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Rapid multiserotype detection of human rhinoviruses on optically coated silicon surfaces

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Abstract

Background: More than 100 immunologically distinct serotypes of human rhinoviruses (HRV) have been discovered, making detection of surface exposed capsid antigens impractical. However, the non-structural protein 3C protease (3Cpro) is essential for viral replication and is relatively highly conserved among serotypes, making it a potential target for diagnostic testing. The thin film biosensor is an assay platform that can be formatted into a sensitive immunoassay for viral proteins in clinical specimens. The technology utilizes an optically coated silicon surface to convert specific molecular binding events into visual color changes by altering the reflective properties of light through molecular thin films. Objective: To develop a rapid test for detection of HRV by developing broadly serotype reactive antibodies to 3Cpro and utilizing them in the thin film biosensor format. Study design: Polyclonal antibodies to 3Cpro were purified and incorporated into the thin film assay. The in vitro sensitivity, specificity and multiserotype cross-reactivity of the 3Cpro assay were tested. Nasal washes from naturally infected individuals were also tested to verify that 3Cpro was detectable in clinical specimens. Results: The 3Cpro assay is a 28-min, non-instrumented room temperature test with a visual limit of detection of 12 pM (picomolar) 3Cpro. In terms of viral titer, as few as 1000 TCID50 equivalents of HRV2 were detectable. The assay detected 45/52 (87%) of the HRV serotypes tested but showed no cross-reactivity to common respiratory viruses or bacteria. The thin film assay detected 3Cpro in HRV-infected cell culture supernatants coincident with first appearance of cytopathic effect. Data are also presented demonstrating 3Cpro detection from clinical samples collected from HRV-infected individuals. The assay detected 3Cpro in expelled nasal secretions from a symptomatic individual on the first day of illness. In addition, 9/11 (82%) concentrated nasal wash specimens from HRV infected children were positive in the 3Cpro test. Conclusion: We have described a novel, sensitive thin film biosensor for rapid detection of HRV 3Cpro. This test may

Abbreviations: HRV, human rhinovirus; 3Cpro, 3C protease; OIA, optical immunoassay; HRP, horseradish peroxidase; TMB, tetramethylbenzidine; MOI, multiplicity of infection; CPE, cytopathic effect.

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be suitable for the point of care setting, where rapid HRV diagnostic test results could contribute to clinical decisions regarding appropriate antibiotic or antiviral therapy. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Rhinovirus detection; Optical immunoassay; OIA; Thin film biosensor; 3C protease

1. Introduction

Most respiratory diseases are viral in origin, and specific antiviral compounds are becoming available for treatment of these infections. Significant progress has been made recently toward developing highly specific anti-rhinovirus compounds (Wang et al., 1998; Matthews et al., 1999; Patick et al., 1999; Schiff and Sherwood, 2000) and specific influenza treatments are currently available. Since the symptoms of many viral respiratory infections overlap but the therapies are virus specific, rapid diagnostic tests performed at the point of care setting may contribute to appropriate administration of these new drugs. In addition, identification of a viral infection may reduce inappropriate use of antibiotics. Currently approximately 50% of patients diagnosed with colds or upper respiratory tract infection receive antibiotics, even though this treatment is unlikely to change the course of disease (Gonzales et al., 1997).

Human rhinoviruses (HRV) are the most common cause of acute upper respiratory illness. Children experience 8–12 colds annually and adults have 2–3 colds per year (Winther et al., 1998). HRV has also been associated with lower respiratory tract disease in high risk populations, such as patients with asthma or other airway inflammation (Krilov et al., 1986; Gern and Busse, 1999; Rakes et al., 1999; Papadopoulos et al., 2000).

Development of a rapid assay for HRV is a challenging task because over 100 immunologically distinct serotypes exist (Uncapher et al., 1991). In addition, only a small fraction of nasal epithelial cells are infected at the peak of illness (Turner et al., 1982, 1984; Winther et al., 1986; Arruda et al., 1995). Broad multiserotype reactivity is essential for an HRV diagnostic because epidemiological studies have shown that the predominant serotypes change from year-to-year, multiple serotypes circulate in a community concurrently, and the serotypes vary by geographical location (Monto and Cavallaro, 1972; Calhoun et al., 1974; Fox et al., 1975, 1985; Monto et al., 1987).

