ANTIGEN phosphatase

to the immobilized antigen. The test procedure involves:

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 Conjugate goat anti-human IgG is added to the wells and the plate is incubated. The Conjugate will react with antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized horseradish 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.

SIGNIFICANCE AND BACKGROUND

Anti-neutrophil cytoplasmic antibody (ANCA) was initially described by Davies, et al in 1982 (1). Since this initial discovery, ANCA has been found to be associated with a number of Systemic Vasculitides (SV). ANCA is now recognized to include two primary specificities: c-ANCA directed against Proteinase-3 (PR-3), and p-ANCA directed against Myeloperoxidase (MPO). Testing for both p-ANCA and c-ANCA is highly recommended in the laboratory workup of patients who present with clinical features suggestive of SV. The clinical syndromes most frequently associated with ANCA are as follows:

- Wegener’s Granulomatosis (2)
- Polyrteritis (3)
- "Overlap" Vasculitis (4)
- Idiopathic Crescentic Glomerulonephritis (ICGN) (5)
- Kawasaki Disease (6)

Although the initial identification of c-ANCA and p-ANCA was based on the indirect immunofluorescence procedures, further identification and purification of PR-3 and MPO has resulted in the development of enzyme immunoassays (ELISA) for both PR-3 and MPO.

PRINCIPLE OF THE ASSAY

The ZEUS ELISA ANCA Screen Test System is designed to detect IgG class antibodies to MPO and/or PR3 in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with a mixture of MPO and PR3 antigen. The test procedure involves three incubation steps:

1. Plate: 96 wells configured in twelve, 1x8-well, strips coated with a mixture of MPO and PR3 antigens. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific). One, 15mL, white-capped bottle. Ready to use.
3. Positive Control (Human Serum): One, 0.35mL, red-capped vial.
4. Calibrator (Human Serum): One, 0.5mL, blue-capped vial.
5. Negative Control (Human Serum): One, 0.35mL, green-capped vial.
6. SAVe Diluent®: One, 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered saline. Ready to use. Note: The SAVe Diluent® will change color when combined with serum.
7. TMB: One, 15mL, amber-capped, amber bottle containing 3, 3', 5', 5' tetramethylbenzidine (TMB). Ready to use.
8. Stop Solution: One, 15mL, red-capped, bottle containing 1M H$_3$SO$_4$, 0.7M HCl. Ready to use.
9. 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.

NOTES:

1. The following components are not Test System Lot Number dependent and may be used interchangeably with 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 HIV and HCV by approved test methods. However, since no test method can offer complete assurance that infectious agents are present, 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 odors 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>Condition</th>
<th>Storage Temperature</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coated Microwell Strips</td>
<td>2°C - 8°C</td>
<td>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>Conjugate – DO NOT FREEZE.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unopened Test System, Calibrator, Positive Control, Negative Control, TMB, SAVe Diluent®</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop Solution</td>
<td>2°C - 25°C</td>
<td>Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days. 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 for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three 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.
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 - 100µL/well.

3. Incubate 25 ± 5 minutes.

4. Wash.

5. Add Conjugate - 100µL/well.

6. Incubate 25 ± 5 minutes.

7. Wash.

8. Add TMB - 100µL/well.


10. Add Stop Solution - 50µL/well - Mix.

11. READ within 30 minutes.

**QUALITY CONTROL**

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included.

2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.

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

<table>
<thead>
<tr>
<th>OD Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control ≤0.250</td>
</tr>
<tr>
<td>Calibrator ≥0.300</td>
</tr>
<tr>
<td>Positive Control ≥0.500</td>
</tr>
</tbody>
</table>

   a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤0.9.
   b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.
   c. 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. **Calculations:**
   a. **Correction Factor:** The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System box.

   \[ \text{Cutoff OD Value} = \text{CF} \times \text{Mean OD of Calibrator} \]

   b. **Cutoff OD Value:** To obtain the Cutoff OD Value, multiply the CF by the mean OD of the Calibrator determined above.

      \[ \text{Cutoff OD Value} = \text{CF} \times \text{Mean OD of Calibrator} \]

   c. **Index Values/OD Ratios:** Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step b.
2. **Interpretations:** Index Values/OD Ratios are interpreted as follows.

