton wool swabs spiked with varying concentrations of sample suspensions and submitted either with or without DNA stabilizers. Swabs were subjected to different storage temperatures (4 °C or 25 °C), and DNA was extracted at 0, 24, 48, and 72 h using an automated system, followed by qPCR analysis. DNA from both species remained detectable on damp cotton-wool swabs under all conditions, indicating resilience to transport delays and cold-chain interruptions. Although MagNA Pure Bacteria Lysis Buffer and MagNA Pure DNA Tissue Lysis Buffer provided acceptable stabilization, specimen submission without stabilizers was analytically more sensitive, detecting *Moraxella* DNA at higher dilutions and yielding higher inferred DNA concentrations (lower Cq values). These results indicate that *Moraxella* specimens can be packaged for transport without stabilizing buffers.

Bovine keratoconjunctivitis (BK) is the most common inflammatory eye condition of cattle (Dennis and Kneipp, 2021), with a multifactorial pathogenesis involving the ocular surface, conjunctival microbiota—particularly *Moraxella bovis* and *Moraxella bovoculi*—and environmental stressors like ultraviolet radiation (Brown et al., 1998; Loy et al., 2021a; Maartens et al., 2025).

Pathogenic strains of *M. bovis* expressing type IV pili and an RTX-like cytotoxin can disrupt the epithelial barrier, independently of environmental stressors (Loy et al., 2021b). Although *M. bovoculi* is less pathogenic than *M. bovis*, certain strains are associated with BK under field conditions (O'Connor et al., 2021).

While laboratory confirmation is not typically required for treatment, accurate detection of *Moraxella* spp. is essential for epidemiological studies (Schnee et al., 2015; Kneipp et al., 2021). Both species are readily culturable (Loy and Maier, 2022), but isolation sensitivity may be compromised by overgrowing contaminants and loss of viability during transport. In such cases, qPCR offers a more reliable alternative (Shen et al., 2011; Zheng et al., 2019).

Maintaining the cold chain during fieldwork in remote areas can be challenging (Lowe et al., 2020). Although dehydrated DNA is generally stable (Bonnet et al., 2009), the stability of moist specimens is less predictable. Currently, there is no clear guidance on best practices for handling, packaging, and transporting field swabs for *Moraxella* detection, including acceptable storage temperatures, maximum delays to analysis, and whether DNA stabilizers are required to preserve sample integrity. This study therefore evaluated the stability of *M. bovis* and *M. bovoculi* DNA on damp cotton wool swabs stored in different transport media under suboptimal conditions.

The preservation of *Moraxella bovis* and *M. bovoculi* DNA on moist cotton wool swabs was assessed in three experiments, using commercial nucleic acid stabilization buffers, compared to an unbuffered control. The three buffers, selected for compatibility with the testing facility's equipment, included Buffer-1 (MagNA Pure Bacteria Lysis Buffer, Roche South Africa, Ref. 04659180001), Buffer-2 (MagNA Pure LC RNA Isolation Tissue buffer, Ref. 03604721001), and Buffer-3 (MagNA Pure DNA Tissue Lysis Buffer, Ref. 04805160001).

* Corresponding author at: Discipline of Veterinary Science, James Cook University, Townsville, Australia.

E-mail addresses: louis.maartens@my.jcu.au.edu, louism@deltamune.co.za (L.H. Maartens).

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Four hemolytic field isolates from clinically affected cattle—two *M. bovis* (#0003, #0322) and two *M. bovoculi* (#2149, #2184)—were cultured aerobically on 5% sheep blood tryptose agar at 37 °C for 24–48 h. Single colonies were suspended in 1 ml PBS (pH 7.4), and cotton wool swabs were immersed in these suspensions before being transferred to Eppendorf tubes containing 600 µl of the respective buffers.

In Experiment 1, four sets of swabs were prepared from each of the four isolates, implying a total of 16 samples. Three sets were treated with the individual stabilizing buffers and the fourth served as an unbuffered control. All samples were tested using undiluted bacterial suspensions. Experiment 2 omitted the least effective buffer and evaluated DNA preservation for one *M. bovis* and one *M. bovoculi* isolate, across a tenfold dilution series (undiluted to 10⁻⁴). Each isolate was tested under the two remaining buffer conditions and an unbuffered control, at five dilution steps, giving a total of 30 samples. In both experiments, sterile water swabs were included as negative controls to rule out cross contamination. The specimens were packed on ice and shipped overnight, from a satellite facility to the testing laboratory, in IATA-compliant polystyrene containers (“Aulaxsa 03B”, Aulax Distributors, www.aulaxsa.co.za).

