Direct Detection of Rhinoviruses by an Enzyme-Linked Immunosorbent Assay

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This paper describes the first enzyme-linked immunosorbent assay for the detection of rhinovirus antigens in clinical specimens (nasal washings), either directly or following overnight cell culture amplification. The assay takes approximately 48 hours to perform and utilizes the same rabbit antirhinovirus hyperimmune serum as both the capture and detecting antibody. The latter has been biotin-labelled and is detected via a streptavidin β -galactosidase preformed complex. This new assay has been found to be very sensitive, detecting human rhinovirus (HRV)-EL and HRV-2 at titres as low as $10^{1.8}$ TCID₅₀ 100 μ l⁻¹ and $<10^1$ TCID₅₀ 100 μ l⁻¹, respectively. Furthermore, when 57 different human rhinovirus serotypes were tested in both the HRV-EL and HRV-2 ELISA systems a total of 49 (86%) were found to be cross-reactive. Of 36 clinical specimens tested by virus isolation, cell-culture-amplified (CCA) ELISA, and direct ELISA, 15 were positive by isolation, 11 by CCA-ELISA, and 11 by direct ELISA. The overall correlation of the CCA and direct ELISA techniques with virus isolation was found to be 88.9% and 66.7%, respectively. The present study demonstrates that the ELISA system developed is a sensitive technique for the diagnosis of rhinovirus infections.

Key words: ELISA, biotin, streptavidin, β -galactosidase

INTRODUCTION

Rhinoviruses are the major causative agent of the common cold and could be responsible for at least half of all acute respiratory infections in humans [Mogabgab, 1968; Higgins et al, 1970]. Most rhinovirus infections are trivial and self-limiting in that only a minor upper respiratory tract illness of some 2–5 days duration occurs. However, in a proportion of cases, particularly in immunocompromised individuals and children with a history of bronchitis and allergy, infection can be more serious [Craighead et al, 1969]. In addition, there is some evidence to suggest that rhinovirus infections are associated with exacerbations of bronchitis and asthma [Gregg, 1983]. Furthermore, Krilov et al have recently detected rhinoviruses in 9 out of 53 (17%) specimens from infants with lower

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respiratory tract infection [Krilov et al, 1986]. Rhinoviruses are therefore appropriate targets for treatment with antiviral chemotherapeutic agents.

Rapid diagnosis of rhinovirus infection will be necessary so that antiviral treatment can be initiated. However, at present, virus isolation and identification, in an appropriate cell-culture system, may take up to 2–4 weeks to complete. We have therefore developed an enzyme-linked immunosorbent assay (ELISA) so that this time interval can be reduced to approximately 48 hours. In this test the capture and the detecting antibody are both prepared from the same rabbit hyperimmune antirhinovirus serum. The latter, however, is labelled with biotin and is then detected using a streptavidin- β -galactosidase preformed complex. This system was proven to be both sensitive and specific.

In this paper we describe an assay which can be used to detect rhinoviral antigens both directly in nasal washings and following overnight cell culture amplification of the same specimen. The results obtained were compared with those yielded by conventional virus isolation procedures.

MATERIALS AND METHODS

Antigens

The rhinovirus used to establish the ELISA procedure was the untyped human rhinovirus EL. Although a member of the rhinovirus genus according to its physical and biochemical characteristics, HRV-EL has not yet been assigned a serotype number in the collaborative scheme [Kapikian et al, 1967; Kapikian et al, 1971].

HRV-EL was grown to high titre by infecting confluent monolayers of Ohio human epithelial carcinoma (HeLa) cells, in 20-oz medical flats, overlaid with 25-ml of HeLa maintenance media (BME containing 2% foetal calf serum, 0.13% tryptose phosphate broth, 0.088% NaH CO₃, 30 mM MgCl₂, and antibiotics). After the cytopathic effect had developed, the virus was released from the cells by three cycles of freezing and thawing. The tissue culture fluid containing the virus was then clarified by low-speed centrifugation (2,000 rpm for 10 minutes) and stored at -70°C . Control antigen was prepared in the same way except that the cells were not infected.

