

Effect of freezing and storage time on culture yields of body site swabbing samples from dairy heifers

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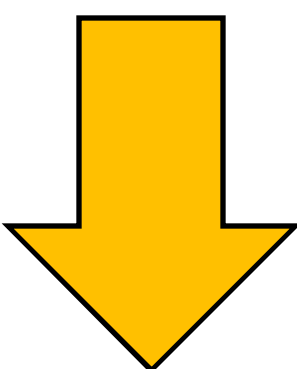

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Background

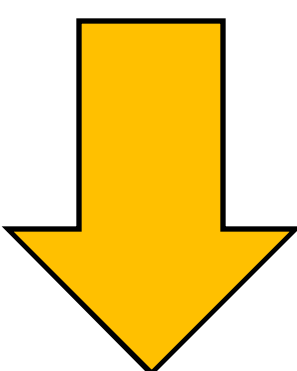
- Coagulase-negative staphylococcal (CNS) species are a major contributor to intramammary infection (IMI) in dairy heifers.
- Current research has identified teat and inguinal skin colonization as potential sources of CNS IMI in dairy heifers.
- Currently it is unknown how long body site swabbing samples can be stored before storage conditions and time negatively impact culture yield.
- We hypothesized that there would be no difference in the colony counts between fresh and frozen body site samples.

Materials and Methods

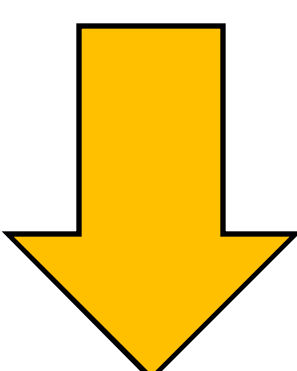
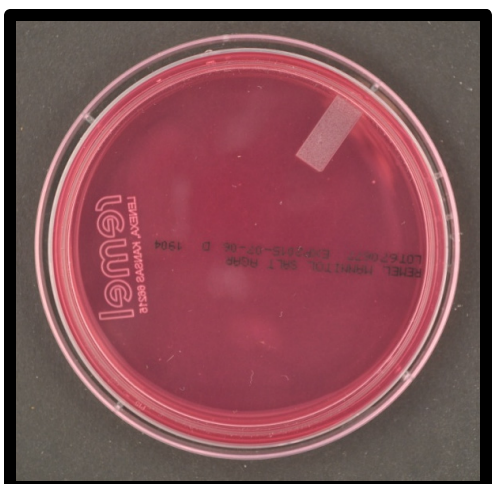
- Teat and inguinal body site swabbing samples (n= 30 samples; 15 body site and 15 inguinal) were taken from 15 dairy heifers using clean gloves and sterile electrostatic dusters.



- Samples were placed in whirl pack bags and placed in a cooler for transport to the laboratory.



- On the day of collection, samples were immersed in 10 ml of 0.9% saline, agitated, and a 10 µl aliquot of the solution was inoculated onto Mannitol Salt Agar (MSA).



- Colonies were counted at 24 hours and 48 hours. Samples were frozen at -20°C for 28 d and 15 samples were randomly selected for plating; the remaining 15 samples were stored at -20°C for a total 56 days. The 15 samples that were thawed and studied at 28 d were again studied at 56 d to assess freeze-thaw effects on colony counts.
- At the 28 and 56 d time points, the assigned samples were thawed, agitated, and 10 µl were inoculated onto MSA.

