REF 362760
4 mL Draw Capacity
(13 x 100mm tube Size)

REF 362761
8 mL Draw Capacity
(16 x 125mm tube Size)

BD Vacutainer® CPT™

Cell Preparation Tube with Sodium Citrate

For the Separation of Mononuclear Cells from Whole Blood

Sterile Interior

For In Vitro Diagnostic Use

Intended Use

The BD Vacutainer® CPT™ Cell Preparation Tube with Sodium Citrate is an evacuated tube intended for the collection of whole blood and the separation of mononuclear cells. The cell separation medium is comprised of a polyester gel and a density gradient liquid. This configuration permits cell separation during a single centrifugation step. The separated sample can be transported without being removed from the BD Vacutainer® CPT™ Tube since the gel forms a stable barrier between the cell layers.

Summary and Explanation

Isolation of mononuclear cells from whole blood is a first step for many *in vitro* assays. One currently accepted technique for mononuclear cell separation, referred to as the FICOLL™ Hypaque™ method, employs a liquid density gradient medium of FICOLL™ 400 and sodium metrizoate or sodium diatrizoate solution [1,2,3]. The procedure uses anticoagulated blood, collected by routine phlebotomy, which is diluted with a buffered solution, and then carefully layered onto the medium. This preparation is then centrifuged to isolate the mononuclear cells above the medium. The cells are harvested by carefully pipetting them from the liquid interface.
The BD Vacutainer® CPT™ Cell Preparation Tube with Sodium Citrate combines a blood collection tube containing a citrate anticoagulant with a FICOLL™ Hypaque™ density fluid and a polyester gel barrier which separates the two liquids. The result is a convenient, single tube system for the collection of whole blood and the separation of mononuclear cells. The BD Vacutainer® CPT™ Cell Preparation Tube with Sodium Citrate reduces the risk of sample contamination and eliminates the need for additional tubes, pipettes, and reagents. Samples can be transported without removing them from the tube.

TEST PRINCIPLES

The BD Vacutainer® CPT™ Cell Preparation tube with Sodium Citrate is an evacuated blood collection tube system containing 0.1 M sodium citrate anticoagulant and blood separation media composed of a thixotropic polyester gel and a FICOLL™ Hypaque™ solution.

The blood separation media takes advantage of the relatively low density of mononuclear cells to isolate them from whole blood. The separation occurs during centrifugation when the gel portion of the medium moves to form a barrier separating the mononuclear cells and plasma from the denser blood components. The mononuclear cells can be collected by pipetting the cell layer, or the cells can be resuspended into the plasma by gentle inversion to improve cell viability if the sample is to be transported.

REAGENTS, SUPPLIES AND EQUIPMENT

Reagents Provided:

BD Vacutainer® CPT™ Cell Preparation Tubes with Sodium Citrate.

REF 362760
4 mL Draw Capacity (13 x 100 mm tube Size)
Sterile Tube Interior

Contains:
- 0.45 mL of 0.1 Molar Sodium Citrate Solution (Top Fluid Layer)
- 1.8 gm of Polyester Gel (Middle Layer)
- 1.0 mL of Polysaccharide/Sodium Diatrizoate Solution (FICOLL™ Hypaque™ solution, Bottom Fluid Layer)
- Silicone Coated Glass Tube
- Silicone Lubricated Rubber Stopper

REF 362761
8 mL Draw Capacity (16 x 125 mm Tube Size)
Sterile Tube Interior

Contains:
- 1.0 mL of 0.1 Molar Sodium Citrate Solution (Top Fluid Layer)
- 3.0 gm of Polyester Gel (Middle Layer)
- 2.0 mL of Polysaccharide/Sodium Diatrizoate Solution (FICOLL™ Hypaque™ solution, Bottom Fluid Layer)
- Silicone Coated Glass Tube
- Silicone Lubricated Rubber Stopper

Reagents Not Provided:

Reagent
- Phosphate Buffered Saline (PBS) without Ca++ or Mg++.

