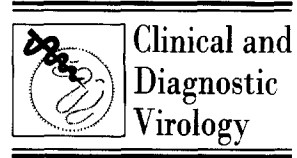




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Comparison of monoclonal biotin-avidin enzyme immunoassay and monoclonal time-resolved fluoroimmunoassay in detection of respiratory virus antigens

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Abstract

Background: Detection of respiratory viruses by time-resolved fluoroimmunoassay based on monoclonal antibodies were developed in our laboratories in the late 1980s and they have been successfully used in daily diagnosis for more than seven years. Later, similar Biotin-EIAs were developed but the sensitivities were unsatisfactory.

Objectives: Further optimization of monoclonal Biotin-EIAs and comparison of the optimized assays with TR-FIAs.

Study design: Variations in test format, diluents, incubation times and temperatures, and different monoclonal antibodies were tested, and the final comparisons were made with TR-FIA using stored nasopharyngeal aspirates.

Results: The improvements in Biotin-EIA featured four changes which increased sensitivity in the assay: (a) test diluent contained diethylenetriamino-pentaacetic acid; (b) antigen and biotinylated detector antibody were added simultaneously; (c) reaction time was extended from 1 h at 37°C to overnight at 4°C; (d) from the thirteen monoclonal antibodies used in TR-FIA, ten were optimal also in Biotin-EIA, but in the parainfluenza 1 and 2 assays other monoclonals proved more sensitive. Out of 257 originally positive specimens tested in the comparison studies, 192 (74.7%) were again positive and 54 (21.0%) were negative in both assays; nine were negative in TR-FIA but positive in Biotin-EIA, while two specimens were negative in Biotin-EIA but positive in TR-FIA. The overall agreement between the two assays was 95.7%.

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Conclusions: All monoclonal Biotin-EIAs can be optimized to the same sensitivity as TR-FIAs for the detection of respiratory viruses.

Laboratories which have no TR-FIA expertise may use Biotin-EIA in the diagnosis of acute respiratory infections.

Key words: Respiratory virus; Enzyme immunoassay; Time-resolved fluoroimmunoassay; Biotin; Nasopharyngeal aspirate; Monoclonal

1. Introduction

Acute respiratory viral infections are one of the most common causes of seasonal morbidity and of massive absence from work, with resulting economic repercussions. Many groups have reported a number of methods for the rapid diagnosis of such viral respiratory infections (Grandien et al., 1985) as respiratory syncytial virus (Clayton et al., 1987; Obert and Beyer, 1988; Brucková et al., 1989), adenovirus (Lehtomäki et al., 1986; Brucková et al., 1989), influenza virus (Bucher et al., 1987; Coonrod et al., 1988; Havlickova et al., 1990), and rhinovirus (Al-Nakib et al., 1989) infections. Moreover, commercially available kits for the detection of respiratory syncytial virus have been evaluated (Freymuth et al., 1986, 1991; Halstead et al., 1990).

Our groups have previously developed polyclonal and monoclonal EIAs and TR-FIAs for detecting respiratory viruses directly in clinical specimens (Sarkkinen et al., 1981a, b; Halonen et al., 1983, 1985; Walls et al., 1986; Hierholzer et al., 1987, 1989, 1990). These tests have proved to be very successful in diagnostic service. Since 1987, seven respiratory virus TR-FIAs (influenza A and B, parainfluenza 1–3, respiratory syncytial virus, and adenoviruses) have been used in day-to-day diagnostic service at the Department of Virology, University of Turku, and more than 3000 nasopharyngeal aspirate specimens have been tested each year, more than 18,000 altogether. A panel of twelve TR-FIAs has been widely used at CDC, Atlanta, Georgia, for detecting respiratory viruses including parainfluenza 4 and mumps (Hierholzer et al., 1993), coronavirus types 229E and OC43 (Hierholzer et al., 1994), and two enteroviruses causing acute hemorrhagic conjunctivitis (enterovirus 70 and coxsackie A 24 variant) (Hierholzer et al., 1990).

