

# FROM TUBE TO TUBELESS VIROLOGY: A QUALITY IMPROVEMENT INITIATIVE

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## Abstract:

**Background:** Conventional roller-tube (RT) cultures used for the detection of viruses in clinical specimens rely upon recognizable cytopathic effect (CPE), and are time consuming. In order to improve the relevancy of viral test results, more rapid and specific methods were tested. We examined the advantages of conversion to a "tubeless" system for culturing all specimen types for viruses, including: 1) potential reduced turn-around-time; 2) workflow; 3) ability to absorb volume increases; and reliability.

**Method:** Three test systems were validated using tissue cultures in shell vials (SV) followed by fluorescent monoclonal antibody staining (F.A.). Results were compared with current methods (RT) and/or historical data.

**Study 1:** R-Mix Too™ SV in combination with D3 Ultra Respiratory Virus Screening and ID Kit™ (Diagnostic Hybrids Inc. [DHI]) was evaluated. Specimens included a Respiratory Virus Training Panel (DHI, 24 samples; 14 positive, 10 negative) and an in-house panel (12 positives). Isolates included all 7 commonly isolated respiratory viruses.

**Study 2:** Super E-Mix™ SV (DHI) in combination with IMAGEN™ Enterovirus F.A. staining (DakoCytomation). Specimens included an Enterovirus Training Panel (DHI, 24 samples; 13 positive, 11 negative) and an in-house panel (22 positive, 20 negative).

**Study 3:** H&V Mixed Fresh Cells™ (DHI, SV) performance was compared to our present method for detection of Herpes Simplex Virus (HSV), Cytomegalovirus (CMV), and Varicella-zoster virus (VZV). Specimens included an H&V Training Panel (DHI, 24 samples; 18 positive, 6 negative) and an in-house panel (10 positive, 9 negative). An HSV typing kit (DHI) was also evaluated.

**Results:** For each of the three studies results confirmed the use of mixed cell cultures (DHI; Super E-Mix, H&V Mix, R-Mix TOO) in SV format alone, used in combination with virus-specific monoclonal F.A. stains at 24-72 hours post inoculation performed well when compared to conventional RT methods or combinations of RT and SV.  
**Conclusion:** Conversion to "tubeless" cultures in a large viral laboratory setting is warranted; providing timely results, labor efficiency, improved patient care, and cost savings.

## Result:

R-MixToo™ (DHI)

DHI Training Panel:

At 24 Hours: 12/14 Positive

At 48 Hours: 14/14 Positive

No false positives

Of 12 in-house frozen specimens tested 8

duplicated previous results, 4 were non-viable in SV or RT. Freeze-thaw may have produced too low a titer to detect. There were no false positives detected.

## Introduction:

Conventional RT cultures are held from 7 to 28 days depending upon the virus sought and the specimen type. Centrifugation enhanced SV cultures have been shown to reduce turn-around-time (TAT) for HSV, CMV, and respiratory viruses.

Viral testing is most often performed using a combination of RT and SV tissue cultures, with final results reported weeks after arrival in the laboratory.

Use of mixed cell monolayers in SV format may allow recovery of clinically significant viruses for all specimen types within 5 days; reducing both TAT and labor.

## Method:

A single protocol was used with H&V, R-MixToo and Super E-Mix shell vials:

Shell vials were kept at room temperature until day of use. Re-feed media was kept refrigerated between uses.

Shell vials and corresponding re-feed media were pre-warmed at 37C for two hours prior to inoculation.

Maintenance media was removed from each shell vial and replaced with 1-2 mls of the appropriate re-feed media.

200 microliters of prepared specimen was inoculated into shell vials. Positive and negative controls were included in each run.

Vials were centrifuged at 2000 RPM for one hour, then incubated at 37C for 1-5 days.

Monolayers were fixed using cold acetone(100%) and stained according to each manufacturer's guidelines.

Coverslips were examined for the presence of apple-green fluorescent cells exhibiting staining patterns consistent for the particular virus. To reduce hands-on time vials were read using an inverted F.A. scope and a 12-vial carrier. Coverslips were not removed from vials.

## Super-E Mix™ (DHI)

At 24 Hours: 13/13 DHI panel and 12/22 in-house were positive. At 5 Days: 34/35 positive. 1 non-viable in SV or RT.

Possible cross-reaction with Infl B and VZV under investigation for 2 negatives.

## H&V Mixed Fresh Cells™ (DHI)

HSV1: 0/6 pos at 24 hrs; 6/6 pos at 48 hrs.

HSV2: 3/6 pos at 24 hrs; 5/6 pos at 48

hrs. 1 frozen specimen non-viable in MRC-5

CMV: 5/8 pos at 24 hrs; 6/8 pos at 48 hrs. 2

frozen specimens non-viable in MRC-5

VZV: 12/13 pos in 48 hrs; 1 non-viable at

72 hrs in H&V and MRC-5 SV.

All DHI Training Panel isolates produced expected results (Pos and Neg).

## CONCLUSION:

All three SV types performed well. The TAT compared to RT was reduced by more than 50%. Tubeless testing appears to be a viable method for rapid, reliable and cost-effective virus isolation.

12 month review: Of those specimens set on RT and SV, greater than 95% were SV positive.