Developments in Tissue Culture Detection of Respiratory Viruses

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KEYWORDS

- Viral culture • Shell vial viral culture • Influenza virus
- Respiratory syncytial virus • Parainfluenza virus • Adenovirus

There are now several technologies available for detection of viruses, including those viruses which frequently cause respiratory tract infections. The available methods range from insensitive, but technically simple and inexpensive, point-of-care tests to highly sensitive, but technically challenging and expensive, nucleic acid amplification tests (NAAT). While viral culture is moderately technically challenging, it is simpler than NAAT and it has advantages over immunoassays in sensitivity for some viruses and for the types of viruses that can be readily detected.

The focus of this article is on improvements in the utility of viral culture for detection of respiratory viruses. Detection of viruses from clinical specimens is the main subject, but the use of viral culture for testing the susceptibility of influenza A and B viruses to some anti-viral drugs is also discussed. Viral culture is also a method for production of viruses, and has current and potential applications in research and vaccine development; however, it is beyond the scope of this article.

PROBLEMS WITH CONVENTIONAL VIRAL CULTURE FOR RESPIRATORY VIRUSES

For this article, we consider conventional viral culture for respiratory viruses to mean culture of respiratory samples in tubes of several types of mammalian cells that are chosen to support detection of influenza viruses, respiratory syncytial viruses (RSV), parainfluenza viruses, adenoviruses, and sometimes, rhinoviruses.[1] Typically, three or four cell types are inoculated with the specimen (sometimes in duplicate) and these cultures are incubated for 10 to 14 days, or longer in a few laboratories. Each tube is periodically checked for the presence of replicating virus by hemadsorption (HA) for

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influenza and parainfluenza viruses, and by microscopy for cytopathic effect (CPE) for the other viruses. Once HA or CPE has been detected, the presence of the suspected viruses is confirmed by staining the cells by immunofluorescence.

This simple description of conventional viral culture makes the problems with this method clear: it is slow, it is a lot of work, and the supplies are expensive. The length of time it takes to detect viruses by conventional viral culture reduces the clinical utility of the result. Reporting the presence of influenza virus in a day will have more clinical impact than reporting it in a week.

Why perform viral culture on respiratory specimens at all? Even the fastest methods of viral culture take one or two days, while immunoassays can be performed in 1 hour or less. NAAT are more sensitive than viral culture and can be performed in a few days or less, depending on the workflow. All of these tests have reasonable specificity when they are correctly performed. Nevertheless, the modern methods of viral culture for respiratory viruses described in this article will continue to be a good choice for many laboratories. The advantages of viral culture are first, that it is more sensitive than immunoassays for most respiratory viruses, and second, that it is far simpler and cheaper than NAAT. Until NAAT become easier to perform, probably through automation, and less expensive, viral culture for respiratory viruses will continue to be useful.

In the rest of this article, we discuss newly described cell lines that can be used for viral culture and the use of shell-vial culture for detection of respiratory viruses.

NEWLY DESCRIBED CELL LINES FOR CULTURE OF RESPIRATORY VIRUSES

Genetically Modified Madin-Darby Canine Kidney Cells

Madin-Darby canine kidney (MDCK) cells are an established cell line that can be used to grow and detect influenza viruses.[1] They are a convenient alternative to primary monkey kidney (PMK) cells for influenza virus culture. MDCK cells have been genetically modified to express higher levels of the molecule bound by the viral hemagglutinin and neuraminidase proteins present on the surface of influenza virus strains adapted to growth in human hosts. Influenza viruses bind to host cells through the binding of viral hemagglutinin protein to sialyloligosaccharides on the host-cell surface.[2] After replication, exit of influenza virus from the host cell depends on cleavage of sialic acid from the saccharides on the host cell. The structure of the sialyloligosaccharides differs between different animal species, and the influenza viruses that grow in a given host species preferentially bind and cleave the specific sialyloligosaccharides found in that species. Most of the terminal N-acetyl sialic acid residues on human respiratory epithelial cells are bound to the saccharide in an α2,6-linkage (called NeuAcα2,6Gal), and so influenza viruses from humans would be expected to replicate well in cells with surface NeuAcα2,6Gal.[2] While MDCK cells do have NeuAcα2,6Gal on their surface, they also have a second type of cell-surface sialic acid (NeuAcα2,3Gal).[3] By increasing the level of NeuAcα2,6Gal and decreasing the level of NeuAcα2,3Gal present on the surface of MDCK cells, one might be able to make MDCK cells better able to support replication of influenza viruses from human infections.