Efforts to develop antibodies with multiserotype recognition have not been successful because the epitopes targeted were exterior regions of the viral capsid (Al-Nakib et al., 1986; Dearden and Al-Nakib, 1987; McCray and Werner, 1987). These regions are highly variable and antibodies to the most immunodominant epitopes are often serotype specific (Sherry and Rueckert, 1985; Sherry et al., 1986). Nucleic acid detection methods targeting the conserved 5’ non-coding region of the viral RNA have been successful in identification of HRV RNA from clinical samples (Mori and Clewley, 1994; Arruda et al., 1997; Santti et al., 1997; Hyypia et al., 1998; Andeweg et al., 1999). RT-PCR has proven to be both sensitive and specific but the method is currently too complex for the physician office setting, where timely therapeutic decisions must be made.

We have developed a simple, rapid assay for HRV based on 3C protease (3Cpro) detection. The 3Cpro is responsible for the majority of cleavage events necessary for processing the viral polyprotein into the functional structural and enzymatic proteins required for viral replication. A significant degree of 3Cpro amino acid homology exists among diverse HRV serotypes, especially in the active site and RNA binding domain (Matthews et al., 1994; Walker et al., 1995; Meador et al., 1998; Palmenberg, 1998). Although 3Cpro is required throughout all stages of viral replication, it is not packaged into the mature virion (Rueckert, 1996). Therefore, the diagnostic clinical sample must contain infected cells or free 3Cpro originating from lysis of infected cells. The rapid test described in this study detects 3Cpro in expelled nasal secretions from HRV infected individuals and from virally infected cell culture lysates.

The optical immunoassay (OIA®) described here is based on thin film detection using optically
coated silicon surfaces. Specific 3Cpro antibody is bound to the optical surface. An immune complex forms on the silicon surface between the surface capture antibody, 3Cpro, and horseradish peroxidase (HRP) labeled detector antibodies. Addition of a precipitating substrate for HRP transduces the molecular surface binding events to mass deposition on the surface, creating a thin film and producing a visible color change. The increase in thickness alters the reflective properties of the optical surface such that the predominant reflective wavelengths shift from yellow and red to purple and blue (Ostroff et al., 1999b), indicating a positive result.

Thickness changes as small as 10 Å can be visualized with this system (Ostroff et al., 1999a). Recent clinical studies comparing thin film assay detection of group A streptococcus or influenza virus to culture have demonstrated OIA test sensitivity to be equivalent to or more sensitive than routine bacterial or viral culture (Gerber et al., 1997; Covalciuc et al., 1999). We have applied the thin film technology to detection of HRV 3Cpro. In this study we demonstrate multiserotype recognition and detection of HRV from in vitro and clinical specimens.

2. Material and methods

2.1. Recombinant 3Cpro purification

HRV2 and HRV16 3C proteases (3Cpro) were purified using the same procedure from E. coli strain, BL21/DE3 (Webber et al., 1996). The expression plasmid used was designed in-house. Cells were disrupted using a microfluidizer and cleared of cell debris using an ultracentrifuge. After a 40% ammonium sulfate precipitation, the 3Cpro was resuspended with 25 mM Tris-Cl, pH 7.5, 1 mM EDTA, and 1 mM dithiothreitol. It was further purified using chromatography on a Pharmacia Blue-Sepharose fast flow column, followed by gel filtration chromatography on a 2-liter Sephacryl S-100 column (Pharmacia, Piscataway NJ).

2.2. 3Cpro antibody

Rabbits were immunized with HRV16 3Cpro and total IgG was recovered from the serum by Protein G affinity purification (Pierce, Rockford, IL). Specific 3Cpro antibody was purified using a HiTrap NHS activated column (Pierce) with HRV2 3Cpro linked by primary amines. Specific antibody was eluted with 0.1 M glycine buffer, neutralized with 1 M Tris buffer, pH 9 and dialyzed against three changes of PBS.

