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Fetal Red Cell Detection; Approved Guideline

This document provides guidance for the quantitation of fetal red blood cells in blood and other biologic fluids. The performance characteristics of various flow cytometric and microscopic assays are reviewed, recommendations are made for control usage, and principles for distinction of F cells and fetal red cells are discussed.

A guideline for global application developed through the NCCLS consensus process.

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Fetal Red Cell Detection; Approved Guideline

Abstract

NCCLS document H52—*Fetal Red Cell Detection*, provides guidance for the performance of fetal red blood cell (RBC) counting in blood and other human biologic samples. The various flow cytometric, static cytometry, and microscopic methods for the detection of fetal RBCs are reviewed, with discussion of the calibration, relative imprecision, and limitations of the various assays. Recommendations for the proper use of quality controls for the assays are presented in the context of the medical and diagnostic utility of the various methods. Additional topics discussed include screening assays for fetomaternal hemorrhage and identification of F cells (HbF containing adult red blood cells) for assessment of hemoglobinopathies and other hematopoietic diseases.

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Contents

Foreword

The detection and quantitation of fetal red blood cells (RBCs), like the reticulocyte count, are determined by most clinical laboratories using manual microscopic visual methods. The performance of this assay has been repeatedly shown to have poor precision and interlaboratory correlations, despite the fact that this assay is a major determinant in the therapeutic administration of Rh immune globulin with fetomaternal hemorrhage (FMH).[1-5] Flow cytometric methods suitable for clinical practice have recently been developed using antigenic differences or quantitative assessment of fetal hemoglobin (HbF) to distinguish fetal RBCs from adult RBCs. These methods are more precise, less subjective, and require less of the technologist's time compared to the microscopic or Kleihauer-Braun-Betke assay.[6-14]

The principles, performance variability, and quality control of flow cytometric and static cytometry methods are outlined in this guideline, along with the microscopic Kleihauer-Braun-Betke (KBB) and rosette or agglutination screening assays. Sources of interferences and the relative limitations inherent with each methodology are discussed. Even though the KBB method has limitations in precision, sensitivity, and standardization, it will likely continue to be utilized in many clinical laboratories where a less precise quantitation of FMH satisfies the clinical needs, lacking access to flow cytometry, and for calibration of more automated methods. Hence, this guideline provides methodologic details on the KBB method in an attempt to improve performance.

Many of the methods used to quantitate fetal RBCs for detection of FMH also identify nonfetal or adult RBCs that contain lower levels of HbF, the so-called "F cells." Recognition of F cells was formerly of concern as a source of false-positive results with the KBB assay and in the evaluation of patients with suspected hereditary persistence of HbF (HPFH).[15-18] However, recognition of the therapeutic benefit of increasing levels of F cells in hemoglobinopathies, such as sickle cell anemia, has recently increased the interest in assays that allow quantitation of F cells.[19-21] Additionally, there is evidence that F-cell quantitation may provide prognostic information in myelodysplastic syndrome.[22-24] Accordingly, this proposed guideline provides a discussion of the challenges in standardizing F-cell counting methods, as well as the importance in distinguishing F cells from fetal RBCs. The subject of fetal cell identification for purposes of genetic testing, although a technologically challenging and potentially important area of medical diagnosis,[25] is outside the scope of this document and is not discussed.

Key Words

Erythrocytes, erythropoiesis, F cells, fetal cells, fetal hemoglobin, fetomaternal hemorrhage, flow cytometry, hematopoiesis, hemoglobinopathy, hemolytic disease of the newborn, maternal transfusion, myelodysplastic syndrome, quality control, red cells, reference method, sickle cell anemia

Fetal Red Cell Detection; Approved Guideline

1 Introduction

The motivation for developing this guideline was twofold: the advent of flow cytometric and other automated methods for fetal red blood cell (RBC) detection and the desire to provide a document to assist in the standardization and quality control of these new techniques. The limitations of the manual microscopic visual counting method for fetal RBCs, the Kleihauer-Braun-Betke (KBB) or acid elution technique are well documented,[1-5] but given the therapeutic need for directing Rh immune globulin (RhIG), administration in fetomaternal hemorrhage (FMH) and the lack of alternative methodology, this method has lingered on in clinical practice. The arrival of flow cytometric and static cytometry alternatives now promises to greatly improve the laboratory's ability to more accurately detect and enumerate fetal RBCs in FMH.[6-13] These automated methodologies have the potential to more reliably and reproducibly count F cells/nonfetal RBCs that contain lower levels of hemoglobin F (HbF), for which a clinical need is becoming established.

This guideline reviews the performance characteristics of all available methods for fetal cell detection and enumeration, cites sources of potential interference, and provides recommendations for quality control.

2 Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be treated as infectious and handled according to "standard precautions." Standard precautions are new guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. 1996;Vol 17;1:53-80), (MMWR 1987;36[suppl 2S]2S-18S), and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials and for recommendations for the management of blood-borne exposure, refer to the most current edition of NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections*.

3 Scope

This document outlines the methodologies currently available and caveats for interpretation utilized in fetal RBC counting. Included are manual microscopic screening and quantitative techniques and more automated flow cytometric and static cytometric methods. Methods to ensure clinically acceptable precision and accuracy in calibration and quality control are outlined. The KBB method is currently designated the Class B reference method in this document, due to the anticipation that flow cytometric methods based upon HbF detection by monoclonal antibodies will gain acceptance as the more appropriate reference method, due to greater accuracy. The relationship to fetal RBC counting results and therapeutic administration of RhIG in the treatment of FMH in Rh_o or D antigen-negative women is also provided. Although the enumeration of F cells is not the primary focus of this proposed guideline, potential methods for F-cell counting are discussed, primarily in an effort to guide the subsequent standardization of this clinically useful measurement.

