Copan M-Swab, a viral and bacterial preservation medium for Nucleic acids amplification assays and culturing methods.

R.Botrugno[1], A.Giambra[1], A.Squassina[1], A. Gervasoni[1], B. Massetti[1], S. Castriciano[1,2]
[1]Copan Italia SpA, Brescia, Italy. [2]St. Joseph's Healthcare, Hamilton, Canada

ECCMID 2013 BERLIN Poster P1919

Background

Dedicated transport media are available for collection of clinical specimens for bacteria culture and other for viruses culture. A medium that supports culture and nucleic acid detection for viruses and bacteria would improve sample procurement for centralized microbiology laboratory.

Copan developed M-Swab, a new pre-analytic device for the collection, transportation and processing of clinical specimens that supports aerobic and anaerobic facultative Gram positive bacteria and viruses culture and can be used for both nucleic extraction with extraction platforms or as an extraction-free direct testing by real-time PCR.

Objectives

To validate the M-Swab medium for:

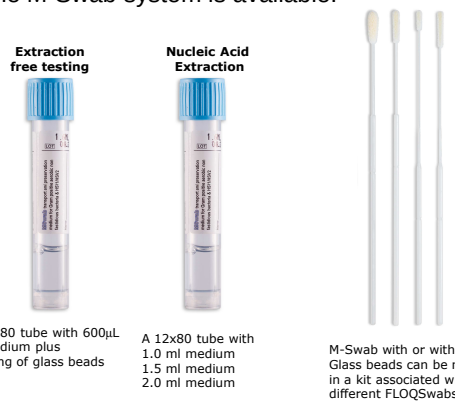
1) bacteria and viruses viability with culture methods.

2) viruses and bacteria nucleic acid preservation for:

- Extraction-free nucleic acid detection by real time PCR;
- Nucleic acid extraction with commercial platforms for molecular amplification assays.

Materials

The M-Swab system is available:



A 12x80 tube with 600µL of medium plus 150 mg of glass beads

A 12x80 tube with 1.0 ml medium 1.5 ml medium 2.0 ml medium

M-Swab with or without Glass beads can be made in a kit associated with different FLOQSwabs™

Methods

For this study, MRSA and SA ATCC bacteria strains as per CLSI M40 guidelines, viruses stock from ATCC strains of HSV1, HSV2, and clinical specimens were used.

For bacteria culture validation, serial dilutions of a fresh isolate of MRSA and SA were prepared and 100 µL of the dilution that was giving 30 to 300 colonies (10^{-4} dilution) were used to inoculate both M-Swab and ESwab tubes.

Inoculated tubes were held at room temperature (20-25 ^{0}C) and 4^{0}C for T=24h and T=48h. At T=0, and after T=24h and T=48h, 100 µL of both inoculated M-Swab and ESwab tubes were seeded onto Blood Agar plates and incubated for 24-30 h at 35^{0}C.

For viruses culture validation, dilutions of a frozen stock of HSV1 and HSV2 were prepared, as per previous titration, and 200 µL of a high and low dilution were inoculated into a M-Swab and UTM media tubes. A regular flocked swab was added to each tube.

M-Swab and UTM mocked samples were vortexed and 200 µL were inoculated in shell vials cultures at 0 time, and after 24 and 48 hours at 4 ^{0}C and room temperature storage.

Shell vials were incubated in the appropriated environment for 48 hours, fixed, DFA stained and infected cells were recorded.

200 µL of the MRSA, SA, HSV 1 and HSV 2 of the mock M-Swab, ESwab and UTM sample were used for nucleic acid extraction.

Clinical specimens (90 for HSV and 60 for respiratory) collected in M-Swab were tested by culture, and a subset was used for molecular testing using nucleic extraction with extraction platforms or as an extraction-free direct testing by real-time PCR.

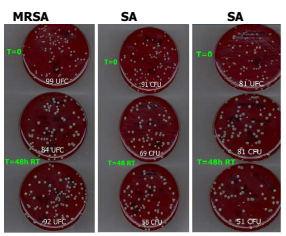
After vortexing and swab removal, an aliquot of the M-swab mocked and clinical after vortexing, was boiled for 5 minutes, cooled to room temperature and briefly vortexed and spun at high speed prior to nucleic acid testing.

