Comparison of Complement Fixation With Two Enzyme-Linked Immunosorbent Assays for the Detection of Antibodies to Respiratory Viral Antigens

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Key Words: Complement fixation; Enzyme-linked immunosorbent assay; ELISA

Abstract
We compared complement fixation (CF) for the measurement of antibodies against influenza A, influenza B, respiratory syncytial virus (RSV), human adenovirus, and parainfluenza viruses 1, 2, and 3 (para-1, para-2, and para-3) with 2 enzyme-linked immunosorbent assays (ELISA kits, A and B). The IgG ELISA kits compared very well with each other except for the influenza A and B IgG ELISAs. The IgG ELISAs, in general, did not agree with CF. In contrast, the IgM ELISAs compared well with CF and each other, except for the consensus parainfluenza panel from ELISA B. The poor agreement of the IgG ELISAs with the CF test can be explained by the increased sensitivity of the ELISAs and differences between CF antigens and the ELISA antigens. The influenza A and influenza B ELISA antigens differed between both kits, which may explain their poor agreement. The ELISA is a suitable replacement for CF, providing greater sensitivity, isotype specificity, and ease of use.

Seasonal respiratory viral infections of the upper and lower respiratory tracts are a yearly event. The most common are caused by influenza A, influenza B, respiratory syncytial virus (RSV),1 human adenovirus,2 and parainfluenza viruses 1, 2, and 3 (para-1, para-2, and para-3).3,5 Infections cause morbidity in the general population and bring about more severe disease in patients with underlying respiratory problems or heart disease and in infants and elderly people.4 The orthomyxoviruses (influenza A and influenza B) and the paramyxoviruses (para-1, para-2, para-3, and RSV) are similar and thought to have descended from a common single-stranded RNA virus.6 Adenovirus is in a separate family of DNA viruses and consists of 51 human serotypes.2,7,8 Determination of current, recent, or past infections by these viruses has an important role in patient care and is useful in respiratory viral epidemiology.9-13 Recently, fear of recombinant (antigenic shift) forms of viruses, including influenza A and coronaviruses that seem to be lethal to humans, has initiated an intensified surveillance of new and emerging viral respiratory diseases.4,5,14-17 In addition, the recent failure in the ability to produce sufficient influenza vaccine7 to protect at risk populations has sensitized health care personnel to the need for close monitoring of respiratory viral infections.18-20

Methods for viral antigen detection in immunocompetent patients include culture, staining with fluorescent monoclonal antibodies (direct fluorescent and immunofluorescent assays), and polymerase chain reaction (PCR) and reverse transcription (RT)-PCR.21 These genome detection techniques can be complex to perform and are expensive.22 They are most effective for diagnosis during the acute stage of respiratory viral infection and are powerful tools for identifying viral genomes that do not clear the body immediately after the acute phase of...
Serologic techniques for specific IgG and IgM detection can be useful for detecting recent and past infection. Most commonly used for surveillance of vaccine conversion or epidemiologic monitoring of infections in the community, serologic techniques may be used as an aid to clinical diagnosis in the absence or failure of isolation by culture, immunofluorescent assay, or molecular techniques (PCR or RT-PCR). Serologic testing generally is not accepted as a diagnostic tool with respiratory viruses but has been used in conjunction with other technology to determine the diagnosis.

We compared two enzyme-linked immunosorbent assays (ELISAs) for the detection of antibodies to 7 respiratory viruses (influenza A, influenza B, para-1, para-2, para-3, RSV, and adenovirus) with our current complement fixation (CF) assay. One of the limitations of CF is that complement-fixing antibodies include IgM and, to some extent, IgG, whereas the ELISA kits can differentiate the IgG, IgM, and IgA classes. ELISA technology is more sensitive than CF, less time-consuming, and does not require laboratory personnel trained in high-complexity testing. CF frequently is found as a method in the testing menus of clinical reference laboratories. The measurement of isotype-specific IgG or IgM antibodies can help distinguish acute from past infection. ELISA also is analytically more sensitive than CF and has the feature of eliminating false IgM-positive results due to interfering specific IgG antibodies and rheumatoid factor (RF) by use of kit-specific absorbent reagents.

Materials and Methods

Serum Specimens

We included 190 serum specimens that were submitted for respiratory viral serology testing between October 2002 and April 2004 in the study. Samples originally were tested by using an in-house CF assay for influenza A, influenza B, adenovirus, RSV, para-1, para-2, and para-3. Serum was the only specimen type used for this study. After initial testing by CF, specimens were archived and stored frozen (−20°C) until further testing by ELISA and CF for viruses not originally assayed. Samples were kept in original containers owing to the minimal volumes available. Freeze-thaws were kept to a minimum (<3).

