KAISER PERMANENTE®
COLORADO LABORATORY

COMPLETE WHOLE BLOOD COUNT ON THE SYSMEX® XT-1800*i*
(Principle and sample operating procedure)

PRINCIPLE

The Sysmex XT-Series is a quantitative automated hematology analyzer for in vitro diagnostic use for determining 24 hematological parameters on the Sysmex XT-1800*i*™ analyzer. Examination of the numerical and/or morphologic findings of the complete blood count are useful in diagnosis of such disease states as anemias, leukemia's, allergic reactions, viral, bacterial, and parasitic infections. The Sysmex XT-1800*i* analyzer directly measures the WBC, RBC, HGB, HCT, PLT, LYMPH#, MONO#, EO#, and BASO #. The remaining parameters are calculated or derived: MCV, MCH, MCHC, NEUT#, RDW-CV, RDW-SD and differential percentages.

The Sysmex XT-1800*i* counts and sizes red blood cells (RBC) and platelets (PLT) using electronic resistance detection enhanced by hydrodynamic focusing. Hematocrit (HCT) is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hemoglobin (HGB) is converted to SLS-hemoglobin, and read photometrically.

WBC count and differential are evaluated using flow cytometry with a semiconductor laser exploiting the differences in cell size, complexity and RNA/DNA content. WBC and basophils (BASO) are treated with an acidic lyse, that lyses RBC and WBC, but not BASO. The remaining WBC nuclei and intact BASO are differentiated by cell size and internal cellular structure. The WBC differential channel classifies lymphocytes (LYMPH), monocytes (MONO), eosinophils (EO), and granulocytes by cellular complexity and nucleic acid content. The differential cell placement is then enhanced utilizing Adaptive Cluster Analysis.

SPECIMEN

A. Specimen requirement

1. Whole blood anticoagulated with EDTA preferred.
2. Check all specimens for obvious clots prior to placing the specimen on the analyzer.

B. Specimen volumes required

1. Optimal draw is a tube drawn to capacity. The collection tube should be filled to a minimum of one-half full for acceptable results.
2. A minimum of 1 mL whole blood is required for automode analysis.
3. An EDTA micro-container filled above the 250 µL line is adequate for testing in the open mode.

C. Unacceptable specimens, including those listed below, must be redrawn:

1. Clotted samples or those containing clots or fibrin strands. All specimens will be checked visually by the operator for obvious clots prior to sampling by the analyzer.
2. Grossly hemolyzed samples
3. Samples drawn above an IV

D. Characteristics that may affect test results: lipemia, icterus, and cold agglutinins, warm agglutinins, hemolysis electrolyte, imbalances, megakaryocytes and WBC fragments. See MCHC protocol.

E. Stored Specimen Stability

1. Stored at 4°C, EDTA blood samples with normal results may be analyzed up to 36 hours without significant loss of differential stability.
2. Sample stability at room temperature is 24 hours. Samples stored at room temperature may exhibit an increase in MCV after 24 hours; this may be minimized by refrigeration.
3. Allow refrigerated samples to come to room temperature and mix well before analysis.

F. **Do not place samples on a mechanical rocker. Constant rocking may cause PLT clumping and alter white cell membranes resulting in false interpretive messages**.

> **WARNING**: All patient specimens should be considered potentially infectious and must be handled with precautions used for human blood, as described in CDC recommendations and in compliance with the Federal OSHA Bloodborne Pathogen Standard, 29 CFR part 1910.1030. Follow specimen handling as outlined by laboratory safety policy.
>
> **Recommended**: Wear gloves, a lab coat and safety glasses, if there is a risk of splash.

SUPPLIES & REAGENTS

A. Supplies

1. De-ionized water
2. Test tubes
3. Plastic squeeze bottles
4. Clorox Ultra™ bleach (Use when Cellclean is indicated)
5. Sysmex™ reagents

- Five Sysmex reagents and bleach are used on the Sysmex XT-Series. These are ordered through the Kaiser Permanente facility buyer – see KPID below.
- All reagents are stored at room temperature and are to be used within the manufacturer's expiration date on each container.
- Record date received and date opened on container. Reagent lot, expiration and open date stability are all recorded in the Sysmex IPU.

- All reagents are azide free, and intended for in vitro diagnostic use only, **do not** ingest.

Reagent	Abbreviation	Open Expiration	KPID
Sysmex CELLPACK™	**EPK**	60 days	0005-5381
Sysmex STROMATOLYSER-4DL™	**FFD**	60 days	0023-6256
Sysmex STROMATOLYSER-4DS™	**FFS**	60 days	0023-6257
Sysmex STROMATOLYSER-FB™	**FBA**	60 days	0024-9688
Sysmex SULFOLYSER™	**SLS**	90 days	0001-4947

DILUENTS

1. **Sysmex CELLPACK (EPK)** is a whole blood diluent for use in the determination of hemoglobin and electric counting and sizing of blood cells. Sysmex CELLPACK also forms a laminar sheath flow around the diluted sample for hydrodynamic focusing the RBC and PLT.