All the other rhinoviruses used in this study were passaged once from laboratory stocks. Ohio HeLa cells, grown to confluence in 25-cm² plastic tissue culture flasks (Sterilin), were infected with virus and overlaid with 10-ml HeLa maintenance media. The tissue culture fluids were then harvested as for HRV-EL and stored at -70°C .

The titre of all the rhinovirus stocks were estimated by titration in Ohio HeLa cells.

Antisera

The high titre HRV-EL, prepared as an antigen, was further purified by fluorocarbon extraction. One volume of Arklone (ICI) was shaken, at room temperature, with two volumes of antigen; and the mixture was then centrifuged at 2,000 rpm for 10 minutes. The aqueous layer was harvested and centrifuged at 30,000 rpm for 2 hours. The virus pellet was then resuspended in a small volume at 0.3 M NaCl/0.1 M Tris buffer (pH 7.5) and mixed with an equal volume of Freund's incomplete adjuvant. Rabbits were immunized with the purified antigen by inoculating 0.5 ml of the emulsion intramuscularly in each hind leg followed by 1 ml intravenously 4 weeks later. The animals were bled 1 week later, and the hyperimmune serum was found to have a neutralisation titre of 1:32,000 against 100 TCID₅₀ of the homologous virus.

A hyperimmune serum to HRV-2 was prepared following the same schedule and was found to have a neutralisation titre of 1:128 against 50 TCID₅₀ of the homologous virus.

Biotinylated Antibody

A small aliquot of the hyperimmune serum was dialyzed overnight at 4°C against three changes of 0.1 M NaHCO₃ (pH 8.0) (B.D.H.). In 1 ml of dimethylsulphoxide (Sigma) 1.4 mg of biotin (N-hydroxy-succinimidobiotin) (Sigma) was dissolved. Two hundred microlitres of the biotin preparation was added to each millilitre of the hyperimmune sera, and the mixture was allowed to react for 4 hours, mixing slowly on a laboratory stirrer, at room temperature. The mixture was then dialyzed overnight at 4°C against three changes of 0.1 M phosphate-buffered saline (pH 7.2) (Difco) [Gary et al, 1983].

Human Specimens

Eighteen volunteers inoculated intranasally with HRV-EL or saline, who took part in a separate trial at the Common Cold Unit [Zerial et al, 1985] provided samples for this study. Nasal washings were collected on days 2–6 after virus challenge by instilling 5 millilitres of Hanks' balanced salt solution into each nostril. The expelled fluid was mixed with an equal volume of nutrient broth and stored at –70°C. Virus in nasal washings was isolated in Ohio HeLa cells.

Streptavidin Wash and Preformed Complex

The streptavidin wash (Amersham) was supplied as a 1 mg ml⁻¹ solution in 50 mM phosphate buffer (pH 7.4) containing 0.05% w/v sodium azide and was diluted to a working concentration (1/200) with phosphate buffered saline (pH 7.2) (Difco) for the test.

The streptavidin preformed complex was supplied as streptavidin-biotinylated β -galactosidase complex (Amersham) and was diluted to a working concentration (1/200) with phosphate buffered saline (pH 7.2) (Difco) for the test.

Substrate

Ortho nitrophenyl- β -D-galactosidase (ONPG) (Sigma), a water-soluble yellow product that absorbs light at 410 nm, was used as the basis of the substrate and prepared for the test as follows: 0.9 mg ml⁻¹ ONPG, 10 mM MgCl₂ (B.D.H.), and 0.1 M 2-mercaptoethanol (Sigma) in phosphate buffered saline (pH 7.5) (Difco) [Craven et al, 1965].

ELISA Procedure

All assays were performed in rigid, nonsterile, U-bottomed, 96-well polystyrene plates (Gibco/Nunc). Wells were coated overnight at 4°C with 100 μ l of rabbit hyperimmune antirhinovirus-EL serum diluted 1/4,000 with 0.05 M carbonate-bicarbonate buffer (pH 9.6) (ELISA coating buffer, Don Whitley Scientific Ltd). Plates were emptied and 100 μ l of 1% v/v bovine serum albumin (BSA) (Sigma) in PBS was added for 2 hours at 37°C in order to block any nonspecific binding. The HRV-EL antigen, which had a titre of approximately 10⁶ TCID₅₀ 100 μ l⁻¹, was diluted in log₁₀ steps in PBS-Tween + 0.1% v/v BSA + 5% v/v control antigen. The control antigen (uninfected tissue culture fluid) was also diluted in the same way. Plates were washed three times with PBS-Tween (200 μ l