Previously, all-monoclonal Biotin-EIAs were compared with all monoclonal TR-FIAs and the sensitivity of TR-FIAs was always higher (Hierholzer et al., 1987, 1989). Because time-resolved fluorometry has not yet been widely adapted to diagnostic virus laboratories, we have further optimized the Biotin-EIAs using an identical test format and assay diluent as in TR-FIA, and reevaluated the sensitivities and specificities of these optimized assays. The present report indicates that the monoclonal antibodies which are optimal in TR-FIA are not always optimal in Biotin-EIA, and that the sensitivity obtained with clinical samples is almost identical with these two assays.

2. Materials and methods

2.1. Specimens

Nasopharyngeal aspirate specimens (NPA) used in this study were selected from the specimen library in Turku. They were sent to the diagnostic unit mainly from hospitalized children with an acute respiratory infection.

In the laboratory, the NPA specimens were diluted 1:5 in PBS containing 20% inactivated fetal calf serum and 2% Tween 20, then sonicated, and further diluted 1:2 in 0.5% gelatine in 0.05 M Tris-HCl, pH 7.75, 0.15 M NaCl, 0.05% NaN₃, 0.01% Tween 40. Then specimens were tested by monoclonal TR-FIA for seven respiratory viruses (Walls et al., 1986; Hierholzer et al., 1987, 1990) and stored at –20°C up to four years. During the storage period the specimens were thawed many times.

Altogether, 257 specimens were included in the study that had been positive when freshly tested: 51 for adenovirus, 51 for influenza A virus, 45 for influenza B virus, 18 for parainfluenza type 1 virus, 15 for parainfluenza type 2 virus, 40 for parainfluenza type 3 virus, and 37 for respiratory syncytial virus (RSV). Moreover, 21 negative NPA specimens, 3 for each virus tested, were tested for the standardization of the tests. All specimens were retested by TR-FIA simultaneously with Biotin-EIA against the appropriate antibody.

2.2. Monoclonal antibodies

Monoclonal antibodies (MAbs) used in the evaluation of the Biotin-EIA were purified from ascites fluids obtained by intraperitoneal injection of hybridoma cells in pristane-primed BALB/c mice. The hybridoma cell lines employed to produce MAbs and their source are indicated in Table 1. The adenovirus monoclonal antibodies were anti-hexon, the influenzavirus and the RSV ones were anti-nucleoprotein, the parainfluenzavirus 1 ones were anti-fusion, the parainfluenzavirus 2 and 3 ones were anti-HN. Immunoglobulin G (IgG) was purified from ascites fluids by fast anion-exchange chromatography on a Q-Sepharose FF HR10/10 column (Pharmacia, Uppsala, Sweden) as described by Waris et al. (1988). NaCl was added to 0.4 M to the clarified ascites fluid and then passed through a column containing 1 ml of Q-Sepharose FF (Pharmacia, Uppsala, Sweden) in 20 mM triethanolamine hydrochloride, 0.35 M NaCl, pH 7.7. The sample was collected into a test tube by low-speed centrifugation of the column. The ascites fluid was then passed through a PD-10 prepacked column of Sephadex G-25 (Pharmacia, Uppsala, Sweden) for desalting to 20 mM triethanolamine hydrochloride, pH 7.7, passed through Millex-AA and Millex-GV filters (pore size 0.8 and 0.2 µm, respectively) (Millipore, S.A., Molsheim, France) and finally through a Q-Sepharose FF column equilibrated with 20 mM triethanolamine hydrochloride, pH 7.75. The elution buffer was 20 mM triethanolamine hydrochloride, pH 7.7, with a 160 ml linear gradient of 0 to 0.35 M NaCl. The peak of IgG was collected and the concentration measured spectrophotometrically using an extinction coefficient at 280 nm of 1.4 ml/mg per cm.

