Evaluation of Sigma-VCM® in the Storage and Transportation of *Mycoplasma hominis*

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Introduction

Mycoplasma hominis, a notoriously fastidious organism, is sensitive to environmental factors (1). Transport medium and conditions are important to successful detection by culture in clinical assays. This study aims to evaluate the stability of *M. hominis* in Sigma-VCM® universal transport medium at different storage temperatures over 192 hours.

Materials

Materials
R85 Hardy Diagnostic medium
A8 agar (Experience plc)
Phosphate Buffer Solution
Sigma-VCM® transport medium for viruses, chlamydia & mycoplasma (MWE)

Methods

We inoculated clinical isolates of M. hominis (1989a, 2005a, 2012a and 2013a) into transport medium. Our negative control was set up by washing the transport tubes with phosphate buffer solution (PBS). The transport medium was replaced with 1mL of PBS.

Viability was measured at 24hr intervals by colour change in R85 Hardy Diagnostic medium and colony-forming units in A8 agar (Mycoplasma experience plc)

Samples were kept at room temperature (RT), 4°C, -20°C and -80°C. Frozen samples were subject to daily freeze-thaw cycles for viability testing. Samples in PBS were stored at RT and -80°C.

The influence of the presence of either ∑-Swab® or Purflock® was also assessed.

Four millilitres of transport medium were inoculated with 40 µL of log-phase growth clinical isolate, resulting in a baseline titre of ≈3.5 x 10^5 cfu/ml. The transport medium was separated into four swab containers allocated to 4 separate temperature conditions (2 clinical isolates randomly allocated to each swab type and swab absence). This was repeated for the negative control samples.

Viability was measured (both broth and agar) daily in triplicate over 192 hours. Viability and survival curves were analysed by Prism Graphpad and integrated statistical packages.

Sigma VCM™ products for this study were provided by MWE. This presentation was supported by MWE

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Controlled variables	Independent variables	Dependent variable
• Concentration of Isolate • Transport tube with glass beads	• Storage temperature • Presence of Swab® or Purflock®	• Colour change in R85 Hardy Diagnostic medium • Colony-forming units in A8

Results

Viability trends took one of two patterns: Room temp (RT) & -20°C generally produced reduced viability than 4°C & -80°C. Repeated measures ANOVA showed a significant difference in all agar growth sets (P=<0.005) (Figure 1A). Increased variability for broth growth titres obscured the statistical significance in difference between data sets, although the general trends were identical to agar growth titration (Figure 1B).

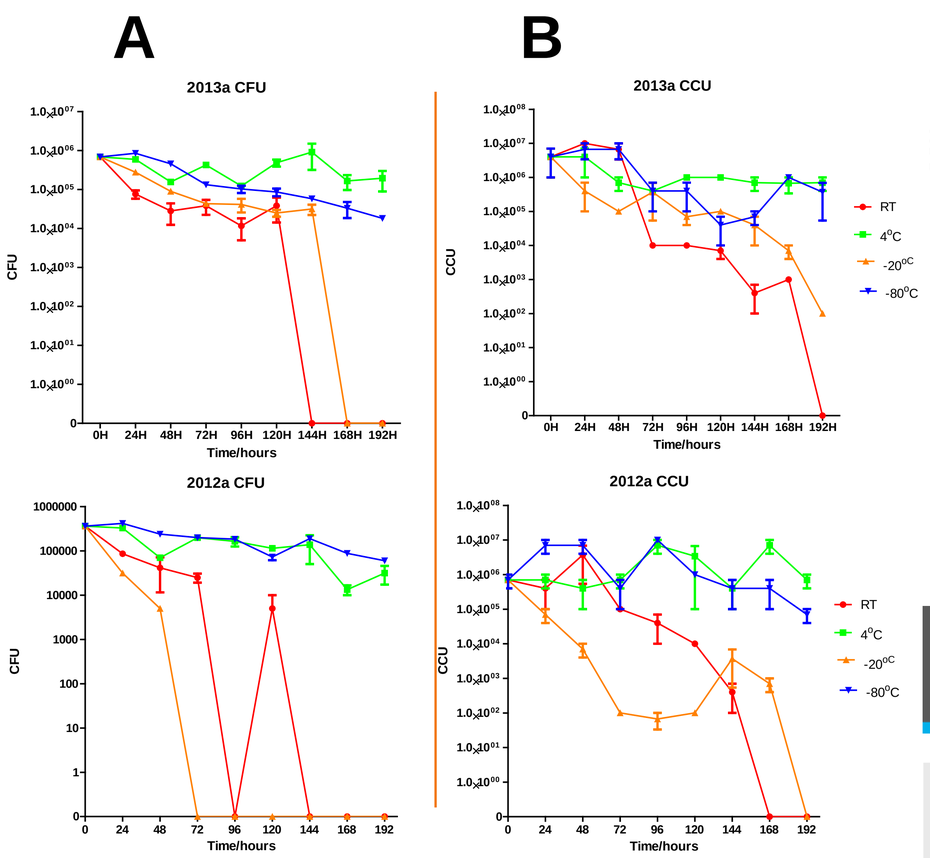

*Figure 1 – **A** Viability of isolates 2013a and 2012a measured by colony forming units grown on A8 agar. Viability was measured over 192 hours after storage at 4 different temperatures. **B** Viability of isolates 2013a and 2012a measured by colour changing units grown in R85 Hardy Diagnostic medium. Viability was measured over 192 hours at 4 different temperatures.*

