An investigation into manually drawn up “flush” as a culture medium.

Ebony Smith [BSc (Hons), RVN], Hayley Carne [BSc (Hons) Grad Dip VN, PG Cert Vet Ed, RVN] and Dr Andrew Mead [PhD MRes BSc(Hons) BA(Hons)]

Introduction

Flushing is essential for intravenous catheter (IVC) maintenance - reducing blockages, decreasing drug interactions, and prevent blood from flowing-back by “locking”.
Injectables and the IVC system can be a source of nosocomial infection so ensuring the sterility of injectables (such as flush) improves patient welfare by risk reduction.
In the authors experience, manually drawn up flush is kept for varying time periods depending on clinic standard operating procedures.

Aim

To investigate bacterial contamination and growth dynamics in sterile saline over time.

Objectives

1. Establish the **bacterial content** of saline over time, as drawn up into syringes from a non-sealed saline bag at a single point, and cultured at set intervals over a period of up to 62 days post-collection.
2. Evaluate the growth, persistence, or decline of *Staphylococcus pseudintermedius* and *Staphylococcus aureus* in saline by comparing the bacterial load in unaltered sterile saline (SS) syringes, with syringes inoculated with these bacteria over a similar extended time period.

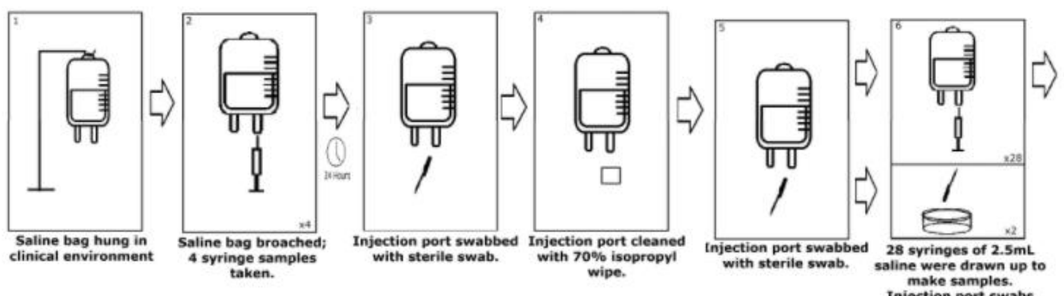
Hypothesis

1. Saline will remain sterile within the syringe and no bacteria will grow on the agar plates, as it is not in contact with any contaminating sources, for the 62-day period.
2. The quantity of bacteria within the inoculated samples will change, either increasing as bacteria grows or decreasing as they die.

Methods

Manual sterile saline (MSS) syringes

A 1L sterile saline bag was **broached** and left for 24hrs in a clinical environment. After this, 28, 3ml syringes of saline were drawn up to **create samples**, which were labelled 1-28 and randomly assigned to a predetermined time point. A **sample size** of 4 per time period was used, calculated using the difference between two means. **Time points** assessed were immediate (within 1 hour), 24hours, 48hours, 7 days, 14 days, 30 days, and 62 days.
Syringes were **stored** needle down in a pencil pot, with needle and cap on the syringe, until testing.
At the assigned time point, **spreader plates** were made from each syringe using 0.1 mL of sample solution. These were incubated for 24hrs at 37°C and then colonies were counted.
The rest of the sample had **enrichment broth** added to encourage growth. These were incubated in an incu-shaker for 24hrs at 37°C before being observed for turbidity. Additional spreader plates were then made and incubated at 37°C for 24 hours.
Colony counts were performed and recorded to provide numerical data.



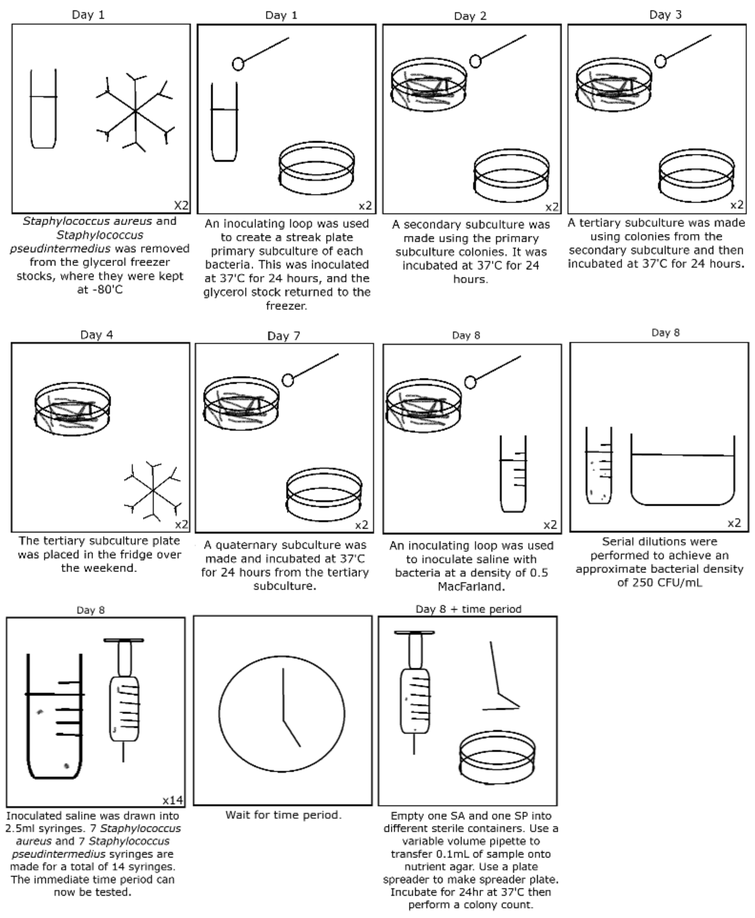
Inoculated Samples - *Staphylococcus pseudintermedius (SP) & Staphylococcus aureus (SA)*

