Dried Blood Spot Metals Analysis Feasibility Study Report **February 2005**

*** Utah Report for Rocky Mountain Bio-monitoring Consortium**

1. Study Goal:

The purpose of this study is to determine the feasibility of using dried blood spots collected for routine neonatal screening to study baseline levels of metals contaminant in human. The metals of concern are : Arsenic, Cadmium, Chromium, lead, mercury, and selenium.

2. Background:

Mercury, lead, cadmium, chromium and selenium are considered to be toxic and are of health concern to the states in the consortium. Levels of these metals in human at the time of birth may be a good indicator of levels to expect if humans were not exposed to of these toxicants. Although high levels in newly born babies can be explained as an indication of maternal exposure to toxicants. Trace levels of lead have been analyzed in blood samples collected on PKU (phenylketourea) cards from newborn babies. Study assessed utilizing these cards to obtain information on lead levels in new born babies [1]. The use of blood spots dried on filter paper has been studied as an alternative to liquid blood for analysis of lead in blood [2-8]. Migration of Cr –51 was measured in spotted blood. [9]. These studies discuss the advantage and disadvantage of using filter paper blood spots to determine lead levels in blood. Advantages of using filter paper blood dots have been determined to be: relatively small amount of sample need, use of readily available filter paper blood samples, stable and easy transportation, long holding time, six months compared with two weeks for liquid blood sample. Concerns expressed include contamination from filter paper, sample handling, environment, punch size and spread of metal onto the filter paper which may bias results[1-9, 11]. Not much information is available about mercury, arsenic, selenium and cadmium on blood dots.

3. Study Design:

Our study evaluates the filter papers, before and after exposure to external environment to determine if it is feasible to use blood collected on filter papers from newborn babies to obtain unbiased information on trace metals.

The known toxic level of these metals in human blood (Table 1) was used as a marker to determine expected level of detection for the analytical method developed.

The study consists of evaluation of the following steps:

1. Filter paper evaluation for homogeneity
2. Filter paper analysis
3. Analysis of unexposed blank filter papers
4. Analysis of blank spots from filter papers exposed to hospitals environment
5. Analysis of blood dots received from hospitals

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4. Experimentation :

a) Filter paper evaluation

Three lots of filter paper were obtained. Filter paper cards S&S Lot # W030 and # Lot # W011 was obtained from the manufacturer. 25 cards from Whatman lot # 1488 were from our consortium Partner State in Montana. Lot # W011, from S&S, came as a single sheet which was cut into pieces about the same size as the card sections which had the five available dots on them. 16 cards from each lot were chosen for evaluation. Then, with the aid of a random number generator, 20 dots were punched from each lot into clean 15 mL polypropylene tubes and weighed using a Mettler Toledo AX205 balance capable of measuring to the fifth decimal place. Half of the dots were punched and weighed on one day with the remainder being completed the following day. This was done so that the masses reported would contain as many variance components as possible including the inter-day variance component. Data were analyzed using Minitab 13.1, which utilizes a double precision (64 bit) algorithm for the computation and storage of numbers.

b) Analytical Method:

Unexposed filter papers are those that was obtained from the vendor or laboratory. Filter paper exposed to hospital environment are those that was received from hospital containing blood dots. Blank spots adjacent to the blood spots were analyzed. A few blood spots were also analyzed. Hospital firsts are those that contained new born blood taken within three days of birth. Hospital seconds are new born blood taken about three weeks from birth.
All Filter papers: unexposed, exposed hospital firsts, exposed hospital seconds and blood dots were analyzed following procedure described below.

Extraction Procedure

Dots ¼ inch (6.35 mm) diameter were punched directly into 15 mL polypropylene tubes. 1 mL of extraction solution was added. The solution was a 50/50 mixture of 2% HNO_3 and 10 ppb Ir and Rh in 2% HNO_3. The tubes were then vortexed for 1 minute, allowed to sit for 10 minutes, and then vortexed again for 1 minute. The effect of extraction time was evaluated by varying the contact time of the filter paper with acid from 5 minutes until over night. The dots were then removed from the tubes using a 5 ½ inch Pasture pipette. In those cases where the dots were too mechanically unsound to remove by this method, such as where the dots started to disintegrate using the pipette, then the tube was centrifuged for 5 minutes at 5000 RPM in an Eppendorf 5804 centrifuge. The solution was then decanted into a different tube. The unused blank, blank dots from hospital cards containing blood dots and blood dots , were all processed similiarly.
It should be noted that while each metal was analyzed separately, all the metals were spiked as a mixture. This was done because in normal blood metals can be expected to be mixed and, as noted by Son *et al.*[12], there is selective competition among different metals for sorption sites on cellulose.

Procedure for Instrumental Analysis

Extracted samples were analyzed utilizing an Inductive Coupling Plasma (ICP)with Mass Spectrometer (MS), manufactured by Perkin Elmer Sciex Elan DRC II ICP/MS. The dynamic reaction cell (DRC) was not utilized for the analyses in this work. Calibration curves were prepared by spiking blank filter paper with the appropriate standards and allowed to dry overnight. Blanks for the calibration curves were prepared in a similar manner. Dots were then punched and extracted as indicated above and analyzed. Initial experiment used aqueous calibration standards to quantitate metals in filter paper. The two ions were chosen based upon which gave the best calibration curves from earlier work at low concentrations. Two ions for each metal were scanned with exception of As where only one isotope exists. The two ions chosen were based upon which gave the best calibration curves. Two replicate readings were taken for each m/z and the calibration curves were constructed using simple linear regression. In no cases were the intercepts forced through the origin. Background blank dots were analyzed in this manner except a diluent subtraction was employed to allow total background determination.

Method detection limits (MDLs), when possible were estimated utilizing a variant of the method proposed by the EPA [13]. In this particular case, 7 spiked dots were extracted and analyzed and the standard deviation (s) estimated instead of spiked reagent water. The MDL was then calculated utilizing MDL = s $t_{(n-1,\ 1-\alpha)}$ where s is the standard deviation estimate and $t_{(n-1,\ 1-\alpha)}$ is Student's t at a specified number of degrees of freedom and confidence level, in this particular case the latter being 99%. The limit of quantitation (LOQ), as used in this report is defined as LOQ = 10s. The detection limit (DL), as defined in this report, is the concentration of the aqueous metal solution which was spiked onto filter paper and a recovery of 90% or better was obtained.

c) Quality Assurance & Quality Control:

All samples were analyzed by staff trained center of disease control (CDC) to analyze heavy metals using ICP/MS. To assure quality control of experiments, with every batch of dots analyzed following quality control tests were performed: a diluent blank to assure no contamination from instrument or glassware, a method blank, check standards, and a dot spiked with known concentration of metals was analyzed to assure quality of all the samples analyzed in a batch.
In addition, to assure quality in analytical procedure, Utah State laboratory participated in monthly performance tests (PT) for lead in blood dots administered by Wisconsin State Laboratory of Hygiene Proficiency Testing program.

