

Sysmex[®] XS-Series Automated Hematology Analyzer

Flagging Interpretation Guide

Document Number: 1269-LSS

September 2016

© 2016, Sysmex America, Inc. All rights reserved.

The contents of this manual, including all graphics and photographs are the property of Sysmex America, Inc. Information in this document is subject to change without notice. Sysmex is not liable for technical or editorial errors or omissions contained herein.

No part of this document may be reproduced or transmitted in any form or by any means, electronic or mechanical, for any purpose, without the express written permission of Sysmex America, Inc.

Sysmex is a registered trademark of Sysmex Corporation.

This manual was created by Sysmex America, Inc. Questions or comments regarding the content of this manual can be directed to:

Sysmex America, Inc. Attention: Technical Assistance Center 577 Aptakisic Road, Lincolnshire, IL 60069 USA

1-800-379-7639

No part of this publication may be reproduced in any form or by any means without prior written permission.

Table of Contents

I.	Introduction	5
II.	WBC IP Messages <u>Abnormal, WBC Abn Scattergram</u>	7 10 12 14 16 18
III.	RBC IP Messages <u>Abnormal, RBC Abn Distribution</u> <u>Abnormal, Dimorphic Population</u> <u>Suspect, RBC Agglutination</u> ? <u>Suspect, Turbidity/HGB Interference</u> ? <u>Suspect, Iron Deficiency</u> ? <u>Suspect, HGB Defect</u> ? <u>Suspect, Fragments?</u>	20 22 25 28 30 30 31
IV.	PLT IP Messages <u>Abnormal, PLT Abn Distribution</u> <u>Suspect, PLT C(S)?</u> <u>Suspect, PLT Clumps?</u>	33 35 36
V.	Action Messages Count DIFF-CH	39 39
VI.	Interfering Substances	41
VII.	References	42

Key Words and Phrases Select the link below to go directly to the specific topic:

Bands

Cold Agglutinin

Correcting CBC Parameters for Dilution Factor When Doing Manual Dilution

Interfering Substances

MCHC Troubleshooting Chart

NRBC Correction

Plasma Replacement

Platelet Clumping

Pseudothrombocytopenia and Approaches for Managing Platelet Clumping

Sodium Citrate Anticoagulant (Blue Top Tubes) and Platelet Clumping

Vortexing of Sample and Platelet Clumps

Warm Agglutinin

Introduction

The XS-Series Flagging Interpretation Guide is designed to serve many objectives including:

- Providing users with an explanation of criteria used for the XS-Series Interpretive Program (IP) Messages.
- Suggesting actions to be taken when samples generate IP Messages.
- Suggesting actions to resolve sample related problems.

The following sections introduce the IP Messages. Definitions and examples of each message are presented with suggested actions to be taken by qualified personnel to verify the presence of specific cell types and obtain a correct result when interference occurs. These action steps are merely suggested guidelines and not requirements. Always follow your local laboratory procedures for repeat testing or confirmation of results.

The XS-Series analyzers are designed to aid in the separation of specimens into POSITIVE and NEGATIVE categories according to preset criteria. The system bases its judgments on comprehensive surveys of numerical data, particle size distributions, and scattergrams and provides easy-to-understand flags and messages indicating the analyzer's findings. These flags and messages are referred to as IP Messages. The IP Messages may be classified as either Suspect IP Messages or Abnormal IP Messages. The IP Messages generated by the analyzer determine if the sample is judged as POSITIVE or NEGATIVE.

Suspect IP Messages are generated by analyzer software algorithms. Abnormal IP Messages are based on numerical user defined settings.

A specimen is judged NEGATIVE when there are no IP messages generated. NEGATIVE does not necessarily indicate a normal sample; however, the results are generally reported without review.

The XS-Series analyzers will generate a POSITIVE when an IP Message is present. ERROR will be generated when there is an analysis error. These judgments indicate the possibility of sample abnormality. These results should be reviewed carefully and may require further examination in accordance with your local laboratory protocol. All analyzer flags, error messages and results must be interpreted together and in consideration of the patient's clinical condition prior to results being reported from the laboratory. Any asterisk (*) next to a parameter indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting. Protocols for comparison of current results to previous results (delta checking) as well as critical value alerts are also useful for identifying potentially erroneous results prior to reporting to the clinician.

NOTE: Discrete test selection may determine availability of certain IP messages. Refer to the Instructions for Use for detailed list of IP Messages.

Abnormal, WBC Abn Scattergram

The WBC Abn Scattergram IP message is generated whenever clustering in the DIFF scattergram is abnormal. Dashes may appear in place of data that was not calculated.

In the example below, clustering failed between the Monocytes and the Neutrophils on the DIFF scattergram.

💮 IPU - [Data Bro	owser]									
File(F) Edit	(E) View(V) I	Record(R) Actio	on(A) Report(P) Setting(S) Windov	v(W) Help(H)	Ver.:00-1	9 User Name:a	idmin		
F1 S F2 MANU	JAL	F4 F5 Menu QC	Files Work list	F7 F8 F8 Explorer Browser		F11 Validate	Out	0 Upper	Lower	Last20 Delete
Positiv Valida	e ated	© [№] .	05/11/20	6611211702 16 10:33:53		9			1	6
Main (Graph Cu	umulative	Q-Flags	Service Rese	arch erential				WB	c
Item	Data	Unit	LL	UL Item	Data	Unit	LL	UL		
WBC RBC HGB HCT MCV MCH MCHC PLT RDW-SD RDW-CV MPV	45.22 \$ 3.26 - 9.0 - 28.4 - 87.1 27.6 31.7 - 85 - 59.9 22.3 - 9.6 \$	<pre>10^3/uL 10^6/uL 9/dL % fL pg g/dL 10^3/uL fL % fL</pre>		NEUT# LYMPH# MONO# EO# BASO# Item NEUT% LYMPH% MONO% EO% BASO%	2.78 * 3.66 * 0.10 * 6.1 * 8.1 * 0.2 *	10^3/uL 10^3/uL 10^3/uL 10^3/uL 10^3/uL Unit % % % % %		UL ++++++++++++++++++++++++++++++++++++		PSC C T T 40FL IFF
Flag(s)- WBC Blasts Imm Gr Left S NRBC? WBC Ab Mono+ Leuko+	? an? hift? n Scg	RBC		PLT	*		\uparrow		55 	SSC

