UNOPETTE

Microcollection system

Product Circular for Reorder No. 365854 and 365855

WBC/PLATELET DETERMINATION FOR MANUAL METHODS
FOR IN VITRO DIAGNOSTIC USE

INFORMATION AND PROCEDURES

PRODUCT INFORMATION

INTENDED USE

UNOPETTE Test 36584/365855 is a stable in vitro diagnostic reagent system for the enumeration of leukocytes and platelets in whole blood.

SUMMARY

This method is based on the procedure developed by Brecher and Cronkite.[1] The ammonium oxalate diluent is modified by the addition of Sorensen's phosphate buffer to maintain the pH of the reagent, and thimerosal to act as an antibacterial agent.

PRINCIPLE

Whole blood is added to the diluent, the tonicity of which lyses red cells but preserves platelets, leukocytes and reticulocytes. When erythrocytes are completely lysed, the solution will be clear red and counting can proceed. The diluted blood is placed in a hemacytometer according to accepted technique. Cells are allowed to settle for ten (10) minutes before leukocytes and platelets are counted.

Under 100X magnification using bright-light microscopy, leukocytes appear refractile. At this magnification some red cell stroma will be evident but will not interfere with the counting procedure.

Under 430X magnification using bright-light microscopy, platelets appear oval or round and frequently have one or more dendritic processes. Under the same magnification using phase microscopy, they appear morphologically the same as with bright-light microscopy but are uniformly opaque with a pink or purple sheen.

Leukocyte counts obtained with UNOPETTE Test 365854/365855 compare favorably with those obtained using the Thoma White Cell Pipette with 3% acetic acid.

Platelet counts obtained with UNOPETTE Test 365854/365855 also compare favorably with results obtained with the Coulter Counter* Model F electric counting method.

*Coulter Counter is a registered trademark of Coulter Electronics, Inc. Hialeah, Florida.

REAGENTS

1. UNOPETTE Reservoir containing 1.98 ml of diluent mixture:
   Ammonium oxalate..... 11:45 gm
   Sorensen's phosphate buffer..... 1.0 gm
   Thimerosal..... 0.1 gm
   Purified water..... qs to 1 liter
2. UNOPETTE Capillary Pipette..... 20µl capacity

WARNING

Thimerosal, a preservative added to both the jar and the reservoir reagent, may cause skin irritation. If reservoir is squeezed too hard, the specimen may be expelled through the top of the overflow chamber, resulting in contamination of the fingers. All specimen handling, diluting, and handling of the filled hemacytometer should be performed wearing gloves.

DILUTION RATIO

Sample to total volume..... 1:100

REAGENT STABILITY

The reagent system is stable if stored below 30°C (86°F), protected from sunlight, and used before the expiration date under the stated conditions of the test. Do not use if reagent solution is not clear

EQUIPMENT

1. Microscope equipped for 100X and 430X magnification. Phase contrast for platelet counts is optional.
2. Hemacytometer. Neubauer hemacytometer recommended.
3. Petri dish. Bottom should be lined with moist filter paper.

SPECIMEN

Free flowing capillary or thoroughly mixed anticoagulated venous blood. EDTA is the anticoagulant of choice. Use of an appropriate VACUTAINER® Brand Tube with EDTA is recommended for collection of venous specimens. (See TECHNICAL NOTES 1 and 2). The diluted specimen is stable for three (3) hours at room temperature.

INTERFERENCES

Bacteria and other debris can interfere with platelet counts. However, bacteria, dirt, and crystals can usually be properly identified by their refractility and lack of pink or purple sheen under phase microscopy. Care must be taken to differentiate platelets from extrinsic particles under bright-light microscopy.

STORAGE INSTRUCTIONS

If count cannot be performed immediately after blood is diluted, store reservoir at room temperature. Perform count within three (3) hours of making dilution.

PROCEDURE

1. PUNCTURE DIAPHRAGM

Using the protective shield on the capillary pipette, puncture the diaphragm of the reservoir as follows:

a. Place reservoir on a flat surface. Grasping reservoir in one hand take pipette assembly in other hand and push tip of pipette shield firmly through diaphragm in neck of reservoir, then remove.
b. Remove shield from pipette assembly with a twist.