Supplies and Equipment Not Provided:

Specimen Collection
- BD Vacutainer® and BD Vacutainer® Needle or BD Vacutainer® Blood Collection Set.

- Alcohol Swab.
- Dry Sterile Gauze.
- Tourniquet.
- Adhesive Bandage.
- Gloves appropriate for the protection of the person collecting specimen.
- Sharps disposal system.

Specimen Processing

- 15 mL Size Plastic Conical Centrifuge Tubes with Caps.
- Pasteur Pipettes.
- Centrifuge with Swinging Bucket Rotor and Tube Carriers/Adapters for 13 x 100mm and/or 16 x 125mm Tube Size.

NOTE: Centrifuge must be capable of generating at least 1500 RCF at the tube bottom.

- Gloves appropriate for the protection of the person processing specimen.

WARNINGS AND PRECAUTIONS FOR *IN VITRO* DIAGNOSTIC USE

1. Do not re-use BD Vacutainer® CPT™ Tubes.

2. Do not use tubes after expiration date printed on the tube label.

3. Do not use tubes if the clear liquid solutions above and/or below the gel layer become discolored or form precipitates.

4. Do not use tubes for collection of materials to be injected into patients.

5. Since this BD Vacutainer® CPT™ Tube contains chemical additives, precautions should be taken to prevent possible backflow from the tube during blood drawing (see Prevention of Backflow section).

6. Excessive centrifugation speed (over 2000 RCF) may cause tube breakage, exposure to blood, and possible injury.

7. Remove and reinsert stopper by either gently rocking the stopper from side to side or by grasping with a simultaneous twisting and pulling action. A "thumb roll" procedure for stopper removal is not recommended, as tube breakage and injury may result.

8. **CAUTION:**

- All glass has the potential for breakage, therefore, precautionary measures should be taken during handling.
- Handle all biologic samples and blood collection "sharps" (lancets, needles, and blood collection sets) in accordance with the policies and procedures of your facility.
- Obtain appropriate medical attention in the event of any exposure to biologic samples (for example, through a puncture injury) since the samples may transmit HBV (hepatitis), HIV (AIDS), or other infectious diseases.
- Utilize any built-in used needle protector, if the blood collection device provides one. Becton Dickinson does not recommend reshielding used needles, but the policies and procedures of your facility may differ and should always be followed.
- Discard all blood collection "sharps" in biohazard containers approved for their disposal.
- Filling the tubes from a hypodermic syringe while the stopper is in place is not recommended. Forcefully depressing the syringe plunger without removing the stopper can create positive pressure in the tube causing the stopper and specimen to fly out with explosive force.

9. Centrifugation:

CAUTION: If tubes with cracks or chips are used or if excessive speed is used in centrifugation, a tube may break causing the release of sample, droplets, and possibly an aerosol into the centrifuge bowl. The release of these potentially hazardous materials can be mitigated by using specially designed sealed containers in which tubes are held during centrifugation. The use of special containment vessels is not recommended for routine purposes.

Centrifuge carriers and inserts should be of the size specific to the tubes used. Use of carriers too large or too small for the tube may result in breakage. Care should be taken to ensure that tubes are properly seated in the centrifuge cup. Improperly seated tubes may catch on centrifuge head resulting in breakage. Tubes must be balanced in the centrifuge head to minimize the possibility of glass breakage. Always allow centrifuge to come to a complete stop before attempting to remove tubes. When centrifuge head has stopped, open lid and examine for possible broken tubes. If breakage is indicated, use mechanical device such as forceps or hemostat to remove tubes. **Caution: Do not remove broken tubes by hand.**

STORAGE

Store BD Vacutainer® CPT™ Tubes upright at room temperature (18-25°C). Protect tubes from direct light. Shelf life at 18-25°C is one year from the date of manufacture.

