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Comparison of a Multiplex Reverse Transcription-PCR-Enzyme Hybridization Assay with Conventional Viral Culture and Immunofluorescence Techniques for the Detection of Seven Viral Respiratory Pathogens

LISA LIOLIOS,[1]* ADAM JENNEY,[2] DENIS SPELMAN,[1,2] TOM KOTSIMBOS,[3] MICHAEL CATTON,[4] AND STEVE WESSELINGH[1]

Infectious Diseases Unit[1] and Departments of Microbiology[2] and Respiratory Medicine,[3] Alfred Hospital, Prahran 3181, and Victorian Infectious Diseases Reference Laboratory, North Melbourne 3051,[4] Victoria, Australia

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A multiplex reverse transcription-PCR-enzyme hybridization assay (RT-PCR-EHA; Hexaplex; Prodesse Inc., Waukesha, Wis.) was used for the simultaneous detection of human parainfluenza virus types 1, 2, and 3, influenza virus types A and B, and respiratory syncytial virus types A and B. One hundred forty-three respiratory specimens from 126 patients were analyzed by RT-PCR-EHA, and the results were compared to those obtained by conventional viral culture and immunofluorescence (IF) methods. RT-PCR-EHA proved to be positive for 17 of 143 (11.9%) specimens, whereas 8 of 143 (5.6%) samples were positive by viral culture and/or IF. Eight samples were positive by both RT-PCR-EHA and conventional methods, while nine samples were RT-PCR-EHA positive and viral culture and IF negative. Eight of the nine samples with discordant results were then independently tested by a different multiplex RT-PCR assay for influenza virus types A and B, and all eight proved to be positive. In comparison to viral culture and IF methods, RT-PCR-EHA gave a sensitivity and a specificity of 100 and 93%, respectively. Since RT-PCR-EHA was able to detect more positive samples, which would otherwise have been missed by routine methods, we suggest that this multiplex RT-PCR-EHA provides a highly sensitive and specific means of diagnostic detection of major respiratory viruses.

Respiratory infections caused by human parainfluenza virus (HPIV) type 1 (HPIV-1), HPIV-2, and HPIV-3, influenza virus types A and B, and respiratory syncytial virus (RSV) types A and B produce upper and lower respiratory tract diseases and are major causes of croup, bronchiolitis, and pneumonia in infants and young children (15, 16, 20, 21, 26). However, these childhood viruses may cause significant morbidity and even mortality in adults, especially among elderly and immunocompromised individuals (4, 14).

Conventional testing for the detection of these seven respiratory pathogens involves the isolation of intact virus particles in cell culture (viral culture) and/or viral antigen detection by immunofluorescence (IF). Viral culture has been recognized as the "gold standard" for the testing of these pathogens; however, this method is generally slow, often taking up to 14 days before results are available (3, 23). Viral antigen detection by IF provides rapid results, but it often lacks sensitivity in detecting some viruses and further confirmation by viral culture may sometimes be required (8, 17). Although the combination of both of these techniques can provide an increase in the proportion of positive results, it has been reported that a significant number of specimens still remain negative, despite clinical and epidemiological suspicions of viral infection (6, 10, 12, 25).

To overcome these limitations, there has been a keen interest in the development of new nucleic acid-based assays. Reverse transcription-PCR (RT-PCR) assays have been shown to be rapid, sensitive, and specific for the detection of respiratory viruses (2, 25). However, monospecific RT-PCR assays requiring separate amplification of each virus of interest are potentially expensive and resource intensive, especially since respiratory pathogens may cause similar clinical syndromes. Multiplex RT-PCR has a significant advantage in that it permits simultaneous amplification of several viruses in a single reaction (1, 5, 9, 13, 22, 27), facilitating cost-effective diagnosis and perhaps improved clinical management (e.g., antiviral therapy for influenza virus type A and B infections).

In the study described here we compared a commercially available multiplex RT-PCR-enzyme hybridization assay (RT-PCR-EHA; Hexaplex; Prodesse Inc., Waukesha, Wis.) with conventional viral culture and IF methods for the detection of seven respiratory viruses.

