SOP: Measure Reticulocyte Count

DEFINITION

A bioassay to measure the number of reticulocytes (immature red blood cells).

PURPOSE

Reticulocyte count measures how fast red blood cells are made. Elevated reticulocyte counts are associated with blood loss, hemolysis and hemolytic anemia, and in response to the anemia of clinical conditions such as sickle cell disease. Reticulocyte counts can also be used to measure efficacy of treatments (e.g., iron replacement therapy).

DESCRIPTION OF PROTOCOL

This protocol provides instructions for drawing, processing and storing blood according to the National Health and Nutrition Examination Survey (NHANES) methods. Because there are many comparable assays and instruments for measuring reticulocyte count, the protocol also provides basic guidelines to aid comparability among different studies.

SPECIFIC INSTRUCTIONS

The National Health and Nutrition Examination Survey (NHANES) instructions for drawing, processing, and storing blood provide a standard methodology used successfully for many years to ensure comparable results across study sites. However, the Sickle Cell Disease Cardiovascular, Pulmonary, and Renal Working Group notes that certain aspects (e.g., exclusion criteria) of the NHANES protocol are study specific and might not be applicable to all types of studies (e.g., sickle cell disease). Investigators who want to include participants that have hemophilia or have received cancer chemotherapy in the last 4 weeks will need to implement special venipuncture procedures.

Reticulocyte count can be combined with other indirect markers of hemolysis (Aspartate Aminotransferase Level, Haptoglobin Level, Lactate Dehydrogenase Level, and Bilirubin Level) to derive a hemolytic component for sickle cell disease patients. Reticulocyte count analysis is performed on anticoagulated blood, collected in an EDTA tube.

PROTOCOL

The following is a summary version of the full National Health and Nutrition Examination Survey 2011-2012 protocol.

Exclusion Criteria

Persons will be excluded from this component if they:

- Report that they have hemophilia; or

- Report that they have received cancer chemotherapy in the last 4 weeks

SP = Sample Person.

1. Do you have hemophilia?

Yes [ ]
No [ ]
Don't know [ ]

If the SP answers, "Yes," the SP is excluded from the blood draw.
If SP answer "No" or "Don't Know," blood is drawn from the SP.

2. Have you received cancer chemotherapy in the past four weeks or do you anticipate such therapy in the next four weeks?

Yes [ ]
No [ ]
Don't know [ ]

If the SP answers, "Yes," the SP is excluded from the blood draw.
If SP answer "No" or "Don't Know," blood is drawn from the SP.

Venipuncture Procedures

See Protocol for CBC above

Recording the Results of the Venipuncture Procedure

See Protocol for CBC above

Laboratory Assay for Reticulocyte Count

The Sickle Cell Disease Cardiovascular, Pulmonary, and Renal Working Group notes that there are a number of different assays and instruments that are appropriate to measure the reticulocyte count from serum. Once an assay is chosen for a particular study, the Working Group recommends that no changes in the protocol be made over the course of the study. To aid comparability, the Working Group recommends that the investigator record the make and manufacturer of equipment used and the repeatability and coefficients of variation for the assay.

Reference Ranges for Reticulocyte Count:

Children: 3 - 6%
Adults: 0.5 - 1.5%

Percentage of reticulocytes and absolute reticulocyte counts (ARC) are given by the following formulas:

Reticulocyte Percentage = [Number of Reticulocytes (thousands/μL) x RBC count (millions/μL)] X 100

Absolute reticulocyte count (thousands/μL) = reticulocyte % x RBC count (millions/μL) x 10

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