St. Joseph's Healthcare Hamilton

McMaster University

Universal Transport Medium (UTM - RT) With Flocked Swabs and Real - Time PCR Detects Viral Nucleic Acids

***Santina Castriciano*, A. Petrich, M. Smieja, and M. Chernesky**

Departments of Pathology & Molecular Medicine, and Paediatrics McMaster University, St. Joseph's Healthcare, Hamilton, ON, Canada

ABSTRACT:

Background: Nucleic acid amplification assays are becoming widely used for the laboratory diagnosis of viral infections. A versatile transport medium and collection system, effective for all laboratory technologies is necessary.

Objective: To compare a room temperature universal transport medium [UTM-RT] and flocked swabs [FS] (Copan) to M4-RT (Remel) transport medium and Micro Gent Stainless Steel/Plastic swabs (MG) for the detection of *HSV1, HSV 2, VZV, CMV, Influenza A, Influenza B*, and *Echovirus 11* in contrived specimens. To test clinical samples collected into UTM-RT with FS in RT-PCR and by DFA/culture.

Methods: Laboratory strains of *HSV1, HSV 2, VZV, CMV, Influenza A, Influenza B*, and *Echovirus 11* were serially diluted ten fold in UTM-RT and M4-RT from 10^{-1} to 10^{-10}. One ml of each dilution for both media was aliquoted into sterile vials (contrived specimens). A swab, provided with each transport media, was added to the respective vials. Suspensions were vortexed, swabs were removed, 0.2 ml was inoculated into 2 shell vial cell cultures each. For RT-PCR nucleic acid was extracted from 0.2ml using a manual magnetic silica method and extracted nucleic acids were tested in duplicate in the LightCycler using artus RealArt^{tm}-LC PCR kits (Qiagen). A total of 48 culture positive clinical samples collected by FS into UTM-RT were tested by RT-PCR, after freezing and thawing. Infected cell cultures were stained using FITC monoclonal antibodies.

Results: Endpoints of detection of the contrived specimens were equivalent for the combination of FS with UTM-RT to the MG in M4-RT for *HSV1* and *2, CMV, VZV* and *Influenza A*. Endpoint determinations were enhanced 10-fold for *Influenza B* and *Echovirus 11* by the FS/UTM-RT. The artus LC real-time PCR was more sensitive than shell vial culture with both swab/transport systems with contrived specimens. RT-PCR detected 46 /48 of clinical positives from UTM-RT.

Conclusions: The FS/UTM-RT could serve as a universal system for detecting viruses by real time PCR.

BACKGROUND:

Nucleic acid amplification assays are becoming widely used for the laboratory diagnosis of viral infections. A versatile transport medium and collection system, effective for all laboratory technologies is necessary.

OBJECTIVE:

1) To compare two room temperature universal transport media systems for the detection of viral nucleic acids in contrived specimens by real time PCR.

-The UTM-RT and flocked swabs (Copan)
-The M4-RT (Remel) transport medium and Stainless Steel/Plastic swabs.

2) To test the ability to detect viral nucleic acids by real time PCR in clinical specimens collected into UTM-RT with FS, that were already defined positive by DFA/culture.

UTM-RT (Universal Transport Media - Room Temperature) and Flocked Swab

(Copan Diagnostics Inc.)

M4-RT and steel/plastic swabs (Remel)

