This report has been corrected for the following responses on page 1: Note added to the Program Update.

COLLEGE of AMERICAN PATHOLOGISTS

Surveys and Anatomic Pathology Education Programs

Comprehensive Hematology with Automated Differential FH6-A 2017

Participant Summary 0.5 Hours of Self-Reported Training Available

This document is available on e-LAB Solutions.

© 2017 College of American Pathologists. The College does not permit reproduction of any substantial portion of the material in this Report without its written authorization. The College hereby authorizes participants in the program to use the material in this Report solely for educational purposes within their own institutions. The College prohibits use of the material in the Report — and any unauthorized use of the College's name or logo — in connection with promotional efforts by marketers of laboratory equipment, reagents, materials, or services. Data from this program do not necessarily indicate the superiority or inferiority of instruments, reagents, or other materials used by participating laboratories. Use of these data to suggest such superiority or inferiority may be deceptive and misleading. The College will take all steps open to it under the law to prevent unauthorized reproduction of substantial portions of the material in this Report, deceptive use of any such material, and any unauthorized use of the College's name or logo in connection with promotional efforts by marketers of laboratory equipment, reagents, material, and any unauthorized use of the Section of substantial portions of the material in this Report, deceptive use of any such material, and any unauthorized use of the College's name or logo in connection with promotional efforts by marketers of laboratory equipment, reagents, materials, or services.

TABLE OF CONTENTS

Program Update	1
Evaluation Criteria	2
White Blood Cell Count	4
Red Blood Cell Count	5
Hemoglobin	6
Hematocrit	7
MCV	8
MCH	9
	10
Platelet Count	11
MPV	12
RDW	13
Red Cell Distribution Width Discussion	14
Neutrophils/Granulocytes	15
Lymphocytes	17
Monocytes	19
Eosinophils	21
Basophils	23
Blood Cell Identification - Graded	25
Discussion	30
Blood Cell Identification - Ungraded	31
- Discussion	36
Actions Labs Should Take When a PT Result is Not Graded	38
Self Reported Training	39

HEMATOLOGY AND CLINICAL MICROSCOPY RESOURCE COMMITTEE

Sherrie L Perkins, MD, PhD, FCAP, Chair Eric D. Hsi, MD, FCAP, Vice Chair

Parul Bhargava, MD, FCAP	Horatiu Olteanu, MD, PhD, FCAP
Chung-Che (Jeff) Chang, MD, PhD, FCAP	Jay L. Patel, MD, FCAP
David R. Czuchlewski, MD, FCAP	Natasha M. Savage, MD, FCAP
Yuri D. Fedoriw, MD, FCAP	Lauren B. Smith, MD, FCAP
John L. Frater Jr., MD, FCAP	Amy Thommasen, MD, BSc, FCAP
Michael R. Lewis, MD, FCAP	Lawrence Tsao, MD, FCAP
Etienne R. Mahe, MD, MSc, FCAP	Maria Vergara-Lluri, MD, FCAP
Megan O. Nakashima, MD, FCAP	Roberta L. Zimmerman, MD, FCAP
Jennifer L. Oliveira, MD, FCAP	Maria A. Proytcheva, MD, FCAP, ICSH, Liaison

2017 FH6-A PARTICIPANT SUMMARY

Program Update

The CAP appreciates your participation in the ungraded, online wildcard challenge, included in the Hematology Automated With Differential Series (FH series), Basic Hematology (HE), and Blood Cell Identification (BCP/BCP2) 2017 A mailing programs. The purpose of the challenge is to pilot the use of online images, rather than paper photographs, for cell identification. Data from this challenge will not appear in the PSR and will only be reviewed internally by the CAP.

Beckman Coulter users: For optimal proficiency testing (PT) grading, your laboratory should be enrolled in the Surveys program recommended for your instrument system, as follows:

Coulter Instrument System	Recommended Survey				
Councer matument System	FH3	FH6	FH13		
DxH 500	Х				
Gen-S, HmX, LH 500, MAXM series, STKS, VCS		Х			
LH 700 series, UniCel DxH			Х		

Don't Miss Out on this Educational Opportunity!

With your participation in CAP's Surveys programs, *every member of your team* can take part in education activities: earn Continuing Education (CE) credits or receive Self-Reported Training* at no additional charge.

This Survey mailing includes a Self-Reported Training activity. By reviewing the discussion that begins on page 30, your laboratory staff can earn **0.5 education hours** that can be used towards fulfilling education and certification of maintenance requirements. For your convenience, a form has been included to document your staff's participation in the activity. See page 39.

*CAP Self-Reported Training activities do not offer CE credit, but can be used towards fulfilling requirements for certification of maintenance by agencies such as the American Society of Clinical Pathology (ASCP). Please verify with your certifying agency to determine your education requirements.

Evaluation Criteria

Analytes regulated for proficiency testing appear in **bold** type.

Quantitative		
Analyte	Target Value	Evaluation Criteria
Basophils*	Peer Group	\pm 3 SD or \pm 1.0 (whichever is greater)
Eosinophils*	Peer Group	\pm 3 SD or \pm 1.0 (whichever is greater)
Hematocrit	Peer Group	± 6%
Hemoglobin	Peer Group	± 7%
Lymphocytes*	Peer Group	\pm 3 SD or \pm 1.0 (whichever is greater)
MCH	Peer Group	± 3 SD
MCHC	Peer Group	\pm 3 SD
MCV	Peer Group	\pm 3 SD
MPV	Peer Group	\pm 3 SD
Monocytes*	Peer Group	\pm 3 SD or \pm 1.0 (whichever is greater)
Neutrophils/Granulocytes*	Peer Group	\pm 3 SD or \pm 1.0 (whichever is greater)
Platelet Count	Peer Group	± 25%
RDW	Peer Group	\pm 3 SD
Red Blood Cell Count	Peer Group	± 6%
White Blood Cell Count	Peer Group	± 15%

Qualitative

.

<u>Analyte</u>	Evaluation Criteria
Blood Cell Identification*	80% referee or participant consensus

*Blood cell identification results are included in the CMS performance summary. In the event that Blood Cell Identification is not performed, results from the flow through differential will be reported.

The quantitative data tables provided in the Participant Summary report include the mean, SD, and %CV. Data are not included for methods used by fewer than 10 laboratories. The limits of acceptability are located on your participant evaluation report.

