EVALUATION OF THREE COMMERCIAL TRANSPORT MEDIA COMPARED TO IN-HOUSE TRANSPORT MEDIA, DEMONSTRATED BY THE RECOVERY OF VIRUSES , CHLAMYDIAE AND MOLLICUTES.

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ABSTRACT

Objective: To compare the recovery of *Influenza A, Herpes simplex 2 (HSV 2), Chlamydia trachomatis (CT), Ureaplasma urealyticum (UU), Mycoplasma hominis (MH) and Mycoplasma pneumoniae (MP)*, from three commercially prepared multi-organism transport media, (Flex-Trans/Bartels, M4/ Remel , MT S160/ Starplex Scientific, Inc.) and organism specific, in-house transport media.

Method: Laboratory stock cultures (ATCC strains) of *Influenza A*, *HSV2*, *CT* (8 serotypes), *UU* (3 serotypes), *MH* and *MP*, were titrated in serum-free medium and inoculated into appropriate culture systems to establish endpoints. After determination of endpoints each organism was diluted in selective media to obtain sufficient volumes of stock dilution capable of demonstrating endpoint when titrated 10^{-1} – 10^{-3}. Using standard specimen inoculation guidelines, 100_uL of each dilution for : *Influenza A* was inoculated into Primary Rhesus Monkey Kidney RTs. *HSV2* was inoculated into Primary Rabbit Kidney RTs. *CT* was inoculated into McCoy cell monolayers in 96 well micro-plates. *UU, MH* and *MP* were inoculated into selective agar mini-plates.
In order to simulate transport conditions, inoculations were made at "0", "24" and "48" hours. All dilutions in selective media were stored at 4°C between inoculations and observation for organism growth followed 48 hours – 5 days incubation.

Results: No significant differences in viral growth were observed for "0", "24" and "48" hour inoculations for *HSV2* and *Influenza A*. CPE was detected at 10^{-2} for *Influenza A* after 72 hours incubation, for all transport media. *HSV2* showed CPE at 10^{-3} at "0" time for all transport media and 10^{-2} for "24" and "48" hour inoculations. Inclusions for *CT* (8 serovars) were quantified with significant growth differences between in-house and the three commercial transport media at 10^{-1}. Differences were less significant at 10^{-2} and – 10^{-3}. Similarly for *UU, MH* and *MP*, the in-house media recorded slightly more colonies than the commercial transport media after 48 hours incubation. MT S160 and M4 media showed better performance than the Flex-Trans media for *UU*8 and *MP*.

Conclusion: With the exception of initial dilutions, only slight but no significant differences between in-house and MT S160 and M4 transport media, were recorded for all organisms.
Based on this study the Starplex Scientific Inc. Multitrans Media S160 and Remel M4 media are acceptable alternative commercial transport media for the transport of *Influenza A, HSV2, Ureaplasma urealyticum, Chlamydia trachomatis, Mycoplasma hominis and Mycoplasma pneumonia*

Introduction

The Ontario Public Health Laboratories use routine culture methods for the detection of Viruses, Chlamydiae and Mollicutes, from specimens received from all regions of the Province. Currently, considerable resources are required to ensure that in-house transport media are optimally prepared, quality controlled and stored appropriately prior to use. In order to reduce constraints placed on efficient and timely delivery of various media, an evaluation of three commercial multi-organism transport media systems, Flex-trans/Bartels (FT), M4/Remel (M4), MT S-160/Starplex Scientific (MT S-160), will provide the basis for choosing an alternative to using numerous specific, in-house transportation media. In a previous study it was determined that MT S-160/Starplex Scientific transport media, stored for a period of one year at either 4°C or room temperature, is suitable for the recovery of similar organisms.

Objectives

- Compare the recovery of *Herpes simplex Virus 2, (HSV2), Influenza A, Chlamydia trachomatis*, *CT (8 serovars)*, *Ureaplasma urealyticum*, *(UU3, UU5, UU8)*, *Mycoplasma hominis*, *(MH)* and *Mycoplasma pneumoniae*, *(MP)* from three commercial multi-organism transport media Vs in-house transport media.
- Evaluate simulated transportation by comparing organism recovery rates at '0', '24' and '48' hour inoculation times.

