QUANTA Lite® RF IgM ELISA 708690

For In Vitro Diagnostic Use
CLIA Complexity: High

Intended Use
QUANTA Lite® RF IgM is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of IgM rheumatoid factor (RF) antibodies in patient sera. The presence of these antibodies, when considered in conjunction with other laboratory and clinical findings, is an aid in the diagnosis of rheumatoid arthritis (RA).

Summary and Explanation of the test
Rheumatoid factors (RF) are immunoglobulins of any isotype with antibody activity directed against antigenic sites on the Fc region of human or animal IgG. IgM-RF is the main isotype identified by clinically available diagnostic assays for RF detection. The most consistent serological finding in patients with Rheumatoid Arthritis (RA) is an increase in the concentration of RF IgM in blood and synovial fluid. RF IgM has been reported to occur in approximately 70-80% of patients with confirmed RA. The concentration of RF tends to be highest when the disease peaks and tends to decrease during prolonged remission. RF IgM is found in 1 to 4% of the general population. RF is present in 75% of adult RA patients with the highest incidence of RF occurring in persons over 65 years of age. Increased titers may accompany a variety of acute immune responses, particularly viral infections and a number of other diseases (infectious mononucleosis, tuberculosis, leprosy, various parasitic diseases, liver disease, sarcoidosis, and systemic lupus erythematosus).

In addition to RF IgM, raised levels of RF IgG and IgA have been reported in patients with RA. Several groups have reported that a high level of IgA RF is prognostic for a more severe disease outcome. When RF isotype levels are compared with radiological abnormalities of the joints, the strongest correlation is with raised levels of RF IgA. High levels of RF IgA within three years of the onset of symptoms have been associated with a more severe disease after six years of onset. Studies from as early as 1984 suggest that the detection of RF IgA in early disease indicates poor prognosis and justifies a more aggressive course of treatment.

Two different groups demonstrated that raised levels of RF IgG are virtually confined to the sera of patients with rheumatoid arthritis and not other arthritides. The most striking clinical association with RF IgG appears to be RA vasculitis.

Conventional methods for the measurement of RF IgM have depended upon the agglutination of particles (e.g. latex, charcoal, bentonite, or erythrocytes) coated with human or animal IgG. The latex agglutination test is sensitive, but it can result in a rather high number of false positives. Non-specific agglutination of latex particles by sera from normal individuals is not uncommon. Quantitative serological tests such as EIA, RIA, and nephelometry have the advantage of objective instrument measurement on a single sample dilution. EIA methods have the added advantage of being able to simultaneously detect RF of IgG and IgA subclasses in addition to RF IgM and are not susceptible to prozone. It has become apparent that the specificity and predictive value of the RF test is substantially increased by the detection of all three RF isotypes.

Principles of the Procedure
The QUANTA Lite® RF IgM ELISA is a solid phase microwell ELISA designed to detect RF. Microwells are coated with rabbit IgG as rabbit material has been shown to be more specific for diagnosing RA than when human IgG is used on the solid phase. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any RF IgM antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgM conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgM to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgM, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the wells of a multi-point standard curve. The assay can be evaluated by comparing the color that develops in the patient wells with the color in the wells of a multi-point standard curve calibrated against a WHO international reference preparation (64/2).

Reagents
1. Polystyrene microwell ELISA plate coated with a purified rabbit IgG antigen (12-1 x 8 wells), with holder in foil package containing desiccants
2. ELISA Negative Control, 1 vial of buffer containing preservative and human serum with no human antibodies to RF IgM, prediluted, 1.2mL
3. RF IgM ELISA Calibrator A, 1 vial of buffer containing preservative and human serum antibodies to RF IgM, prediluted, 1.2mL
4. RF IgM ELISA Calibrator B, 1 vial of buffer containing preservative and human serum antibodies to RF IgM, prediluted, 1.2mL
5. RF IgM ELISA Calibrator C, 1 vial of buffer containing preservative and human serum antibodies to RF IgM, prediluted, 1.2mL
6. RF IgM ELISA Calibrator D, 1 vial of buffer containing preservative and human serum antibodies to RF IgM, prediluted, 1.2mL
7. RF IgM ELISA Calibrator E, 1 vial of buffer containing preservative and human serum antibodies to RF IgM, prediluted, 1.2mL
8. RF IgM ELISA Control, 1 vial of buffer containing preservative and human serum antibodies to RF IgM, prediluted, 1.2mL
9. HRP Sample Diluent, 1 vial – colored pink containing Tris-buffered saline, Tween 20, protein stabilizers and preservative, 50mL
10. HRP Wash Concentrate, 1 vial of 40x concentrate - colored red containing Tris-buffered saline and Tween 20, 25mL. Refer to the Methods Section for dilution instructions.
11. HRP IgM Conjugate, (goat), anti-human IgM, 1 vial – colored green containing buffer, protein stabilizers and preservative, 10mL
12. TMB Chromogen, 1 vial containing stabilizers, 10mL
13. HRP Stop Solution, 0.344M Sulfuric Acid, 1 vial – colorless, 10mL