Results

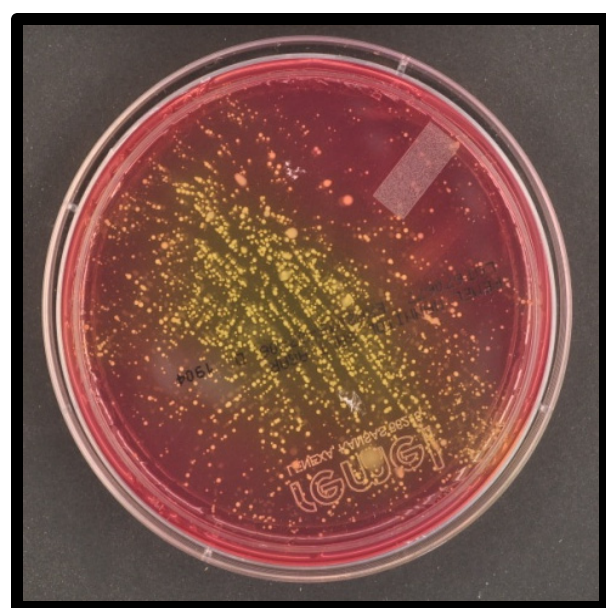
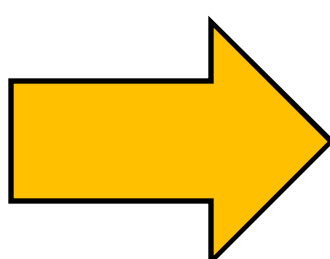
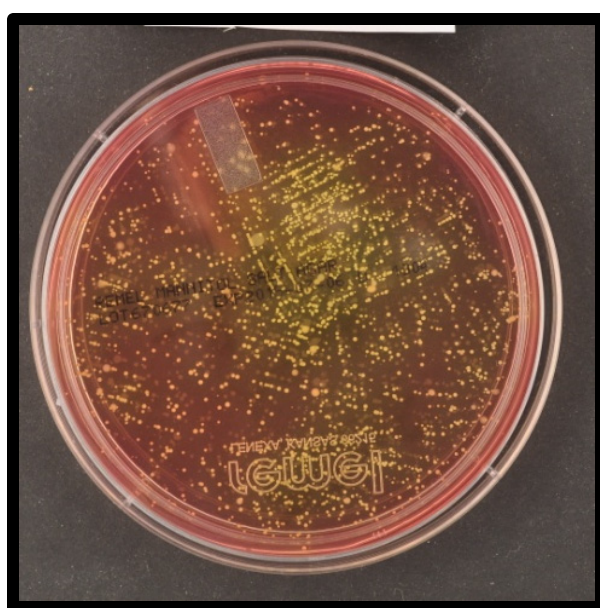


Figure 1 : Sample from heifer teat skin; 24 h growth of sample plated on day of collection (left) and 24 h growth of the same sample stored at -20°C for 28 d (right).

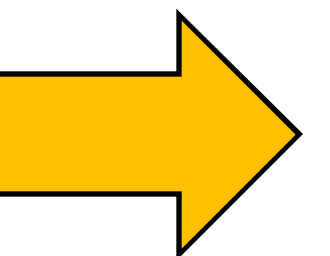
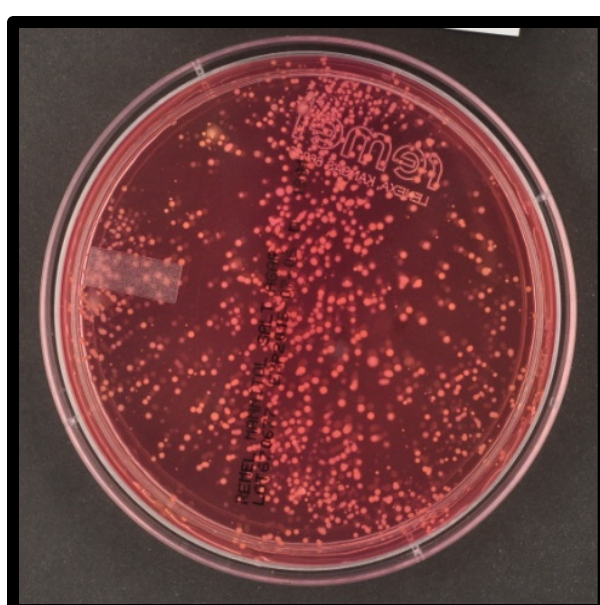


Figure 2 : Sample from heifer inguinal skin; 24 h growth of sample plated on day of collection (left) and 24 h growth of the same sample stored at -20°C for 28 d (right).

