Poster BAMA-P07 Scientific Spring Meeting KNVM & NVMM 2017

Evaluation of Sigma-VCM® in the Storage and Transportation of *Ureaplasma spp.*

William Ridge | Dr O.B. Spiller | Cardiff University

Introduction

A common sexually transmitted infection, *Ureaplasma,* can come in two species, *Ureaplasma parvum* and *Ureaplasma urealyticum,* as well as multiple serovars. Infection with *Ureaplasma* can result in premature births, making its effective diagnosis and treatment imperative.

Given the delicate nature of *Ureaplasma,* the effective transport of clinical samples so that viability of the bacteria is maintained is an essential part of an effective diagnosis. The Clinical and Laboratory Standards Institute Standard M40-A2 gives a minimum basic requirement of 48 hours for bacteria to survive in transport devices at ambient or refrigerated temperatures.

This study evaluates the stability of *Ureaplasma* in MWE's Σ-VCM transport devices with standard Sigma foam swabs or PurFlock swabs at 4 different temperatures over 264 hours, to ensure they meet the basic requirements and to study how *Ureaplasma* copes with different conditions and freeze-thaw cycles.

Methods

Initially, two 1ml vials of Σ-VCM medium (without swabs) were inoculated with ATCC prototype strains serovar 3 (*U. parvum*) and 8 (*U. urealyticum*) and a 1:10 dilution titration (in triplicate) was used to measure infectious units of microbes, by colour change of *Ureaplasma* Selective Medium (Mycoplasma Experience, plc.).

Log-phase growth of *Ureaplasma* produces high pH that is detected by pH indicator (measured in colour change units; CCU). The inoculated media were divided into 4 vials and stored at room temperature (RT), 4˚C, -20˚C or -80˚C. Over 96 hours, daily residual viability was measured by dilution titration (in triplicate) and compared to initial inoculation levels. CCU for remaining viable *Ureaplasma* after 48 hours incubation at 37ºC and infectious units per ml were calculated and analysed using Graphpad Prizm software.

The influence of swabs in the medium was then evaluated for these two strains as well as 2 clinical isolates (HPA5 and W11). Identical methodology was utilised except that the initial transport medium was increased to four 4 ml vials before inoculation with separate strains. Each 4ml vial was then separated into four 1ml aliquots in swab transport vials (one for each temperature): Serovar 8 and HPA5 were stored with the PurFlock swab, and serovars 3 and W11 were stored with the Sigma foam swab. Viability testing for these samples was extended to 264 hours.

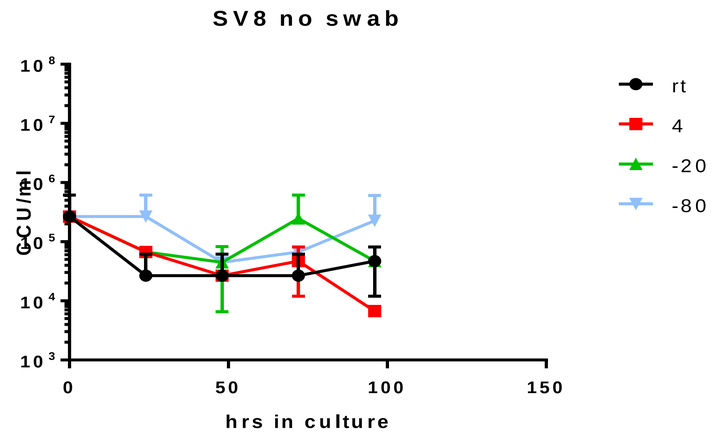
Sigma VCM™ products for this study were provided by MWE. This presentation was supported by MWE

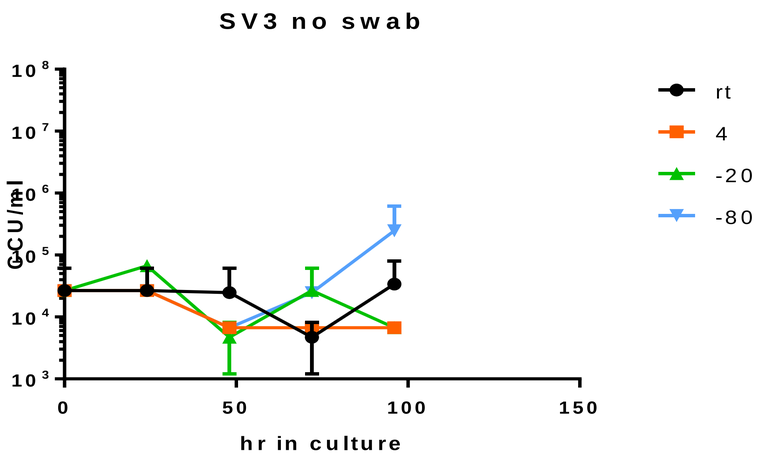
Results

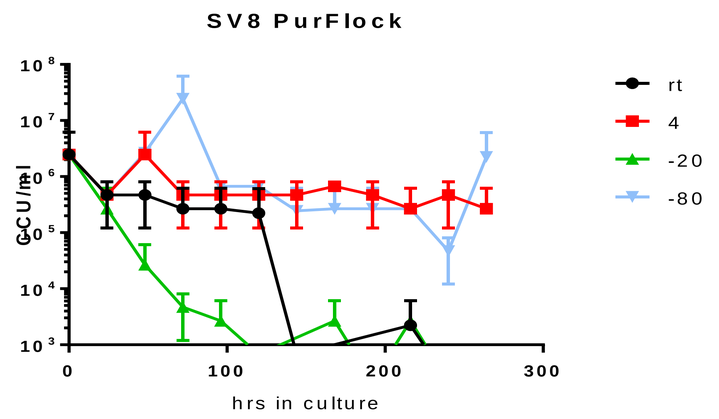
The results are presented in graphs showing the change in the colour changing units per millilitre (CCU/ml) over time. This gives a measurement of the stability of the *Ureaplasma*, with a decreasing line indicating loss of viability of the bacteria and termination of the line before the end of the experiment at 264 hours indicating death of the bacteria.

