RF IgM Test System

**INTENDED USE**
The ZEUS ELISA Rheumatoid Factor (RF) IgM Test System is intended for the qualitative and semi-quantitative detection of RF IgM class antibody in human sera. The test is intended to be used as an aid in the diagnosis of rheumatoid arthritis and is for In Vitro diagnostic use.

**SIGNIFICANCE AND BACKGROUND**
Rheumatoid arthritis (RA) is a chronic, usually progressive inflammatory disorder of the joints. RA is a highly variable disease that ranges from a mild illness of brief duration to a progressive, destructive polyarthritis associated with a systemic vasculitis (1). The disease has been recently estimated to occur in one to two percent of the general population (2), and two times more likely to occur in women than in men (1).

Clinical features of the early disease include lymphadenopathy, anorexia, weakness, fatigue, and morning stiffness or generalized aching (1, 3). RA is associated with a number of attributes which are measurable within the laboratory setting (4). The most common laboratory findings associated with RA include rheumatoid factor (RF), antinuclear antibodies (ANA), immune complexes, and characteristic complement levels (3). Measurement of serum RF IgM plays an important role in the diagnosis of RA, and has more recently been implicated with disease prognosis (6).

RF belongs to a group of immunoglobulins typically defined as polyclonal antibodies which react to the Fc portion of human (and some other species of) IgG molecules (1, 4). RF antibodies are of three major immunoglobulin classes; IgM, IgG, and IgA; however, IgE RF has also been described (5). IgM and IgG RF is the most common (1), with IgM RF being present in 75% of patients diagnosed with RA (4). RF has also been associated with some bacterial and viral infections such as hepatitis and infectious mononucleosis and some chronic infections such as tuberculosis, parasitic disease, subacute bacterial endocarditis, and cancer (1). In addition, elevated levels of RF may be seen in 15% of the population greater than 65 years of age (4).

Historically, RF was measured using various agglutination tests such as sensitized sheep cell test, latex agglutination, and bentonite flocculation (1), but more recently, newer, more sensitive methods, such as nephelometry, RIA, and ELISA, have been developed. ELISA provides a simple and accurate means to measure RF. Additionally, ELISA provides the distinct advantage of being class specific and non-susceptible to prozone, two common draw-backs of agglutination based test systems.

**PRINCIPLE OF THE ASSAY**
The ZEUS ELISA Rheumatoid Factor IgM Test System is designed to detect IgM class antibodies to RF in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with RF antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgM is added to the wells and the plate is incubated. The Conjugate will react with the antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

**TEST SYSTEM COMPONENTS**
Materials Provided:
Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. NOTE: The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator, and SAVe Diluent®.

<table>
<thead>
<tr>
<th>PLATE</th>
<th>CONJ</th>
<th>CONTROL +</th>
<th>CAL A</th>
<th>CAL B</th>
<th>CAL C</th>
<th>CAL D</th>
<th>CONTROL -</th>
<th>DIL SPE</th>
<th>SOLN TMB</th>
<th>SOLN STOP</th>
<th>WASHBUF 10X</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.</td>
<td>2.</td>
<td>3.</td>
<td>4.</td>
<td>5.</td>
<td>6.</td>
<td>7.</td>
<td>8.</td>
<td>9.</td>
</tr>
<tr>
<td>Plate</td>
<td></td>
<td>Positive Control (Human Serum): One, 0.35mL, red-capped vial.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calibrator A (Human Serum): One, 0.5mL, white-capped vial.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calibrator B (Human Serum): One, 0.5mL, yellow-capped vial.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calibrator C (Human Serum): One, 0.5mL, orange-capped vial.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calibrator D (Human Serum): One, 0.5mL, blue-capped vial.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative Control (Human Serum): One, 0.35mL, green-capped vial.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SAVe Diluent®: One, 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Ready to use.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TMB: One, 15mL, amber-capped, amber bottle containing 3, 3', 5', 5'-tetramethylbenzidine (TMB). Ready to use.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stop Solution: One, 15mL, red-capped, bottle containing 1M H2SO4, 0.7M HCl. Ready to use.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. One, 100mL, clear-capped, bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). NOTE: 1X solution will have a pH of 7.2 ± 0.2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTES:**
1. The following components are not Test System Lot Number dependent and may be used interchangeably within the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer. SAVe Diluent® may be used interchangeably with any ZEUS ELISA Test System utilizing Product No. 005CC.
2. Test System also contains a Component Label containing lot specific information inside the Test System box.

