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Molecular efficacy of two commercially available liquid transport collection devices for downstream testing on the GeneXpert® platform and the Roche FLOW® system

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Introduction

Simple workflow methodologies are prerequisites for efficiency and reflex testing which often incorporates molecular testing. In this context, and given the increased awareness of the importance of both molecular and traditional testing methodologies, selection of the most versatile collection device is paramount.

Objective

To evaluate the performance of the eSwab™ (Copan, Italy) and ∑-Transwab® (foam tip)(MWE, UK), ∑-Transwab® PF (PurFlock® tip) (MWE, UK) for the transport and maintenance of Influenza A H3N2 Virus, Influenza B Virus , Respiratory Syncytial Virus (RSV-A) Type A, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 12344 and *Neisseria gonorrhoeae* ATCC 43069 for molecular testing, and to compare its performance with the gold standard of ∑- Virocult® medium (MWE) for virus.

Fig 1: Copan Diagnostics eSwab®

Fig 2: MWE ∑- Transwab®

Method

Phase 1

Detection of ZeptoMetrix controls and ATCC cultured organisms was assessed for the collection devices utilising the Roche FLOW® and Cepheid GeneXpert® XVI systems with the Fast Track Diagnostics (FTD) RESP 21 CE marked multiplex PCR assay; Xpert® MRSA/SA SSTI kit (GXMRSA/SA-SSTI-CE); Xpert® Strep A (XPRSTREPA-CE-10) and Xpert® CT/NG (XCT/NG-CE-10).

- A 10^5 U/mL pooled solution of Flu A, Flu B and RSV was prepared (Zeptometrix NATtrol™ Influenza/RSV Positive Control (NATFLURSV-6L)
- Each swab type was inoculated in duplicate with 100µL of the pooled solution prepared.
- Collection devices were held for 24h at room temperature (21°C).
- After the holding period , the collection devices transport media were tested in duplicate for Flu A, Flu B and RSV using the Roche FLOW system and the Fast Track Diagnostics (FTD) RESP 21 CE marked multiplex PCR assay
- Results obtained from eSwab™ (Copan, Italy) and ∑-Transwab® (foam tip)(MWE, UK), ∑-Transwab® PF (PurFlock® tip) (MWE, UK) and Sigma Virocult® (MWE, UK) were analysed and compared using PCR Ct values.
- The Sigma Virocult® media (MWE) was seen as the "gold standard"
- Sensitivity of the different collection devices was assessed by using a dilution of 1:100 of the pooled solution

Phase 2

The hypothesis to be tested in phase two was the impact of bacterial cultures on the target molecular efficacy of the collection devices when all targets were pooled together and left at a holding period of 24 hrs at room temperature.

- Each swab type was inoculated in duplicate with 200µL of the pooled solution prepared containing know clinical positives of Flu A, B and RSV with bacterial cultures of:
- *Streptococcus pyogenes* ATCC 12344
- *Neisseria gonorrheae* ATCC 43069
- *Staphylococcus aureus* ATCC 25923
- The known clinical positive material of Flu A, Flu B and RSV had previously been tested from Sigma Virocult® medium
- Collection devices were held for 24 hours at room temperature (21°C).
- After the holding period , the collection devices transport media were tested in duplicate for Flu A, Flu B and RSV using the Roche FLOW system and the Fast Track Diagnostics (FTD) RESP 21 CE marked multiplex PCR assay (Appendix 1_kit insert) targeting influenza A (FA), influenza A (H1N1), influenza B (FB), coronaviruses NL63, 229E, OC43 and HKU1 (C63, C229, C43, HKU), parainfluenza 1,2,3,4, (PF1,2,3,4), human metapneumovirus A and B (MPV), rhinovirus (RHI), respiratory syncytial viruses A and B (RSV), adenovirus (ADE), enterovirus (ENT), parechovirus (PARE), bocavirus (BOCA) and *Mycoplasma pneumoniae* (MYCO) including internal control (IC) (Equine arteritis virus).
- The collection devices were also tested on the Cepheid GeneXpert® XVI system utilising the Xpert® MRSA/SA SSTI kit (GXMRSA/SA-SSTI-CE); Xpert® Xpress Strep A (XPRSTREPA-CE-10) and Xpert® CT/NG (XCT/NG-CE-10).
- Manufacture instructions were followed for the Roche FLOW and GeneXpert® XVI systems
- Results obtained from eSwab™ (Copan, Italy), ∑-Transwab® Foam (MWE), ∑-Transwab® PurFlock (MWE) and ∑-Virocult® media (MWE) were analyzed and compared using PCR Ct values.

