1. INTENDED USE

BD Retic-Count™ reagent is intended for determining reticulocytes as a percentage of total erythrocytes in human peripheral blood by a BD FACSCalibur™, BD FACSort™, BD FACScan™, or BD FACStrak™ flow cytometer. The BD Retic-Count reagent is for in vitro diagnostic use.

2. SUMMARY AND EXPLANATION

Reticulocytes are young red blood cells from which the nucleus has been lost yet contain residual organelles (ribosomes and mitochondria). The presence of these organelles differentiates reticulocytes from mature erythrocytes. The organelles contain residual ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), which are absent from mature erythrocytes.

BD Retic-Count is a trade name for 1-methyl-4[(3-methyl-2(3H)-benzothiazolylidine) methyl]-quinolinium 4-methyl benzene sulfonate (Thiazole Orange), a reagent synthesized by BD. BD Retic-Count reagent is functionally similar to acridine orange. Both compounds bind DNA with a ratio of 1.2 dye molecules per base pair, and have the same dissociation constant, positive charge, and membrane permeability. The animal carcinogenicity of acridine orange is indefinite (distinguished from negative). BD Retic-Count reagent can also be potentially carcinogenic and should be handled with care. The reagent binds to both RNA and DNA, forming a fluorescent nucleotide-reagent complex which exhibits an absorption band at 475 nm (blue light) and a fluorescence emission band at 530 nm (green light). This property makes it suitable for use with flow cytometers equipped with a 488-nm laser such as the
In the normal (non-anemic) individual, the reticulocyte matures within three days in bone marrow and is found in peripheral circulation during the fourth day. The reticulocyte count is a measure of erythropoietic activity. This measure is not perfect, however, since increased erythropoiesis tends to shorten marrow maturation time and lengthen blood maturation time. Reticulocytes that are one to three days old ("shift" reticulocytes) can be found in the peripheral blood and will be counted as day-four reticulocytes. To gauge erythropoiesis accurately, the count should be corrected for "shift" reticulocytes.1

The standard method for counting reticulocytes employs the vital stain New Methylene Blue. Blood samples are stained with this reagent, a wedge smear is prepared, then the stained cells are counted using a light microscope.4 However, this method is time consuming and limited by the sampling error inherent in a thousand-cell count. When deviations from the published standard method are employed, the reproducibility of the microscopic method is further diminished.5 Other methods for reticulocyte assay employ a flow cytometer (for example, the BD FACSTM brand cell analyzer) with the supravital stain thioflavin-T.

Using BD Retic-Count with the BD FACSCalibur, BD FACSort, BD FACScan, or BD FACStrak flow cytometer offers significant advantages over the New Methylene Blue method. Ten times more cells are counted in the flow cytometric method, thus providing greater sampling accuracy.6 A typical count requires less than a minute of operator time, and cells are counted in suspension, thus eliminating the concern for distributional errors in slide preparations.

Additional advantages can be obtained using BD Retic-Count software—operator variability is reduced by automatic marker setting, results are automatically computed and printed, and the unstained control tube is not required.

CAUTION It is highly recommended that a complete blood count (CBC) and Wright-Giemsa–stained blood smear are performed on all patient samples. If a BD Retic-Count value is obtained for a patient sample which is unexpected in light of other clinical information, review the CBC and blood smear and refer to Section 9, Limitations, for more information.

Clinical Applications
The reticulocyte count is clinically indicated for assessment of erythroid cell production within the bone marrow compartment. It can be useful for the differential diagnosis of anemia.7 In an anemic patient, an elevated reticulocyte count is consistent with normal bone marrow function and can suggest the presence of a hemolytic blood disorder such as sickle cell anemia, thalassemia, spherocytosis, glucose-6-phosphate dehydrogenase deficiency, immune hemolytic disease, and hypersplenism. The reticulocyte count also increases in response to blood loss, and can be an indication of covert bleeding which might not be evident from either clinical examination or hematocrit changes.8,9,10,11
In contrast, anemias accompanied by normal or decreased numbers of blood reticulocytes are associated with impaired erythropoiesis. The pathogenesis of these disorders results from disturbances in red cell maturation or proliferation within the bone marrow, for example, immunologic or drug-induced red blood cell aplasia; metastatic carcinoma; decreased erythropoietin production; and iron, folate, or B12 deficiency anemias.8,9

The reticulocyte count is also useful in the management of patient therapy. It can be used to follow idiopathic myelofibrosis,12 evaluate a patient's responsiveness to folate, iron, or B12 supplementation, follow the progress of bone marrow transplant,10 and assess the impact of chemotherapy and radiation on hematopoietic function.13

3. PRINCIPLES OF THE PROCEDURE

When BD Retic-Count reagent is added to human whole blood, the reagent binds to the residual RNA and DNA found within the reticulocyte.