**PRECAUTIONS**
1. For In Vitro diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips potentially biohazardous materials and handle accordingly.
4. The Controls are potentially biohazardous materials. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition; and OSHA’s Standard for Bloodborne Pathogens (9).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The SAVe Diluent®, Controls, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate’s enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong oxidizers from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm. NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.
2. Pipettes capable of accurately delivering 10 - 200µL.
3. Multichannel pipette capable of accurately delivering 50 - 200µL.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite).

STORAGE CONDITIONS

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>2°C - 8°C</td>
<td>Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening - strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue.</td>
</tr>
<tr>
<td></td>
<td>Conjugate – DO NOT FREEZE.</td>
</tr>
<tr>
<td></td>
<td>Unopened Test System, Calibrator, Positive Control, Negative Control, TMB, SAVe Diluent®</td>
</tr>
<tr>
<td>2°C - 25°C</td>
<td>Stop Solution: 2 - 25°C</td>
</tr>
<tr>
<td></td>
<td>Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days.</td>
</tr>
<tr>
<td></td>
<td>Wash Buffer (10X): 2 - 25°C</td>
</tr>
</tbody>
</table>

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (7, 8). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store test sera at −20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (10).

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
2. Determine the number of microwells needed. Allow seven Control/Calibrator determinations (one Blank, one Negative Control, four Calibrators and one Positive Control) per run. Run a Reagent Blank on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 - 8°C.

<table>
<thead>
<tr>
<th>EXAMPLE PLATE SET-UP</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Blank</td>
<td>Patient 2</td>
</tr>
<tr>
<td>B</td>
<td>Negative Control</td>
<td>Patient 3</td>
</tr>
<tr>
<td>C</td>
<td>Calibrator A</td>
<td>Patient 4</td>
</tr>
<tr>
<td>D</td>
<td>Calibrator B</td>
<td>Etc.</td>
</tr>
<tr>
<td>E</td>
<td>Calibrator C</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Calibrator D</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Positive Control</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Patient 1</td>
<td></td>
</tr>
</tbody>
</table>

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVe Diluent®) of the Negative Control, Calibrator, Positive Control, and each patient serum. NOTE: The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the diluent.

4. To individual wells, add 100µL of each diluted Control, Calibrator and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.

5. Add 100µL of SAVe Diluent® to well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.

6. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.

7. Wash the microwell strips 5 times.
   a. Manual Wash Procedure:
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps 1 and 2 for a total of 5 washes.
      4. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day’s run.
   b. Automated Wash Procedure:
      If using an automated microwell wash system, set the dispensing volume to 300 - 350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

8. Add 100µL of the Conjugate to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.

9. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.

10. Wash the microwells by following the procedure as described in step 7.

11. Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.

12. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.

13. Stop the reaction by adding 50µL of Stop Solution to each well, including the Reagent Blank well, at the same rate and in the same order as the TMB. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.

14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the Reagent Blank. Read the plate within 30 minutes of the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell - 100µ/L/well.
3. Incubate 25 ± 5 minutes.
4. Wash.
5. Add Conjugate - 100µ/L/well.
6. Incubate 25 ± 5 minutes.
7. Wash.
8. Add TMB - 100µ/L/well.
10. Add Stop Solution -50µ/L/well - Mix.
11. READ within 30 minutes.

QUALITY CONTROL

1. Each time the assay is performed, a Reagent Blank, Negative Control, Positive Control and Calibrators A – D must also be included.

2. The mean OD value for the Calibrator, Positive Control, and Negative Control should fall within the following ranges:

<table>
<thead>
<tr>
<th>Positive Control</th>
<th>OD Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>Must be &gt;15 IU/mL</td>
</tr>
<tr>
<td></td>
<td>Must be &lt;5 IU/mL</td>
</tr>
</tbody>
</table>

a. The OD of the Negative Control divided by the OD of the Positive Control should be ≤0.9.

b. If the above conditions are not met the test should be considered invalid and should be repeated.

4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure, but will not ensure precision at the assay Cutoff.

5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

INTERPRETATION OF RESULTS

1. Calibrator:
   Based upon testing of normal sera, disease state sera, and the World Health Organization (WHO) international standard, a maximum normal IU/mL value has been determined by the manufacturer and correlated to the calibrators. The calibrators will allow the user to determine the unit value for each of the test samples evaluated. The unit values are determined for each lot of kit produced, and are printed on the Componen List included with each kit.

2. Quality Control
   Refer to the specification sheet included with each kit. This sheet describes the lot specific specifications for each of the calibrators. If any of the calibrators are out of range, the results are considered invalid, and the patient results may not be reported.