2.3. Labeling of MAbs

Biotinylation of MAbs was done by Enzotint reagent (N-biotinyl-w-aminocaproic acid-N-hydroxysuccinimide ester) at pH 8.5 according to the manufacturer's instruc-

Table 1

Designation and source of monoclonal antibodies (MAbs) and the IgG concentrations used in biotin-avidin enzyme immunoassay (Biotin-EIA) and time-resolved fluoroimmunoassay (TR-FIA) in the detection of adenovirus group-reacting hexon antigen (Adenovirus), influenza A virus (Influenza A), influenza B virus (Influenza B), parainfluenza 1 virus (Parainfl. 1), parainfluenza 2 virus (Parainfl. 2), parainfluenza 3 virus (Parainfl. 3), and respiratory syncytial virus (RSV)

Virus	TR-FIA		Biotin-EIA	
	Catching MAb ($\mu\text{g}/\text{well}$)	Labeled MAb (ng/well)	Catching MAb ($\mu\text{g}/\text{well}$)	Labeled MAb (ng/well)
3				
4				
5				
Adenovirus	20/11 ^a (0.5)	2/6 ^a (10)	20/11 (0.5)	2/6 (90)
Influenza A	A3 ^a (0.5)	A1 ^a (12.5)	A3 (0.5)	A1 (70)
Influenza B	B2 ^a (0.5)	B2 (25)	B2 (0.5)	B2 (50)
Parainfl. 1	877 ^b (0.5)	877 (50)	253-9D ^a (0.5)	251-12A ^a (450)
Parainfl. 2	2G5 ^c (0.5)	2G3 ^c (50)	233-4D ^a (0.5)	233-4D (240)
Parainfl. 3	341-7H ^a (0.5)	343-5G ^a (50)	341-7H (0.5)	343-5G (100)
RSV	RSV-4 ^d (0.5)	NC-4 ^d (50)	RSV-4 (0.5)	NC-4 (370)

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tions (Enzo Biochem. Inc., New York, N.Y.). Purified MAbs were dialyzed overnight at 4°C against two changes of 0.1 M sodium bicarbonate. 120 μl of Enzotrin reagent, dissolved in dimethylsulfoxide at the same concentration as the proteins to label, was added per milliliter of MAbs. This mixture was left to react at room temperature for 4 hr and then dialyzed overnight at 4°C against two changes of 0.01 M phosphate saline buffer, pH 7.2 (PBS). Biotinylated MAbs were divided into aliquots and stored at -70°C until used.

MAbs used in TR-FIA were labeled by europium chelate (Hemmilä et al., 1984) (Wallac OY, Turku, Finland) following the method described by Waris et al. (1988). An aqueous solution of europium chelate (20.7 nmol/ml) was added in a 50-fold molar excess to 1–2 mg of MAbs. The mixture was incubated at 4°C overnight and then passed over a Trisacryl GF-2000 column equilibrated with 50 mM Tris, pH 7.75, to separate the uncoupled europium chelate ions. Bovine serum albumine treated with diethylene triaminepentaacetic acid (Wallac Oy, Turku, Finland) was added to the labeled IgG to a final concentration of 0.1%. The europium-labeled MAbs were stored in aliquots at -70°C until used.

2.4. TR-FIA

The concentrations of capture and europium-labeled MAbs were determined in previous experiments (Table 1). Twelve-well polystyrene microstrips (Eflab Oy, Helsinki, Finland) were coated with 0.5 µg/well of monoclonal immunoglobulin in 200 µl of 0.1 M NaHCO₃, pH 9.6. Strips were incubated overnight at room temperature. After washing twice with 5 mM Tris-HCl, 0.15 M NaCl, 0.05% Tween 20, pH 7.75 (TR-FIA wash solution), wells were postcoated overnight at room temperature with 250 µl/well of 0.1% gelatine in 0.05 M Tris-HCl, 0.15 M NaCl, 0.01% Tween 40. Strips were then used immediately or stored at 4°C for several months. Before use, the strips were washed twice with TR-FIA wash solution. 100 µl of treated NPS was pipetted into duplicate wells and immediately thereafter 100 µl/well of labeled MAbs diluted in TR-FIA assay buffer (0.5% gelatin, in 0.05 M Tris-HCl, pH 7.75, 0.15 M NaCl, 0.05% NaN₃, 0.01% Tween 40, 20 µM N'-diethylene triamine-pentaacetic acid (DTPA)) were added. The strips were incubated 1 hr at 37°C and then washed six times with TR-FIA washing solution. 200 µl of DELFIA™ enhancement solution (Wallac Oy, Turku, Finland) was added to each well, and the strips were shaken at room temperature for 10 min. Fluorescence was measured with a 1230 Arcus fluorometer (Wallac Oy, Turku, Finland) and values were expressed as counts per second (cps). The fluorometer was programmed to a previous cut-off value of two times the mean of six negative controls. Specimens were then tested, and the definitive cut-off was calculated adding six times the standard deviation of the negative specimens to their mean. All samples with a cps value over this second cut-off were regarded as positive.