Warnings
1. WARNING: This product contains a chemical (0.02% chloramphenicol) in the sample diluent, controls, and conjugate known to the State of California to cause cancer.
2. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the RF IgM ELISA Control, RF IgM ELISA Calibrator and ELISA Negative Control should be handled in the same manner as potentially infectious material.18
3. Sodium Azide is used as a preservative. Sodium Azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
4. The HRP conjugate contains a dilute poisonous/corrosive chemical, which may be toxic if ingested in large amounts. To prevent possible chemical burns, avoid contact with skin and eyes.
5. TMB Chromogen contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
6. The HRP Stop Solution consists of a dilute sulfuric acid solution. Avoid exposure to bases, metals, or other compounds, which may react with acids. Sulfuric acid is a poison and corrosive, which may be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
7. Use appropriate personal protective equipment while working with the reagents provided.
8. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions
1. This product is for In Vitro Diagnostic Use.
2. Substitution of components other than those provided in this system may lead to inconsistent results.
3. Incomplete or inefficient washing and insufficient liquid removal from the ELISA well strips will cause poor precision and/or high background.
4. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
5. A variety of factors influence the assay performance. These include the starting temperature of the reagents, the ambient temperature, the accuracy and reproducibility of the pipetting technique, the thoroughness of washing and liquid removal from the wells of the ELISA strips, the photometer used to measure the results, and the length of the incubation times during the assay. Careful attention to consistency is required to obtain accurate and reproducible results.
6. Strict adherence to the protocol is recommended.
7. Incomplete resealing of the zip-lock pouch containing microwell strips and desiccants will result in antigen degradation and poor precision.
8. Unacceptably low absorbencies may be observed following two or more uses from a single bottle of HRP conjugate over a period of time. It is important to follow all recommended HRP conjugate handling procedures to prevent this occurrence.
9. Chemical contamination of the HRP conjugate can result from improper cleaning or rinsing of equipment or instruments. Residues from common laboratory chemicals such as formalin, bleach, ethanol or detergent will cause degradation of the HRP conjugate over time. Thoroughly rinse all equipment or instruments after the use of chemical cleaners/disinfectants.

Storage Conditions
1. Store all the kit reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
2. Unused antigen coated microwell strips should be resealed securely in the foil pouch containing desiccants and stored at 2-8°C.
3. Diluted wash buffer is stable for 1 week at 2-8°C.
Specimen Collection
This procedure should be performed with a serum specimen. Addition of azide or other preservatives to the test samples may adversely affect the results. Microbiologically contaminated, heat-treated, or specimens containing visible particulate should not be used. Grossly hemolyzed or lipemic serum or specimens should be avoided.

Following collection, the serum should be separated from the clot. CLSI Document H18-A2 recommends the following storage conditions for samples: 1) Store samples at room temperature no longer than 8 hours. 2) If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C. 3) If the assay will not be completed within 48 hrs, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.

Procedure
Materials provided
1. RF IgM ELISA microwell plate (12-1 x 8 wells), with holder
2. 1.2mL prediluted ELISA Negative Control
3. 1.2mL prediluted RF IgM ELISA Calibrator A
4. 1.2mL prediluted RF IgM ELISA Calibrator B
5. 1.2mL prediluted RF IgM ELISA Calibrator C
6. 1.2mL prediluted RF IgM ELISA Calibrator D
7. 1.2mL prediluted RF IgM ELISA Calibrator E
8. 1.2mL prediluted RF IgM ELISA Control
9. 50mL HRP Sample Diluent
10. 25mL HRP Wash Concentrate, 40x concentrate
11. 10mL HRP IgM Conjugate, (goat), anti-human IgM
12. 10mL TMB Chromogen
13. 10mL HRP Stop Solution, 0.344M Sulfuric Acid

Additional Materials Required But Not Provided
Micropipets to deliver 5, 100, 200-300 and 500µL
Disposable micropipet tips
Test tubes for patient sample dilutions, 4mL volume
Distilled or deionized water
1L container for diluted HRP Wash Concentrate
Microwell plate reader capable of measuring OD at 450nm (and 620nm for dual wavelength readings)

