



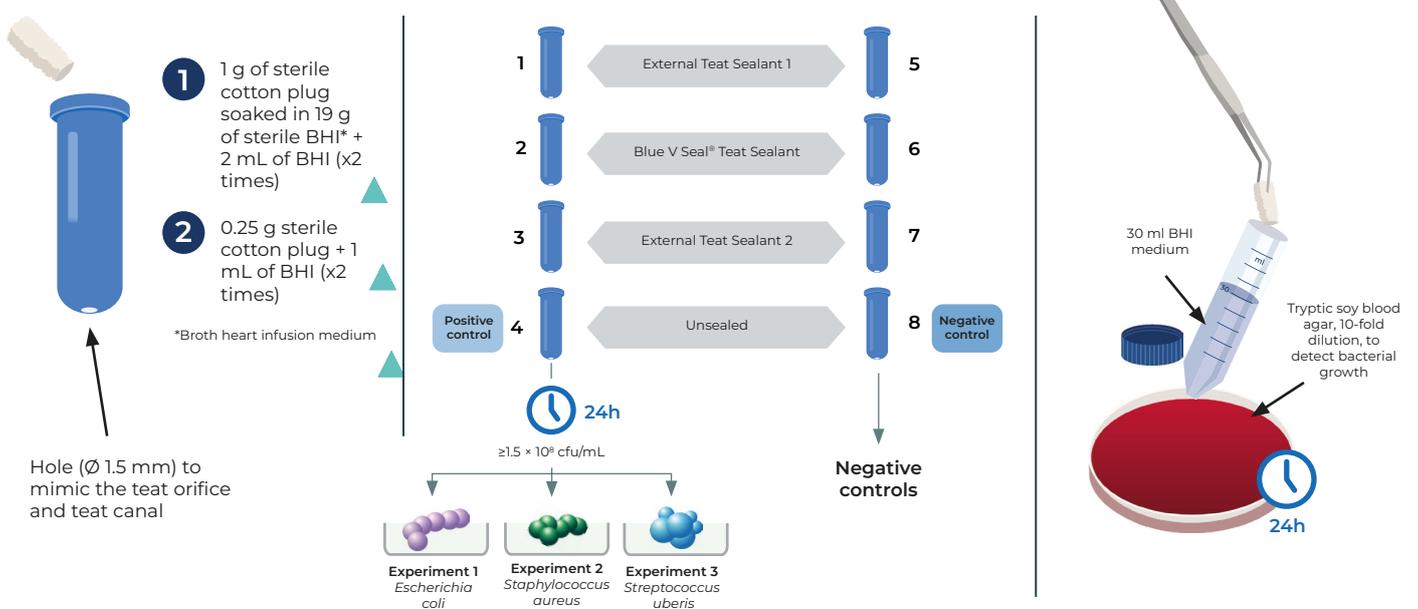
Protective effect of an external teat sealant against intramammary infections

Objective

To evaluate the barrier characteristics of an external teat sealant for dry cows (Blue V Seal®) in preventing mastitis-causing bacterial penetration.

Study design

Three separate *in vitro* experiments with rubber calf-feeding nipples.



Results

Bacteriological culture results from the cotton swabs used to swab the interiors and the exteriors of the rubber teats and the cotton plugs collected from the rubber teats after 24 h of incubation at 37 °C for three experiments.

Experiment 1 *Escherichia coli* (ATCC 25922)

Teat number	Immersed in bacterial suspension	Teat dip	Cotton swabs (teat interior, cfu)	Cotton swabs (teat exterior, cfu)	Cotton plug (cfu/mL)
1	Yes	External Teat Sealant 1	Negative	>150 ¹	>150 ¹
2	Yes	Blue V Seal®	Negative	>150 ¹	>150 ¹
3	Yes	External Teat Sealant 2	>150 ¹	>150 ¹	8.0 × 10 ⁷¹
4	Yes	Control	>150 ¹	>150 ¹	1.0 × 10 ⁸¹
5	No	External Teat Sealant 1	Negative	Negative	Negative
6	No	Blue V Seal®	>150 ¹	Negative	1.5 × 10 ⁵³
7	No	External Teat Sealant 2	Negative	Negative	Negative
8	No	Control	Negative	Negative	Negative

Experiment 2

Staphylococcus aureus (ATCC 25923)

