Evaluation Of The Starplex Multitrans Media For Maintaining The Viability Of Clinical Samples

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ABSTRACT

Objective:

In the wake of laboratory centralization and service regionalization, the integrity of transport media, a key component of quality of diagnostic virology, has been put to the test. The Starplex S160 transport media was evaluated for its ability to maintain virus viability under various conditions including room temperature storage and expiry issues.

Method:

In order to mimic the conditions that may occur during routine clinical sample submission, viral transport media was spiked with laboratory quality control stock viruses and tested continuously for viability. Various stock virus control material was tested under various conditions to establish the efficacy of this transport media. Routine cell line quality control protocol was followed with the exception of spiking transport media with equivalent viral loads and testing for viability over time. Transport media was evaluated at a storage temperature of 4° C and room temperature, as well as in date (long expiry) versus expired media.

Results:

Testing was conducted over a period of 34 days with the last inoculation of tissue cultures occurring 30 calendar days after creating the spiked QC material. Our results indicated that the expired media was very comparable to the in-date lot for keeping the viruses viable with the viral recovery diminishing only slightly, starting at the 14 day mark. A comparison of room temperature to 4°C storage showed identical results for viral recovery until day 27 and only slight percentage decreases for HSV and Parainfluenza at that time. As suspected the more fastidious viruses like Cytomegalovirus became completely negative first, by day 25, while some conditions for Parainfluenza (expired media at room temperature) also became irretrievable prior to the last day of inoculation. The other viruses HSV and Echo 2 retained a degree of positivity until the experiment was final.

Conclusions:

Sample quality plays an integral part of the diagnostic process in patient care. After proper collection techniques have been implemented, transport media then becomes the backbone of sample integrity. Today this is becoming increasingly important with the trend to amalgamate smaller lab services into more citywide or regional operations, affecting transportation times as an end result. The Starplex Multitrans Media is able to maintain virus viability over long periods of time and at a range of temperatures.

PROCEDURE:

Optimized cell lines (Diagnostic Hybrids, Athens, Ohio) were used for the isolation of the selected viral pathogens studied. R-Mix FreshCells[™] is a mixed monolayer of human adenocarcinoma cells (strain A549) and mink lung cells (strain Mv1Lu) and was chosen to detect Parainfluenza Type 2. Hs27 (HFF) FreshCells[™] is a standard monolayer of fibroblast cells, which isolates Cytomegalovirus efficiently. H&V-Mix FreshCells[™] is a mixed monolayer of African Green Monkey (strain CV1) and human embryonic lung cells (strain MRC-5) for the isolation of Herpes Simplex Virus. Enteroviruses were isolated with the use of Super E-Mix FreshCells[™], which is a mixed monolayer of A549 and Buffalo Green Monkey Kidney (BGMK) cells, both of which are permissive to Enteroviruses.

Pooling multiple cryovials of in-house control strains of HSV, CMV, Echo 2 and PF2 into single vessels and mixing well, prepared control material. Two lot numbers of Starplex Multitrans Media (4J21A exp. Sept/05 & 5D18A exp. Apr/06) were spiked with identical volumes of control material. Each virus was inoculated into four VTM containers and the remaining material was aliquoted into two vessels to provide baseline data for comparison. Equal numbers of expired and fresh media vessels and raw material was stored at either room temperature or 4° C and continuously used as source material over the next 30 days to be evaluated using our standard shell vial protocols.

Set Up & Harvest:

- 1. Label each shell vial appropriately on the plastic cap and on the side of the glass (also include date)
- 2. Add 0.2 ml of specimen or controls (Negative Control = VTM/no spike)
- 3. Centrifuge at 700 x g (1800 rpm) for 40 minutes at 20-30°C.
- Incubate at 37°C in 5% CO2. [CMV is harvested at 16 & 72 hours incubation, PF2 at 48 hours and HSV/Echo 2 observed for CPE and blind stained at 5-7 days in absence of CPE.]
- 5. Remove the supernatant from each shell vial using the aspiration equipment.
- Wash each shell vial twice with 1 ml PBS per vial (removing PBS in same manner as supernatant).
- 7. After removal of the last PBS wash, add 1 ml of Methanol.
- Allow the Methanol to fix the coverslip in each shell vial for 10 minutes.
- 9. Use a syringe/needle and forceps to remove the coverslip from the shell vial. (Try to manipulate only the outer rim of each coverslip)
- 10. Allow slips to air dry on paper toweling divided into sections labeled with each identification number.
- 11. Mount the slips on appropriately labeled frosted end glass slides with a drop of Diatex/Toluene per slip.
- 12. Allow mounted slips to air dry on slides for 30 minutes before staining.
- 13. Place 25 ul of appropriate stain reagent on each coverslip. Incubate for 15-30 minutes at room temperature or 37° C.
- 14. Rinse coverslips in PBS.
- 15. Air dry, mount coverslips and read at 100 and 250X magnification.



CONCLUSIONS

The maintenance of viability of virus in clinical samples during transportation to the diagnostic laboratory is a critical determinant in the ability of the lab to identify or confirm a viral etiology of disease, allowing for the most appropriate clinical management. Viruses vary greatly in their ability to survive outside of the host environment, with the Herpes simplex group and Enteroviruses being relatively hardy and viruses such as Parainfluenza, Respiratory Syncytial virus and Cytomegalovirus being less robust. Viral transport media have been created to maintain a stable environment that allows for minimal loss of viability of viruses during the transportation process. Serum albumin, antibiotics and antifungals, pH balance and the addition of glass beads to the transport medium have all helped to improve viral recovery. Sucrose in the medium has been shown to preserve virus viability when specimens are stored in a frozen state. The ability to keep samples long term is important in diagnostic virology as further investigations may be needed on the original sample at a later date.

In addition to the key role this product plays in viral culture, many laboratories including our own, utilize this sample collection system for molecular amplification techniques. The analysis for Bordetella pertussis is currently only performed by PCR and a single respiratory sample can be submitted for both viral DFA & Culture, as well, PCR testing if requested. Although currently only the B. pertussis assay is performed routinely on these samples, the laboratory is investigating other potential screening/diagnostic molecular tests to aid clinicians and Infection Control on potential pathogens which the lab may prefer not to culture.

The Starplex Multitrans Media system incorporates all the needed components of a transport medium to maximize the likelihood of virus isolation and is ideally suited for use when transit times of specimens are increasing due to centralization of diagnostic virology services. The ability of the sample to be stored frozen, in the medium, without significant loss of virus viability of many types of viruses also makes such samples of greater potential use in the investigation of new and emerging viral infections.