MDCK cells have been genetically modified to express the gene for an enzyme that catalyzes the α-2,6-sialylation of N-acetyllactosamine (the SIAT1 gene).[3] Compared with control MDCK cells, the MDCK-SIAT1 cells have approximately twofold higher levels of NeuAcα2,6Gal and twofold lower levels of NeuAcα2,3Gal.[3] As expected, an influenza A (H1N1) strain adapted to growth in humans binds with greater affinity to the MDCK-SIAT1 cells than to control MDCK cells. When the same strain of influenza A virus was adapted to growth in embryonated chicken eggs, it did not have the preferential affinity for the MDCK-SIAT1 cells. These data suggest that

increased expression of NeuAcα2,6Gal would make the MDCK-SIAT1 cells better able to support replication of influenza viruses from human infections.

MDCK-SIAT1 cells were created for use in a functional assay for resistance of influenza virus to neuraminidase inhibitors (eg, oseltamivir, brand name Tamiflu).[3] Neuraminidase inhibitors are used to reduce the illness caused by influenza virus infection, but resistance can develop during treatment. During the winters of 2007 to 2008 and 2008 to 2009 many isolates of influenza A (H1N1) were resistant to oseltamivir.[4] Cell lines used for influenza virus culture, including MDCK cells, do not give accurate results in functional assays for oseltamivir resistance, probably because expression of low levels of NeuAcα2,6Gal lead to apparent oseltamivir insensitivity even when the virus is susceptible to the drug.[3] Unlike MDCK cells, MDCK-SIAT1 cells allow ready detection of oseltamivir resistance in a bioassay.[3] Matrosovich and colleagues used two wild-type oseltamivir susceptible influenza A strains and their corresponding oseltamivir resistant mutants in a bioassay with MDCK-SIAT1 cells and found that the resistant mutants demonstrated 100-fold, or greater, resistance to oseltamivir.[3] Hatakeyama and colleagues tested a larger number of influenza virus isolates (six H3N2, six H1N1 and five influenza B) in a bioassay for oseltamivir susceptibility with independently generated MDCK engineered to express increased levels of NeuAcα2,6Gal (which they called ST6Gal I cells).[5] They found that reduction of plaque size by 50% in greater than 90% of plaques, rather than plaque number, accurately reflected the susceptibility of the viruses to oseltamivir. The gold standard for oseltamivir susceptibility in this study was an in vitro assay for the concentration of oseltamivir which inhibited 50% of the sialidase activity of neuraminidase. Taken together, these studies demonstrate that MDCK-SIAT1 is a good bioassay for oseltamivir susceptibility in influenza A and B viruses.

There are intriguing data which indicate that MDCK-SIAT1 cells could be used in a sensitive cell culture assay for influenza viruses. Hatakeyama and colleagues compared ST6Gal I cells with control MDCK cells for detection of influenza virus in 20 clinical specimens known to contain influenza viruses.[5] The clinical specimens were titrated with the control MDCK cells and ST6Gal I cells, cultured with media containing trypsin (to enhance plaque formation), and then stained for viral plaques after 2 or 3 days. Eight specimens had influenza A (H3N2), seven had influenza A (H1N1), and five had influenza B. Influenza virus was detected in all 20 specimens using the ST6Gal I cells, but in only 10 of 20 specimens using the MDCK cells (three influenza A (H3N2), six influenza A (H1N1), and one influenza B). Furthermore, the replication efficiency of influenza viruses previously grown in MDCK cells was greater in ST6Gal I cells than in MDCK cells, and plaques formed in the transfectants were larger and easier to visualize.

