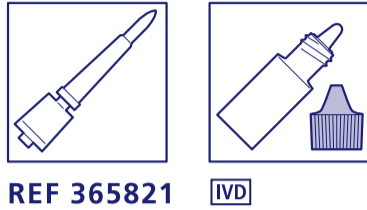


BD Unopette™ System Tests



REF 365821

IVD

Product Information

Intended Use

BD Unopette™ System Tests 365821 is a stable *in vitro* diagnostic reagent system for the enumeration of reticulocytes in whole blood.

Summary

This method is based on the accepted reticulocyte enumeration procedure using new methylene blue N as the stain.¹ Potassium oxalate is added to the staining solution to prevent coagulation and to obtain a staining solution that is isotonic.¹

Principle

Whole blood is mixed with new methylene blue N, a supravital stain which stains RNA in young erythrocytes (reticulocytes) deep blue. A smear of the blood stain mixture is prepared in a glass slide. Under an oil immersion lens, reticulocytes are counted. Reticulocytes are often larger than mature erythrocytes.

When reticulocytes are stained with new methylene blue N the reticulum stains as deep-blue granules or threads in sharp contrast to the light greenish-blue background. Mature erythrocytes in the same preparation stain the same greenish-blue background color, but without any granules or threads.

Reticulocyte counts obtained with BD Unopette™ System Tests 365821 compare favorably with those obtained using fresh new methylene blue N stain mixed 1:1 with whole blood.

Reagents

- BD Unopette™ dropper bottle containing 7.00 ml of diluent mixture:
 New methylene blue N 5.0 gm
 Potassium oxalate 16.0 gm
 Purified water qs to 1 liter
- BD Unopette™ Capillary Pipette 25 µl capacity

Warning

Avoid contact of reagent or blood-stain mixture with skin and clothes because staining will occur. Specimen collection, dilution and transfer should be performed wearing gloves. After use, always recap bottle.

Dilution Ratio

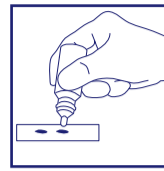
Blood to dye mixture 1:1

Reagent Stability

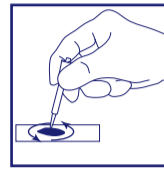
The reagent system is stable if stored below 30°C (86° F), bottle recapped after every use, and used before the expiration date under the stated conditions of the test.

Procedure

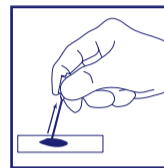
1. Stain Specimen



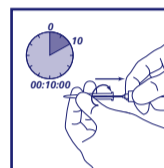
- Place a drop of fresh capillary or anticoagulated venous blood on a clean glass slide. Beside it place an equal volume of new methylene blue N stain.



- Mix blood and stain with tip of capillary pipette shield, as shown. Wipe tip of shield to remove blood-stain mixture.
- Remove shield from pipette assembly with a twist.

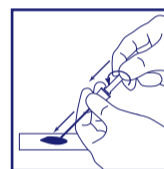


- Collect blood-stain mixture in capillary. This may be facilitated by tilting the slide slightly.



- Cover capillary pipette with shield. Blood-stain mixture is automatically forced into overflow chamber. Allow to stand at room temperature ten (10) minutes.

2. Prepare Slide



- Remove shield from capillary pipette. Holding tip of capillary over glass slide, fit shield onto open end of overflow chamber. Advance shield with a twisting motion to deliver blood-stain mixture onto slide.



- Mix blood-stain mixture on slide again, using tip of glass capillary.
- Prepare thin blood-stain film in usual manner and allow to air dry.

3. Count And Calculate

Reticulocytes are counted as follows:

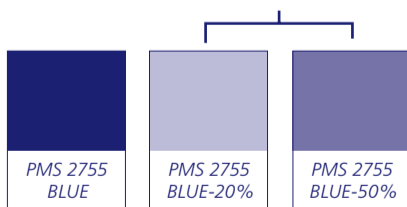
- Using oil immersion lens, count 1000 erythrocytes and record the number of reticulocytes seen.
- Calculate the percent of reticulocytes using the following formula:

$$\frac{\text{Number reticulocytes} \times 100}{1000 \text{ erythrocytes}} = \text{percent reticulocytes}$$

EXAMPLE: If 40 reticulocytes are counted per 1000 erythrocytes, the percent reticulocytes would be:

$$\frac{40 \times 100}{1000} = 4\% \text{ reticulocytes}$$

Tints used for illustration, page 1, and chart, page 2.



Color Break Information

Dimensions: Length: 9.5"
Width: 8.5"

Folded dimension: Length: 4.25"
Width: 3.167"

All Text and Graphics prints PMS 2755 Blue except where noted. Document prints double sided.

Follow directions at bottom of page for fold guide.

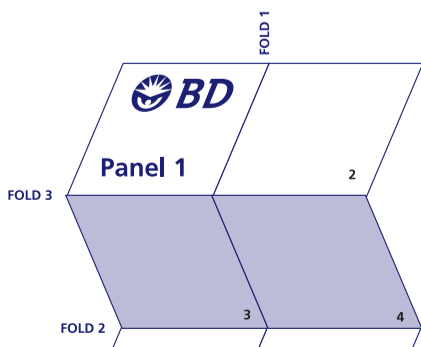
Panel 1 should be on the outside.

Dimensional Tolerances: ± 0.03125"

Material: Paper, 60 lb. offset white opaque stock, or equivalent

Note:

- Reference Quality Inspection Document VT10066.