per well) using a 12-outlet minimicrowash (Skatron) and 100 μ l of HRV-EL or control antigen was added to a set of wells and plates were then incubated overnight at 4°C. After washing as before, 100 μ l of the streptavidin wash, diluted 1/200 with PBS, was added to the wells in order to block any nonspecific binding to endogenous biotin in the specimens. The plates were incubated at room temperature for 10 minutes, followed by five washes with 200 μ l per well of PBS-Tween. One hundred microlitres of biotinylated antiserum, diluted to its optimal working dilution with PBS-Tween + 0.1% v/v BSA + 5% v/v control antigen, was added and the plates were incubated at 37°C for 2 hours. After three washes as before with PBS-Tween, 100 μ l of streptavidin β -galactosidase preformed complex, diluted 1/200 with PBS-Tween + 0.1% v/v BSA, was added to the plates, which were again incubated at 37°C for 2 hours. Plates were then washed five times with PBS-Tween, 200 μ l per well, and 100 μ l of substrate (prepared approximately 20 minutes prior to use) was added to all the test wells plus a row of wells, not used in the test, as a blank. Plates were left covered at 37°C and read hourly at 410 nm using an automatic plate reader (Titertek, Multiscan, Flow Laboratories) until the colour intensity exceeded its maximum. As each dilution of HRV-EL or control antigen was added to a duplicate set of wells, the mean optical density of each set was calculated. A positive result was recorded when the mean optical density of the test wells was ≥ 1.5 times the mean optical density of a similar dilution of control antigen. This "cutoff" value was set after experiments were performed in which a series of negative and positive specimens at various dilutions were assayed. Mean optical densities recorded for the negative specimens never exceeded 1.5 times the mean optical density of the control antigen (undiluted), whereas the mean positive specimen optical densities were always >1.5 times the mean control antigen optical density.

A similar assay for HRV-2 was also developed using the same procedure as described for HRV-EL.

The optimal dilutions of the reagents used in the ELISA systems were determined by checkerboard titrations. Experiments to determine optimal incubation times and temperatures were performed once the reagent concentrations had been standardized.

Cross-Reactivity Studies

Cross-reactivity experiments were performed with a total of 57 different human rhinoviruses and four other control viruses (influenza A/Eng/40/83, coronavirus 229E, echovirus 1, and coxsackie A21) plus control antigen (uninfected tissue culture fluid) as controls. The titre of all the viruses was adjusted to approximately $10^{4.0}$ TCID₅₀ ml⁻¹ by diluting with PBS-Tween + 0.1% v/v BSA + 5% v/v control antigen, whereas the control antigen was used undiluted in the test. The antigens were then tested in both the HRV-EL and HRV-2 ELISA systems according to the described protocol. The "cutoff" value was calculated as stated in the ELISA procedure. Therefore, a positive result was again recorded when the mean optical density (OD) of a duplicate set of sample wells was ≥ 1.5 times the mean optical density calculated for the control antigen (undiluted).

Testing of Clinical Specimens

Nasal washings, taken on consecutive days, from the 18 volunteers challenged with HRV-EL or saline were tested for the presence of HRV-EL antigen in the HRV-EL ELISA system.

Initially a cell culture amplification procedure was employed in which 50 μ l of each nasal washing was inoculated, in duplicate, into monolayers of Ohio HeLa cells grown in

microtitre plates (Nunc Microwell plate 96F with lid). After 90 minutes of adsorption at 33°C, the nasal washings were removed and 200 μl HeLa maintenance media was added to each well. The plates were incubated at 33°C overnight. After one cycle of freezing and thawing 100 μl from each test well was removed and placed in the corresponding well of a precoated ELISA plate. The plates were then incubated overnight at 4°C, and the test continued according to the ELISA protocol.