Peptide antibody was generated to the amino acid sequence VLKLDREKFRDIR, which is in the conserved RNA binding domain of HRV 3Cpro. The peptide was conjugated to keyhole limpet hemocyanin (KLH) (Pierce) and injected into rabbits. Specific antibody was bound to a peptide affinity column and eluted with glycine buffer as described above.

2.3. Thin film assay

Silicon wafers were optically coated with 500 Å of silicon nitride by vapor deposition. T-polymer (polydimethylsiloxane) (United Chemical Technologies, Bristol, PA) was applied by spin coating. The silicon nitride layer is responsible for the thin film interference effect. The T-polymer layer is required for passive adsorption of antibody to the silicon surface.

HRV16 3Cpro antibody (5 µg/ml) was coated onto the silicon surfaces in 0.1 M Heps (Research Organics, Cleveland, OH) buffer, pH 8.0 at room temperature for 72 h. Both HRV16 3Cpro and peptide antibody were conjugated to HRP (Boeringer Manheim, Indianapolis, IN) by traditional methods (Nakane, 1975; Ettinger et al., 1999) to produce the detector antibodies. Detector antibodies were diluted in 0.1 M Mopso (3-[N-morpholino]-2-hydroxypropane sulfonic acid) buffer, pH 7.5 containing 3.5% casein, 20% bovine serum and 0.03% Tween 20, prior to use.

To begin the assay, expelled nasal secretions were absorbed onto a rayon swab and the swab was immersed in 150 µl of extraction reagent for 3 min. Alternatively, non-clinical samples were tested without swabs and aliquots were mixed 1:1 with extraction reagent for 3 min.
HRP labeled detector antibodies were added (0.01 mg/ml) to the sample and further incubated for 5 min. A 20-μl aliquot was placed onto the antibody coated silicon surface, incubated for 10 min, and washed with a stream of 10 mM phosphate buffer, pH 7.2 for 3 s. Next, 35 ul of tetramethylbenzidine (TMB) substrate (BioFX, Randallstown, MD) was added to the silicon chip for 10 min and washed. As many as 10 assays can be run simultaneously in the current format. All incubations were at room temperature. The results were interpreted visually or quantitated with a CCD camera.

2.4. Image analysis

An 8-bit CCD camera (Sony Electronics, Park Ridge, NJ) with a white light illuminator (Dolan-Jenner Industries, Inc. Lawrence, MA) was used to quantitate the visual signal. The CCD camera has an output of three color components for each pixel (red, green, and blue) which were analyzed with image processing software to calculate the color difference (CD) and quantify the color change of the assay. The analysis of the ratio of red, green, and blue for the reactive spot and unreacted surrounding gold background surface was used to calculate the CD value. Multiple readings were taken for each assay. The results are presented as the mean CD value for duplicate experiments ± 1 S.D. The color difference was defined as:

\[
CD = \sqrt{(R_s - R_b)^2 + (G_s - G_b)^2 + (B_s - B_b)^2}
\]

\[(R_b + G_b + B_b)/3\]

where \(R_s, G_s, B_s, R_b, G_b,\) and \(B_b\) refer to the red, green and blue measurement on the reactive spot and the background unreactive area, respectively. Both the CCD camera and the human eye discriminate low positive and negative results with approximately the same level of sensitivity. A CD value of 0.01 corresponds to the visual limit of detection.

2.5. HRV lysates

H1 HeLa cells (ATCC) were grown in Minimum Essential Medium with Earle’s salts (EMEM) with 10% fetal calf serum (FCS) (Gibco BRL Products, Rockville, MD) at 37°C and inoculated with each HRV serotype in EMEM with 2% fetal calf serum at a multiplicity of infection (MOI) of 0.01 at 33°C. Cytopathic effect (CPE) was monitored microscopically and viral lysates were harvested when 80% of the cells exhibited significant CPE, which ranged from 3 to 5 days after infection depending on serotype. Lysates were prepared by three cycles of freeze/thaw (−70°C) of the cells followed by centrifugation of the lysate at 1200 rpm for 15 min and storage at −70°C. The lysates were titered to determine the TCID₅₀ value (Reed and Muench, 1938). A 20-μl aliquot was tested in duplicate for reactivity in the 3Cpro assay.