Method

Viruses Laboratory stock culture of *HSV2* and *Influenza A* were titrated in serum-free Minimum Essential Medium, (MEM) to determine endpoint. Optimized stock dilutions, (capable of producing endpoint at 10^{-2}) were prepared in FT, M4, MT S-160 and in-house media. Further titrations, 10^{-1}, 10^{-2}, 10^{-3}, were prepared in sufficient volumes for '0', '24' and '48' hour inoculations. Using standard inoculation guidelines, 100_uL of each dilution of *HSV2* was seeded into Primary Rabbit Kidney, (PRK) tube cultures and 100_uL of each dilution of *Influenza A* was seeded into Rhesus Monkey Kidney, (RMK) tube cultures at '0', '24' and '48' hours. All inoculated cultures were incubated at 36°C and read daily for Cytopathic Effect, (CPE). CPE was graded semi-quantitatively 0-4+.

Chlamydiae Laboratory stock cultures of *CT*, (8 serovars) were prepared in 2SP in-house transport medium and titrated to endpoint. Optimized stock dilutions were prepared, (as in viruses) in FT, M4, MT S-160 and 2SP transport media. Further titrations, 10^{-1}, 10^{-2}, 10^{-3} were prepared similarly to viruses and using standard inoculation guidelines all dilutions were inoculated onto McCoy cell monolayers in 96 well microplates at '0', '24', '48' hours. Inoculated microplates were incubated at 36°C/5%CO2 for 48 hours. Plates were fixed with absolute alcohol and stained with fluorescent conjugate to determine inclusion body growth by IFA.

Mollicutes - Laboratory stock cultures of *MP, MH, UU3, UU5* and *UU8* were titrated in selective media to determine endpoint. Optimized stock dilutions, (as inViruses and Chlamydiae) were prepared in FT, M4, MT S-160 and Mycoplasma in-house transport media. Further titrations were prepared similarly to Viruses and Chlamydiae and using standard inoculation guidelines, 100_uL of each dilution was inoculated onto selective agar at '0', '24' and '48' hours.

Please see over

Results

***Herpes simplex Type* 2** – Readings at 48 hours incubation of typical cytopathic effect (cpe)

Dilution	Remel MT4			Bartels Flextrans			Starplex Scientific MTS160			In-House		
	'O' Time	24 hours	48 hours	'O' Time	24 hours	48 hours	'O' Time	24 hours	48 hours	'O' Time	24 hours	48 hours
10^{-0}	4+ 4+	4+ 4+	4+ 4+	4+ 4+	4+ 4+	4+ 4+	4+ 4+	4+ 4+	4+ 4+	4+ 4+	4+ 4+	4+ 4+
10^{-1}	3+ 2+	3+ 3+	2+ 2+	4+ 4+	2+ 1+	1+ 1+	4+ 4+	3+ 3+	2+ 1+	4+ 4+	3+ 3+	2+ 2+
10^{-2}	1+ 1+	2+ 1+	1+ 1+	3+ 2+	1+ 1+	+ 0	4+ 3+	1+ 1+	+ +	3+ 3+	1+ 1+	0 0
10^{-3}	0 1+	0 0	0 0	1+ 0	0 0	0 0	1+ 1+	0 0	0 0	2+ 1+	+ 0	0 0

Influenza A – Readings at 72 hours incubation of typical cytopathic effect (cpe)

Dilution	Remel MT4			Bartels Flextrans			Starplex Scientific MTS160			In-House		
	'O' Time	24 hours	48 hours	'O' Time	24 hours	48 hours	'O' Time	24 hours	48 hours	'O' Time	24 hours	48 hours
10^{-0}	3+ 3+	4+ 4+	3+ 3+	3+ 3+	4+ 3+	3+ 3+	4+ 4+	4+ 4+	3+ 3+	4+ 4+	4+ 4+	3+ 3+
10^{-1}	2+ 2+	3+ 3+	2+ 2+	3+ 2+	3+ 3+	2+ 2+	4+ 4+	3+ 3+	1+ 1+	3+ 3+	3+ 3+	2+ 2+
10^{-2}	2+ 0	2+ 2+	1+ 1+	1+ 0	2+ 0+	1+ 1+	2+ 0+	1+ 1+	0 0	0 0	2+ 0	+ 0
10^{-3}	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