Secondly, a direct ELISA system was developed in which the nasal washings or control antigen (uninfected tissue culture fluid) were added directly to each of a set of duplicate ELISA plate wells coated with either pre- or postchallenge rabbit anti HRV-EL hyperimmune serum. Plates were then incubated overnight at 4°C and the test continued as described above. Test results were calculated by subtracting the mean optical density of a specimen tested in duplicate pre-serum-coated wells from the mean optical density of the same specimen tested in duplicate post-serum-coated wells. Results for the control uninfected tissue culture fluid were also calculated in this way. A nasal washing was considered positive when the test result was ≥ 1.5 times that of the control uninfected tissue culture fluid. This calculation was found to be the most appropriate method of controlling the considerable variation in background optical densities obtained with different nasal wash specimens. The “cutoff” value was set after a series of positive and negative specimens had been assayed using the direct ELISA as described in the ELISA procedure.

RESULTS

Background Levels in the ELISA

A 1% BSA block and a streptavidin wash stage were incorporated in the test in order to block unoccupied sites on the surface of the ELISA plate and endogenous biotin in clinical specimens, respectively. Experiments were performed in which each stage of the test was omitted in turn and the results compared with that obtained from a complete test. Figure 1 shows that the 1% BSA block and streptavidin wash were necessary stages in the ELISA test in order to keep background levels to a minimum.

Limits of Detection

With the optimal test conditions established, experiments were performed in order to determine the limits of detection of the assay for both HRV-EL and HRV-2 antigen detection. The optical density, recorded at 410 nm, decreased with increasing dilutions of the HRV-EL and HRV-2 stocks and remained approximately the same for all dilutions of control antigen. At a titre of $10^{1.8}$ TCID₅₀ 100 μl^{-1} and $<10^1$ TCID₅₀ 100 μl^{-1} HRV-EL and HRV-2, respectively, the mean optical density was approximately equal to 1.5 times that recorded for control antigen. Therefore the limits of detection of the assay were concluded to be $10^{1.8}$ TCID₅₀ 100 μl^{-1} and $<10^1$ TCID₅₀ 100 μl^{-1} for HRV-EL and HRV-2, respectively.

Cross-Reactivity Studies

Studies on the cross-reactivity between the various rhinovirus serotypes are summarised in Table I. Briefly, of the 57 different human rhinoviruses tested, 5 (8.8%) showed strong cross-reactivity, 28 (49.1%) showed a lesser degree of cross-reactivity, 6 (10.5%) were weakly cross-reactive, whilst 18 (31.6%) showed no reaction at all with HRV-EL. All

Fig. 1. Comparison of ELISA performed with each stage of the assay individually excluded in turn with complete test in order to evaluate the blocking procedures employed in the ELISA. **A** = complete ELISA; **B** = no hyperimmune capture sera; **C** = no BSA block; **D** = no streptavidin wash; **E** = no biotinylated hyperimmune sera; **F** = no streptavidin complex; **G** = substrate control.

four control viruses (echovirus type 1, coronavirus 229E, coxsackie A21 and influenza A/Eng/40/83) were not cross-reactive. Control antigen also showed no reaction.

In the HRV-2 ELISA system, 14 (24.6%) of the 57 rhinoviruses tested (including HRV-2) showed strong cross-reactivity. Twelve (21.1%) showed a lesser degree of cross-reactivity, 4 (7.0%) were weakly cross-reactive, whilst 27 (47.4%) showed no reaction. Of the controls, coronavirus 229E, coxsackie A21, influenza A/Eng/40/83, and control antigen did not cross-react with HRV-2. However, echovirus type 1 reacted strongly in the test, thus suggesting a degree of cross-reactivity with HRV-2. These results were reproduced several times.

Clinical Specimens

Figure 2 shows the results obtained with nasal washings, collected from three volunteers on consecutive days following HRV-EL or saline challenge, tested in both the cell-culture-amplified (CCA)-ELISA and direct ELISA systems. The figure also illustrates the good correlation obtained between CCA-ELISA, direct ELISA, and virus isolation in detecting positive or negative specimens from these three volunteers.