2.6. Analytical sensitivity

3Cpro was serially diluted in phosphate buffer containing 0.1% BSA, 0.1% Tween 20. The HRV2 viral lysate (TCID₅₀ titer of 1 × 10⁷/ml) was diluted in EMEM. All samples were tested with the 3Cpro assay as described. Each data point is a result of two experiments performed in duplicate. Error bars are ± 1 S.D.

2.7. Time course of 3Cpro detection in culture

An HRV clinical isolate, confirmed by acid lability and RT-PCR (Giranda et al., 1992; Santti et al., 1997), was recovered from nasal secretions of a symptomatic donor by immersing the sample in 3 ml of viral transport media (Becton Dickinson, Sparks, MD) and inoculating 200 μl into H1 HeLa cell culture. Characteristic CPE (Couch, 1996) was observed and viral lysates were harvested as described above. This first passage virus was used to inoculate an 80% confluent lawn of H1 HeLa cells at an MOI of 0.001 in duplicate. A 100-μl aliquot of each culture supernatant was removed daily for testing in the 3Cpro assay. Each assay analyzed a 15-μl aliquot of culture supernatant.
2.8. Cross-reactivity panel

Bacteria were grown on solid media by standard methods (Murray et al., 1999). Growth from multiple plates was harvested with a rayon swab and resuspended in sterile saline. An A_{540} of a dilute sample was measured to estimate CFU/ml, which ranged from $10^8$ to $10^{10}$ CFU/ml. Viral panel members were commercially obtained from cell culture lysates (Novavax, Inc., Rockville, MD) with titers ranging from $10^6$ to $10^8$ TCID_{50}/ml. Cross-reactivity was assessed by testing 100 μl of each suspension with the 3Cpro assay.

2.9. Clinical sample testing

Expelled nasal secretions were collected from a symptomatic individual into plastic wrap. A rayon swab was immersed in the secretions and tested in the 3Cpro assay. Nasal wash specimens from symptomatic pediatric patients were obtained from Dr. W. Greene, Hershey Medical Center, PA. The patient age range was 2 months to 3 years old, with the exception of one influenza specimen from a 95-year-old patient. All specimens were confirmed as rhinovirus positive by temperature sensitive growth and characteristic CPE in MRC5 cells. A 0.1-ml aliquot of each specimen was concentrated fivefold by evaporative centrifugation and tested in the 3Cpro assay.

3. Results

3.1. 3Cpro antibody generation

Several viral proteins were considered as potential targets for a rhinovirus diagnostic test. The lack of sequence conservation in externally exposed capsid regions eliminated antigenic sites on the surface of the virion as diagnostic candidates. Among the non-structural proteins, 3Cpro was attractive because the amino acid sequence is highly conserved, especially in regions essential for enzyme function. The enzyme is required throughout the viral replication cycle, so 3Cpro is expected to be present throughout the course of infection. However, 3Cpro is not thought to be packaged in the virion, so the quantity of 3Cpro present in a clinical sample was unknown.

The RNA binding domain is essential for 3Cpro function (Leong et al., 1993) and appears to be highly conserved among HRV serotypes, thus making this region a suitable target for peptide antibody generation. Two 3Cpro polyclonal antibodies were generated, one to an 14 amino acid peptide sequence (3Cpep) from the RNA binding domain and the second to the whole 3Cpro molecule. Examination of available sequence data (Palmenberg, 1998) revealed that within the 3Cpep sequence, 10/14 amino acids were identical, and two of the remaining positions were conservative changes. Based on crystallographic data, the 3Cpep region of 3Cpro is on the opposite side of the molecule from the active site and is likely to be solvent exposed (Matthews et al., 1994).

Specific 3Cpro antiserum was generated by immunizing rabbits with recombinant HRV16 3Cpro. Specific antibody was purified on an HRV2 3Cpro affinity matrix. The strategy of immunizing with one serotype and purifying antibody cross-reactive to a different HRV serotype was intended to select for antibodies to conserved epitopes and increase multiserotype recognition. The recognition of recombinant 3Cpro by each antibody was evaluated by Western blotting. Both antibodies generated in this study recognized a 20-kDa band consistent with 3Cpro (data not shown).