Your results are evaluated based upon a range of acceptability. The range is determined using a target value and a limit. There must be at least 10 laboratories in the peer group. If a peer group of 10 is not established, your results may be evaluated against the **Instrument** group mean.

To provide a timely evaluation of your results, statistics presented in this Participant Summary reflect participant data received by the due date.

Evaluation CriteriaThe CAP is required to submit PT results to the Centers for Medicare and Medicaid
Services (CMS) for all labs that have provided a CLIA identification number. If you do
not notify the CAP that your lab has discontinued testing of a regulated analyte,
a score of zero will be given. Your reporting preferences are outlined on the CMS
Analyte Reporting Selections document. If new products are ordered and/or
canceled, this may affect your reporting selections, so it is recommended that you
periodically check this report on e-LAB Solutions™, which will always reflect the most
up-to-date information. This information can also be obtained by calling the
Customer Contact Center at 800-323-4040, Option 1 (domestic) or 001-847-832-
7000, Option 1 (international).

In the event a result is not graded, a numeric code will appear next to your result. A definition of the code will appear on the first page of your evaluation. Please see "Actions Laboratories Should Take when a PT Result is Not Graded" on page 38.

White Blood Cell Count - x 10⁹/L

	No. Labs	Mean	S.D.	C.V.
Coulter HmX	125	11.95	0.27	2.2
Coulter LH 500	200	11.95	0.25	2.1
Coulter HmX	124	7.02	0.14	2.0
Coulter LH 500	202	7.00	0.15	2.1
Coulter HmX	125	3.28	0.13	3.9
Coulter LH 500	201	3.27	0.10	3.1
Coulter HmX	124	15.50	0.30	2.0
Coulter LH 500	198	15.42	0.32	2.0
Soulter HmX	124	5.05	0.13	2.7
Coulter LH 500	201	5.05	0.12	2.3

Red Blood Cell Count - x 10¹²/L

	No. Labs	Mean	S.D.	C.V.
Coulter HmX	124	3.411	0.075	2.2
Coulter LH 500	199	3.402	0.064	1.9
Coulter HmX	125	4.334	0.063	1.5
Coulter LH 500	199	4.324	0.067	1.5
Coulter HmX	123	2.926	0.058	2.0
Coulter LH 500	198	2.925	0.057	1.9
Coulter HmX	124	5.330	0.092	1.7
Coulter LH 500	199	5.308	0.102	1.9
Soulter HmX	124	3.448	0.066	1.9
Coulter LH 500	200	3.438	0.064	1.9

Hemoglobin

	No.	g/c	۱L		g/L		
	Labs	Mean	S.D.	C .V.	Mean	S.D.	
	1						
Instrument							
Coulter HmX	122	9.22	0.13	1.4	92.24	1.31	
O Coulter LH 500	199	9.21	0.13	1.4	92.13	1.26	
Instrument							
S Coulter HmX	124	11.93	0.17	1.4	119.28	1.70	
Coulter LH 500	200	11.88	0.18	1.5	118.76	1.81	
Æ							
Instrument							
Coulter HmX	123	6.92	0.11	1.6	69.17	1.10	
Coulter LH 500	200	6.92	0.11	1.6	69.25	1.09	
τ.							
Instrument							
Coulter HmX	123	16.66	0.22	1.3	166.55	2.22	
Coulter LH 500	200	16.56	0.22	1.3	165.60	2.15	
L.							
	400	40.50	0.45		405.05	4 50	
Coulter I H 500	123	10.53	0.15	1.4	105.35	1.50	
	133	10.51	0.14	1.5	103.00	1.40	

Hematocrit

	No.	%	, D		L/L		
	Labs	Mean	S.D.	C .V.	Mean	S.D.	
	1	1					
Coulter HmX Coulter LH 500	125 199	26.400 26.377	0.696 0.598	2.6 2.3	0.264 0.264	0.007 0.006	
Coulter HmX Coulter LH 500	123 197	34.878 34.863	0.595 0.636	1.7 1.8	0.349 0.349	0.006 0.006	
Coulter HmX Coulter LH 500	125 200	20.400 20.440	0.524 0.598	2.6 2.9	0.204 0.204	0.005 0.006	
					·		
Coulter HmX Coulter LH 500	123 199	48.041 47.960	0.909 1.014	1.9 2.1	0.480 0.480	0.009 0.010	
		1					
Soulter HmX Coulter LH 500	124 199	30.371 30.367	0.727 0.660	2.4 2.2	0.304 0.304	0.007 0.007	

MCV – Femtoliters (fL)

	No. Labs	Mean	S.D.	C.V.
Coulter HmX	123	77.25	0.81	1.0
Coulter LH 500	197	77.34	0.81	1.0
S-912 Coulter HmX Coulter LH 500	123 199	80.42 80.64	0.76 0.81	0.9 1.0
Coulter HmX	124	69.46	0.72	1.0
Coulter LH 500	198	69.69	0.71	1.0
Instrument Coulter HmX Coulter LH 500	124	89.98	0.91	1.0
	199	90.29	0.91	1.0
Soulter HmX	123	87.94	0.79	0.9
Coulter LH 500	199	88.21	0.84	0.9

MCH – Picograms (pg)

	No. Labs	Mean	S.D.	C.V.
Instrument				
Coulter HmX Coulter LH 500	123 198	27.03 27.10	0.55 0.54	2.0 2.0
Instrument				
Coulter LH 500	123 194	27.48 27.48	0.45 0.44	1.7 1.6
Coulter HmX Coulter LH 500	124 196	23.61 23.68	0.46 0.45	2.0 1.9
Coulter HmX Coulter LH 500	124 195	31.25 31.22	0.56 0.52	1.8 1.7
Coulter HmX Coulter LH 500	125 199	30.53 30.60	0.67 0.64	2.2 2.1