In a separate study, Oh and colleagues used MDCK-SIAT1 cells and MDCK for detection of influenza virus from a large number (125) of specimens known to contain influenza virus (39 influenza A (H1N1), 53 influenza A (H3N2), and 33 influenza B).[6] After 4 days of growth, the supernatant was tested for influenza virus by hemagglutination. Two thirds of the isolates were detected from cell lines, one third were detected only from the MDCK-SIAT1 cells, and none were detected only from the MDCK cells. The titer of virus obtained from clinical specimens was higher in the MDCK-SIAT1 cells than in MDCK cells for influenza A isolates, but the two cell lines yielded similar titers of influenza B. The repeated passage of influenza viruses in MDCK and MDCK-SIAT1 cells does lead to mutations in the genes encoding the viral hemagglutinin protein, but the frequency of mutation was the same regardless of which cell was used.[6]

To date, there are no studies that we are aware of that determine whether MDCK cells modified to express high levels of NeuAcα2,6Gal are superior to unmodified

MDCK cells, or to primary monkey kidney cells, when used for routine methods of detection of influenza virus from clinical specimens. The studies discussed earlier use small numbers of samples or methods of viral detection that are unlikely to be used in routine diagnostic laboratories (eg, plaque assays). However, these studies do demonstrate that most influenza A and B isolates that have been tested grow to higher titer in the engineered MDCK cells than in control MDCK cells, and they provide promising, if limited, data suggesting that these cells could be routinely used in a sensitive influenza virus assay.

HuH7 Cells

HuH7 cells are human hepatocellular carcinoma cells. These cells, and other human hepatocellular carcinoma cell lines, have the interesting feature that they produce little or no type I interferons (IFN-α and IFN-β) in response to viral infection.[7] Interferons are cytokines that are produced in response to viral infection and that cause neighboring cells to reduce permissiveness for viral replication. In addition to producing little or no type I interferons, HuH7 cells show reduced viral replication only with very high levels of type I interferons.[7] These findings suggest that HuH7 cells might support viral replication well, and could be useful in detection of viruses by viral culture.

Freymuth and colleagues have described the use of HuH7 cells for viral culture with respiratory viruses.[8,9] They cultured HuH7 cells in 24 well plates to 80% confluence, and inoculated the cells with specimen by centrifugation, as would be done with shell-vial cultures. The cells were examined for CPE for 4 days and then analyzed by either direct fluorescence assay (DFA) or by NAAT on the culture supernatant. They tested the ability of HuH7 cells to support replication of culture-adapted isolates of influenza A, influenza B, RSV, human metapneumovirus, coronaviruses, parainfluenza viruses types 1, 2, 3, and 4, adenoviruses, enteroviruses and rhinoviruses. CPE was detectable with all tested isolates of influenza viruses, parainfluenza viruses, coronavirus, rhinovirus and most (19 of 21) adenovirus. There was no detectable CPE with RSV or human metapneumovirus, although replication of these viruses could be detected by immunofluorescence or reverse transcription polymerase chain reaction, respectively. Although the rate of replication of influenza viruses in HuH7 cells was slower than in MDCK cells, all isolates of influenza viruses could be detected with the HuH7 cells.

These authors also described the use of HuH7 cells as part of routine viral culture.[8] They used HuH7 cells for viral culture of specimens that were negative by immunofluorescence for influenza A and B viruses, RSV, parainfluenza viruses 1, 2, and 3, and adenovirus. Viral culture with HuH7 cells detected a large number of influenza A and B viruses that were missed by immunofluorescence (approximately 30% more of each detected), and parainfluenza 1 (31.7%), parainfluenza 2 (55.5%) and adenovirus (21.1%). A smaller yield was found with HuH7 viral culture for detecting RSV (1.2% more detected than IF) and parainfluenza 3 (11.4%), but given that immunofluorescence is sensitive for these two viruses, this is not surprising. Several viruses that were not tested for immunofluorescence, but that were detected by HuH7 viral culture, included rhinovirus, enterovirus, and coronavirus. HuH7 cells also support replication of coronaviruses, including the severe acute respiratory syndrome (SARS) coronavirus.[9,10]

HuH7 cells appear to be a good alternative to other cell lines for detection of influenza viruses, parainfluenza viruses, and adenoviruses, and also allow detection of at least some isolates of rhinovirus, enterovirus, and coronavirus. Unfortunately, there are no comprehensive comparisons of the sensitivity of HuH7 cells to alternative cell lines, such as MDCK or A549 cells for detection of all of these viruses. Such

a comparison would be very useful in determining how these cells would be best used in the diagnostic virology laboratory.