200 µL aliquot of inoculated M-Swab and UTM clinical samples, were used to extract nucleic acids with easyMAG (bioMérieux).

Purified nucleic acids (5 µL) were tested by RT-PCR for HSV1&2 (Qiagen), Path-MRSA (Primer design) on the Rotorgene and Influenza A and B and other respiratory viruses with the Xtag RVP assay (Luminex).

Results

MRSA , SA and HSV1 and HSV2 Culture Stability and Molecular Results



ATCC Strains	T=0	T=48	
	0 time	Plate 1	Plate 2
MRSA 43300	91	69 79	58 69
SA 25923	130	68 74	99 87
SA 6538	84	51 54	49 60

Media	HSV1 Dilutions	Inoculation times		
		0 Time	48h 4ºC	48h RT
M-Swab	Dil 1	250	134	120
	Dil 2	50	30	25
UTM	Dil 1	250	205	213
	Dil 2	50	36	39

Media	HSV2 Dilutions	Inoculation times		
		0 Time	48h 4ºC	48h RT
M-Swab	Dil 1	200	150	100
	Dil 2	40	20	30
UTM	Dil 1	200	165	170
	Dil 2	40	31	26

M-Swab Clinical Samples	Clinical Results	Shell vial Cell culture After room temperature storage		
		24 h	72 h	7 days
HSV1	29	28	22	16
HSV2	6	5	1	0
Negative	56	57	67	74
Total	90	90	90	90

Sample #	Virus	EasyMag Extraction	Extraction free
2	Influenza A H1N1	Pos	Pos
3	Influenza A H3N2	Pos	Pos
2	H1N1 A swine	Pos	Pos
3	Influenza B	Pos	Pos
2	Para 1	Pos	Pos
1	Para 2	Pos	Pos
2	Para 3	Pos	Pos
3	RSV	Pos	Pos
2	hMPV	Pos	Pos
2	Adeno	Pos	Pos
38	Negative	Neg	Neg

5	MRSA	Pos	Pos
5	SA	Pos	Pos
5	HSV1	Pos	Pos
5	HSV2	Pos	Pos
5	Negative	Neg	Neg

Results

After 48 hours at RT the colony counts culture results of the M-Swab MRSA and SA samples were in accordance with the CLSI M40 guidelines.

After 48 hours at RT shell vial culture had equivalent numbers of infected FA cells present in the M-Swab HSV1 and HSV2 samples as the UTM reference medium.

Same culture results similar as reference (35 positive and 55 negative) UTM medium were found in M-Swab specimens; after room temperature storage in the 29 HSV1 positive, 28 were stable after 24 hours, 22 after 72 hours and 16 after 7; in the 6 HSV2 positive, 5 were stable after 24 hours and 1 after 72 hours.

Same results (38 negative and 22 respiratory positive, 5 HSV1 and HSV2, MRSA, SA and negative each) were obtained when testing M-Swab samples using nucleic acid extracted with easyMAG and extraction-free direct testing by real-time PCR.

Conclusions

The data obtained in this study demonstrated that the Copan M-Swab medium supports MRSA and SA for culture up to 48 hours at room temperature.

M-Swab can be used with both nucleic acid extraction platforms and exrtraction free for the detection of bacteria and viruses with molecular amplification assays.

M-Swab eliminates the need for chemical extraction, shorten results turn around time and eliminate the cost of extraction reagents.

The Copan M-swab can be used for the collection of clinical specimens for a centralized bacteriology and virology laboratory.

References

CVS 2009 Poster:
DIRECT NUCLEIC ACID TESTING FOR INFLUENZA WITHOUT EXTRACTION BY REAL-TIME PCR
K. Luinstra[1], S. Castriciano[2*], A. Giambra[2], M. Ackerman[1], A. Petrich[1], S. Chong[1], J. Mahony[1], M. Smieja[1].[1]St. Joseph's Healthcare, Hamilton, ON, Canada, [2]Copan Italia, Brescia, Italy.

Presenting Author Romolo Bothrugno email:romolo.botrugno@copanitalia.com