XS-Series Results

Abnormal DIFF Scatter (Close-Up)



Normal DIFF Scatter (Reference)



Manual Differential Results

Neutrophils		73%
Band Neutrop	ohils	12%
Metamyelocy	tes	5%
Lymphocytes	i	5%
Monocytes		5%
NRBC	1/100	WBC

Abnormal, WBC Abn Scattergram (continued)

The suppressed differential values can be found in the Research Tab of the Browser Screen if needed.

IPU - [Data Browser]													
File(F) Edit(E) View(V)	Re	cord(R) Action	i(A) Report(P)	Setting(S)	Wi	ndow(W) Help	(H) Ve	::00-19 User I	Nam	e:admin		
F1 S F2 Help MANU	IAL	F4	Menu QC F	F6	⁷ F ⁸ Explorer Br	ows	er	F11- Vali	date Out	1.	Upper Lo	Lower Last20	
Positiv Valida	Na 6611211702 O 05/11/2016 10:33:53												
Main G	iraph 0	Cun	nulative	Q-Flags S	ervice Diffe	Re	esearch ntial——				52	WBC	1
Item	Data		Unit	Item	Data		Unit	Item	Data		Unit	\land	
WBC	45.22	3'r	10^3/uL	IG#	2.85		10^3/uL	IG%	6.3	_	%		
WBC-C	43.99		10^3/uL	NEUT#&	35.64		10^3/uL	NEUT%&	78.9		%		
WBC-D	45.22		10^3/uL	LYMP#&	2.48	x	10^3/uL	LYMP%&	5.4	x	%	FSC	
RBC	3.26	-	10^6/uL	MONO#	3.66	x	10^3/uL	MONO%	8.1	×	%	RBC	
HGB	9.0	-	g/dL	EO#	0.10	x	10^3/uL	E0%	0.2	×	%	\wedge	
HCT	28.4	-	%	BASO#	0.19	ste	10^3/uL	BASO%	0.4	¥.	%	/	
MCV	87.1		fL	OTHER#	0.30	3'0	10^3/uL	OTHER%	0.7	3c	%		
MCH	27.6		pq									250fL	
MCHC	31.7	-	g/dL	I								PLT	
PLT	85	22	10^3/uL										
RDW-SD	59.9		fL										
RDW-CV	22.3	+	%									40fL	
PDW	10.5	+	fL										
MPV	9.6		fL									DIFF	
P-LCR	23.1	+	%									s	
PCT	0.08		%										
Flag(s)-			PRC]	DI T			1				- 1	
WBC	2		KBC		PLI			-					
Imm Gr Left S NRBC?	an? Shift?	• •		* -			* -					SSC	
		_				_				_		37	1

NOTE: Results from the Research Tab of the Browser are not directly reportable by the laboratory and must be confirmed prior to being released.

Abnormal, WBC Abn Scattergram (continued)

Suggested Action Steps:

- 1. Dashes (— —) in place of numeric data:
 - Verify WBC and differential results according to your laboratory's policy. Possible actions may include:
 - o repeating the sample
 - o performing a manual differential
- 2. Asterisk (*) next to results:
 - Verify WBC and differential results according to your laboratory's policy. Possible actions may include:
 - scanning the slide for abnormal cells or platelet clumping and to estimate the WBC count
 - o performing a manual differential if abnormal cells are observed
 - If no abnormalities are found when reviewing the smear and the WBC estimate matches the analyzer reported WBC, the results with asterisks (*) may be reported.

Suspect NRBC?

The NRBC? IP Message is generated when clustering is detected in the NRBC area between the lymphocytes and the RBC ghosts on the DIFF scattergram. An asterisk (*) appears next to the WBC and the % and # for the Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

NOTE: When WBC is < 0.5 x 10³/µL, no WBC Suspect Messages will be generated



XS-Series Results

Abnormal DIFF Scatter (Close-Up)



Normal DIFF Scatter (Reference)



Suspect, NRBC? (continued)

Suggested Action Steps:

- 1. Review results according to your laboratory protocol. This may include scanning the peripheral smear for the presence of NRBCs or other abnormal cells. Report any NRBCs or abnormal cells according to your laboratory protocol.
- 2. If a 100-cell differential is done and NRBCs are counted, the WBC result may be corrected using the following equation if indicated by your laboratory protocol.

Corrected WBC (x 10^3/ μ L) = $\frac{(WBC \times 100)}{(NRBC + 100)}$

NOTE: It is up to the laboratory to determine the NRBC count which would indicate correction of the WBC count.

3. If no abnormalities are found when reviewing the smear, the results with asterisks (*) may be reported.

Suspect, Immature Gran?