Sysmex CELLPACK Active Ingredients

Sodium Chloride	6.38 g/L
Boric Acid	1.00 g/L
Sodium Tetraborate	0.20 g/L
EDTA-2K	0.20 g/L

Sysmex CELLPACK Storage

a. Store at a controlled temperature of 5-30^{o} C.
b. **If frozen**, thaw, mix thoroughly, and allow bubbles to disperse before use.
c. Sysmex CELLPACK is clear and colorless. If there are signs of contamination, instability or color change, do not use.

Sysmex CELLPACK Stability

a. Unopened, expiration is marked on the container
b. Opened, Sysmex CELLPACK is stable for 60 days.

LYSE REAGENTS

1. **Sysmex STROMATOLYSER-4DL (FFD)** is the lyse and diluent for the enumeration of LYMPH, MONO, EO and granulocytes after eliminating RBC stroma.

Sysmex STROMATOLYSER-4DL Active Ingredients

Nonionic surfactant	0.18%
Organic quaternary Ammonium salts	0.08%

Sysmex STROMATOLYSER-4DL Storage

a. Store at a controlled temperature of 2-35^{o} C.
b. **If frozen do not** use.
c. Sysmex STROMATOLYSER-4DL is a clear, odorless liquid. If there are signs of contamination, instability or color change, do not use.

Sysmex STROMATOLYSER-4DL Stability

a. Unopened, expiration is marked on the container.
b. Opened, Sysmex STROMATOLYSER-4DL is stable for 60 days.

CAUTION: Do not ingest. Avoid skin and eyes contact. Flush with plenty of water immediately. Consult with a physician in case of ingestion and/or eyes contact.

2. **Sysmex STROMATOLYSER-FB (FBA)** is a diluent and lyse reagent for enumeration of WBC count and Basophils.

Sysmex STROMATOLYSER -FB Active Ingredients

Nonionic Surfactant	0.4%

Sysmex STROMATOLYSER -FB Storage

a. Store at a controlled temperature of 5-30^{o} C.
b. **If frozen** let stand over 24 hours to thaw, mix thoroughly, and allow bubbles to disperse before use.
c. Sysmex STROMATOLYSER-FB is a clear, odorless liquid. If there is signs of contamination, instability or color change, do not use.

Sysmex STROMATOLYSER -FB Stability

a. Unopened, expiration is marked on the container
b. Opened, Sysmex STROMATOLYSER-FB is stable for 60 days.

CAUTION: Do not ingest. Avoid skin and eyes contact. Flush with plenty of water immediately. Consult with a physician in case of ingestion and/or eyes contact.

3. **Sysmex SULFOLYSER (SLS)** is the RBC lysing reagent that releases the hemoglobin to be measured by SLS hemoglobin method.

Sysmex SULFOLYSER Active Ingredients

Sodium Lauryl Sulfate	1.7 g/L

Sysmex SULFOLYSER Storage

a. Store at controlled temperature of 2-30^{o} C.
b. **If frozen**, may form a white cloudy precipitate. Thaw and warm reagent in a 30^{o} C water bath to dissolve ingredients completely and mix thoroughly before use.
c. Sysmex SULFOLYSER is a clear, odorless liquid. If there are signs of contamination, instability or color change, **do not** use.

Sysmex SULFOLYSER Stability

a. Unopened, expiration is marked on the container
b. Opened, Sysmex SULFOLYSER is stable for 90 days.

CAUTION: Do not ingest. Avoid skin and eyes contact. Flush with plenty of water immediately. Consult with a physician in case of ingestion and/or eyes contact.

STAINING REAGENTS

1. **Sysmex STROMATOLYSER-4DS (FFS)** is used to stain leukocytes in diluted, lysed blood samples for the determination of the 4-part differential including LYMPH, MONO, EO and granulocytes.

Sysmex STROMATOLYSER-4DS Active Ingredients:

Ethylene Glycol	96.9%
Methanol	3.0%
Polymethine dye	0.002%

Sysmex STROMATOLYSER-4DS Storage

a. Store at controlled temperature of 2-35 C^{o}.
b. **If frozen, do not** use.
c. Sysmex STROMATOLYSER -4DS is a blue viscous liquid with a faint odor. If there are signs of contamination, instability or color change, **do not** use.

Sysmex STROMATOLYSER-4DS Stability

a. Unopened, expiration is marked on the container.
b. Opened Sysmex STROMATOLYSER-4DS is stable for 60 days.