All patient samples included in this study were deidentified according to the University of Utah Institutional Review Board–approved protocol (No. 7275) to meet Health Information Portability and Accountability Act patient confidentiality guidelines. The patient population included people living in the United States who may have received influenza vaccines within 2 years of blood collection. Patient ages ranged from younger than 2 weeks to 90 years. It was assumed that samples were from patients with symptoms consistent with current, recent, or past infections with respiratory viruses.

Complement Fixation

CF was performed as previously described. The commercially available antigen reagents (BioWhittaker, Walkersville, MD) are listed in Table 1. Briefly, with CF, patient serum is diluted from 1:8 to 1:64 and combined with antigen. Guinea pig complement is added and incubated overnight (at least 12 hours) at 4°C. Sensitized sheep RBCs are added and incubated at 37°C for 30 to 60 minutes. When antibody to the antigen is present, complement is bound and the RBCs settle out and form a pellet on the bottom of the well. When antibody is absent, unbound complement lyses the RBCs and no pellet is formed. The CF assay takes approximately 16 hours to complete. A CF result was considered positive for both IgG and IgM antibodies at titers of 1:8 or more.

ELISA Kit A

The specimens were tested for IgG and IgM by using VIROTECH ELISA test kits (Genzyme Virotech, Rüssellsheim, Germany [available in the United States from VIROTECH]).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Specific Antigens Used for Complement Fixation, VIROTECH ELISA, and IBL ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>Complement Fixation Antigen</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Influenza A</td>
<td>H3N2/HK/8/1968</td>
</tr>
<tr>
<td>B</td>
<td>B/Mass/3/1966</td>
</tr>
<tr>
<td>Adenovirus Respiratory syncytial virus Parainfluenza Parainfluenza 1 2 3 Panel</td>
<td>Sendai C-35 strain Greer strain C-243 strain —</td>
</tr>
</tbody>
</table>

ELISA, enzyme-linked immunosorbent assay.
SCIMEDX, Denville, NJ) for the 7 viruses (Table 1) according to the manufacturer’s recommendations. Specimens that were positive or equivocal for the presence of IgM antibody were retested after treatment with an absorbing reagent (RF-SorboTech, Genzyme Virotech [SCIMEDX]). RF-SorboTech contains sheep antihuman IgG and precipitates IgG antibodies that can compete with IgM antibodies for antigen binding sites on the ELISA plate, reducing false-positive results due to IgM RFs. The specific viral antigens coating the ELISA plates are listed in Table 1. The IgG and IgM assays took approximately 2 and 4 hours for completion, respectively. ELISA A kits were for research use only.

ELISA Kit B

Specimens were tested for IgG and IgM antibodies by using IBL ELISA test kits (Immuno-Biological Laboratories, Hamburg, Germany [available in the United States from IBL-America, Minneapolis, MN]) according to the manufacturer’s recommendations for the 7 viruses, except that the para-1, para-2, and para-3 were tested for IgG and IgM antibodies in a panel format combining all 3 viruses in 1 assay. Specimens that were positive or equivocal for the presence of IgM antibody were retested after treatment with the RF adsorbent exclusive to ELISA B kits (Immuno-Biological Laboratories [IBL-America]). The specific viral antigens coating the ELISA B plates are listed in Table 1. The IgG ELISA and IgM assays took approximately 2 and 4 hours for completion, respectively.

Because CF measures complement-fixing antibodies (IgG and IgM), direct comparison with the IgG- and IgM-specific ELISAs was not possible. To overcome the inherent difference in the methods, samples with a positive IgG ELISA result were removed from the statistical analysis of the comparison of the CF with the IgM ELISAs. The removal of the IgG-positive results from the calculation of the IgM statistical data is based on the high frequency of IgG antibodies against respiratory antigens in the healthy population. This assumes that the majority of CF-positive samples will be due to the presence of IgG antibodies. ELISA B kits were for research use only.

Statistical Analysis

Statistical comparisons of CF, ELISA A, and ELISA B were done by using an online program calculating 2-way contingency table analysis and reporting $\kappa$ as a measure of concordance. Agreement, sensitivity, specificity, and $\kappa$ results were determined for CF vs ELISA A, CF vs ELISA B, and ELISA A vs ELISA B.