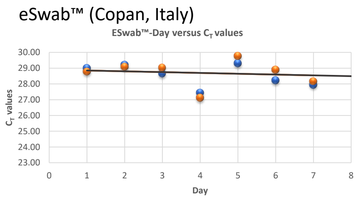
Results

Statistical significant differences in cycle threshold (C_t) values were observed between the Copan eSwab® and Medical Wire and Equipment collection devices utilized in this study when evaluating their efficacy in detecting the ZeptoMetrix control targets.(Phase 1) Furthermore, inhibition in some cases was observed when utilising pooled samples of viral and bacterial targets for the Copan eSwab™.(Phase 2)

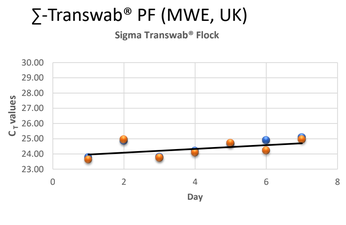
Phase 1

Flu A on the Roche FLOW

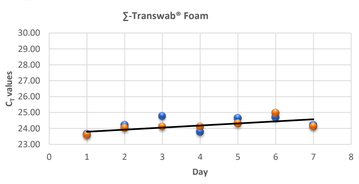
eSwab™ (Copan, Italy)



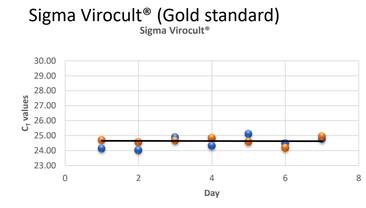
∑-Transwab® PF (MWE, UK)



∑-Transwab® Foam (MWE, UK)



Sigma Virocult® (Gold standard)

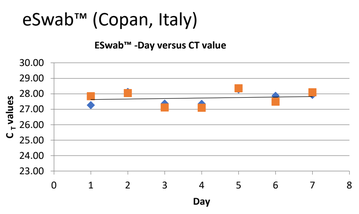


Notes for Flu A

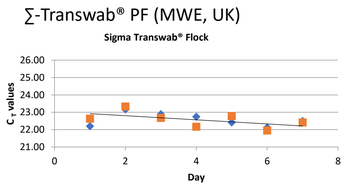
- **Variation within the swabs types: significantly different (p> 0.05) except for Sigma Virocult®.**
- **Overall precision is good for each swab type**
- **Combined SD for ESwab™ is 0.77 while the MWE devices have are 0.43; 0.55 and 0.33, respectively**
- **Paired t-Test shows that is no significant difference between the three MWE devices including the "gold standard" of the Sigma Virocult® (p >0.05).**
- **Significant difference between the eSwab™ and the MWE collection devices (P <0.05). Approx. 5 CT**

Flu B on the Roche FLOW

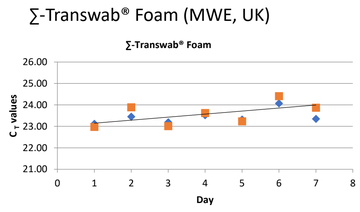
eSwab™ (Copan, Italy)



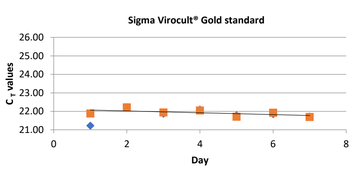
∑-Transwab® PF (MWE, UK)



∑-Transwab® Foam (MWE, UK)



Sigma Virocult® (Gold standard)

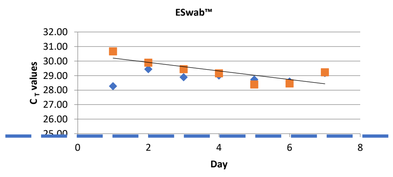


Notes for Flu B

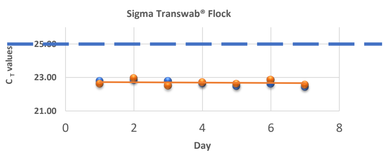
- **Variation within the swabs types: significantly different (p> 0.05) except for Sigma Virocult®.**
- **Overall precision is good for each swab type**
- **Combined SD for ESwab™ is 0.44 while the MWE devices have are 0.43, 0.40 and 0.25, respectively**
- **Paired t-Test shows that is no significant difference between the three MWE devices including the "gold standard" of the Sigma Virocult® (p >0.05).**
- **Significant difference between the eSwab™ and the MWE collection devices (P <0.05) by approx. 5 CT**

