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**Wadsworth Center**

New York State Department of Health

**ERYTHROCYTE PROTOPORPHYRIN**

**Proficiency Test Report**

**Special Event #2, 2011**

**September 16, 2011**

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## **TOXICOLOGY – ERYTHROCYTE PROTOPORPHYRIN**

### **Special Event #2, 2011**

September 16, 2011

Dear Laboratory Director,

This interlaboratory report presents our continuing evaluation of the use of whole caprine blood as a proficiency testing (PT) material for Erythrocyte Protoporphyrin (EP) measurements by hematofluorometer (HF). In this second PT event of 2011, HF participants were shipped the same whole blood PT materials that were shipped to laboratories using the extraction technique (EP11-06 to 10). As before, the goal was to explore whether whole blood obtained from lead-dosed animals might be used as PT materials for HF participants in the future. The caprine blood pools used for this study were also used to produce the glycerolized materials (HF11-06 to 10) that have been previously used for PT purposes. Data obtained for EP11-06 to 10 based on the reference ethyl acetate extraction technique and for HF11-06 to 10 obtained by HF were previously reported in "Erythrocyte Protoporphyrin Proficiency Test Report - Event #2, 2011" released September 1, 2011.

For this Special Event, we requested Aviv and Helena participants to analyze the unprocessed whole blood (EP11-06 to 10) materials for EP. The data and statistical summaries are enclosed. We calculated a mean value, rather than a target value, using data from all participants (peer group). Mean values for Aviv instruments are reported as  $\mu\text{g/dL}$ , assumed hematocrit 35, while those for Helena ProtoFluor-Z instruments are reported as  $\mu\text{mol ZPP/mol heme}$ .

Mean values obtained for the unprocessed whole blood (EP11-06 to 10) based on ethyl acetate extraction technique ( $n=3-4$  after performance of robust statistics and deletion of laboratory values  $>\pm 15\%$ , or  $\pm 6 \mu\text{g/dL}$ ) are compared with those obtained from Aviv HF participants ( $n=26-27$  after deletion of outliers by the Grubbs' test). After correcting the extraction and Aviv HF data for hematocrit, i.e., expressing results as  $\mu\text{g/dL RBC}$ , there is reasonable agreement (Table 1), although the HF population exhibits poorer inter-laboratory precision. We note that the whole blood PT samples were shipped unrefrigerated during a heat wave. Shipment under these conditions may have had an adverse affect on the samples. Indeed, agreement among the five participant extraction laboratories for this event was poorer than observed for EP11-01 to 05 (see "Erythrocyte Protoporphyrin PT Report" released June 2, 2011).

When Aviv HF participant mean values for unprocessed whole blood (EP11-06 to 10) are compared to those obtained for the glycerolized material (HF11-06 to 10), the agreement is reasonable. However, interlaboratory precision is consistently poorer for

unprocessed whole blood (Table 2). This is consistent with literature reports that plasma artifacts can adversely affect HF measurements, and with NYS PT results for HF11-01 to 05 presented in the "Erythrocyte Protoporphyrin PT Report" released June 2, 2011. Since the plasma component is removed during the preparation of the glycerolized materials, we would expect better interlaboratory agreement.

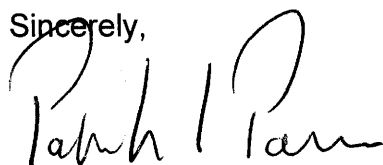
Helena ProtoFluor-Z participants also submitted data for unprocessed whole blood (EP11-06 to 10) and glycerolized PT materials (HF11-06 to 10). A comparison between Helena mean values for the two materials showed somewhat poorer agreement for results reported in  $\mu\text{mol ZPP/mol heme}$ . The reason(s) for the observed discrepancy is unknown but could be due to the more "fragile" nature of caprine red blood cells (RBCs) when treated with the ProtoFluor reagent – caprine RBCs tend to lyse when treated with the reagent. As with the Aviv data, interlaboratory precision is consistently poorer for unprocessed whole blood (Table 3), but consistent with NYS PT results for HF11-01 to 05 presented in the "Erythrocyte Protoporphyrin PT Report" released June 2, 2011.

The results are consistent with our earlier observations for whole blood materials<sup>1</sup>, and with NYS PT results for HF11-01 to 05 presented in the "Erythrocyte Protoporphyrin PT Report" released June 2, 2011. Data collected for the unprocessed whole blood continues to appear as a less desirable product for distribution in an interlaboratory study. Consequently, we will no longer ship whole caprine blood to HF participants in the future.

For the third event of 2011, we will ship only processed glycerolized RBCs for analysis to HF participants. Should you have any questions, feel free to contact us at [trel@wadsworth.org](mailto:trel@wadsworth.org).

Thank you for your participation.

Sincerely,



Patrick J. Parsons, Ph.D.  
Chief,  
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Assistant PT Section Head,  
PT Program for Blood Lead, EP  
and Trace Elements

cc: CLEP

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<sup>1</sup> PJ Parsons, JR Meola and DG Mitchell. Development of Standard Materials for Use with the Hematofluorometer. *Clinical Chemistry* 1988;34:1062-1066.

