

# A MULTIYEAR HISTORY OF CELL CULTURE DURING CONVERSION TO TOTALLY TUBELESS VIROLOGY AND EFFECT ON ISOLATION RATES OF VIRUSES

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**Abstract:** A MULTIYEAR HISTORY OF CELL CULTURE DURING CONVERSION TO TOTALLY TUBELESS VIROLOGY AND EFFECT ON ISOLATION RATES OF VIRUSES

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**Introduction:** Changing medical environments have caused our laboratory to adapt to the need for more rapid viral reports as physicians seek to determine etiology of disease and treat within short time windows. The University of Louisville Hospital Microbiology Laboratory for many years processed viral specimens to up to 5 different cell lines, using traditional tube cultures which were read for cytopathic effect over a period of 7-21 days. This required highly trained laboratory staff capable of processing specimens using diverse and sometimes confusing protocols and reading cellular materials daily to detect changes (CPE) associated with viral infection. Our laboratory elected to make changes in virology that would allow us to take advantage of some of the newer assays developed by Diagnostic Hybrids, Inc. (cultivated and engineered cells), shifting to a rapid culture platform utilizing blind staining with monoclonal antibodies and requiring no CPE reading for more objective results. We were able to use standardized protocols, consume less personnel time in training and processing and report more quickly.

From tube cultures to shell vials. One to two day results are available with DHI's R Mix™, E Mix™, and H and V Mix™ for nearly all respiratory viruses, Enteroviruses, and lesion screens for non genital cultures for Herpes, CMV and VZV viruses. These mixed cells now compliment ELVIS engineered cells used for genital Herpes simplex overnight testing.

## Materials and Methods

All methods were performed in accordance with traditional procedures in use at the University of Louisville following validations over time. Use of Diagnostic Hybrids, Inc. products followed recommended use guidelines for mixed and proprietary cell cultures. Data over time is presented for both transitional cell culture set up and turn around time adjustments appropriate for shell vial technologies following extensive side by side validations.

## Changes in Set Up Past-Present

Source	Traditional Cultures 1998-2002 Cell Lines (all tubes except indicated SV)	Current Method Setup
Respiratory	A549 HF RMIK LLC-MK2 Hep-2 MRC-5 SV	Respiratory 3 R Mix
Genital	A549 HF	Herpes 1 ELVIS
Skin-Surface	A549 HF	Enterovirus 2 E Mix
Stool	A549 HF RMIK LLC-MK2 Hep-2	Stool 1 R Mix
CSF	A549 HF RMIK LLC-MK2 Hep-2	Lesion ELVIS
Urine/BAL	MRC-5 SV HF	CMV 2 MRC-5 SV* HF Tube

## Changes in Incubation to Final Report

Method	HSV	CMV	Respiratory	Enteroviruses	Lesions
Tube	7 days	21 days	14 days	14 days	14 days
Shell Vial	24 hr	16-48 hr	48 hr	5 days	7 days

## Percent Positive Cultures by Year

Year	Total Specimens	No. Pos	% Pos
1998	1433	96	6.7
1999	1691	106	6.3
2000	1266	66	5.2
2001	768	71	9.2
2002	605	71	11.7
2003	577	41	7.1
2004	608	70	11.5
2005	694	82	11.8
2006	944	71	7.5

## Types of Isolates by Year

Year	Total Tests	HSV	CMV	VZV	Adeno.	RSV	Flu A	Flu B	Para	Entero
1998	1433	85	6	0	4	0	0	0	1	0
1999	1691	91	4	0	5	0	1	0	1	4
2000	1266	54	6	0	0	0	2	0	1	2
2001	768	52	3	0	5	0	1	0	3	3
2002	605	58	3	0	2	0	5	0	0	3
2003	577	31	2	1	1	0	6	0	0	0
2004	608	60	1	0	1	1	3	0	2	1
2005	694	55	6	1	2	1	2	1	3	1
2006	944	53	7	0	3*	0	5	0	0	0

\*One Co-infection with Parainfluenza

## Discussion

Ordering patterns for transplant patients changed the positivity rate and isolate mix. Between 2000 and 2001 the physician directed transplant protocol was changed to reduce the number of samples taken from multiple screens per week to once per week per patient. As a shift occurred to fewer transplant cultures and more general medical cultures, more positive cultures were seen, most within 24-48 hours. There was a decrease in 2003 of requested cultures. In 2004 and 2005 the rate of positives increased due to more tests on inpatients and better sample collection practices. Physicians and clinicians requested more cultures in 2006 upon learning of the utility of earlier reporting. Currently transplant patients accounts for 35-40% of all cultures ordered.

Technologist training has greatly improved. Before three months or more was required to train technologists to become proficient in reading of cytopathic effect. A recently trained technologist was proven efficient and accurate after only two weeks training for all platforms using the new cell approach which requires no reading of CPE.

## CONCLUSIONS

Conversion from tube cultures saved 2 to 14 days to final reports from prior conventional methods, improved training time, resulted in improved physician utilization of laboratory services and allowed more work to be done with the same number of staff members. Without the benefit of the improved workflow, we would not have been able to adapt to the increased numbers of cultures recently sent to the laboratory for processing under new transplant guidelines. We are now considering eliminating the final tube used for CMV long term cultures and using only shell vials for 24 and 48 hour testing for CMV pending data review.