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**Wadsworth Center**

**New York State Department of Health**

**ERYTHROCYTE PROTOPORPHYRIN**

**Special Event for Aviv HF Participants**

**December 30, 2008**

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STATE OF NEW YORK  
DEPARTMENT OF HEALTH

Wadsworth Center The Governor Nelson A. Rockefeller Empire State Plaza P.O. Box 509 Albany, New York 12201-0509

Richard F. Daines, M.D.  
*Commissioner*

Wendy E. Saunders  
*Chief of Staff*

December 30, 2008

**Update – Participants using the Aviv ZPP Hematofluorometer to report results under Toxicology Erythrocyte Protoporphyrin**

Dear Laboratory Director:

I want to take this opportunity to provide an update on the status of our proficiency testing (PT) program for erythrocyte protoporphyrin (EP), and the traceability of measurements produced using the Aviv Biomedical Inc. (Aviv) hematofluorometer (HF). Earlier this year, we temporarily suspended the regulatory grading process for EP (for both extraction and HF methods), such that the first PT event of 2008 was considered an “Educational Event.” Please note that the issue addressed below applies only to participants using HF instrumentation from Aviv.

On May 13, 2008, we met with Mr. Jack Aviv in an effort to understand better the process by which Aviv HF instrumentation traceability is maintained and verified. As a result of that meeting, the manufacturer provided us with archived cryo-preserved test materials of human origin for circulation among our PT participants using Aviv HF instruments. Five of these test pools were thawed, mixed, and aliquotted into labeled additive-free evacuated glass tubes, and then circulated to Aviv participants in June as part of our second PT event of 2008. The third PT event of 2008 for EP (for both extraction and HF methods) was shipped as scheduled in October as an “Educational Event” as well, but the summary reports were postponed pending the outcome of our on-going investigations.

The principal reason we postponed the summary reports was our concern with the loss of traceability for participants using the Aviv HF. We are especially apprehensive that the program’s “assigned values”, whether they are called target values or mean values, are being incorrectly perceived as “true” values and, therefore traceable to extraction methods. This ongoing situation has the potential to perpetuate the appearance that all is well with this assay. Yet, many of our Aviv participants, as well as some users that do not participate in the NY PT program, have reported to us that their patient population means are gradually shifting upward. This has caused some alarm among Aviv participants as many more “positive” or abnormal test results are being identified and reported.

Results from the archived cryo-preserved Aviv materials, for which historical target values are available, demonstrate unequivocally, that a positive bias exists for the majority of Aviv users in the field. We have shared these data with the manufacturer, who has agreed with us that this bias must now be resolved. Clearly, a bias adjustment will be required for many of the Aviv HF instruments currently in use, as well as for RBC calibration materials and RBC ZPP controls that are produced and marketed by Aviv.

The attached summary report entitled “Special Event for Aviv HF Participants” dated December 30<sup>th</sup> 2008, documents the results reported by each Aviv participant for the archived cryo-preserved Aviv materials. Note that the assigned values for these five test samples are based on the historical target values assigned previously by Aviv and are traceable to the extraction method. Consequently, each participant can evaluate their own laboratory specific bias. Note that the normalized mean statistic gives an indication of bias across the ranges reported. In

addition to the tabulated data, we have also included a graphed plot of participant data showing reported values as a function of the historical assigned value in units of  $\mu\text{g/dL}$ , hct 35%. The slope of the fit shows that a positive bias of ~13% is evident among participants. Clearly some participants have a greater bias and others less. Participants with an unusually large bias may wish to contact the manufacturer to discuss their HF service history to ensure that there are no other technical issues involved.