МСНС

	No.	No. g/dL			g/L		
	Labs	Mean	S.D.	C .V.	Mean	S.D.	
Instrument							
Coulter HmX	123	34.99	0.86	2.4	349.89	8.55	
Coulter LH 500	199	35.02	0.78	2.2	350.21	7.78	
Instrument							
Coulter HmX	122	34.22	0.63	1.8	342.21	6.31	
Coulter LH 500	198	34.06	0.64	1.9	340.64	6.37	
Instrument							
Coulter HmX	122	34 02	0 70	21	340 17	7 01	
Coulter LH 500	199	33.99	0.75	2.2	339.90	7.49	
Π							
	1	1					
Instrument	400		0.70			- • •	
Coulter HmX	123	34.74	0.70	2.0	347.45	7.01	
	197	34.56	0.62	1.8	345.58	6.19	
Instrument							
2 Coulter HmX	122	34 75	0.79	23	347 52	7 94	
Goulter I H 500	198	34.70	0.75	2.3	347.04	7.47	
	100	00	0.70	2.2	017.01	,	

Platelet Count – x10⁹/L

	No. Labs	Mean	S .D.	C.V.
Coulter HmX	125	445.6	16.1	3.6
Coulter LH 500	200	447.0	14.8	3.3
Coulter HmX	124	228.8	7.9	3.4
Coulter LH 500	200	228.6	7.6	3.3
Coulter HmX	125	83.0	3.5	4.2
Coulter LH 500	199	81.4	3.8	4.7
Instrument Coulter HmX Coulter LH 500	124	586.7	17.9	3.0
	199	596.8	18.4	3.1
Soulter HmX	123	101.9	4.5	4.4
Coulter LH 500	200	99.8	4.1	4.1

MPV – Femtoliters (fL)

	No. Labs	Mean	S.D.	C.V.
Coulter HmX Coulter LH 500	114 158	10.23 10.27	0.16 0.18	1.6 1.8
Coulter HmX Coulter LH 500	114 159	10.22 10.24	0.18 0.20	1.8 1.9
Coulter HmX Coulter LH 500	116 159	10.47 10.53	0.23 0.23	2.2 2.2
InstrumentCoulter HmXCoulter LH 500	114 157	10.29 10.34	0.16 0.18	1.5 1.7
Coulter HmX Coulter LH 500	116 158	10.32 10.34	0.20 0.20	2.0 1.9

RDW-% (RDW-CV)

	No. Labs	Mean	S.D.	C.V.
Coulter HmX	125	14.44	0.22	1.5
Coulter LH 500	196	14.46	0.20	1.4
Coulter HmX	123	13.49	0.21	1.5
Coulter LH 500	198	13.48	0.23	1.7
Coulter HmX	124	16.60	0.20	1.2
Coulter LH 500	198	16.60	0.21	1.3
Instrument Coulter HmX Coulter LH 500	125	13.20	0.20	1.5
	197	13.15	0.19	1.4
Coulter HmX	125	13.61	0.21	1.6
Coulter LH 500	198	13.63	0.20	1.5

RDW-fL (RDW-SD)

Due to fewer than ten labs reporting, no data is available.

Red cell distribution width (RDW-SD vs. RDW-CV) discussion:

The red cell distribution width (RDW) is a calculated value which quantitatively reflects the degree of anisocytosis, or variation in red blood cell size, in a given blood sample. The RDW, in conjunction with the mean cell volume (MCV) and other red cell indices, may be a useful parameter in the laboratory evaluation of anemia and other hematologic conditions. An elevated RDW generally conveys increased variation in red blood cell size, and is seen in a variety of clinical settings including iron deficiency, autoimmune hemolysis, and in some patients with myelodysplastic syndrome.

Many modern automated hematology analyzers produce two distinct RDW measurements. The most commonly used and reported in clinical practice is the coefficient of variation RDW (RDW-CV), which is based on the coefficient of variation of the red blood cell distribution volume. The RDW-CV is calculated using the formula below, and the reference range in adults is typically 11.0-15.0%.

$$RDW - CV = \frac{1SD}{MCV} \times 100$$

Another way of expressing the RDW is the red cell distribution width-standard deviation, or RDW-SD. The RDW-SD is an actual measurement of the width of the red cell distribution curve and provides an absolute value in femtoliters (fL). The RDW-SD more accurately reflects red cell anisocytosis because it is directly measured and is not influenced by the MCV. The reference range for RDW-SD in adults is typically 36-47 fL.

The RDW-CV and RDW-SD are different expressions of the RDW and laboratories should exercise caution so as not to confuse them for purposes of clinical reporting as well as proficiency testing.

Jay Patel, MD Hematology and Clinical Microscopy Resource Committee

References:

- 1. Constantino, BT. The red cell histogram and the dimorphic red cell population. *LabMedicine*. 2011; 42(5):300-308.
- 2. Kjeldsberg CR, Perkins SL, eds. *Practical Diagnosis of Hematologic Disorders*. 5th ed. Singapore: American Society for Clinical Pathology; 2010.
- MediaLab, Inc. Website. <u>http://www.medialabinc.net/spg579122/red_blood_cell_distribution_width_rdw_definition_a.aspx</u>. Accessed June 3, 2013.

Neutrophils/Granulocytes – %

	No. Labs	Mean	S.D.	C.V.
Coulter HmX	124	59.88	0.76	1.3
Coulter LH 500	183	59.91	0.74	1.2
Coulter HmX	124	59.03	0.75	1.3
Coulter LH 500	182	59.08	0.67	1.1
Coulter HmX	124	51.26	0.77	1.5
Coulter LH 500	182	51.34	0.72	1.4
Coulter HmX	123	69.66	0.79	1.1
Coulter LH 500	183	69.75	0.70	1.0
Coulter HmX	123	64.45	0.67	1.0
Coulter LH 500	184	64.45	0.64	1.0

Neutrophils/Granulocytes – x 10⁹/L

	No. Labs	Mean	S.D.	C .V.
Coulter HmX	115	7.165	0.192	2.7
Coulter LH 500	198	7.158	0.178	2.5
Coulter HmX	114	4.144	0.118	2.8
Coulter LH 500	199	4.137	0.108	2.6
Coulter HmX	115	1.664	0.073	4.4
Coulter LH 500	199	1.655	0.083	5.0
Coulter HmX	114	10.810	0.275	2.5
Coulter LH 500	196	10.754	0.250	2.3
Soulter HmX	114	3.256	0.094	2.9
Coulter LH 500	198	3.237	0.107	3.3