In cases where the analyzer has detected abnormal clustering in the region for immature granulocytes in the DIFF scattergram, the Immature Gran flag is displayed. In these cases, an asterisk (*) appears next to the % and # for the Neutrophil, Eosinophil and Basophil. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

🗇 IPU - [Data Bro	owser]	-												
File(F) Edit	(E) View(V)	Re	cord(R) Actio	on(A) Rep	oort(P) Setti	ing(S) Windov	v(W) Help(H)	Ver.:00-1	9 User Nam	ie:sysmex			
F1 S F2 MANU	JAL	F4	Menu QC	Files Wor	k list Explor	er Browser			F11 Validate	F12 Out	0 Upper	U Lower	Last20	Delete
Positiv Valida	e ated			08/25	5725 /2015	3-09 11:27:30	_	11 20)					
Main (Graph 0	Cum	ulative	Q-Fla	gs Serv	vice Rese	arch erenti	al-				_	W/B C	
Ttem	Data		Unit	1.L	UL	Item	Data		Unit	LL	UL		WBC	SS 85
WBC RBC HGB HCT MCV MCH MCH PLT RDW-SD RDW-CV MPV	6.38 2.98 9.7 29.5 99.0 32.6 32.9 456 49.8 14.9 9.5	+ + + +	10^3/uL 10^6/uL g/dL % fL pg g/dL 10^3/uL fL % fL			Item NEUT# LYMPH# MONO# EO# BASO# Item NEUT% LYMPH% MONO% EO% BASO%	4.99 0.39 0.88 0.09 0.03 Data 78.2 6.1 13.8 1.4 0.5	* + * * * * *	10^3/uL 10/3/uL 10/3/uL 10^3/uL 10^3/uL Unit % % % % %					FSC 250fL
Flag(s) WBC Imm Gr Left S	an? hift?	4	RBC		P T	LT	* *					SFL		SSC

XS-Series Results

Abnormal DIFF Scatter (Close-Up)



(IG population circled)

Normal DIFF Scatter (Reference)



Suspect, Immature Gran? (continued)

Suggested Action Steps:

When the Immature Gran flag is displayed:

- 1. Review results according to your laboratory protocol. This may include scanning the peripheral smear for the presence of:
 - immature granulocytes promyelocytes, myelocytes and metamyelocytes
 - band cells in increased numbers
 - toxic granulation or vacuolation of neutrophils
 - other abnormal cells

Report any abnormal cells according to your laboratory protocol.

2. If no abnormalities are found when reviewing the smear, the results with asterisks (*) may be reported.

Suspect, Abn Lympho/Blast?

The Abn Lympho/Blast? IP message indicates that the analyzer has detected abnormal clustering in the region for blasts and abnormal lymphocytes in the DIFF scattergram.

An asterisk (*) appears next to the % and # for the Neutrophil, Lymphocyte and Monocyte. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.



DIFF Scatter (Close-Up)



Normal DIFF Scatter (Reference)



Suspect, Abn Lympho/Blast? (continued)

Suggested Action Steps:

- 1. Review results according to your laboratory protocol. This may include scanning the peripheral smear for the presence of:
 - blasts lymphoblasts, myeloblasts, and myelomonoblasts
 - immature granulocytes promyelocytes, myelocytes, metamyelocytes
 - atypical or immature lymphocytes
 - other abnormal cells

Report any abnormal cells according to your laboratory protocol.

NOTE: Reviewing the feathered edge and sides of the peripheral smear is suggested as blasts and other large cells may migrate to this area during smear preparation.

- 2. If no abnormalities are found, the results with the asterisk (*) may be reported.
- 3. If dashes (— —) are in place of numeric data, verify differential results according to your laboratory's policy. Possible actions may include:
 - repeating the sample
 - performing a manual differential

Suspect, Left Shift?

The Left Shift? IP message indicates that the analyzer has detected abnormal clustering in the region for left shift (bands) in the DIFF scattergram. When bands are present, they are included in the neutrophil population.

An asterisk (*) appears next to the % and # for the Neutrophil and Eosinophil. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.



XS-Series Results

DIFF Scatter (Close-Up)



Normal DIFF Scatter (Reference)



XS-Series Flagging Interpretation Guide Document Number: 1269-LSS, Rev.1, September 2016

Suspect, Left Shift? (continued)

Suggested Action Steps:

- 1. Review results according to your laboratory protocol. This may include scanning the peripheral smear for the presence of:
 - band cells in increased numbers
 - toxic granulation or vacuolation of neutrophils
 - other abnormal cells

Report any abnormal cells according to your laboratory protocol.

- 2. If no abnormalities are found, the results with the asterisk (*) may be reported.
- 3. If dashes (— —) are in place of numeric data, verify differential results according to your laboratory's policy. Possible actions may include:
 - repeating the sample
 - performing a manual differential

Suspect, Atypical Lymph?

The Atypical Lymph? IP message indicates that the analyzer has detected significant clustering in the region for atypical lymphocytes that is located in the upper left lymphocyte region on the DIFF scattergram.

An asterisk (*) appears next to the % and # for the Neutrophil, Lymphocyte and Monocyte. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.



XS-Series Results

DIFF Scatter (Close-Up)



Normal DIFF Scatter (Reference)



Suspect, Atypical Lymph? (continued)

Suggested Action Steps:

- 1. Review results according to your laboratory protocol. This may include scanning the peripheral smear for the presence of:
 - atypical or variant lymphocytes
 - abnormal or atypical monocytes
 - immature lymphocytes, such as seen in ALL or CLL
 - immature monocytes
 - smudge cells
 - other abnormal cells

Report any abnormal cells according to your laboratory protocol.