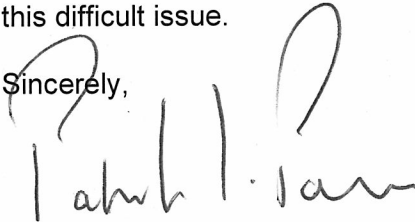
At this point the PT data from the second and third events of 2008 have been released, along with calculated mean values for the Aviv participant group. However, these mean values should not be interpreted as either robust target values or as being traceable. On the other hand, the "assigned" values that are provided in Special Event for Aviv HF Participants are based on historical data provided by the manufacturer.

The manufacturer has informed us that they are working on a plan to implement a bias adjustment during the 1st quarter of 2009. This plan will include a protocol for users to make a calibration adjustment in their own lab, as well as corrected target values for calibrator and RBC control lots currently in circulation. HF users should contact the manufacturer for more details on the unfolding plan to address the calibration bias.

As a PT program provider, we are committed to working with the manufacturer to help correct the bias and return this assay to a firm scientific and technical basis, with full traceability to standards based on protoporphyrin IX, i.e., the extraction methodology.

Should you have any questions, feel free to contact me at [patrick.parsons@wadsworth.org](mailto:patrick.parsons@wadsworth.org) or by telephone at 518-474-8383. Thank you once again for your patience as we attempt to resolve this difficult issue.

Sincerely,



Patrick J. Parsons, Ph.D.  
Section Head, Proficiency Testing Program for Blood Lead, EP and Trace Elements

cc: Richard Jenny, PhD., NYS CLEP  
Mary Verostek, PhD., NYS PT program  
Noel Stanton, WSLH  
M. Jeffery Shoemaker, Ph.D., PA Bureau of Laboratories  
Jack Aviv, Aviv Biomedical, Inc.

**New York State Department of Health**  
**Erythrocyte Protoporphyrin - Special Event for Aviv HF Participants**  
**PERFORMANCE OF PARTICIPATING LABORATORIES**

Lab Code	Method	Results (µg/dL whole blood)					Normalized Mean
		08EDU-1	08EDU-2	08EDU-3	08EDU-4	08EDU-5	
Assigned Values:		27	68	320	174	210	
104	Aviv Hematofluorometry	30	81	386	208	253	1.18
107	Aviv Hematofluorometry	30	79	364	194	235	1.13
109	Aviv Hematofluorometry	30	76	358	194	238	1.12
110	Aviv Hematofluorometry	32	82	363	201	242	1.17
112	Aviv Hematofluorometry	28	76	354	190	229	1.09
114	Aviv Hematofluorometry	28	79	372	197	240	1.13
123	Aviv Hematofluorometry	26	75	360	191	232	1.08
126	Aviv Hematofluorometry	30	78	363	194	237	1.13
131	Aviv Hematofluorometry	28	78	361	194	235	1.11
147	Aviv Hematofluorometry	23	69	325	176	213	0.98
155	Aviv Hematofluorometry	31	76	345	187	227	1.10
156	Aviv Hematofluorometry	31	76	353	193	233	1.12
158	Aviv Hematofluorometry	20	72	353	188	230	1.02
160	Aviv Hematofluorometry	28	76	353	192	230	1.09
164	Aviv Hematofluorometry	33	81	366	205	246	1.18
199	Aviv Hematofluorometry	26	76	351	188	231	1.07
221	Aviv Hematofluorometry	22	79	392	209	255	1.12
251	Aviv Hematofluorometry	28	77	344	184	222	1.07
272	Aviv Hematofluorometry	29	79	345	200	246	1.13
293	Aviv Hematofluorometry	13	53	291	158	185	0.79
305	Aviv Hematofluorometry	35	92	407	224	273	1.30
348	Aviv Hematofluorometry	28	71	347	185	225	1.06

**Notes: Assigned Values:** Previously determined by manufacturer based on comparison with acid extraction method.

**Normalized Mean:** The average of each reported result divided by the corresponding "target" value. It measures bias.

**New York State Department of Health  
Erythrocyte Protoporphyrin - Special Event for Aviv HF Participants  
PERFORMANCE OF PARTICIPATING LABORATORIES**

