

COMPARISON OF CULTURE FOR CYTOPATHIC EFFECT TO SHELL VIAL AND FLUORESCENT ANTIBODY STAIN FOR VIRAL DIAGNOSIS

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Introduction:

Our classic viral culture for diagnosis has consisted of inoculating several monolayers of actively growing human or animal cells, incubating and then observing the cells for the cytopathic effect (CPE). Cultures were maintained, typically, for one to two weeks and sometimes as long as twenty-eight days depending upon the virus. CPE took many forms and was at times difficult to appreciate. Cultures had to be maintained over many days with multiple media changes while carefully preventing cell substrate contamination.

We present a comparison of culture for viruses using long tubes and CPE, reading versus shell vial and fluorescent monoclonal antibody blind staining using the Diagnostic Hybrids (DH) Method.

Material and Methods:

Cell Lines: Cultures of MRC-5 (human embryonic lung), RMK (rhesus monkey kidney), HEp2 (human laryngeal carcinoma), A-549 (human lung carcinoma) were purchased from DH for use in conventional virus cultures.
Basic Design: Known virus samples were purchased (DH), simultaneously inoculated onto both conventional long tubes and shell vials and incubated at 37°C. Positive conventional cultures were determined by CPE, hemagglutination or blind staining. Shell vials were determined to be positive using Diagnostic Hybrids Inc. criteria (see below). The samples were treated as if they were patient specimens. The technology evaluating the results was unaware of which samples were positive and for which virus. For all cultures the transport media was removed at the time of inoculation and replaced by refed and refed twice weekly.

Testing for Various Viruses

Herpes Simplex (HSV): Thirty unknown HSV samples were inoculated onto MRC-5 cells. Concurrently, shell vials containing genetically engineered baby hamster kidney cells, ELVIS™ (DHI) cells, were inoculated. ELVIS cells are engineered with a HSV promoter gene linked to Escherichia coli LacZ reporter gene. In the presence of X-Gal, infected cells turn blue (Shabel et al., 1993; Stabell and Olivo, 1992). The MRC-5 cells were examined daily for up to 7 days for CPE. The ELVIS cells were fixed and stained at 24 hours and examined for the presence of blue cells. Positive MRC-5 cell cultures (Chemicon) stained with a fluorescein isothiocyanate (FITC) labeled monoclonal antibody (Chemicon) to HSV-1 and HSV-2. The ELVIS cultures were stained with a (FITC) labeled monoclonal antibody (DHI) to HSV-2. The presence of any cells fluorescing was considered positive for HSV-2. If no fluorescent cells were detected in the ELVIS samples, they were rinsed and restained with a FITC labeled monoclonal antibody to HSV-1 (DHI). The presence of fluorescence was considered positive for HSV-1.

Respiratory Viruses: Twenty-four unknown respiratory virus samples were inoculated onto RMK, HEp2, and MRC-5 cell lines. Concurrently, shell vials containing R-Mix (DHI) were also inoculated. R-Mix, a mixture of a mink lung line (MvLu) and A-549, has been shown to have excellent sensitivity for respiratory viruses (St. George et al., 2002; Barentanger et al., 2001). Conventional cultures were evaluated twice a week for CPE and tested for hemagglutination on day 10. R-Mix cultures were fixed and stained at 24 hours, 48 hours and 5 days using a FITC labeled pan anti-respiratory virus antibody solution (DHI). Positive cultures of each were further evaluated by staining with staining with specific FITC labeled antibodies (DHI) for influenza A and B, parainfluenza 1, 2 and 3, and Respiratory Syncytial Virus. Adenovirus was incubated with an anti-adenovirus monoclonal antibody (Bartels) followed by a FITC labeled anti-mouse antibody (Bartels).

Enteroviruses: Twenty-four unknown enterovirus samples were inoculated onto RMK, A-549 and MRC-5 cells. Super-E-Mix (DHI) shell vials were also inoculated. Super E-Mix is a mixture of buffalo green monkey kidney cells (BGMK) genetically engineered to contain human decay acceleration factor (hDAF) and A-549 cells (Huang et al., 2002; Buck et al., 2002). Conventional culture tubes were evaluated for CPE twice weekly. Cultures positive for CPE and cultures still negative after 10 days were stained with a pan FITC labeled anti-enterovirus antibody solution (Chemicon). E-Mix cultures were stained with the pan enterovirus antibody solution on days 2 and 5. Cultures that showed fluorescence, both conventional as well as E-Mix cultures, were further evaluated by staining with species specific FITC labeled antibodies (Chemicon) for Coxsackie A and B and Echovirus (Chemicon). Enteroviruses were incubated with an anti-enterovirus monoclonal antibody (Chemicon) followed by a FITC labeled anti-mouse antibody (Bartels).