Results

CF vs ELISA

Despite similar methods, the respiratory virus IgG ELISA A and CF had poor agreement except for influenza A and influenza B. ELISA B and CF had poor agreement for all viruses except adenovirus. ELISA A and ELISA B had good agreement for adenovirus, RSV, and consensus parainfluenzas but poor agreement for influenza A and influenza B. Sensitivity was good in all comparisons except ELISA B and CF with influenza B and RSV. ELISA A and ELISA B had poor sensitivity with influenza B. The compared specificities of the IgG ELISA tests were extremely poor compared with each other and with CF except ELISA A and ELISA B adenovirus, RSV, and consensus parainfluenza.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Agreement (%)</th>
<th>95% CI</th>
<th>Sensitivity (%)</th>
<th>95% CI</th>
<th>Specificity (%)</th>
<th>95% CI</th>
<th>$\kappa$</th>
<th>95% CI</th>
<th>No. of Specimens</th>
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<td></td>
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<td>Flu A</td>
<td>71</td>
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<td>73-87</td>
<td>57</td>
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<td>85-94</td>
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<td>26-46</td>
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CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; Flu, influenza; Para, parainfluenza; RSV, respiratory syncytial virus.

* Consensus.

Table 2

Table 2. Comparison of Complement Fixation and ELISA A Results Using 2-Way Contingency Table Analysis Comparison

Table 3

Table 3.
and Table 4. In contrast with IgG, the agreement for IgM ELISA kits compared well with each other and with the CF test except CF and both ELISAs A and B with consensus parainfluenza. CF and ELISA A had poor sensitivity with influenza A, RSV, parainfluenza 2, and consensus parainfluenza but good sensitivity with influenza B, adenovirus, and parainfluenzas 1 and 3. CF and ELISA B had poor sensitivity with influenza B, RSV, and consensus parainfluenza but had good sensitivity with influenza A and adenovirus. Comparing ELISA A with ELISA B, there was poor sensitivity with RSV and good sensitivity with all other respiratory virus ELISAs. Specificities were good in all IgM comparisons (Tables 2-4).

Statistics

Statistical analysis using 2-way contingency tables generated agreement, sensitivity, specificity, and 95% confidence intervals. The κ also was determined to measure the agreement between different methods (Tables 2-4). In each comparison of CF with ELISA A or B, CF was used as the “gold standard.” When comparing ELISA A with ELISA B, ELISA A was considered the gold standard (Table 4).

Discussion

Recent advances in technology have added to the number of diagnostic tools used for the detection of respiratory viral infections. These include culture, direct specimen testing, direct and indirect immunofluorescent antibody staining of specimens, electron microscopy, RT-PCR, PCR amplification, and serologic testing. For years, culture has been the accepted standard to which all other testing is compared. However, nonserologic tests can be problematic owing to cost, lengthy turnaround times, susceptibility to changes in sample integrity, contamination, complex confirmatory techniques, and the required expertise and specialized equipment.

Serologic testing, although usually not helpful during the acute phase of an infection, can provide useful information about recent or past viral infections within a community at a reasonable cost and in a short turnaround time. Serologic testing can be used diagnostically if acute and convalescent samples are obtained.

Our intention was to find a suitable alternative for antiquated CF assays. We compared 2 enzyme-linked immunosass
with CF for the serodiagnosis and identification of immunoglobulin isotypes (IgG and IgM) for influenza A, influenza B,25,28,37-39 adenovirus,40 RSV,41 and para-1, para-2, and para-3. Previous studies have demonstrated that ELISA is more sensitive than CF.3,12,13,38,39,42,43 The 2 ELISAs were capable of differentiating IgG and IgM, took less time to complete, and were more sensitive than CF. Our results were similar to previous observations based on similar outcomes.3,38,39,42 The ELISA IgM kits also were able to eliminate factors contributing to false-positive results.