Lab Code	Method	Results ( $\mu\text{g/dL}$ whole blood)					Normalized Mean
		08EDU-1	08EDU-2	08EDU-3	08EDU-4	08EDU-5	
Assigned Values:		27	68	320	174	210	
383	Aviv Hematofluorometry	32	87	389	213	256	1.22
386	Aviv Hematofluorometry	37	99	445	243	288	1.40
398	Aviv Hematofluorometry	28	78	362	194	236	1.11
399	Aviv Hematofluorometry	32	83	359	198	230	1.15
406	Aviv Hematofluorometry	27	74	334	179	224	1.05
407	Aviv Hematofluorometry	30	82	373	207	249	1.17
447	Aviv Hematofluorometry	29	74	344	185	223	1.07

**Notes: Assigned Values:** Previously determined by manufacturer based on comparison with acid extraction method.

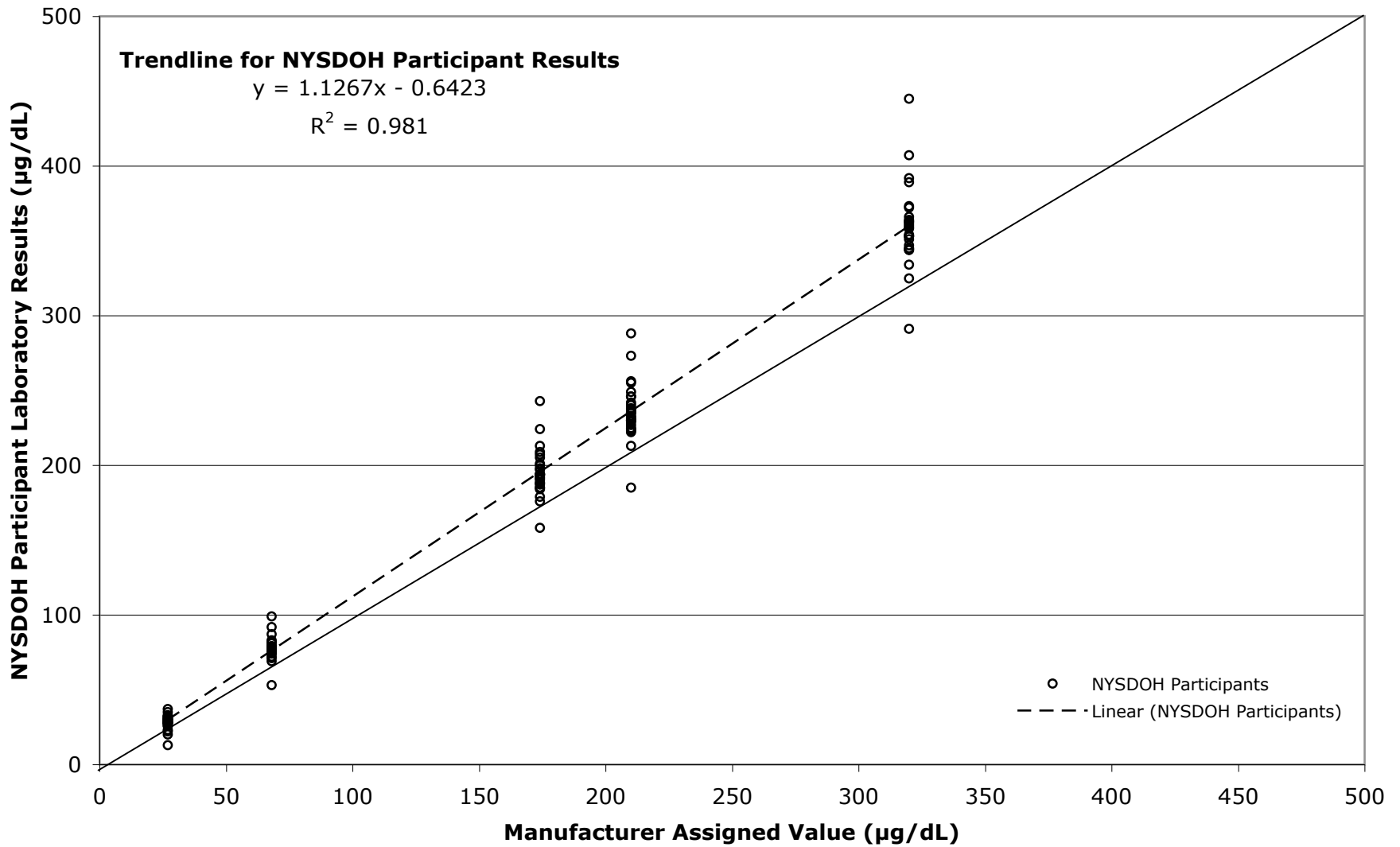
**Normalized Mean:** The average of each reported result divided by the corresponding "target" value. It measures bias.