4. Results & Discussions:

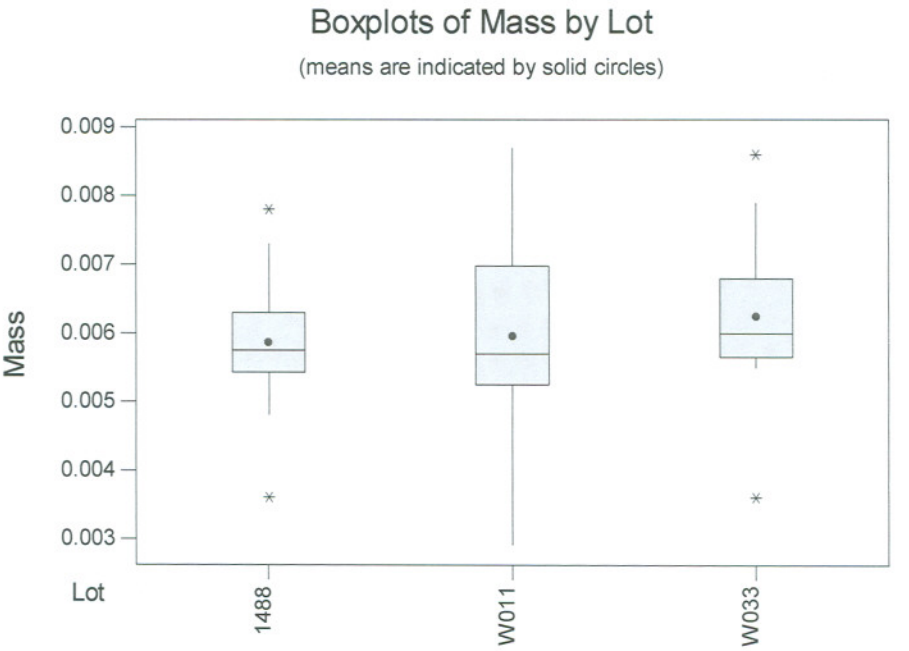
Filter Paper Evaluation

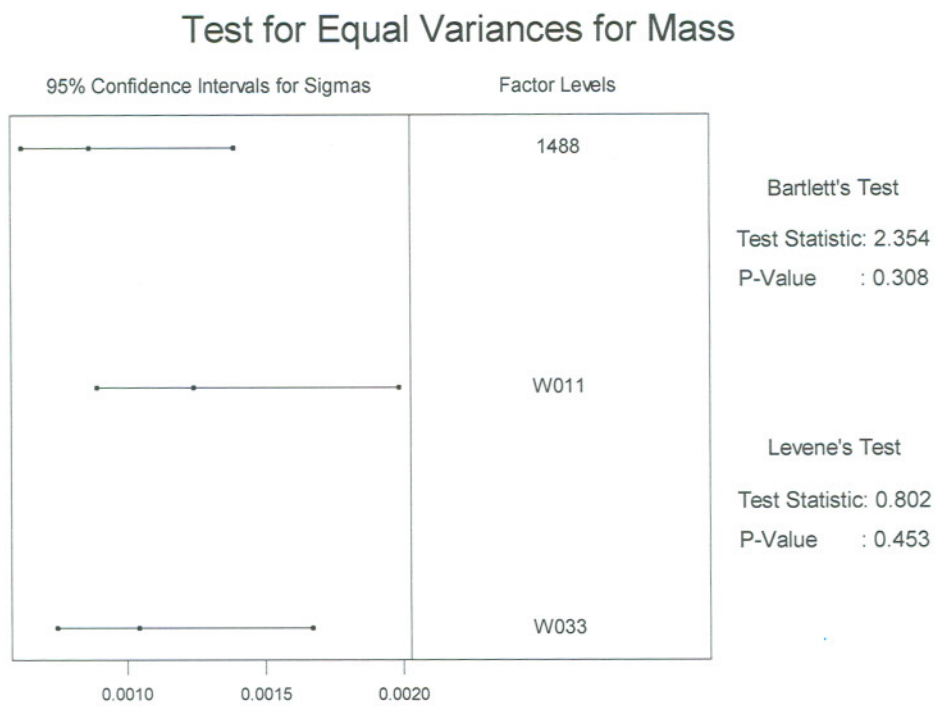
Results

Filter papers were evaluated to determine absorption capability in a particular filter paper dot, which may affect the amount of material absorbed in the dots in filter papers. Homogeneity within filter paper, and between filter papers from different manufactures

and lots were studied. Below are boxplots of the masses for the dots from each lot of filter papers. The solid circles denote the means while the asterisks denote outliers.

From the graphical display all the means appear to be equal. It is particularly noteworthy that the range of masses for each of the lots of filter paper is quite large. Below are graphs depicting the 95% confidence intervals for the standard deviations for all three lots of filter paper. Both Bartlett's and Leven's tests for equal variances yielded p-values of 0.308 and 0.453, respectively, indicating that the null hypothesis of equal variances for all three lots cannot be rejected, or in other words, there is insufficient evidence for suspecting the lot variances to be different.





Summarized below are the results with the associated one-way ANOVA statistics. ANOVA (Analysis of Variance) is a formal statistical test for means, specifically testing if one or more means from a set of means if different. Furthermore, the test is called "one-way" because there is only one factor differentiating the means, it being "lots" for this specific data.

Blood Dot Masses with Associated One-way ANOVA Statistics.

Lot Identification	Mean Mass (g)	Standard Deviation	F*	p-value*
Whatman 1488	0.0059	0.0009	0.68	0.509
S&S W011	0.0060	0.0012		
S&S W033	0.0062	0.0010		

* Values computed using a pooled standard deviation of 0.0011.

The small F-value and the resultant high p-value indicate that the null hypothesis of equal mass means cannot be rejected, or in other words, there is insufficient evidence for suspecting the means to be different.

Although the experiment demonstrates the means to be significantly homogeneous, the high variance associated with each mean is likewise significant, as indicated in the above table. (For example, from the boxplots, also from Table 4, the masses can vary from 3 mg to almost 9 mg.) This would tend to suggest, if we assume the size of the dots to be

homogeneous, that the densities might differ and hence, the amount of blood that might be contained within each dot, might also differ.

Filter paper Analysis for metals:

Filterpapers were extracted as described in the Extraction Procedure section. Appendix Table 1 represents results with contact times of ten and sixty minutes. It is clear that there was no significant difference in the two results. Thus, ten-minute contact was determined optimum for efficiency and to avoid disintegration of filter paper which would make it difficult to obtain a clear extract. Special care had to be taken to carefully decant clear extract for analysis. Problem often was experienced with filter paper particles clogging the auto sampler tube of the ICP/MS.

The performance of Utah State lab in the Filter Paper lead proficiency test for the months of September through December, 2004 is presented in Table1. The table summarizes results obtained, which includes both target values and acceptable range values. It should be noted that Utah passed the test every month participated in the challenge. Examination of the last three months illustrates that they consistently obtained, or came very close to, the target values, which indicates, at least for this metal, that Utah's technique produces excellent results.

Table 1: Lead PT Performance Results for September through December of 2004.

Month	Sample ID	Target Value (µg/dL)	Acceptance Range (µg/dL)	UT Value (µg/dL)	UT Value Status
September	04FP29	1	0 - 5	<2	Accepted
	04FP30	18	14 - 22	15	Accepted
	04FP31	32	28 - 36	29	Accepted
	04FP32	23	19 - 27	21	Accepted
	04FP33	31	27 - 35	25	Outside*
October	04FP34	3	0 - 7	<2	Accepted
	04FP35	27	23 - 31	27	Accepted
	04FP36	27	23 - 31	28	Accepted
November	04FP37	6	2 - 10	6	Accepted
	04FP38	25	21 - 29	23	Accepted
	04FP39	20	16 - 24	20	Accepted
December	04FP40	1	0 - 5	<2	Accepted
	04FP41	23	19 - 27	23	Accepted
	04FP42	15	11 - 19	15	Accepted

* While the single value was outside, the performance-test status for the month was pass.

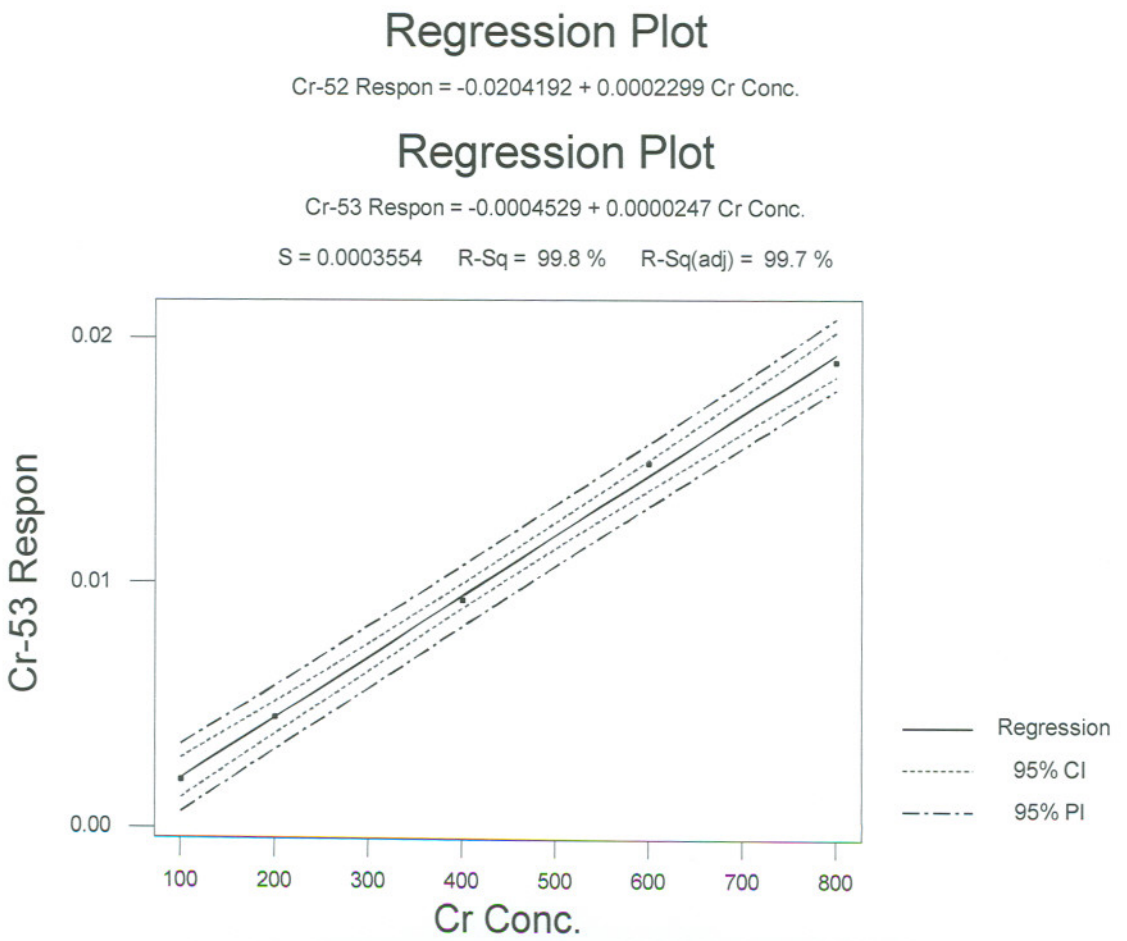
Availability of filter paper from different sources and in sufficient quantities, was a problem. Work presented in this section represents later work performed with filter paper S&S lot #903. Results of initial work is presented in Appendix. Filter papers used in the

initial stages of the study were not available any more to continue the study. Table 4 represents the calculated method detection limit (MDL), limit of quantitation (LOQ) and detection level(DL). Although DLs for Cr and Se looks relatively high, levels down to 1 ppb was detectable for all shown in the Table.