<table>
<thead>
<tr>
<th>Index Value/OD Ratio</th>
<th>Negative Specimens</th>
<th>Equivocal Specimens</th>
<th>Positive Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.91 to 1.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1.10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. An OD ratio ≤0.90 indicates no significant amount of antibodies to MPO or PR-3 were detected.
b. An OD ratio ≥1.10 indicates that antibodies specific to MPO and/or PR-3 were detected. The results of this test system are qualitative; ratio values in the reactive range are not indicative of the amount of antibody present.
c. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested in duplicate. Report any two of the three results which agree. Test repeatedly equivocal specimens by an alternate serologic procedure and/or re-evaluated by drawing another sample one to three weeks later.

**LIMITATIONS OF THE ASSAY**

1. Do not make a diagnosis on the basis of the ZEUS ELISA ANCA Screen Test System results alone. Interpret test results in conjunction with clinical evaluation and results of other diagnostic procedures.

2. The performance characteristics of this device have not been established for lipemic, hemolyzed and icteric specimens; therefore, do not use these specimens types with this assay.

3. Although the ZEUS ELISA ANCA Screen Test System will detect antibodies to both MPO and PR-3, the assay will not differentiate between the two. Test positive ANCA Screen specimens using the individual ZEUS ELISA MPO and PR-3 Test Systems to determine which antibody is present.

4. The results of this assay are not diagnostic proof of the presence or absence of disease. Do not start immunosuppressive therapy based only on a positive result.

**EXPECTED RESULTS**

A study was conducted that evaluated 90 normal donor sera from Northeastern United States for ANCA autoantibodies. Of the 90 tested, one (1.1%) was positive and one (1.1%) was equivocal. In another study using 105 specimens sent to a reference laboratory in the Northeastern United States, fourteen (14/105 = 13.3%) were positive for ANCA antibodies. Taken together, these studies demonstrate that the incidence of ANCA is relatively rare.

**PERFORMANCE CHARACTERISTICS**

1. **Comparative Study:**
   An in-house comparative study was conducted to demonstrate the equivalence of the ZEUS ELISA ANCA Screen Test System to another commercially available ANCA IgG ELISA Test System using 316 specimens; 196 disease-state specimens, 113 specimens sent to a reference laboratory in the Northeastern United States for routine ANCA serology, and 7 specimens previously tested and found to be reactive for ANCA. Tables 1 and 2 summarize the results of the investigation.

**Table 1: Clinical Specimens Summary**

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>Male</th>
<th>Female</th>
<th>High</th>
<th>Low</th>
<th>Mean</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease Category: Wegener’s Granulomatosis</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Disease Category: Idiopathic Necrotizing and Crescentic Glomerulonephritis</td>
<td>22</td>
<td>13</td>
<td>9</td>
<td>3</td>
<td>9</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Disease Category: Microscopic Polyarteritis</td>
<td>20</td>
<td>12</td>
<td>8</td>
<td>2</td>
<td>6</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Disease Category: Pulmonary Renal Syndrome</td>
<td>11</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Vasculitis/Glomerulonephritis Disease Controls, Non-ANCA related vasculitis.</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
<td>1</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Information Not Available</td>
<td>113</td>
<td>68</td>
<td>45</td>
<td>27</td>
<td>14</td>
<td>45.7</td>
<td></td>
</tr>
</tbody>
</table>

