



## The Determination of Mercury in Whole Blood by Cold Vapor Atomic Fluorescence

### Introduction

Mercury is a toxic element that can be inhaled as vapor or pass through the skin and into the blood stream. Compounds of mercury can enter the body through the lungs, skin or by ingestion. Repeated exposure to mercury has adverse health effects whose symptoms are well documented. Individuals at high risk of exposure or who are suspected of mercury intoxication are typically monitored through analysis of blood and urine samples. The mercury blood test will detect all types of mercury but because mercury remains in the bloodstream for only a few days the test should be performed soon after exposure. The urine mercury test only measures inorganic and elemental mercury as organic forms are not excreted.

This application note describes sample preparation and analysis of blood samples using the Hydra AF mercury analyzer. The Hydra AF employs cold vapor atomic fluorescence and is ideally suited for the determination of mercury ranging from  $<10\mu\text{g/L}$  up to the elevated levels sometimes found as a result of occupational exposure.

### Experimental

#### Sample Preparation

0.1 mL of blood was pipetted into a 12mL polypropylene test tube. Next, 3.2mL of concentrated sulfuric acid was added and the mixture allowed to sit at room temperature for 20 minutes to digest the sample. De-ionized water<sup>1</sup> and 5% potassium permanganate were added to each sample tube and tubes were held at 50°C for 30 minutes to oxidize mercury to mercuric (+2) valence. Samples were cooled to room temperature and sodium chloride:hydroxylamine sulfate (12%:12%) solution was added to remove excess permanganate.



<sup>1</sup> The de-ionized water mentioned here was added to samples only and excluded from standard cups. The water added is to compensate for the standard solution added to calibration standard cups. The standard cups contained 4mL of mercury standard solution in 2% hydrochloric acid and no blood. The water added contained 50ppm anti-foaming agent (Leeman Labs #606-00008)

**Table 1: Reagent Additions**

Reagent	Samples	Standards
Mercury standard	0.0mL	4.0mL
Water with antifoam	4.0	0.0
H2SO4	3.2	3.2
5% KMnO4	0.4	0.4
NaCl:NH2OH:SO4	0.5	0.5

**Sample Analysis**

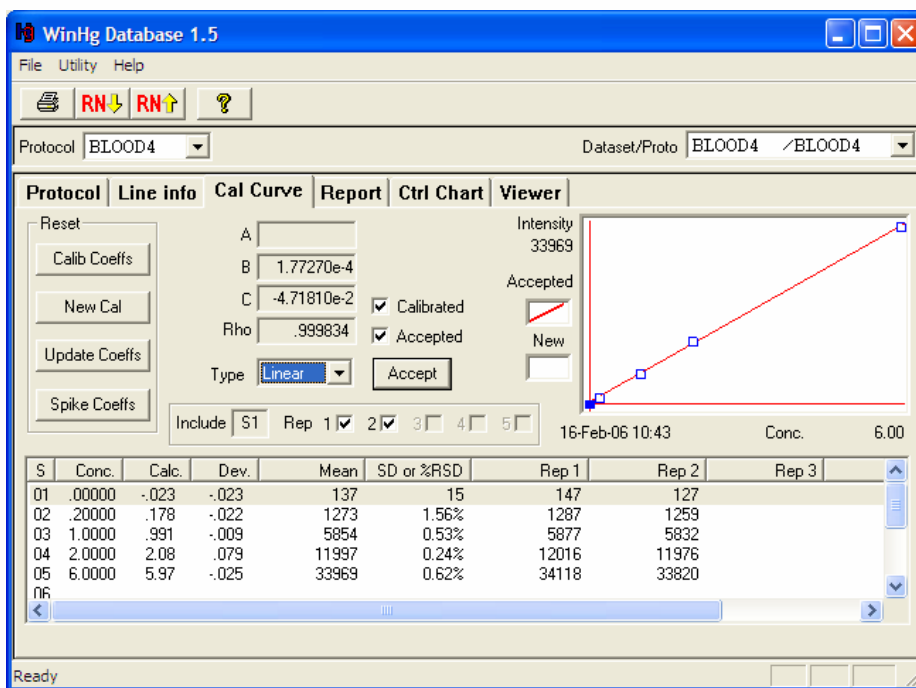
The Hydra AF mercury analyzer is a continuous flow system where sample and reductant, in this case 10% SnCl<sub>2</sub>, are pumped into a gas/liquid separator. There the mercury in the sample is reduced to elemental mercury. It then enters the gas phase and is carried to the spectrometer. Instrument parameters are provided in Table 2.

**Table 2: Instrument Parameters**

Parameter	Value
<b>Gas flow</b>	<b>0.3 LPM</b>
<b>Pump speed</b>	<b>10 mL/min</b>
<b>Rinse time</b>	<b>60 sec.</b>
<b>Uptake time</b>	<b>20 sec.</b>
<b>Integration time</b>	<b>30 sec.</b>

Samples were mixed and then diluted 1:6 with 2% hydrochloric acid prior to analysis. The calibration standard concentrations were entered as the original values before reagent addition and dilution (0.0, 0.2, 1.0, 2.0 & 6.0 µg/mL). Sample concentrations were reported with 40-fold volume corrections applied [0.1ml→4.0 mL]. Figure 1 shows the calibration curve. The fit type is linear with a correlation coefficient of 0.999834. Two replicates run on each standard show excellent precision throughout.

**Figure 1: Linear Calibration**



### Reference Materials

Three control samples of lyophilized whole blood were obtained from Bio-Rad Laboratories, Irvine, CA. Results for samples are provided in Table 3.

**Table 3: Control Samples**

Lyphochek	Lot #	Result	Reference Mean <sup>1</sup>	Reference Range <sup>2</sup>
Level 1	36681	10.7µg/L	9.6µg/L	7.7-11.6µg/L
Level 2	36682	37.8	39	31-47
Level 3	36683	75	73	58-87

### Conclusions

The Hydra AF mercury analyzer provides accurate measurement of Hg in whole blood without the need for microwave digestion of samples.

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<sup>1</sup> Reference data were established for Bio-Rad by inductively coupled plasma mass spectrometry (ICP-MS).