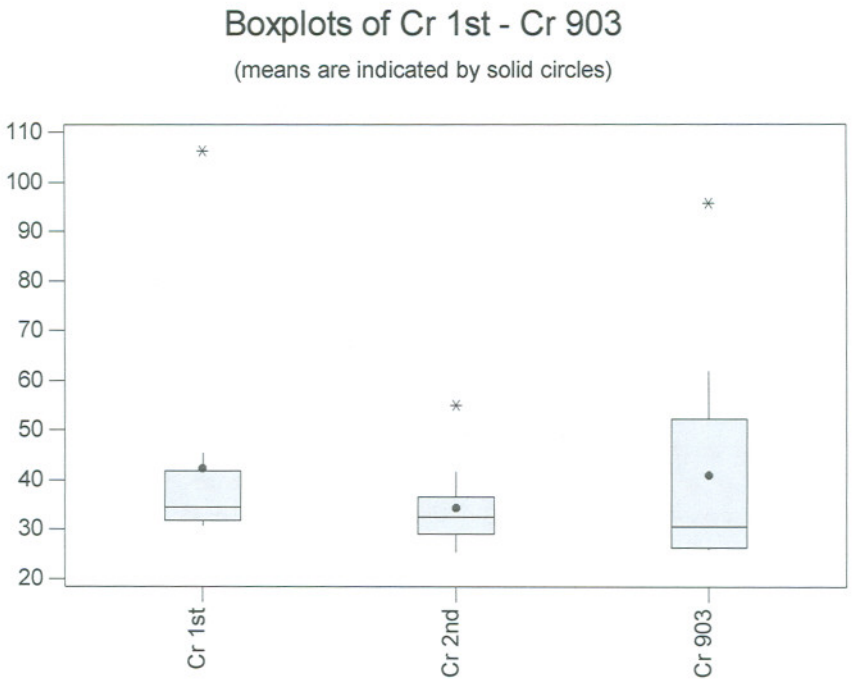
Chromium

Qualitatively, from working with this element we estimated an LOQ of 100 ppb and constructed curves starting from this point. Below are the calibration curves for chromium scanning for m/z 52 and 53. Three features become readily apparent when contrasting the two curves. First, the variance of the chromium-52 curve is greater relative to chromium-53, as evidenced not only by the smaller R^2 value, but also by the wider 95% confidence and prediction intervals. Second, the intercept of the chromium-53 curve is closer by more than an order of magnitude to the origin than chromium-53, and third, the first point of the chromium-52 curve is less than zero. Thus, all quantitation for the extracted blank dots, that which is called the 1st, 2nd, and 903 dots, was performed using ion mass 53.

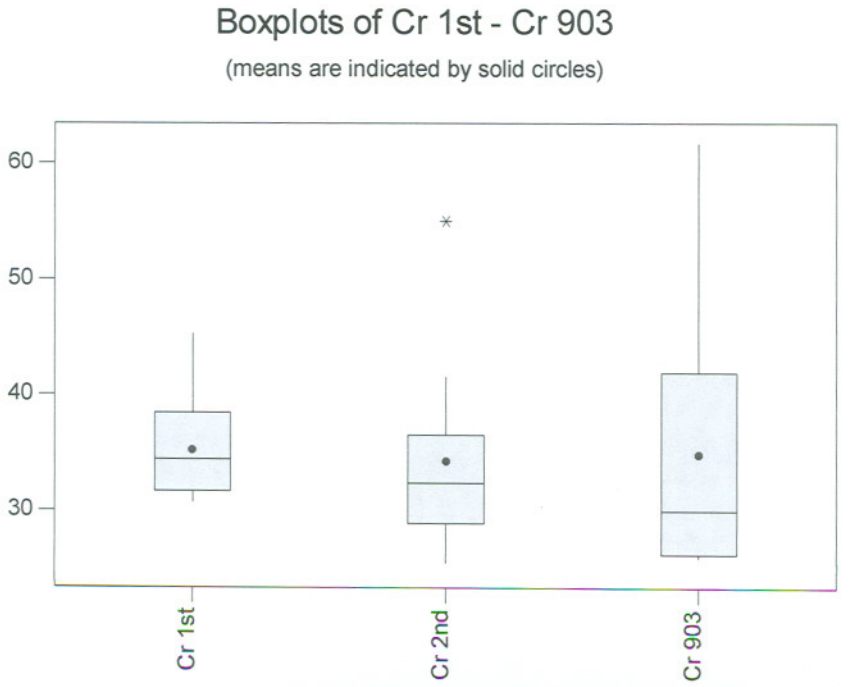
The estimated MDL utilizing the m/z 52 ion was 43.73 ppb while the MDL utilizing the 53 mass ion was 26.23 ppb. The corresponding LOQ's are 130 and 77.9 ppb, respectively. The latter value of 78 ppb comes close to our qualitative estimate of 100 ppb. The average recoveries were 102 ± 13.0 % for the 52 mass ion and 96.4 ± 7.79% for the mass 53 ion. It should be noted that the higher standard deviation for the former value is further demonstrative of the higher error associated with this particular mass.



Below are the box plots for the 1st, 2nd, and 903 blanks. Most values are above the MDL and close to it, but all are well below the LOQ with exception of the noted outliers, so there is considerable error associated with these estimates.



Below are the boxplots of the data with the outliers from the 1st and 903 removed.



The one striking feature, when comparing all three boxplots, is the tendency of the distributions to skew toward the high side even with the previously noted outliers removed. Supposedly this should not be deemed too unusual a feature, for as "...the ubiquitous presence of chromium in laboratories..." [14] has been noted, the argument should not be overly stressed to be made applicable to hospitals and doctors' offices as well. Furthermore, considering the punching devise we used is apparently a chromium-containing alloy, again these results should not be deemed too unusual.

The boxplots also illustrate means that are close to each other. One-way ANOVA, in apparent agreement, suggests that the null hypothesis of equal means cannot be rejected.

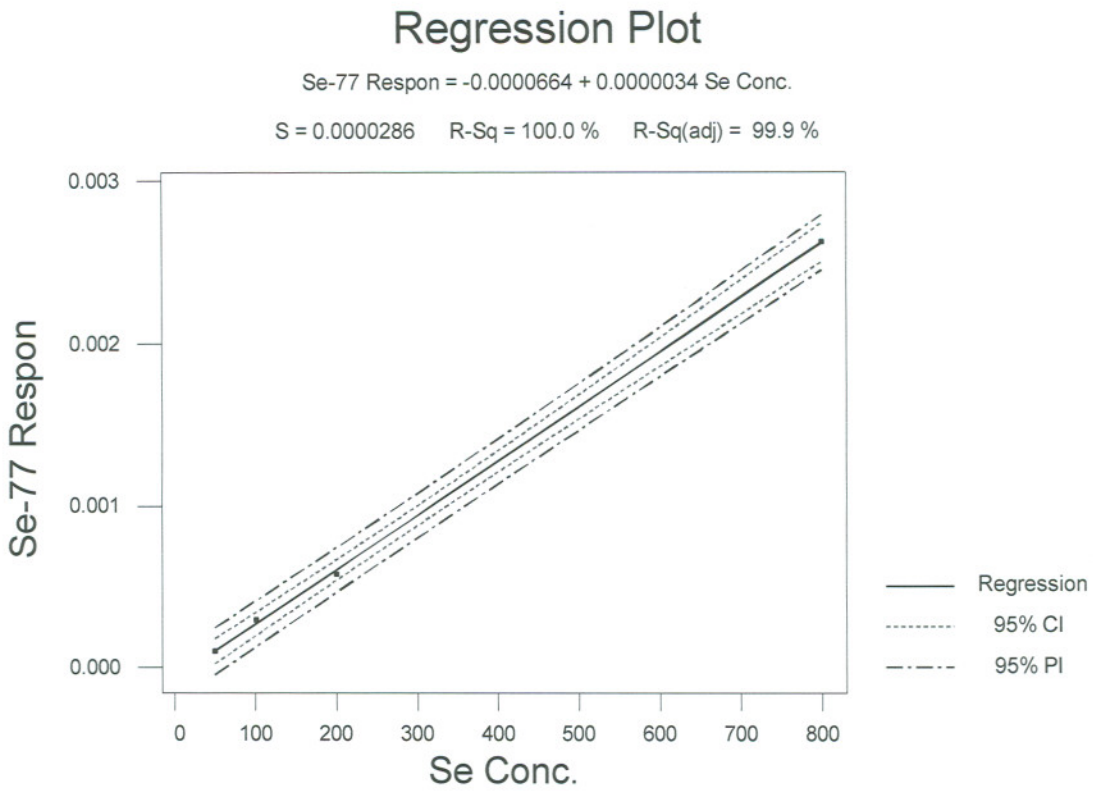
We also analyzed two blood dots and punches from the cards near the blood dot areas. The first blood dot yielded a value of 139 ppb with surrounding punch values of 12.0 and 12.2 ppb. The second blood dot yielded a value of 143 ppb with surrounding punch values of 16.2 and 13.6 ppb. A second blood dot from the same card yielded a chromium value of 161 ppb. Baselt [15] reports an average whole blood chromium concentration of 0.90 ppb among stainless steel workers contrasted against 0.43 ppb among non-welders at the same facility. The highest mean value reported in reference 14 was 6.9 ppb whole blood chromium in workers exposed to chromium (VI). We ascribe the disparity between our blood values and literature values due to signal-enhancing species formed in the argon plasma, specifically, ArC^+, HSO^+,ClO^+, and/or $HClO^+$.