Nasal washings collected 3 or 4 days after inoculation with HRV-EL or saline from 18 volunteers who participated in a trial were investigated by all three methods

TABLE I. Degree of Cross-Reactivity Shown by Tests on 10^4 TCID₅₀ of 57 Different Rhinovirus Serotypes and Four Control Viruses When Tested in Both the HRV-EL and HRV-2 ELISA Systems

Virus	Degree of cross-reactivity			
	Strong ^a	Moderate ^b	Weak ^c	Negative ^d
HRV-EL	12, 44, 45, 55, EL	3, 10, 11, 13, 14, 15, 16, 18, 20, 23, 24, 28, 29, 30, 31, 32, 41, 42, 43, 49, 51, 56, 62, 63, 64, 65, 69, 70	6, 8, 9, 19, 40, 48	1a, 1b, 2, 4, 5, 7, 17, 25, 27, 47, 72, 73, 75, 77, 80, 81, 82, 85, ECHO I, Coronavirus 229E, influenza A/Eng/40/83, coxsackie A21
HRV-2	2, 19, 23, 25, 27, 28, 44, 48, 51, 62, 70, 72, 73, 81, ECHO I	17, 18, 20, 24, 29, 30, 31, 40, 41, 43, 47, 77	13, 45, 80, 82	1a, 1b, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 32, 42, 49, 55, 56, 63, 64, 65, 69, 75, 85, EL, Coronavirus 229E, influenza A/Eng/40/83, coxsackie A21

^aStrong = OD of test sample $\geq 2.5 \times$ the OD of control antigen.

^bModerate = OD of test sample $2-2.5 \times$ the OD of control antigen.

^cWeak = OD of test sample $1.5-2 \times$ the OD of control antigen.

^dNegative = OD of test sample $< 1.5 \times$ the OD of control antigen.

(Table II). Samples from 7 of 18 (38.9%) volunteers gave concordant results by all three procedures for both days. In two cases (AD and AG) specimens were positive by isolation but negative by CCA-ELISA. However, overall there was 88.9% correlation between the CCA-ELISA and virus isolation.

The direct ELISA failed to detect viral antigen in 6 specimens that were positive by isolation and 5 specimens that were positive by both isolation and CCA-ELISA. However, viral antigen was detected in 5 specimens that were negative by isolation and CCA-ELISA. Hence, the direct ELISA showed 66.7% agreement with either virus isolation or CCA-ELISA.

It is noteworthy that all the control nasal washings collected from the four volunteers who were challenged with saline instead of HRV-EL (N.O., A.C., J. Ram, J.R.) were negative by all three procedures (Table II), thus indicating the high specificity of the ELISA techniques.

DISCUSSION

In this paper we describe the first ELISA system for rhinovirus detection. The assay was found to be simple, rapid, and capable of detecting a large number of human rhinovirus serotypes. It is a reliable method for the detection of HRV-EL antigen in clinical specimens, and results have suggested that the limits of detection were as low as $10^{1.8}$ TCID₅₀ $100\mu\text{l}^{-1}$ and $<10^1$ TCID₅₀ $100\mu\text{l}^{-1}$ for HRV-EL and HRV-2 antigen, respectively. The assay offers three distinct advantages over virus isolation in cell culture. Firstly, the test is rapid, requiring only 48 hours to complete once antibody coated plates are available whereas virus isolation and serological identification may take as long as 2–4 weeks. Secondly, the ELISA, as demonstrated in this study, is capable of detecting noninfectious as well as infectious virus (some volunteers showed ≥ 4 -fold rises

Fig. 2. Comparison of CCA-ELISA and direct ELISA with virus isolation in Ohio HeLa cell cultures where **A** denotes a volunteer inoculated with HRV-EL who developed symptoms of a common cold, **B** denotes a symptomless volunteer inoculated with HRV-EL, and **C** denotes a volunteer inoculated with saline. The cutoff value is taken as $1.5 \times$ control antigen optical density.

in antibody titre, were ELISA positive yet did not excrete virus); hence, the need to preserve infectivity of virus in the clinical specimen may not be essential. Thirdly, as tests are read spectrophotometrically evaluation of results is objective rather than subjective.

