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Reliable Detection of Respiratory Syncytial Virus Infection in Children for Adequate Hospital Infection Control Management

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By using a rapid test for respiratory syncytial virus (RSV) detection (Abbott TestPack RSV), a number of patients were observed, showing repeatedly positive results over a period of up to 10 weeks. A prospective study was initiated to compare the rapid test with an antigen capture enzyme immunoassay (EIA) and a nested reverse transcriptase PCR (RT-PCR) protocol for detection of RSV serotypes A and B. Only respiratory samples from children exhibiting the prolonged presence of RSV (≥5 days) as determined by the rapid test were considered. A total of 134 specimens from 24 children was investigated by antigen capture EIA and nested RT-PCR. Using RT-PCR as the reference method, we determined the RSV rapid test to have a specificity of 63% and a sensitivity of 66% and the antigen capture EIA to have a specificity of 96% and a sensitivity of 69% for acute-phase samples and the homologous virus serotype A. In 7 (29%) of 24 patients, the positive results of the RSV rapid test could not be confirmed by either nested RT-PCR or antigen capture EIA. In these seven patients a variety of other respiratory viruses were detected. For general screening the RSV rapid test was found to be a reasonable tool to get quick results. However, its lack of specificity in some patients requires confirmation by additional tests to rule out false-positive results and/or detection of other respiratory viruses.

During the cold season, respiratory viruses substantially contribute to morbidity in infants and toddlers. In these very young children respiratory syncytial virus (RSV) is the main agent causing severe infections in the lower respiratory tract (14). A considerable number of affected children require hospitalization for adequate care because of respiratory distress, oxygen dependency, or apnea. Since RSV is highly contagious and since nosocomial spread to fellow patients at high risk for severe RSV disease, including those with cyanotic, congenital heart disease or underlying pulmonary disease, may be detrimental, appropriate isolation and measures of precaution are mandatory (16, 17). Cell culture is still the best surrogate for contagious patients. However, rapid tests, which detect RSV as reliably as culture, serve as welcome guides for emergency room and hospital ward staff who must establish isolation or cohorting of RSV-infected patients in order to prevent transmission of the virus to fellow patients with compromised cardiac, pulmonary, or immune systems. During the RSV peak season, rapid tests for RSV detection based on enzyme immunoassay (EIA) technology may serve also as a surrogate means for identifying patients who are no longer contagious and thus no longer require application of stringent precautionary measures. This has an significant impact when the number of single rooms or the available space for cohorting of patients in intensive-care units, nurseries, or other wards is limited, because it offers the possibility of avoiding unnecessary, prolonged isolation of patients and unwarranted occupancy of urgently needed space.

The nasopharyngeal excretion of RSV decreases rapidly 1 to 3 days after the onset of symptoms (18). The observation in several patients that a rapid test for RSV yielded repeatedly positive results in sequentially respiratory secretions collected over periods of several weeks prompted us to conduct the prospective study reported here. The aim was to compare the results of the rapid test, potentially resulting in inadequately long cohorting of patients, with the results of other laboratory methods for RSV detection in children who exhibited positive results in the rapid RSV test in at least two follow-up samples over a period of 5 days or longer.

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MATERIALS AND METHODS

Clinical samples. Nasopharyngeal secretions (NPS) were derived from children exhibiting respiratory symptoms who were hospitalized at the University Children's Hospital of Zurich during the RSV season lasting from November 1998 to April 1999. The specimens had been sent to the Infectious Diseases Laboratory with a request for a rapid test for RSV, which was executed within 15 to 30 min. The surpluses of the samples were subsequently stored at 4°C and were transferred to the Institute of Medical Virology once a week, where they were kept at −80°C until further investigation. Samples from patients showing positive results in the rapid test in at least two consecutive specimens collected ≥5 days apart were investigated employing two other methods to detect RSV or other respiratory viruses. Randomly selected specimens from patients testing negative in the rapid test for RSV served as negative controls for this virus.