The prevalence of IgG antibodies to influenza A, influenza B, adenovirus, RSV, and para-1, para-2, and para-3 within a population increases with age. Exposure to and infection with all 7 viruses are common throughout a lifetime, as primary and as secondary infections. Vaccinations for influenza have become routine as a preventive measure, mostly in the Western world.44 In children younger than 12 months, IgG antibodies are seen in 5% or fewer,36 and in the general population (children and adults), prevalence rates of 18%3 to 84.2% have been reported.35 Adenovirus, RSV, and parainfluenza virus IgG antibodies are prevalent in almost 100% of adults if all serotypes are included2,40,45 and fewer if specific serotypes are studied.46

In our general population study group (children and adults), we found the IgG antibody prevalence for influenza A by ELISA A to be 59.3%, 94.8% by ELISA B, and 56.8% and 51.7% by CF in the same comparisons. Influenza B IgG prevalence was 76.9% by ELISA A, 64.3% by ELISA B, and 61.9% and 41.1% by CF. Adenovirus IgG detection resulted in a prevalence of 88.9% by ELISA A, 64.8% by ELISA B, and 41.6% and 48.1% by CF in the same comparisons. We found RSV IgG at 93.8% by ELISA A, 82.0% by ELISA B, and 28.3% and 20.0% by CF. These data are consistent with ranges of respiratory viral IgG antibody prevalence found by others.13

Parainfluenza virus IgG antibodies have been studied as individual viruses and as a group. Parainfluenza viruses are common47 and similar to one other, with para-1 and para-3 more closely related than para-2. In our study, ELISA A and CF detected antibodies to the individual parainfluenza viruses. We found the prevalence of IgG antibodies using ELISA A in 85.5% of samples tested for para-1, 60.1% for para-2, and 92.0% for para-3. The CF assay detected 24.8%, 28.4%, and 29.2% in samples for the 3 viruses, respectively. The ELISA B assay had been designed as a panel to detect all 3 parainfluenza viruses in 1 assay. A consensus result for the 3 individual parainfluenza viruses was determined for the ELISA A and CF for direct comparison with ELISA B. Consensus IgG for the 3 parainfluenza viruses was 93.3% by ELISA A and 49.1% by CF. The ELISA B parainfluenza panel detected 86.7% compared with 53.3% by CF. Our results are consistent with those of previous studies.22,48

The comparisons of ELISA A IgM with CF resulted in good agreement for all viruses except influenza A and influenza B, which were slightly lower than other IgM ELISA A analyses. Although IgM is less specific than IgG, the lower agreement could be a result of differing antigens used in the assays or the enhanced ability of IgM to trigger complement compared with IgG. The ELISA A uses viral antigen strains for influenza A, and influenza B kits have more recently been in circulation in the world population and could be more representative of antibodies in the current population.

Comparison of ELISA B with CF also gave good results for all assays except those for the parainfluenza viruses. The 3 separate antigens used by CF were more sensitive for detecting all parainfluenza IgM than the ELISA B panel kit using a single antigen (para-1 strain). When the 2 ELISAs were compared, agreement was good. The comparisons of ELISA A IgG with CF and of ELISA B with CF gave poor agreement for all kits. This can be explained by the higher analytic sensitivity of ELISA technology owing to the ability to discern smaller changes in increasing antibody titer and the use of influenza strains not representative of recent infectious strains or vaccines.

All comparisons resulted in a large number of CF-negative, ELISA A– and ELISA B–positive samples. Comparing the ELISA kits with each other resulted in good agreement for adenovirus, RSV, and the consensus ELISA A/ELISA B parainfluenza panel. Influenza A and influenza B compared poorly, possibly owing to the different antigens used for coating the respective plates. The use of current or recent antigens circulating in a community seems to have enhanced the sensitivity of the ELISA method. The specificity of the ELISA methods depends greatly on the specific antigens (or combination) used. Cross-reactions observed between related viruses by other serologic methods can be enhanced when ELISA is used.49-51 We did not observe heterologous rises in antibodies for influenza A and B with ELISA A or B, and we did not observe cross-reactivity with ELISA A kits among para-1, para-2, and para-3. ELISA B was a parainfluenza panel and did not specifically identify antibodies to para-1, para-2, or para-3 (data not shown).

The ELISA technology that we describe is a sensitive method for measuring IgG and IgM antibodies to viral antigens from influenza A, influenza B, adenovirus, RSV, and para-1, para-2, and para-3 and likely should replace the outdated CF testing still used in many clinical laboratories. Statistical analysis using \( \kappa \) to measure the agreement between CF and ELISA A revealed poor agreement for all IgG assays and the IgM influenza A. In addition, poor agreement was seen when comparing CF and ELISA B for all IgG and IgM assays except IgM influenza A and adenovirus. In the comparison of ELISA A and ELISA B, \( \kappa \) showed good agreement for adenovirus, RSV, and parainfluenza IgG assays and good agreement for all IgM assays except RSV.
References