SHELL-VIAL CULTURE FOR RESPIRATORY VIRUSES

Shell-vial viral culture has the advantage of giving faster results than conventional (tube) viral culture. Shell-vial culture is performed by centrifuging the specimen onto a monolayer of cells on a coverslip in the bottom of a vial.[1] The culture is incubated for 1 to 4 days before being stained for the presence of virus by immunofluorescence. There is little, if anything, to be gained by reading shell-vial cultures for CPE and this is usually not done. The centrifugation is long (eg, 30 to 60 minutes) and slow (eg, 700 x gravity), and this enhances the viral infectivity.[1] The mechanism by which shell-vial culture increases sensitivity is not known. The selection of which cell types are used, the days that the shell vials are stained, and of course, the specificity of the antibodies used, all determine what viruses can be detected.

As previously mentioned, the main advantage of shell-vial culture is that viruses are detected more quickly than by conventional culture. There is little need for longer incubation times because most viruses are detected within 1 or 2 days. Another advantage of shell-vial cultures is a reduction in labor compared with conventional viral culture, which can reduce the cost of the test. There are two potential disadvantages to shell-vial culture. First, if the method is not optimized, shell-vial culture can be less sensitive than conventional culture, and this insensitivity must be carefully assessed before shell-vial culture is adopted. Second, shell-vial culture will only detect the viruses that are supported by the cell lines used and that are detected by the antibody used. As a result, shell-vial cultures typically detect fewer types of virus than a conventional viral culture. But progress has been made in increasing the number of viruses that a shell vial can detect by using multiple cell types in a single vial, and by staining the monolayer with multiple antibodies.

We briefly review the use of shell-vial culture to detect a single type of respiratory virus, but these methods have become less useful as methods for the detection of multiple respiratory viruses in a single shell vial have become common. The latter is the focus of this section, as this is the method of viral culture likely to be adopted by most laboratories which consider shell-vial culture for respiratory viruses.

Shell-Vial Cultures for Single Respiratory Viruses

Most studies of the use of shell-vial culture for influenza A and B viruses have used PMK cells, as these are the preferred cells for conventional culture of influenza viruses. The sensitivity of shell vial for influenza viruses is lower than conventional culture with PMK cells. In one study, conventional culture detected influenza A or B in 82 samples; only 30 of these were detected by shell vial, and shell vial detected another 12 that were negative in conventional culture.[11] Conventional culture took 3.6 and 4.3 days to detect influenza A and B, respectively, while shell vials were completed at 2 days.[11] Another study using PMK cells found that all specimens positive for influenza A or B were detected by conventional culture, although only 60% of these were detected by shell-vial culture.[12] Cell lines that can be grown for diagnostic use are more convenient for viral culture than primary cells. One study compared three cell lines for use in shell-vial culture detection of influenza virus.[13] MDCK cells were 100% sensitive, which was significantly higher than the sensitivity of Vero cells (71.4%) or MRC-5 cells (57.1%). PMK cells were not included in this study, either in shell-vial or conventional-viral culture, so the relative sensitivities of PMK and MDCK cells in shell-vial testing was not determined.

HEp-2 cells can be used for shell-vial culture of RSV. The sensitivity of shell-vial culture for RSV is equal to, or higher, than conventional culture. Sensitivities of shell-vial cultures for RSV range from 73% to 92%, while the sensitivities of conventional cultures in the same studies range from 58.4% to 90%.[14–17] Out of 46 specimens that were positive for RSV by shell vial in one study, 43 (93.5%) were detected on the first day, 3 (6.5%) more were detected on the second day, and no additional positives were detected on the third day.[16] In the same study, it took 2 to 8 days to detect RSV by conventional culture, with an average of 4.5 days. Immunofluorescence is typically more sensitive than culture for RSV, but if culture for RSV is done, shell-vial culture can be recommended as being as sensitive, or slightly more sensitive, compared with conventional culture, but with shorter turn-around time.