EIAs. (i) Rapid test. The Abbott TestPack RSV (Abbott Laboratories, Abbott Park, Ill.) was used as the rapid test for RSV detection. Mucus was dissolved from a 750-μl specimen of NPS and was processed according to the recommendations of the manufacturer. The total time required to perform a test was roughly 20 min.

(ii) Antigen capture EIA. Antibodies used for antigen capture (guinea pig-derived) and antigen detection (rabbit-derived) were obtained from the Institute of Virology of the University of Turku, Turku, Finland. Anti-RSV antibodies had been induced by a type A strain of RSV. Multiwell plates (Nunc, Roskilde, Denmark) were coated with guinea pig anti-RSV antibodies, incubated overnight at room temperature, washed three times with phosphate-buffered saline, and stored at 4°C. NPS specimens were sonicated, and 100 μl was transferred into each well. Positive and negative controls (antigens provided by MicrobeScope, Rüschlikon, Switzerland) were included in each run. Antigen capture was accomplished during incubation at 37°C overnight. After three washings, rabbit anti-RSV antibodies were added and the plate contents were incubated at 37°C for 1 h. For antigen detection, swine anti-rabbit peroxidase-coupled immuno-

globulin G conjugate (MicrobeScope) was pipetted into each well and the plate contents were incubated for another hour. Substrate (OPD; Sigma, St. Louis, Mo.) was added for color development, and the optical density at 495 nm was determined. Cutoff values were defined as three times the optical density at 495 nm of the negative controls. Test results of specimens were available at noon of the day following collection.

The same technology was used for screening of NPS for other respiratory viruses, including adenoviruses, influenza viruses A and B, and parainfluenza viruses 1 to 3.

PCR analyses. (i) Nested RT-PCR for RSV. RNA was extracted from a 180-μl sample of NPS by using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, Calif.). Two microliters of eluted RNA was transferred to a reverse transcriptase PCR (RT-PCR) mixture of 28 μl of RNase-free water, 10 μl of 5× reaction buffer, 1 μl of deoxynucleoside triphosphate mix, 5 μl of primer mix containing 25 pmol of each primer, 2 μl of $MgSO_4$, and 1 μl of each avian myeloblastosis virus RT and Tfl polymerase (all reagents provided in the Promega RT-PCR kit; Promega, Madison, Wis.). The primers used were those described by Stockton et al. (15) (outer primers: RSV AB1, 5′-GTCTTACAGCCGTGATTAGG-3′; and RSV AB2, 5′-GGGCTTTCTTTGGTTACTTC-3′). The cycling protocol included 1 h of reverse transcription at 48°C, a 5-min activation step of the Tfl polymerase at 95°C and 40 cycles of 15 seconds of denaturation at 95°C, and 30 s of primer annealing at 50°C and 30 s of primer extension at 72°C, followed by a final extension step of 7 min at 72°C in PE Biosystems GeneAmp 2400 thermocyclers. Five microliters of the first-round PCR product was transferred to freshly prepared master mixes containing 26 μl of H_2O, 5 μl of 10× reaction buffer, 5 μl of 10 mM deoxynucleoside triphosphates, 5 μl of primer mix (containing 25 pmol of each inner primer), 4 μl of 12.5 mM $MgCl_2$, and 2 U of hot start *Taq* polymerase (Amplitaq Gold; PE Biosystems, Foster City, Calif.). Type-specific primer pairs for RSV serotype A (RSV A) and RSV B were used for nested PCR (inner primers: RSV A1, 5′-GATGTTACGGTGGGGAGTCT-3′; RSV A2, 5′-GTACACTGTAGTTAATCACA-3′; RSV B1, 5′-AATGCTAAGATGGGGAGTTC-3′; and RSV B2, GAAATTGAGTTAATGACAGC-3′).Cycling conditions of the second round of PCR included a 12-min activation step of the *Taq* polymerase at 94°C, followed by 30 cycles of 30 s of denaturation at 94°C, 30 s of primer annealing at 50°C, and 90 s of primer extension at 72°C for both serotypes. PCR products (first-run products, 836 bp for RSV A and B; second-run products, 334 bp for RSV A and 183 bp for RSV B, respectively) were detected by gel electrophoresis on 1.5% agarose gels and with ethidium bromide staining. Diluted supernatants of uninfected and RSV A-infected Vero cell cultures served as negative and positive controls and were included in every assay.