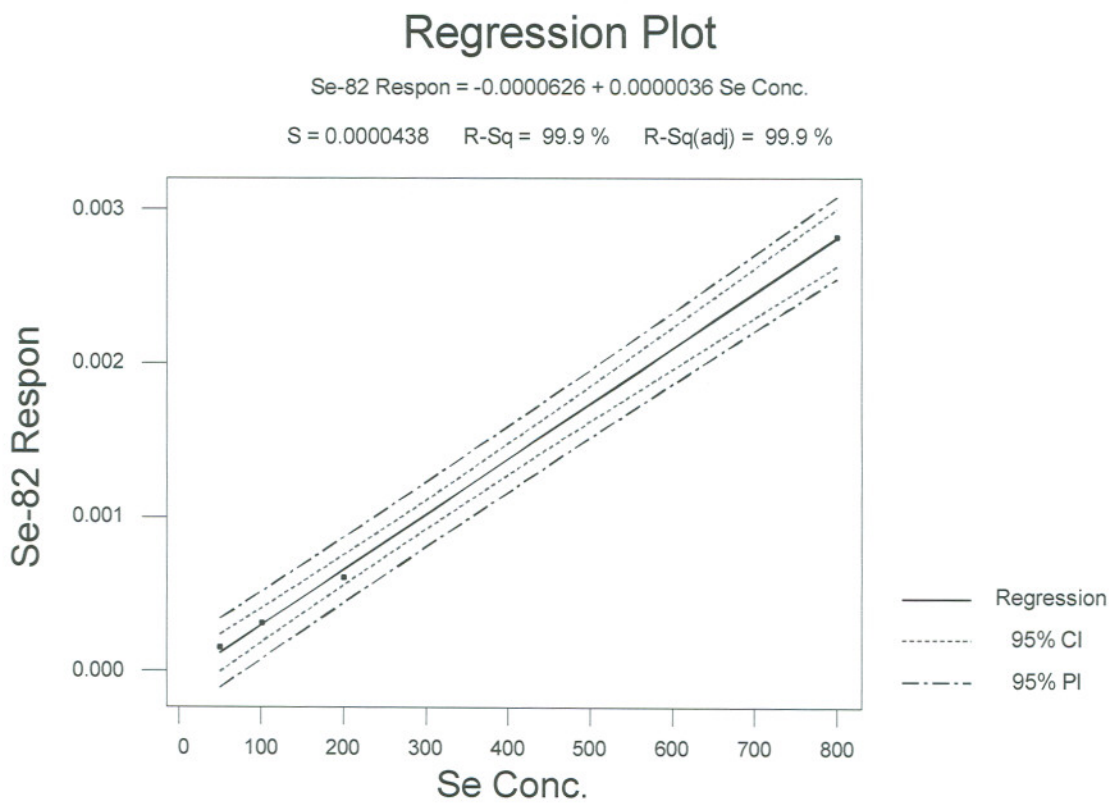
Selenium

Qualitatively, from working with this element we estimated an LOQ of 50 ppb and constructed curves starting from this point. Below are the calibration curves for selenium scanning for m/z 77 and 82, after removal of the 400 ppb standard. Both curves are comparable in the slope, intercept, and R^2 values. However, as indicated by the 95% confidence and prediction intervals, there is greater error about the m/z 82 curve. The standard deviation of the Se-82 curve is about 50% greater than the Se-77 curve.

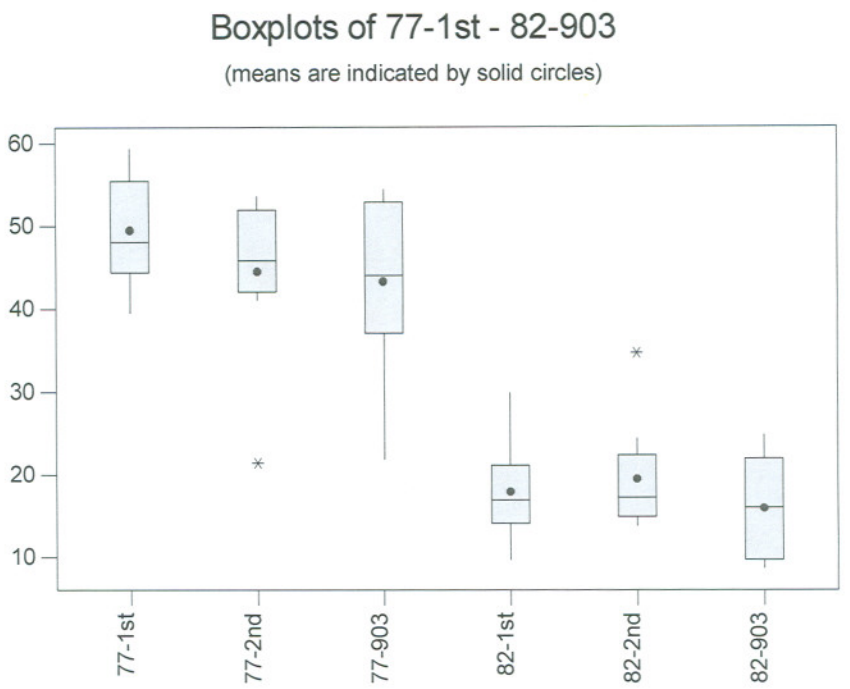
The estimated MDL utilizing the m/z 77 ion and 100 ppb standards was 23.79 ppb while the MDL utilizing the 82 mass ion was 17.26 ppb. The corresponding LOQ's are 75.7 and 54.9 ppb, respectively. The latter value of 55 ppb comes close to our qualitative estimate of 50 ppb but the former value is in error by about 52% of our qualitative estimate. The average recoveries for 100 ppb standards were 96.1 ± 7.6 % for the 77 mass ion and 97.0 ± 5.5% for the mass 82 ion.

Upon closer examination of the mass 77 ion data, one of the points appeared to be quite low. Application of both Dixon's and Grubbs tests for outlying observation [16] however, indicates no statistical reason for rejection of the low value. If however, we disregard the two lowest observations, again realizing no statistical reason to do so, the average recovery for selenium is 99.7 ± 5.0%, leading to an MDL of 18.81 ppb and an LOQ of 50 ppb. While these results have important implications that will be addressed in the next section, disregarding about 28% of the data can be a dangerous proposition and could lead to an artificially deflated estimate of the variance.


Regression Plot
Se-77 Respon = -0.0000664 + 0.0000034 Se Conc.
S = 0.0000286 R-Sq = 100.0 % R-Sq(adj) = 99.9 %
Se-77 Respon
0.003
0.002
0.001
0.000
0
100
200
300
400
500
600
700
800
Se Conc.
Regression
95% CI
95% PI


Regression Plot
Se-82 Respon = -0.0000626 + 0.0000036 Se Conc.
S = 0.0000438 R-Sq = 99.9 % R-Sq(adj) = 99.9 %
Se-82 Respon
0.003
0.002
0.001
0.000
0
100
200
300
400
500
600
700
800
Se Conc.
Regression
95% CI
95% PI

Below are the results obtained for the 1st, 2nd, and 903 blank dot analysis utilizing both the mass 77 and 82 ions.



The most apparent striking feature of this illustration is that almost all the concentrations determined using the m/z 82 ion are considerably lower than those determined using the m/z 77 ion. A similar feature, albeit not so rigidly, was noted in the chromium data.

We analyzed a single blood dot and found a concentration of 149.7 ppb utilizing the mass 77 ion, and a concentration of 46.9 ppb utilizing the mass 82 ion. Punches made from areas around the blood dot yielded 56.16 and 31.51 for m/z 77 and 10.53 and 20.02 for m/z 82. The U.S. Department of Health and Human Services [17] has reported. "In the United States, normal selenium have been reported in blood as 80 – 300 ppb…" The same agency, quoting another reference, reports a mean infant selenium level for the United States as 120 ± 8 ppb. The lowest blood level reported by the above mentioned agency is a mean level of 61 ± 14 ppb in Scandinavian infants. Taking the average of the two blank values for m/z 77 and subtracting it from the 149.7 value yields 105.9 ppb, placing it well within values for the United States. Performing the same procedure for m/z 82 yields a value of 31.6 ppb, placing this value somewhat low for any value reported. Assuming the origin of the blood to be from the United States (a very valid assumption), then this evidence suggests the m/z 77 data to be valid.