MATERIALS AND METHODS

Clinical samples. One hundred forty-three specimens (50 nasopharyngeal aspirate and 93 bronchoalveolar lavage specimens) from 126 patients were screened against all seven viruses by RT-PCR-EHA. A total of 0.5 to 1 ml of specimen was added to viral transport medium (minimal essential medium with 2% fetal bovine serum, penicillin [100 U/ml], streptomycin [100 μg/ml], amphotericin B [20 μg/ml], neomycin [40 μg/ml], $NaHCO_3$ buffer), and the mixture was frozen at −70°C for subsequent analysis by RT-PCR-EHA. Another 1 to 2 ml of each sample was used for viral culture and IF testing.

Viral culture and immunofluorescence. Clinical specimens underwent viral culture and IF by standard methods. Briefly, the specimens were diluted in phosphate-buffered saline (PBS) and centrifuged at 2,000 × *g* for 10 min. The pellets were resuspended in PBS, dotted onto Teflon-coated microscope slides, and then dried and fixed in acetone. Indirect IF was then carried out with a VRK Viral Respiratory kit (Bartels, Issaquah, Wash.) according to the manufacturer's

* Corresponding author. Mailing address: Infectious Diseases Unit, Alfred Hospital, Commercial Rd., Prahran, Victoria, Australia, 3181. Phone: 61-3-9276 3516. Fax: 61-3-9276 3424. E-mail: L.Liolios@alfred.org.au.

instructions. The slides were read with an IF microscope. Specimen supernatants were inoculated into primary monkey kidney, HeLa T, human embryonic lung, and Madin-Darby canine kidney cell lines and incubated at 35°C for 14 days in appropriate culture media. The cells were examined biweekly for cytopathic effect, and terminal, blind hemadsorption was performed on day 14.

Multiplex RT-PCR-EHA. RT-PCR-EHA was performed as described previously (8, 9). Briefly, frozen aliquots of the clinical specimens were allowed to thaw and were centrifuged at 1,000 × *g* for 10 min at 4°C. Viral genomic RNA from 280 μl of supernatant (or plasmid RNA from positive RNA transcripts) was extracted, as recommended by the manufacturer, with the QIAamp Viral RNA Mini kit (Qiagen Inc., Valencia, Calif.). Extracted RNA, random hexamers (Prodesse Inc.), and murine leukemia virus reverse transcriptase (Perkin-Elmer, Foster City, Calif.) were used to synthesize the cDNA (7).

PCR amplification was then performed by adding Super-Mix (Prodesse Inc.) and 2.5 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer) to the newly synthesized cDNA. The Super-Mix contained 6.5 pairs of primers designed from highly conserved regions of genetic sequences for the seven respiratory viruses. These primers specifically targeted the hemagglutinin neuraminidase gene of HPIV-1, -2, and -3, the membrane gene of influenza virus type A, the nonstructural gene of influenza virus type B, and the Ib and nucleocapsid genes of RSV types A and B (9). An initial pre-PCR step of 95°C for 10 min was performed in a DNA thermocycler (9700, Perkin-Elmer), followed by a total of 40 PCR cycles under the following conditions: 2 cycles of 95°C for 60 s, 55°C for 30 s, and 72°C for 45 s and then 38 cycles of 94°C for 60 s, 60°C for 30 s, and 72°C for 30 s. The final cycle was followed by an additional 72°C for 7 min to complete partial polymerizations.

A QIA Quick Purification kit (Qiagen Inc.) was then used to purify the PCR products. A total of 5 μl of purified and denatured PCR product and 65 μl of peroxidase-labeled probe solutions 1 to 7 (Prodesse Inc.) were then added to wells of a 96-well avidin-coated microtiter plate (8, 18). A capture and hybridization reaction was then carried out for 1 h at 42°C. Each well was washed 10 times with 250 μl of 1× Wash Solution (10× Wash Solution; Prodesse Inc.), and then 200 μl of substrate solution was added to each well. After 10 min, the reaction was stopped with the addition of 50 μl of 1 N H_2SO_4, and the optical density (OD) of each well was measured at 450 nm on a spectrophotometer (Dynatech, Guernsey, Channel Islands). The positive cutoff value was calculated to be four times greater than the value for the negative control and had an OD of ≥0.400.