(ii) PCR for adenoviruses and influenza A and B viruses. Selected samples of NPS were screened in addition to RSV for the presence of adenoviruses and influenza A and B viruses by following the PCR protocols of Saitoh-Inagawa et al. (13) and Zhang and Evans (19), respectively.

RESULTS

Frequency of repeatedly positive rapid assays for RSV. During the study period, 732 NPS samples from 441 children (208 females and 223 males) were sent to the Infectious Diseases Laboratory with a request for a rapid RSV detection assay (Fig. 1A). More than one sample was sent for testing from 117 of these children. In 81 children the collection period was ≥5 days. Positive rapid assays for RSV in NPS samples collected over a period of at least 5 days were noted in 24 (29.6%) of these children. Of the 136 samples of these 24 children (range, 2 to 13 samples per child; mean, 5.6), 134 were subjected to further investigations by antigen capture EIA and RT-PCR. Two samples could not be further evaluated due to insufficient volumes.

Comparison of assays detecting RSV. Seventy-four (55%) of the 134 NPS samples, evaluable by different tests, were positive in the rapid assay for RSV, while 60 (45%) were negative (Fig. 1B). Positive results in the rapid assay of 18 samples (seven children) could not be confirmed, either by nested RT-PCR or by antigen capture EIA. Four children with repeatedly positive results in the rapid test never tested positive in any of the other two assays. Three children with a confirmed RSV B infection

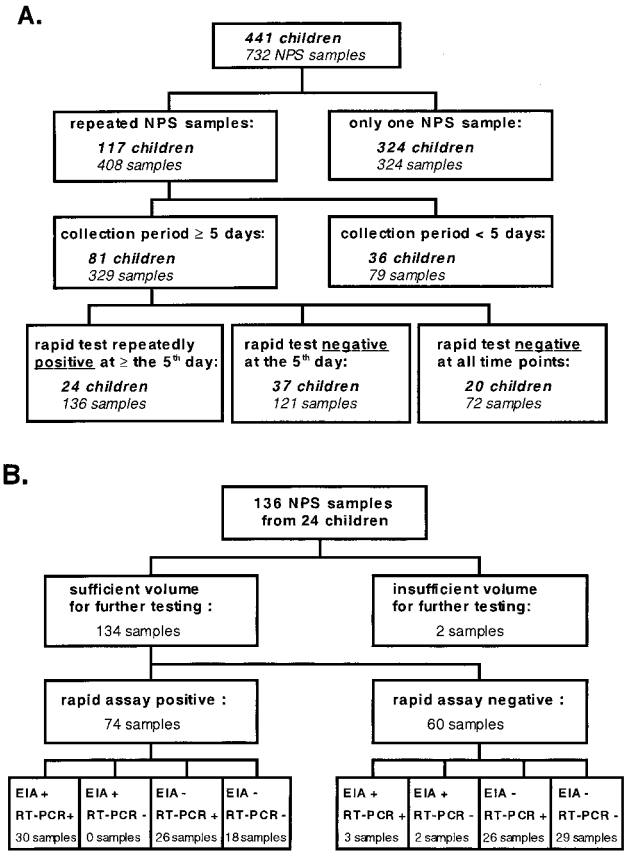

FIG. 1. Study algorithm and sample distribution. (A) The rapid test (Abbott TestPack RSV) was executed with all NPS obtained. Depending on the results, the children were distributed into those with repeated samples and those with only one sample; the latter were excluded from further investigation. Children whose samples were collected over a period of 5 days or more were segregated into those with repeatedly positive results in the rapid assay on the 5th day or later, those who converted to negative results within 5 days and remained negative, and those who never showed a positive result in the rapid assay. Samples of children who showed positive results over a period of at least 5 days were further investigated by antigen capture EIA and nested RT-PCR. (B) One hundred and thirty-four samples of the children that were included in the study were further investigated by nested RT-PCR and antigen capture EIA. Two samples had to be excluded due to insufficient volume to execute all assays. The 134 samples were divided into 74 with a positive result in the rapid assay and 60 with a negative result. These two groups were further segregated into those varying or confirming results in the antigen capture EIA and the nested RT-PCR.

