Particle Concentration Fluorescence Immunoassay: A New Immunoassay Technique for Quantification of Human Immunoglobulins in Serum

Chris MacCrindle, Kathryn Schwenzer, and Michael E. Jolley

A new fluorescence immunoassay technique, particle concentration fluorescence immunoassay (PCFIA), has been developed for quantifying the human immunoglobulins (IgA, IgM, and IgG). In these "two-site sandwich assays," the capture antibody is immobilized on small polystyrene spheres and the tracer is fluorescein-labeled antibody. Polystyrene particles less than 1 μm in diameter make up the solid phase, to which goat anti-human antibody for each respective assay is attached. Serum specimens are diluted (5000-fold for IgA or IgM, 20 000-fold for IgG) placed on the 96-well Pandex assay plate; and mixed with the solid phase and tracer (fluorescein-labeled goat anti-human IgA, IgM, or IgG), which are added automatically by the Pandex Screen Machine™. This instrument incubates the reaction mixture for 17 min at ambient temperature, separates the bound and free label by filtration, washes the solid phase, and determines the total particle-bound fluorescence by front-surface fluorometry or epifluorescence, calculates results, and generates detailed reports. Ninety-six specimens may be analyzed in 29 min or 960 specimens in 136 min. Results by PCFIA for IgA, IgM, and IgG in serum correlated well with those by rate nephelometry.

Additional Keyphrases: IgA · IgM · IgG · proteins · rate nephelometry compared · dual-wavelength fluorometry

Current methods of choice for diagnosing immunoglobulin (lg) abnormalities are immunoelectrophoresis or serum protein electrophoresis, combined with quantification by nephelometry, radial immunodiffusion, or enzyme-linked immunosorbent assay. These techniques provide adequate results, but less-expensive methods are needed to rapidly quantify and characterize protein abnormalities, in particular the Ig disorders (1). A new fluorescence immunoassay technique, particle concentration fluorescence immunoassay (PCFIA) (2), offers the specificity of immunoenzyme and rapid batch quantification of human IgA, IgM, and IgG.

In PCFIA, immunoreactive species are bound to small particles, typically 0.8-μm-diameter polystyrene; the resulting solid phase is dispersed in the sample, and labeled reagent is added. After analyte and labeled reagent bind on the surface of the particles, the mixture is filtered, washed, and the total particle-bound fluorescence determined. By using a reaction well with a 2-mm-diameter filtration area (Figure 1), the fluorescence from all of the solid phase may be detected. Background fluorescence, primarily from stray and scattered light and from the endogenous fluorescence of the filter membrane and solid phase, can be minimized by

---

Additional Notes:

1 Pandex Laboratories, 909 Orchard St., Mundelein, IL 60060.

2 Nonstandard abbreviations: PCFIA, particle concentration fluorescence immunoassay; FITC, fluorescein isothiocyanate; IBS, isotonic buffered saline.

Received April 11, 1985; accepted June 14, 1985.
Results

Standard Curves

Figure 2 illustrates typical standard curves for IgA, IgM, and IgG by PCFIA. The IgA standard curve exhibits a "hook" or prozone effect (the ratio of the intensity decreases) at a concentration greater than 1200 mg/dL. The IgM standard curve exhibits a hook effect at concentrations greater than 1400 mg/dL, the IgG standard curve at concentrations greater than 4500 mg/dL.

The antibody-coated particles are stable for one week at both 37 °C and 45 °C, and are also stable to a freeze–thaw cycle. Given our previous experience with reagent stability at warmer temperatures, the solid phase should be stable for at least two years at 2–8°C. The current FITC tracers, however, will not be similarly stable 2–8°C in the diluted format, and we suggest that they be freshly diluted before use.

Incubation times may be minimized by increasing the concentrations of tracer and analyte in the reaction mixture. In the IgA assay, changing the sample dilution from 5000- to 2000-fold and increasing the tracer concentration from 5 to 30 μg/mL yielded identical standard curves for incubation periods of 7, 10, or 30 min.

Analytical Variables

Specificity. The affinity-purified antibodies were monospecific as determined by various methods. All of the antibodies were tested with purified immunoglobulins by PCFIA. Antibody to IgA, IgM, and IgG used to coat the particles and the FITC-labeled antibodies to IgA, IgM, and IgG were monospecific as determined by double diffusion. All of the antibodies except FITC-labeled anti-IgG were also tested by immunoelectrophoresis. The antibodies to IgA, IgM, and FITC-labeled antibody to IgA were tested by solid-phase RIA with a normal immunoglobulin panel and a myeloma panel.