A viral RNA-positive control (Prodesse Inc.) was used in each run. The positive control included viral RNA transcripts of plasmid containing the viral sequences of interest and did not contain any intact viral particles. A negative control (viral transport medium) containing no nucleic acid was also included in each run to check for any PCR cross contamination and to establish a daily baseline reading for the detection phase of the procedure.

Influenza virus RT-PCR. Respiratory samples that were RT-PCR-EHA positive but IF and viral culture negative for either influenza virus types underwent multiplex RT-PCR for influenza virus types A and B, as described previously (30). The PCR primers used in this assay hybridized to a region of the viral genome different from that to which the primers used in the RT-PCR-EHA hybridized.

RESULTS

Primer and probe specificities. To assess the integrities of the primers and probes used in the RT-PCR-EHA, positive RNA controls from all seven viruses were assayed in the presence of all primer pairs and screened against all seven probes. Typical OD readings for negative controls and typical OD readings for positive controls, were achieved with the specific probes (data not shown). No cross-reactivity was detected among the seven respiratory pathogens, demonstrating the high degree of specificity of this assay.

Viral culture and IF. A total of 143 clinical specimens collected from 126 patients were used in the study. Table 1 presents the patient demographics. Viral culture and/or IF results revealed 8 positive samples of a total of 143 samples tested (5.6%). Five samples were IF positive and viral culture negative (four were positive for influenza virus type A and one was positive for RSV), one sample was IF negative and viral culture positive (for influenza virus type A), one sample was IF positive (for RSV; no viral culture was performed), and one sample was positive by both IF and viral culture methods (for influenza virus type B).

TABLE 1. Patient demographics

Characteristic	Value
Total no. of patients	126
Sex (no. of males/no. of females)	81/45
Age range (yr)	
Male	18–85
Female	22–78
Collection interval	August 1999–March 2000
Total no. of clinical specimens	143
No. of nasopharyngeal aspirate specimens	50
No. of bronchoalveolar lavage specimens	93

Detection of RNA in respiratory samples. The same 143 clinical specimens were screened for the seven respiratory viruses by RT-PCR-EHA. RT-PCR-EHA found a total of 17 positive samples (13 for influenza virus type A, 2 for influenza virus type B, 1 for HPIV-3, and 1 for RSV types A and B) among 143 clinical specimens tested (11.9%). This included all eight samples found to be positive by routine methods and nine additional positive samples. Table 2 compares the results of viral culture and those of IF with multiplex RT-PCR-EHA for the detection of viral RNA in the respiratory samples.

Influenza virus-specific RT-PCR. Eight of the nine samples with discordant results were tested by a second multiplex RT-PCR assay for influenza virus types A and B. All eight samples (seven of which were influenza virus type A positive and one of which was influenza virus type B positive) were found to be positive by this additional testing, supporting the findings of the RT-PCR-EHA results. One sample which was positive for HPIV-3 by RT-PCR-EHA but negative for HPIV-3 by IF and viral culture (Table 3) was not tested by another method. These supplementary investigations confirmed that eight of the nine samples with RT-PCR-EHA-positive results were true positives that would not have been found to be positive by

TABLE 2. Comparison of results of viral culture and IF and those of multiplex RT-PCR-EHA methods for detection of respiratory viruses[a]

RT-PCR-EHA/VC-IF result[b]	No. of specimens with indicated results					
	HPIV-1	HPIV-2	HPIV-3	Inf A[c]	Inf B[d]	RSV
+/+	0	0	0	6	1	1[e]
+/−	0	0	1	7	1	0
−/−	143	143	142	130	141	142
−/+	0	0	0	0	0	0

[a] A total of 143 clinical specimens were screened for each respiratory virus.
[b] VC, viral culture. The four possible combinations of RT-PCR-EHA and viral culture-IF results are listed. +, positive result; −, negative result.
[c] Inf A, influenza virus type A.
[d] Inf B, influenza virus type B.
[e] The subtype was not defined by viral culture or IF. However, the sample was RSV type A and B positive by RT-PCR-EHA.

