Evaluation of the Starplex Multitrans Transport Media S160 stored at room temperature Vs 4°C

G.Blackwell,* F. Jamieson, M. Fearon, L. Burton, K. Rohoman, R. Bosy,
Ontario Ministry of Health and Long Term Care, Laboratories Branch, 81 Resources Rd., Etobicoke On. Ca. M9P 3T1

Abstract

Objective: To compare the recovery of Herpes simplex 1(HSV1), Influenza A, *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma hominis* from the Multitrans system S160 (MT S160) (Starplex Scientific Inc., Etobicoke, Ontario, Canada) stored for one year at room temperature versus refrigerated temperature.
Method: Laboratory stock culture of HSV1was titrated 10^{0}, 10^{-1}, $10^{-1.5}$ and stock Influenza A was titrated 10^{0}, 10^{-1}. 100 *u*L of each dilution was seeded into duplicate tubes containing 2.5 mL of MT S160 media stored at room temperature and 4°C. *C. trachomatis* (15 serovars), *U. urealyticum* (2 strains) and *M. hominis* were titrated 10^{-1},10^{-2}, 10^{-3}. 200 *u*L of each dilution was seeded into duplicate tubes containing 2.5mLof MT S160 media, (4°C and room temperature). Using standard specimen inoculation guidelines, all duplicate sets of dilutions seeded in Multitrans S160 were inoculated as follows: HSV1 into Primary Rabbit Kidney tissue tube culture; Influenza A into Rhesus Monkey Kidney tube culture; Chlamydiae serovar dilutions were inoculated into McCoy tissue monolayers in 96 well microplates and *U. urealyticum* and *M. hominis* inoculated into selective agar mini-plates. Initial inoculation was '0' time and further sets of dilutions were stored at 4°C and inoculated at 24 and 48 hours to simulate delayed specimen transportation times.Observation for organism growth followed 48 hours – 5 days incubation.
Results: No significant differences in viral growth were observed at '0' time and 48 hour inoculations for HSV1 and Influenza A. HSV1 showed 4+ CPE at $10^{-1.5}$ dilution with MT S160 4°C and MT S160 room temperature after 5 days incubation. Influenza A showed 3+ CPE at 10^{-1} dilution with MT S160 4°C and MT S160 room temperature after 48 hours incubation. Inclusions for all Chlamydiae serovars were quantified at 10^{-1} and 10^{-2}, but no significant differences were observed for all dilutions after 48 hours incubation with MT S160 4°C and MT S160 room temperature. Similarly with *U. urealyticum* and *M. hominis*, colonies were quantified for all dilutions but no significant growth differences occurred between MTS1604°C and MT S160 room temperature.
Conclusion: Starplex Multitrans Media S160 stored at 4°C and room temperature for a period of one year prior to use yielded similar results in the recovery of Viruses, Chlamydiae and Mollicutes with only slight but no significant differences.
MultitransMedia S160 stored at either room temperature or 4°C for a period of one year are acceptable for the transportation of the above organisms.

Introduction

The Ontario Public Health Laboratories receive clinical specimens from all regions of the province and these specimens are often subject to less than optimum conditions during transport. Another factor affecting the successful recovery of organisms is the relatively short 'shelf life' of most transport media unless stored at -20°C.This study is intended to demonstrate the effectiveness of the Multitrans Media S160 stored at 4°C and room temperature for a period of one year, in the recovery ofViruses, Chlamydiae andMollicutes, from analytical samples prepared to simulate typical transportation conditions.

Objectives:

- Compare the recovery of Herpes Simplex 1 (HSV1), Influenza A, *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma hominis* from the Multitrans system S160 (MT S160), stored for one year at room temperatureVsrefrigerated temperature.
- Evaluate the effect of 24 and 48 hour simulated transportation time on organism recovery.