Lymphocytes – %

	No. Labs	Mean	S.D.	C.V.
Coulter HmX	124	25.67	0.58	2.3
Coulter LH 500	182	26.00	0.63	2.4
Coulter HmX	123	29.11	0.66	2.3
Coulter LH 500	182	29.46	0.58	2.0
Coulter HmX	126	35.46	0.68	1.9
Coulter LH 500	182	35.86	0.62	1.7
Instrument Coulter HmX Coulter LH 500	124	19.00	0.55	2.9
	181	19.20	0.63	3.3
Soulter HmX	124	25.56	0.65	2.5
Coulter LH 500	184	25.90	0.59	2.3

Lymphocytes – x 10⁹/L

	No. Labs	Mean	S.D.	C.V.
Coulter HmX	115	3.071	0.099	3.2
Coulter LH 500	197	3.108	0.106	3.4
Coulter HmX	113	2.050	0.072	3.5
Coulter LH 500	195	2.062	0.063	3.1
Coulter HmX	116	1.162	0.057	4.9
Coulter LH 500	197	1.174	0.049	4.2
Coulter HmX	114	2.941	0.101	3.4
Coulter LH 500	195	2.966	0.115	3.9
Soulter HmX	114	1.294	0.050	3.9
Coulter LH 500	197	1.308	0.048	3.7

Monocytes – %

	No. Labs	Mean	S.D.	C.V.
Coulter HmX	124	8.377	0.330	3.9
Coulter LH 500	183	8.498	0.347	4.1
Soulter HmX	124	7.788	0.305	3.9
Coulter LH 500	182	7.844	0.330	4.2
Coulter HmX	125	7.753	0.320	4.1
Coulter LH 500	182	7.745	0.324	4.2
Instrument Coulter HmX Coulter LH 500	123	8.801	0.419	4.8
	182	8.963	0.353	3.9
Soulter HmX	123	7.723	0.301	3.9
Coulter LH 500	182	7.838	0.319	4.1

Monocytes – x 10⁹/L

	No. Labs	Mean	S.D.	C.V.
Coulter HmX	114	1.003	0.047	4.7
Coulter LH 500	193	1.016	0.056	5.5
Coulter HmX	114	0.548	0.049	8.9
Coulter LH 500	195	0.547	0.050	9.1
Coulter HmX	115	0.254	0.048	18.8
Coulter LH 500	195	0.250	0.050	19.9
Coulter HmX	113	1.369	0.075	5.5
Coulter LH 500	194	1.383	0.076	5.5
Soulter HmX	112	0.399	0.006	1.5
Coulter LH 500	194	0.400	0.002	0.6

Eosinophils – %

	No. Labs	Mean	S.D.	C.V.
Coulter HmX	123	6.03	0.45	7.5
Coulter LH 500	182	5.60	0.47	8.3
Coulter HmX	125	4.09	0.34	8.4
Coulter LH 500	181	3.60	0.38	10.7
Coulter HmX	125	5.50	0.41	7.4
Coulter LH 500	182	5.07	0.38	7.5
Coulter HmX	122	2.47	0.36	14.6
Coulter LH 500	178	2.05	0.25	12.4
S-999999999999999999999999999999999999	121	2.23	0.34	15.3
	181	1.82	0.23	12.5

Eosinophils – x 10⁹/L

	No. Labs	Mean	S.D.	C.V.
Coulter HmX	114	0.721	0.062	8.6
Coulter LH 500	195	0.663	0.062	9.4
Coulter HmX	108	0.300	0.007	2.3
Coulter LH 500	196	0.253	0.050	19.8
Soulter HmX	112	0.199	0.005	2.7
Coulter LH 500	195	0.186	0.034	18.3
InstrumentCoulter HmXCoulter LH 500	111	0.383	0.063	16.4
	192	0.315	0.046	14.6
Soulter HmX	107	0.101	0.004	3.9
Coulter LH 500	193	0.100	0.000	0.0

Basophils – %

	No. Labs	Mean	S .D.	C.V.*
Coulter HmX	125	0.01	0.03	*
Coulter LH 500	168	0.00	0.00	0.0
Coulter HmX	114	0.00	0.00	0.0
Coulter LH 500	175	0.00	0.00	0.0
Coulter HmX	123	0.01	0.03	*
Coulter LH 500	173	0.00	0.00	0.0
Instrument Coulter HmX Coulter LH 500	112	0.00	0.00	0.0
	166	0.00	0.00	0.0
Coulter HmX	118	0.00	0.00	0.0
Coulter LH 500	177	0.00	0.00	0.0

*When low results are reported on an analyte, a high coefficient of variance (CV) may result.

Basophils – x 10⁹/L

	No. Labs	Mean	S.D.	C.V.
Coulter HmX	112	0.000	0.000	0.0
Coulter LH 500	197	0.000	0.000	0.0
Coulter HmX	114	0.000	0.000	0.0
Coulter LH 500	196	0.000	0.000	0.0
Soulter HmX	113	0.000	0.000	0.0
Coulter LH 500	197	0.000	0.000	0.0
Instrument Coulter HmX Coulter LH 500	109	0.000	0.000	0.0
	197	0.000	0.000	0.0
Souter HmX	113	0.000	0.000	0.0
Coulter LH 500	197	0.000	0.000	0.0

Case History

This peripheral blood smear is from a 34-year-old woman presenting with systemic sclerosis. Laboratory data include: WBC = $3.8 \times 10E9/L$; RBC = $4.42 \times 10E12/L$; HGB = 13.3 g/dL; HCT = 39.8%; MCV = 90 fL; and PLT = $215 \times 10E9/L$. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

To access the online Hematology Glossary, please click the hyperlink below:

http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution%20Folders/WebContent/pdf/hematolog y-glossary.pdf



1	
	ļ
•	
Ć	5
ĥ	1

	Refe	erees	Partic	ipants	
Identification	No.	%	No.	%	Evaluation
Neutrophil containing Pelger-Huët nucleus (acquired or congenital)	86	90.5	5092	90.1	Good
Neutrophil, segmented or band	9	9.5	492	8.7	Unacceptable