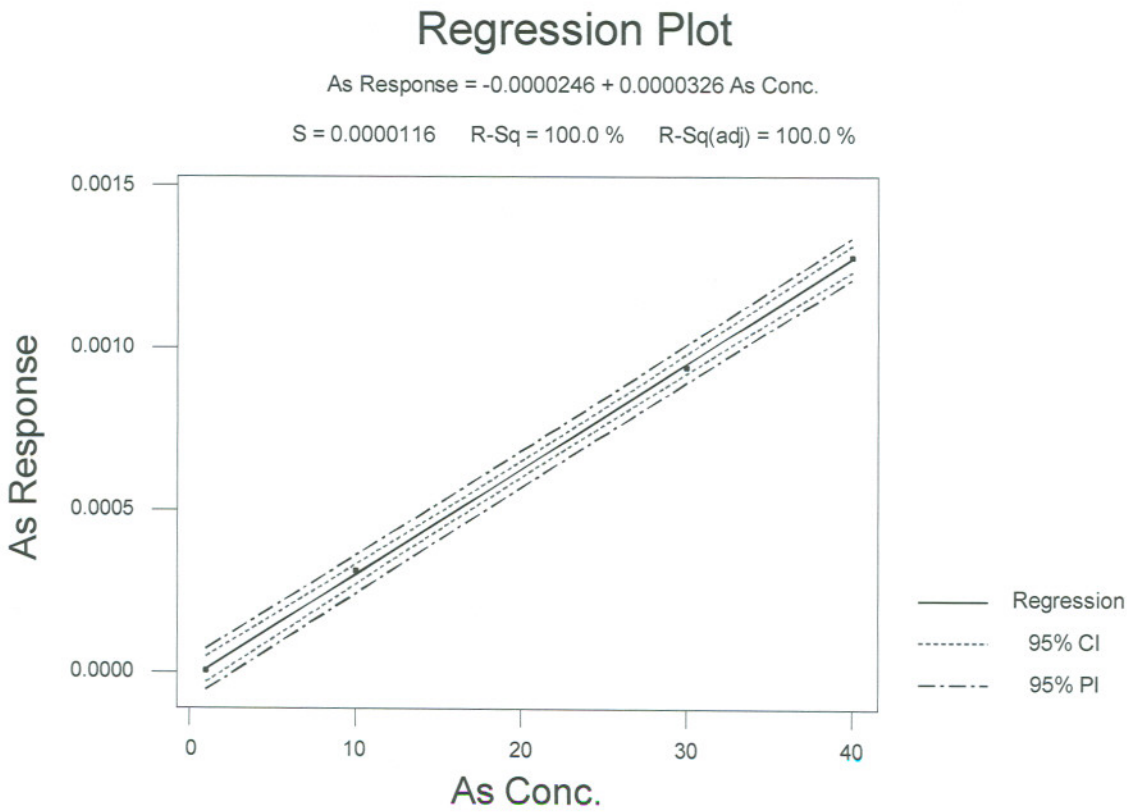
The mean blank dot values are 49.49, 44.49 and 43.23 ppb for the first, second and 903 punches, respectively. If our original LOQ estimate of 76 ppb is true, then these values

are quite far from the LOQ and it should be suspected that a fair amount of error might be contained within the estimates. However, if as previously mentioned the LOQ is truly about 50 ppb, then the mean estimates, being quite close to this value, may be deemed reliable. We calculated the 95% confidence interval for σ (the standard deviation population parameter) and found it to be between 4.87 and 16.7, which translates to the LOQ being between 49 and 167 ppb. Because this interval contains 50 ppb, the possibility that the true value is 50 ppb, at least statistically, cannot be discounted altogether. Furthermore, if the few outliers as noted are ignored, then the distribution about each mean if fairly symmetrical and normal, thus lending further credence, at least broadly, to the 50 ppb LOQ supposition.

Lastly, one-way ANOVA calculations indicate that the null hypothesis of equal means cannot be rejected. As a side note, while Se has major peaks at masses 78 and 80, Gürleyük, et al. [18] have noted that these signals are "...overlapped by...massive argon dimer peaks ($^{40}Ar^{40}Ar^{+}$, $^{40}Ar^{38}Ar^{+}$) generated by the plasma gas...," which may explain why our original calibration curves were not too good at these masses. They circumvented the problem, without resorting to hydride generation, by utilizing DRC's (dynamic reaction cells) during the analyses.

Arsenic

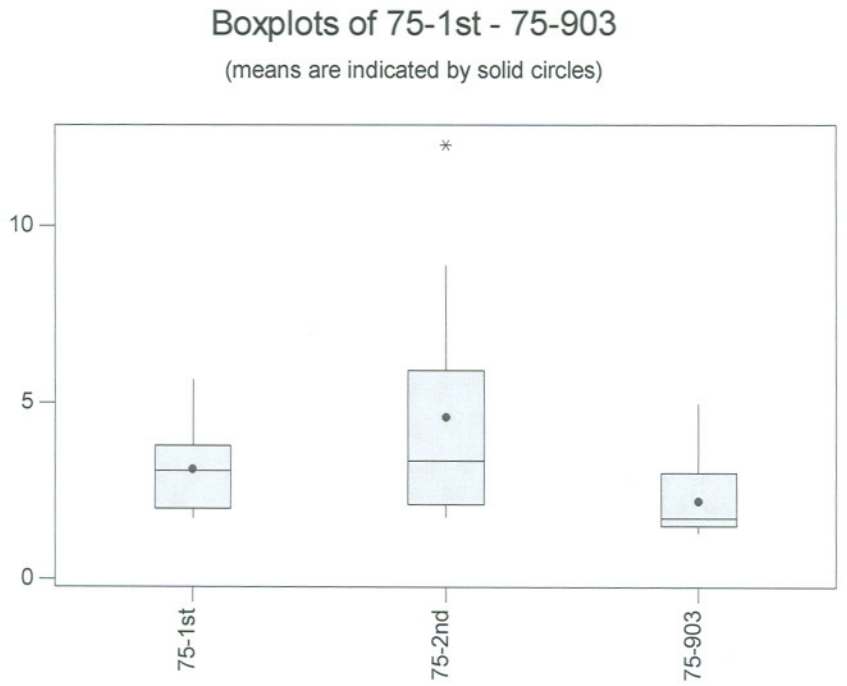
Qualitatively, we estimated an LOQ of 1.0 ppb and constructed calibration curves starting at this point. Below is the calibration curve for arsenic with its respective 95% confidence and prediction intervals with the 20 ppb discarded.



Perhaps the most striking feature of the calibration curve is the overall small standard deviation as illustrated by the close proximity of the confidence and prediction intervals to the mean line and further supported by an R^2 value close to unity.

The estimated MDL utilizing, 1.0 ppb standards, was 0.59 ppb with a corresponding LOQ estimate of 1.84 ppb. Average recovery of the 1ppb standards was 100.3 ± 18.4%. Examination of the data showed both 1 high point and 1 low point in the set. Grubbs test [16] suggests the low value could be rejected if a 10% false rejection probability is allowed. Rejection of this value leads to a mean recovery of 106.0 ± 11.2% with an MDL of 0.40 ppb and a corresponding LOQ of 1.12 ppb, close to our qualitative estimate of 1.0 ppb. If the high value is rejected as well, then the mean recovery is 102.4 ± 7.8% with an MDL of 0.29 ppb and a corresponding LOQ of 0.77 ppb, again being quite close to our original estimate.

Below are the results for the 1st, 2nd and 903 blank dot analyses for arsenic.



Similar to the observations made with chromium, it is noted that the data have tendency to be skewed high. One-way ANOVA demonstrates that the null hypothesis of equal means cannot be rejected, albeit the low p-value (8.2%) suggests there might be a tendency for one to be significantly different. This we attribute to a couple of high values in the 2nd set of punches pulling the mean high and causing it to appear to be more separated from the 903 mean distribution than might be warranted. The mean values for the 1st, 2nd, and 903 punches are 3.10, 4.49, and 2.21 ppb, respectively.