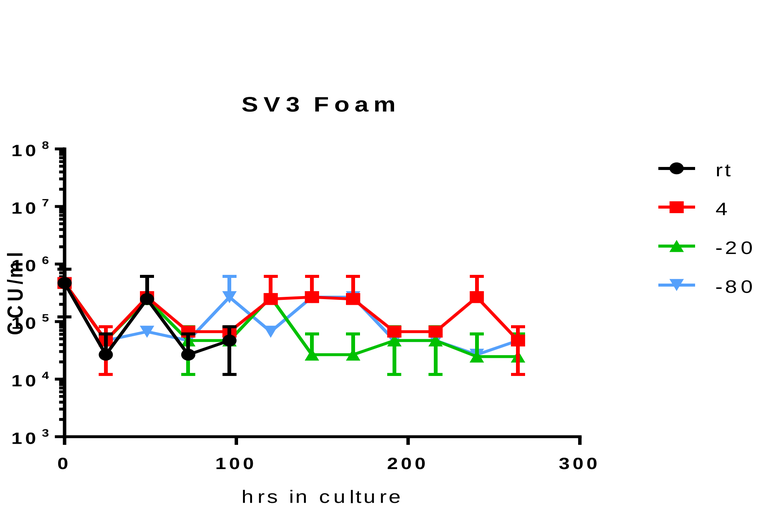
Serovar 3 and HPA5 strains (*U. parvum*) showed no loss of viability at all temperatures until 96 hours, however, viability declined linearly to zero when stored at room temperatures for 120 hours. When stored at 4˚C, -20˚C and -80˚C both of these strains showed negligible viability loss up to 264 hours when the study ended.

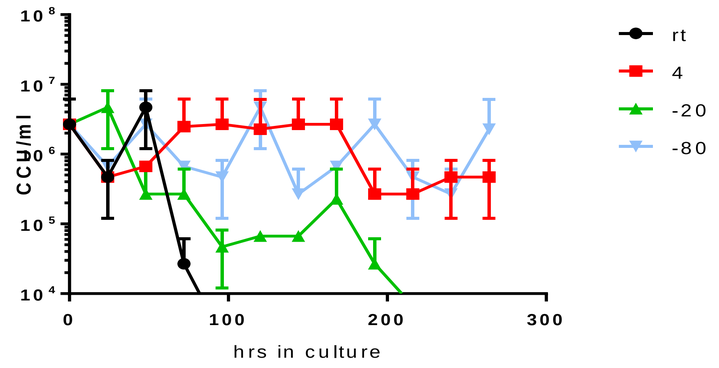
Serovar 8 and W11 (*U. urealyticum*) isolates showed similar results. However, serovar 8 still showed residual viability up to 264 hours when stored at room temperature, and both serovar 8 and W11 strains showed a loss of viability when stored at -20˚C.

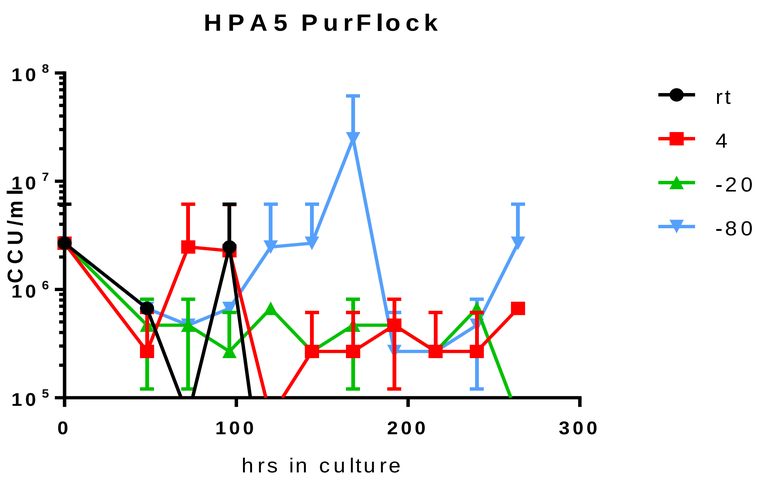












Conclusion

Viability of *Ureaplasma* species in Σ-VCM transport medium was stable for 4 days at room temperature. Viability could be extended to at least 264 hours if kept at 4˚C. Unlike *M. hominis* (see related abstract BAMA-P10), *Ureaplasma* species were more stable at -20˚C with freeze thaw cycles in transport medium, suggesting the physiology of *Ureaplasma spp.* is more robust than *Mycoplasma hominis*.

The extended viability shown with freeze-thaw cycles is of particular importance as it allows *Ureaplasma* to be stored for an extended length of time, allowing important specimens to be kept for research purposes and investigated at a later date. This study has shown that Σ-VCM transport medium has met and exceeded the basic requirements for transport of bacteria set out by the Clinical and Laboratory Standards Institute Standard M40-A2, and extended viability is possible through freeze-thaw cycles.

REFERENCE

Clinical and Laboratory Standards Institute (CLSI). Quality Control of Microbiological Transport Systems; Approved Standard- Second Edition. CLSI document M40-A2. Wayne, PA: CLSI; 2014.