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Efficacy and Downstream Compatibility of the Respiratory Sigma Collection Device

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Introduction

The pre-analytical phase of the total laboratory testing process is where the majority of laboratory errors occur and represents the most labour intensive, least standardized aspect of the process. Microbiology involves the collection of a wide diversity of samples from the patient to determine the aetiology of diseases resulting in a complex pre-analytical process. Specimen processing is one of the most important steps in the pre-analytical phase as downstream reading, interpretation and reporting by technical staff is heavily dependent on the quality of specimen setup. Automated specimen processors integrated within total laboratory automation, such as the InoqulA® (Becton Dickinson Kiestra) and the liquid-based microbiology (LBM) concept have been introduced to microbiology laboratories. The key providers of liquid collection devices in the United Kingdom are Medical Wire & Equipment (MWE) and Copan both offering a comprehensive range of liquid swabs for the various specimen types found in Microbiology.

The recent paradigm shift from manual to automated processing with vast amounts of investments in pre-analytical has begged the question the continued manual manipulation of sputum samples. There is also considerable interest in automation that could potentially lessen labour demands for specimen processing due to efficiency savings required within laboratories. Respiratory samples such as bronchoalveolar lavage (BAL), bronchial aspirate, (BAS) and sputum (SP) represent a significant proportion of routine microbiological specimens and are very important for the management of critically ill patients. Routine culture of sputum typically requires collection of specimen within a wide bore universal, a liquefaction step and inoculation of agar media. The viscosity of these specimens reduces the ability to reproducibly and media and precludes placing them on automated, liquid handling systems. For microbiology to unleash efficiencies in specimen processing and the potential for automation, a liquid based microbiology approach is needed; but this goal can only be achieved first with innovations in specimen devices that will automatically transform samples into liquid in standardized containers.

The availability of the Sigma SP™ device which contains a novel mucolytic agent along with pre-analytical automation fundamentally addresses these issues and provides an automated solution to sputum processing. Furthermore, this novel agent is fundamentally a safer and more stable agent than the traditional Dithiothreitol (Cleland's reagent). This study investigates the efficacy of such a solution and associated benefits, as well as comparing the efficacy of the collection device with the Copan SL™ solution collection device in downstream testing in the area of mycobacterium through liquid culture as well as molecular systems.

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Results:

The utilisation of the Sigma SP™ swab corresponded well versus the manual methodology and demonstrated superior streaking and plate utilisation characteristics. No statistical differences were noted in the number of pathogens isolated and or the quantity of upper respiratory track flora observed.

Table 1: Manual versus Sigma SP™ /automation observed differences

Characteristics	Manual pre and post analytical	Sigma SP and total lab automation
Spread pattern	Poor utilisation of plate surface	Automated methodology resulted in good plate surface utilisation
No of single colonies	Fewer single colonies when compared to corresponding automated/Sigma SP™ result	Greater number of single colonies when compared to manual methodology
No of pathogens isolated	Strong correspondence across both methodologies	Strong correspondence across both methodologies
Amount of upper respiratory tract flora	Strong correspondence across both methodologies	Strong correspondence across both methodologies

Reproducibility studies: (specimens were repeated 3 times the FA mode of the InoqulA)

MW& E Sigma SP collection device and Kiestra automation (FA mode) (Reproducibility)				
number of single colonies	Run1	Run 2	Run 3	
sample 1	69	63	67	
sample 2	72	76	68	
sample 3	69	71	69	
sample 4	62	64	67	
sample 5	69	76	71	
sample 6	71	75	79	
sample 7	67	75	73	
sample 8	75	67	67	
sample 9	78	79	75	
sample 10	70	72	76	

Manual methodology			
number of single colonies	run 1	run 2	run 3
sample 1	45	28	32
sample 2	34	24	21
sample 3	62	42	34
sample 4	28	21	34
sample 5	45	29	41
sample 6	21	28	35
sample 7	40	38	23
sample 8	32	28	40
sample 9	38	36	33
sample 10	42	28	19

Fig. 3: Reproducibility studies of the number of single colonies isolated utilizing manual methodology

Reproducibility studies demonstrated a clear improvement in CV values for the automated methodology versus the manual methodology.