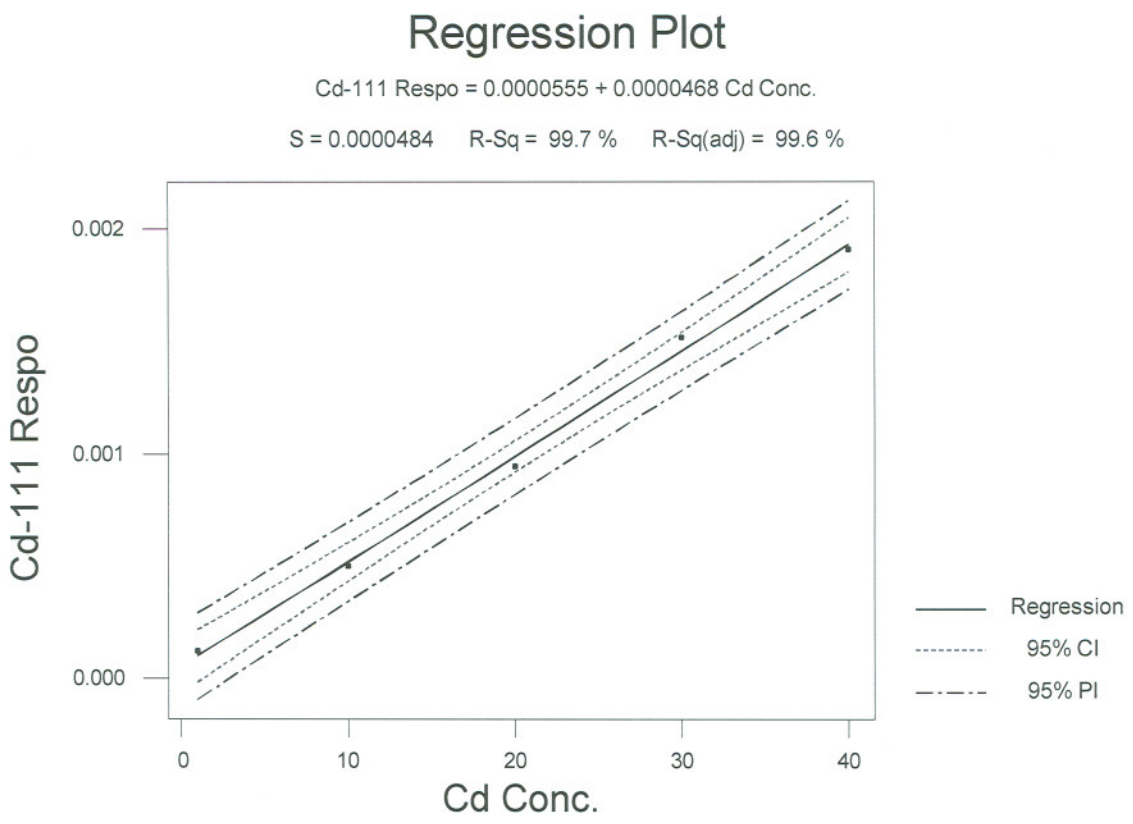
Six blood dots were extracted and analyzed for arsenic. All values were between 24 and 27 ppb. Baselt [19], quoting the work of others, reports "...[Arsenic] blood concentrations in normal subjects vary due to dietary and environmental influence and have been found to range from...[2 – 62 ppb]...in some populations..." Our reported values certainly fall within this range. However, as Baselt also reports that "[a]rsenic is...probably bound to proteins..." in tissues, we reasoned if the same might not also hold true for blood borne proteins and if 10 minutes of extraction time with 2% HNO_3 was sufficient to release the bound element if it was truly bound in the above suggested manner. We also reasoned further that if the arsenic was not being released, then the signals we observed and quantitated were artifacts probably arising from the polyatomic $^{40}Ar^{35}Cl^{+}$ ion.

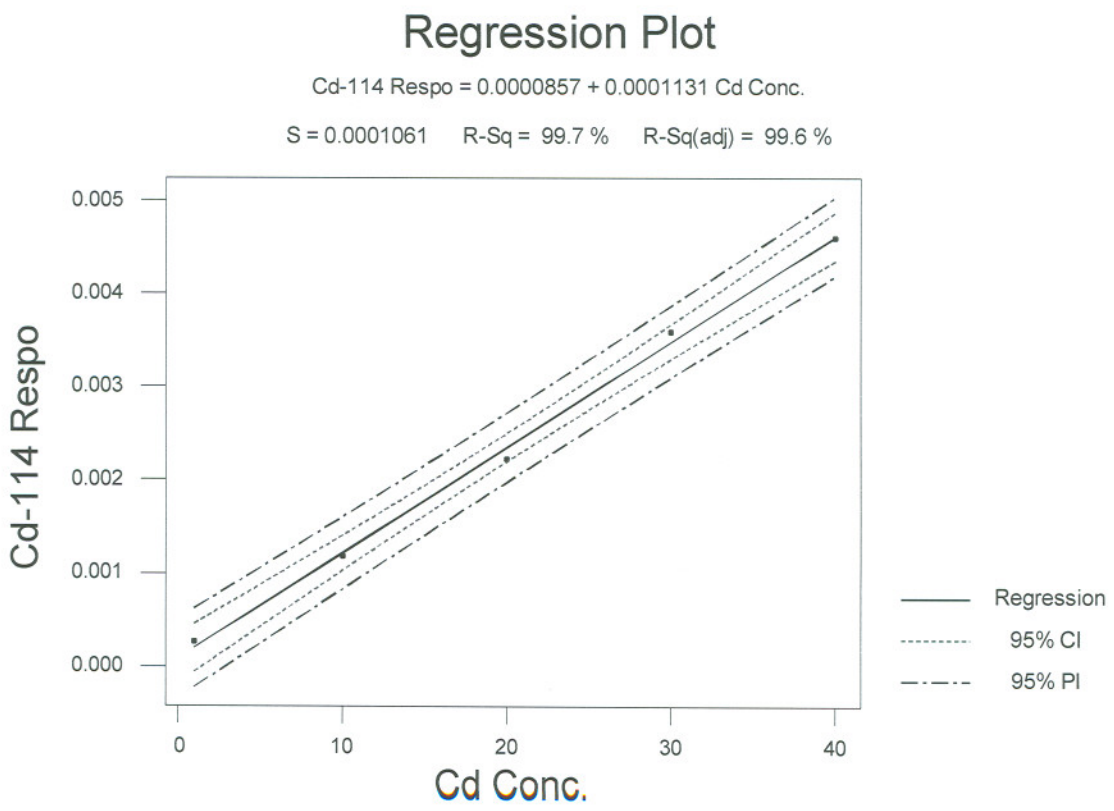
As an indirect test, we took 2 blood dots and 2 punches from the dots' surrounding periphery and extracted them for 10 minutes in 1 mL of 18.1 MΩ water. These solutions, in conjunction with a sample of the reagent water, were analyzed on a Dionex DX-500 ion chromatography system equipped with a GP40 gradient pump, a CD20 conductivity detector, a Waters 717+ autosampler, and an AS4A column. Significantly high chloride ion concentrations were found. For example, in the resulting solutions from the periphery dots, chloride ion concentrations of 524 and 586 ppb were found, indicating 0.524 and 0.586 μg of chloride, respectively, were extracted from these dots. While this is quite significant, the blood dot solutions themselves had 35,789 and 37,383 ppb concentrations of chloride ion, indicating that about 35.789 and 37.383 μg of chloride, respectively, was extracted from the blood dots. Assuming 12 μL of blood is contained in and on each dot, this translates to the blood containing 2982 and 3115 **ppm** chloride ion, respectively. With the reagent water only containing only about a 52 ppb concentration of chloride ion, the amount found in the in the blood samples was very significant indicating that the signals, and hence, the arsenic concentrations found the blood dots, must be held suspect. However, if it is also assumed that chloride ion concentrations are more or less constant in the filter paper, then the amount of arsenic found in the blank dots might reflect an actual concentration as the interfering species would be effectively subtracted via the blank.

Cadmium

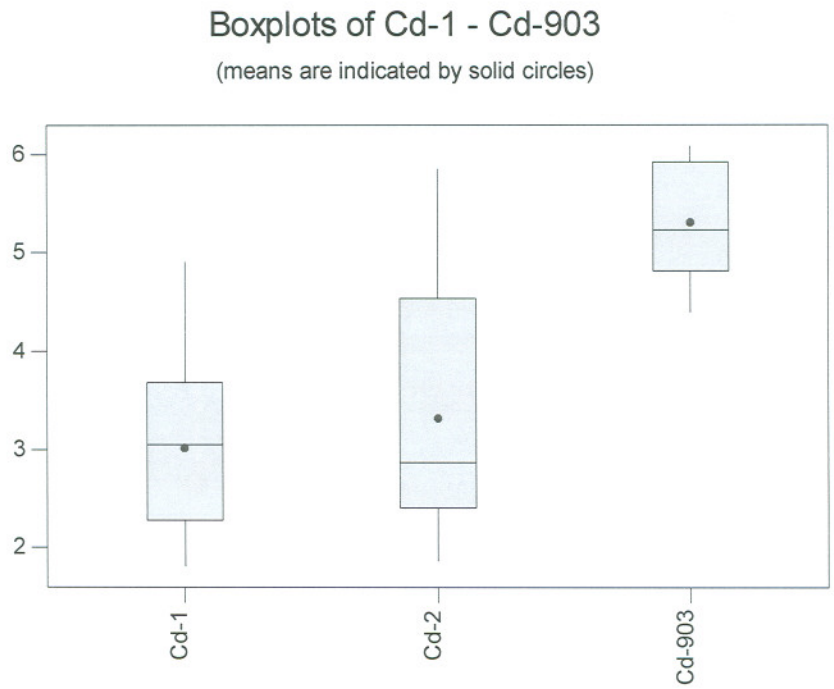
Below are the calibration curves for cadmium for both the m/z 111 and 114 mass ions with their respective 95% confidence and prediction intervals. Both curves are comparable to each other in terms of R^2 values and the amount of spread about the mean regression line as indicated by the confidence and prediction intervals.

The estimated MDL utilizing the m/z 111 ion from a 1 ppb standard was 0.88 ppb with a corresponding LOQ of 1.94ppb. The estimated MDL utilizing the m/z 114 ion was 1.26 ppb with a corresponding LOQ of 2.78 ppb. The MDL estimates, admittedly, are high relative to the LOQs. This is because a few data points were high and rejected. The last high points were retained and Grubbs test revealed the point could be rejected with less than 1% probability for false rejection. Thus, with fewer data points, higher Student t-values had to be employed to calculate the MDLs. Lastly, the LOQ estimates are somewhat high relative to our qualitative estimate of 1.0 ppb.