Materials and Methods:

Herpes Simplex 1, Influenza A

Laboratory stock cultures of HSV1 and Influenza A were prepared in serum-free Minimum Essential Medium (MEM-E), to simulate clinical specimens. HSV1 was titrated 10^{0}, 10^{-1}, $10^{-1.5}$ and 100 *u*L of each dilution was seeded into duplicate tubes containing 2.5 mL MT S160 stored at room temperature and 4°C. Using standard specimen inoculation guidelines, all duplicate sets of HSV1 dilutions were inoculated into Primary Rabbit Kidney (PRK), tissue tube culture at '0' time and 48 hours. Influenza A was titrated 10^{0}, 10^{-1} and 100 *u*L of each dilution was seeded into duplicate tubes of MT S160 stored at room temperature and 4°C. Duplicate sets of dilutions were inoculated into Rhesus Monkey Kidney (RMK), tube cultures at '0' time and 48 hours. All inoculated cultures for HSV1 and Influenza A were incubated at 36°C and read daily for typical cytopathic effect (cpe).Cpe was graded semi-quantitatively (0-4+) and viral growth was recorded on day 2 for Influenza A and on days 3 and 5 for HSV1. For QC purposes Influenza A and HSV1 were titrated in serum-free MEM-E and 100 *u*L was inoculated as per trial study to determine the viability of each organism.

Chlamydia trachomatis

Laboratory stock cultures of *C. trachomatis* (15 serovars), were prepared in 2SP Chlamydiae transport medium to simulate clinical specimens. All 15 serovars of *C. trachomatis* were titrated 10^{-1}, 10^{-2}, 10^{-3} and 200 *u*L of each dilution was seeded into duplicate tubes containing 2.5 mL MT S160 stored at room temperature and 4°C. Using standard specimen inoculation guidelines, all duplicate sets of *C .trachomatis* were inoculated into McCoy cell monolayers in 96 well microplates at '0' time, 24 hours and 48 hours. After 48 hours incubation the microplates were fixed with ethanol for 20 minutes and stained with fluorescein-conjugated monoclonal antibody to identify Chlamydiae in tissue culture. Inclusion bodies were quantitated for each dilution and recorded. All Chlamydiae serovars were similarly titrated in 2SP and inoculated as per trial study for QC purposes.

Mollicutes

Laboratory stock cultures of *Mycoplasma hominis* and *Ureaplasma urealyticum* (UU3, UU8), were prepared in Argenine broth and U10C broth respectively, to simulate clinical specimens. *M. hominis* and *U. ureaplasma* were titrated 10^{-1}, 10^{-2}, 10^{-3} and 200 *u*L of each dilution was seeded into duplicate tubes containing 2.5 mL MT S160 stored at room temperature and 4°C. Using standard specimen inoculation guidelines all duplicate sets of *M. hominis* were inoculated into Hayflick's #1 mini-plates and UU3 and UU8 were inoculated into A8 mini-plates at '0' time, 24 hours and 48 hours. After 48 hours incubation at 36°C in anaerobic jars, the mini-plates were examined to determine colony counts. *M. hominis* and *U. ureaplasma* were similarly titrated in Argenine broth and U10C broth and inoculated as per trial study for QC purposes.

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Results:

HSV1 (cpe)

	Inoculated same day				Inoculated at 48 hours			
	Reading @ 3 days		Reading @ 5 days		Reading @ 3 days		Reading @ 5 days	
MT 4^0C	10^0	4+ 4+	10^0	4+ 4+	10^0	4+ 4+	10^0	4+ 4+
	10^{-1}	3+ 0	10^{-1}	4+ 0	10^{-1}	4+ +	10^{-1}	4+ 4+
	$10^{-1.5}$	0 0	$10^{-1.5}$	4+ 0	$10^{-1.5}$	4+ 4+	$10^{-1.5}$	4+ 4+
MT Room Temp	10^0	4+ 4+	10^0	4+ 4+	10^0	4+ 4+	10^0	4+ 4+
	10^{-1}	+ 0	10^{-1}	4+ 0	10^{-1}	4+ 2+	10^{-1}	4+ 4+
	$10^{-1.5}$	0 0	$10^{-1.5}$	4+ 0	$10^{-1.5}$	+ 0	$10^{-1.5}$	4+ 0

Influenza A (cpe)

	Inoculated same day		Inoculated at 48 hours	
	Reading @ 2 days		Reading @ 2 days	
MT 4^0C	10^0	3+ 3+	10^0	3+ 3+
	10^{-1}	3+ 3+	10^{-1}	3+ 3+
MT Room Temp	10^0	3+ 3+	10^0	3+ 3+
	10^{-1}	3+ 3+	10^{-1}	3+ 3+

***Chlamydia trachomatis* inclusion quantification (stock serovars were diluted to yield 200 300 inclusions in order to facilitate counts)**