Analytical recovery. We determined analytical recovery by assaying commercially available standards and controls from several sources, measuring all three immunoglobulins (IgA, IgM, and IgG) by PCFIA. The correlations and slopes (Figure 3) approximately equal 1.0, demonstrating very good recovery in the immunoglobulin assays, especially within the normal range. We also supplemented 10 IgA-containing clinical specimens with 1050 mg of IgA per liter, and measured an average recovery of 93.71% (984, SD 111, mg/L). The average recovery was 101.60% (589, SD 59, mg/L) for 10 IgM-containing specimens supplemented with 560 mg of IgM per liter, and 101.29% (5310, SD 628, mg/L) for 10 IgG-containing specimens with 5240 mg of added IgG per liter.

Sensitivity. Sensitivity, the lowest concentration that can be detected with 95% confidence, was determined by assaying five replicates of the zero standard. We calculated the sensitivity as that concentration detectable at 2 SD from the mean for the zero standard. Our results for this analytical system were as follows: 14 mg/dL for IgA, 10 mg/dL for IgM, and 11 mg/dL for IgG.

Precision. We determined within-assay-plate reproducibility by running eight replicates of low, medium, and high controls for each immunoglobulin (Table 1). The within-plate CVs were <10% for all the assays. We currently (April 1985) recommend including a standard curve on every assay plate with the unknown samples.

Also, the PCFIA system includes a fluorescent internal standard in the antibody-coated particle solution, which compensates for errors in pipetting particles, differences in assay plates, differences between well positions, and varia...
Asigned 0.08 25.14 100 an 08 3.23 4
Bscltman SD 1 200 (mg IQ.
PCFI A internal comparison control from signal sion. The concentration PCFIA +13.5.09 PCFIA average = 148 mg/dL, assigned average = 135 mg/dL. For IgG PCFIA = 0.947 assigned value +135.09 (n = 26, r = 0.948, PCFIA average = 1241 mg/dL, assigned average = 1167 mg/dL)

**Table 1. Precision**

<table>
<thead>
<tr>
<th>Conc, g/L</th>
<th>Mean</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>5.60</td>
<td>0.12</td>
<td>2.15</td>
</tr>
<tr>
<td></td>
<td>3.64</td>
<td>0.13</td>
<td>3.52</td>
</tr>
<tr>
<td></td>
<td>1.27</td>
<td>0.02</td>
<td>1.68</td>
</tr>
<tr>
<td>IgM</td>
<td>3.23</td>
<td>0.08</td>
<td>2.62</td>
</tr>
<tr>
<td></td>
<td>1.83</td>
<td>0.02</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>0.80</td>
<td>0.01</td>
<td>1.24</td>
</tr>
<tr>
<td>IgG</td>
<td>25.14</td>
<td>2.48</td>
<td>9.85</td>
</tr>
<tr>
<td></td>
<td>15.50</td>
<td>1.03</td>
<td>6.66</td>
</tr>
<tr>
<td></td>
<td>5.23</td>
<td>0.31</td>
<td>5.93</td>
</tr>
</tbody>
</table>

Correlation with commercially available assays. Figure 4 summarizes the correlation between PCFIA results for IgA, IgM, and IgG and those measured by the Beckman ICS. The correlations, slopes, intercepts, and averages between the PCFIA and the Beckman ICS assays indicate that PCFIA is a clinically useful tool for determining immunoglobulin concentrations in clinical specimens. With the PCFIA 96 tests may be run in 29 min and 10 plates (960 tests) may be run in 136 min.

**Discussion**

Determining IgA in 40 specimens with the Beckman ICS requires approximately 30 min of attended time; the PCFIA analysis requires a similar amount of attended time, because of the need for specimen dilution. Total assay time for 40 specimens is approximately 2 h with the Beckman ICS, whereas the PCFIA requires 29 min. As the number of specimens increases, the time saved by PCFIA analysis increases (i.e., 960 tests may be completed in 136 min). If it is desirable to increase the speed of reaction, incubation times for PCFIA analysis of the immunoglobulins can be decreased from 17 to 7 min by increasing the concentration of the tracer and decreasing the dilution of the specimen, so that 40 specimens can be assayed in 19 min.

The PCFIA immunoassay technique is rapid for large batch analysis, and is sensitive and accurate. The polysty-
rene particles allow for faster reaction rates than do other technologies: their high surface-area-to-volume ratio and their natural brownian motion keep the reagents and analytes continuously mixing and suspended in solution for an extended period.

The large dilutions of patient's serum (5000- and 20 000-fold) required for these assays demonstrate the high sensitivity of the system, especially for high-molecular-mass analytes, which, until now, have been the domain of enzyme immunoassay or solid-phase RIA. We believe that any possible interferences in the immunoglobulin assays will be negligible at the large dilutions used. In another assay system under development in our laboratories (unpublished data), in which 20 μL of a 50-fold dilution of specimen is used, we saw no problems with icteric, hemolytic, or lipemic specimens. The good precision, recovery, and correlation with an existing method for quantifying immunoglobulins demonstrate the accuracy and clinical utility of PCFIA.

References