Poster BaMa-P12 Scientific Spring Meeting KNVM & NVMM 2018

Evaluation of *Mycoplasma hominis* transfer by different swabs to Sigma-VCM™ transport medium

Akanksha Kiran and Dr. Owen B. Spiller

Cardiff University School of Medicine, Cardiff, UK

Introduction

Mycoplasma hominis (*M.hominis*), is a fastidious opportunistic bacteria, causing genitourinary infections in women.

With rising rates of *M.hominis* antibiotic resistance, it is becoming more imperative to carry out antimicrobial susceptibility testing.

Currently all diagnostic samples must be transported to a central London reference laboratory; therefore, this study aims to evaluate bacterial capture and release of various clinical swabs used for *M.hominis* isolation and transport.

Materials

R85 Hardy Diagnostic Medium
A8 Agar (Experience Plc)
Phosphate buffer solution
Sigma-VCM™ Transport Medium

Methods

Each swab was used to absorb droplets of varying volume and concentration which was placed on a non-absorbent sterile surface.

The swab was placed into Sigma-VCM™ Transport Medium to allow transfer of bacteria.

Following this, the different release methods were investigated by either allowing the swab to stand in the media at room temperature or vortexing the media with the swab, both of which were standardised to 2 minutes.

Swab was discarded and bacterial load in the transport media was immediately measured. The media was titred to form 10 fold dilutions in R85 Hardy Diagnostic medium followed by inoculation of Mycoplasma Experience Mycoplasma selective agar with 2µl of the 1st tier of dilutions.

After incubation for 48hrs at 37˚C, growth in the diagnostic medium growth was evaluated by a colour change from yellow to pink, in addition to colony counting dilutions on agar plates under a microscope (4X objective).

Results were compared to a maximum transfer value which was obtained by measuring the bacterial load in the transport media following direct inoculation of the same volume/bacterial load in the droplet, without a swab being utilised.

Results were analysed for statistical significance using ANOVA with Bonferroni's correction to allow for multiple post-hoc comparisons.

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

www.mwe.co.uk

Controlled variables	Independent variables	Dependent variable

• Sigma-VCM™ Transport Medium
• Direct inoculation of bacteria into transport medium
	• Swab
	  • Flock tip (PurFlock®)
	  • Foam tip (∑-Swab®)
	  • Copan Rayon dry tip
	  • Cobas PCR
	• Release Method
	  • Non-Vortex
	  • Vortex
	• Threshold
	  • Bacterial load
	  • Droplet Volume
	  • Dry-time post pick up
		• Colour change in R85 Hardy Diagnostic medium
		• Colony-forming units in A8 Agar

Figure 4. Taken from ELITech Group Solutions. 2017. Image depicts the classic fried egg appearance of *Mycoplasma Hominis* on an agar plate.

Results

No statistically significant difference in the mean percentage of bacteria transferred to the transport medium between the ∑-Swab® (30.79 ± 6.15%) (Mean±SD) and the Purflock® (18.14 ± 2.21%) (Fig 1), but these were much better than Copan Rayon dry tip and Cobas PCR swabs (p≤0.001 and p≤0.01 Fig 1)

Larger droplet volumes of 500µl led to decreased transfer of viable bacteria to transport medium compared to 50 µl droplets for Purflock® (p≤0.01) and ∑-Swab® (p≤0.0001). However no further decrease was observed for the already low values for the Copan Rayon dry tip swabs (Fig 2).

Vortexing for 2 minutes did not increase the release of bacteria to Sigma-VCM™ transport medium from any of the swabs (Fig 3).

Delay (potential drying) in transferring the swab for 30 minutes also did not reduce viable bacterial transfer (Fig 5).

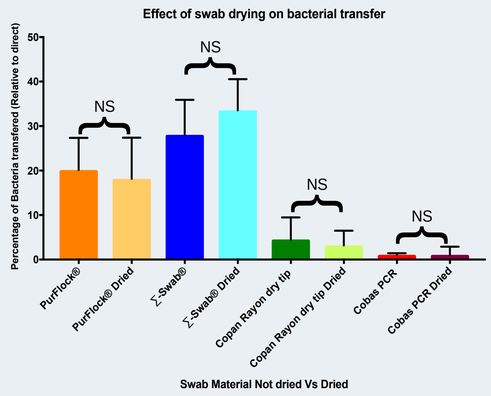

Figure 5. Effect of swab drying on bacterial transfer. Graph calculated by combining percentage transfer of bacteria by each swab material both dried and not dried for Leeds and DF48 strains. One way-ANOVA with Bonferroni correction:
PurFlock® Vs PurFlock® dried= Non-significant
∑-Swab® Vs ∑-Swab dried = Non-significant
Copan Rayon dry tip Vs Copan Rayon dry tip dried= Non-significant
Cobas PCR Vs Cobas PCR dried= Non-significant