Whole blood samples are stained with BD Retic-Count reagent and acquired on a flow cytometer. During acquisition the cells travel past the laser beam and scatter the laser light. The scatter signals provide information about the cell's size and internal granularity. The stained cells fluoresce when excited by the laser beam.

Analysis is restricted to the red blood cell (RBC) population falling within a forward-scatter (FSC) versus side-scatter (SSC) gate (see Figure 2 in Section 7, Procedure). Cells within this RBC gate are analyzed to determine the amount of bound BD Retic-Count reagent that is measured by the fluorescence-1 (FL1) detector.

The BD FACSCalibur, BD FACSort, or BD FACScan flow cytometer used with a BD FACStation™ computer system can collect up to 50,000 events. A minimum of 10,000 events should be collected. The data files should be saved in list mode and given logical names to aid in retrieval for subsequent analysis by BD Retic-Count software or BD CellQuest™ software.

4. REAGENT

Reagent Provided, Sufficient for 200 Tests

BD Retic-Count reagent, sufficient for 200 tests, is provided in a 200-mL bottle. The solution contains Thiazole Orange in phosphate-buffered saline (PBS) with 0.1% sodium azide.

Concentration values are listed in the following table:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiazole Orange</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Precautions

- For in vitro diagnostic use.
- The BD Retic-Count reagent is light sensitive and must be stored in the dark at 2°C–8°C. Avoid unnecessary exposure of the reagent or reagent/cell mixture to light.
- Incubation time or temperatures other than those specified can be a source of error.
- The reagent bottle must be kept well sealed when not in use.
- When stored at 2°C–8°C, the reagent is stable until the expiration date shown on the label. Do not use after the expiration date.

**WARNING** The reagent solution contains a nucleotide-reactive material
and can be carcinogenic. Handle with care. Avoid contact with skin and mucous membranes.

- The BD Retic-Count reagent contains sodium azide as a preservative; however, care should be taken to avoid microbial contamination, which can cause erroneous results.

**WARNING** All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection\(^{14,15}\) and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

5. INSTRUMENT

The following instrument system is recommended:

- Flow cytometer equipped with a 488-nm argon-ion laser, FSC, SSC, and fluorescence detectors, and appropriate analysis software. The FSC and SSC detectors must have logarithmic amplifiers. Performance has been established for the following flow cytometers equipped with the corresponding computer systems:
  - BD FACSCalibur and a BD FACStation computer system
  - BD FACSort and a BD FACStation computer system
  - BD FACScan and a BD FACStation computer system
- BD FACSComp™ software. For detailed information on use, refer to the appropriate software user’s guide.

One of the following software programs is recommended for acquisition and analysis. Refer to the appropriate software user’s guide for detailed information.

- BD Retic-Count software
- BD CellQuest software

6. SPECIMEN AND COLLECTION PREPARATION

Collect blood aseptically by venipuncture\(^{16,17}\) into a sterile BD Vacutainer® EDTA blood collection tube. A minimum of 5 µL of whole blood is required for this procedure when using BD Retic-Count software and a minimum of 10 µL of whole blood is required when not using BD Retic-Count software. Blood should be stained within 48 hours of venipuncture for optimal results. Anticoagulated blood can be stored at room temperature (20°C–25°C) for up to 48 hours until ready for staining.

**CAUTION** Use standard precautions when obtaining, handling, and disposing of all human blood samples and potentially carcinogenic reagents.

7. PROCEDURE

**Reagent Provided**

See Reagent Provided, Sufficient for 200 Tests and Precautions in Section 4, Reagent.