Cytomegalovirus (CMV) and Varicella Zoster Virus (VZV): Thirty-four unknown CMV and VZV samples were simultaneously inoculated onto MRC-5 cells and H&V-Mix (DHI). H&V-Mix is a mixture of MRC-5 cell and CV-1 cells (African green monkey kidney cells) (Huang et al., 2002). CMV conventional culture tubes were observed daily for CPE while VZV cultures were evaluated 3 times a week. Positive CMV conventional cultures and those still not positive on day 28 were stained with FITC labeled monoclonal antibodies to CMV (Chemicon). Positive VZV cultures and those still negative on day 14 were stained with a FITC labeled monoclonal antibody to VZV (Merflur). H&V-Mix cultures for CMV were stained on days 2 and 3 and for CMV with a FITC labeled monoclonal antibody (DHI). VZV cultures were stained on days 2 and 5 for VZV with a FITC labeled monoclonal antibody (Merflur)

Observations and Comparison Charts

Respiratory Cultures: R-Mix cultures correctly identified all positive respiratory samples (14 of 14) within 24 hours with no false negatives (10 of 10) (Table 2). Conventional cultures also identified all positive samples, however, some conventional cultures required as long as 10 days to become positive.

Number	Expected Result		Shell Vial		Long Tube	
	Result	Day	Result	Day	Result	Day
Resp 1	Neg	5	Neg	5	Neg	10
Resp 2	Neg	5	Neg	5	Neg	10
Resp 3	Flu B	1	Flu B	1	Flu B	1
Resp 4	RSV	1	RSV	1	RSV	3
Resp 5	Parainfl 2	1	Parainfl 2	1	Parainfl 2	3
Resp 6	RSV	5	RSV	5	RSV	10
Resp 7	RSV	3	RSV	3	RSV	3
Resp 8	Flu A	1	Flu A	1	Flu A	3
Resp 9	Adeno	1	Adeno	1	Adeno	3
Resp 10	Neg	5	Neg	5	Neg	10
Resp 11	RSV	1	RSV	1	RSV	3
Resp 12	Flu A	1	Flu A	1	Flu A	3
Resp 13	Flu A	1	Flu A	1	Flu A	3
Resp 14	Parainfl 1	1	Parainfl 1	1	Parainfl 1	3
Resp 15	Parainfl 1	1	Parainfl 1	1	Parainfl 1	3
Resp 16	Parainfl 3	1	Parainfl 3	1	Parainfl 3	3
Resp 17	Neg	5	Neg	5	Neg	10
Resp 18	Neg	5	Neg	5	Neg	10
Resp 19	Adeno	1	Adeno	1	Adeno	3
Resp 20	Flu B	1	Flu B	1	Flu B	1
Resp 21	Neg	5	Neg	5	Neg	10
Resp 22	Neg	5	Neg	5	Neg	10
Resp 23	Neg	5	Neg	5	Neg	10
Resp 24	Flu A	1	Flu A	1	Flu A	3

Number	Expected Result		Shell Vial		Long Tube	
	Result	Day	Result	Day	Result	Day
Entero 1	Neg	5	Neg	5	Neg	7
Entero 2	Neg	5	Neg	5	Neg	7
Entero 3	Echovirus	2	Echovirus	2	Echovirus	2
Entero 4	Echovirus	2	Echovirus	2	Echovirus	2
Entero 5	Echovirus	2	Echovirus	2	Echovirus	2
Entero 6	Neg	5	Neg	5	Neg	10
Entero 7	Neg	5	Neg	5	Neg	10
Entero 8	Poliovirus	2	Poliovirus	2	Poliovirus	2
Entero 9	Poliovirus	2	Poliovirus	2	Poliovirus	2
Entero 10	Neg	5	Neg	5	Neg	10
Entero 11	Poliovirus	2	Poliovirus	2	Poliovirus	2
Entero 12	Poliovirus	2	Poliovirus	2	Poliovirus	2
Entero 13	Poliovirus	2	Poliovirus	2	Poliovirus	2
Entero 14	Neg	5	Neg	5	Neg	10
Entero 15	Poliovirus	2	Poliovirus	2	Poliovirus	2
Entero 16	Poliovirus	2	Poliovirus	2	Poliovirus	2
Entero 17	Neg	5	Neg	5	Neg	10
Entero 18	Neg	5	Neg	5	Neg	10
Entero 19	Poliovirus	2	Poliovirus	2	Poliovirus	2
Entero 20	Poliovirus	2	Poliovirus	2	Poliovirus	2
Entero 21	Neg	5	Neg	5	Neg	10
Entero 22	Neg	5	Neg	5	Neg	10
Entero 23	Neg	5	Neg	5	Neg	10
Entero 24	Echovirus	2	Echovirus	2	Echovirus	2