In Experiment 3, the two *M. bovis* and two *M. bovoculi* isolates were pairwise combined into four isolate mixtures. Two of the isolate mixtures contained both *Moraxella* species and two contained a single species. Four sets of swabs were prepared from the isolate mixtures (16 swabs per isolate mixture, *n* = 64) and equally allocated among four condition–temperature combinations: Unbuffered at 4 °C, Unbuffered at 25 °C, Buffer-1 at 4 °C, and Buffer-1 at 25 °C. Representative swabs from every condition–temperature combination were analyzed at four consecutive timepoints: 0, 24, 48 and 72 h (Table 1).

The specimens were briefly vortexed in 600 µl of the relevant buffer or in PBS (pH 7.4) for unbuffered swabs. DNA was extracted using the MagNA Pure 24 system (Roche, <https://lifescience.roche.com>; cat. no. 07290519001) with the Total NA Isolation Kit (cat. no. 7658036001) in 200 µl reaction volumes. Eluted DNA (100 µl) was stored at 4 °C and analyzed within 24 h.

Agent-specific DNA was detected via qPCR on the Roche LightCycler® Nano thermocycler. Primers and probes were adapted from Zheng et al. (2019), synthesized by TIB Molbiol (www.tib-molbiol.de), and applied with amplification conditions as per Maartens et al. (2025).

Positive controls were synthesized by GenScript (www.genscript.com), by cloning DNA fragments (80–130 bp) encompassing the qPCR target regions of *M. bovis* and *M. bovoculi* into plasmid pUC57Simple. The analytical sensitivity of the assay was ~30 plasmid copies for each target.

Quantification cycle (Cq) values from the LightCycler® Nano were converted to log-transformed bacterial concentrations (CFU/ml) using standard curves constructed in Microsoft® Excel® (Version 2402) from serial tenfold dilutions of quantified suspensions. Cq values were regressed against CFU/ml to obtain slope and intercept parameters, and unknown concentrations were inferred using the linear equation. Standard curves were validated in triplicate across the dynamic range and assessed for linearity and PCR efficiency. For *M. bovis* (slope: -3.08, intercept: 38.95) and *M. bovoculi* (slope: -3.48, intercept: 39.71), the reaction efficiencies were 111% and 94%, respectively, with R² > 0.99.

Statistical analyses were performed in Stata® BE 18.0 (StataCorp,

College Station, TX). For Experiment 2, a linear regression model was fitted with stabilization method, sample dilution, and *Moraxella* species as predictors. In Experiment 3, a panel data structure was defined using the *xtset* function, and a generalized estimating equations (GEE) model was applied with a Gaussian family, identity link function, autoregressive correlation structure, and robust standard errors. Combined and stratified analyses were conducted to evaluate trends in equivalent bacterial concentration during storage.

In Experiment 1, species-specific detection of *M. bovis* and *M. bovoculi* was achieved using the unbuffered method, Buffer-1 and Buffer-3, while Buffer-2 supported detection of *M. bovoculi* only. In Experiment 2, which included Buffer-1 and Buffer-3, both targets were consistently detected across all dilutions using the unbuffered method (Table S1, Supplementary Material). Buffer-3 yielded significantly lower equivalent bacterial concentrations (*P* = 0.01), with *M. bovoculi* detectable only at the 10⁻³ dilution. Similarly, Buffer-1 showed reduced concentrations (*P* < 0.01) for both targets, while detection was limited to the 10⁻³ dilution for both organisms (Fig. 1).

In Experiment 3, both the unbuffered method and Buffer-1 enabled species-specific detection of *M. bovis* and *M. bovoculi* on mixed-isolate swabs (Table S2, Supplementary Material). The unbuffered method yielded significantly higher bacterial concentrations than Buffer-1 (β = 0.3, 95% CI: 0–0.6, *P* = 0.04) (Table 2). Storage at 25 °C resulted in higher concentrations than at 4 °C (β = 0.7, 95% CI: 0.4–0.9, *P* < 0.01), while *M. bovoculi* targets were associated with lower concentrations than *M. bovis* (β = -1.7, 95% CI: -2.0 to -1.4, *P* < 0.01). Storage time significantly affected qPCR results (*P* < 0.05). Subset analysis showed a decline in concentration over time for Buffer-1 (48 h: β = -0.4, *P* = 0.01; 72 h: β = -0.4, *P* = 0.02), but not for the unbuffered method (*P* > 0.2; Tables S3 & S4, Supplementary material).