3.2. Detection of 3Cpro

The 3Cpro assay was a 28-min, room temperature procedure. Detector antibodies were mixed with the sample and added to the antibody coated optical surface. Binding of 3Cpro was visualized through an enzymatic reaction that deposited mass on the surface, generating a thickness change. The thin film optics of the surface transduced the thickness increase into a visible color change (Fig. 1). We determined the limit of detection for purified recombinant HRV2 3Cpro (Fig. 2A). The visual limit of detection was 12 pM, or 0.3 fmol/assay.
Next, the sensitivity of the 3Cpro assay was determined with a more complex sample, rhinovirus infected cell lysates. Serial dilutions of the HRV2 lysate were prepared and tested in the 3Cpro assay and the limit of visual detection was determined to be 1000 TCID₅₀/test (Fig. 2B).

Fig. 1. Optically coated silicon surface and biological thin film formation. The unreacted surface is composed of an optically coated silicon wafer plus capture antibody. The reacted surface illustrates binding of antigen and HRP conjugated antibody, followed by HRP dependent mass deposition, resulting in altered light reflection and a visible color change.

| HRV Serotype | TCID₅₀/test |
|--------------|------------|
| 1B           | 2 x 10⁵    |
| 9            | 2 x 10⁴    |
| 12           | 2 x 10⁴    |
| 58           | 2 x 10⁴    |
| 65           | 2 x 10⁵    |
| 78           | 2 x 10⁵    |
| HeLa Cell Lysate | 0         |

Fig. 3. Representative 3Cpro visible reactions with infected cell lysates from selected HRV serotypes. Last chip is the negative result from uninfected control HeLa cell lysate. Viral titer/test is listed below the chip.
3. Multiserotype detection

A clinically relevant HRV assay must detect the majority of HRV serotypes. We tested the extent of multiserotype reactivity in our assay with cell lysates from 52 different HRV serotypes. The lysates were harvested from infected HeLa cells 3–5 days post-infection. The titers of the lysates ranged from $10^4$ to $10^7$ TCID$_{50}$/ml. A 20-μl aliquot of each lysate was tested for reactivity in the 3Cpro assay. The results in Table 1 and show that 45/52 (87%) of the tested serotypes were detected. The analytical sensitivity level varied between
serotypes, presumably depending on sequence conservation of the dominant 3Cpro epitopes and relative affinity of the 3Cpro antibodies.

The HRV serotypes are divided into two groups, based on cellular receptor specificity. The major group viruses attach to intercellular adhesion molecule 1 (ICAM-1) and the minor group binds to the low density lipoprotein receptors (LDLR) to initiate infection (Marlovits et al., 1998). One HRV serotype, HRV 87, binds to an unknown cellular receptor to initiate infection (Uncapher et al., 1991). A total of 45 major receptor group serotypes were tested, and 39 (87%) were positive. Six minor receptor group serotypes were tested, and all six (100%) were positive. The seven serotypes that were not detected by the 3C pro assay may be phylogenetically distinct from the HRV16-like viruses, based on available sequence data (Horsnell et al., 1995; Meador et al., 1998; Palmenberg, 1998).

3.4. Cross-reactivity studies

To evaluate the specificity of the 3Cpro assay, we tested for cross-reactivity to a panel of common respiratory viruses and bacteria. The panel consisted of 20 viruses and 11 bacterial species. The samples were either virally infected cell lysates or bacterial cell suspensions. Concentrations ranged from $10^6$ to $10^8$ TCID$_{50}$/ml for the viruses and $10^8$ to $10^{10}$ CFU/test for the bacteria (Table 2). No cross-reactivity was observed. The lack of reaction with the six tested enteroviruses is especially noteworthy.

3.5. Time course of in vitro 3Cpro detection

To examine the availability of 3Cpro throughout the course of viral replication, a time course study in culture was undertaken with an HRV clinical isolate. This isolate was cultured from nasal secretions of a symptomatic individual and had been passaged in culture once before the time course experiment. The isolated virus was identified as HRV by RT-PCR and temperature sensitive growth in HeLa cells. Data from a natural isolate was expected to result in a more accurate representation of in vivo 3Cpro temporal detection than a laboratory adapted serotype.