***Ureaplasma, Mycoplasma hominis* and *Mycolasma pneumoniae* –** colony counts

Type/Sub-type - dilution	Bartels Flextrans			Remel M4			Starplex Scientific MT-S160			In-house		
	"0" hrs	"24" hrs	"48" hrs	"0" hrs	"24" hrs	"48" hrs	"0" hrs	"24" hrs	"48" hrs	"0" hrs	"24" hrs	"48" hrs
UU-3 10^{-1}	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
UU-3 10^{-2}	>100	>100	>100	>100	>100	>100	>100	>100	>100	>150	>150	>150
UU-3 10^{-3}	17	15	29	9	9	9	13	19	20	>100	>100	>100
UU-5 10^{-1}	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
UU-5 10^{-2}	>100	>80	>80	>75	>75	>65	>100	>100	>100	>175	>175	>175
UU-5 10^{-3}	12	10	12	3	2	5	7	4	3	>100	>100	>100
UU-8 10^{-1}	no growth	no growth	no growth	no growth	5	2	no growth	6	3	8	24	0
UU-8 10^{-2}	no growth	no growth	no growth	no growth	1	no growth	no growth	no growth	no growth	2	2	no growth
UU-8 10^{-3}	no growth	no growth	no growth	no growth	no growth	no growth	no growth	no growth	no growth	no growth	no growth	no growth
M. hominis 10^{-1}	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
M. hominis 10^{-2}	>100	>100	>100	>100	>50	>50	>100	>100	>100	>100	>100	>100
M. hominis 10^{-3}	14	21	7	4	4	4	22	10	10	>75	>75	>50
M. pneumonia 10^{-1}	>25	>50	>25	>200	>200	>200	>200	>200	>200	>200	>200	>200
M. pneumonia 10^{-2}	>8	>5	>5	>50	>50	>25	>100	>100	>100	>200	>100	>100
M. pneumonia 10^{-3}	0	0	1	3	1	1	8	6	1	>100	>50	>20

***C.trachomatis* –** EB counts

Serotype & Dilution	REMEL M4			STARPLEX SCIENTIFIC MT-S160			BARTELS FT			2SP C.T.M.		
	0 hrs	24 hrs	48 hrs	0 hrs	24 hrs	48 hrs	0 hrs	24 hrs	48 hrs	0 hrs	24 hrs	48 hrs
D 10^{-1}	138	76	48	79	35	12	103	69	34	>150	>150	63
D 10^{-2}	16	4	6	9	2	0	5	7	4	32	14	8
D 10^{-3}	0	0	1	0	0	0	2	0	1	3	3	1
E 10^{-1}	36	16	9	8	7	0	19	10	7	78	63	46
E 10^{-2}	4	4	0	1	0	0	1	0	0	4	4	1
E 10^{-3}	0	0	0	1	0	0	0	0	1	1	0	0
F 10^{-1}	29	10	3	21	6	1	23	8	3	83	52	21
F 10^{-2}	1	0	0	2	0	0	1	3	0	11	3	0
F 10^{-3}	0	0	0	0	0	0	0	0	0	0	0	0
G 10^{-1}	18	11	5	4	5	0	9	9	3	34	41	4
G 10^{-2}	4	2	0	0	0	0	1	0	1	5	7	0
G 10^{-3}	1	0	1	0	0	0	0	0	0	1	0	0
H 10^{-1}	7	4	1	7	6	1	7	2	0	14	16	4
H 10^{-2}	1	0	0	2	0	0	2	0	0	0	2	0
H 10^{-3}	1	0	0	0	0	0	0	0	0	0	0	0
I 10^{-1}	75	38	11	20	22	8	40	29	15	>150	114	54
I 10^{-2}	3	7	1	3	2	1	3	5	2	17	9	3
I 10^{-3}	0	0	0	2	0	1	0	0	0	1	1	0
J 10^{-1}	125	58	3	63	27	7	56	22	14	>150	>150	73
J 10^{-2}	9	5	0	2	2	0	7	2	1	26	21	14
J 10^{-3}	0	0	0	3	0	0	0	0	1	2	0	1
K 10^{-1}	45	25	17	45	26	10	106	69	28	>150	143	36
K 10^{-2}	6	4	0	7	4	0	5	6	4	16	13	0
K 10^{-3}	1	0	1	1	0	0	0	2	1	1	2	0

Conclusion

Based on this study, Starplex Scientific, Multitrans media S-160 and Remel M4 media were determined to be acceptable alternative commercial transport media for the transport of *Influenza A, Herpes simplex 2, Chlamydia trachomatis, Mycoplasma hominis* and *Mycoplasma pneumoniae*. With the exception of initial dilutions, only slight but no significant differences were observed between in-house, MT S-160 and M4 transport media. Bartels FT showed acceptable recovery rates for *Chlamydia trachomatis* and *Mycoplasma hominis* but showed reduced organism recovery rate for *Mycoplasma pneumoniae* and no organism recovery for *UU8*, when compared to the other media.

References

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