S[1] Type	Dilution	Starplex RT			Starplex 4^0C		
		0 Time	24 hr	48 hrs	0 Time	24 hr	48 hrs
A	10^{-1}	25	12	2	5	2	Neg
	10^{-2}	3	1	Neg	Neg	Neg	Neg
	10^{-3}	Neg	Neg	Neg	Neg	Neg	Neg
B	10^{-1}	14	4	1	7	2	Neg
	10^{-2}	3	1	Neg	1	Neg	Neg
	10^{-3}	Neg	Neg	Neg	Neg	Neg	Neg
Ba	10^{-1}	26	5	1	10	3	2
	10^{-2}	6	Neg	Neg	Neg	Neg	1
	10^{-3}	Neg	Neg	Neg	Neg	Neg	Neg
C	10^{-1}	>200	>200	50	>200	>200	8-10/field
	10^{-2}	10-15/field	74	6	164	74	25
	10^{-3}	22	3	1	24	5	1
D	10^{-1}	98	45	3	82	38	17
	10^{-2}	11	3	Neg	9	4	1
	10^{-3}	Neg	Neg	Neg	Neg	Neg	Neg
E	10^{-1}	8	4	1	3	1	Neg
	10^{-2}	Neg	Neg	Neg	1	Neg	Neg
	10^{-3}	Neg	Neg	Neg	Neg	Neg	Neg
F	10^{-1}	16	Neg	Neg	15	2	3
	10^{-2}	4	Neg	Neg	1	Neg	Neg
	10^{-3}	1	Neg	Neg	Neg	Neg	Neg
G	10^{-1}	5	Neg	Neg	3	1	1
	10^{-2}	Neg	Neg	Neg	Neg	Neg	Neg
	10^{-3}	Neg	Neg	Neg	Neg	Neg	Neg
H	10^{-1}	10	2	Neg	6	3	1
	10^{-2}	Neg	Neg	Neg	Neg	Neg	Neg
	10^{-3}	Neg	Neg	Neg	Neg	Neg	Neg
I	10^{-1}	73	25	2	27	14	1
	10^{-2}	9	2	Neg	5	3	Neg
	10^{-3}	1	Neg	Neg	1	Neg	Neg
J	10^{-1}	47	11	1	38	18	3
	10^{-2}	4	2	2	5	1	2
	10^{-3}	Neg	Neg	Neg	Neg	Neg	Neg
K	10^{-1}	87	32	5	26	46	23
	10^{-2}	3	1	Neg	4	3	3
	10^{-3}	1	Neg	Neg	Neg	Neg	1
L1	10^{-1}	25	8	Neg	19	11	1
	10^{-2}	Neg	1	Neg	2	1	1
	10^{-3}	Neg	Neg	Neg	Neg	Neg	Neg
L2	10^{-1}	18	3	Neg	14	6	4
	10^{-2}	2	1	Neg	1	Neg	1
	10^{-3}	Neg	Neg	Neg	Neg	Neg	Neg
L3	10^{-1}	6	2	Neg	3	1	1
	10^{-2}	Neg	1	Neg	Neg	Neg	Neg
	10^{-3}	Neg	Neg	Neg	Neg	Neg	Neg

***U. urealyticum, M. hominis* (colony counts)**

		O Time		24 hours		48 hours	
		R.T.	4^0C	R.T.	4^0C	R.T.	4^0C
UU 3	10^0	>500	>500	>500	>500	287	>500
	10^{-1}	181	185	65	110	69	35
	10^{-2}	16	20	7	14	7	6
UU 8	10^0	433	290	355	335	242	308
	10^{-1}	85	34	20	16	12	27
	10^{-2}	7	5	NG	1	NG	NG
MH	10^0	>1000	>1000	>1000	>1000	>1000	>1000
	10^{-1}	800-1000	800-1000	>500	>500	>500	>500
	10^{-2}	146	131	108	140	108	75

Comments and Conclusion

Starplex Multitrans Media S160 stored at room temperature and 4^0C for a period of one year prior to use yielded similar results in the recovery of Viruses, Chlamydiae andMollicutes with only slight but no significant differences.

MultitransMedia S160 stored at either room temperature or 4^0C for a period of one year are acceptable for the transportation of the above organisms.