showed positive results in the rapid assay in samples taken up to 70 days apart that were not confirmed by any of the other tests. Twenty-six samples (12 children) that tested positive by nested RT-PCR showed no positive results, either in the rapid assay or the antigen capture EIA. False-positive PCR results due to contamination were ruled out by appropriate controls and by the fact that positive results for these samples were obtained in independent PCR runs. In 6 of 8 children suffering from RSV B and in 2 of 12 with RSV A, the antigen capture EIA gave negative results, sometimes repeatedly, although both Abbott TestPack RSV and RT-PCR showed positive reactions. In summary we determined a sensitivity of 66% and a

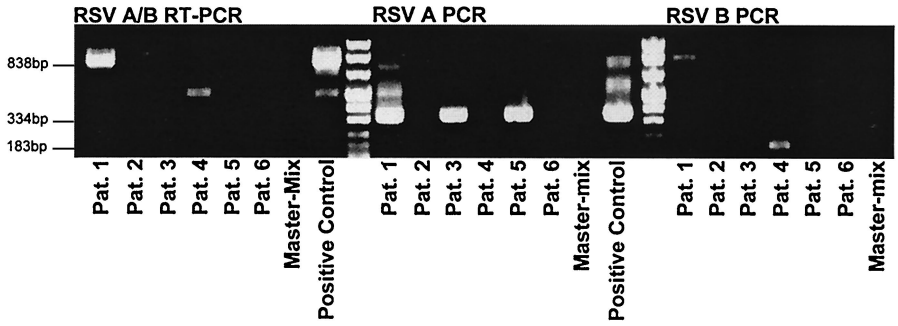

FIG. 2. RSV typing by RT-PCR. Reverse transcription and a first run of PCR are executed in a one-step protocol using primers that allow amplification of RSV A as well as RSV B (RSV A/B RT-PCR). These amplification products are used in a second PCR (nested PCR) with serotype A (RSV A PCR)- and B (RSV B PCR)-specific primer sets. A positive control (RSV A) and a negative control (master mix) are included. For detection, gel electrophoresis on a 1.5% agarose gel is executed.

specificity of 63% for the rapid assay in our study group, using RT-PCR as the reference. The antigen capture EIA exhibited an overall sensitivity of 39% and a specificity of 96%. Taking in account only the homologous serotype A and the first sample in the acute infection, the calculated sensitivity of the antigen capture EIA was 69%.

Further investigations. Serotypes were determined by using specific RT-PCR primer sets (Fig. 2). The 85 samples that tested positive in the nested RT-PCR divided up into 65 samples (12 children) of RSV A and 20 samples (8 children) of RSV B. RSV A was detectable by RT-PCR as long as 30 days maximum with a mean of 12.8 days, while RSV B tested positive in RT-PCR as long as 10 days with a mean of 5.8 days (Fig. 3). Available samples of children with an unconfirmed positive result in the rapid assay were further investigated for other respiratory viruses, including adenoviruses, parainfluenza viruses 1 to 3, and influenza viruses A and B, by using an antigen capture EIA for all of these viruses and/or nested RT-PCR for adenoviruses and influenza viruses. Employing these methods, NPS from one child was found to harbor both influenza A and adenoviruses. In the NPS of two other children, positive results for parainfluenza viruses and adenoviruses were determined. The NPS of a fifth child tested positive for influenza B virus, while the NPS of three further children contained adenoviruses (Table 1).