Discussion: The shell vial techniques using the Diagnostic Hybrid 5 ELVIS cell (HSV), R-Mix cells (respiratory viruses), Super E-Mix cells (enteroviruses) and H&V-Mix cells (CMV and VZV) were able to correctly identify 100% of sample viruses tested. Conventional viral cultures correctly identified 100% of the HSV, respiratory viruses, enteroviruses and CMV but missed 2 VZV positive samples. Neither technique, in this study, falsely identified a negative sample as positive.

The major advantage of the Diagnostic Hybrid 5 shell vial technique is decreased turn around time. HSV cultures decreased turn around time to 24 hours compared to 2-7 days using the conventional culture method. Respiratory cultures had a decreased turn around time to 24 hours compared to 3-10 days. Enteroviruses had a decrease to 48 hours compared to 2-10 days. CMV cultures were positive within 48 hours with the Diagnostic Hybrids technique compared to 3-8 days for the conventional technique. Negative cultures were held for observation for 28 days. VZV turn around time was reduced to 48-72 hours compared to 14 days for conventional cultures.

Cost Analysis

A comparison of the cost (Table 6) shows that per sample, in our laboratory, the Diagnostic Hybrids techniques are slightly less expensive than our previous methods using CPE as an end point. Barentanger et al. (2001) found that using R-Mix was slightly more expensive than conventional cultures and respiratory cultures. The shell vial technique is easier to learn, takes much less training time and end points are less subjective than CPE.

Method	Expected Result		Shell Vial		Long Tube	
	Result	Day	Result	Day	Result	Day
Resp 1	Neg	5	Neg	5	Neg	10
Resp 2	Neg	5	Neg	5	Neg	10
Resp 3	Flu B	1	Flu B	1	Flu B	1
Resp 4	RSV	1	RSV	1	RSV	3
Resp 5	Parainfl 2	1	Parainfl 2	1	Parainfl 2	3
Resp 6	RSV	5	RSV	5	RSV	10
Resp 7	RSV	3	RSV	3	RSV	3
Resp 8	Flu A	1	Flu A	1	Flu A	3
Resp 9	Adeno	1	Adeno	1	Adeno	3
Resp 10	Neg	5	Neg	5	Neg	10
Resp 11	RSV	1	RSV	1	RSV	3
Resp 12	Flu A	1	Flu A	1	Flu A	3
Resp 13	Flu A	1	Flu A	1	Flu A	3
Resp 14	Parainfl 1	1	Parainfl 1	1	Parainfl 1	3
Resp 15	Parainfl 1	1	Parainfl 1	1	Parainfl 1	3
Resp 16	Parainfl 3	1	Parainfl 3	1	Parainfl 3	3
Resp 17	Neg	5	Neg	5	Neg	10
Resp 18	Neg	5	Neg	5	Neg	10
Resp 19	Adeno	1	Adeno	1	Adeno	3
Resp 20	Flu B	1	Flu B	1	Flu B	1
Resp 21	Neg	5	Neg	5	Neg	10
Resp 22	Neg	5	Neg	5	Neg	10
Resp 23	Neg	5	Neg	5	Neg	10
Resp 24	Flu A	1	Flu A	1	Flu A	3

Conclusion

Conclusion: Diagnostic Hybrids shell vial technique reduces turn around time without sacrificing sensitivity or specificity, has a cost that is roughly equivalent if not less expensive, and is easier to learn with decreased training time and a less subjective end point than conventional cultures using CPE.

References:

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