Part A

A **sample size** of 1 per time period for each bacteria. 7 syringes with SA, 7 with SP.
Sample creation involved
First bacteria was **subcultured**. From this, saline was **inoculated** at a density of 0.5 McFarland's and then **serial diluted** to achieve a density of 250 CFU/mL. The samples were then drawn up into 3 ml syringes, and **stored** needle and cap on, placed needle down in a pencil pot.
The **time points** assessed were immediate (within 10 minutes), 24 hours, 48 hours, 7 days, 14 days, 30 days, and 62 days.
Spreader plates were made from each syringe once they reached their preassigned time period using 0.1 mL. These were **incubated** for 24hrs at 37°C and then **colonies were counted**.

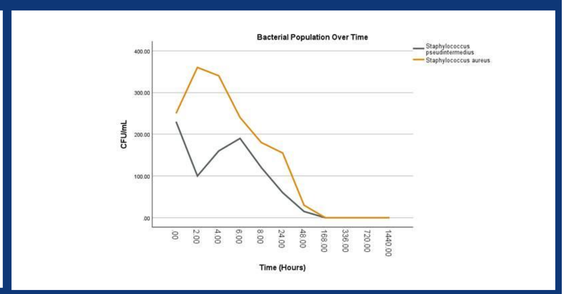
Part B

Repeat of Part A with different time points **because** colony growth stopped at the 7-day mark, further testing would illustrate the growth curve in more detail.
Testing was done at: immediate (within 10 minutes), 2 hours, 4 hours, 6 hours, 8 hours, 24 hours and 48 hours.



Results

1 - All colony counts were *negative*. Using a binomial model, the true contamination rate in the samples is <0.0018, indicating a high probability that the samples are sterile and that the chance of contamination being present and not detected is low (with 95% confidence).
2 - SA and SP underwent an overall *decline in population*, with CFU/mL dropping to 0 by 168 hours (7 days). There was a *statistically strong negative correlation* between the CFU/mL of SA and hours since inoculation (Spearman's’ correlation coefficient = -0.95; p<0.001), indicating that as time increased the population of SA present decreased. There was a statistically strong negative correlation between the CFU/mL of SP and hours since inoculation (Spearman's’ correlation coefficient = -0.91; p<0.001), indicating that as time increased the population of SP decreased.



Discussion

- Inoculation methodology may not be representative of real contamination levels.
- CFU infection concentration is unknown in animals - 15 CFU/mL at catheter tip in humans.
- Contamination may have been missed but this is improbable.
- Samples are agitated - syringes are often not. Means bacterial dispersal is increased and procedure increases chance of bacterial detection.
- Spike in SP/SA - may be due to enrichment transference from agar plates into samples.
- Further research on production, storage, and usage of flush would be beneficial.

Conclusions

- With aseptic technique, flush syringes can be stored and remain uncontaminated for up to a 62-day period.
- *Staphylococcus pseudintermedius* and *Staphylococcus aureus* decrease in quantity when stored in syringes of saline.
- Veterinary practices may wish to examine current flush procedures.

References

Aryal, S., 2021. McFarland Standards- Principle, Preparation, Uses, Limitations. [online] *Microbe Notes.* Available at: <https://microbenotes.com/mcfarland-standards/> [Accessed 3 February 2025].
Gahlot, R., Nigam, C., Kumar, V., Yadav, G., Anupurba, S., Gahlot, R., Nigam, C., Kumar, V., Yadav, G. and Anupurba, S., 2014. Catheter-related bloodstream infections. *International Journal of Critical Illness and Injury Science*, [online] 4(2), p.162. https://doi.org/10.4103/2229-5151.134184.
Goossens, G.A., 2015. Flushing and Locking of Venous Catheters: Available Evidence and Evidence Deficit. *Nursing Research and Practice*, [online] 2015, pp.1–12. https://doi.org/10.1155/2015/985686.
Lee, P.T. and Terry, J., 2021. Changing practice to using pre-filled syringes for flushing IV cannulas. *British Journal of Nursing*, [online] 30(14), pp.S14–S22. https://doi.org/10.12968/BJON.2021.30.14.S14/ASSET/IMAGES/LARGE/BJON.2021.30.14.S14_T05.JPEG.