2. **Table 2: Calculation of Relative Sensitivity, Specificity, and Agreement**

<table>
<thead>
<tr>
<th>Commercial ANCA ELISA Test System</th>
<th>Positive</th>
<th>Negative</th>
<th>Equivocal*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZEUS ELISA ANCA Screen Test System</td>
<td>148</td>
<td>8</td>
<td>0</td>
<td>156</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>113</td>
<td>4</td>
<td>120</td>
</tr>
<tr>
<td>Equivocal*</td>
<td>15</td>
<td>22</td>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>166</td>
<td>143</td>
<td>7</td>
<td>316</td>
</tr>
</tbody>
</table>

*Equivocal specimens were excluded from all calculations.
Relative Sensitivity = 148/156 = 94.9% 95% Confidence Interval** = 91.4 to 98.3%
Relative Specificity = 113/116 = 97.4% 95% Confidence Interval** = 94.5 to 100%
Relative Agreement = 261/272 = 96.0% 95% Confidence Interval** = 93.6 to 98.3%
**95% confidence intervals calculated using the exact method.

2. **Precision and Reproducibility:**
To evaluate both intra-assay and inter-assay reproducibility, six specimens were tested, eight replicates each, on each of three days. These results were then used to calculate mean unit values, standard deviations, and percent CV. Two of the specimens were strong positives, two were clearly negative, and two were near the assay cutoff. The results of the study have been summarized below.

**Table 3: ZEUS ELISA ANCA Screen Test System Precision Testing Results**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Intra-Assay Reproducibility</th>
<th>Inter-Assay Reproducibility</th>
<th>All Days Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
</tr>
<tr>
<td>1</td>
<td>7.40</td>
<td>0.25</td>
<td>3.4</td>
</tr>
<tr>
<td>2</td>
<td>5.89</td>
<td>0.20</td>
<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>0.07</td>
<td>6.7</td>
</tr>
<tr>
<td>4</td>
<td>0.97</td>
<td>0.07</td>
<td>7.6</td>
</tr>
<tr>
<td>5</td>
<td>0.17</td>
<td>0.01</td>
<td>5.3</td>
</tr>
<tr>
<td>6</td>
<td>0.08</td>
<td>0.01</td>
<td>6.0</td>
</tr>
</tbody>
</table>

ZEUS ELISA ANCA Screen Test System
3. **Cross Reactivity:**

To evaluate the potential cross-reactivity to other autoantibodies, eight specimens which were positive for antibodies to nuclear antigens (ANA) on HEp-2 cells were tested. Two of the specimens demonstrated a homogenous pattern, two demonstrated a nucleolar pattern, two the centromere pattern, and two a speckled pattern. The results of this study have been summarized in Table 4 below. The results of this investigation indicate that cross-reactivity with other antinuclear antibodies is not likely.

### Table 4: Results of the Cross Reactivity Investigation

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Pattern</th>
<th>Endpoint Titer</th>
<th>ANA HEp-2 IFA Results</th>
<th>ZEUS ELISA ANCA Screen Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Homogenous</td>
<td>1:1280</td>
<td>0.066</td>
<td>0.36</td>
</tr>
<tr>
<td>2</td>
<td>Homogenous</td>
<td>1:640</td>
<td>0.019</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>Speckled</td>
<td>1:2560</td>
<td>0.044</td>
<td>0.24</td>
</tr>
<tr>
<td>4</td>
<td>Nucleolar</td>
<td>1:1280</td>
<td>0.101</td>
<td>0.56</td>
</tr>
<tr>
<td>5</td>
<td>Centromere</td>
<td>1:1280</td>
<td>0.050</td>
<td>0.28</td>
</tr>
<tr>
<td>6</td>
<td>Centromere</td>
<td>1:1280</td>
<td>0.035</td>
<td>0.19</td>
</tr>
<tr>
<td>7</td>
<td>Speckled</td>
<td>1:5120</td>
<td>0.051</td>
<td>0.28</td>
</tr>
<tr>
<td>8</td>
<td>Nucleolar</td>
<td>1:10240</td>
<td>0.028</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**REFERENCES**