- 2. If no abnormalities are found, the results with the asterisk (*) may be reported.
- 3. If dashes (— —) are in place of numeric data, verify differential results according to your laboratory's policy. Possible actions may include:
 - repeating the sample
 - performing a manual differential

Abnormal, RBC Abn Distribution

The RBC Abn Distribution IP Message is generated when the histogram pattern from the RBC channel is abnormal or when RBC < $0.50 \times 10^{6}/\mu$ L.

Dashes appear in place of affected results. For example, if there are multiple peaks present on the RBC histogram, there would be dashes in place of results for the RDW-SD and RDW-CV. Sometimes this IP Message can cause the RDW-SD and RDW-CV to be marked with an asterisk (*). The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.



XS-Series Results

Abnormal, RBC Abn Distribution (continued)

Suggested Action Steps:

- 1. Review results according to your laboratory protocol. This may include scanning the peripheral smear for the presence of abnormal RBC morphology such as:
 - 1. increased anisocytosis
 - 2. multiple RBC populations
 - 3. fragmented RBCs
 - 4. poikilocytosis
 - 5. rouleaux or RBC agglutination (refer to suggested action for "RBC Agglutination?" if present)

Report any abnormal RBC morphology according to your laboratory protocol.

- 2. If no abnormalities are found, the results with the asterisk (*) may be reported.
- 3. If dashes (— —) are in place of numeric data, verify results according to your laboratory's policy. Possible actions may include repeating the sample or reporting RBC morphology from smear review.
- 4. If the RBC morphology is normal and the MCHC is abnormal (<30 or >37.5 g/dL) an interfering substance or condition may be present. Refer to the suggested guidelines for the HGB/Turbidity Interference? IP Message.

Abnormal, Dimorphic Population

The Dimorphic Population IP Message is generated when there are multiple peaks in the RBC histogram pattern. This message may occur with the RBC Abn Distribution IP Message.

Dashes appear in place of results for the RDW-SD and RDW-CV. This message may cause certain RBC parameters to be marked with an asterisk (*). The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.



XS-Series Results

RBC Histogram (Close-Up)



Normal RBC Histogram (Reference)



Abnormal, Dimorphic Population (continued)

The RBC count and MCV for the two populations shown on the RBC histogram can be found in the Service/RBC/PLT tab of the Browser Screen if needed.

telp	J UAL	F4 Menu	F5 V	Work list	Zxplorer	r	F11 Validat	e Out	0 Upper	Lower	Last20	Delet
valio	ve lated		№. ⊙ 0	5 8/27/201	72726950 5 11:59:2	2 21 ©	ψ V					
ain	Graph C	umulat	ive Q-	-Flags S	ervice Re	search						
RBC.	PLT	-		Ni	ckname:							
Serv	vice Data	1				1						
P	BC and B	T Samr	ling	Data		Analyzed	Data					-
			r mg	Data		R-MEV	87.7	fL	РСТ	0.14	%	
	823 KB0		-	865	0	PDW-CV	1000.0		I -RBC	2 54	1046/01	
	853	0	-	807	0	S-RBC	1 91	1046/01		112 0	fi	
	870	0	-	923	0	S NOV	65.6	10.10/ UL	L-MCV	112.0	1	
	855	0		873	0	S-MCV	0.0	TL C	L-RDW	0.0	TL	_
	884	0		921	0	S-RDW	0.0	TL .	PLT-I	130	10^3/uL	
	868	0		892	0	P-MFV	9.1	fL				
	848	0		882	0	Discri						
	865	0		899	0	RBC-LD	5		PLT-LD	1		
	881	0		875	0	RBC-MD	16					
	830	0		852	0		40			22		
	431	0		433	0	RBC-00	49		PLI-UD	23	-	
	0	0		0	0	HGB						
	0	0		0	0	Sample	6577		Blank	5379		
	0	0	L	0	0	RBC						
		9011 (*9)			9316 (*3)	Clog	98					
	~					Distribu	ition—					1
						RBC		MP	PLT			

NOTE: Results from the Service Tab are not directly reportable by the laboratory and must be confirmed prior to reporting.

Abnormal, Dimorphic Population (continued)

Suggested Action Steps:

- 1. Review results according to your laboratory protocol. This may include scanning the peripheral smear for the presence of abnormal RBC morphology such as:
 - increased anisocytosis
 - multiple RBC populations
 - fragmented RBCs
 - poikilocytosis
 - rouleaux or RBC agglutination (refer to suggested action for "RBC Agglutination?" if present)

Report any abnormal RBC morphology according to your laboratory protocol.

- 2. If no abnormalities are found, the results with the asterisk (*) may be reported.
- 3. If dashes (— —) are in place of numeric data, verify results according to your laboratory's policy. Possible actions may include repeating the sample or reporting RBC morphology from smear review.
- 4. If the RBC morphology is normal and the MCHC is abnormal (<30 or >37.5 g/dL) an interfering substance or condition may be present. Refer to the suggested guidelines for the HGB/Turbidity Interference? IP Message.

Suspect, RBC Agglutination?

The RBC Agglutination? IP Message is determined by calculation and size comparison of certain RBC items (MCHC, MCH, RBC, Upper RBC histogram discriminator [RU%] *).

*The RU% is not a reportable parameter, but it is used in the RBC Agglutination algorithm.