2.5. Biotin-EIA

Optimal concentrations of the catching and of the labeled antibody (Table 1) were determined in checkerboard titrations against control antigens. Eight-well polystyrene microstrips (Eflab Oy, Helsinki, Finland) were coated with 0.5 µg/well of MAbs in 200 µl of carbonate buffer, pH 9.6. The strips were incubated overnight at room temperature and then washed twice in PBS, 0.1% Tween 20 (PBS-T). Wells were postcoated as in TR-FIA. If not used immediately, strips were stored at 4°C for several months. Before use, the strips were washed twice in PBS-T, and 100 µl of NPA was pipetted into duplicate wells. 100 µl of biotinylated MAbs, diluted to the appropriate concentration in assay buffer, were added to each well and the strips were incubated overnight at 4°C. The next day, the strips were warmed up to 37°C for 10 min and then washed twice in PBS-T. 200 µl of horseradish peroxidase labeled avidin (Vector Laboratories Inc., Burlingame, CA) diluted 1:6000 in assay buffer were added to each well and strips were incubated 30 min at 37°C. After washing three times in PBS-T and twice in PBS, 200 µl/well of 1,2-o-phenylenediamine in sodium citrate buffer, pH 5.0, containing 0.03% hydrogen peroxide was added. After 30 min incubation at room temperature in the dark, the reaction was stopped by adding 33 µl/well of 6 M HCl. The optical density (OD) was measured at 492 nm by a Multiskan Plus spectrophotometer (Labsystem, Helsinki, Finland). Specimens were considered positive if their absorbance values were higher than three times the mean value of negative controls; the minimum cut-off value was 0.250.

3. Results

Monoclonal antibodies employed in both assays were the same for five out of the seven respiratory virus antigens tested, with the exceptions of parainfluenza type 1 virus and parainfluenza type 2 virus (Table 1). Since monoclonal antibodies used in TR-FIA for these two viruses were not satisfactory in Biotin-EIA, other monoclonal antibodies were tested. Clones 253-9D and 251-12A for parainfluenza type 1 virus, and 233-4D for parainfluenza type 2 virus were found to be best in Biotin-EIA.

In the optimization tests, semipurified virus preparations and two TR-FIA positive and three negative specimens were included. The use of TR-FIA assay buffer increased the P/N ratio of the Biotin-EIAs at least twofold compared with the standard EIA assay buffer, which was PBS with 0.5% BSA and 0.2% Tween 20. DTPA was the most essential reagent in the assay buffer which improved the Biotin-EIA performance. With semipurified virus, TR-FIA was always more sensitive than Biotin-EIA, but with clinical specimens no difference was observed. No quantitative tests were done with highly purified virus preparations, so the sensitivities could not be expressed as ng/ml values.

Next, we selected 257 nasopharyngeal aspirate specimens to test simultaneously with Biotin-EIA and TR-FIA. The results are summarized in Table 2. The overall agreement was 95.7%: 192 specimens (74.7%) were positive and 54 (21%) were negative in both assays. From 11 specimens with discrepant results, 9 were positive in Biotin-EIA but negative in TR-FIA, and 2 were positive in TR-FIA but negative in EIA. The range of OD values and cps are shown in Table 3.

The sensitivity of Biotin-EIA and TR-FIA was further tested by titrating three specimens positive for each virus. All specimens tested were positive in each assay at least up to a 1:500 dilution (data not shown).

The specificity of Biotin-EIA was confirmed by selecting 10 NPA specimens positive in Biotin-EIA and testing them against the six other respiratory viruses by Biotin-EIA. None were positive in these cross-matched tests.