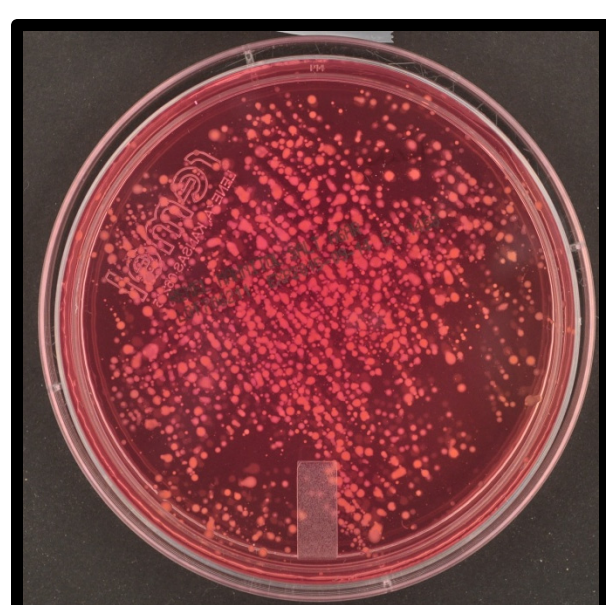
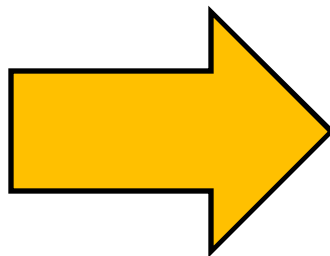
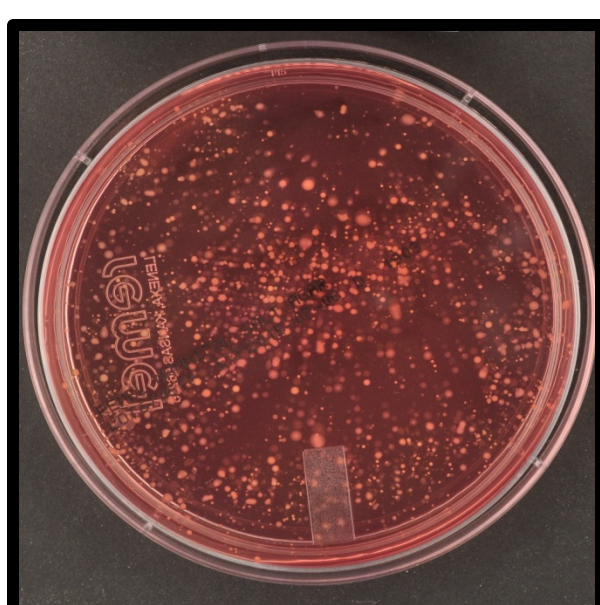


Figure 3 : Sample from heifer inguinal skin; 24 h growth of sample plated on day of collection (left) and 24 h growth of the same sample stored at -20°C for 56 d (right).

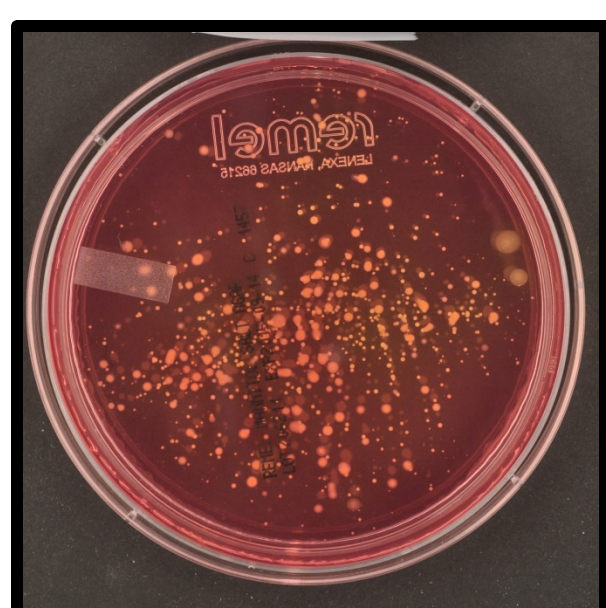
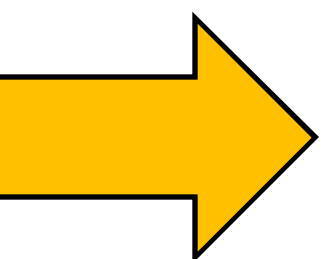


Figure 4 : Sample from heifer teat skin; ; 24 h growth of sample plated on day of collection (left) and 24 h growth of the same sample stored at -20°C for 56 d (right).