CAUTION: Do not ingest. Avoid skin and eyes contact. In the case of skin contact, wash immediately with plenty of soap and water. In case of contact with the eyes, rinse immediately with water or normal saline. Consult with a physician in case of ingestion and/or eyes contact.

Recommended: Wear gloves, a lab coat and safety glasses for protection.

CLOROX® ULTRA

Clorox Ultra bleach is recommended for use in cleaning and shutdown of the Sysmex XT-1800*i* analyzer whenever CELLCLEAN is indicated.

Clorox Ultra Ingredients

Sodium Hypochlorite	6.0%

Clorox Ultra Storage

Stable under normal use and storage conditions

WARNING: Clorox Ultra. Avoid acidification or contact with ammonia containing products, which can generate hazardous chlorine gas. Contains a strong oxidizing agent. Causes substantial but temporary eye injury. May irritate skin. May cause nausea and vomiting if ingested. Exposure to vapor or mist may irritate nose, throat and lungs.

Recommended: Wear gloves, a lab coat and safety glasses for protection.

REAGENT REPLACEMENT

1. Replace reagents when the analyzer gives the "Replace Container XYZ" message.
2. Verify which reagent to replace and bring the new container to the analyzer.
3. Remove the cap and tubing from the empty reagent container. Use caution not to contaminate the tubing during the process.
4. Insert the tubing into the new reagent container.
5. Initial and date the new reagent container. When replacing reagents foil packs, dispose of packs in a biohazard container.
6. On the reagents replacement dialog box, highlight the correct reagent.
7. Scan in the lot number and other information with the bar code wand or enter all information manually.
8. Click **[OK]**

Note: Run 1 level of commercial control on the closed mode after changing any of the reagents to ensure the system is working properly.

PATIENT SAMPLE PROCESSING

NOTE: <u>DO NOT</u> place specimens on a mechanical rocker before processing on the XT1800*i* analyzer.

A. **<u>Sampler Mode with Barcodes</u>** (**150 µL aspirated sample volume**). A minimum of 1.0 cc of blood is required in the tube for the sampler mode.

1. Place specimens in a rack with barcodes **facing the front of the rack**. Ensure that labels are smooth with no loose edges.
2. Load up to 5 racks at one time (50 samples). A new rack may be added to the right rack pool at any time.
3. On the IPU, click on the "**Sampler**" icon or press the F3 function key. The Sample number dialog box displays.
4. Click **[SAMPLET START]** and **[OK]**.
5. The Sysmex XT-1800*i* automatically mixes the sample 10 times, aspirates, and analyzes the sample according to the barcode discrete order. Results print as they are completed if auto-output is employed.

Note: *If Barcodes are not used, the sample number will increment by 1 as each sample is analyzed. Also, the discrete test to be performed must be selected in the Sampler dialog box.*

B. **<u>Open Manual Mode</u> (85 µL aspirated sample volume)**

1. Click the "**Manual**" icon or press the F2 function key
2. Enter the specimen number (up to 15 Alpha/numeric characters) by using the barcode scanner or typing the accession number into the ID field.
3. Click on the discrete panel to be performed on the sample.
4. Click **[OK]**.
5. Mix the patient sample and uncap the tube.
6. Place sample under the sample probe.
7. Press the **Start** switch. Remove the sample when 3 beeps sound, or the green Ready LED stops blinking. Remove the sample. Patient results print as samples are completed.
8. When Ready LED is on, repeat steps 1-7 for each additional sample.

C. **<u>Capillary Mode</u>** (**85 µL aspirated volume of 1:5 diluted sample**)

1. Prepare a 1:5 dilution using CELLPACK diluent by pipetting 200 µL of CELLLPACK into a test tube, then add 50 µL of whole blood. Because of the dilution factor reduces the reliability of the differential, differential results are suppressed on capillary samples. Run the diluted sample on the analyzer as soon as possible. Diluted samples are stable for up to 15 minutes at room temperature.
2. Click on the "Manual" icon or press on the F3 function key.

3. Enter the specimen number (up to 15 Alpha/numeric characters).
4. Click on the discrete panel to be performed on the sample.

5. Click on the "Capillary" Mode. Only CBC discrete panel is available for capillary specimens.
6. Click **[OK]**.
7. Place the well-mixed dilution up to the sample probe.
8. Press the **Start** switch. Remove the sample when two beeps sound, or the green Ready light stops flashing. The analyzer will multiply by 5 and display the results. **Do not** analyze an undiluted sample.
9. Results print as tests are completed.
10. Reset the "Capillary" mode to "Manual" following the steps above

WARNING: Potential biohazard exposure when handling open patient specimens. Follow Standard precautions outlined by laboratory safety guidelines.

Recommended: Wear gloves, a lab coat and safety glasses. Use plastic lined gauze when opening specimen tubes.

CALCULATIONS

A. If making a 1:5 dilution of patient specimen and NOT running in the capillary mode, multiply measured parameters by 5, recalculate indices.