Biotin-EIA was also tested by one-hour incubation format. The preliminary results obtained showed a slight decrease in the absolute optical density values, but the P/N ratios were similar to those obtained with the overnight format.

Table 2

Comparison of Biotin-EIA and TR-FIA in the detection of seven respiratory viruses in 257 selected nasopharyngeal specimens from hospitalized children with acute respiratory disease

TR-FIA	BIOTIN-EIA													
	Adeno		Infl. A		Infl. B		Parainfl. 1		Parainfl. 2		Parainfl. 3		RSV	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-
+	46	0	47	0	35	0	5	2	4	0	36	0	19	0
-	1	4	1	3	2	8	2	9	0	11	0	4	3	15

Table 3

Range of optical density values and counts per second obtained testing 257 selected nasopharyngeal specimens by Biotin-EIA and TR-FIA

	BIOTIN-EIA ¹	TR-FIA ²
Adeno	0.027–3.631	1,126–894,205
Infl. A	0.024–3.855	1,316–441,120
Infl. B	0.022–3.452	1,278–393,473
Parainfl. 1	0.011–3.053	1,096–103,421
Parainfl. 2	0.024–3.016	1,001–203,343
Parainfl. 3	0.017–3.543	1,322–692,344
RSV	0.020–3.641	1,340–956,826

¹ Optical density values.

² Counts per second.

4. Discussion

The results of the present study indicate that the all-monoclonal biotin-avidin enzyme immunoassays can be optimized to the same sensitivity as the all-monoclonal TR-FIAs for the detection of respiratory virus antigens in nasopharyngeal aspirate specimens of patients with acute respiratory disease. The important details in these optimization experiments included simultaneous incubation of the specimen with biotinylated monoclonal detector antibody in microtiter wells coated with catching antibody. Apparently, the liquid phase antigen-antibody reaction and immediate binding of the complexes on the solid phase catching antibody is more efficient than step-wise reaction (first, the antigen on catching antibody on solid phase and then, after washing, the binding of the biotinylated detector antibody on the antigen). Additional improvements of the test were obtained by overnight incubation of detector antibody with specimen, and by using the TR-FIA diluent containing 20 μ M *N*'-diethylene triaminepentaacetic acid. The effect of this chelating agent on an EIA test is not clear, but it should be evaluated in other antigen/antibody EIA systems.

Another interesting finding was the difference in avidity of the parainfluenza 1 and 2 monoclonal antibodies after biotinylation and Eu-chelate labeling. Biotinylated antibodies resulted in low sensitivity, even though the coupling reaction is similar in both labeling procedures. Both involve primary amino groups, and the molecular weights of biotin and Eu-chelate are similar: 400 and 450, respectively. To overcome this problem, we screened other MAbs available against parainfluenza 1 and 2 viruses and found different clones that were more optimal. A change in the capture antibody was also necessary to optimize these two assays. These clones were not tested in TR-FIA, so information about how efficient they are after Eu-labelling is not available.

The Biotin-EIA tests we developed longer incubation times and an additional pipetting step (stopping solution) compared with TR-FIA. However, even with these inconveniences, many laboratories having no TR-FIA expertise or equipment may find these Biotin-EIA formats to be superior to other tests for detecting respiratory

viruses in clinical specimens. Overnight incubation was not extensively compared with the customary 1 h incubation at 37° C with clinical specimens, but did improve the sensitivity of the tests. With semipurified control antigens, the overnight incubation was significantly better. The use of the overnight format and the higher concentration of the biotinylated monoclonals in biotin-EIA could explain the 9 'false negative' results we had in TR-FIA. As regards the 54 negative specimens that we found using both methods, we suppose they could be due to the fact that, especially in the cases of influenza B and parainfluenza specimens, all the aspirates were stored for more than 2 years at –20°C and were frozen and thawed many times before we tested them, so the viral proteins, particularly the nucleoproteins, may have been denatured.