HeLa H1 cells were inoculated with this isolate at an MOI of 0.001 and monitored for 5 days. Small aliquots (15 µl from a total of 3 ml) of the culture supernatant were tested daily for 3Cpro and the results were compared to an uninfected control culture. The assay detected 3Cpro beginning on day 2 (Fig. 4), when CPE was visible, and continued to detect 3Cpro through day 5. The

### Table 1

| Serotypes detected | Serotypes not detected |
|--------------------|------------------------|
| Major receptor group | 8, 9, 10, 11, 12, 13, 14, 15, 16, 19, 21, 22, 23, 24, 25, 26, 27, 28, 32, 36, 38, 39, 40, 50, 53, 54, 56, 58, 59, 63, 65, 67, 68, 73, 75, 78, 81, 84, 85, 89, 95, Hanks | None |
| Minor receptor group | 2, 29, 31, 49, 1A, 1B | None |
| Non-major, non-minor group | None | 87 |

*Infected cell lysates of each serotype were tested with the 3Cpro assay. Serotypes were categorized based on receptor type (Uncapher et al., 1991). Reactivity of representative serotypes is shown in Fig. 2.*

### Table 2

| Cross-reactivity panel |
|------------------------|
| **Viruses**          | **Bacteria**            |
| Adenovirus types 5, 7A | Neisseria mucosa        |
| Coronavirus 229E       | Neisseria sicca         |
| Coxsackievirus types A9, A21, B3, B5 | Pseudomonas aeruginosa |
| Cytomegalovirus       | Proteus mirabilis       |
| Echovirus 11          | Salmonella minnesota    |
| Enterovirus 70        | Staphylococcus aureus   |
| Herpes simplex types 1, 2 | Streptococcus agalactiae |
| Influenza types A, B  | Streptococcus anginosus |
| Parainfluenza types 1, 2, 3 | Streptococcus pneumoniae |
| Respiratory syncytial virus | Streptococcus pyogenes  |
| Rubella               | Group G streptococcus   |
| Varicella zoster      |                        |
Fig. 4. Time course of 3Cpro detection from HRV infected culture supernatant. (A) CD values from days 1 through 5 of both infected and uninfected culture supernatants. Error bars represent ± 1 S.D. (B) Daily 3Cpro assay images from infected and uninfected lysates.

Fig. 5. Detection of 3Cpro from expelled nasal secretions. (A) 3Cpro assay CD values from daily sampling of an HRV infected individual. (B) Image of 3Cpro assay results.
peak for detection was day 3, when CPE was well established. Uninfected lysates remained negative in the 3Cpro assay throughout the experiment.

Detection of 3Cpro in culture supernatants correlated with CPE in this experiment. However, lysis of the infected cells prior to the appearance of CPE resulted in earlier detection of 3Cpro. Initial experiments with HRV39 infected cells demonstrated that if cells were lysed by freeze/thaw, the visual signal increased, making detection possible as early as day 1 (data not shown). This observation is consistent with cell lysis releasing intracellular 3Cpro produced early in the viral infection, before substantial damage to the monolayer has occurred.

3.6. Detection of rhinovirus in nasal secretions from an infected individual

The next step in assay characterization was to detect 3Cpro directly from an infected individual. Consideration of appropriate sample type was based on reports documenting the site of viral replication as focal infection of epithelial cells in the posterior nasopharynx (Turner et al., 1984; Gwaltney, 1995). Expelled nasal secretions were chosen for this study because the collection method was simple and the specimen originated from the physical site of viral replication.

Expelled nasal secretions were collected from an individual with severe rhinorrhea, nasal obstruction and general malaise on four consecutive days, beginning within 24 h of symptom onset. The presence of rhinovirus in the secretions was confirmed by RT-PCR. The secretions were also tested in the 3Cpro assay. Viral protease was detected on each day, with day 3 yielding the strongest reaction (Fig. 5). Day 3 also coincided with the peak day of combined symptom severity as determined by subjective scoring of rhinorrhea, nasal obstruction, cough and malaise. Thus, 3Cpro was detected throughout the course of illness, with the strongest signals correlating with the peak of respiratory symptoms.