BD Diagnostics – Preanalytical Systems, Franklin Lakes, NJ						
REV LEVEL	ALOA #	ECO #	DESCRIPTION	DESIGNER	DATE	SAP #
00-02			Refer to archived version/s of this document for description of prior rev level/s, ECO#s incorporated revision/s, designer/s, date/s and SAP#s. New titleblock format reflects updated VO8-817 procedure.			
03	00307-01	ECO68424	Added Note: Reference to Quality Inspection document VT10066. Update title block to reflect current revision date.	J. BOUGHTON	9/05/07	8079884

Print Date: 11/11/2008
Release Date: 09/29/2007

Version: 3.0 Status: Released

Releasing ECO: ECO68424

PACKAGING LEVEL

PRODUCT CIRCULAR - 1 OF 2

DRAWING #

VDP40017-03

Equipment

1. Microscope equipped with oil immersion lens.
2. Clean glass slides.
3. Lens immersion oil.

Specimen

Free-flowing capillary or thoroughly mixed anticoagulated venous blood. With free flowing capillary sample, it may be desired to make an immediate blood-stain mixture on slide directly from patient. For venous blood samples, EDTA is the anticoagulant of choice. Use of an appropriate BD Vacutainer® Tube with EDTA is recommended for collection of venous specimens. The mixed preparation of whole blood and stain should be allowed to stand ten (10) minutes before slide is made.¹

Interferences

It has been reported that sodium salts² inhibit the uptake of stain by reticulocytes. In addition, crenation of erythrocytes (including reticulocytes) may possibly obstruct the passage of stain into these cells.³

Siderotic granules in siderocytes stain in the presence of new methylene blue N are indistinguishable from the reticulum of reticulocytes. If a large number of siderotic granules are present or suspected, the reticulocyte-stain preparation may be fixed with absolute methyl alcohol, stained for iron, and counter-stained with Safranin O. With this technique, reticulum stains red and siderotic granules stain blue.¹

With high-titer cold agglutinins, erythrocyte clumping may occur, masking reticulocytes. To eliminate clumping, warm the blood-stain mixture in a 37°C water bath and quickly make slide preparation.

Storage Instructions

Reticulocyte preparations may fade on prolonged storage. Faded preparations can be restored by fixation in methyl alcohol and restaining with Wright's stain.¹

Limitation Of Procedure

Particles of stain adhering to the surface of mature red blood cells may be a serious source of error.¹ The ten (10) minute incubation time for blood-stain mixture must be strictly observed. The longer the blood-stain mixture is allowed to stand beyond this time limit, the greater the possibility of stain adhering to mature blood cells and causing erroneous interpretations.

Expected Performance

Precision of reticulocyte counts on one patient using fresh new methylene blue N stain and BD Unopette™ System Tests 365821.

% Reticulocytes

	BD Unopette™ System Tests 365821	Fresh-New Methylene Blue-N-Stain
Determinations (n)	40	40
Mean	1.0	1.0
Standard Deviation	±0.104	±0.098
Coefficient of Variation	10.4%	9.8%

References

1. Cartwright GE: Diagnostic Laboratory Hematology, ed 4, New York, Grune & Stratton, 1968, p 156, 157.
2. Heath CW, Daland GA: Staining of reticulocytes by brilliant cresyl blue, Arch Int Med 48:133, 1931.
3. Davidson LSP: The basophilic substance of the erythrocyte, Edinburgh Med J 37:425 (Aug) 1930.

Symbol & Mark Key

REF Catalog Number	Upper Limit Of Temperature
Use By	Keep Away From Sunlight
LOT Batch Code	Manufacturer
IVD In Vitro Diagnostic Medical Device	EC REP Authorized Representative
Do Not Reuse	Fragile, Handle With Care
Caution, Consult Accompanying Documents	This End Up
Consult Instructions For Use	Keep Dry

Caution

Handle all biologic samples and blood collection products according to the policies and procedures of your facility. All glass has the potential for breakage; take precautionary measures during handling. Obtain appropriate medical attention in the event of any exposure to biologic samples (e.g., through a puncture injury) since samples may transmit viral hepatitis, HIV (AIDS), or other infectious diseases. Discard all products contaminated with blood in biohazard containers approved for their disposal.

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8079884 9/2007

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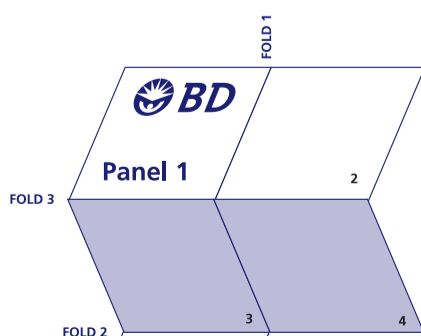
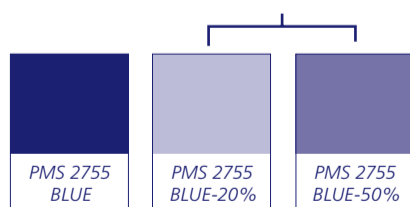
Dimensional Tolerances: ± 0.03125"

Material: Paper, 60 lb. offset white opaque stock, or equivalent

Note:

1. Reference Quality Inspection Document VT10066.

Tints used for illustration,
page 1, and chart, page 2.



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03	00307-01	ECO68424	Added Note: Reference to Quality Inspection document VT10066. Update 4/2005 to 9/2007. Update title block to reflect current revision date.	J. BOUGHTON	9/05/07	8079884

Print Date: 11/11/2008
Release Date: 09/29/2007

Version: 3.0 Status: Released

Releasing ECO: ECO68424

PACKAGING LEVEL

PRODUCT CIRCULAR - 2 OF 2

DRAWING #

VDP40017-03