Shell-Vial Cultures for Multiple Respiratory Viruses

Several shell-vial viral culture systems have been tested for the detection of panels of respiratory viruses. Typically, these are designed to detect influenza A and B viruses, RSV, adenovirus, and parainfluenza viruses (types 1, 2, and 3). To be adequately sensitive for each of these viruses, two cell lines are used. Most cell types will support proliferation of each of the viruses to differing degrees, but in general the shell-vial cultures include a cell line that supports replication of RSV and adenovirus (eg, HEp-2, A549) and another cell line to support replication of influenza, parainfluenza, and adenovirus (eg, PMK, MDCK). Separate shell vials are usually used for each cell type, but one commercial product combines multiple cell lines in a single shell vial (see later discussion). The number of shell vials of each type inoculated will depend on the number of time points on which vials are harvested for staining by immunofluorescence (usually one or two time points), and the staining strategy, as discussed next.

Immunofluorescence for detection of multiple viruses can be done in two general ways. First, the cells can be scraped off the cover slip, spotted in multiple wells of a slide, and then dried and fixed. Next, one well can be stained by immunofluorescence for each of the viruses separately, for a total of approximately seven stains. Alternatively, the cells can be stained for immunofluorescence using a pool of antibodies against all the viruses to be detected. If a pooled reagent with a single fluorochrome is used, negative results are obtained directly from the results of the pooled stain. If the pooled stain is positive, however, then individual stains must be done for each of the individual viruses included in the pool. There are now commercial reagents that are a pool of antibodies in which one antibody has a different fluorochrome than all the other antibodies. Millipore markets SimulFluor Pooled DFA reagent, which differentiates RSV from other viruses in the pool, and Diagnostic Hybrids markets the D3 Duet Respiratory Virus Screening Kits, which have differential staining for either influenza A or RSV in pooled reagents. This differentiation allows the specimens positive for the virus with the unique fluorochrome to be reported directly from the results of the pooled stain. Staining with pooled immunofluorescent reagents can result in a savings in labor, although this will depend on which viruses are present in the specimens a laboratory tests and the fraction of specimens that are negative for those viruses.

Shih and colleagues evaluated the utility of MDCK cells in shell-vial culture at 24 hours, and also determined the gain of testing an additional vial at 48 hours and the gain of using HEp-2 cells at 24 and 48 hours (**Table 1**).[18] Shell-vial cultures were stained with a commercial pool of antibodies, followed by staining with individual antibodies if the pool was positive (Bartels, WA, USA). The reference standard was conventional culture with MDCK, MK-2, MRC-5 and HEp-2 for up to 30 days. The

Table 1
Sensitivity of shell-vial cultures for respiratory viruses

Cell Lines	Screening Stain (Fluorescence Assay)	Days Stained	Influenza A %	Influenza B (%)	RSV (%)	Adenovirus (%)	Parainfluenza 1 (%)	Parainfluenza 2 (%)	Parainfluenza 3 (%)	References
MDCK	IFA, Bartels	1	81.5	26.7	82.6	79.6	100[a]	25[a]	73.3	Shih et al[18]
MDCK and A549	IFA, Bartels	3, 7	100	ND	72	66[a]	100[a]	100[a]	100[a]	Lee et al[19]
PMK and A549	IFA, Bartels	2, 3	94[b]							Matthey et al[20]
PMK and A549	IFA, Bartels	2	ND	94	75	83	33[a]	75[a]	79	Rabalais et al[21]

Abbreviation: ND, not determined because this virus was not detected in any specimens.
[a] Fewer than 10 specimens contained this virus.
[b] The data were pooled in the original report and so sensitivities for some viruses are indicated by combination of the columns.