Epidemiological studies in remote areas often face logistical challenges, including long-distance sample transport, processing delays, and cold chain disruptions—making reliable submission methods essential. In this three-stage investigation, *M. bovis* and *M. bovoculi* DNA remained stable on cotton wool swabs even without stabilizing buffers. Swabs spiked with bacterial isolates and sealed in plastic sleeves—without prior drying—yielded consistent qPCR results despite delays of up to three days and exposure to conditions simulating cold-chain failure. This stability likely reflects the physical properties of cotton wool, which provides a cellulose matrix that absorbs bacterial cells and nucleic acids while permitting excess liquid and soluble compounds to drain away (Adamowicz et al., 2014; Jansson et al., 2020). Although this passive separation supports DNA preservation until analysis, it may also hinder recovery from the cellulose matrix and thereby reduce the amount of DNA available for extraction.

While cotton wool swabs can preserve DNA effectively, recovery efficiency is also influenced by the ease with which cells release their nucleic acids during extraction. Gram-negative bacteria such as *Moraxella* spp. possess relatively fragile cell walls that are readily disrupted by bead beating in the MagNA Pure system, enabling high recovery rates of absorbed DNA even without lysis buffers (Santaus et al., 2019). In contrast, Gram-positive organisms, with their thick peptidoglycan layers, typically require more aggressive lysis strategies, and recovery from cotton wool may be less efficient under the same conditions

Table 1

Experimental layout for qPCR assessment on specimens varying by *Moraxella* species composition, storage temperature and storage duration.

Sample solution	Agent composition on specimens				Storage temperature		Storage duration			
	<i>M. bovis</i> #0003	<i>M. bovis</i> #0322	<i>M. bovoculi</i> #2149	<i>M. bovoculi</i> #2184	4 °C	25 °C	0 h	24 h	48 h	72 h
	S3.01	✓	✓	–	–	✓	✓	✓	✓	✓
S3.02	–	✓	–	✓	✓	✓	✓	✓	✓	✓
S3.03	✓	–	✓	–	✓	✓	✓	✓	✓	✓
S3.04	–	–	✓	✓	✓	✓	✓	✓	✓	✓

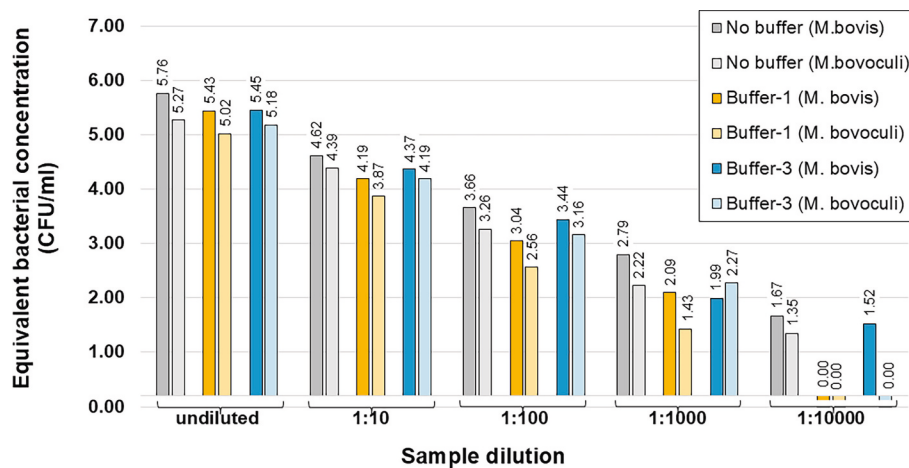


Fig. 1. Bacterial concentrations inferred from Cq-values obtained by qPCR on serial tenfold dilutions of *Moraxella bovis* and *M. bovoculi* sample suspensions.

Table 2

Generalized estimating equations (GEE) analysis of log-transformed equivalent bacterial concentrations detected by qPCR under varying experimental conditions.