**Reagents and Materials Required But Not Provided**

- BD Calibrite™ Beads (Catalog No. 349502) can be used for setting the photomultiplier tube (PMT) voltages, setting the fluorescence compensation, checking instrument sensitivity, and monitoring the performance of the flow cytometer.
cytometer. For information on use, refer to the BD Calibrite Beads instructions for use (IFU).

- BD Vacutainer EDTA blood collection tubes or equivalent.
- Falcon® disposable 12 x 75-mm polystyrene test tubes or equivalent.
- Vortex mixer.
- Micropipettor with tips
- Repipettor dispenser (optional): Dispenses 1-mL volumes or fixed volume.
- BD FACSFlow™ sheath fluid (Catalog No. 342003) or equivalent.

CAUTION Use only BD FACSFlow™ sheath fluid diluent to dilute BD Calibrite beads.

Additional Materials Required When Not Using BD Retic-Count Software

- PBS (1X) (Dulbecco’s modified, pH 7.2 ±0.2; 0.01 mol/L PO₄; and 0.15 mol/L NaCl).¹ This reagent does not contain calcium, magnesium, phenol red, or sodium azide. Filter PBS through a 0.2-µm filter before use. Store at 2°C–8°C.

Staining and Fixing Cells When Using BD Retic-Count Software

1. Label one 12 x 75-mm polystyrene tube for each donor sample.
   
   NOTE Only one tube is required if BD Retic-Count software is used.

2. Add 1 mL of BD Retic-Count reagent to the 12 x 75-mm tube.

3. Add 5 µL of well-mixed whole blood to the tube. Cap and vortex gently.

4. Incubate the sample at room temperature for 30 minutes in the dark. The sample is now ready for analysis. Mix by gently vortexing immediately prior to analysis.

   NOTE Samples should be run within 3.5 hours after incubation (step 4). See Section 9, Limitations.

Staining and Fixing Cells When Not Using BD Retic-Count Software

1. Label two 12 x 75-mm polystyrene tubes for each donor sample. One tube is for the stained sample and one is for the unstained (control) sample.

2. Add 1 mL of BD Retic-Count reagent to one 12 x 75-mm tube (for stained sample).

3. Add 1 mL of 1X PBS with 0.1% sodium azide to the second 12 x 75-mm tube (for unstained sample).

4. Add 5 µL of well-mixed whole blood from the same donor to each tube. Cap and vortex gently.

5. Incubate both samples at room temperature for 30 minutes in the dark. Samples are now ready for analysis. Mix by gently vortexing immediately prior to analysis.

   NOTE Samples should be run within 3.5 hours after incubation (step 5). See Section 9, Limitations.

Flow Cytometry

Run samples within 3.5 hours after the 30-minute incubation. Mix samples by

¹ Falcon is a registered trademark of Corning Incorporated.
gently vortexing immediately prior to analysis.

We recommend using BD Calibrite beads and the appropriate software such as BD FACSComp for setting PMT voltages, setting fluorescence compensation, and checking instrument sensitivity prior to use. Refer to the BD FACSComp Software User’s Guide and the BD Calibrite Bead IFU for information.

Data acquisition and analysis are performed automatically by BD Retic-Count software. Refer to the BD Retic-Count Software User’s Guide for instructions.

The following method describes acquisition, manual gating, and analysis procedures using BD CellQuest software. Refer to the appropriate software user’s guide for instructions.

1. Set FSC and SSC amplifier gains to log mode. If necessary, increase the FSC threshold to exclude noise and debris (see Figure 1). Set compensation to 0.

![Figure 1](image)

**Figure 1** (a) FSC vs SSC dot plot with noise and debris included (FSC threshold equals 64). (b) FSC vs SSC dot plot with noise and debris excluded (FSC threshold raised to 200).

2. Acquire up to 50,000 events from the unstained sample tube using the LO flow rate (for BD FACStrak press the Retic button). Save the list-mode data.

3. Acquire up to 50,000 events from the stained sample tube using the LO flow rate. Save the list-mode data.

**Data Analysis**

1. Display an FSC vs SSC dot plot from the unstained (control) sample data and set a gate around the red blood cell population excluding platelets (see Figure 2 for gating example).
2. Display a fluorescence 1 (FL1) histogram of the gated data acquired from the unstained sample tube (see Figure 3). This histogram displays the autofluorescence generated by red blood cells in the unstained sample. Set two marker sets on the histogram:

- Set the first marker set to include the majority of the autofluorescence peak—the left marker at the beginning of the FL1 scale (channel 1, log scale), the right marker where the peak meets the baseline.
- Set the second marker set to include the remainder of the FL1 scale—the left marker where the peak meets the baseline, the right marker at the end of the FL1 scale (far right).

