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Evaluation of a new PCR-based platform for the detection and identification of viruses or bacteria from swab transport devices

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ABSTRACT

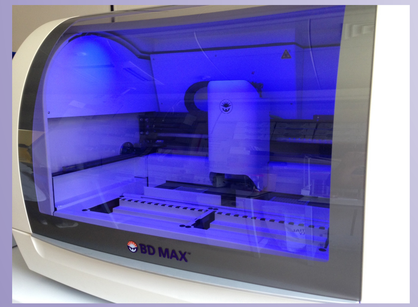
Objectives
Significant technological advances are being made in diagnostic microbiology. An example is BD Max®, a platform that uses PCR methods for the rapid detection and identification of bacteria and viruses. Most of the transport devices in current use were developed for use with culture methods. The best devices will maintain microorganisms in a viable condition, but this may not guarantee compatibility with molecular platforms. The present study has been designed to evaluate some novel devices to assess their suitability for use with the BD Max® platform.
Fecal Transwab® (Medical Wire) is a rectal swab based device for recovery of enteric pathogens from fecal specimens. The device using either PurFlock® flocked swabs or Sigma polyurethane foam swabs was tested with stool specimens previously shown to be positive for Salmonella, Shigella,or Campylobacter.
Sigma Virocult® (Medical Wire) is a virus transport swab. Clinical specimens which had previously tested positive on SmartCycler® for either Herpes Simplex Virus Type 1 or Herpes Simplex Virus Type 2 were retested on the BD Max®.

Methods
In this study the performance of Fecal Transwab (MWE) with a BD Max® Fecal Panel (for detection of Salmonella, Shigella, Campylobacter and or E. coli O157) was investigated. Swabs with buds of either polyurethane foam (PU) or PurFlock® Polyester (PF) were inoculated from known positive stool samples. The Sigma Virocult® swabs were all clinical specimens which had previously been tested on the SmartCycler® platform (Biomerieux)

Results
With the BD Max Fecal Panel, all Fecal Transwab specimens were correctly identified, with no difference between PurFlock® or polyurethane foam bud swabs. With the HSV Panel, all Sigma Virocult specimens were correctly identified using the BD Max HSV panel. The Ct values were lower than the equivalent values for SmartCycler® indicating greater sensitivity.

Conclusions
The new BD Max® PCR platform correctly identified all specimens, whether enteric bacteria from Fecal Transwab®, or herpes simplex viruses from Sigma Virocult®.

Introduction
Significant technological advances are being made in diagnostic microbiology. An example is BD Max®, an open platform that uses PCR methods for the rapid detection and identification of bacteria and viruses. Most of the transport devices in current use were developed for use with culture methods. The best devices will maintain microorganisms in a viable condition, but this may not guarantee compatibility with molecular platforms. The present study has been designed to evaluate some novel devices to assess their suitability for use with the BD Max® platform.



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Methods
Fecal Transwab® (Medical Wire) is a rectal swab based device for recovery of enteric pathogens from fecal specimens. The device was tested with stool specimens previously shown by direct testing to be positive for Salmonella, Shigella, Campylobacter, and also for Shiga Toxin. The device was tested in its current format with a Sigma Purfoam Swab (polyurethane foam tip), and also in a development format with a PurFlock Swab (multilength multifilament flocked fibre).
In this study the performance of Fecal Transwab® with a BD Max® Fecal Panel (for detection of Salmonella, Shigella, Campylobacter or E. coli O157) was investigated. Swabs with buds of PurFlock® Polyester (PF) were inoculated from known positive stool samples. 38 confirmed positive specimens were tested, together with 6 confirmed negative stool specimens, and 6 uninoculated devices.
Sigma Virocult® (Medical Wire) is a virus transport swab. 36 clinical specimens using these swabs which had previously tested positive on SmartCycler® for either Herpes Simplex Virus Type 1 or Herpes Simplex Virus Type 2 were retested on the BD Max®.

Results

Table 1. Results from first run* using Fecal Transwab® and BD Max.

Sample ID	Culture result	PurFlock® Bud				PurFoam Bud			
		SH	ST	CA	SA	SH	ST	CA	SA
A1/2	Shigella	+	-	-	-	+	-	-	-
A3/4	Shigella	+	-	-	-	+	-	-	-
A5/6	Salmonella	-	-	-	+	-	-	-	-
A7/8	Salmonella	-	-	-	+	-	-	-	-
A9/10	Campylobacter	-	-	+	-	-	-	+	-
A11/12	Campylobacter	-	-	+	-	-	-	+	-
B1/2	Campylobacter	-	-	+	-	-	-	+	-
B3/4	Campylobacter	-	-	+	-	-	-	+	-

SH=Shigella, ST=Shiga Toxin, CA= Campylobacter. SA=Salmonella
**Two further runs were completed using additional samples as noted. In all cases PCR results were in agreement with the results from culture.*

Fecal Transwab
Results from BD Max for known positive stools retested using Fecal ®Transwab with PurFlock® bud.