Statistical analysis for the number of single colonies produced:

Data was assessed for normality for each batch. It was concluded from the analysis with a confidence limit of 95% that the populations did not deviate significantly from normality

Results of Paired T-Test and CI: (Sigma SP™ collection device & Kiestra) versus manual method.

	Normality	Mean	Standard Deviation	SE Mean
16K31	10	75.20	6.83	2.16
Manual method	10	38.70	11.21	3.54
Difference	10	36.50	13.50	4.27

The improvement team in the laboratory believes the utilization of a new Sigma SP™ liquid collection device coupled with automated inoculating Kiestra technology delivers a number of improvements

The hypothesis was:

H0: Null hypothesis: There is no difference in the mean number of colonies isolated for the traditional versus liquid-automated methodologies

H1: Alternative hypothesis: There is a difference in the mean number of colonies isolated for the traditional versus liquid-automated methodologies

95% CI for mean difference: (26.84, 46.16)

T-Test of mean difference = 0 (vs ≠ 0): T-Value = 8.55 P-Value = 0.000

Result

As P is low we reject the Null hypothesis as there is sufficient evidence to suggest that there is a difference between the numbers of single colonies produced between the two methodologies with a confidence level of 95%

The boxplot of data clearly demonstrates visually the improvement in the number of single colonies

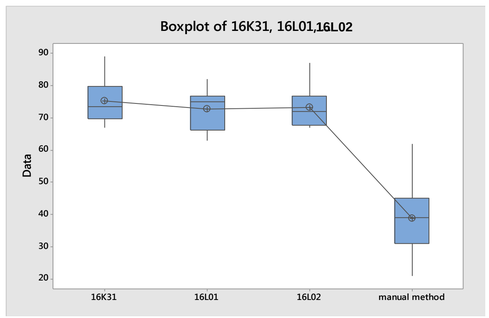


Fig. 4: Number of singles colonies produced utilzing different batch number of sigma SP collection devices (3) versus manual method

Tukey Pairwise Comparisons: Clearly shows the significant difference between manual and automated/SigmaSP™ liquid methods.

Grouping Information Using the Tukey Method and 95% Confidence

Factor	Normality	Mean	Grouping
16K31	10	75.20	A
16L02	10	73.20	A
16L01	10	72.80	A
Manual Method	10	38.70	B

Means that do not share a letter are significantly different. i.e. Manual method (B) is significantly different to the automated batches (16K31, 16L02 and 16L01)

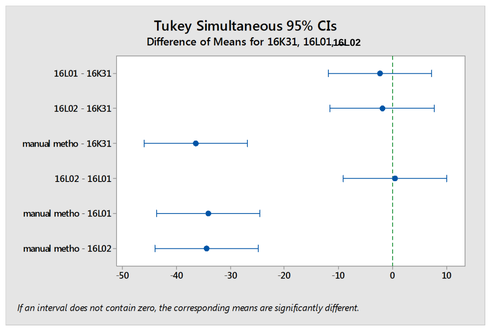


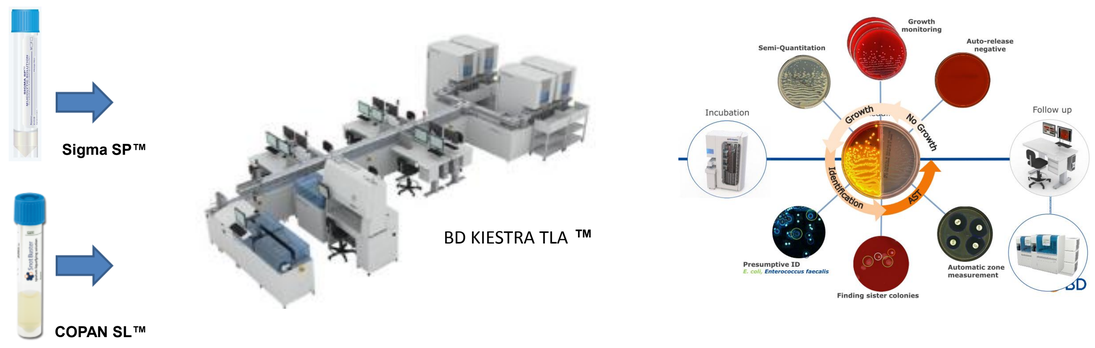
Fig. 5: Tukey Pairwise Comparisons of the number of single colonies between manual and automated/SP liquid swabs

Means that do not share a letter are significantly different. This data clearly demonstrates that there is a significant difference between the automated/Sigma SP collection device and the manual traditional method of processing sputum.