Storage of isolates at RT produced a loss in viability of less than 10 fold over 24-48 hours. Storage at 4°C & -80°C showed a loss in viability of less than 10 fold over 192 hours (except for isolate 2005a). Post-hoc Tukey testing showed that for 1989a and 2012a, 4°C & -80°C results were significantly different to that of RT & -20°C storage groups (p=<0.05). In all data sets 4°C was significantly different and superior to RT and -20°C (p=<0.05).

Broth culture was much better at detecting viability than agar. However, there was much greater daily variance which has been substantiated by statistical analysis. Broth culture produced results 48-72 hours beyond the point that isolates formed colonies on agar.

When compared to PBS, Sigma VCM® showed significant efficacy in the storage of samples at -80°C (P<0.05).

Additionally, storage of the isolates in Sigma-VCM® universal transport medium at room temperature showed greater efficacy than PBS. Further to this, viability was maintained past 72 hours.

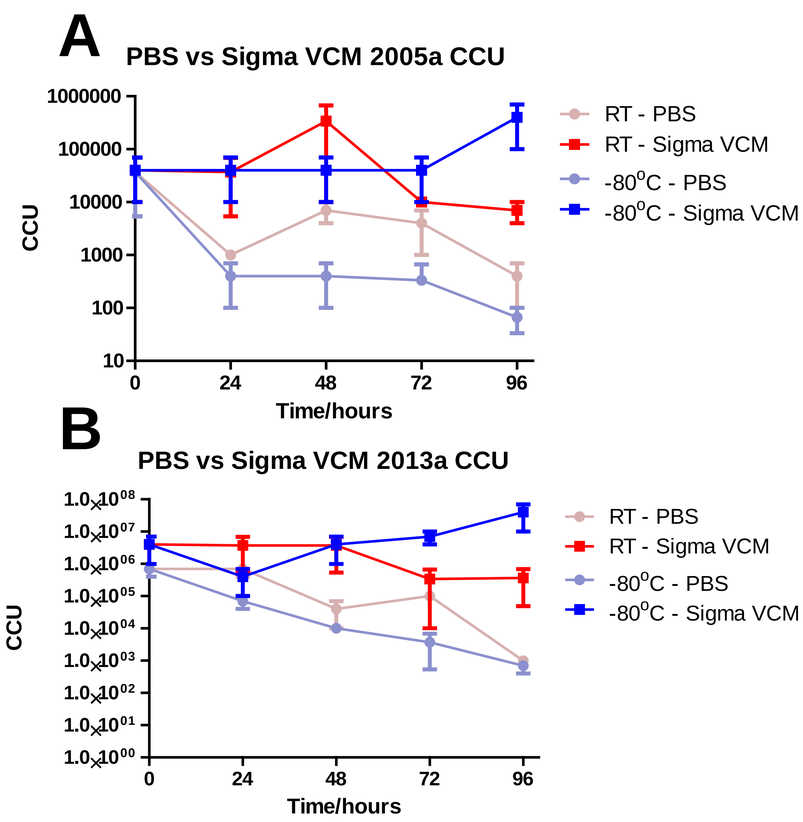

*Figure 1 – **A** Viability of isolate 2005a measured by colour changing units grown in R85 Hardy Diagnostic medium. Viability was measured over 96 hours at RT and -80°C. Samples were stored in either Sigma VCM or PBS (Sigma VCM indicated by line colour brightness) **B** Viability of isolate 2013a measured by colour changing units grown in R85 Hardy Diagnostic medium. Viability was measured over 96 hours at RT and -80°C. Samples were stored in either Sigma VCM or PBS (Sigma VCM indicated by line colour brightness)*

Conclusion

Sigma-VCM® has proven effective in preserving the viability of *Mycoplasma hominis*. Viability at RT showed no significant decrease over 48-72 hours, however transport at 4°C significantly improves viability. Therefore, Sigma-VCM® would be valuable in the clinical setting where there is often a delay in refrigeration of samples. Furthermore, we have shown Sigma VCM to be superior to phosphate buffer solution.

Despite 8 freeze-thaw cycles from -80°C to RT, 3 of the 4 strains maintained near baseline viability. This feature of Sigma-VCM® provides a significant improvement on currents methods of laboratory storage of *M. hominis.*

With respect to sample isolation, no significant difference between results obtained from ∑-Swab® or Purflock® containing transport tubes were observed.

References

1. Nulens E, Reynders M. Mycoplasma hominis: More than just An Innocent Bystander. J Antimicro. 2016;2(114):2472-1212.

Analysis performed using GraphPad Prism version 5.01 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com