The arrowed cell is a neutrophil with Pelger-Huët nucleus, as correctly identified by 90.5% of referees and 90.1% of participants. Neutrophils with abnormally unilobed or bilobed nuclei in the pince-nez conformation (two round nuclear lobes connected by a distinct thin filament) are designated as neutrophils with Pelger-Huët nuclei or as Pelger-Huët cells. They occur as an inherited autosomal dominant abnormality of nuclear segmentation referred to as Pelger-Huët anomaly. The nuclear chromatin in Pelger-Huët cells is generally denser than in normal cells. This feature helps to differentiate Pelger-Huët cells from band neutrophils and immature granulocytes such as myelocytes or metamyelocytes which may be seen in the context of a granulocytic left-shift and show more open or lightly staining chromatin. Neutrophils with identical nuclear features are occasionally observed in association with other clinical conditions, including myelodysplastic syndrome (MDS), infection and drug effect. The proportion of nuclei affected in these situations is variable but typically only a small subset of cells are affected, which is a clue since individuals with true Pelger-Huët anomaly usually demonstrate the morphologic abnormality in the majority of their neutrophils. When these cells are seen outside of the context of the congenital abnormality, they are usually referred to as neutrophils with dysplastic nuclei or pseudo-Pelger-Huët cells. However, for proficiency testing purposes, cells with pseudo-Pelger-Huët nuclei are best defined as Pelger-Huët cells.

		Proper	ty of the CAP		
Identification	Refe No.	rees %	Partic No.	ipants %	Evaluation
Eosinophils, any stage	95	100.0	5651	100.0	Good

The arrowed cell is an eosinophil, as correctly identified by 100.0% of referees and 100.0% of participants. The eosinophil is characterized by coarse, orange-red granules of uniform size and is similar to a neutrophil in diameter (10 to 15 μ m). Normally, the nucleus demonstrates condensed chromatin and nuclear segmentation with two or three nuclear lobes. This arrowed eosinophil is bilobed, but unilobate forms may also be seen due to the aforementioned Pelger-Huët anomaly.



The arrowed cell is a basophil, as correctly identified by 100.0% of referees and 99.7% of participants. Basophils are the least common circulating granulocytes. Unlike neutrophils with 3-5 lobed nuclei and fine pink or eosinophilic granules, basophils typically have only two prominent nuclear lobes and cytoplasm with numerous dense purple or basophilic granules, often obscuring the nuclear detail. Basophils are an important part of the allergic immune response, and infrequently circulate in appreciable number (typically representing <0.3% of peripheral leukocytes).

BCP-03



4	
Ω	
0	
~	

	Refe	rees	Partic	ipants	
Identification	No.	%	No.	%	Evaluation
Lymphocyte	90	94.7	5452	96.4	Good
Lymphocyte, reactive	4	4.2	62	1.1	Unacceptable
Nucleated red cell, normal or abnormal morphology	1	1.1	127	2.3	Unacceptable

The arrowed cell is a lymphocyte, as correctly identified by 94.7% of referees and 96.4% of participants. This cell shows features of mature, non-reactive lymphocytes and is a normal constituent of peripheral blood. The typical lymphocyte is slightly larger than a normal red blood cell with scant to moderate pale blue cytoplasm, round nuclear contours, mature chromatin and inconspicuous nucleoli.



	Refe	rees	Partic	ipants	
Identification	No.	%	No.	%	Evaluation
Ovalcyte (elliptocyte)	92	96.8	5619	99.4	Good
Erythrocyte, normal	2	2.1	16	0.3	Unacceptable
Stomatocyte	1	1.1	2	0.0	Unacceptable

The arrowed cell is an ovalocyte (elliptocyte), as correctly identified by 96.8% of referees and 99.4% of participants. The term ovalocyte is used interchangeably with the term elliptocyte, since these red blood cell types have similar disease associations and overlapping morphologic characteristics that make their distinction difficult. Classically, elliptocytes are described as elongated red blood cells with parallel or nearly parallel sides and a concentration of hemoglobin at the ends. Ovalocytes also have an elongated appearance as well but may be differentiated from elliptocytes by having slightly to moderately round rather than straight sides. Central pallor is preserved. Ovalocytes are encountered in a variety of conditions including thalassemia, megaloblastic and iron deficiency anemia, and sickle cell disease. Rare ovalocytes may also be observed in blood smears from normal individuals or as an artifact of smear preparation.

Case Presentation:

This peripheral blood smear is from a 34-year-old woman presenting with systemic sclerosis. Laboratory data include: WBC = $3.8 \times 10E9/L$; RBC = $4.42 \times 10E12/L$; HGB = 13.3 g/dL; HCT = 39.8%; MCV = 90 fL; and PLT = $215 \times 10E9/L$.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Pelger-Huët Anomaly

The Pelger-Huët anomaly refers to a genetic defect which results in characteristically bilobed or unilobed mature granulocyte nuclei. Dr. Karl Pelger, a Dutch hematologist, first described the morphologic features in 1928. Pediatrician G.J Huët established the inherited nature of the abnormality in 1932 when he recognized it in a young girl along with several of the child's relatives. The general incidence of the Pelger-Huët anomaly varies from 0.1-0.01% but may be slightly higher in certain European populations. Neutrophils are most prominently affected and classically show a bilobed nucleus with the lobes separated by a delicate thin filament (so-called spectacle-like or pince-nez formation). The chromatin of affected cells is typically clumped and appears denser than that of normal granulocytes. This feature helps to differentiate Pelger-Huët cells from neutrophil bands which are commonly seen in a granulocytic left-shift and have more open or lightly staining chromatin. Cytoplasmic granulation is usually normal. Other cell lineages, such as monocytes and lymphocytes, are unaffected.

The morphologic phenotype is causally related to mutations in *LBR*, the gene that encodes the lamin B receptor. Lamin B receptor is a constituent of the neutrophil nuclear membrane and is required for normal morphologic development. The Pelger-Huët anomaly is inherited in an autosomal dominant fashion. In the heterozygous state, most of the neutrophils have bilobed nuclei. Individuals with homozygous Pelger-Huët associated gene mutation are very rare and typically demonstrate unilobed nuclei in mature neutrophils. Heterozygous individuals with concurrent infection or systemic inflammation due to granulocytic left-shift may mimic the homozygous state. Detection of Döhle bodies or toxic granulation provides clues to the presence of a left-shift. Notably, individuals with Pelger-Huët anomaly do not appear to be at increased risk for infection, as their neutrophils retain normal functional capability.