What is the advantage of Biotin-EIA or TR-FIA compared with the immunofluorescence technique (Gardner and McQuillin, 1980; Grandien et al., 1985) in rapid diagnosis of respiratory infections? The answer depends on the location of the laboratory, the number of specimens tested daily, the availability of reagents, and the previous expertise of the laboratory. Solid phase immunoassays have two definite advantages. One is the possibility for bulk testing, because a technician can test up to 40 specimens per day for seven respiratory viruses with TR-FIA and it takes only minutes for a virologist to read the test. Another advantage is the stability of the specimens for Biotin-EIA or TR-FIA, since no intact cells are required in the specimens and the viral proteins in the virion or in soluble form are relatively stable, at least if they are not frozen and thawed several times; thus, if necessary, the specimens can be sent to the laboratory without cooling, even by mail. However, these advantages do not apply to smaller hospital laboratories where a limited number of specimens is tested daily and transportation is not a factor.

The availability of immunoreagents is one of the greatest problems for laboratories interested in Biotin-EIA or TR-FIA for the detection of respiratory virus antigens. Hopefully, biotinylated reagents or EIA/TR-FIA kits will become commercially available for the major respiratory viruses in the near future.

References

- Al-Nakib, W., Dearden, C.J. and Tyrrell, D.A.J. (1989) Evaluation of a new enzyme-linked immunosorbent assay (ELISA) in the diagnosis of rhinovirus infection. *J. Med. Virol.*, 29, 268–272.
- Brucková, M., Grandien, M., Pettersson, C.-A. and Kunzová, L. (1989) Use of nasal and pharyngeal swabs for rapid detection of respiratory syncytial virus and adenovirus antigens by enzyme-linked immunosorbent assay. *J. Clin. Microbiol.*, 27, 1867–1869.
- Bucher, D.J., Kharitonov, I.G., Khan, M.W., Palo, A., Holloway, D. and Mikhail, A. (1987) Detection of influenza viruses through selective adsorption and detection of the M-protein antigen. *J. Immunol. Methods*, 96, 77–85.
- Clayton, A.-L., Albert, Z.I. and Chantler, S.M. (1987) The selection and performance of monoclonal and polyclonal anti-respiratory syncytial virus (RS) antibodies in capture ELISAs for antigen detection. *J. Virol. Methods*, 17, 247–261.
- Coonrod, J.D., Karathanasis, P., Betts, R.F. and Donofrio, J.C. (1988) Enzyme-linked immunosorbent assay of core antigens for clinical diagnosis of influenza. *J. Med. Virol.*, 25, 399–409.
- Freytmuth, F., Brooks, W., Petitjean, J., Daon, F. and Constantini, S. (1991) Evaluation of an enzyme