**New York State Department of Health**  
**Erythrocyte Protoporphyrin - Educational Test Results, 2011 Event #2**  
**SUMMARY**

Lab Code	Method	Results ( $\mu\text{g/dL}$ whole blood)				
		EP11-06	EP11-07	EP11-08	EP11-09	EP11-10
104	Aviv Hematofluorometry	85	180	60	131	50
107	Aviv Hematofluorometry	81	136	53	106	45
110	Aviv Hematofluorometry	94	162	55	126	50
114	Aviv Hematofluorometry	80	154	45	114	36
123	Aviv Hematofluorometry	75	130	44	101	37
126	Aviv Hematofluorometry	102	179	57	135	49
147	Aviv Hematofluorometry	105	190	59	140	49
155	Aviv Hematofluorometry	110	201	67	108	54
156	Aviv Hematofluorometry	83	150	47	110	38
158	Aviv Hematofluorometry	86	151	54	114	44
160	Aviv Hematofluorometry	87	192	55	118	50
164	Aviv Hematofluorometry	76	154	46	106	39
199	Aviv Hematofluorometry	94	158	48	126	37
221	Aviv Hematofluorometry	70	132	42	98	35
272	Aviv Hematofluorometry	110	193	55	140	53
293	Aviv Hematofluorometry	54	119	14	78	6
305	Aviv Hematofluorometry	82	160	46	109	42
383	Aviv Hematofluorometry	70	136	40	96	34
386	Aviv Hematofluorometry	76	142	44	104	37
398	Aviv Hematofluorometry	115	185	58	140	49
406	Aviv Hematofluorometry	76	146	49	106	43
407	Aviv Hematofluorometry	95	185	61	140	49
447	Aviv Hematofluorometry	71	134	40	99	33
451	Aviv Hematofluorometry	90	171	55	132	60
454	Aviv Hematofluorometry	71	133	41	100	34
459	Aviv Hematofluorometry	83	157	52	110	43
467	Aviv Hematofluorometry	81	153	51	114	41
Number of Sample Measurements:		27	27	27	27	27
<b>Mean values:</b>		<b>85</b>	<b>159</b>	<b>50</b>	<b>115</b>	<b>42</b>
Standard Deviation:		14.3	22.8	10.0	16.3	10.2
RSD (%):		16.8	14.4	20.2	14.2	24.1

**notes:** Results reported as less than the detection limits are treated as zero for statistical and grading purposes.

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**Erythrocyte Protoporphyrin - Educational Test Results, 2011 Event #2**  
**SUMMARY**

Lab Code	Method	Results ( $\mu\text{mol ZPP/mol heme}$ )				
		EP11-06	EP11-07	EP11-08	EP11-09	EP11-10
110	Helena ZPP	160	276	94	205	75
112	Helena ZPP	101	209	64	129	54
131	Helena ZPP	82	153	45	110	37
197	Helena ZPP	100	178	67	129	54
201	Helena ZPP	146	265	89	192	71
204	Helena ZPP	101	178	56	127	50
206	Helena ZPP	125	262	79	173	64
208	Helena ZPP	142	250	83	194	68
290	Helena ZPP	113	227	69	150	60
333	Helena ZPP	106	215	65	135	50
401	Helena ZPP	99	183	58	128	46
405	Helena ZPP	124	243	62	173	63
460	Helena ZPP	150	289	101	188	56
Number of Sample Measurements:		13	13	13	13	13
<b>Mean values:</b>		<b>119</b>	<b>225</b>	<b>72</b>	<b>156</b>	<b>58</b>
Standard Deviation:		24.1	43.1	16.4	32.2	10.7
RSD (%):		20.2	19.2	22.8	20.6	18.6

**notes:** Results reported as less than the detection limits are treated as zero for statistical and grading purposes.

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Erythrocyte Protoporphyrin - Educational Test Results**

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**Table 1. Extraction Technique vs. Aviv Hematofluorometer**

<b>Extraction</b> <i>Unprocessed Whole Blood</i>				<b>Aviv</b> <i>Unprocessed Whole Blood</i>			
<b>Results</b> ( $\mu\text{g/dL}$ whole blood)			<b>Results</b> ( $\mu\text{g/dL}$ RBC) <i>(Hematocrit corrected)</i>	<b>Results</b> ( $\mu\text{g/dL}$ RBC) <i>(Hematocrit corrected)</i>			
	n*	Mean	RSD (%)	Mean	n*	Mean	RSD (%)
EP11-10	3	30	10.0	23	26	34	16.8
EP11-08	4	49	6.0	41	26	43	14.2
EP11-06	4	103	3.5	103	27	86	16.8
EP11-09	4	119	8.9	103	27	99	14.2
EP11-07	4	193	6.7	188	27	155	14.4

**Table 2. Aviv Hematofluorometer**

<b>Aviv</b> <i>Unprocessed Whole Blood</i>				<b>Aviv</b> <i>Glycerolized Product</i>			
<b>Results</b> ( $\mu\text{g/dL}$ 35)				<b>Results</b> ( $\mu\text{g/dL}$ 35)			
	n*	Mean	RSD (%)		n*	Mean	RSD (%)
EP11-10	26	44	16.8	HF11-10	26	43	9.3
EP11-08	26	51	14.2	HF11-08	26	54	8.1
EP11-06	27	85	16.8	HF11-06	26	96	8.3
EP11-09	27	115	14.2	HF11-09	27	128	7.4
EP11-07	27	159	14.4	HF11-07	26	173	8.8

**Table 3. Helena ProtoFluor-Z Hematofluorometer**

<b>Helena</b> <i>Unprocessed Whole Blood</i>				<b>Helena</b> <i>Glycerolized Product</i>			
<b>Results</b> ( $\mu\text{mol}$ ZPP/mol heme)				<b>Results</b> ( $\mu\text{mol}$ ZPP/mol heme)			
	n*	Mean	RSD (%)		n*	Mean	RSD (%)
EP11-10	13	58	18.6	HF11-10	12	49	5.3
EP11-08	13	72	22.8	HF11-08	12	64	7.3
EP11-06	13	119	20.2	HF11-06	12	108	7.1
EP11-09	13	156	20.6	HF11-09	12	146	8.0
EP11-07	13	225	19.2	HF11-07	12	196	8.2

\*Outliers omitted after performance of Grubbs' test.

+Reported value(s) not used after performance of robust statistics