Regression Plot
Cd-111 Respo = 0.0000555 + 0.0000468 Cd Conc.
S = 0.0000484 R-Sq = 99.7 % R-Sq(adj) = 99.6 %
Cd-111 Respo
0.002
0.001
0.000
0
10
20
30
40
Cd Conc.
Regression
95% CI
95% PI


Regression Plot
Cd-114 Respo = 0.0000857 + 0.0001131 Cd Conc.
S = 0.0001061 R-Sq = 99.7 % R-Sq(adj) = 99.6 %
Cd-114 Respo
0.005
0.004
0.003
0.002
0.001
0.000
0
10
20
30
40
Cd Conc.
Regression
95% CI
95% PI

Below are the boxplots for the 1st, 2nd and 903 blank dot analyses for cadmium. We noted that the agreement for the two ions was quite remarkable, and we speculate this is due to the fact that all the values were close to or exceeding the LOQ estimates. As a result, the average of the values was calculated and used to estimate the values of the blank dots.



The mean values for the 1st, 2nd, and 903 dots were 3.02, 3.32, and 5.30 ppb, respectively. The 1st and 2nd dots show some tendency to be skewed high overall the distributions appear to be more-or-less symmetrical. Quite noteworthy is the fact that the 903 distribution is higher relative to the other two. One-way ANOVA confirms that the null hypothesis of equal means can be rejected and the alternate hypothesis of at least one mean being significantly different, accepted. Thus, at least in this instant, cadmium amounts across different filter papers appear to be significantly different.

We also extracted blood dots and dots from their respective, surrounding periphery. Baselt [20] states that "[m]ean serum cadmium concentrations in healthy unexposed persons have ranged from 0.5 – 2.0 μg/L in separate studies; whole blood contains nearly twice as much cadmium as serum…," indicating normal blood cadmium concentrations should be in the range of about 1.0 – 4.0 ppb. Ellenhorn and Barceloux [21] seem to indirectly concur by stating that "[l]evels above…[7.0 ppb]…indicate significant exposure."

Two blood dots were analyzed from 1st punch cards and yielded values (averaged from both ions) of 1.10 and 1.94 ppb. Their corresponding periphery values were 2.03 and 3.91 ppb, respectively. Both periphery blank values are nearly twice the values contained in the blood. However, two blood dots were also analyzed from 2nd punch cards and yielded values of 2.92 and 3.76 ppb with corresponding periphery values of 1.55 and 1.04 ppb, respectively, with the blood containing almost three times the values found in the blanks. With this limited data set we can only provide speculative explanations for these observations.

It should be noted that while all these blood values fall within the above mentioned range for normal levels (ignoring their respective periphery values), none exceed all the average blank values displayed in the boxplots. The only explanation that seems plausible at this time is that different cards contain different amounts of cadmium and not necessarily homogeneously. These facts, taken together, in conjunction with the fact that we have observed cadmium in all the filter papers suggests that cadmium is rather ubiquitous in the environment.

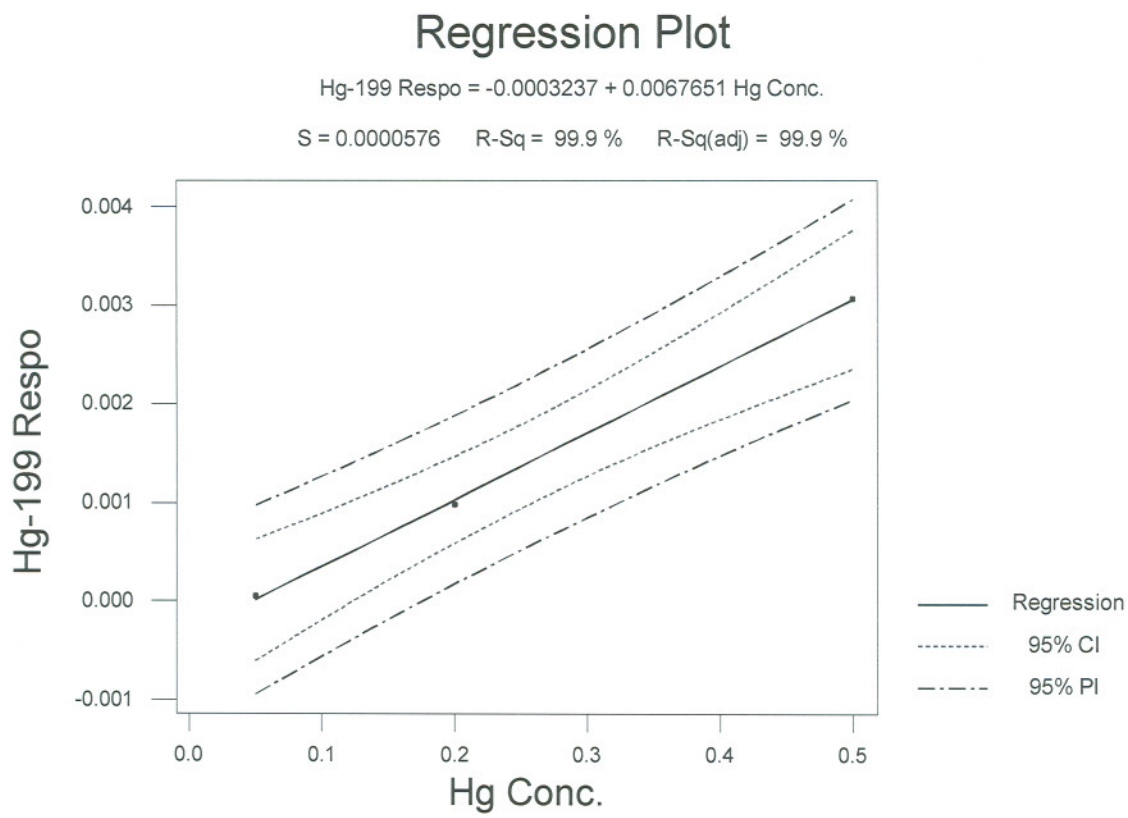
Gürleyük, et al. [18] have run cadmium via ICP/MS and ICP/DRC/MS on rainwater samples; both methods yielded similar values indicating no interfering species were being generated in these matrices. We know of no interfering species other than $^{114}Sn^{+}$ and MoO^{+} for the m/z 114 mass. Given the fact that the 111 mass was in excellent agreement with the 114 mass ion, we infer no known (uncorrected) interference, as of yet, from the samples and specimens we analyzed. This is not say the potential does not exist. For example, Baselt [22] states that mean normal blood levels for tin are 0.14 ppm, adding however, that "…not all specimens contain detectable amounts of the metal."

Mercury

From our previous work, we have found Hg to be "unstable" via ICP/MS analysis. Specifically, the calibration curves produced might be good initially, but degrade over time using the same solution, or sometimes the calibration curves produced might not be very good initially even with fresh standards. Our search of the literature indicates two mechanisms in a single paradigm exist to explain these observations. The EPA [23] states that, "Some researchers believe that mercury ions bind to the reactive sites on the surface of…high density polyethylene (HDPE)…containers. Mercury ions are thought to be reduced at these sites. The elemental mercury is lost on or through the wall of the plastic bottles. Mercury vapor may also be lost when the bottles are uncapped." The latter mechanism holds that elemental mercury is lost to the atmosphere while the former, also seemed to be held by Wilbur [24] and Schmidt [25], is that mercury is sorbed on the surface of tubing and glass parts leading to what is commonly called, "memory effects."

In reviewing the excellent work of the Minnesota Department of Health [26], they corrected for these problems by adding mercaptoethanol and L-Cysteine in their diluent and using a 4%/0.001% Triton X-100 in 2% HCl as the rinse solution. ARUP Laboratories [27] likewise uses L-Cysteine for mercury analysis via ICP/MS, adding

however, they are switching to the use of Au for such analyses. EPA [23] uses gold (III) chloride because, "[t]he gold acts as a strong oxidizing agent that converts or maintains mercury as mercuric ion which remains in solution." Schmidt [25] also recommends using it in the rinse solution as well. We prepared aqueous standards of mercury in 2% HNO_3 /200 ppb Au. Gold was likewise added to the rinse solution. Below is the calibration curve utilizing the m/z 199 ion.



The starting aqueous standard was a 0.05 ppb standard which would correlate to 4 ppb in whole blood. The curve is fairly linear with 99.9 % of variance being explained by the regression. The 95% confidence and prediction intervals are wide because there are only three points defining the curve. Additional points would be expected to shrink the intervals.

As a side note, we have noted the potential interference from W in this analysis (presumably from the $^{184}W^{16}O^{+}$ polyatomic ion) at the m/z 200 mass. In our earlier work, we have found that addition of tungsten to a mercury solution can raise the mercury signal.

Currently, we are continuing this work to incorporate the use of gold in analyzing for mercury in dried blood dots, on filter paper.

Table 3. Blank Spot values and Calculated Means for each Metal.

