MICROHEMATOCRIT DETERMINATION

**Principle**

Anticoagulated whole blood is centrifuged and the volume occupied by the erythrocytes is expressed as a percentage of the total volume (packed cell volume, PCV).\(^1\,^2\,^3\)

**Reagents and Equipment**

1. Capillary tubes (75 ± 0.5 mm in length, 1.155 ± 0.085 mm in bore)  
   Plain tubes for anticoagulated blood  
   Heparinized tubes for capillary blood
2. Clay or critoseal
3. Microhematocrit centrifuge  
   Specifications:
   a) radius should be greater than 8.0 cm  
   b) capable of reaching maximum speed in 30 seconds  
   c) sustains a RCF of 10,000-15,000 x g for five minutes without exceeding a  
      temperature of 45° C
4. Microhematocrit reading device

**Quality Control**

A quality control program for microhematocrit determinations should include:
1. Centrifuge calibration:
   a) The centrifuge timer should be checked for accuracy and reproducibility  
      with a stopwatch.
   b) The centrifuge speed should be checked with a properly calibrated  
      tachometer.
   c) The minimum time to achieve optimal packing of the red cells should be  
      checked with the following procedure. Choose two fresh EDTA-  
      anticoagulated blood samples (one sample should have a Hct > 50%).  
      Perform duplicate microhematocrit determinations at increasing times,  
      beginning at two minutes. Centrifuge times should be increased by 30-  
      second intervals. Record duplicate values at each time interval. Continue  
      to increase centrifuge time until the value remains the same for two  
      consecutive time intervals. The second time interval is the minimum time  
      for optimal packing of red cells.
2. Centrifuge brushes should be checked regularly and replaced when the brushes are less  
   than half their original size.
3. Quality control material should be run periodically. The frequency is determined by each  
   laboratory's workload. For instance, quality control material may be run at the beginning  
   of each eight-hour shift.
Specimen

Whole blood, anticoagulated with EDTA, is required for the plain capillary tubes. The EDTA-anticoagulated blood should be analyzed within six hours of collection when stored at room temperature.

If capillary blood is to be used, heparinized capillary tubes should be used for the collection of the capillary blood and centrifuged soon after collection.

Procedure

1. Fill two capillary tubes approximately 2/3 to 3/4 full with the well-mixed blood sample.
2. Seal the dry end of the capillary tube by placing it into the sealing clay at a 90° angle.
3. Place the capillary tubes in the microhematocrit centrifuge with the sealed end toward the periphery. Duplicate tubes should be opposite each other for balance.
4. Centrifuge for five minutes.
5. Using a microhematocrit reading device, determine the hematocrit. Results should be recorded to the nearest whole number. Duplicates should agree within 1 unit (1%).

Reference Intervals

<table>
<thead>
<tr>
<th></th>
<th>Conventional Units (%)</th>
<th>SI Units (L/L)</th>
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<tbody>
<tr>
<td>Adult Males:</td>
<td>41-53%</td>
<td>0.41-0.53</td>
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<tr>
<td>Adult Females:</td>
<td>36-46%</td>
<td>0.36-0.46</td>
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</tbody>
</table>

Comments

1. OSHA requires the use of mylar-wrapped capillary tubes or plastic capillary tubes to minimize risk of capillary tube injuries.⁴
2. Inclusion of the buffy coat (leukocytes and platelets) in the hematocrit reading will result in falsely elevated hematocrit results.
3. Sources of technical error for the microhematocrit procedure are given in Web Table 7-5.
4. Sources of physiologic error include:
   a) A small amount of plasma remains trapped in the erythrocyte portion even when the specimen is properly centrifuged. This trapped plasma may result in a falsely elevated hematocrit. Increased amounts of trapped plasma are associated with hypochromasia, macrocytosis, spherocytosis, thalassemia, sickle cell anemia, and polycythemia. As a result of trapped plasma, the spun microhematocrits are 1-3% higher than hematocrits calculated by electronic counters.
   b) Hemoconcentration as a result of prolonged use of a tourniquet will result in falsely increased values.
   c) Difficult venipuncture or skin puncture resulting in dilution with interstitial fluid causes a falsely decreased value.
   d) Hemolysis results in a falsely decreased hematocrit.
   e) Dehydration resulting in a decreased plasma volume will increase the hematocrit.
f) After acute blood loss, the hematocrit is not a reliable assessment of the degree of anemia. In this condition, the plasma volume is replaced faster than erythrocyte volume. The net result is a falsely low hematocrit until the plasma volume and erythrocyte volume equalize.

References