TABLE 3. Comparison of results for 17 clinical specimens positive by either viral culture-IF, multiplex RT-PCR-EHA, or multiplex RT-PCR methods for detection of respiratory viruses[a]

Sample no.	Specimen	VC-IF[a] result	RT-PCR-EHA result	RT-PCR result[b]
1	NPA	Flu A+	Flu A+	NT
2	NPA	RSV +	RSV +	NT
3	NPA	Flu A+	Flu A+	NT
4	NPA	Flu A+	Flu A+	NT
5	NPA	Flu A+	Flu A+	NT
6	NPA	Flu A+	Flu A+	NT
7	NPA	Flu A+	Flu A+	NT
8	NPA	Flu B+	Flu B+	NT
9	NPA	ND	Flu A+	Flu A+
10	BAL	ND	Flu A+	Flu A+
11	BAL	ND	Flu A+	Flu A+
12	NPA	ND	Flu A+	Flu A+
13	NPA	ND	Flu A+	Flu A+
14	NPA	ND	Flu A+	Flu A+
15	NPA	ND	HPIV-3+	NT
16	NPA	ND	Flu A+	Flu A+
17	NPA	ND	Flu B+	Flu B+

[a] Abbreviations and symbols: VC, viral culture. NPA, nasopharyngeal aspirate; BAL, bronchoalveolar lavage; NT, Not tested; ND, not detected; Flu A, influenza virus type A; Flu B, influenza virus type B; +, positive result.
[b] Multiplex RT-PCR for influenza virus types A and B only (non-RT-PCR-EHA).

routine methods and that therefore would have otherwise been missed.

Sensitivity and specificity. Initial comparison of RT-PCR-EHA results to those of IF and/or viral culture as a gold standard generated a sensitivity, a specificity, a positive predictive, and a negative predictive value of 100, 93, 47, and 100%, respectively, for RT-PCR-EHA (Table 4). When eight of the nine samples with initial discordant results were considered true positives, the specificity and positive predictive value increased to 99 and 94%, respectively.

DISCUSSION

Detection of respiratory pathogens by RT-PCR-EHA proved to be better than that by conventional IF or viral culture methods. These findings are consistent with those of other studies previously reported, which have used monospecific or multiplex RT-PCR assays for the detection of viral infections (2, 11, 12, 25). Although oligonucleotide hybridization confirms the specificity of the amplified PCR product, there is also evidence of improved sensitivity of PCR assays with oligonucleotide hybridization compared to the sensitivity of PCR assays with agarose gels for the detection of infection (10, 28). RT-PCR-EHA incorporates both the RT-PCR technology and microplate hybridization for confirmation of results, with the combination of both techniques potentially augmenting the sensitivity of this assay.

TABLE 4. Comparison of results of viral culture and IF and those of multiplex RT-PCR-EHA for detection of seven respiratory viruses

RT-PCR-EHA result	No. of specimens with the following viral culture-IF results:	
	Positive	Negative
Positive	8	9
Negative	0	126
Total	8	135

In our study, RT-PCR-EHA detected all viral culture- and IF-positive clinical samples and additional positive samples which would otherwise have been missed by routine methods. These results highlight the superiority of the sensitivity and specificity of RT-PCR-EHA compared with those of conventional methods. The finding of viral culture- and IF-negative but RT-PCR-EHA-positive samples may be due to the amount and viability of the viruses present in the nasopharyngeal aspirate and bronchoalveolar lavage specimens. The advantage of the RT-PCR methodology for the detection of viruses from clinical specimens is that the method can detect the virus genome when it is present at a low titer or when the virus is not replication competent. The brevity of the infection, localization to the respiratory tract, and temporal association with clinical symptoms characteristic of viral shedding make it relatively easy to ascribe clinical significance to the detection of viral nucleic acid. The possibility that the additional samples detected by RT-PCR-EHA represent samples with false-positive results is unlikely since eight of the nine samples were confirmed to be positive by another RT-PCR method. This high degree of sensitivity of RT-PCR compared to those of viral culture and IF for the detection of currently circulating influenza strains (influenza A/Sydney IS/97-like and influenza B/Beijing/184/93-like) is in line with our experience over two respiratory seasons of parallel testing by culture, IF and influenza virus-specific RT-PCR (data not shown). The result for the one sample positive for HPIV-3 by RT-PCR-EHA but whose result could not be confirmed by other methods is, we believe, unlikely to represent a false-positive result due to the rigorous attention given to optimal PCR work practices (19) and the integrity of the no-target controls carried through each step of the assay process. The result is biologically plausible, as HPIV-3 did circulate in the community during the study.