B. If correcting the HGB or HCT due to interfering substances (i.e. icterus, lipemia, etc) recalculate and correct the affected indices: See MCHC protocol.

MCHC = HGB/HCT x 100

MCH = HGB/RBC x 10

MCV = HCT/RBC x 10

C. If a sodium citrate tube is used for EDTA induced platelet clumping, multiply the citrate platelet count by 1.11 to correct for anticoagulant dilution.

REFERENCE RANGE

Refer to Reference Ranges posted to the web site

PROCEDURE NOTES

A. Nucleated RBC's. Perform manual differential and the LIS will automatically correct Sysmex XT-1800*i* WBC count for the NRBC's per 100 WBC's counted.

WBC = XT WBC count x 100 / 100 + NRBC

B. Megakaryocytes. When megakaryocytes are present, perform a WBC and PLT estimate.

C. Capillary Analysis

1. Use when insufficient patient sample is available for aspiration in the open mode (<85 µL) or a sample has a parameter above the linearity limits of the analyzer.
2. Because of the dilution factor, the reliability of the differential is reduced; therefore, only a CBC discrete panel can be selected.
3. If there are marked changes in plasma constituents, (i.e. Very low sodium or very high glucose, prepare a 1:5 dilution and allow to equilibrate for 5 minutes (no longer than 15 minutes) before analysis.

D. Analysis of the specimen on the Sysmex XT1800i is recommended before removing the cap to make a smear.

E. **Do not** place samples on a mechanical rocker. Excessive mixing may induce platelet clumping and alter white cell membranes resulting in false interpretive messages.

F. Clorox Ultra, a filtered bleach is recommended for use in cleaning. If Clorox Ultra is not available generic bleach may be used, but must be 5% Sodium Hypochorite concentration and be free of particles that may cause background contamination when used on the analyzer. Clorox Ultra must be diluted from 6% by volume (straight from the bottle) to 5% before preparing further dilutions recommended for maintenance.

G. For troubleshooting specifics refer to the Sysmex XT-1800*i* Instructions for Use Manual, Section 13.

LIMITATIONS OF PROCEDURE

A. Sysmex XT-Series MANUFACTURER STATED LINEARITY:

Parameter	Range	Units
WBC	0-310.0	$x10^3/\mu L$
RBC	0-8.00	$x10^6/\mu L$
HGB	0-25.0	g/dL
HCT	0-60.0	%
PLT	0-2000	$x10^3/\mu L$

1. Parameters that exceed these limits are flagged with @ beside the result. The sample must be diluted, rerun and multiplied by the dilution factor, or dilute 1:5 and use the capillary mode.
2. Note the use of dilution on the instrument printout only.

B. KNOWN INTERFERING SUBSTANCES

1. Specimens must be free of clots and fibrin strands.
2. Sodium Citrate may be used when EDTA platelet clumping or platelet satellitism is noted on the EDTA specimen. Use Sodium Citrate results for platelet counts and WBC counts. Multiply instrument PLT and WBC result by 1.11 to correct for anticoagulant dilution.
3. Marked changes in plasma constituents (e.g., low sodium, extremely elevated glucose) may cause cells to swell or shrink. The blood to anticoagulant ratio is important.

4. Red cell fragments, microcytic RBC's or white cell cytoplasmic fragments may interfere with automated platelet counts.
5. Cold agglutinins produce spurious macrocytosis, elevated MCH's, MCHC's, falsely decreased RBC counts and HCT's. Rare Warm agglutinins produce the same spurious results as a cold agglutinin.
6. Extremely elevated WBC's may cause turbidity and increase the hemoglobin.
7. Severely hemolyzed samples (in vitro) falsely decrease RBC and hematocrit. Recollect hemolyzed specimens.
8. Giant platelets and clumped platelets may falsely elevate the WBC count and falsely decrease the platelet count.
9. Platelet clumping and/or "platelet satellitism" can occur in specimens collected in EDTA. This may falsely elevate the WBC and falsely decrease the platelet count. Recollect the specimen in Sodium Citrate anticoagulant and multiply by 1.11 dilution factor.
10. Abnormal paraproteins found in Multiple Myeloma patients can falsely increase the HGB. To correct HGB perform plasma replacement.
11. Lipemia falsely elevates the HGB and MCHC. Perform a plasma replacement.
12. Severely Icteric samples may falsely elevate the HGB value and related indices. Make a 1:5 dilution with Sysmex CELLPACK.
13. Rocking specimen excessively may affect the WBC differential.
14. Megakaryocytes may falsely increase WBC counts on automated hematology analyzers.
15. Abnormal proteins as seen in Multiple Myeloma and Waldenstrom's macroglobulinemia may falsely increase the WBC, PLT or Hgb count.

REFERENCES

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