- membrane immunoassay (Directigen RSV) for the rapid diagnosis of respiratory syncytial virus infections. *Path. Biol.*, 39, 283–286.
- Freytmuth, F., Quibriac, M., Petitjean, J., Amiel, M.L., Pothier, P., Denis, A. and Duhamel, J.F. (1986) Comparison of two new tests for rapid diagnosis of respiratory syncytial virus infections by enzyme-linked immunosorbent assay and immunofluorescence techniques. *J. Clin. Microbiol.*, 24, 1013–1016.
- Gardner, P.S. and McQuillin, J. (1980) In: P. Gardner and J. McQuillin (Eds.), *Rapid virus diagnosis. Application of immunofluorescence*, Butterworths, London.
- Grandien, M., Pettersson, C.A., Gardner, P.S., Linde, A. and Stanton, A. (1985) Rapid viral diagnosis of acute respiratory infections: comparison of enzyme-linked immunosorbent assay and the immunofluorescence technique for detection of viral antigens in nasopharyngeal secretions. *J. Clin. Microbiol.*, 22, 757–760.
- Halonen, P., Meurman, O., Lövgren, T., Hemmila, I. and Soini, E. (1983) Detection of viral antigens by time-resolved fluoroimmunoassay. *Curr. Top. Microbiol. Immunol.*, 104, 133–146.
- Halonen, P., Obert, G. and Hierholzer, J.C. (1985) Direct detection of viral antigens in respiratory infections by immunoassays: a four year experience and new developments. In: L.M. de la Maza and E.M. Peterson (Eds.), *Medical virology*, 4, Lawrence Earlbaum, Hillsdale, N.J., pp. 65–83.
- Halstead, D.C., Todd, S. and Fritch, G. (1990) Evaluation of five methods for respiratory syncytial virus detection. *J. Clin. Microbiol.*, 28, 1021–1025.
- Havlickova, M., Pljusun, A.Z. and Tumova, B. (1990) Influenza virus detection in clinical specimens. *Acta Virol.*, 34, 449–456.
- Hemmilä, I., Dakubu, S., Mukkala, V.M., Siitari, H. and Lövgren, T. (1984). Europium as label in time-resolved immunofluorometric assays. *Anal. Biochem.*, 137, 335–343.
- Hierholzer, J.C., Anderson, L.J. and Halonen, P.E. (1990). Monoclonal time-resolved fluoroimmunoassay: sensitive systems for the rapid diagnosis of respiratory virus infections. In: L.M. de la Maza and E.M. Peterson (Eds.), *Medical virology*, 9, Plenum Press, New York and London, pp. 17–42.
- Hierholzer, J.C., Bingham, P.G., Castells, E. and Coombs, R.A. (1993) Time-resolved fluoroimmunoassay with monoclonal antibodies for rapid identification of parainfluenza type 4 and mumps viruses. *Arch. Virol.*, 130, 335–352.
- Hierholzer, J.C., Bingham, P.G., Coombs, R.A., Johansson, K.H., Anderson, L.J. and Halonen, P.E. (1989) Comparison of monoclonal antibody time-resolved fluoroimmunoassay with monoclonal antibody capture biotinylated detector enzyme immunoassay for respiratory syncytial virus and parainfluenza virus antigen detection. *J. Clin. Microbiol.*, 27, 1243–1249.
- Hierholzer, J.C., Halonen P.E., Bingham, P.G., Coombs, R.A. and Stone, Y.O. (1994) Antigen detection in human respiratory coronavirus infections by monoclonal time-resolved fluoroimmunoassay. *Clin. Diagn. Virol.*, 2, 165–179.
- Hierholzer, J.C., Johansson, K.H., Anderson, L.J., Tsou, C.J. and Halonen, P.E. (1987) Comparison of monoclonal time-resolved fluoroimmunoassay with monoclonal capture-biotinylated detector enzyme immunoassay for adenovirus antigen detection. *J. Clin. Microbiol.*, 25, 1662–1667.
- Lehtomäki, K., Julkunen, I., Sandelin, K., Salonen, J., Virtanen, M., Ranki, M. and Hovi, T. (1986) Rapid diagnosis of respiratory adenovirus infections in young adult men. *J. Clin. Microbiol.*, 24, 108–111.
- Obert, G. and Beyer, C. (1988) An enzyme-linked immunosorbent assay using monoclonal antibodies for the detection of respiratory syncytial virus in clinical specimens. *Arch. Virol.*, 26, 1103–1105.
- Sarkkinen, H.K., Halonen, P.E., Arstila, P.P. and Salmi, A.A. (1981a) Detection of respiratory syncytial virus, parainfluenza type 2, and adenovirus antigens by radioimmunoassay and enzyme immunoassay on nasopharyngeal specimens from children with acute respiratory disease. *J. Clin. Microbiol.*, 13, 258–265.
- Sarkkinen, H.K., Halonen, P.E. and Salmi, A.A. (1981b) Type-specific detection of parainfluenza viruses by enzyme immunoassay and radioimmunoassay in nasopharyngeal specimens of patients with acute respiratory disease. *J. Gen. Virol.*, 56, 49–57.
- Walls, H.H., Johansson, K.H., Harmon, M.W., Halonen, P.E., and Kendal, A.P. (1986) Time-resolved fluoroimmunoassay with monoclonal antibodies for rapid diagnosis of influenza infections. *J. Clin. Microbiol.*, 24, 907–912.
- Waris, M., Halonen, P., Ziegler, T., Nikkari, S. and Obert, G. (1988) Time-resolved fluoroimmunoassay compared with virus isolation for rapid detection of respiratory syncytial virus in nasopharyngeal aspirates. *J. Clin. Microbiol.*, 26, 2581–2585.