3. Conversion of Optical Density to IU/mL
   Optical densities of the samples are determined from the standard curve generated from the calibrators. A standard curve should be generated using the paired data points for each of the four calibrators (OD on the Y axis and corresponding IU/mL value on the X axis). Using the best-fit point to point curve, determine the
IU/mL value for each of the specimens tested by extrapolation. **NOTE:** It is permissible to use the reagent blank as a fifth calibrator. In such cases, the reagent blank OD after subtraction of the reagent blank OD (therefore zero OD) may be used as a fifth calibrator and should have a value of 0 IU/mL assigned to it. If this optional fifth calibrator is employed, it will allow for interpretation of any specimen or control that happens to have an OD less than that of Calibrator D.

4. **Interpretations:**

Using normal healthy individuals, disease-state specimens, and the WHO standard, the manufacturer has established the following guidelines for interpretation of patient results:

- <6.0 IU/mL Negative
- 26.0 IU/mL Positive
- ≥25 IU/mL Strongly Reactive, Indicative of Rheumatoid Arthritis

**LIMITATIONS OF THE ASSAY**

1. Do not make a diagnosis solely on the basis of the ELISA result. Interpret the test results for RF IgM in conjunction with clinical evaluation and results of other diagnostic procedures.

2. A negative result does not exclude rheumatoid arthritis. Approximately 25% of patients with a diagnosed case of rheumatoid arthritis may present with a negative result for RF.

3. Certain non-rheumatoid conditions, connective tissue disorders and a variety of other disease states such as hepatitis may elicit a positive RF test result.

4. RF exists in three major immunoglobulin classes: IgA, IgG, and IgM. This test will only detect IgM class RF antibodies.

5. Reproducible results with an ELISA system require careful pipetting, strict adherence to incubation periods and temperatures requirements, as well as thorough washing of the test wells and thorough mixing of all solutions.

6. Hemolytic, icteric, or lipemic samples may interfere with this ELISA. Avoid the use of these types of specimens.

**EXPECTED RESULTS**

A study was conducted evaluating 162 normal donor sera, from Southeastern United States, for RF IgM antibodies. Of the 162 tested, 150 (92.6%) were found to be negative, six (3.7%) were positive, and six (3.7%) were strongly reactive.

**PERFORMANCE CHARACTERISTICS**

1. **Comparative Study:**

A comparative study was conducted to demonstrate the equivalence of the ZEUS ELISA RF IgM Test System to another commercially available RF IgM ELISA test system. Technicians evaluated performance using 232 specimens; 182 assorted normal donor sera, 25 specimens previously tested and found to be RF IgM positive, and 25 disease-state specimens from clinically diagnosed rheumatoid arthritis patients. Table 1 summarizes the results of the investigation.

<table>
<thead>
<tr>
<th>Commercial ELISA Test System</th>
<th>ZEUS ELISA RF IgM Test System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>160</td>
</tr>
<tr>
<td>Equivocal*</td>
<td>3</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>164</td>
</tr>
</tbody>
</table>

*Five samples were equivocal on the Commercial assay. These equivocal specimens were excluded from all calculations.

Relative Sensitivity = 62/63 = 98.5%  
95% Confidence Interval** = 95.3 to 100%

Relative Specificity = 160/164 = 97.6%  
95% Confidence Interval** = 95.2 to 99.9%

Relative Agreement = 222/227 = 97.8%  
95% Confidence Interval** = 95.9 to 97.7%

** 95% confidence intervals calculated using the exact method.

2. **Precision and Reproducibility:**

To evaluate both intra-assay and inter-assay reproducibility, ZEUS Scientific tested eight specimens, eight replicates each, on each of three days. These results were then used to calculate mean IU/mL values, standard deviations, and percent CV. One of the eight specimens was negative, three of the specimens were near the cut off zone, and four of the specimens were strongly reactive. Table 2 summarizes the results of this study.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Intra-Assay Reproducibility</th>
<th>Inter-Assay Reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td></td>
<td>Mean (IU/mL)</td>
<td>StD</td>
</tr>
<tr>
<td>1</td>
<td>37.4</td>
<td>3.00</td>
</tr>
<tr>
<td>2</td>
<td>0.3</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>5.6</td>
<td>0.15</td>
</tr>
<tr>
<td>4</td>
<td>9.4</td>
<td>0.40</td>
</tr>
<tr>
<td>5</td>
<td>34.3</td>
<td>1.40</td>
</tr>
<tr>
<td>6</td>
<td>29.2</td>
<td>0.54</td>
</tr>
<tr>
<td>7</td>
<td>28.8</td>
<td>1.50</td>
</tr>
<tr>
<td>8</td>
<td>8.2</td>
<td>0.50</td>
</tr>
</tbody>
</table>
REFERENCES