RSV on the Roche FLOW

eSwab™ (Copan, Italy)

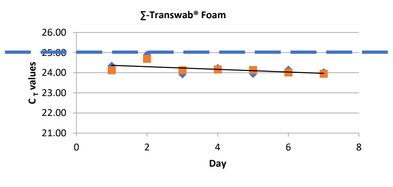


∑- Transwab® PF (MWE, UK)

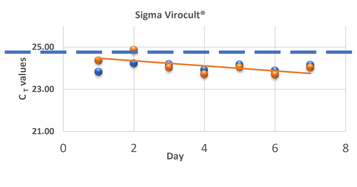


CT = 25

∑-Transwab® Foam (MWE, UK)



Sigma Virocult® (Gold standard)



Notes for RSV

- **Variation within the swabs types: significantly different (p> 0.05) except for Sigma Virocult®.**
- **Overall precision is good for each swab type**
- **Combined SD for ESwab™ is 0.64 while the other MWE devices have are 0.28 ; 0.15 and 0.30, respectively**
- **Paired t-Test shows that is a significant difference between the three MWE devices including the "gold standard" of the Virocult® (p >0.05). But not in PCR terms**
- **Significant difference between the ESwab™ and the MWE collection devices (P <0.05) by approx. 5 CT**

Phase 2

Pooled Bacterial and Viral Targets* (GeneExpert & Roche FLOW)

	eSwab			∑-Transwab® Foam			∑-Transwab® PurFlock			∑-Virocult®		
Day	**Target Flu A**	**Average CT**	**Median CT**	**Target Flu A**	**Average CT**	**Median CT**	**Target Flu A**	**Average CT**	**Median CT**	**Target Flu A**	**Average CT**	**Median CT**
1	inhibited	-	**27.83**	Detected	24.11	**24.39**	Detected	23.72	**23.78**	Detected	22.73	**22.68**
2	Detected	28.17		Detected	24.11		Detected	23.74		Detected	22.68	
3	Detected	27.64		Detected	24.40		Detected	23.89		Detected	22.59	
4	inhibited	-		Detected	24.57		Detected	23.73		Detected	22.60	
5	Detected	27.62		Detected	24.43		Detected	23.79		Detected	22.70	
	Target Flu B	**Average CT**	**Median CT**	**Target Flu B**	**Average CT**	**Median CT**	**Target Flu B**	**Average CT**	**Median CT**	**Target Flu B**	**Average CT**	**Median CT**
1	Inhibited	-	**29.70**	Detected	25.12	**24.82**	Detected	24.20	**24.07**	Detected	23.01	**23.01**
2	Detected	29.67		Detected	24.65		Detected	23.88		Detected	23.00	
3	Detected	29.97		Detected	24.98		Detected	24.06		Detected	23.09	
4	Inhibited			Detected	24.89		Detected	23.93		Detected	22.97	
5	Detected	29.55		Detected	24.66		Detected	24.31		Detected	22.93	
	Target RSV	**Average CT**	**Median CT**	**Target RSV**	**Average CT**	**Median CT**	**Target RSV**	**Average CT**	**Median CT**	**Target RSV**	**Average CT**	**Median CT**
1	Inhibited	-	**28.58**	Detected	23.48	**23.44**	Detected	23.24	**23.40**	Detected	22.69	**22.58**
2	Inhibited	28.86		Detected	22.94		Detected	23.38		Detected	22.46	
3	Detected	28.58		Detected	22.84		Detected	23.30		Detected	22.64	
4	Detected	-		Detected	23.73		Detected	23.48		Detected	22.44	
5	Detected	28.06		Detected	23.57		Detected	23.75		Detected	22.60	
	Target *Staphylococcus aureus*	**Average CT**	**Median CT**	**Target *Staphylococcus aureus***	**Average CT**	**Median CT**	**Target *Staphylococcus aureus***	**Average CT**	**Median CT**	**Target *Staphylococcus aureus***	**Average CT**	**Median CT**
1	Detected	23.07	**23.09**	Detected	17.87	**17.86**	Detected	16.69	**16.47**	Detected	16.17	**16.25**
2	Detected	23.20		Detected	17.69		Detected	16.42		Detected	16.10	
3	Detected	23.13		Detected	17.93		Detected	16.43		Detected	16.26	
4	Detected	22.98		Detected	17.68		Detected	16.51		Detected	16.29	
5	Detected	23.08		Detected	18.02		Detected	16.56		Detected	16.30	
	Target *Neisseria gonorrhoeae*	**Average CT**	**Median CT**	**Target *Neisseria gonorrhea***	**Average CT**	**Median CT**	**Target *Neisseria gonorrhea***	**Average CT**	**Median CT**	**Target *Neisseria gonorrhea***	**Average CT**	**Median CT**
1	Inhibited	-	**18.52**	Detected	17.56	**17.44**	Detected	16.91	**16.94**	Detected	15.44	**15.32**
2	Detected	18.50		Detected	17.15		Detected	16.96		Detected	15.53	
3	Detected	18.71		Detected	17.51		Detected	16.99		Detected	15.39	
4	Detected	18.68		Detected	17.49		Detected	17.01		Detected	15.34	
5	Inhibited	18.37		Detected	17.53		Detected	16.91		Detected	15.24	
	Target *Streptococcus pyogenes*	**Average CT**	**Median CT**	**Target *Streptococcus pyogenes***	**Average CT**	**Median CT**	**Target *Streptococcus pyogenes***	**Average CT**	**Median CT**	**Target *Streptococcus pyogenes***	**Average CT**	**Median CT**
1	Detected	20.48	**20.44**	Detected	20.10	**19.98**	Detected	18.59	**19.56**	Detected	17.96	**17.76**
2	Detected	20.28		Detected	19.92		Detected	18.69		Detected	17.43	
3	Detected	20.43		Detected	19.90		Detected	19.89		Detected	17.90	
4	Detected	20.37		Detected	20.10		Detected	19.77		Detected	17.73	
5	Detected	20.90		Detected	19.49		Detected	19.56		Detected	17.64	