Method
Before you start
1. Bring all reagents and samples to room temperature (20-26°C) and mix well.
2. Dilute the HRP Wash Concentrate 1:40 by adding the contents of the HRP Wash Concentrate bottle to 975mL of distilled or deionized water. If the entire plate will not be run within this period, a smaller quantity can be prepared by adding 2.0mL of the concentrate to 78mL of distilled or deionized water for every 16 wells that will be used. The diluted buffer is stable for 1 week at 2-8°C.
3. Preparation of standard curve: For a 5 point standard curve, use PREDILUTED RF IgM ELISA Calibrators A through E directly from the vial. The five point standard curve has the following values:

<table>
<thead>
<tr>
<th>RF IgM Units</th>
<th>RF IgM ELISA Calibrator</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>Prediluted RF IgM ELISA Calibrator A</td>
</tr>
<tr>
<td>B</td>
<td>Prediluted RF IgM ELISA Calibrator B</td>
</tr>
<tr>
<td>C</td>
<td>Prediluted RF IgM ELISA Calibrator C</td>
</tr>
<tr>
<td>D</td>
<td>Prediluted RF IgM ELISA Calibrator D</td>
</tr>
<tr>
<td>E</td>
<td>Prediluted RF IgM ELISA Calibrator E</td>
</tr>
</tbody>
</table>
4. Prepare a 1:101 dilution of each patient sample by adding 5µL of sample to 500µL of HRP Sample Diluent. Diluted samples must be used within 8 hours of preparation. DO NOT DILUTE the RF IgM ELISA Control, RF IgM ELISA Calibrators and ELISA Negative Control 1:101.
5. Determination of the presence or absence of RF IgM using arbitrary units requires two wells for each of the three controls and one or two wells for each patient sample. It is recommended that samples be run in duplicate.

Assay procedure
1. ALL REAGENTS MUST BE BROUGHT TO ROOM TEMPERATURE (20-26°C) PRIOR TO BEGINNING THE ASSAY. Place the required number of microwells/strips in the holder. Immediately return unused strips to the pouch containing desiccants and seal securely to minimize exposure to water vapor.
2. Add 100µL of each of the diluted RF IgM ELISA Calibrators, the prediluted RF IgM ELISA Control, the ELISA Negative Control and the diluted patient samples to the wells. Cover the wells and incubate for 30 minutes at room temperature on a level surface. The incubation time begins after the last sample addition.
3. Wash step: Thoroughly aspirate the contents of each well. Add 200-300µL of the diluted HRP Wash buffer to all wells then aspirate. Repeat this sequence twice more for a total of three washes. Invert the plate and tap it on absorbent material to remove any residual fluid after the last wash. It is important to completely empty each well after each washing step. Maintain the same sequence for the aspiration as was used for the sample addition.
4. Add 100µL of the HRP IgM Conjugate to each well. Conjugate should be removed from the bottles using standard aseptic conditions and good laboratory techniques. Remove only the amount of conjugate from the bottle necessary for the assay. TO AVOID POTENTIAL MICROBIAL AND/OR CHEMICAL CONTAMINATION, NEVER RETURN UNUSED CONJUGATE TO THE BOTTLE. Incubate the wells for 30 minutes as in step 2.
5. Wash step: Repeat step 3.
6. Add 100µL of TMB Chromogen to each well and incubate in the dark for 30 minutes at room temperature.
7. Add 100µL of HRP Stop Solution to each well. Maintain the same sequence and timing of HRP Stop Solution addition as was used for the TMB Chromogen. Gently tap the plate with a finger to thoroughly mix the wells.
8. Read the absorbance (OD) of each well at 450nm within one hour of stopping the reaction. If bichromatic measurements are desired, 620nm can be used as a reference wavelength.

Quality Control
1. The RF IgM ELISA Control, the RF IgM ELISA Calibrator and the ELISA Negative Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
2. Note that since the RF IgM ELISA Control, the RF IgM ELISA Calibrator and the ELISA Negative Control are prediluted, they do not control for procedural methods associated with dilution of specimens.
3. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at < -20°C.
4. In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated.
   a. The absorbance of the prediluted RF IgM ELISA Calibrator A must be greater than the absorbance of the prediluted RF IgM ELISA Control, which must be greater than the absorbance of the prediluted ELISA Negative Control.
   b. The prediluted RF IgM ELISA Calibrator A must have an absorbance greater than 1.0 while the prediluted ELISA Negative Control absorbance cannot be over 0.2.
   c. The RF IgM ELISA Control absorbance must be more than twice the ELISA Negative Control or over 0.25.
   d. The RF IgM Control concentration must be within the range stated on its label.
   e. The user should refer to CLSI Document C24-A for additional guidance on appropriate QC practices.