Neutrophils with identical nuclear features are occasionally observed as an acquired abnormality in association with various other clinical conditions and in such settings they are referred to as pseudo-Pelger-Huët cells. These include myeloid malignancies such as myelodysplastic syndrome, acute myeloid leukemia, and chronic myelogeneous leukemia. In addition, pseudo-Pelger-Huët cells may be detected in patients with infection and have been linked to HIV, influenza, and mycoplasma. Lastly, a variety of drugs including sulfonamides, colchicine, valproic acid, mycophenolate mofetil, and tacrolimus have been associated with pseudo-Pelger-Huët cells. The proportion of nuclei affected in these situations is variable, but normally segmented neutrophils are usually identifiable.

Jay L. Patel, MD Hematology and Clinical Microscopy Resource Committee

References:

- 1. Kjeldsberg CR, Perkins SL, eds. *Practical Diagnosis of Hematologic Disorders*. 5th ed. Singapore: American Society for Clinical Pathology; 2010.
- 2. Pereira I, George TI, Arber DA, eds. *Atlas of Peripheral Blood: The Primary Diagnostic Tool.* Philadelphia: Wolters Kluwer; 2012.
- 3. Colella R, Hollensead SC. Understanding and recognizing the Pelger-Huët anomaly. *American Journal of Clinical Pathology.* 2012;137:358-366.

Case History

This peripheral blood smear is from a 65-year-old woman with past medical history of breast carcinoma presenting with fatigue. Laboratory data includes: WBC = 14.7 × 10E9/L; RBC = 2.52 × 10E12/L; HGB = 7.6 g/dL; HCT = 22.7%; MCV = 93 fL; PLT = 52 × 10E9/L; and MPV = 7.2 fL. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

To access the online Hematology Glossary, please click the hyperlink below:

http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution%20Folders/WebContent/pdf/hematologyglossary.pdf



The arrowed cells are tear drop cells (dacrocytes), as correctly identified by 100.0% of referees and 99.7% of participants. Red cells appearing in the shape of a teardrop or a pear with a single, short or long, often blunted or rounded end are called teardrop cells. These are commonly seen in primary myelofibrosis but may also be seen in pernicious anemia, anemia of renal disease, hemolytic anemias, and other forms of severe anemia. These cells are often associated with an abnormal spleen or bone marrow. Bone marrow infiltration with hematologic and non-hematologic malignancies may also be accompanied by dacrocytosis. Teardrop cells may be seen as an artifact of slide preparation; such dacrocytes are usually easily recognized from the fact that their "tails" all point in the same direction.



The arrowed cell is a neutrophil, myelocyte, as correctly identified by 87.4% of referees and 87.0% of participants. The transition from promyelocyte to myelocyte occurs with the end of production of azurophilic (primary) granules and the beginning of production of lilac or pale orange/pink (specific) granules. Myelocytes are usually confined to the marrow where they constitute approximately 10% of the nucleated cells. In pathologic states, myelocytes are seen in blood. The myelocyte is smaller than the earlier precursors, usually 10 to 18 µm. The cells are round to oval in shape and have a nuclear-to-cytoplasmic ratio of 2:1 to 1:1. The nucleus is slightly eccentric, lacks a nucleolus, and begins to demonstrate chromatin clumping; one side often shows slight flattening. Sometimes a clear space or hof is seen adjacent to the nucleus, indicating the location of the Golgi apparatus. The cytoplasm is relatively more abundant than in earlier precursors and is amphophilic. Both azurophilic and specific granules are present in the cytoplasm with specific granules coming to predominate as maturation progresses. See BCP-08 for discussion of a promyelocyte.

BCP-07



	Referees		Participants		
Identification	No.	%	No.	%	Evaluation
Neutrophil, promyelocyte	75	79.0	4503	81.9	Educational
Neutrophil, promyelocyte abnormal containing/lacking Auer rod(s)	12	12.6	399	7.3	Educational
Neutrophil containing dysplastic nucleus and/or hypogranular cytoplasm	1	1.1	2	0.0	Educational
Lymphocyte, large granular	1	1.1	5	0.1	Educational
Malignant lymphoid cell (other than blast)	1	1.1	17	0.3	Educational

The arrowed cell is a neutrophil, promyelocyte, as correctly identified by 79.0% of referees and 81.9% of participants. Promyelocytes are round to oval cells that are generally slightly larger than myeloblasts; the diameter is 12 to 24 μ m. They are normally confined to bone marrow, where they constitute less than 2% of nucleated cells; but like the myeloblast, they can be seen in the blood in pathologic states. The nuclear -to-cytoplasmic ratio is high – 5:1 to 3:1. The nucleus is round to oval, has fine chromatin, and contains distinct nucleoli. The cytoplasm is basophilic, more plentiful than in a myeloblast, and contains multiple distinct azurophilic (primary) granules. A paranuclear hof or cleared space may be present.

The arrowed cell is a "neutrophil, promyelocyte" and distinct from a "neutrophil, promyelocyte abnormal", which is the neoplastic cell in acute promyelocytic leukemia (APL). An abnormal promyelocyte differs from a promyelocyte in several respects. The abnormal promyelocyte nucleus is usually folded, bilobed, or reniform, often with overlapping nuclear lobes; a distinct Golgi zone is typically absent. Cytoplasmic granules, while abundant in the classic hypergranular form of APL, may differ in appearance, often being coarser or finer than those seen in normal promyelocytes and slightly darker or more reddish in color. In the microgranular variant of APL, very few granules may be visible and those granules present may be very fine. Finally, the abnormal promyelocyte of APL frequently contains numerous overlapping Auer rods. The arrowed cell in this question has normal nuclear contours and a distinct Golgi zone. Moreover, the granules have a typical appearance in regards to color, number, and texture. Lastly no Auer rod is seen.



The arrowed cell is a nucleated red blood cell (nRBC), as correctly identified by 100.0% of referees and 99.1% of participants. The term *nucleated red blood cell* is used to state the presence of normoblasts in the peripheral blood and includes all normoblasts regardless of the stage of maturation. Typically, the circulating nucleated red cell is at the orthochromic stage of differentiation. Both megaloblastic and dysplastic changes can be seen in these circulating red cells, reflecting simultaneous erythroid maturation abnormalities present in the bone marrow. Caution should be used in classifying a circulating nucleated red cell as dysplastic on the basis of abnormal nuclear shape (lobated or fragmented), as these changes may occur during their egress from the marrow space and may not be present in the maturing erythroids precursors present in the marrow. For the purposes of proficiency testing, it is adequate to identify a cell as a nucleated red cell when it is present in the peripheral blood, be it normal or abnormal (ie. exhibits megaloblastic or dysplastic changes).