If samples with true-positive results are defined as those which are culture and/or IF positive or culture and IF negative but positive by RT-PCR-EHA and the second RT-PCR method, then the specificity of RT-PCR-EHA is 99%, with a positive predictive value of 94%. The negative predictive value was 100%, with no false-negative results obtained by RT-PCR-EHA.

Although the viruses tested for in the present study have previously been reported to be significant pathogens, especially in immunocompromised bone marrow and lung transplant recipients (24, 29), the patient population in our study was not specifically targeted to assess the incidence of these infections through a winter period. Future work would aim at a more targeted population. We found that the majority of our positive samples were positive for influenza virus type A (76%), followed by influenza virus type B (12%), which is indicative of our adult patient population. The incidence of childhood virus infections (RSV and HPIV infections) was low.

Although RT-PCR-EHA is capable of simultaneously detecting seven respiratory pathogens from one clinical specimen, no multiple infections were detected in the present study. However, one sample was positive for both RSV type A and

RSV type B by RT-PCR-EHA. This is due to the ability of the RSV type A-specific probe to bind to both RSV type A and RSV type B nucleic acid material. Hence, in the presence of RSV type B, a positive signal is also observed for RSV type A and the possibility of a dual infection with RSV type A and RSV type B cannot be ruled out.

The clinical relevance of detection of seven viruses in the population tested depends on several factors. These include the association between virus detection and the clinical disease syndrome that may be caused by the virus, the ability of the host to eradicate the virus without going into respiratory failure, and the availability of timely treatment interventions. A large number of our patients tested were immunocompromised hosts. We believe that the identification of these viruses as causes of respiratory disease in these patients is the first step in determining how frequently they may cause serious problems and, hence, how hard we should push with both accepted treatments such as those for influenza virus infection (either empirical or targeted treatment) and more controversial treatments such as those for RSV and parainfluenza virus infections (ribavirin and RSV hyperimmune globulin).

Only clinical specimens (nasopharyngeal aspirate and bronchoalveolar lavage specimens) were assayed in the present study. However, RT-PCR-EHA is able to detect the seven respiratory viruses in various body fluids, including washes (nasal, throat, and tracheal washes), swabs (nasopharyngeal and throat swabs), aspirates (tracheal, lung, and throat aspirates), lung biopsy specimens, and cerebrospinal fluid.

The speed of diagnosis of viral infection by RT-PCR-EHA is intermediate between the speeds of detection by viral culture and rapid IF methods. The assay requires approximately 10 h of processing time, and clinical specimens can simultaneously be screened against seven respiratory pathogens with comparatively little effort. It should be noted that a chosen cocktail can be used with this kit to target a particular virus, for example, influenza virus types A and B only; however, this will not result in any significant cost savings or a loss of technical time. The cost-effectiveness is yet to be established, but batch testing and an increase in throughput of specimens would certainly decrease the unit cost.

In conclusion, RT-PCR-EHA constitutes a more specific and sensitive alternative to conventional viral culture and IF methods, making this multiplex assay well suited for use in epidemiological studies and beneficial for a respiratory disease diagnostic service. Specific and sensitive assays, such as the RT-PCR-EHA described here, which are able to provide rapid (with turnaround times of 24 to 36 h) and defined results for virus detection are critical in the clinical setting. Such assays may potentially reduce nosocomial transmission to high-risk patients, limit unnecessary antibiotic use, and improve clinical management as a result of the use of appropriate and directed therapy following diagnosis of infection with a specific virus. The results of the present study indicate that multiplex RT-PCR assays have great potential for use in the detection of common respiratory pathogens.