Calculation of Results
1. Determine a mean value for all duplicate readings.
2. Plot the mean absorbance (OD) of the samples in the standard curve against their values in Units. Use a linear regression curve fit, log/log scale. The units assigned to the calibrators are found on the calibrator vial.
3. Determine the unknown RF IgM concentration from the "X" axis by reading corresponding absorbance on the "Y" axis.

Interpretation of Results
The ELISA assay is very sensitive to technique and is capable of detecting even small differences in patient populations. The values shown below are suggested values only. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

<table>
<thead>
<tr>
<th>Units</th>
<th>Negative</th>
<th>Positive</th>
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<tr>
<td></td>
<td>≤6</td>
<td>&gt;6</td>
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1. A positive result indicates the presence of RF IgM antibodies and suggests the possibility of rheumatoid arthritis.
2. A negative result indicates no RF IgM antibody or levels below the negative cut-off of the assay.
3. It is suggested that the results reported by the laboratory should include the statement: “The following results were obtained with the INOVA QUANTA Lite® RF IgM ELISA. RF IgM values obtained with different manufacturers’ assay methods may not be used interchangeably. The magnitude of the reported IgM levels cannot be correlated to an endpoint titer.”

Limitations of the Procedure
1. The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.
2. Diagnosis cannot be made on the basis of RF results alone. These results must be interpreted in conjunction with physical findings.
3. Treatment must not be initiated on the basis of a positive RF titer alone. Supportive clinical indications must also be present.
4. RF can appear transiently during many infections. Patients positive for RF should be retested following an appropriate wait.
5. Results of this assay should be used in conjunction with clinical findings and other serological tests.
6. The assay performance characteristics have not been established for matrices other than serum.

**Expected Values**

**Normal Range**
In a study conducted at the research laboratories of INOVA Diagnostics, 193 random normal samples were tested for IgM RF. There were 58 males and 135 females. The population ranged in age from 20 – 69 years with a mean of 36.3 years. Seven (3.6%) of these samples were found positive. Only 1 of these seven positive samples was above 10 units.

**Relative Sensitivity and Specificity**
The QUANTA Lite™ RF IgM ELISA kit was compared to another commercially available RF IgM ELISA kit. Performance was evaluated using 163 samples submitted for routine RF testing. The results are summarized below.

<table>
<thead>
<tr>
<th>Reference</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>INOVA</td>
<td>110</td>
<td>4</td>
</tr>
<tr>
<td>Relative Sensitivity</td>
<td>91.6%</td>
<td></td>
</tr>
<tr>
<td>Relative Specificity</td>
<td>91.3%</td>
<td></td>
</tr>
<tr>
<td>Relative Efficiency</td>
<td>91.6%</td>
<td></td>
</tr>
</tbody>
</table>

Of the 163 samples tested, 110 were positive and 38 samples negative by both tests. There were 4 samples positive on the INOVA test yet negative by the reference method. These 4 samples were weakly reactive at 6, 7, 7 and 9 units each, just over the 6 unit cutoff. There were 11 samples positive with the reference method yet negative with the INOVA kit. Six of these 11 positive samples were under 10 units. The strongest sample had a value of 31 units. All 11 samples were, however, found to be negative for IgM RF by nephelometry.

One hundred and eighteen samples positive for antinuclear antibodies (ANA) were tested for IgM RF using the QUANTA Lite™ kit. Eleven of these samples (9.3%) were found positive. The positives ranged from 6 to 17 units with only 5 of the eleven being above 10 units.

**Precision and Reproducibility**
The precision and reproducibility of the assay was measured by running six replicates each of negative, weak positive and strong positive samples in six separate assays. The mean of the strong positive was 52.8, the weak positive was 11.9 and the negative was 2.1. The standard deviation and coefficient of variation for each sample are summarized below.

<table>
<thead>
<tr>
<th></th>
<th>Negative</th>
<th>Weak Positive</th>
<th>Strong Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>0.19</td>
<td>0.23</td>
<td>1.67</td>
</tr>
<tr>
<td>CV</td>
<td>9.2%</td>
<td>1.9%</td>
<td>3.2%</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Run</td>
<td>0.15</td>
<td>0.24</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>7.1%</td>
<td>2.0%</td>
<td>2.1%</td>
</tr>
<tr>
<td>Between Run</td>
<td>0.15</td>
<td>0.24</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>7.0%</td>
<td>2.0%</td>
<td>3.3%</td>
</tr>
</tbody>
</table>
References


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