The Sigma SP collection devices demonstrated exceptional reproducibility when integrated into the work-flow of the Inoqula FA and digital imaging protocols. The manual methodology reproducibility demonstrated a large degree of variability on the number of single colonies and spread pattern

Methods

- In this study, the Copan SL™ solution device (Copan Italia; Brescia, Italy) and the MWE Sigma SP™ liquid collection device containing a novel mucolytic agent were used. The Copan SL™ solution is a ready to use system for liquefying sputum specimens (it contains dithiothreitol as the active principle). SL solution is used to pre-treat mucus-rich respiratory specimens for culturing, and molecular tests. Copan developed the SL-Solution (SL), a ready-to-use mucus-dissolving solution in tubes with 1.0 ml aliquots. The Sigma SP™ device contains a novel patented mucolytic agent.
- Anonymised clinical samples were split and processed via a routine non-automated methodology including streaking and manual incubation, and in parallel through a separate work stream utilizing the Sigma SP™ liquid collection device and the BD Kiestra pre-analytical InoqulA FA automation and automated incubation with digital reading.
- Reproducibility studies were also conducted in which the manual method and Sigma SP™ with full automation (FA)were repeated in triplicate across ten different specimens.
- Statistical analysis was performed utilising Minitab® 17 software. Normality, equal variances, paired T-Test and Tukey Pairwise Comparisons were performed on the data.
- The compatibility of the Sigma SP™ collection device was tested with the Biomerieux FilmArray® system utilizing NATtrol RP Multimarker Control Pack (product code MDZ001) control strains.
- The study specifically looked at inhibition rates for *Mycobacterium tuberculosis* complex (MTB) detection with the Sigma SP™ collection device (MWE) and the Copan SL™ solution collection device utilizing the Xpert® MTB/RIF (Cepheid). Standard protocols were followed as per manufacturer's guidance for the Xpert®.
- Additionally, time to detection of spiked positive *M. tuberculosis* complex (MTB) as well as atypical strains utilizing the Sigma SP™ and Copan SL™ collection devices were monitored utilizing the Becton Dickinson MGIT™ system.



Figs 1 & 2

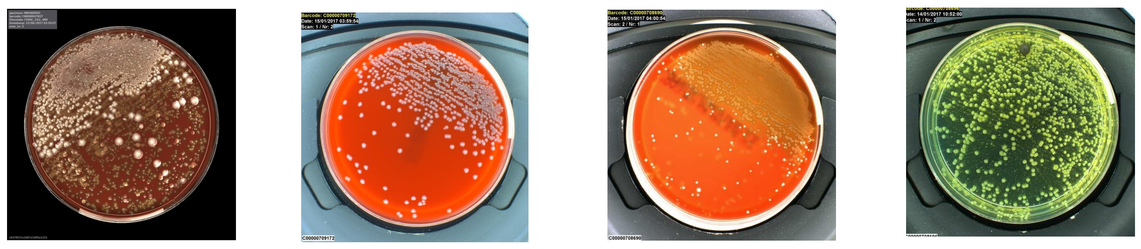
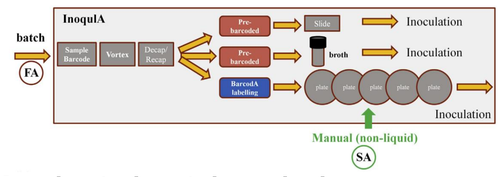


Fig. 6: The images above demonstrate clear evidence that the whole surface area of the plate was utilised. This results in an increase number of single colonies available for secondary testing such as MALDI-TOF

Utilisation of the MWE Sigma SP™ collection devices allows for the full integration of sputum samples onto the Kiestra Total Lab automation system (TLA) as per workflow represented below. This integration is key in reducing labour costs, standardization, superior streaking and linked to requirements of sample and process traceability as per ISO 15189 standards