use of an MDCK-shell vial at 24 hours was surprisingly sensitive (see **Table 1**) for most viruses, but it was insensitive for detection of influenza B viruses. Incubation of the MDCK for an additional 24 hours (48 hours total) more than doubled the number of influenza B-positive specimens detected, but this only raised the sensitivity to approximately half that of conventional tube culture.[18] The addition of HEp-2 cells increased the sensitivity for RSV from 82.6% to 100%, and also increased detection of parainfluenza viruses and adenoviruses, although only small numbers of these were detected in the study.[18]

One report suggests that the use of MDCK and A549 shell vials can be more sensitive than conventional culture for detection of some respiratory viruses (see **Table 1**).[19] Three and 7 days after initiation, shell-vial cultures were stained with a commercial pool of antibodies, followed by staining with individual antibodies if the pool was positive (Bartels, WA, USA). The reference standard was conventional culture with HEp-2 and PMK for 7 days. For all respiratory viruses analyzed, the sensitivity of the shell-vial culture was 81.9% versus 72.3% for conventional culture (calculations by the present authors, not included in the reference). The shell vials were more sensitive than conventional cultures for influenza A (100% and 42% respectively) and also detected a few more isolates of parainfluenza viruses, although the total number of parainfluenza viruses was small.[19] Conventional culture was more sensitive than shell-vial culture for RSV (86% and 72% respectively).

The use of PMK and A549 cells in shell-vial culture has been evaluated in two studies (see **Table 1**).[20,21] Rabalais and colleagues incubated shell vials for 3 days and stained with the same pooled antibody reagent as those discussed earlier, with staining with individual antibodies if the pool was positive (Bartels, WA, USA).[21] The reference standard was conventional culture with PMK, A549 and HEp-2 cells for up to 14 days. The overall sensitivity of the two methods was similar; shell vial detected 79% of all positive specimens, while conventional culture detected 80% of all positives specimens. Shell-vial culture results were complete in 2 days, while the results of conventional culture took a mean of 7.6 days, with a range of 1 to 14 days. The shell-vial culture was sensitive for influenza B virus, detecting more positive specimens than did conventional culture (no influenza A positive specimens were detected by either method). The two methods were of comparable sensitivity for RSV (75% for shell-vial culture, 70% for conventional culture, calculations by the present authors, not included in the reference). Although the methods were somewhat different, Matthey and colleagues reached similar conclusions, in that shell-vial culture had an overall high sensitivity (94%).[20] Out of 10 specimens positive for influenza A virus, 9 were detected by conventional culture and all 10 were detected by shell-vial culture.

A useful commercial product for shell-vial culture of respiratory viruses, R-Mix (Diagnostic Hybrids, Inc, Athens, Ohio), contains a mixture of a mink lung cell line (Mv1Lu) with A549 cells. Because the cell lines are combined in the same shell vial, fewer shell vials need to be used. The rationale for selecting these cell lines is that A549 should support replication of RSV, adenovirus and parainfluenza viruses, while Mv1Lu will support replication of influenza viruses. In practice, it appears that both the cell lines work well for each of the viruses.[22]

Several studies have been done to determine the sensitivity of R-Mix for detection of respiratory viruses (**Table 2**).[23–26] Each of these studies included some type of conventional culture in the reference standard, and some also included direct antigen testing,[24,26] or another shell-vial culture system.[23] Most studies found that R-Mix is sensitive for detection of influenza A and B viruses, with reported sensitivities of 89% to 98% and 94.7% to 100%, respectively.[24–26] In one study, the sensitivity for

Table 2
Sensitivity of R-Mix shell-vial cultures for respiratory viruses

Cell Lines	Screening Stain (FA)	Days Stained	Influenza A (%)	Influenza B (%)	RSV (%)	Adenovirus (%)	Parainfluenza 1 (%)	Parainfluenza 2 (%)	Parainfluenza 3 (%)	References
Mv1Lu and A549 (R-Mix)	D3, Diagnostic Hybrids	1, 2	78[a]		73	45	83[a]			LaSala et al[23]
Mv1Lu and A549 (R-Mix)	Pooled Reagent, Bartels	1, then CPE to 10 for HA	98.8	94.7	86.7	68.6	86.7	100[b]	83.3[b]	Dunn et al[24]
Mv1Lu and A549 (R-Mix)	None (Individual FA Used)	1, 2, 5	89[b]	100[b]	86	25	100[b]	80[b]	67	Weinberg et al[25]
Mv1Lu and A549 (R-Mix)	Respiratory Virus Screen, Chemicon	1	96	ND	ND	ND	ND	ND	ND	Fong et al[26]
Mv1Lu and A549 (R-Mix ReadyCells)	D3, Diagnostic Hybrids	1, 3	100	93	91	90	100			Kim et al[27]