Asterisks (*) appear next to the RBC, HCT, MCV, MCH and MCHC parameters. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

🚳 IPU - [Data Brows	erl	_											
File(F) Edit(E)	View(V)	Rec	ord(R) Actio	n(A) Rep	ort(P) Set	ting(S) Windov	/(W) Help(H)	Ver.:00-1	9 User Nan	ne:sysmex			
F1 S F2 MANUAL		F4	Aenu QC I	Files Work	list Explo	F8		Flight Validate	F12 Out	0 Upper	Lower	Last20	Delete
Positive Validat	ed		Ĩ ⊘	02/04,	630 /2016	4386102 15:22:45		υ γυ					
Main Gra	aph C	lumi	ulative	Q-Flag	js Ser	vice Rese _[WBC Diff	arch erential	÷				WBC	
Item [Data		Unit	LL	UL	Item	Data	Unit	LL	UL			n Shi AZ A Shi K
WBC RBC HGB HCT MCV MCH PLT RDW-SD RDW-CV MPV	5.85 0.62 10.2 7.1 114.5 164.5 143.7 377 9.5	* * * * * *	10^3/uL 10^6/uL g/dL % fL pg g/dL 10^3/uL fL % fL			I CCM NEUT# LYMPH# MONO# EO# BASO# Item NEUT% LYMPH% MONO% EO% BASO%	3.95 1.26 0.40 0.20 0.04 Data 67.6 21.5 6.8 3.4 0.7	10^3/uL 10^3/uL 10^3/uL 10^3/uL 10^3/uL 10^3/uL 0/3/uL					250fL
Flag(s) WBC		*	RBC RBC Ag Turb/H RBC Ab Dimorp	glut? GB? n Dst h Pop	F T	PLT	*				5 5	DIFF	ssc

XS-Series Results (initial run)

Suspect, RBC Agglutination? (continued)

XS-Series Results (after warming at 37°C)



Suggested Action Steps:

- 1. Follow your laboratory protocol and scan the peripheral smear for the presence of agglutinated RBCs or visually check the sample tube for agglutination.
- If agglutinated RBCs are present, warm the sample at 37°C for 15-30 minutes according to your laboratory policy. Reanalyze the warmed sample in the manual mode after mixing by manual inversion 10 times. Make a new peripheral smear from the warmed sample if agglutination is severe and WBCs and PLTs cannot be accurately assessed.
- NOTE: Sometimes agglutination can be so severe that warming the sample does not enable accurate analysis.

Suspect, RBC Agglutination? (continued)

- 3. In cases with high cold agglutinin titers, a plasma replacement using warm CELLPACK[®] may be necessary to reduce the interference from the antibody. Further warming post-plasma replacement may also be necessary.
 - a. To perform a plasma replacement
 - i. Centrifuge an aliquot of blood from the primary tube to separate the cells from the plasma.
 - ii. Using a pipette, remove a measured amount of plasma removing as much plasma as possible without disturbing the buffy coat.
 - iii. Add back the same amount of CELLPACK as the volume of plasma removed in step ii. (Example: If 0.5 mL of plasma is removed then add back 0.5 mL of CELLPACK.)
 - iv. Cap the tube and mix the sample by manual inversion until the cells are fully re-suspended in the CELLPACK.
 - v. Reanalyze the sample in the manual mode.
- 4. In cases where a warm-reacting antibody has caused agglutination, a plasma replacement may reduce the interference from the antibody. Room temperature CELLPACK may be used to replace the plasma.

Suspect, Turbidity/HGB Interference?

The Turbidity/HGB Interference? IP Message occurs when the MCHC is >36.5 g/dL and indicates that turbidity may be present in the diluted and lysed sample. This turbidity could interfere with the HGB detection light path and falsely increase the HGB value. Other interfering substances or conditions may impact the hematocrit and also cause an increased MCHC.

Asterisks (*) appear next to the HGB, MCH and MCHC parameters. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.

🗇 IPU - [Data Bro	owser]	100											
File(F) Edit	(E) View(V)	Record(R)	Action(A) R	eport(P) Setti	ing(S) Windov	v(W) Help(I	H)	Ver.:00-1	9 User Nan	ne:admin			
F1 S F2 Help MANU	JAL	F4 III Menu	F5 e F6 QC Files W	ork list Explor	er Browser			F11 Validate	F12 Out	0 Upper	Lower	Last20	Delete
Positiv Valida	e ated		No. ⊘ 05/1	6619 9/2016	187802 10:06:42		N SC						
Main G	iraph	Cumulat	ive Q-F1	ags Serv	vice Rese WBC_Diff	arch erentia	a]-					WBC	1
Item WBC RBC HGB HCT MCV MCH MCH PLT RDW-SD RDW-CV MPV	Data 4.47 3.37 12.3 32.7 97.0 36.5 37.6 219 48.6 17.8 9.0	Un - 10^3 - 0/06 * 9/dL - % + fL * pg g/dL 10^3 + % - fL * %	it LL //uL //uL //uL //uL //uL		Item NEUT# LYMPH# MONO# EO# BASO# Item NEUT% LYMPH% MONO% EO% BASO%	Data 3.24 0.55 0.51 0.14 0.03 Data 72.5 12.3 11.4 3.1 0.7	- + -	Unit 10^3/uL 10^3/uL 10^3/uL 10^3/uL Unit % % % % %					FSC 250fL
Flag(s) WBC		RBC	rb/HGB?	• P	LT	*					5		550

XS-Series Results

NOTE: An MCHC up to 37.5 g/dL may indicate a normal specimen on the high end of normal range in which case no action is needed. This may occur more often in samples with higher hemoglobin and hematocrit results.

Consider the MCHC and the MCV together when evaluating results and the reasons for the interference. Refer to the following table for possible interferences and corrective actions.