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REFERENCES

1. **Aguilar, J. C., M. P. Pérez-Breña, M. L. Garcia, N. Cruz, D. D. Erdman, and J. E. Echevarria.** 2000. Detection and identification of human parainfluenza viruses 1, 2, 3, and 4 in clinical samples of pediatric patients by multiplex reverse transcription-PCR. J. Clin. Microbiol. **38:**1191–1195.
2. **Atmar, R. L., B. D. Baxter, E. A. Dominiguez, and L. H. Taber.** 1996. Comparison of reverse transcription-PCR with tissue culture and other rapid diagnostic assays for detection of type A influenza virus. J. Clin. Microbiol. **34:**2604–2606.
3. **Betts, R. F.** 1995. Influenza virus, p. 1546–1567. *In* G. L. Mandell, R. G. Douglas, Jr., and J. E. Benett (ed), Principles and practice of infectious diseases, 4th ed. Churchill Livingstone, Inc., New York, N.Y.
4. **Dowell, S. F., L. J. Anderson, H. E. Gary, Jr., D. D. Erdman, J. F. Plouffe, T. M. File, Jr., B. J. Marston, and R. F. Breiman.** 1996. Respiratory syncytial virus is an important cause of community-acquired lower respiratory infection among hospitalized adults. J. Infect. Dis. **174:**456–462.
5. **Echevarría, J. E., D. D. Erdman, E. M. Swierkosz, B. P. Holloway, and L. J. Anderson.** 1998. Simultaneous detection and identification of human parainfluenza viruses 1, 2, and 3 from clinical samples by multiplex PCR. J. Clin. Microbiol. **36:**1388–1391.
6. **Ellis, J. S., D. M. Fleming, and M. C. Zambon.** 1997. Multiplex reverse transcription-PCR for surveillance of influenza A and B viruses in England and Wales in 1995 and 1996. J. Clin. Microbiol. **35:**2076–2082.
7. **Fan, J., Z. Bass, and J. L. Fahey.** 1993. Elevated IFN-γ and decreased IL-2 gene expression are associated with HIV infection. J. Immunol. **151:**5031–5040.
8. **Fan, J., and K. J. Henrickson.** 1996. Rapid diagnosis of human parainfluenza virus type 1 infection by quantitative reverse transcription-PCR-enzyme hybridization assay. J. Clin. Microbiol. **34:**1914–1917.
9. **Fan, J., K. J. Henrickson, and L. L. Savatski.** 1998. Rapid simultaneous diagnosis of infections with respiratory syncytial viruses A and B, influenza viruses A and B and human parainfluenza virus types 1, 2, and 3 by multiplex quantitative reverse transcription-polymerase chain reaction-enzyme hybridisation assay (Hexaplex). Clin. Infect. Dis. **25:**1397–1402.
10. **Freymuth, F., G. Eugene, A. Vabret, J. Petitjean, E. Gennetay, J. Brouard, J. F. Duhamel, and B. Guillois.** 1995. Detection of respiratory syncytial virus by reverse transcription-PCR and hybridization with a DNA enzyme immunoassay. J. Clin. Microbiol. **33:**3352–3355.
11. **Freymuth, F., A. Vabret, F. Galateau-Salle, J. Ferey, G. Eugene, J. Petitjean, E. Gennetay, J. Brouard, M. Jokik, J. F. Duhamel, and B. Guillois.** 1997. Detection of respiratory syncytial virus, parainfluenzavirus 3, adenovirus and rhinovirus sequences in respiratory tract of infants by polymerase chain reaction and hybridization. Clin. Diagn. Virol. **8:**31–40.
12. **Gilbert, L. L., A. Dakhama, B. M. Bone, E. E. Thomas, and R. G. Hegele.** 1996. Diagnosis of viral respiratory tract infections in children by using a reverse transcription-PCR panel. J. Clin. Microbiol. **34:**140–143.
13. **Gröndahl, B., W. Puppe, A. Hoppe, I. Kühne, J. A. I. Weigl, and H. J. Scmitt.** 1999. Rapid identification of nine microorganisms causing acute respiratory tract infections by single-tube multiplex reverse transcription-PCR: feasibility study. J. Clin. Microbiol. **37:**1–7.
14. **Gross, P. A., M. Rodstein, J. R. LaMontagne, R. A. Kaslow, A. J. Saah, S. Wallenstein, R. Neufeld, C. Denning, P. Gaerlan, and G. V. Quinnan.** 1988. Epidemiology of acute respiratory illness during an influenza outbreak in a nursing home: a prospective study. Arch. Intern. Med. **148:**559–661.
15. **Hall, C. B.** 1987. Respiratory syncytial virus, p. 1653–1676. *In* R. D. Feigin and J. D. Cherry (ed.) Textbook of paediatric infectious diseases, 2nd ed. The W. B. Saunders Co., Philadelphia, Pa.
16. **Henrickson, K. J.** 1998. Viral pneumonia in children. Semin. Pediatr. Infect. Dis. **9:**217–233.
17. **Henrickson, K. J., S. M. Kuhn, L. L. Savatski, and J. Sedmak.** 1994. Recovery of human parainfluenza virus types one and two. J. Virol. Methods. **46:**189–206.
18. **Holodniy, M., A. Katzenstein, M. Israelski, and T. C. Merigan.** 1994 Reduction in plasma human immunodeficiency virus ribonucleic acid after dideoxynucleoside therapy as determined by the polymerase chain reaction. J. Clin. Investig. **88:**1755–1759.
19. **Kwok, S., and R. Higuchi.** 1989. Avoiding false positives with PCR. Nature **339:**237–238.
20. **Monto, A. S.** 1973. The Tecumseh study of respiratory illness. V. Patterns of infection with the parainfluenzaviruses. Am. J. Epidemiol. **97:**338–348.
21. **Murry, A. R., and S. F. Dowell.** 1997. Respiratory syncytial virus: not just for kids. Hosp. Pract. **32:**87–98.
22. **Osiowy, C.** 1998. Direct detection of respiratory syncytial virus, parainfluenza virus, and adenovirus in clinical respiratory specimens by a multiplex reverse transcription-PCR assay. J. Clin. Microbiol. **36:**3149–3154.
23. **Paton, A. W., J. C. Paton, A. J. Lawrence, P. N. Goldwater, and R. J. Harris.** 1992. Rapid detection of respiratory syncytial virus in nasopharyngeal aspirates by reverse transcription and polymerase chain reaction amplification. J. Clin. Microbiol. **30:**901–904.
24. **Rabella, N., P. Rodriguez, R. Labeaga, M. Otegui, M. Mercader, M. Gurgui, and G. Prats.** 1999. Conventional respiratory viruses recovered from immu-