3.7. Detection of 3Cpro in nasal washes

We examined the sensitivity of the 3Cpro assay in a set of nasal wash specimens from culture confirmed natural infections. The specimens were concentrated fivefold prior to testing to mimic the 3Cpro concentration in undiluted nasal secretions. Of the 11 specimens tested, 9 (82%) were visually positive. In addition, four influenza positive specimens tested negative in the 3Cpro assay. These results confirm that 3Cpro is present in nasal secretions and may be a sensitive and specific marker of rhinovirus infection.

4. Discussion

In this study we describe a novel, sensitive assay for HRV 3Cpro with a sensitivity limit of 12 pM, or 1000 TCID_{50}/test. This level of sensitivity is consistent with viral titers from experimentally infected volunteers on the peak days for symptom severity (Douglas et al., 1966). This assay is based on thin film detection of 3Cpro bound to specific antibody immobilized on an optically coated silicon surface. The assay identified 3Cpro in nasal secretions of HRV infected, symptomatic individuals throughout the course of illness. It is also a useful tool for monitoring in vitro viral growth. We have demonstrated specific detection of 87% of the HRV serotypes tested without cross-reactivity to other common respiratory viruses or bacteria, including enteroviruses. This multiseroype detection is unique among immunologically based HRV detection methods.

A number of systems have been described for HRV detection, including immunoassays using hyperimmune rabbit serum or peptide antigens from capsid proteins (Al-Nakib et al., 1986; McCray and Werner, 1987). The reported serotype recognition of these antibodies ranged from 52 to 68% of the serotypes tested. Another approach to HRV detection utilized soluble ICAM-1, the cell surface receptor for the major subgroup of HRV, as both the capture and detection reagent in a 4-h format (Last-Barney et al., 1991). While this assay would be predicted to recognize all major subgroup serotypes, it may not be specific for HRV because all ICAM-1 binding molecules would give a positive result.

Several HRV nucleic acid detection methods
have been described (Mori and Clewley, 1994; Arruda et al., 1997; Santti et al., 1997; Hyyppia et al., 1998; Andeweg et al., 1999). Although these assays are both sensitive and specific, they are not currently suitable for the point of care setting, where therapeutic decisions are made. The 3Cpro assay described in this report is a 28-min test with visual detection sensitivity down to 12 pM. No instrumentation is required, making the format suitable for the point of care setting. The test result could be used to limit prescription of antibiotics or guide specific antiviral therapy.

The clinical sample type used in this study, expelled secretions, is easily collected. Studies carried out by Turner demonstrated that rhinoviral infection of the nasal epithelium was focal and sporadic, with a higher concentration of infected cells in the posterior portion of the nasopharynx (Turner et al., 1984; Gwaltney, 1995). Therefore, sampling of the nasopharynx with a small swab was expected to be an inefficient method for collection of infected material. Nasal washes are often collected in laboratory evaluations of respiratory infections, but this method of sample collection dilutes the specimen and is not practical in the physician office setting. Patient expelled secretions are a convenient collection method and sample material is released from infected cells throughout the length of the nasopharynx.

Another application of the 3Cpro test is culture confirmation of HRV infection. HRV is often diagnosed in culture by characteristic CPE and verified by either acid lability or temperature-dependent replication (Couch, 1996). This process may take 3–14 days to complete. We have shown that the 3Cpro assay detects 3Cpro in virally infected culture supernatants as early as 48 h after inoculation. The specificity of the test allows for HRV culture identification without additional confirmatory testing.

We have presented preliminary data indicating that 3Cpro can be detected from HRV infected patients throughout the course of illness. This report is the first demonstration of 3Cpro as a marker for in vivo and in vitro HRV infection. We have provided evidence that although 3Cpro is not a structural viral protein, the concentration present in nasal secretions is sufficient for use of this protein as a diagnostic target. Rapid point of care viral assays may guide appropriate antiviral therapy in the near future. The 3Cpro assay described in this report is a first step in the direction of rapid identification of HRV in clinical specimens in a simple format suitable for the point of care setting. Further clinical validation is required to confirm these results and establish the utility of this assay in rapid early diagnosis of HRV infection.

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