VENIPUNCTURE TECHNIQUE AND SAMPLE COLLECTION

Prevention of Backflow

Since this BD Vacutainer® CPT™ Tube contains chemical additives, it is important to prevent possible backflow from the tube with its attendant possibility

of adverse reactions to the patient. To guard against backflow, the following precautions should be taken when drawing blood into the tube:

1. Keep patient's arm in the downward position during the collection procedure.
2. Hold the tube with the stopper uppermost.
3. Release the tourniquet as soon as the blood starts to flow into the tube, or within 2 minutes of application.
4. Make sure the tube contents do not touch the stopper or the end of the needle during the collection procedure.

Correct Position of Patient's Arm and Tube Assembly to Reduce the Possibility of Backflow

Tourniquet is released as soon as blood starts to flow.

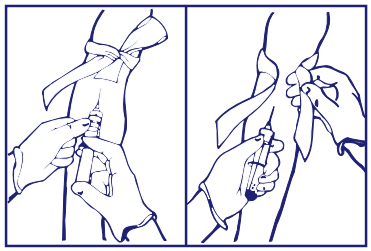

Figure 1

General Instructions

NOTE: Gloves should be worn for venipuncture procedure.

1. Select the tubes appropriate for samples desired.
2. Open needle package but do not remove needle shield. Thread needle onto holder.
3. Insert tube into holder. LEAVE IN THIS POSITION.
4. Select site for venipuncture.
5. Apply tourniquet. Prepare venipuncture site with an appropriate antiseptic. DO NOT PALPATE VENIPUNCTURE AREA AFTER CLEANSING. Allow site to dry.
6. Remove needle shield. Perform venipuncture WITH PATIENT'S ARM IN A DOWNWARD POSITION AND TUBE STOPPER UPPERMOST (see Figure 1). This reduces the risk of backflow of any anticoagulant into the patient's circulation.
7. Push tube onto needle, puncturing diaphragm of stopper.
8. REMOVE TOURNIQUET AS SOON AS BLOOD APPEARS IN TUBE, within 2 minutes of venipuncture. DO NOT ALLOW CONTENTS OF TUBE TO CONTACT THE STOPPER OR THE END OF THE NEEDLE DURING THE PROCEDURE.

If no blood flows into the tube or if blood ceases to flow before an adequate sample (approximately 3.0 mL as minimum blood volume for 4 mL draw and approximately 6.0 mL minimum blood volume for 8 mL draw) is collected, the following steps are suggested to complete satisfactory collection:

a. Confirm correct position of needle cannula in vein.
b. If a multiple sample needle is being used, remove the tube and place a new tube into the holder.
c. If the second tube does not draw, remove needle and discard in appropriate disposal device. DO NOT RESHIELD. Repeat procedure from step 1.

NOTE: When using a blood collection set, a reduced draw of approximately 0.5 mL will occur on the first tube. This reduced draw is due to the trapped air in the blood collection set tubing which enters the first tube.

9. When first tube has filled to its stated volume, remove it from holder.
10. Place succeeding tubes in holder, puncturing diaphragm to initiate flow.
11. While each successive tube is filling invert previous tube 8 to 10 times to mix anticoagulant additive with blood. DO NOT SHAKE. Vigorous mixing can cause hemolysis.
12. As soon as last tube is filled and mixed as above, remove needle from vein. Apply pressure to puncture site with dry, sterile gauze until bleeding stops.
13. Apply bandage, if desired.
14. After venipuncture, the top of the stopper may contain residual blood. Proper precautions should be taken when handling tubes to avoid contact with this blood. Any needle holder that becomes contaminated with blood should be considered hazardous.
15. After collection, dispose of needle using an appropriate disposal device. DO NOT RESHIELD.

PROCEDURE

1. The BD Vacutainer® CPT™ Tube with Sodium Citrate should be at room temperature (18-25°C) and properly labeled for patient identification.
2. Collect blood into the tube using the standard technique for BD Vacutainer® Evacuated Blood Collection Tubes (see Venipuncture Technique & Sample Collection section and Prevention of Backflow section).
3. After collection, store tube upright at room temperature until centrifugation. Blood samples should be centrifuged within two hours of blood collection for best results.