DISCUSSION

In the present prospective study, the observation could be confirmed that some patients show positive laboratory results for RSV over a period of up to 10 weeks. This affects patient management, number of hospital days, and costs, all of which can be positively influenced by reasonably applied laboratory methods. In this context, the choice of methods for RSV detection in the hospital routine, its performance, the resulting consequences, and the recommendations for patient management need to be considered.

When methods for RSV detection are being compared, defining a "gold standard" that works for daily routine is difficult (10). Isolation in cell culture has proven to be sensitive and specific and, in contrast to other methods, does not target a single virus. Poor specimen quality and/or inappropriate specimen handling, however, severely decreases the sensitivity of cell culture, giving rise to false-negative results (8). In this study cell culture was not undertaken due to inappropriate storage conditions and transport of samples. In addition, isolation in cell cultures is not sufficiently rapid to influence patient management (2). Analysis of NPS by EIA technology represents an alternative method for virus detection combining

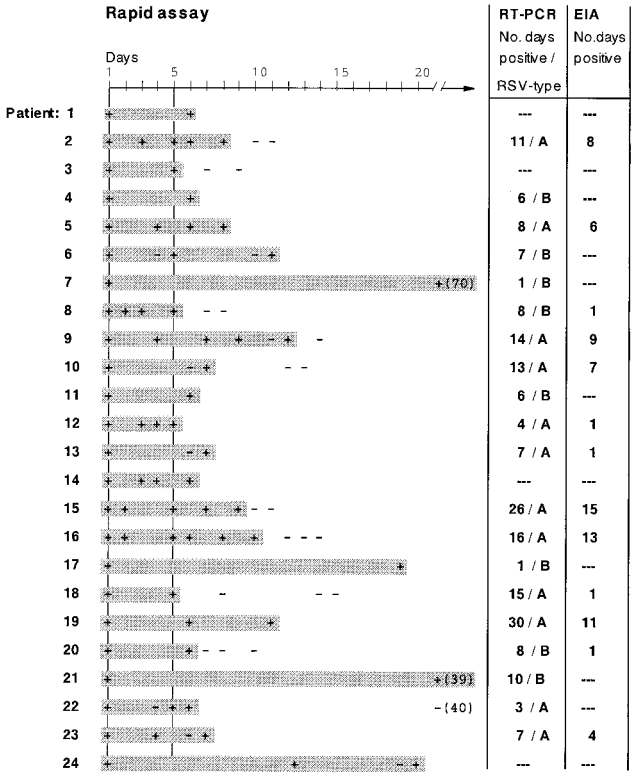

FIG. 3. Duration of RSV presence using different detection assays. The results in the rapid assay of the 24 children included in the study are given in context to the time when the samples were obtained. The 5th day is marked since it represents the main inclusion criteria of the study. The periods in which positive results in the rapid assay were obtained are shaded gray. The number of days in which a positive result was found in the antigen capture EIA and in the nested RT-PCR is given at the right; serotypes as determined by PCR are included. – – –, no positive result was found.

TABLE 1. Screening for respiratory viruses[a]

Patient	Day	Results for different viruses obtained by using various tests							
		RSV A and B			Adenovirus		Parainfluenza viruses 1–3	Influenza viruses A and B	
		Test-Pack	PCR	EIA	PCR	EIA	EIA	PCR	EIA
A	1	**+**	**−**	**−**	+		−		−
	6	**+**	**−**	**−**					
B	1	**+**	**−**	**−**	+	+	−		−
	5	**+**	**−**	**−**	+	+	+3		−
C	1	+	+B	−	+	(+)	(+)3		−
	70	**+**	**−**	**−**	+	−	−		−
D	1	**+**	**−**	**−**	−			+B	
	3	**+**	**−**	**−**	−	−	−		−
	4	**+**	**−**	**−**		−	−		−
	6	**+**	**−**	**−**		−	(+)2		−
E	1	+	+B	−	+	−	−		−
	18	**+**	**−**	**−**	−	−	−		−
F	1	**(+)**	**−**	**−**	+			−	
	2	−	−	−					
	3	−	−	−					
	35	+	+B	−	+			−	
	44	−	+B	−	+			−	
G	1	**+**	**−**	**−**	+			+A	
	12	**+**	**−**	**−**					
	20	**+**	**−**	**−**					