	Refe	erees	Partic	ipants	
Identification	No.	%	No.	%	Evaluation
Blast cell	81	85.3	4806	87.5	Educational
Lymphocyte, reactive	3	3.2	124	2.3	Educational
Monocyte, immature (promonocyte, monoblast)	3	3.2	85	1.6	Educational
Malignant lymphoid cell (other than blast)	3	3.2	106	1.9	Educational
Lymphocyte, large granular	1	1.1	10	0.2	Educational

The arrowed cell is a blast cell, as correctly identified by 85.3% of referees and 87.5% of participants. A blast is a large, round to oval cell, 10 to 20 µm in diameter. In the blood film, the cell may appear flattened or compressed by adjacent red cells. The nuclear-to-cytoplasmic ratio is high, varying from 7:1 to 5:1. The blast often has a round to oval nucleus, but sometimes it is indented or folded; and it has fine, lacy or reticular chromatin. One or more prominent nucleoli may be seen. The cytoplasm is variably basophilic and typically agranular. The morphologic features of a blast cell do not permit determination of the cell lineage, ie. myeloblast versus lymphoblast. The one exception is the presence of Auer rods, which are diagnostic of myeloid lineage (ie. myeloblast). Other cells may have the appearance of a blast, including some lymphoma cells. In the absence of Auer rods, immunophenotyping by flow cytometry, immunohistochemistry on tissue sections or, less commonly, cytochemical staining (eg. peroxidase or Sudan black B reactivity) is required to determine the lineage of a given blast cell. As blasts are quite variable in appearance, it is often impossible to correctly classify an individual cell based on the morphology alone. Blasts can be morphologically indistinguishable from lymphoma cells. For identification purposes, one should classify individual cells exhibiting this type of morphology as blast cells when additional confirmatory information is unavailable.

Case Presentation:

This peripheral blood smear is from a 65-year-old woman with past medical history of breast carcinoma presenting with fatigue. Laboratory data includes: WBC = $14.7 \times 10E9/L$; RBC = $2.52 \times 10E12/L$; HGB = 7.6 g/dL; HCT = 22.7%; MCV = 93 fL; PLT = $52 \times 10E9/L$; and MPV = 7.2 fL.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Myelophthisic Smear

The peripheral blood smear is remarkable for mild leukocytosis with granulocyte left shift including blast cells, normocytic anemia with presence of nucleated red blood cells and many dacrocytes (tear drop cells), and moderate thrombocytopenia. These findings along with the provided clinical history are consistent with myelophthisic anemia. Myelophthisic anemia is defined as anemia secondary to marrow infiltration. This may include overt leukoerythroblastosis with immature granulocytes (often times myelocytes and metamyelocytes but sometimes even myeloblasts) and nucleated red blood cells in the peripheral blood or may present with only a few tear drop cells (dacrocytes) in the smear. Although leukoerythroblastosis may be alarming and raise suspicion of a marrow infiltrative process, several conditions may result in this finding in peripheral blood smear. These include premature infants or newborns, severe infection/trauma, and regeneration after marrow insult/injury including chemotherapy among other etiologies. However, leukoerythroblastosis with associated prominent dacrocytes (tear drop cells) is more ominous and suggestive (although not definitive) of a marrow infiltrative process.

Marrow infiltrative processes include granulomas such as those seen in sarcoidosis or miliary tuberculosis, storage disorders with histiocyte proliferations including Gaucher disease, and metastatic malignancy. Regarding metastasis, most patients present with bicytopenia or pancytopenia with anemia being the most common finding. The anemia may be a result of anemia of chronic disease, nutritional deficiency, microangiopathic process (disseminated intravascular hemolysis is often associated with mucin producing tumors), recent anti-neoplastic therapy, and/or marrow replacement. Interestingly, the mean platelet volume (MPV) can predict likelihood of marrow metastasis in patients with thrombocytopenia and known solid tumor. Specifically, a MPV of < 7.4 fL was found to have significant predictive value and correlates with bone marrow metastasis. Metastatic tumor cells are very rarely seen in the peripheral blood, but when noted are often in the feathered edge and may have the appearance of a lymphoma or blast cell. Ultimately, bone marrow biopsy is needed in patients with myelophthisic anemia to determine exact etiology and multiple and bilateral biopsies may be needed to sample a potentially patchy process.

The likely identification of the metastatic malignancy varies depending on age and sex of the patient. In children, neuroblastoma is the most common metastatic cause by far, but other small round blue cell tumors are reported. In adult females, breast and lung carcinoma (oftentimes small cell carcinoma) predominate. In males, prostate and again lung carcinoma are most frequent. In addition, gastrointestinal adenocarcinomas are reported with some frequency. Rarely sarcomas and melanoma can be seen infiltrating the bone marrow.

Lastly, leukoerythroblastosis and dacrocytes can be seen in patients with hematopoietic neoplasms, with primary myelofibrosis (PMF) being the prototype. However, other hematopoietic neoplasms may also be seen including other myeloproliferative neoplasms, myelodysplastic syndromes with fibrosis, and acute leukemias with fibrosis. Moreover, lymphoma including classical Hodgkin lymphoma can cause marrow replacing lesions with accompanying fibrosis. The finding of large abnormal platelets may suggest PMF among other myeloid neoplasms; the presence of overtly dysplastic granulocytes may also support that a myeloid neoplasm as opposed to a non-hematopoietic neoplasm is inducing a myelophthisic anemia. Finally, overtly malignant myeloblasts such as those containing Auer rods would also confirm a myeloid neoplasm as opposed to a metastasis resulting in myelophthisic anemia.