Pattern of Resul	ts	Encountered in					
Low or Normal MCV High MCHC (>37.5 g/dL)		 Hemolysis Plasma electrolyte abnormalities (i.e., low sodium) affecting hematocrit results Severe lipemia Icterus Severe leukocytosis affecting hemoglobin measurement Abnormal plasma protein precipitation affecting hemoglobin measurement 					
High MCV High MCHC (>37.5 g/dL)		 RBC Aggl Rouleaux Refer to 1 	utination Froubleshooting Chart				
Always follow your loca	Troublesh	ooting Chart	ng or rejection of samples				
Low Sodium Affecting Hematocrit? 1. Perform a 1:5 dilution of sample with CELLPACK 2. Allow the dilution to equilibrate for ten to fifteen	RBC Aggluti 1. Prewa for fift minut 2. Sever agglu roulez	nation? arm at 37°C een to thirty es then rerun re cold tinins or	Severe Lipemia, Icterus, Abnormal Protein or Leukocytosis Affecting Hemoglobin Measurement or Hemolysis? 1. Perform a 1:5 dilution of sample with CELLPACK 2. Repeat diluted sample 3. Correct results for				
 minutes 3. Rerun after equilibration 4. Correct results for dilution factor prior to reporting. NOTE: MCV, MCH, MCHC, RDW-SD, RDW- CV, MPV and differential percent results are unaffected by dilution and do not require correction. 	requir plasm replac CELL 3. For se agglu additi incub may b follow or pla replac	e dilution or na cement with PACK. evere cold tinins, onal ation at 37°C oe necessary ring dilution sma cement.	 Confect results for dilution factor prior to reporting. Lipemia or Icterus Only Perform a plasma replacement procedure Hemolysis: Recollect a new sample. 				

Suspect, Turbidity/HGB Interference? (continued)

Suspect, Iron Deficiency?

The Iron Deficiency? IP Message is determined by calculation and size comparison of certain RBC items (MCV, RDW-CV).

NOTE: It is suggested to use this flag as an alert to evaluate the MCV and RDW-CV results. If your laboratory protocol does not require further action based on the MCV and RDW-CV results, results may be reported without further investigation.

Suspect, HGB Defect?

The HGB Defect? IP Message is determined by calculation and size comparison of certain RBC items (MCV and RDW-CV).

NOTE: It is suggested to use this flag as an alert to evaluate the MCV and RDW-CV results. If your laboratory protocol does not require further action based on the MCV and RDW-CV results, results may be reported without further investigation.

Suspect, Fragments?

The Fragments? IP Message is determined from calculation and size comparison of certain RBC and PLT items (MCV, RDW-SD, MCHC, RBC Lower Discriminator [RL]*, PLT Upper Discriminator [PU]*, PLT Upper Discriminator % [PU%]*).

*RBC lower discriminator, PLT upper discriminator, % of the PLT upper discriminator. These parameters are not reportable, and are used only in the algorithm for the Suspect, Fragments flag.

Asterisks (*) appear next to the RBC, HCT, MCV, MCH, MCHC, RDW-SD and RDW-CV parameters. The PLT parameter may also have an asterisk. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.



XS-Series Results

Platelet Histogram (Close-Up)



Normal Platelet Histogram (Reference)



XS-Series Flagging Interpretation Guide Document Number: 1269-LSS, Rev.1, September 2016

Suspect, Fragments? (continued)

Suggested Action Steps:

- 1. Scan the peripheral smear for the presence of fragmented RBCs and other poikilocytosis according to your local laboratory protocol.
- 2. Report the presence of any clinically significant RBC morphology according to your local laboratory protocol.

Abnormal, PLT Abn Distribution

The PLT Abn Distribution IP Message is generated by calculation and size comparison of certain PLT items (PDW*, % of PLT lower discriminator [PL%] *, % of upper discriminator [PU%] *, platelet mean-frequent volume [PMFV]*, platelet large cell ratio*, MPV, platelet upper discriminator [PU]*).

*These are all non-reportable parameters that are used as part of the flagging algorithm.

Dashes may appear in place of data for the MPV or the MPV may be marked with an asterisk (*). The asterisk (*) indicates these results may be unreliable and should be confirmed according to your local laboratory protocol prior to reporting.

🗇 IPU - [Data Browser] File(F) Edit(E) View(V) Record(R) Action(A) Report(P) Setting(S) Window(W) Help(H) Ver.:00-19 User Name:sysmex F4 F6 F5 🐳 🏈 F12 P 1 0 MANUA Browser Menu QC Files Work list Explorer Validate Out Last20 Delete Upper Lower 087007172 Positive No. \odot 03/28/2013 15:58:00 Not Validated Main Graph |Cumulative Q-Flags Service Research Items WBC Differential WBC Unit LL LL UL Item Data UL Item Data Unit WBC 6.73 10^3/uL NEUT# 4.43 10^3/uL 8.64 @ 10^6/uL RBC LYMPH# 1.74 10^3/uL 25.8 @ g/dL HGB MONO# 0.26 10^3/uL RBC 75.5 @ % FO# 0.29 10^3/uL HCT MCV 87.4 fL BASO# 0.01 10^3/uL 29.9 MCH pq MCHC 34.2 g/dL * 10^3/uL Unit Item Data LL UL PLT 33 PI T 47.4 NEUT% 65.8 % RDW-SD fL + % * fL LYMPH% 25.9 % RDW-CV 18.0 3.9 % MONO% MPV 11.1 % FO% 4.3 BASO% 0.1 % DIFF Flag(s) WBC RBC PLT PLT Abn Dst Thrombo

XS-Series Results

Platelet Histogram (Close-Up)



Normal Platelet Histogram (Reference)



Abnormal, PLT Abn Distribution (continued)

Suggested Action Steps:

- 1. Review results according to your local laboratory protocol. Possible actions include:
 - a. Scan the peripheral smear to estimate the platelet count and review for the presence of abnormal RBC or PLT morphology such as:
 - large or giant platelets
 - small platelets
 - platelet clumps
 - fragmented RBCs
 - microcytic RBCs
 - parasites
 If abnormal RBC, PLT or other morphology is noted, report according
 to your local laboratory protocol.