[a] Unconfirmed results for children [−, negative result; +, positive result; (+), weakly positive result] in the Abbott TestPack RSV are boldfaced. Available samples were screened for other respiratory viruses: adenoviruses (nested PCR and/or EIA, any serotype), parainfluenza viruses 1 to 3 (EIA, serotypes indicated by numbers 1 to 3), influenza viruses A and B (EIA and/or nested RT-PCR, serotype indicated by A or B).

high specificity and sufficient sensitivity with time requirements shorter than those of culture techniques. However, the merely moderate sensitivity of the antigen capture EIA restricts the method to acute-phase samples from children, who shed significantly higher amounts of respiratory viruses than do adults (4, 7). Based on EIA technology, a rapid test for RSV detection (Abbott TestPack RSV) was developed and shown to have satisfying sensitivity (86.8 to 97%) and specificity (88.1 to 98%) in comparison with cell culture isolation (1, 5, 10), immunofluorescence assays (6), and in-house EIAs (9). This rapid test is largely used for testing for RSV at admission to the hospital and for bedside testing. Several publications have reported the clinical use of RT-PCR for detecting respiratory viruses such as influenza A and B viruses (3, 11, 15), RSV (3, 12, 15), and the parainfluenza viruses (3, 15). The main benefit of molecular methods is their extreme sensitivity and a high specificity depending on appropriate primer selection. One of their drawbacks up to now has been that the majority of PCR protocols target only a single virus for identification. In addition, PCR and especially nested protocols of PCR are expensive and extremely prone to contamination, thus requiring high technical laboratory standards. Therefore, nucleic acid amplification methods are not yet routine in clinical diagnostic laboratories. The lack of conformity in technology between individual laboratories and the missing availability of external quality controls permits only a generalized assessment of their efficacy and usefulness.

In our study we compared the results of the rapid test with antigen capture EIA and RT-PCR. Since RT-PCR was the most sensitive method for detection of RSV used in the study, the results of the other methods were measured against those obtained by RT-PCR. Compared to RT-PCR, the rapid test was negative in 32 out of 145 samples, which had to be expected when taking into account the generally lower sensitivity of the rapid test. In contrast, the rapid test was positive in 18 samples, which could not be confirmed by any of the other methods and therefore must be regarded as a lack of specificity. However, one has to keep in mind that a group of hospitalized and thus preselected patients was investigated, which might reduce the specificity found in our study (63%) relative to the specificity given by the manufacturer (95.3%). The antigen capture EIA, in comparison to RT-PCR, exhibited a satisfying specificity (96%) but only a moderate overall sensitivity, thus restricting its usefulness to samples taken early after the onset of clinical symptoms in children. When serotypes of RSV, as determined by RT-PCR, were taken in account, however, the antigen capture EIA yielded the expected sensitivity of 69% for RSV A in acute-phase samples but yielded a very low and unsatisfying sensitivity of 22% for RSV B. Since the antibodies used for the EIA are induced from a strain of RSV A, this is easily explained.

Based on our findings, recommendations for rapid and reliable detection of RSV for efficient patient management would have to be streamlined, in order to prevent nosocomial infec-

tions or unjustified use of antibiotics and/or inappropriate isolation measures.

The rapid test, with its satisfying sensitivity of 94.3% and specificity of 95.3% (data given by the manufacturer), represents a useful tool for routine testing in emergency rooms. For special conditions, i.e., immunocompromised patients or those at high risk for severe disease due to underlying diseases, however, the rapid test as shown here may exhibit substantially lower sensitivity (66%) and specificity (63%). Thus, the rapid test, showing repeatedly positive results in follow-up samples obtained after 5 days or more, needs to be confirmed by additional methods for detection of RSV. If the positive rapid test results cannot be confirmed by an alternative method, we would suggest screening for other respiratory viruses. In our hands, nested RT-PCR for confirmation of RSV infection proved to be useful with the additional ability of subtyping.

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