4. Centrifuge tube/blood sample at room temperature (18-25°C) in a horizontal rotor (swing-out head) for a minimum of 20 minutes at 1500 to 1800 RCF (Relative Centrifugal Force).

NOTE: Remix the blood sample immediately prior to centrifugation by gently inverting the tube 8 to 10 times. Also, check to see that the tube is in the proper centrifuge carrier/adapter.

WARNING: Excessive centrifuge speed (over 2000 RCF) may cause tube breakage and exposure to blood and possible injury. To calculate the correct centrifuge speed for a given RCF, use the following formula:

$$\text{RPM Speed Setting} = \sqrt{\frac{(\text{RCF}) \times (100{,}000)}{(1.12) \times (r)}}$$

Where ***r*** (expressed in centimeters) is the radial distance from the centrifuge center post to the tube bottom, when the tube is in the horizontal position and RCF is the desired centrifugal force, 1500–1800 in this case.

Layering of Formed Elements in the BD Vacutainer® CPT™ Tube

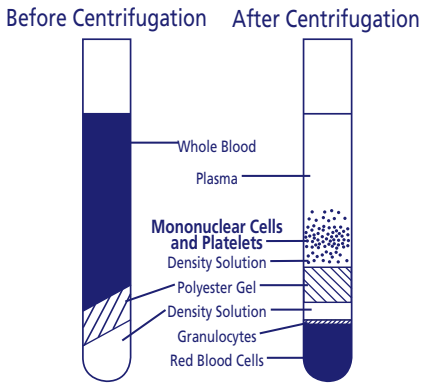

Figure 2

5. After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (see Figure 2). Aspirate approximately half of the plasma without disturbing the cell layer. Collect cell layer with a Pasteur pipette and transfer to a 15 mL size conical centrifuge tube with cap. Collection of cells immediately following centrifugation will yield best results.

6. An alternative procedure for recovering the separated mononuclear cells is to resuspend the cells into the plasma by inverting the unopened BD Vacutainer® CPT™ Tube gently 5 to 10 times. This is the preferred method for storing or transporting the separated sample for up to 24 hours after centrifugation. To collect the cells, open the BD Vacutainer® CPT™ Tube and pipette the entire contents of the tube above the gel into a separate vessel.

Suggested Cell Washing Steps:

1. Add PBS to bring volume to 15 mL. Cap tube. Mix cells by inverting tube 5 times.
2. Centrifuge for 15 minutes at 300 RCF. Aspirate as much supernatant as possible without disturbing cell pellet.
3. Resuspend cell pellet by gently vortexing or tapping tube with index finger.
4. Add PBS to bring volume to 10 mL. Cap tube. Mix cells by inverting tube 5 times.
5. Centrifuge for 10 minutes at 300 RCF. Aspirate as much supernatant as possible without disturbing cell pellet. Resuspend cell pellet in the desired medium for subsequent procedure.

LIMITATIONS

Volume of Blood

The exact quantity of blood drawn will vary with the altitude, ambient temperature, barometric pressure, and venous pressure. The minimum volume of blood that can be processed without significantly affecting the recovery of mononuclear cells is approximately 3.0 mL for 4 mL draw and approximately 6 mL for 8 mL draw. However, hematological parameters such as a low hematocrit or a low mean corpuscular hemoglobin concentration may also adversely affect product performance, with increased red blood cell contamination above gel barrier.

Temperature

Since the principle of separation depends on a density gradient, and the density of the components varies with temperature, the temperature of the system should be controlled between 18-25°C during separation.