2. ADD SAMPLE

Fill capillary with whole blood and transfer to reservoir as follows:

a. Holding pipette *almost* horizontally, touch tip of pipette to blood. Pipette will fill by capillary action. Filling is complete and will stop automatically when blood reaches end of capillary bore in neck of pipette.
b. Wipe excess blood from outside of capillary pipette, making certain that no sample is removed from capillary bore
c. Squeeze reservoir slightly to force out some air. Do not expel any liquid. Maintain pressure on reservoir.
d. Cover opening of overflow chamber of pipette with index finger and seat pipette securely in reservoir neck.
e. Release pressure on reservoir. Then remove finger from pipette opening. Negative pressure will draw blood into diluent.
f. Squeeze reservoir *gently* two or three times to rinse capillary bore, forcing diluent up into *but not out of*, overflow chamber, releasing pressure each time to return mixture to reservoir. CAUTION: See WARNING Section
g. Place index finger over upper opening and gently invert several times to thoroughly mix blood with diluent
h. Let stand for ten (10) minutes to allow red cells to hemolyze. Leukocyte and platelet counts should then be performed within three (3) hours.

3. CHARGE HEMACYTOMETER

Mix diluted blood thoroughly by inverting reservoir (see 2g above) to resuspend cells.

a. Convert to dropper assembly by withdrawing pipette from reservoir and reseating securely in reverse position.
b. To clean capillary bore, invert reservoir, gently squeeze sides and discard first three or four drops.
c. Carefully charge hemacytometer with diluted blood by gently squeezing sides of reservoir to expel contents until chamber is properly filled.
d. Place hemacytometer on moistened filter paper in Petri dish. Cover Petri dish and allow to stand ten (10) minutes to permit cells to settle. (Moistened filter paper retards evaporation of diluted specimen while standing.)

4. COUNT AND CALCULATE

A leukocyte count is performed with a Neubauer hemacytometer as follows:

a. Under 100X magnification, leukocytes are counted in all nine (9) large squares of the counting chamber.
b. Add 10 percent of count to total number of cells counted. (See TECHNICAL NOTE 4).
c. Multiply this figure by 100 to get total leukocyte count.

EXAMPLE: If 70 cells are counted, total count is: (70 + 7) X 100 = 7700 leukocytes/cu mm.

A platelet count is performed with a Neubauer hemacytometer as follows:

a. Under 430X magnification using bright-light or phase microscopy, platelets are counted in all 25 small squares within the large center square.
b. Multiply number of platelets counted by 1000 to get total platelet count.

EXAMPLE: If 150 platelets are counted, total count is: 150 X 1000 = 150,000 platelets/cu mm.

NOTE: If another hemacytometer is used, calculations appropriate to the particular type should be employed.

LIMITATION OF PROCEDURE

A highly elevated leukocyte or platelet count may make accurate counting difficult. In either instance, a secondary dilution should be made. When calculating the total count, adjust the formulas to allow for secondary dilution.

EXPECTED PERFORMANCE

1. Comparison of platelets counts of 526 specimens of blood, performed in duplicate by Coulter Counter Model F and UNOPETTE Test 365854/365855.

	Platelets per cu mm	
	UNOPETTE Test 365854/365855	Coulter Counter Model F
Mean	244,900	207,700
Precision to one standard deviation by comparison of duplicates	± 15,400	± 15,700
Coefficient of variation	6.3%	7.6%

2. Precision of leukocyte counts and platelets counts using UNOPETTE Test.

Leukocyte Count

Determination (n)	40
Mean	5221 Leukocytes / cu mm
Standard deviation	± 382 Leukocytes / cu mm
Coefficient of variation	7.3%

Platelet Count

Determination (n)	18
Mean	303,000 Leukocytes / cu mm
Standard Deviation	± 18,000 Leukocytes / cu mm
Coefficient of variation	5.9%

TECHNICAL NOTES

1. Platelets become activated by surface contact and will aggregate. If samples are collected into evacuated tubes, tubes must be inverted 8-10 times immediately after filling. Delay in mixing may result in platelet clumping and erroneous results.
2. Excessive tissue trauma during collection of blood may also result in platelet clumping and reduce the accuracy of the platelet count. When clumps of platelets are observed in a hemacytometer, another sample of blood must be collected.
3. Cells and diluent must be adequately mixed and counting chambers should be properly filled if errors in manual counting procedure are to be avoided.
4. This step simplifies the calculation which actually entails dividing the number of cells by the number of squares counted and multiplying by 10 to correct for the depth of the chamber.

REFERENCES

1. Brecher G. Cronkite EP: Morphology and enumeration of human blood platelets, J Appl Physiol 3:365 (Dec.) 1950.

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.