Metal	Blank spot Hospital First (µg/L, ppb)	Blank spot Hospital Second (µg/L, ppb)	Blank spot Unexposed (903) (µg/L, ppb)
Arsenic	1.916	2.180	3.169
	1.715	8.872	1.533
Mean 1st: 3.10 ppb	3.428	3.817	1.402
	5.646	4.686	1.799
	2.838	2.839	4.972
Mean 2nd: 4.59 ppb	3.857	1.860	1.690
	3.246	1.740	1.532
	2.135	2.698	1.754
Mean 903: 2.21 ppb	*****	12.329	1.305
	*****	4.911	2.929
Chromium	31.801	29.095	29.063
	31.823	34.874	31.069
Mean 1st: 35.22 ppb	31.517	28.080	34.865
	45.305	25.384	25.803
	40.489	32.643	29.944
Mean 2nd: 34.30 ppb	30.659	31.658	61.762
	34.417	32.580	25.989
	36.431	32.139	26.331
Mean 903:34.87 ppb	34.518	41.523	48.978
	*****	55.046	*****
Cadmium	2.4350	4.935	4.958
	2.1720	5.855	6.089
Mean 1st: 3.02 ppb	3.0460	2.931	5.503
	1.8030	2.415	5.944
	3.0925	1.853	4.965
Mean 2nd: 3.32 ppb	3.7170	3.354	4.399
	4.9070	2.399	4.674
	2.3650	2.801	4.862
Mean 903: 5.30 ppb	3.6470	*****	5.916
	*****	*****	5.720
Selenium	46.014	21.463	21.827
	39.444	43.141	41.023
Mean 1st: 49.49 ppb	46.702	41.019	28.902
	43.744	46.044	39.791
	57.938	50.903	52.393
Mean 2nd: 44.49 ppb	44.711	53.642	54.408
	52.763	45.860	42.809
	54.665	45.154	54.511
Mean 903:43.23 ppb	59.430	53.201	45.180
	49.532	*****	51.473

***** Outlier values removed.

Table 4. Mass of Blank Filter Paper Dots and their Associated Means.

Lot W011 (mass,g)	Lot W033 (mass,g)	Lot 1488 (mass,g)
0.0059	0.0079	0.0057
0.0054	0.0086	0.0056
0.0051	0.0055	0.0036
0.0066	0.0074	0.0062
0.0074	0.0036	0.0063
0.0087	0.0065	0.0064
0.0060	0.0059	0.0065
0.0056	0.0069	0.0058
0.0064	0.0060	0.0057
0.0071	0.0072	0.0078
0.0073	0.0060	0.0048
0.0054	0.0060	0.0054
0.0056	0.0062	0.0054
0.0075	0.0056	0.0073
0.0029	0.0056	0.0056
0.0058	0.0059	0.0054
0.0052	0.0065	0.0063
0.0054	0.0063	0.0059
0.0052	0.0055	0.0061
0.0049	0.0058	0.0055
Mean = 0.0059 ± 0.0009 g	Mean = 0.0060 ± 0.0012 g	Mean = 0.0062 ± 0.0010 g

Table 5. Metal Content in Hospital Blood Spots and Surrounding Periphery Dots.

Metal	Blank spot Hospital Firsts (µg/L, ppb)	Blood spot Hospital Firsts (µg/L, ppb)	Blank spot Hospital Seconds (µg/L, ppb)	Blood spot Hospital Seconds (µg/L, ppb)
Cadmium	1.91	0.81	1.45	2.95
	1.63	0.81	1.10	2.42
	1.81	1.83	0.79	3.96
	1.56	1.53	0.70	3.10
Chromium	55.8	72.7	69.6	124
	36.6	164	40.8	185
	37.2	90.5	55.1	-*
	36.8	167	38.1	157
Selenium	47.7	161	2.80	163
	19.3	140	4.63	81.7
	4.8	108	146	4.3
	7.5	158	***	***

5. Conclusions

Our initial results suggest that with exception of mercury (as we have no conclusive data yet), there are detectable amounts of arsenic, chromium, cadmium, and selenium on the filter paper. Furthermore, with exception of cadmium, while the ANOVA results for each of the metals indicated no difference in the means, there is a tendency, as illustrated in table 3, for the unexposed filter paper means to be either equal or less than the means of the exposed filter papers. With cadmium, the high mean noted on the "unexposed" filter paper could be due to the fact that it is a different lot or manufactured in a different environment.

These initial results also suggest that chromium is not suitable for analysis by this method as both the MDL and LOQs are above what normally can be expected in humans. We are also concerned about the "high" amounts of chromium found in the blank filter papers.

Selenium appears to be a viable metal to analyze by this method albeit further study is warranted. We stated that only our m/z 77 results were reliable based upon published blood levels. The background levels, found in the blank filter papers, based upon this result, were quite high (about 50% of normal U.S. blood levels) and should be of concern. However, if DRCs were utilized, then the argon dimer peaks ($^{40}Ar^{40}Ar^{+}$, $^{40}Ar^{38}Ar^{+}$) could be reduced and the greater abundant isotopes for selenium could be utilized and thus, in theory, greater sensitivity achieved.

Arsenic appears to be a viable element to analyze by this method albeit the high chloride concentrations found in the blood samples should be of great concern. The chloride interferon could likewise be eliminated with the use of DRCs. The amounts of arsenic found in the blank filter papers should also be of concern but not tremendously so.

Cadmium does not appear to be very viable by this method given its very ubiquitous nature, in the environment, as indicated by the high amounts found in the blank filter papers. The means, as noted in table 3, are all higher than the levels that might be expected in normal blood.

Mercury looks very promising is still under study for viability under this analytical paradigm.

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APPENDIX :

Appendix Table 1

Punch #	Card.Location	Lot	Group	Cr 53 ppb	Se 77 ppb	Cd 114 ppb	Pb 208 ppb
43	9.4	1488	A	0.306	0.434	0.001	0.020
47	10.2	1488	A	0.307	0.459	0.001	0.033
4	1.6	1488	A	0.291	0.446	0.001	0.020
48	10.4	1488	A	0.334	0.460	0.000	0.035
28	6.4	1488	A	0.296	0.470	0.000	0.036
73	15.4	1488	B	0.282	0.465	0.001	0.029
33	7.4	1488	B	0.273	0.457	0.001	0.018
51	11	1488	B	0.274	0.437	0.001	0.036
61	13	1488	B	0.277	0.462	0.004	0.049
38	8.4	1488	B	0.252	0.474	0.000	0.016
68	14.4	1488	A	0.269	0.485	0.027	0.020
			Average	**0.287**	**0.459**	**ND(.06)**	**ND(.10)**
100	20.8	W011	B	0.309	0.419	0.003	0.022
156	32	W011	A	0.324	0.426	0.003	0.033
141	29	W011	B	0.306	0.464	0.003	0.027
138	28.4	W011	B	0.282	0.447	0.003	0.039
135	27.8	W011	A	0.274	0.456	0.003	0.024
111	23	W011	A	0.240	0.467	0.003	0.014
93	19.4	W011	A	0.455	0.461	0.003	0.030
			Average	**0.313**	**0.449**	**ND(.06)**	**ND(.10)**
211	43	W031	A	0.345	0.408	0.008	0.031
216	44	W031	A	0.279	0.419	0.007	0.039
224	45.6	W031	A	0.286	0.420	0.008	0.033
234	47.6	W031	A	0.270	0.435	0.007	0.027
185	37.8	W031	B	0.303	0.433	0.007	0.030
226	46	W031	B	0.257	0.460	0.007	0.036
209	42.6	W031	B	0.256	0.477	0.012	0.056
			Average	**0.285**	**0.436**	**ND(.06)**	**ND(.10)**

	Extraction Sequence
Group A	1 minute vortex, 60 minute soak, 1 minute vortex, 2 minute centrifuge, transferred to new sample tube
Group B	1 minute vortex, 10 minute soak, 1 minute vortex, 2 minute centrifuge, transferred to new sample tube

4. Summary

Below are tables summarizing the overall results as they currently stand.

Table 1. Blood Spot Reference Values*.

Metal	Normal level ug/L	Smoker/Other level
Arsenic	70	
Cadmium	0.4-1.0	1.4-4.5**
Chromium		
Lead	100	70***
Mercury	15	2.3***
Selenium	0.1	1.33-7.5****

* Additional values for correlation and comparisons can be found throughout the report.
** Smoker
*** CDC's Second National Report on Human Exposure to Environmental Chemicals.
****Whole blood selenium in individuals

Table 2. Summary of DLs, Recoveries, and Calculated MDLs and LOQs for Metals of Interest.

Metal	Calculated MDL (µg/L, ppb)	Calculated LOQ (µg/L, ppb)	DL (µg/L, ppb)	Percent Recovery of DL (%)
Arsenic	0.59*	1.84*	1	100.3
Chromium	26.23	77.90	100	96.4
Cadmium	1.07**	2.36**	1	158**
Selenium	23.79***	75.70***	50	96.1
Mercury	****	****	****	****

* Values based upon all data. Actual values may be lower if outliers are removed.
** Values are averages based upon mass ions 111 and 114. Extreme outliers removed. Given the apparent, ubiquitous nature of cadmium, the high recovery value is likely due to contamination of the filter paper.
*** Values based upon all data for mass ion 77. Actual values may be lower if outliers are removed.
****No data currently available.