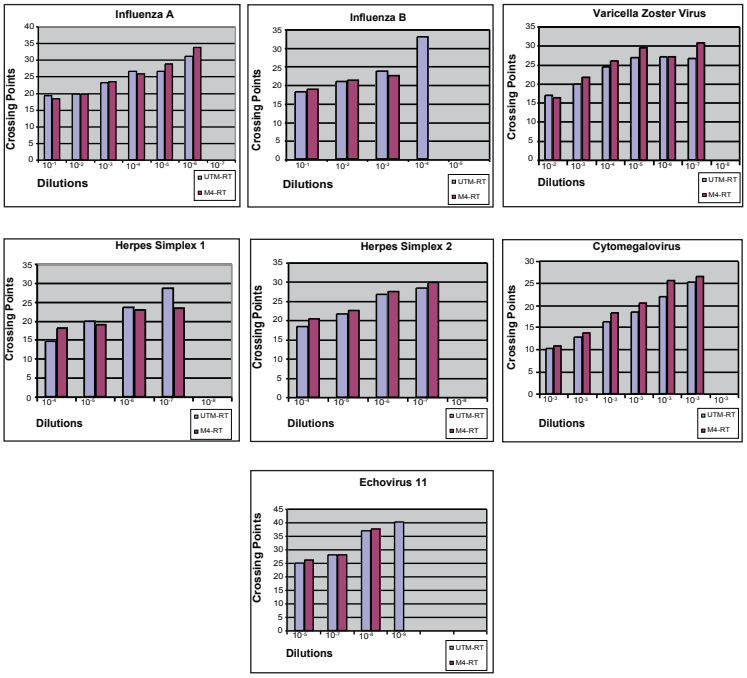
METHODS

- Laboratory strains of HSV1, HSV 2, VZV, CMV, *Influenza A*, *Influenza B*, and *Echovirus 11* were serially diluted in UTM-RT and M4-RT from 10^{-1} to 10^{-10}. to prepare contrived specimens.
- A total of 48 culture positive clinical samples collected by FS into UTM-RT were tested by RT-PCR, after freezing and thawing. The clinical samples were defined positive by culture and identification with type specific FITC monoclonal antibodies.
- Contrived and clinical specimens were tested with the artus RealArt^{tm}-LC PCR kits (Qiagen).
- For the contrived specimens preparation, one ml of each virus dilution for both media was aliquoted into sterile vials.
- A flocked swab was added to each virus dilution in UTM-RT
- A Steel/Plastic swabs was added to each virus dilution in M4-RT.
- All contrived specimens were vortexed, and swabs were removed before testing.
- 0.2 ml of each contrived specimens was inoculated into 2 shell vial cell cultures. R-Mix cells for the *Influenza A*, *B,* and *Echo 11*. H and V Mix cells for *HSV1* and *2*, *CMV* , *VZV*.
- For RT-PCR 0.2 ml volume of all contrived and frozen clinical specimens was used to extract the nucleic acid.
- A manual magnetic silica method was used for the extraction.
- 5 ml of each extracted nucleic acids were tested in duplicate in the LightCycler using artus RealArt^{tm}-LC PCR kits (Qiagen).
- Infected cell cultures were stained using FITC monoclonal antibodies.

RESULTS:

- Endpoints of detection of the contrived specimens were equivalent for the combination of FS with UTM-RT to the MG in M4-RT for *HSV1* and *2*, *CMV, VZV* and *Influenza A*.
- Endpoint determinations were enhanced 10-fold for *Influenza B* and *Echovirus 11* by the FS/UTM-RT. The artus LC real-time PCR was more sensitive than shell vial culture with both swab/transport systems with contrived specimens.
- RT-PCR detected 46/48 of clinical positives from UTM-RT.

REAL TIME PCR RESULTS OF CONTRIVED SPECIMENS



CONTRIVED SPECIMENS CULTURE/PCR RESULTS

Viruses	FS/UTM-RT		SP/M4-RT	
	Culture	PCR	PCR	Culture
Influenza A	10-5	10-6	10-5	10-6
Influenza B	10-3	10-4	10-2	10-3
Echo 11	10-6	10-8	10-5	10-7
HSV 1	10-5	10-7	10-5	10-7
HSV 2	10-5	10-7	10-4	10-7
VZV	10-5	10-7	10-4	10-7
CMV	10-6	10-8	10-5	10-8

CLINICAL SAMPLES PCR RESULTS

Positive		
Viruses	Clinical samples (collected in UTM-RT with Flocked) swabs	
	RT-PCR +/-	Culture +
VZV	9/0	9
Echo11	6/2*	8
Influenza A	13/0	13
Influenza B	10/0	10
CMV	10/0	10
Total	46	48

* Due to freezing and thawing.

CONCLUSIONS:

- The FS/UTM-RT could serve as a universal system for detecting viruses by real time PCR. The FS/UTM-RT could serve as a universal system for detecting viruses by real time PCR in combination with the artus RealArt^{tm}-LC PCR kits.
- The contrived specimens prepared with flocked swabs in UTM-RT enhanced PCR endpoint determinations 10-fold for *Influenza B* and *Echovirus 11*.
- PCR is more sensitive then culture by 1 to 2 log dilution for most of the viruses.