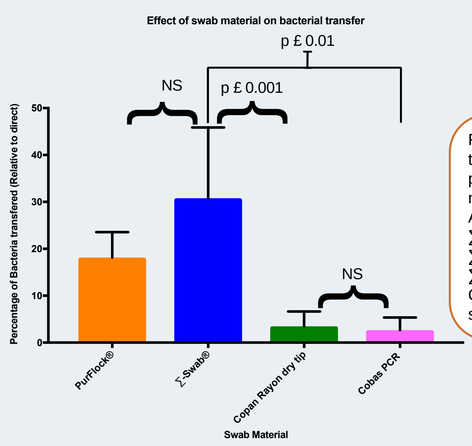

Figure 1. Effect of swab material on bacterial transfer. Data calculated by combining percentage transfer of bacteria by each swab material for both Leeds and DF48. One way-ANOVA with Bonferroni correction:
∑-Swab® vs PurFlock® = Non-significant
∑-Swab® vs Copan Rayon dry tip= p ≤ 0.001
∑-Swab® vs Cobas PCR= p ≤ 0.01
Copan Rayon dry tip vs Cobas PCR= Non-significant

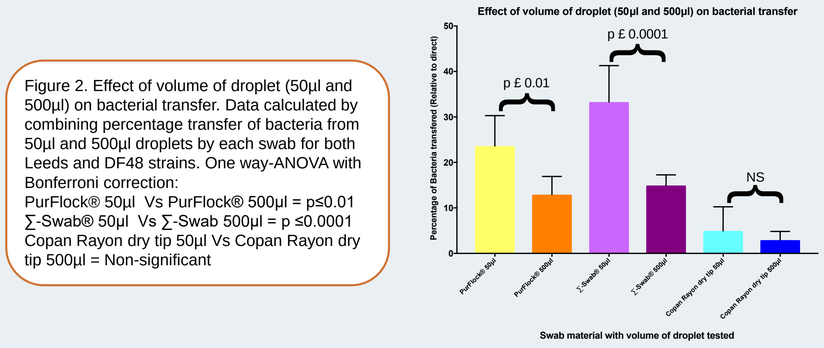

Figure 2. Effect of volume of droplet (50µl and 500µl) on bacterial transfer. Data calculated by combining percentage transfer of bacteria from 50µl and 500µl droplets by each swab for both Leeds and DF48 strains. One way-ANOVA with Bonferroni correction:
PurFlock® 50µl Vs PurFlock® 500µl = p≤0.01
∑-Swab® 50µl Vs ∑-Swab 500µl = p ≤0.0001
Copan Rayon dry tip 50µl Vs Copan Rayon dry tip 500µl = Non-significant

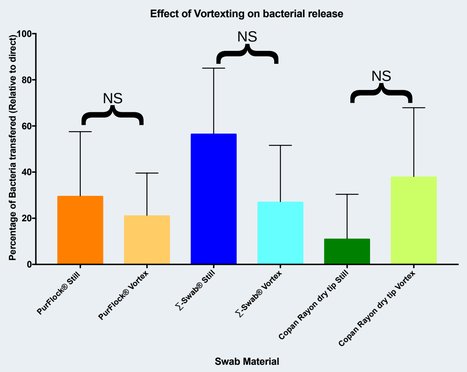

Figure 3. Effect of Vortexing on bacterial release. Data calculated by combining percentage transfer of bacteria by each swab material both vortexed and still for Leeds and DF48 strains. One way-ANOVA with Bonferroni correction:
PurFlock® still Vs PurFlock® Vortexed= Non-significant
∑-Swab® still Vs ∑-Swab Vortexed = Non-significant
Copan Rayon dry tip Still Vs Copan Rayon dry tip Vortexed = Non-significant

Conclusion

No statistically significant superiority between MWE ∑-Swab® and Purflock® for viable bacterial transfer into the Sigma-VCM™ Transport Medium.

MWE ∑-Swabs® superior to Copan Rayon dry tip and Cobas PCR swabs.

Neither vortexing nor drying the swabs alters the amount of viable bacterial transfer into the Sigma-VCM™ Transport Medium for any of the swab materials investigated.

Percentage transfer of viable bacteria was better for droplet volumes less than 500µL, however this was not influenced by bacterial concentration.

References

- Mattila, P. S., Carlson, P., Sivonen, A., Savola, J., Luosto, R., Salo, J. & Valtonen, M. 1999. Life-threatening Mycoplasma hominis mediastinitis. Clin Infect Dis, 29, pp. 1529-37.
- SPILLER, O. B. 2017. Emerging Pathogenic Respiratory Mycoplasma hominis Infections in Lung Transplant Patients: Time to Reassesses it's Role as a Pathogen? EBioMedicine, 19, pp. 8-9.
- Razin S. 1996. Mycoplasmas. In: Baron S, ed. Medical Microbiology. 4th ed.. Galveston (TX): University of Texas Medical Branch at Galveston. Available from: https://www.ncbi.nlm.nih.gov/books/NBK7637/
- ELITech Group Solutions. 2017. A7 Agar. [image] Available at: https://www.google.co.uk/url?sa=i&rct=j&q=&esrc=s&source=images&cd=&ved=0ahUKEwj_n5Tu96rXAhXDCcAKHQk_C3kQjxwIAw&url=https%3A%2F%2Fwww.elitechgroup.com%2Fproduct%2Fa7-agar%2F&psig=AOvVaw3D4xqOpb7oLao351k3xsqd&ust=1510091602548163 [Accessed 6 Nov. 2017].
- Templates Medical Research Council [Internet]. Medical Research Council. [cited 12 March 2018]. Available from: https://www.mrc.ac.uk/about/information-standards/mrc-brand-guidelines/templates/