A biotin-avidin ELISA utilising the enzyme β -galactosidase was developed in this study because of the following advantages over conventional ELISA. The high affinity of avidin for biotin (affinity constant = 10^{15} mol^{-1}) [Green, 1975] ensures that the biotin-avidin complexes are not easily dissociated by the washing steps in the test and hence this may result in enhanced sensitivity [Guesdon et al, 1979]. Furthermore; one molecule of avidin is capable of binding four molecules of biotin and biotin can be covalently linked to antibody without affecting the antigen binding capacity [Guesdon et al, 1979]. It is more specific as the enzyme β -galactosidase prepared from *Escherichia coli* and used in this assay has characteristics different from those found in human tissues under the conditions of the assay. Therefore by employing a β -galactosidase-based substrate (ONPG), high

TABLE II. Correlation of Results of Tests of Nasal Washings Collected From 18 Volunteers 3 and 4 Days After Inoculation by Three Methods

Volunteer	Inoculum: virus (V) or saline (S)	Results on nasal washings collected on indicated day and tested by:					
		Virus isolation		CCA-ELISA		Direct ELISA	
		3	4	3	4	3	4
B.P.	V	+	+	+	+	+	+
S.S.	V	+	+	+	+	+	NT
S.D.	V	+	+	+	+	+	0
N.A.	V	+	+	+	+	0	+
J.D.	V	+	+	+	+	NT	0
D.J.	V	+	+	0	+	+	0
S.J.	V	0	+	+	+	+	0
A.D.	V	+	0	0	0	+	+
A.G.	V	+	0	0	0	0	0
J.G.	V	0	0	0	0	+	+
J.W.	V	0	0	0	0	+	0
K.G.	V	0	0	0	0	+	0
K.S.	V	0	0	0	0	0	0
M.R.	V	0	0	0	0	0	0
A.C.	S	0	0	0	0	0	0
J.R.	S	0	0	0	0	0	0
J.Ram.	S	0	0	0	0	0	0
N.O.	S	0	0	0	0	0	0

background readings—as seen with alkaline phosphatase or horseradish peroxidase—were avoided.

The data presented in this paper demonstrate that a large number of human rhinovirus serotypes are cross-reactive in both the HRV-EL and HRV-2 ELISA systems. This would suggest that an antirhinovirus serum is capable of binding not only its homologous virus but also a number of heterologous viruses. However, the degree of cross-reactivity depends on how close the serological relationship is between the test virus and that against which the hyperimmune serum was prepared. Nevertheless, our data clearly suggest that it may be possible to prepare a hyperimmune serum capable of detecting many rhinoviruses by including a number of rhinovirus serotypes in the initial inoculum. Indeed, in this study we demonstrated that by using the two ELISAs (for HRV-EL and HRV-2) we were able to detect a large number of the human rhinoviruses investigated ($OD \geq 2 \times OD$ of control antigen). Alternatively, it may be possible to develop hyperimmune sera or monoclonal antibodies with C-type reactivity capable of detecting an even larger number of rhinovirus serotypes.

Although we would like to emphasise that our data are preliminary, both the direct and CCA-ELISAs gave a good correlation with virus isolation when used to detect rhinovirus antigens in nasal washings (obtained from 18 volunteers challenged with either HRV-EL or saline). However, the CCA-ELISA showed better correlation (88.9%) with virus isolation than the direct assay (66.7%). This was not totally unexpected as both virus

isolation and the CCA-ELISA depend on the presence of viable virus rather than antigen. The inclusion of an amplification stage in the ELISA system may be necessary when virus is present at a low concentration ($<10^2$ TCID₅₀ ml⁻¹), as is often found with nasal washings obtained from adults. The addition of an amplification stage in the assay does not, in our opinion, increase the work or time load of the test, as whilst the ELISA plates are precoated overnight with the capture antibody nasal washings are inoculated into monolayer cell cultures. Recent studies with cytomegalovirus (CMV) and influenza virus have suggested that cell culture amplification followed by immunofluorescent staining was necessary for the detection of virus present at low concentration in urine [Griffiths et al, 1984; Alpert et al, 1985] or throat swabs [Espy et al, 1986], respectively. Since rhinoviruses are also excreted at low concentrations, particularly by adults, and as nasal washings (although the optimal method of collecting a rhinovirus specimen for virus isolation) contribute further to the dilution of the excreted virus, we feel that the CCA-ELISA may be the method of choice for the detection of rhinoviruses when present at low concentrations.