Variable	Coefficient	Robust SE	95% Confidence interval	P-value
Transport method				
Unbuffered method	(base)			
Buffer-1	-0.3	0.15	-0.6 to 0	0.04
Storage time				
0 h	(base)			
24 h	-0.3	0.14	-0.6 to 0	0.05
48 h	-0.1	0.13	-0.4 to 0.1	0.34
72 h	-0.2	0.12	-0.5 to 0	0.05
Storage temperature				
2 to 8 °C	(base)			
20 to 25 °C	0.7	0.15	0.4 to 0.9	< 0.01
PCR target				
<i>M. bovis</i>	(base)			
<i>M. bovoculi</i>	-1.7	0.15	-2.0 to -1.4	< 0.01

(Packer et al., 2013). These factors likely contributed to the favorable results obtained with unbuffered specimens.

In addition to the demonstrated stability of *Moraxella* DNA on unbuffered swabs, a modest but statistically significant increase in inferred bacterial concentration relative to Buffer-1 ($\beta = 0.3$) indicates that this method performs at least comparably, and potentially more effectively, in recovering target DNA. While the magnitude of this increase is unlikely to be of practical diagnostic relevance, the combination of DNA stability, comparable-to-superior recovery, and the practical advantage of simpler handling without preservatives supports the use of unbuffered swabs as the preferred approach.

The reason why MagNA Pure LC RNA Isolation Tissue Buffer yielded acceptable results for the demonstration of *M. bovoculi* DNA on the swabs but not *M. bovis* DNA remains unclear. PCR products with higher GC content are more likely to form stable secondary structures that may inhibit amplification in PCR reactions (McDowell et al., 1998; Frey et al., 2008), and RNA-optimized buffers may be less effective for DNA preservation (Hornig et al., 2018). Additionally, 1,4-dithiothreitol in the formulation may induce DNA damage via reactive oxygen species under certain conditions (Fjelstrup et al., 2017).

Unexpectedly, specimens stored at room temperature yielded higher equivalent bacterial concentrations than those refrigerated. Although bacterial replication could occur in unbuffered samples at ambient temperature, this is unlikely in the presence of Buffer-1. A more plausible explanation is that cell lysis occurred more readily at room

temperature, rendering the target DNA more accessible and promoting more effective extraction. While the precise mechanism remains unclear, this observation supports the stability of *Moraxella* DNA on moist cotton wool swabs even under suboptimal storage conditions.

While this study evaluated several sample-handling methods for *Moraxella* strains recovered from clinical cases of keratoconjunctivitis, the assessments were limited to a small number of isolates. A more substantial limitation is that swabs were spiked with pure cultures of *M. bovis* or *M. bovoculi*, whereas bovine conjunctival specimens in practice contain diverse microbial communities and biologically active components from lacrimal secretions that may influence sample integrity and qPCR performance.

Based on the findings and limitations of this study, cotton wool swabs collected from bovine lacrimal secretions and intended for DNA extraction via bead beating in the MagNA Pure system should be submitted without lysis buffers, as none of the evaluated formulations improved recovery compared to the unbuffered approach. To preserve sample integrity, contamination should be minimized and submission expedited. Future work should expand the evaluation of DNA extraction methods and cell-disruption techniques, include a broader range of *Moraxella* strains, and incorporate field specimens to better capture the microbial diversity and biological complexity encountered in clinical sampling.

This study demonstrated that *M. bovis* and *M. bovoculi* DNA remain stable on moist cotton wool swabs and are resilient to cold chain interruptions. Although stabilization buffers may be useful under conditions of prolonged delay or heavy contamination, the unbuffered approach—transporting swabs on ice in insulated containers—represents a practical, cost-effective protocol for field studies, provided samples are processed within three days.

CRedit authorship contribution statement

Louis H. Maartens: Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Bruce Gummow:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **John D. Grewar:** Writing – review & editing, Supervision. **Peter N. Thompson:** Writing – review & editing, Supervision, Funding acquisition. **Jacqueline A. Picard:** Writing – review & editing, Supervision, Methodology, Formal analysis.

Ethical statement

This study did not involve experimentation on animals or human subjects. Ethical approval was not required.

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Declaration of competing interest

The authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rvsc.2026.106069>.

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