Events falling within marker set 2 represent a small number of cells whose autofluorescence intensity falls within the range of the fluorescent signal generated by BD Retic-Count. Find the number of autofluorescent cells falling within marker set 2 by looking at the statistics for the histogram (see Table 1). This value will be subtracted from the number of BD Retic-Count positive cells in the stained sample (step 3).

Table 1 Single histogram statistics parameters for the unstained sample: FL1 gated events—9,050 and total events—10,000

<table>
<thead>
<tr>
<th>Left</th>
<th>Right</th>
<th>Events</th>
<th>% Gated</th>
<th>% Total</th>
<th>Mean</th>
<th>Mode</th>
<th>Peak</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>7.56</td>
<td>9,032</td>
<td>99.8</td>
<td>90.3</td>
<td>2.58</td>
<td>1.00</td>
<td>320</td>
<td>44.5</td>
</tr>
<tr>
<td>7.84</td>
<td>10,000.00</td>
<td>18</td>
<td>0.2</td>
<td>0.2</td>
<td>8.15</td>
<td>8.42</td>
<td>8</td>
<td>3.5</td>
</tr>
</tbody>
</table>
Typically, an unstained sample contains less than 0.3% positive cells.

3. Keeping the marker settings in place, generate an FL1 histogram for data acquired from the sample stained with BD Retic-Count reagent. Find the number of events falling within marker set 2 by looking at the statistics for the histogram (see Table 2). These events represent both BD Retic-Count positive cells (reticulocytes), unstained erythrocytes with an increased auto-fluorescence, and can include some other formed elements (see step 4).

The equation for calculating the percent positive reticulocyte is:

\[ A - B = C \]

where:

- \( A \) = % gated stained tube
- \( B \) = % gated unstained tube
- \( C \) = % reticulocytes

4. Other formed elements besides reticulocytes stain positively with BD Retic-Count reagent. These include white blood cells, nucleated red blood cells, Howell-Jolly bodies, platelets, giant platelets, cabot rings, malarial parasites, and the inclusions that give rise to basophilic stippling. The resulting FL1 histogram should be examined for any unusual fluorescence distributions (as shown in Figure 4).

Figure 4 Examples of normal and unusual fluorescence distributions: (a) FL1 histogram showing a normal fluorescence distribution, (b) FL1 histogram from chronic lymphocytic leukemia (CLL) patient showing an unusual fluorescence distribution, and (c) FL1 histogram from patient

---

Table 2 Single histogram statistics for the stained sample: FL1 gated events—9,089 and total events—10,000

<table>
<thead>
<tr>
<th>Left</th>
<th>Right</th>
<th>Events</th>
<th>% Gated</th>
<th>% Total</th>
<th>Mean</th>
<th>Mode</th>
<th>Peak</th>
<th>CVb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>7.56</td>
<td>8,837</td>
<td>97.2</td>
<td>88.4</td>
<td>2.58</td>
<td>1.00</td>
<td>320</td>
<td>44.5</td>
</tr>
<tr>
<td>7.84</td>
<td>10,000.00</td>
<td>252</td>
<td>2.8</td>
<td>2.5</td>
<td>240.31</td>
<td>8.42</td>
<td>8</td>
<td>338.8</td>
</tr>
</tbody>
</table>

a. Unstained tube statistics: The number of positive events in the unstained sample within marker set 2 is 18. This is 0.2% of the gated events (9,050) and 0.2% of the total events (10,000).
b. CV = coefficient of variation
with Howell-Jolly bodies showing an unusual fluorescence distribution.

If an unusual FL1 distribution is observed, review the CBC and blood smear and refer to Section 9, Limitations, for more information.