SH = Shigella,
ST = Shiga Toxin,
CA = Campylobacter.
SA = Salmonella

Samples N1 - N6 are confirmed culture negative stool specimens
Samples B1-B6 are uninoculated devices

With the BD Max Fecal Panel, all 50 Fecal Transwab® specimens were correctly identified. There was no difference between devices with Purfoam swabs and those with the PurFlock swabs. The identified specimens included 10 Shigella, 20 Campylobacter, 4 Salmonella, 4 Shiga Toxin, 6 confirmed negative specimens, and 6 uninoculated devices.

Sigma Virocult
Results from BD Max for known positive specimens of HSV 1 and HSV2 using Sigma Virocult® Results are shown as Ct values.

With the HSV Panel, all Sigma Virocult® PF specimens were correctly identified using the BD Max HSV panel. The Ct values were lower than the equivalent values for SmartCycler® indicating greater sensitivity.

Table 2. Results from first run* using Sigma Virocult®
BD MaxResults are shown as Ct values

Sample ID	SmartCycler		BD Max		
	Result	Ct value	HSV 1	HSV 2	CONTROL
A	HSV 2	34.1		28.6	27.4
B	HSV2	33.1		25.9	25
C	HSV2	32.2		27.8	27.6
D	HSV2	23.7		22.4	25.8
E	HSV2	22.6		19.1	26.4
F	HSV2	24.8		20.5	-
G	HSV2	26.3		21.8	25.2
H	HSV1	33.3	17.7		26.3
J	HSV1	29.4	21.4		27.3
K	HSV1	29.2	20.8		26
L	HSV1	33.5	25.3		26.3
M	HSV1	25.2	14		27.6

**Two further runs were completed using additional samples as noted. In all cases BD Max results were in agreement with the results from SmartCycler.*

Additional data to assess compatibility of Sigma Transwab® with BD Max MRSA and SA Test System
In addition to the main experiments reported here, further tests were run to assess the compatibility of Sigma Transwab® with the BD Max™System. The targets chosen for this study were MRSA and Staphylococcus aureus, two of the organisms most widely investigated by culture of Sigma Transwab®. Sigma Transwab® is a transport swab with liquid Amies medium, routinely available with PurFoam applicators. For this study some dilutions were also tested using PurFlock® applicators.

A 0.5McFarland suspension of an MRSA control strain was prepared and serially diluted. 100µl aliquots of 10-2 dilution and 10-3 dilution were dispensed into the wells of a microtitre plate. This was repeated for a control strain of Staphylococcus aureus (SA).

For MRSA, 60 Sigma Transwabs® (with PurFoam or PurFlock® applicators) were inoculated by immersing the swab into the microtitre well and absorbing as much as possible of the suspension. 48 swabs were tested with 10-2 dilution , and 12 with 10-3 dilution. Each swab was then placed into its tube containing 1ml of liquid Amies medium. The swab was snapped off, and the cap replaced. The tube was vortexed, after which the tube was uncapped and 100µl withdrawn and placed into the PCR tube for processing on BD Max. The MRSA samples were tested with BD MAX MRSA XT. For Staphylococcus aureus, 24 Sigma Transwabs® (with PurFoam applicators) were inoculated with 10-2 dilution and tested with BD Max SR. 6 negative controls (uninoculated Sigma Transwab® tubes) were also tested.

Overall 90 Sigma Transwab® devices were tested on the BD Max platform. All tests gave the correct results.

Results

Target	Dilution	Applicator	No of Tests	MRSA Positive	MRSA Negative	SA Positive	SA Negative
MRSA	10-2	PU	24	24	0	0	24
	10-2	PF	24	24	0	N/A	N/A
	10-3	PF	12	12	0	N/A	N/A
SA (Staphylococcus aureus)	10-2	PU	24	0	24	24	0
Negative Control			6	0	6	0	0

PU = PurFoam, PF = PurFlock®

Conclusions
Fecal Transwab
The new BD Max® PCR platform correctly identified all specimens of enteric bacteria from Fecal Transwab®.
Sigma Virocult
The new BD Max® PCR platform correctly identified all specimens of herpes simplex viruses from Sigma Virocult®.
Sigma Transwab®
The new BD Max® PCR platform correctly identified all specimens of MRSA and SA from Sigma Transwab®.

Fecal Transwab , Sigma Virocult® and Sigma Transwab® were all shown to be compatible the BD Max Platform. While all users must still validate the use of the swabs on any particular system, the results demonstrate that these devices can be used on a PCR based platform.