*** Targets Flu A, Flu B, & RSV tested with Roche FLOW; Targets *S. aureus, N. gonorrhoeae* & *S. pyogenes* tested with GeneXpert**

Sensitivity for Roche FLOW

Target Flu A (1:100 dilution)	R1 C_T value	R2 CT value	Average	Median
eSwab™				
Detected	37.94	38.28	38.11	**38.75**
Detected	37.82	37.09	37.46	
Detected	38.67	38.83	38.75	
Detected	37.12	37.28	37.20	
Not Detected	39.48	39.28	39.38	
Not Detected	39.43	39.91	39.67	
Not Detected	39.95	39.17	39.56	
∑-Transwab®				
Detected	26.98	27.18	27.08	**27.25**
Detected	27.21	27.02	27.12	
Detected	27.28	27.12	27.20	
Detected	27.38	27.32	27.35	
Detected	27.65	27.52	27.59	
Detected	27.71	27.58	27.65	
Detected	27.13	27.1	27.12	

Target Flu A (1:100 dilution)	R1 C_T value	R2 CT value	Average	Median
∑-Transwab® PF				
Detected	27.15	27.52	27.34	27.34
Detected	27.26	27.76	27.51	
Detected	27.11	27.36	27.24	
Detected	27.28	27.13	27.21	
Detected	27.47	27.32	27.40	
Detected	27.89	27.53	27.71	
Detected	27.13	27.45	27.29	
∑- Virocult®				
Detected	27.01	26.86	26.94	**27.09**
Detected	26.84	26.97	26.91	
Detected	27.23	27.14	27.19	
Detected	26.98	27.04	27.01	
Detected	27.12	27.18	27.15	
Detected	27.15	27.06	27.11	
Detected	27.16	27.31	27.24	

Discussion

Microbiology has seen huge transformation in the analytical and post analytical steps of the process improving both sensitivity and specificity. Sample collection quality is crucial for the quality of the subsequent analytical steps and therefore, any improvements of this first step will benefit the whole diagnostic process.

Although leading to inferior diagnostic quality, sample collection utilising swabs is the preferred technique for most clinicians because of its performance ease and swiftness. Thus, if the general approach for sample collection cannot be changed, it appears prudent to optimize swabs and swabbing techniques especially with respect to test sensitivity. As a prerequisite for optimization, test sensitivities of presently available swabs should be quantified under conditions close to natural circumstances.

Conclusion

This study extends and validates the multipurpose use of the ∑-Transwab® foam and ∑-Transwab® PurFlock beyond bacteriological investigations to include viral targets. The ∑-Transwab® Foam (MWE) and ∑- Transwab® PurFlock (MWE) are a truly open swab platform suitable for automation, gram stains, traditional culture, and molecular assays. The study has highlighted a number of potential issues with the Copan eSwab™ in a direct comparison to the MWE ∑-Transwab® foam and ∑- Transwab® PurFlock.

This study was supported by MWE

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