NOTE: Reviewing the feathered edge and sides of the peripheral smear is suggested as platelet clumps and fibrin strands may migrate to this area during smear preparation.

- 2. If platelet estimate confirms accuracy of analyzer count, it may be reported.
- 3. If platelet estimate does not confirm accuracy of analyzer count, confirm with an alternate method such as a manual platelet count according to your local laboratory protocol. Depending on the source of the interference, the analyzer PLT count may be falsely increased or decreased. Report any clinically significant RBC and/or PLT morphology according to your local laboratory protocol.
- 4. If platelet clumps have interfered, perform one of the alternate procedures recommended in the section Suggested Actions for PLT Clumps? IP Message.

Suspect, PLT C(S)?

The PLT C(S)? IP Message is generated by calculation and size comparison of certain PLT items (PDW*, % of PLT lower discriminator [PL%] *, % of upper discriminator [PU%] *, platelet mean-frequent volume [PMFV]*, platelet large cell ratio*, MPV, platelet upper discriminator [PU]*).

*These are all non-reportable parameters that are used as part of the flagging algorithm.

Asterisks (*) will appear next to the PLT results. Dashes may appear in place of data for the MPV or the MPV may be marked with an asterisk (*). The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.



XS-Series Results

Suggested Action Steps:

- 1. Follow your local laboratory protocol.
- 2. Refer to the <u>suggested action steps for the PLT Clumps? IP Message</u> for further information.

Suspect, PLT Clumps?

The PLT Clumps? IP Message is determined by abnormal clustering in the DIFF scattergrams.

Asterisks (*) will appear next to the PLT result. Dashes may appear in place of data for the MPV or the MPV may be marked with an asterisk (*). The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting.



XS-Series Results

Suspect, PLT Clumps? (continued)

Suggested Action Steps:

- 1. Follow your local laboratory protocol. Possible actions may include:
 - a. Checking the sample for the presence of clots
 - b. Scanning the peripheral smear, especially the feathered edge, for the presence of abnormal morphology including:
 - fibrin strands
 - platelet clumps
 - i. If any of the above are present, verify the WBC and PLT by a manual slide estimate.
 - ii. If the WBC and PLT estimates match the analyzer counts, report the results according to your local laboratory protocol.
 - iii. If the estimates do not match the analyzer counts, refer to the next step to obtain an accurate count.

NOTE: Reviewing the feathered edge and sides of the peripheral smear is suggested as platelet clumps and fibrin strands may migrate to this area during smear preparation.

- 2. If platelet clumps or fibrin strands have interfered, perform one of the following alternate procedures to obtain an accurate count:
 - a. Re-draw specimen in EDTA and sodium citrate tubes if possible. Analyze re-drawn EDTA tube. If the repeat run has no PLT Clumps? IP Message, report these results.
 - b. If there is still a PLT Clumps? IP Message and platelet clumps are present on smear review it could be an in vitro reaction with EDTA. Analyze the sodium citrate tube. Obtain only the WBC and PLT counts from the sodium citrate tube as sodium citrate alters RBC morphology and indices.
 - c. Multiply the WBC and PLT results from the sodium citrate tube by the factor used by your laboratory.
 - d. If recollection is not possible or if platelet clumps persist when using sodium citrate, estimate the platelet count and report as decreased, adequate or increased and comment on the platelet clumps according to your local laboratory protocol.

Suspect, PLT Clumps? (continued)

NOTES:

- There are different methods for handling samples with platelet clumps. These
 methods include vortexing of the original sample, recollection and analysis of a
 new sample in sodium citrate or adding amikacin to the original sample and
 reanalyzing. Individual facilities should define and validate the method,
 anticoagulant type, and any dilution factors used.
- 2. Some samples with severe platelet clumping may not be resolved (or only be partially resolved) using any method. In such samples, the only option is to not report the numeric platelet result and instead report a platelet estimate from a review of a stained smear.
- 3. The incidence of completely unflagged instances of pseudothrombocyopenia is very low. However, to identify samples with pseudothrombocytopenia due to platelet clumping, multiple approaches must be employed together. Approaches for detecting platelet clumping may include:
 - a. Smear review based on analyzer generated or user defined flags such as "Thrombocytopenia" or "Platelet Abnormal Distribution", etc.
 - b. Use of delta checks comparing the current result against previous results
 - Review of smears based on both PLT and Mean Platelet Volume (MPV) results. (Low PLT with high MPV may indicate the presence of platelet clumps.)
 - d. Use of review criteria for results that fall between the "thrombocytopenia" threshold and a critical low value that might warrant transfusion support.

Action Messages

Count DIFF-CH

In the CBC discrete test selection, WBC measurement is performed using blood lysed with STROMATOLYSER[®] 4-DL and FSC (Forward Light Scatter) measurement. This message is generated in the CBC discrete test selection when interference is suspected in the WBC histogram based on the FSC measurement.