Centrifugation

Since the principle of separation depends on the movement of formed elements in the blood through the separation media, the "RCF" should be controlled at 1500 RCF to 1800 RCF. The time of centrifugation should be a minimum of 20 minutes. (As noted in the trouble shooting section, some specimens may require up to 30 minutes for optimal separation.) Centrifugation of the BD Vacutainer® CPT™ Tube up to 30 minutes has the effect of reducing red blood cell contamination of the mononuclear cell fraction. Centrifugation beyond 30 minutes has little additional effect. The BD Vacutainer® CPT™ Tube may be recentrifuged if the mononuclear "band" or layer is not disturbed.

Time

Blood samples should be centrifuged/separated within two hours of blood drawing. Red blood cell contamination in the separated mononuclear cell fraction increases with longer delays in sample separation. Mononuclear cell recovery decreases with increased time delay before centrifugation, falling to approximately 40% mononuclear cell recovery at 24 hours.

Cell Separation

As with other separation media, density gradient separation using BD Vacutainer® CPT™ Tubes may alter the proportion of some lymphocyte subsets (e.g., T and B cells) from those in unseparated whole blood [4,5]. This alteration is believed to be relatively insignificant in normal cases. However, in cases where the subject is leucopenic or lymphopenic, the selective loss of one subset may alter proportions significantly.

Certain disease states and/or drugs may also alter cell density and therefore affect separation using BD Vacutainer® CPT™ Tubes[6].

Microbial Contamination

Microbial contamination of reagents may alter the results obtained on cells separated using BD Vacutainer® CPT™ Tubes.

Separated Cell Assays

For determinations other than those described in the results section, the user should establish to his or her satisfaction that the values obtained meet his or her criteria for his or her application.

Platelet Contamination

Studies[7] indicate that mononuclear cell samples separated by the BD Vacutainer® CPT™ Tube method have approximately 1.3 times the platelet concentration obtained using the FICOLL™ Hypaque™ method.

EXPECTED NORMAL DONOR STUDY RESULTS (Using 4 mL Draw Capacity)

Table 1 shows the cell percentages obtained from 45 blood specimens from 32 normal healthy adults using the FICOLL™ Hypaque™ and the BD Vacutainer® CPT™ Tube cell separation methods. Recovery and Purity percentages were determined from the mean of duplicate values obtained using the Coulter Counter® Model S PLUS IV (Coulter Electronics, Inc., Hialeah, FL). Viability percentages were determined by Acridine Orange/Ethidium Bromide staining[7]. Red blood cell percentages were determined by hemocytometer count under a light microscope. Both viability and red blood cell contamination were single determinations for each specimen.

Table 1

Cell Percentages, BD Vacutainer® CPT™ Citrate Tube Method and FICOLL™ Hypaque™ (FH) Method

PARAMETER		MEAN	SD	CV
Recovery	CPT	71.7	10.5	14.7
	FH	80.9	8.5	10.5
Purity				
Total Mononuclear	CPT	98.0	1.8	1.8
Cells	FH	98.7	0.8	0.9
Lymphocytes	CPT	85.9	4.3	5.0
	FH	87.3	4.0	4.5
Monocytes	CPT	12.1	3.3	27.7
	FH	11.4	3.4	29.7
Viability	CPT	99.9	0.3	0.3
	FH	99.7	0.5	0.5
RBC Contamination	CPT	14.3	9.4	65.9
	FH	6.8	5.5	81.3
PMN Contamination	CPT	2.0	1.8	88.7
	FH	1.2	0.8	68.1

FOOTNOTES:

Recovery – Number of recovered mononuclear cells expressed as a % of the total number of mononuclear cells contained in the original whole blood sample.

Purity – Number of mononuclear cells expressed as a % of the mononuclear cells (lymphocytes and monocytes) in the separated white blood cell fraction.

Viability – Number of mononuclear cells expressed as a % of the total mononuclear cells recovered.

RBC Contamination – Number of red blood cells expressed as a % of the number of separated cells.

Granulocyte (PMN) – Number of granulocytes expressed as a % of the Contamination total number of separated white blood cells.