5. Absolute reticulocyte counts can be obtained using the following formula:
   \[ X = \frac{Y}{100} \times Z \]
   where:
   - \( X \) = Absolute reticulocyte counts
   - \( Y \) = % reticulocyte
   - \( Z \) = RBC count per liter

6. Correcting for shift reticulocytes is performed by using the hematocrit value and the following formula:
   \[ D = \frac{E}{F} \times G \]
   where:
   - \( D \) = Corrected % Reticulocytes
   - \( E \) = Patient hematocrit
   - \( F \) = 45 (average normal hematocrit)
   - \( G \) = % Reticulocytes

Quality Control
To ensure the accuracy of BD Retic-Count test results, the following quality-control procedures should be followed:

- Include a normal sample or commercial sample with a known reticulocyte value in each reticulocyte run to serve as a positive control.
- Use BD Calibrite beads and BD FACSComp software to adjust the PMT voltages and to check the instrument’s signal-to-noise discrimination.

8. RESULTS
Cells that react with BD Retic-Count reagent will fluoresce and be detected in the FL1 channel, and exhibit similar patterns as shown in Figure 3.

9. LIMITATIONS

- Unstained blood, when stored at room temperature (20°C–25°C), can be analyzed up to 48 hours after venipuncture without appreciable change in the percentage of reticulocytes.
- In some patients with very high reticulocyte counts, the markers set with the unstained tube can have more than 0.3% positive events. In these cases, the marker should still be set at the base of the unstained peak.
- Blood samples stained with BD Retic-Count reagent produce stable values for at least 3.5 hours after incubation if the sample is kept in the dark at room temperature.
temperature. For many specimens the BD Retic-Count data are constant during this time. However, certain specimens can show a small, clinically insignificant variation. When following a patient over the course of time, choose a consistent analysis time up to 3.5 hours following the 30-minute incubation.

- Correct gating (removal of nonerythroid elements) is essential. BD Retic-Count reagent will also stain white blood cells and platelets. Reticulocyte counts from patients with abnormally high platelet or white blood cell counts should be confirmed by an alternate method.
- BD Retic-Count reagent should not be used with hemolyzed blood samples.
- Patients with macrothrombocytes (giant platelets) can give anomalous results with BD Retic-Count. Reticulocyte counts from patients with macrothrombocytes should be confirmed by an alternative method.
- BD Retic-Count reagent is a nucleotide marker. It will stain erythrocyte inclusions which contain DNA or RNA. Examples of these include the nucleus of nucleated red blood cells (NRBCs), Howell-Jolly bodies, cabot rings, malarial parasites, and the inclusions that give rise to basophilic stippling. Any red blood cell containing such inclusions can be stained and counted as a reticulocyte. Refer to a Wright-Giemsa–stained peripheral blood smear for confirmation of the presence of red cell inclusions when a suspect reticulocyte value is obtained.
- In patients with grossly elevated white blood cell counts (as seen in CLL), a population of lymphocytes will appear in the red blood cell gate. In FL1 histogram analysis after BD Retic-Count staining, these lymphocytes can appear as a peak usually above $10^2$ on the FL1 log scale using the BD FACScan flow cytometer. For patients with elevated white blood cell (WBC) counts, the reticulocyte count should be confirmed by an alternative method.
- Patients being treated with erythropoietin can have increased percentages of reticulocytes. Some patients can also have increased numbers of NRBCs in the peripheral blood, which can interfere with the reticulocyte evaluation. The presence or absence of NRBCs can be confirmed by reviewing a Wright-Giemsa–stained peripheral blood smear.

10. EXPECTED VALUES

The following normal range (central 95%) of reticulocyte values was determined using blood samples from 99 normal individuals ranging from 18 to 55 years of age. Results are shown in Table 3. Each laboratory should establish its own normal range for reticulocyte enumeration with BD Retic-Count reagent. The expected range given in this IFU is for information only. These values compare well to the values reported in Williams, et al:11 males 1.6% ±0.5 and females 1.4% ±0.5. Newborn infants younger than two weeks have higher counts.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sex</th>
<th>n</th>
<th>Mean</th>
<th>95% range</th>
</tr>
</thead>
<tbody>
<tr>
<td>% reticulocytes</td>
<td>Male</td>
<td>40</td>
<td>1.67</td>
<td>0.92–2.71</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>59</td>
<td>1.42</td>
<td>0.61–2.20</td>
</tr>
</tbody>
</table>
11. PERFORMANCE CHARACTERISTICS

All performance characteristics were determined using a BD FACScan flow cytometer.