**New York State Department of Health**  
**Erythrocyte Protoporphyrin - Special Event for Aviv HF Participants**  
**STATISTICAL SUMMARY BY METHOD**

	Results ( $\mu\text{g/dL}$ whole blood)				
	08EDU-1	08EDU-2	08EDU-3	08EDU-4	08EDU-5
<b>Aviv Hematofluorometry</b>					
Number of Sample Measurements:	29	29	29	29	29
Mean:	28.4	77.9	360.7	195.6	236.7
Standard Deviation:	4.6	7.7	27.0	15.4	18.5
RSD (%):	16.3	9.9	7.5	7.9	7.8
<b>All Laboratories</b>					
Number of Sample Measurements:	29	29	29	29	29
Mean:	28.4	77.9	360.7	195.6	236.7
Standard Deviation:	4.6	7.7	27.0	15.4	18.5
RSD (%):	16.3	9.9	7.5	7.9	7.8

**notes:** ? Insufficient data for calculation.

### Special Event for Aviv HF Participants



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**METHOD NOTES**

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***ATOMIC SPECTROMETRY METHODS***

- A-1 ETAAS-Z (Electrothermal atomic absorption spectrometry with Zeeman background correction)
- A-2 ETAAS other (i.e., D<sub>2</sub>, S-H background correction)
- A-3 FAAS (Flame atomic absorption spectrometry)
- A-4 CV-AAS (Cold vapor atomic absorption spectrometry)
- A-5 HG-AAS (Hydride generation atomic absorption spectrometry)
- A-6 AFS (Atomic fluorescence spectrometry)
- A-7 Other

***INDUCTIVELY COUPLED PLASMA***

- P-1 ICP-MS (Inductively coupled plasma - mass spectrometry)
- P-2 DRC/CC-ICP-MS (ICP-MS used in the Dynamic Reaction Cell or Collision Cell mode)
- P-3 ICP-AES/OES (ICP atomic/optical emission spectrometry)
- P-4 HR-ICP-MS (High resolution ICP-MS)
- P-5 ETV-ICP-MS (Electrothermal vaporization ICP-MS)
- P-6 ID-ICP-MS (Isotope dilution ICP-MS)
- P-7 Other

***ELECTROCHEMICAL METHODS***

- E-1 ASV (Anodic stripping voltammetry without digestion)
- E-2 ASV-LeadCare® (Anodic stripping voltammetry using the ESA LeadCare® system)
- E-3 Fluoride specific electrode
- E-4 Other

***MOLECULAR FLUORIMETRY***

- F-1 EtOAc (Ethyl acetate-acetic acid extraction method for determination of erythrocyte protoporphyrin)
- F-2 Aviv hematofluorometry (for determination of EP at hematocrit 35)
- F-3 Helena ZPP (for determination of zinc protoporphyrin in  $\mu\text{mol ZPP/mol heme}$ )
- F-4 Other

***OTHER METHODS***

If your method is not listed in the above list, please describe it briefly.

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