Downstream testing for *Mycobacterium tuberculosis*

Compatibility with FilmArray®:

Initial studies demonstrated all NATtrol RP Multimarker Control Pack targets were detected direct from the Sigma SP collection device

Compatibility with Xpert® MTB/RIF (Cepheid)for Sigma SP™ collection device and Copan SL™ solution device

The recovery of *M. tuberculosis* DNA at a constant level (CT 15-18) following storage of up to 48hrs at both room temperature and 4^0C was achieved for the MWE Sigma SP™ collection device. The Copan SL™ solution device showed variable results at both room temperature and at 4^0C. This indicates potential inhibitors within the Copan SL™ device for the detection of molecular targets. Fontana et al., 2013 indicated a 10% inhibitory rate for the Copan SL™ collection device however this was with a 2:1 ratio of sputum to mucolytic reagents i.e. 2 ml:1 ml. This study focused on a 1:1 ratio of sample to mucolytic reagent. This may explain the greater inhibitory rate demonstrated here. The majority of inhibited samples were sputum, which are especially difficult to treat because they are rich in mucus. The may indicate a greater efficacy of the mucolytic reagent utilised in the MWE Sigma SP™ collection device that the traditional dithiothreitol contained in the Copan SL™ solution collection device.

Initial studies utilizing Sigma SP™ collection device for the detection of mycobacterium with the BD MGIT system has demonstrated excellent concordance versus the traditional method of collection.

Table 5: Percentage of spiked MTB complexes collection devices at holding periods of 0, 24 and 48hrs

Detected (percentage)	0 hrs	48hrs	72hrs
Sigma SP device (RT)	100	100	100
Sigma SP device (4^0C)	100	100	100
Copan SL device (RT)	50	50	40
Copan SL device (4^0C)	60	50	50

Conclusion

The Sigma SP™ coupled with total lab automation leads to a more standardized approach to traditional sputum processing. It minimises the interaction of the human from the process and thus mistakes proofing it. It allows for complete audit tracking of the pre-analytical phase. It saves on traditional biomedical support worker pay costs and ensures that the assets are utilised to their maximum i.e. The InoqulA FA module.

The novel mucolytic agent demonstrated good mucolytic action on the Kiestra InoqulA FA system. It enables the emulsification of sputum and mucus resulting in a homogenous suspension allowing for easier, more consistent and reproducible planting and streaking of specimens.

The study demonstrated an improvement in the quality of the pre-analytical phase i.e. better utilization of the plate surface and increased number of single colonies versus the manual methodology thereby improving downstream reading, interpretation and reporting by technical staff. In addition, the Sigma SP™ collection device demonstrated compatibility with the FilmArray® system, as well as the BD MGIT ™ system and Cepheid GeneXpert ® for the detection of MTB complex. The compatibility of a single collection device with additional investigations such as mycobacterium whether it is a standard liquid culture approach or molecular is of particular importance. Additional non-tangible advantages are time savings for medical or nursing staff (less confusion in collection device selection and fewer samples being collected); time savings for laboratory staff (fewer samples to access and handle for individual investigations); and patient comfort improvement (multiple sample collection can be avoided).

'Eighty-five percent of the reasons for failure to meet customer expectations are related to inefficiencies in systems and process… rather than the employee.

The role of management is to change the process rather than badgering individuals to do better.

References:

1. Fontana C, Favaro M and Favalli C (2013) "How Liquid Based Microbiology Can Change the Workflow in the Microbiology Laboratories," Advances in Microbiology, Vol. 3, pp. 504-510.
2. Jones G, Matthews R, Cunningham R and Jenks P (2011) "Comparison of Automated Processing of Flocked Swabs with Manual Processing of Fiber Swabs for Detection of Nasal Carriage of Staphylococcus aureus," Journal of Clinical Microbiology, Vol. 49, No. 7, pp. 2717- 2718.
3. Van Horn K, G, Audette C, Tucker K and Sebeck D (2008) "Comparison of 3 Swab Transport Systems for Direct Release and Recovery of Aerobic and Anaerobic Bacteria," Diagnostic Microbiology and Infectious Diseases, Vol. 62, No. 4, pp. 471-473.

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