Natasha M. Savage, MD Hematology and Clinical Microscopy Committee

References:

- 1. Aksoy S, Kilickap S, Hayran M, Harputluoglu H, Koca E, Dede DS, Erman M, Turker A. Platelet size has diagnostic predictive value for bone marrow metastasis in patients with solid tumors. *J Lab Hematol.* 2008;30(3):214-219.
- 2. Bain BJ. Blood Cells: A Practical Guide. Carlton, Victoria (Australia): Blackwell Publishing; 2006; p. 233.
- 3. Hutchison RE. *Nonhematopoietic Neoplasms of the Bone Marrow.* In: Jaffe ES, Harris NL, Vardiman JW, Campo E, Arber DA, eds. Hematopathology. St. Louis, Missouri (USA): Elsevier Saunders; 2011; p. 939-950.
- 4. Reichard K. *Metastatic Lesions in the Bone Marrow*. In: Foucar K, Reichard K, Czuchlewski D, eds. Bone Marrow Pathology. Chicago, Illinois (USA): ASCP Press; 2010; p. 686-701.

Actions Laboratories Should Take when a PT Result is Not Graded

The College uses Exception Reason Codes that signify the proficiency testing (PT) for an analyte has not been graded. The Exception Reason Code is located on the evaluation report in brackets to the right of the result. Your laboratory must identify all of the analytes with an Exception Reason Code and investigate the acceptability of performance with the same rigor as if it were an unacceptable performance. The actions accredited laboratories should take include but are not limited to:

Code	Exception Reason Code Description	Action Required
11	Unable to analyze.	Document why the specimens were not analyzed (eg, instrument not functioning or reagents not available). Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and
		extent that would have been tested.
20	No appropriate target/response; cannot be graded.	Document that the laboratory performed a self-evaluation using the data presented in the Participant Summary and compared its results to a similar method, all method, or all participant statistics if provided. If comparison is not available, perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
21	Specimen problem.	Document that the laboratory has reviewed the proper statistics supplied in the Participant Summary. Perform and document alternative assessment for the period that commercial PT was not tested to the same level and extent that would have been tested. Credit is not awarded in these cases.
22	Result is outside the method/ instrument reportable range.	Document the comparison of results to the proper statistics supplied in the Participant Summary. Verify detection limits.
24	Incorrect response due to failure to provide a valid response code.	Document the laboratory's self-evaluation against the proper statistics and evaluation criteria supplied in the Participant Summary. Perform and document the corrective action of any unacceptable results. Document corrective action to prevent future failures.
25	Inappropriate use of antimicrobial.	Document the investigation of the result as if they were unacceptable and review the proper reference documents to gain knowledge of the reason your response is not appropriate.
26	Educational challenge.	Response to the CAP is not required. Laboratory should document its review.
27,31	Lack of participant or referee consensus.	Document that the laboratory performed a self-evaluation and compared its results to the intended response when provided in the Participant Summary. If comparison is not available, perform and document alternative assessment (ie, split samples) for the period that commercial PT reached non-consensus to the same level and extent that would have been tested.
28	Response qualified with a greater than or less than sign; unable to quantitate.	Document that the laboratory performed a self-evaluation and compared its results to the proper statistics supplied in the Participant Summary. Verify detection limits.
30	Scientific Committee decision.	Document that the laboratory has reviewed the proper statistics supplied in the Participant Summary.
33	Specimen determined to be unsatisfactory after contacting the CAP.	Document that the laboratory has contacted the CAP and no replacements specimens were available. Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
40	Results for this kit were not received.	Document why results were not received, corrective action to prevent recurrence and
41	Results for this kit were received past the evaluation cut-off date.	the laboratory's self-evaluation of the results by comparing results to the proper statistics and evaluation criteria supplied in the Participant Summary. If PT specimens were not analyzed, perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
42	No credit assigned due to absence of response.	The Participant Summary indicates which tests are graded (see evaluation criteria) and which tests are Not Evaluated/Educational. Updates to grading will also be noted. If a test is educational, the laboratory is not penalized for leaving a result(s) blank. The code 42 that appears on the evaluation is not a penalty. However, if a test is graded (regulated and non-regulated analytes) and your laboratory performs that test, results cannot be left blank. The laboratory is required to submit results for all challenges within that test or use an appropriate exception code or indicate test not performed/not applicable/not indicated. Exceptions may be noted in the Kit Instructions and/or the Result Form. Document corrective actions to prevent future failures.
44	This drug is not included in our test menu. Use of this code counts as a correct response.	Verify that the drug is not tested on patient samples and document to ensure proper future reporting.
45	Antimicrobial agent is likely ineffective for this organism or site of infection.	Document that the laboratory performed a self-evaluation of written protocols and practices for routine reporting of antimicrobial susceptibility reports to patient medical records. Document that routine reporting of this result to clinicians for patient care is compliant with specific recommendations of relevant Medical Staff and Committees (eg, infectious Diseases, Pharmacy and Therapeutics, Infection Control). Response to the CAP is not required.
77	Improper use of the exception code for this mailing.	Document the identification of the correct code to use for future mailings.
91	There was an insufficient number of contributing challenges to establish a composite grade.	Document the investigation of the result as if it were an unacceptable result. Perform and document the corrective action if required.
35, 43, 88, 92	various codes.	



Attestation of Participation for Self-Reported Training*

We the participants below have completed the review of the CAP $\frac{FH6-A\ 2017}{Product\ Mailing,\ Year}$ Participant Summary/Final Critique report, and can self report the recommended $\frac{0.5}{Education\ Hours}$ hours towards

fulfilling education and certification of maintenance requirements.

Participant	Date	Participant	Date

Director (or Designee) Signature - I have verified that the individuals listed Date above have successfully participated in this activity.

Retain this page for record-keeping and auditing purposes.

Individuals can also track their participation of educational activities through the CAP Learning Management System (LMS).

- 1. Log in to <u>www.cap.org</u>, using your User ID and Password. If you don't have an online account, you will need to create one.
- 2. Click Learning, select Learning Transcript
- 3. Click 'Add My Own Activity'
- 4. Enter the required information, and click **Save** when complete

For assistance, call our Customer Contact Center at 800-323-4040 or 847-832-7000 option 1.

*CAP Self-Reported Training activities do not offer CE credit, but can be used towards fulfilling requirements for certification of maintenance by agencies such as the American Society of Clinical Pathology (ASCP). Please verify with your certifying agency to determine your education requirements. 49215



325 Waukegan Road Northfield, IL 60093-2750 800-323-4040 847-832-7000 (Country code: 001)

© 2017 College of American Pathologists. All rights reserved.