Within-Sample Reproducibility

Blood samples were drawn from five patients after hospital admission. Ten replicate tests from each sample were evaluated. Results are shown in Table 4.

<table>
<thead>
<tr>
<th>Patient Sample</th>
<th>Average % Reticulocyte</th>
<th>SD a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.8</td>
<td>0.38</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>14.3</td>
<td>0.29</td>
</tr>
<tr>
<td>4</td>
<td>3.8</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>0.3</td>
<td>0.04</td>
</tr>
</tbody>
</table>

a. SD = standard deviation

Between-Instrument Reproducibility

Ten replicates of the same abnormal sample were run sequentially on two instruments. Results are shown in Table 5.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Range</th>
<th>Average</th>
<th>SD a</th>
</tr>
</thead>
<tbody>
<tr>
<td>FACScan #1</td>
<td>13.3–15.3</td>
<td>14.5</td>
<td>0.6</td>
</tr>
<tr>
<td>FACScan #2</td>
<td>13.4–14.7</td>
<td>14.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

a. SD = standard deviation

Stability of Instrument

Channel separation for signal-to-noise in fluorescence and light-scatter channels was monitored over 4 months on the same instrument using BD Calibrite beads. Results are shown in Table 6.

<table>
<thead>
<tr>
<th>Date</th>
<th>FSC</th>
<th>SSC</th>
<th>FL1 (Green)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12/22/86</td>
<td>134</td>
<td>229</td>
<td>137</td>
</tr>
<tr>
<td>1/20/87</td>
<td>137</td>
<td>228</td>
<td>136</td>
</tr>
<tr>
<td>2/19/87</td>
<td>143</td>
<td>225</td>
<td>135</td>
</tr>
<tr>
<td>3/17/87</td>
<td>140</td>
<td>228</td>
<td>135</td>
</tr>
</tbody>
</table>

BD Retic-Count versus Comparative Method

Seventy-five samples were analyzed in parallel comparing the New Methylene Blue method (average of two counts per sample) with BD Retic-Count (single determination per sample). Figure 5 shows the correlation between the two methods.

Figure 5 Comparison of the percentage reticulocytes determined on human peripheral blood by the New Methylene Blue method to the BD Retic-Count staining with the BD FACScan flow cytometer (normal subjects plus hospital admissions).
Stability of Stained Cells

Samples were stained with BD Retic-Count reagent and reticulocyte determinations were made following a 30-minute incubation. The same stained samples were reanalyzed after 3.5 hours of storage in the dark at room temperature (20°C–25°C). The results were comparable, indicating that samples can be analyzed up to 3.5 hours following the 30-minute incubation.

Linearity of Determinations

The response is linear within the range of 0 to 35% reticulocytes.

Stability of Blood

Blood samples from five normal individuals were drawn into BD Vacutainer EDTA blood collection tubes. The blood was stored at room temperature (20°C–25°C) and sets of two aliquots were stained at 20 minutes, then 8, 24, and 48 hours after venipuncture. As shown in Table 7, most values increased slightly with time. This slight increase is statistically significant but its magnitude appears clinically insignificant in most applications.

Table 7 Blood stability

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>20 minutes</th>
<th>8 hours</th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>2.38, 2.73</td>
<td>2.80, 2.86</td>
<td>2.46, 2.86</td>
<td>2.94, 3.11</td>
</tr>
<tr>
<td>FR</td>
<td>1.81, 1.89</td>
<td>2.06, 2.10</td>
<td>2.39, 2.67</td>
<td>2.85, 3.02</td>
</tr>
<tr>
<td>MP</td>
<td>1.23, 1.32</td>
<td>1.16, 1.19</td>
<td>1.44, 1.50</td>
<td>1.58, 1.63</td>
</tr>
<tr>
<td>JP</td>
<td>2.56, 2.89</td>
<td>2.37, 2.63</td>
<td>2.61, 2.77</td>
<td>2.47, 3.03</td>
</tr>
<tr>
<td>VS</td>
<td>1.49, 1.49</td>
<td>1.23, 1.46</td>
<td>1.23, 1.24</td>
<td>1.58, 1.99</td>
</tr>
</tbody>
</table>

a. Readings taken at 5 minutes instead of 20 minutes.

REFERENCES