MEAN – Arithmetic Average

SD – Standard Deviation

CV – Coefficient of Variation (%)

(4 mL Draw Capacity, n = 45)
Mean Blood Draw Volume = 3.83 mL (SD 0.08)
Mean Absolute Mononuclear Cell Count
Recovered = 6.54×10^6 cells per tube (SD 1.99×10^6)
With a Range of 3.36 to 10.54 x 10^6 cells

(8 mL Draw Capacity, n = 10)
Mean Blood Draw Volume = 7.58 mL (SD 0.08)
Mean Absolute Mononuclear Cell Count
Recovered = 12.72×10^6 cells per tube (SD 4.64×10^6)
With a Range of 7.02 to 21.44 x 10^6 cells.

PERFORMANCE CHARACTERISTICS

Table 2 shows the reproducibility of separated sample quality using the BD Vacutainer® CPT™ Tube system which was tested and compared to the FICOLL™ Hypaque™ method. Ten samples of one donor's blood were centrifuged and assayed in duplicate for each method. No final washing steps were performed. The samples were resuspended to approximately equal final volumes. Between tube variation was calculated by differencing the mean of the duplicate readings for each tube.

Table 2

Reproducibility Study: Recovery, Purity, Viability, Red Blood Cell (RBC) and Granulocyte (PMN) Contamination** for BD Vacutainer® CPT™ Citrate Tube Method and FICOLL™ Hypaque™ (FH) Method.

PARAMETER		NUMBER	MEAN****	SD	CV%
Recovery*	CPT	9***	70.3	9.7	13.8
	FH	10	82.6	5.5	6.7
Purity*					
Total Mononuclear	CPT	10	98.3	0.6	0.6
Cells	FH	10	99.0	0.3	0.3
Lymphocyte	CPT	10	84.4	1.3	1.5
	FH	10	84.8	0.9	1.1
Monocytes	CPT	10	13.9	1.4	10.4
	FH	10	14.2	0.8	5.9
Viability	CPT	10	100.0	0.0	0.0
	FH	10	99.8	0.4	0.4
RBC Contamination	CPT	10	7.4	1.6	21.7
	FH	10	2.6	1.0	39.0
PMN Contamination	CPT	10	1.7	0.6	34.5
	FH	10	1.0	0.3	30.7

FOOTNOTES:

*Recovery and Purity are based on the Mean of 2 readings.
**Parameters defined in Table 1.
***Sample lost due to spillage, Recovery could not be calculated.
****Using an F-Statistic (2-tailed) at the 5% level, no significant difference was detected between the methods.

MEAN – Arithmetic Mean

SD – Standard Deviation

CV – Coefficient of Variation (%)

N – Number of Tubes

Table 3 shows the cell percentage obtained from 4 groups of 5 patients each, using the FICOLL™ Hypaque™ and the BD Vacutainer® CPT™ Tube cell separation methods. Recovery and purity percentage were determined from the mean of duplicate values obtained using the Coulter® STKR cell counting method. Viability percentages were determined by Acridine Orange/Ethidium Bromide staining[7].

Table 3

Results from Patient Sample Study

SAMPLE NUMBER	TOTAL MONONUCLEAR RECOVERY (%) CPT	FH	PURITY (%) CPT	FH	VIABILITY (%) CPT	FH
Leukemia						
1 CLL	70.5	76.2	98.0	99.4	96.4	97.4
2 CLL	67.8	70.6	99.0	98.5	99.2	98.9
3 CLL	79.7	83.2	97.6	99.5	96.0	97.1
4 CLL	71.4	68.3	99.1	99.3	97.5	98.0
5 CLL	75.6	82.4	99.7	99.7	99.4	100.0
HIV Positive						
6 HIV	62.1	67.3	98.4	97.8	98.5	98.0
7 HIV	59.0	63.7	97.4	98.3	100.0	99.5
8 HIV	68.3	65.6	99.2	96.9	99.2	100.0
9 HIV	63.9	73.0	98.2	97.9	99.3	100.0
10 HIV	70.7	74.8	99.2	98.5	97.2	99.4
Diabetes						
11 DIAB	58.1	64.3	96.7	97.0	99.5	97.8
12 DIAB	66.4	72.3	98.9	99.3	98.9	99.6
13 DIAB	70.2	67.5	97.5	99.0	100.0	99.7
14 DIAB	64.9	70.7	98.5	96.5	99.7	98.2
15 DIAB	62.7	67.7	97.0	98.2	97.8	98.3
Auto Immune						
16 SLE	62.3	64.5	96.4	95.7	97.5	99.0
17 SLE	60.5	63.4	97.0	96.3	99.2	97.3
18 SLE	65.6	62.3	95.7	96.9	96.5	99.0
19 RA	71.5	66.5	96.2	97.2	99.2	100.0
20 RA	69.5	75.6	95.7	97.0	98.9	99.2