Teat number	Immersed in bacterial suspension	Teat dip	Cotton swabs (teat interior, cfu)	Cotton swabs (teat exterior, cfu)	Cotton plug (cfu/mL)
1	Yes	External Teat Sealant 1	Negative	>150 ⁴	Negative
2	Yes	Blue V Seal®	Negative	>150 ⁴	Negative
3	Yes	External Teat Sealant 2	>150 ⁴	>150 ⁴	Negative
4	Yes	Control	>150 ⁴	>150 ⁴	7.5 × 10 ² ⁴
5	No	External Teat Sealant 1	Negative	Negative	Negative
6	No	Blue V Seal®	Negative	Negative	Negative
7	No	External Teat Sealant 2	Negative	Negative	Negative
8	No	Control	Negative	Negative	Negative

Experiment 3

Streptococcus uberis (BAA-854)

Teat number	Immersed in bacterial suspension	Teat dip ¹	Cotton swabs (teat interior, cfu)	Cotton swabs (teat exterior, cfu)	Cotton plug (cfu/mL)
1	Yes	External Teat Sealant 1	Negative	>150 ⁴	2.4 × 10 ⁴ ⁶
2	Yes	Blue V Seal®	Negative	>150 ⁴	Negative
3	Yes	External Teat Sealant 2	>150 ⁴	>150 ⁴	1.6 × 10 ⁵
4	Yes	Control	>150 ⁴	>150 ⁴	2.0 × 10 ⁴ ⁷
5	No	External Teat Sealant 1	Negative	Negative	Negative
6	No	Blue V Seal®	Negative	Negative	Negative
7	No	External Teat Sealant 2	Negative	Negative	Negative
8	No	Control	Negative	Negative	Negative

¹ Pure culture of the original *E. coli* reference strain, as confirmed by random amplification of polymorphic DNA (RAPD) strain typing.

² Pure culture of *S. hominis*, as confirmed by MALDI-TOF MS.

³ Mixed culture of *S. hominis*, *S. warneri*, and *M. luteus*, as confirmed by MALDI-TOF MS.

⁴ Pure culture of the original *S. aureus* reference strain, as confirmed by RAPD strain typing.

⁵ Pure culture of the original *S. uberis* reference strain, as confirmed by testing via bile esculin agar and antimicrobial sensitivity pattern.

⁶ Mixed culture of *S. hominis* and *M. luteus*, as confirmed by MALDI-TOF MS.

⁷ Mixed culture of the original *S. uberis* reference strain with *S. hominis*, *S. warneri*, and *M. luteus*, as confirmed by MALDI-TOF MS.

- ▶ All cotton plugs from rubber teats exposed to mastitis pathogens with **Blue V Seal®** or external teat sealant 1 remained **culture-negative**.
- ▶ In contrast, the cotton plugs from teats sealed with external teat sealant 2 and exposed to *E. coli* or *S. uberis* became culture-positive for those strains, and remained culture-negative for *S. aureus*.
- ▶ Cotton plugs from unsealed teats (teats 4 & 8) became culture-positive for the same pathogen strains, and with one exception, plugs from teats not exposed to any bacterial suspension (teats 5-8) remained culture-negative.
- ▶ Results from cotton swabs used to sample the interior and exterior of the rubber teats were consistent with those from the cotton plugs. Notably, while the cotton plug from rubber teat 3 (treated with external teat sealant 2) in experiment 2 was culture-negative for *S. aureus*, the interior swab was culture-positive for the same strain.
- ▶ Despite the use of sterile gloves and regular disinfection, contamination cannot be excluded, as observed in teat 4 (experiment 3) and teat 6 (experiment 1).

Conclusions

Under *in vitro* conditions using rubber calf-feeding nipples, **Blue V Seal®** showed strong barrier properties against *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus uberis*.

Although this method has not been validated to predict sealant adherence, duration, or intramammary infection risk under field conditions, the experimental setup – overnight incubation at 37 °C in BHI medium, high bacterial exposure, non-contractile teat ends, and strong bacterial growth in positive controls – provides a valuable starting point for model development.

Reference

Piepers, S., Van Den Brulle, I., Mertens, K. and De Vlieghe, S. Short communication: Barrier characteristics of 3 external teat sealants to prevent bacterial penetration under *in vitro* conditions using rubber calf-feeding nipples. *Journal of Dairy Science* 2020;103(7):6569-6575.