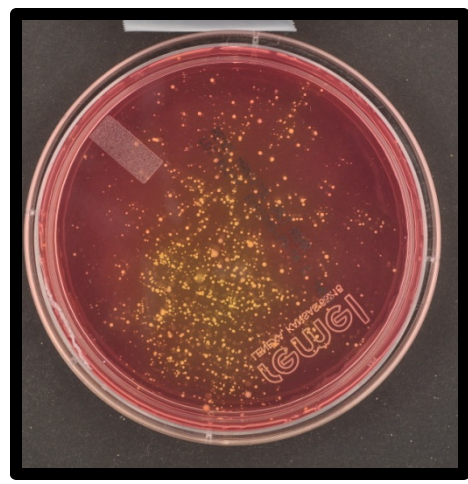
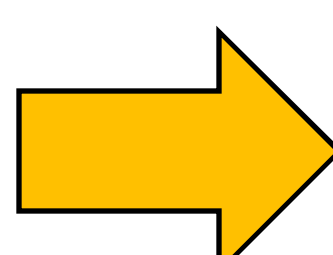
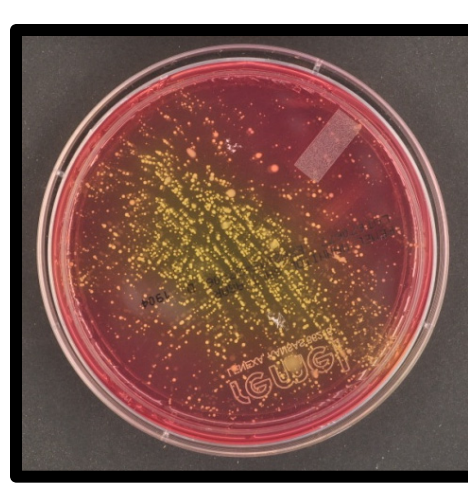
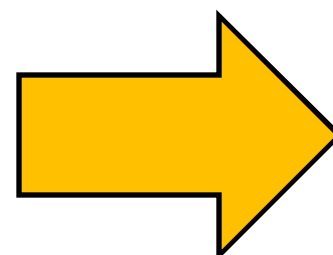
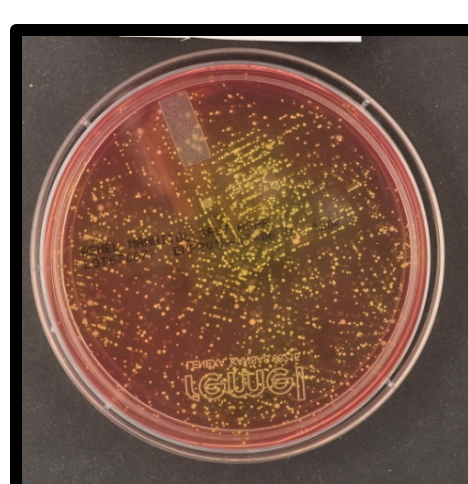


Figure 5: Sample from heifer teat skin; 24 h growth of sample plated on day of collection (left), 24 h growth of the same sample stored at -20°C for 28 d (middle), and 24 h growth of the same sample stored at -20°C for a further 28 d after thawing and refreezing (right).

Table 1 – Median (range) colony counts at each of the studied time points; NA = not applicable.

| | Median (range) 24 h | Median (range) 48 h | Median (range) 24 h | Median (range) 48 h |
|-------------------------|------------------------|------------------------|------------------------|------------------------|
| Baseline | 459 (26-2347) | 723 (52-2480) | 379 (125-2468) | 742 (140-2996) |
| 28 d | 561 (11-3334) | 617 (34-3367) | NA | NA |
| 56 d | NA | NA | 680 (176-2637) | 754 (278-2890) |
| 56 d after thaw at 28 d | 407 (31-2122) | 587 (60-2308) | NA | NA |

- Repeated measures ANOVA found differences ($P < 0.001$) in colony counts between time periods, but all differences were between 24 h and 48 h counts, and overall there was no impact of storage time on counts ($P \geq 0.053$) with the exception that samples that were stored for 56 d had a higher 24 h count than the 24 h count on the same sample when fresh ($P < 0.001$).

Conclusions

- Overall, storage for 28 d and 56 d did not negatively impact colony counts.
- Changes in species diversity caused by storage was not studied, but is a future aim.
- This work will benefit mastitis research workers in their efforts to unravel the epidemiology and ecology of CNS on dairy farms by providing knowledge on sample handling for large studies where immediate sample processing is not possible.

Acknowledgements

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