Asterisks (*) will appear next to the WBC result. The asterisk (*) indicates these results may be unreliable and should be confirmed according to your laboratory protocol prior to reporting. This action message may appear with the WBC Abnormal Scattergram flag.

The message is printed on the report and can be displayed on the Browser screen by selecting the red Action box as shown below.

💮 IPU - [Data Browser]		
File(F) Edit(E) View(V) Re	cord(R) Action(A) Report(P) Setting(S) Window(W) Help(H)	Ver.:00-19 User Name:sysmex
F1 S F2 F2 Help MANUAL	Image: Second system F5 Weights F6 Weights F7 Weights F8 Weights <	F1 F1 Validate Out Upper Lower Last20 Delete
Positive Action Validated	Na 5727366702 3-09 √27/2015 14:12:17 STAT ORDER	
Main Graph Cu Items Item Data WBC 2.84 * RBC 3.18 - HGB 9.4 - HCT 27.8 - MCV 87.4 MCV 87.4 MCH 29.6 MCHC 33.8 PLT 87 * RDW-SD 47.1 RDW-CV 15.4 + MPV 13.2 * Flag(s) WBC Abn Scg	Action Message(s) Action Message(s) Count DIFF-CH	

XS-Series Results

WBC Histogram (Close-Up)



Normal WBC Histogram (Reference)



XS-Series Flagging Interpretation Guide Document Number: 1269-LSS, Rev.1, September 2016

Action Message COUNT DIFF-CH (continued)

Suggested Action Steps:

- 1. Rerun the sample using the CBC+DIFF discrete test selection.
- 2. Verify WBC and differential results according to your laboratory's protocol and flagging which occurs on the CBC+DIFF run. Possible actions may include:
 - a. Scanning the slide for abnormal cells and to estimate the WBC count.
 - b. Performing a manual differential if abnormal cells are observed. If no abnormalities are found when reviewing the smear and the WBC estimate matches the analyzer reported WBC, the results may be reported according to your laboratory's protocol.

Interfering Substances

Some abnormal samples may interfere with automated cell counting methods. The following is a list from the Sysmex XS-Series Instructions for Use of possible substances that may interfere with these parameters.

- NOTE: Compromised samples, such as those not properly collected, stored, transported, or containing clots may cause misleading results. Always use good laboratory practices for inspecting specimens for acceptability and verifying results.
- WBC: Leukocyte aggregation, platelet aggregation, lyse resistant erythrocytes, erythrocyte aggregation (cold agglutinin), erythroblasts (nucleated red blood cells), cryoprotein, cryoglobulin, fibrin, giant platelets (Platelets > 1,000,000/µL)
- RBC: Erythrocyte aggregation (cold agglutinin), microcytosis, fragmented RBCs, leukocytosis (lymphocytes > 100,000/µL), giant platelets (Platelets > 1,000,000/µL)
- HGB: Leukocytosis (lymphocytes > 100,000/µL), lipemia, abnormal protein. The effect of abnormal proteins and lipemia may be removed by plasma replacement or plasma blank procedures.
- HCT: Erythrocyte aggregation (cold agglutinin), microcytosis, fragmented RBCs, leukocytosis (lymphocytes > 100,000/µL), severe diabetes (hyperglycemia), uremia, spherocytosis
- PLT: PLT aggregation, pseudothrombocytopenia, giant platelets, microcytosis, fragmented RBCs, fragmented leukocytes, cryoprotein, cryoglobulin
- NOTE: The Sysmex XS-Series Analyzer is designed to flag abnormal samples that may contain interfering substances. These results should be reviewed carefully and may require further examination in accordance with your local laboratory protocol.

References

BD TechTalk, Volume 8, No. 3, BD Diagnostics Preanalytical Systems, August 2010.

Brown, Barbara A. *Hematology: Principles and Procedures*, Sixth Edition, Lippincott Williams & Wilkins, Media, PA, 1993, pp. 102 – 107, 111 - 116.

CAP Today, Letters to the Editor, "Platelet Clumping", July 2014.

Cornbleet Joanne, M.D., "Spurious Results from Automated Hematology Cell Counters." *Laboratory Medicine*, Vol. 14, No. 8, August 1983.

Gulati GL, Asselta A, Chen C. Using vortex to disaggregate platelet clumps, Laboratory Medicine, 28:665, 1997.

Harmening, Denise M. Clinical Hematology and Fundamentals of Hemostasis, 3rd Edition, F. A. Davis Company, Philadelphia, PA, 1997.

Hagerman, R., "Ethylenediamainetetraacetic acid (EDTA) – Dependant Pseudothromocytopenia: A Case Report of an Incidental but Important Finding."www.priory.com/med/casepresentations. Accessed October 9, 2014

Koepke, John, "Lipemia and Hemoglobin Determinations," *Medical Laboratory Observer*, Vol. 15, No. 1, January 1983.

Rodak, Bernadette, *Diagnostic Hematology*, W. B. Sunders Company, Philadelphia, PA, 1995, pp. 618-619.

Schwartz RS, Silberstein LE, Berkman EM. "Autoimmune Hemolytic Anemias.": *Hematology: Basic Principles and Practice*, (eds. Hoffman R, et al). Churchill Livingston Inc. 1995; 710-729.

Stewart, Charles and Koepke, John. Basic Quality Assurance Practices for Clinical Laboratories, Van Nostrand Reinhold, 1989, p 189.

XS-1000*i*/XS-800*i* Instructions for Use, (North American Edition), March 2013.

Zhou X, Xiaoli W. Amikacin Can Be Added to Blood to Reduce the Fall in Platelet Count, American Journal of Clinical Pathology, 136:646-652, 2011.