It is noteworthy that the time required to investigate serial specimens from 18 volunteers using conventional virus isolation and neutralisation techniques is some 3 to 4 weeks. However, when our ELISAs are employed this time interval is reduced to 48 hours. In addition, by reacting the same clinical specimen with both a pre-immunisation and a hyperimmune rabbit serum as capture antibodies, as in the direct method, the ELISA can offer an in-built confirmation stage. It is therefore our aim to further modify the ELISAs described in this paper in order to improve sensitivity and decrease the time required to obtain results to within a few hours [same day diagnosis]. These developments will be essential if antiviral chemotherapy becomes available and a rapid diagnosis is therefore required in order to initiate treatment in individuals who may be at risk of developing more serious lower respiratory tract complications following rhinovirus infection.

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REFERENCES

- Alpert G, Mazon M, Colimon R, Plotkin S (1985): Rapid detection of human cytomegalovirus in the urine of humans. *Journal Infectious Diseases* 152(3):631–633.
- Craighead JE, Meier M, Cooley MH (1969): Pulmonary infection due to rhinovirus type 13. *New England Journal of Medicine* 281:1403–1404.
- Craven GR, Steers E, Jr, Anfinsen CB (1965): Purification, composition, and molecular weight of the β -galactosidase of *Escherichia coli* K12. *Journal of Biological Chemistry* 240(6):2468–2477.
- Espy MJ, Smith TF, Harmon MW, Kendal AP (1986): Rapid detection of influenza virus by shell vial assay with monoclonal antibodies. *Journal of Clinical Microbiology* 24(4):677–679.
- Gary GW, Jr, Kaplan JE, Stine SE, Anderson LJ (1983): Detection of norwalk virus antibodies with a biotin-avidin immunoassay. *Journal of Clinical Microbiology* 22(2):274–278.
- Green NM (1975): Avidin. *Advances in Protein Chemistry* 29:85–131.

- Gregg I (1983): Provocation of airflow limitation by viral infection: Implication for treatment. *European Journal of Respiratory Diseases* 64 [Suppl 128]:369–379.
- Griffiths PD, Panjwani DD, Stirk PR, Ball MG, Ganczakowski M, Blacklock HA, Prentice HG (1984): Rapid diagnosis of cytomegalovirus infection in immunocompromised patients by detection of early antigen fluorescent foci. *Lancet* ii:1242–1244.
- Guesdon JT, Ternyck T, Avrameas S (1979): The use of avidin-biotin interaction in immunoenzymatic techniques. *Journal of Histochemistry and Cytochemistry* 27:1131–1139.
- Higgins PG, Ellis EM, Woolley DA (1970): Viruses associated with acute respiratory infections in Royal Air Force personnel. *Journal of Hygiene* 6:647–654.
- Kapikian AZ, Conant RM, Hamparian VV, Chanock RM, Chapple PJ, Dick EC, Hopler JC, Jordan WS, Jr, Lennette EH, Meinick JL, Mogabgab WJ, Mufson MA, Phillips CA, Schieble JH, and Tyrrell DAJ (1967): Rhinoviruses: A numbering system. *Nature (London)* 213:761–762.
- Kapikian AZ, Conant RM, Hamparian VV, Chanock RM, Dick EC, Gwaltney JM, Jr, Hamre D, Jordan WS, Jr, Kenny GE, Lennette EH, Melnick JL, Magabgab WJ, Phillips CA, Schieble JH, Stott EJ, Tyrrell DAJ (1971): A collaborative report: Rhinoviruses—extension of a numbering system. *Virology* 43:524–526.
- Krilov L, Pierik L, Keller E, Mahan K, Watson D, Hirsch M, Hamparian V, McIntosh K (1986): The association of rhinoviruses with lower respiratory tract disease in hospitalized patients. *Journal of Medical Virology* 19:345–352.
- Mogabgab WJ (1968): Acute respiratory illness in university (1962–1966), military and industrial (1962–1963) populations. *American Review of Respiratory Disease* 98:359–379.
- Zerial A, Werner GH, Phillpotts RJ, Willman JS, Higgins PG, Tyrrell DAJ (1985): Studies on 44081 R.P., a new antirhinovirus compound, in cell cultures and in volunteers. *Antimicrobial Agents and Chemotherapy* 27:846–850.