

Measurement of Rheumatoid Factor by NEPHELOstar® Plus microplate reader

Lisa Mack and Anne Barton
ARC-Epidemiology Unit, Stopford Building, University of Manchester, UK

- NEPHELOstar® Plus provides higher throughput and cost effectiveness for the detection and quantification of rheumatoid factors
- Results are concordant with large scale IMMAGE® Immunochemistry System

Introduction

Rheumatoid Factors (RF) are immunoglobulins which react to antigenic sites on the Fc region of human or animal immunoglobulin G (IgG). The main isotype is IgM although others occur (IgA, IgD and IgG). RF are found more commonly in patients with rheumatoid arthritis (RA) than the general population but can be found in patients with other connective tissue diseases, following infection and in a number of other diseases such as sarcoidosis and liver disease. However, presence of RF is one of the classification criteria for RA and presence of RF is associated with a poorer prognosis such as increased risk of developing erosive disease.

RF may be measured by a number of methods. These include the particle agglutination test, which relies on the agglutinating properties of IgM class of RF. IgG, usually either human or rabbit, is bound to a carrier such as latex and the presence of RF is detected by flocculation. Doubling dilutions of the serum until the flocculation can no longer be visualized allows a semi-quantitative measurement of antibody titre. However, the assay requires large amounts of serum, is time-consuming and observer dependent.

An alternative method of measuring RF uses nephelometry. Patient serum is added to a microplate containing a fixed amount of antigen (IgG). Any RF present will form an antibody-antigen complex with the IgG and the concentration of the RF antibody can be determined by light dispersion. Potential advantages offered by this system are better throughput, objective reading on a single sample dilution, use of smaller volumes of serum and cost-effectiveness.

In clinical diagnostic settings, measurement is usually performed using an IMMAGE® Immunochemistry Systems but such platforms are expensive to purchase and maintain. This application note investigates a method for testing RF using the BMG LABTECH NEPHELOstar Plus microplate reader.

Materials & Methods

Principle

Nephelometric testing of rheumatoid factor levels is based upon antigen-antibody reactions. RF binds to IgG to form immune complexes. As the number of immune complexes grows, the solution becomes more visibly "cloudy". The NEPHELOstar Plus microplate reader passes a light beam through the well in order to measure the turbidity of the sample. The more RF present, the more immune complexes are formed and the more light scattering will occur. The amount of light scattered is directly proportional to the number of immune complexes and therefore RF particles suspended in the sample.

Materials

- NEPHELOstar Plus plate reader and software (BMG LABTECH)
- RF-PAIA kit, including set of 5 calibrants, reagent and buffer (Orion Diagnostica, Finland)
- 96-well clear microplates (Bibby-Sterilin Ltd, UK)

Operating Procedure

1. Plate out 10 µL of undiluted calibrators 1 to 5, a known RF positive and negative serum and the serum samples to be tested.
2. Add 280 µL of RF buffer.
3. Pipette up and down gently to mix the solution, whilst trying to avoid bubbling*.
4. Insert the plate into the NEPHELOstar Plus microplate reader and adjust the settings for plate mode kinetic as shown:
 - Gain: 85
 - Pos. delay: 0.1 s
 - No. of cycles: 2
 - Meas. start time: 0 s
 - Meas. time/well: 1 s
 - Laser intensity: 50 %
 - Beam focus: 2 mm
 - Shaking time: 20 s
 - Shaking width: 4 mm
 - Shaking before each cycle
5. Begin the test. Cycle 1 will shake the plate and take the background reading.
6. When prompted, remove the microplate and add 10 µL of reagent to each well, pipetting up and down to aid mixing*.
7. Insert the plate again and press continue. Cycle 2 will shake the plate once more, and take the final reading.

* Mixing using a pipette should be performed with care. Generation of bubbles during mixing will interfere with the assay and may lead to inaccurate results.

Results & Discussion

80 samples with five calibrants and a positive and negative control can be measured in approx. 2 minutes. Including pipetting of all solutions (multichannel for buffer, all others singly pipetted), the run takes approximately 2 hours.

Results obtained for serum samples tested by the NEPHELOstar Plus microplate reader were compared with those from a Beckman Coulter IMMAGE® Immunochemistry System using the same serum samples (Fig. 1). Regression analysis showed that the correlation between the readings was 94.1%.



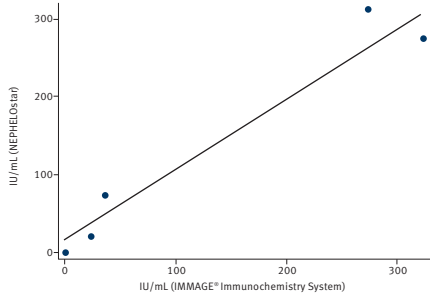


Fig. 1: Measurement of five RF-PAIA calibrants on the IMMAGE® Immunochemistry System vs NEPHELOstar *Plus* microplate reader.

Conclusion

Through this independent analysis, we have shown that the NEPHELOstar *Plus* plate reader can be used to accurately determine both RF antibody status and titre, which is critical to the diagnosis of rheumatoid arthritis and sub group definition of juvenile idiopathic arthritis. Thus this RF assay is a viable and inexpensive assay that can be used in medium to high throughput labs.