FOOTNOTES:

CLL – Chronic Lymphocytic Leukemia

HIV – Human Immunodeficiency Virus Positive

DIAB – Type I Diabetes

SLE – Systemic Lupus Erythematosis

RA – Rheumatoid Arthritis

Recovery, Purity, and Viability Parameters defined in Table 1.

TROUBLESHOOTING

PROBLEM	POSSIBLE	CAUSE SOLUTION
Granulocyte Contamination Greater than 10%.	Centrifuge not at proper speed.	Adjust centrifuge speed to produce 1500-1800 RCF.
	Centrifuge or BD Vacutainer® CPT™ Tube not at room temperature (18-25˚C).	Allow centrifuge and BD Vacutainer® CPT™ Tube to come to room temperature (18-25˚C).
	Delay in centrifugation.	Centrifuge as soon as possible after obtaining blood specimen.
	Abnormal sample with high ratio of granulocytes to mononuclear cells.	Subsequent separation step using standard FICOLL™ Hypaque™ method.
Red blood cell contamination.	BD Vacutainer® CPT™ Tube or centrifuge not at room temperature (18-25˚C).	Allow centrifuge or BD Vacutainer® CPT™ Tube to come to room temperature (18-25˚C).
	Centrifugation time too short.	Increase time of centrifugation (up to 30 minutes).
	MCHC below normal[8].	Increase time of centrifugation (up to 30 minutes).
Too few cells.	Leucopenia.	Collect additional BD Vacutainer® CPT™ specimens as required.
Platelet excess.	High platelet count.	Wash separated cells two times for 15 minutes at 100 RCF.
No defined or distinct mononuclear layer.	Adapter incorrect size.	Use 16 x 125mm centrifuge tube adapter.
	Centrifuge not calibrated correctly.	Have centrifuge calibrated.
	Centrifuge speed too low.	Increase centrifuge speed to produce 1500-1800 RCF.
	Centrifuge time too short.	Increase time of centrifugation (up to 30 minutes).
	Hyperlipemic Sample.	Obtain fasting blood specimen.
No gel movement.	Centrifuge speed too low.	Increase centrifuge speed to produce 1500-1800 RCF.
	Centrifuge temperature less than 18˚C.	Increase centrifuge setting to 18-25˚C.

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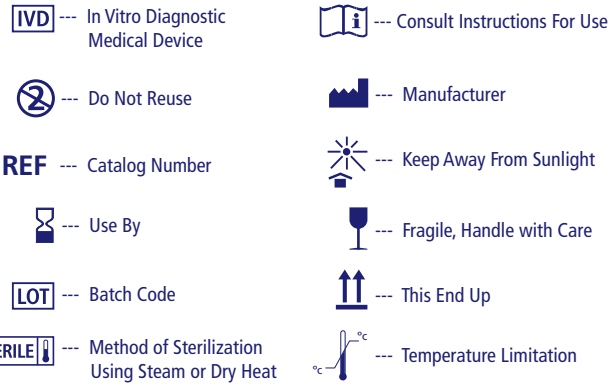
Symbol & Mark Key:



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