Abbreviations: ND, not determined because this virus was not detected in any specimens; FA, Fluorescence assay.
[a] The data were pooled in the original report and so sensitivities for some viruses are indicated by combination of the columns.
[b] Fewer than ten specimens contained this virus.

influenza A and B (combined) was somewhat lower at 78%.[23] The reported sensitivity of R-Mix culture for RSV is moderate, with a range of 73.0% to 86.7%. Most of these studies did not include DFA in the gold standard for RSV, and since DFA is usually more sensitive than viral culture for RSV, they might overestimate the sensitivity of R-Mix culture. R-Mix has moderately low sensitivity for adenovirus, with a range of 25% to 68.6%. In each study where the comparison was made using fresh clinical specimens, the sensitivity of R-Mix for adenovirus was significantly lower than the sensitivity of conventional culture. Most studies did not include enough specimens with parainfluenza viruses to give a robust estimate of sensitivity, but in general R-Mix appears to be reasonably sensitive for these viruses. The combined sensitivity for parainfluenzas 1, 2 and 3 was 83% in one study,[23] and separate studies found sensitivities of 87.6% and 67% for parainfluenzas 1 and 3, respectively.[24,25]

A major advantage of R-Mix shell vials is the shorter turn-around time for positive and negative results. The time to detection of viruses is shorter with R-Mix than with conventional culture. LaSala and colleagues found that the mean time to detection of influenza virus was 1.1 days and 4.3 days for R-Mix and conventional culture, respectively.[23] For RSV, these authors found that the mean time to detection was 1.6 days and 10.2 days for R-Mix and conventional culture, respectively. The time to issue a negative report for R-Mix viral culture will depend on the days on which the shell vials are stained. Practice varies between the published studies, but in most studies the shell vial culture was complete within 3 days,[23,26] but some do hold them as long as 5 to 10 days.[24,25] In our laboratory, we stain R-Mix shell vials at 24 and 48 hours, allowing a maximum turn-around time of 2 days. Cryopreserved R-Mix shell vials with a shelf life of up to 6 months are also available. These shell vials have been validated in one study (see **Table 2**).[27] They are reported to have a high sensitivity for influenza viruses, RSV, adenovirus, and parainfluenza viruses when analyzed on day 3.

The practice of using R-Mix shell-vial culture along with an additional shell vial with PMK has been evaluated.[28] These authors harvested R-Mix after a minimum of 20 hours incubation, and performed terminal HA on PMK shell vials after 10 to 14 days. Ninety-five percent of influenza A and B, and parainfluenza types 1, 2 and 3 were detected by R-Mix and only an additional 5% were detected by the terminal HA.[28] The detection of a small number of additional positives at 10 to 14 days is of limited value. R-Mix culture alone was adequate, at least for the HA positive viruses.

SUMMARY

Recently there have been several important improvements in viral culture. MDCK-SIAT1 cells are a useful addition to the available cell lines for influenza viral culture. They can be used in an accurate bioassay for the response of influenza isolates to neuraminidase inhibitors, and also show promise for use in detection of influenza viruses from clinical specimens. HuH7 cells are another promising cell line for use in viral culture for respiratory viruses, although definitive data supporting the use of these is needed. Shell-vial culture is a sensitive method for detection of respiratory viruses. An important advantage of shell-vial culture is the markedly reduced turn-around time for the culture. While shell vials containing a single cell line can be used to detect respiratory viruses, the total number of shell vials needed, and the labor of the culture, can be reduced using commercial R-Mix shell vials, which have a combination of two cell lines. This combination allows a single type of shell vial to be used for respiratory virus culture.

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