nocompromised patients: clinical considerations. Clin. Infect. Dis. **28:**1043–1048.
25. **Tantivanich, S., K. Suphanaranonda, K Balachanda, and R. Anderson.** 1995. Detection of respiratory syncytial virus from clinical specimens: comparison between reverse transcription polymerase chain reaction and tissue culture. Southeast Asian J. Trop. Med. Public Health **26:**684–688.
26. **Vainionpää, R., and T. Hyypiä.** 1994. Biology of parainfluenza viruses. Clin. Microbiol. Rev. **7:**265–275.
27. **Valassina, M., A. M. Cuppone, M. G. Cusi, and P. E. Valensin.** 1997. Rapid detection of different RNA respiratory virus species by multiplex RT-PCR: application to clinical specimens. Clin. Diagn. Virol. **8:**227–232.
28. **Van Milaan, A. J., M. J. W. Sprenger, P. H. Rothbarth, A. H. Bradenburg, N. Masurel, and E. C. J. Claas.** 1994. Detection of respiratory syncytial virus by RNA-polymerase chain reaction and differentiation of subgroups with oligonucleotide probes. J. Med. Virol. **44:**80–87.
29. **Whimbey, E., R. E. Champlin, R. B. Couch, J. A. Englund, J. M. Goodrich, D. Przepiorka, V. A. Lewis, N. Mirza, H. Yousuf, J. J. Tarrand, and G. P. Bodey.** 1996. Community respiratory virus infections among hospitalized bone marrow transplant recipients. Clin. Infect. Dis. **22:**778–782.
30. **Zhang, W., and D. W. Evans.** 1991. Detection and identification of human influenza viruses by the polymerase chain reaction. J. Virol. Methods **33:**165–189.