Analysis of Mercury Content in Dried Urine of Individuals with Amalgam Fillings Through the Utilization of Direct Mercury Analysis

by
Suchit Kapur

A PROJECT

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Biology
(Honors Scholar)

Presented April 27, 2015
Commencement June 2015

AN ABSTRACT OF THE THESIS OF

Suchit Kapur for the degree of Honors Baccalaureate of Science in Biology presented on April 27, 2015. Title: Analysis of Mercury Content in Dried Urine of Individuals with Amalgam Fillings Through the Utilization of Direct Mercury Analysis.

Abstract approved: _____

Dr. Adrian Gombart

Mercury exposure is widely common throughout the world, ranging from sources such as dental amalgams, fish and seafood consumption, industrial mining, and medicine. A straightforward, efficient, and affordable protocol to measure urine mercury content would greatly contribute to assessing one's mercury exposure and in categorizing populations that are more vulnerable to such exposure. The common current methods of mercury testing require a large amount of sample digestion time, sample quantity, and are extremely costly. Therefore, this project developed an assay that utilizes dried urine strips for direct mercury analysis, evading the limitations of time, cost, and sample quantity. To test the effectiveness of the direct mercury analysis method, dried urine samples were collected from individual's with various numbers of amalgam fillings and tested for mercury concentration. Results demonstrated that there is a direct correlation between number of amalgams and urine mercury content and that the direct mercury analysis assay is ideal for measuring urine mercury content and for further clinical applications and research.

Key Words: Mercury, Amalgams, Dental, Direct Mercury Analysis

Corresponding e-mail address: suchit.kapur@yahoo.com

Analysis of Mercury Content in Dried Urine of Individuals with Amalgam Fillings Through the Utilization of Direct Mercury Analysis

by
Suchit Kapur

A PROJECT

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Biology
(Honors Scholar)

Presented April 27, 2015
Commencement June 2015

Honors Baccalaureate of Science in Biology project of Suchit Kapur presented on April 27, 2015.

APPROVED:

Dr. Adrian Gombart, Mentor, representing Department of Biochemistry and Biophysics

Dr. Marc Curtis, Committee Member, representing Department of Botany and Plant Pathology

Dr. Devon Quick, Committee Member, representing Department of Integrative Biology

Toni Doolen, Dean, University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Suchit Kapur, Author

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude to Dr. Adrian Gombart, for his guidance and mentorship throughout the thesis writing process. Additionally, I would like to thank Dr. Marc Curtis and Dr. Devon Quick for their time, encouragement, and support throughout my thesis process and throughout the last few years of my undergraduate studies. I would also like to thank ZRT Laboratory for the opportunity to conduct this research in a professional environment. My gratitude further extends to the University Honors College and to my honors advisor Rebekah Lancelin, who has provided me with her guidance and enthusiasm over the past four years. I thank all of my friends and colleagues at Oregon State University who have continued to surround me with their optimism throughout my tenure. Finally, the greatest appreciation goes to my father, mother, and younger brother, who have always motivated me to attain all my aspirations.

TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION

i. Background

Mercury, a liquid element at room temperature, is an extremely toxic metal present both in the environment and in common daily settings. Due to the pervasive presence of this element in the environment, human beings are extremely susceptible to being exposed. Most commonly, individuals and the environment are exposed to mercury through its release in three different forms: elemental (metallic), inorganic (mercuric chloride), and organic (methyl, ethyl). Sources of possible mercury contamination include volcanic explosions, combustion of fuels, ritual and folk medicine, photography solutions, disinfectants, thermometers, various lighting, cement production, coal factories, seafood consumption, vaccine preservatives, fish consumption and waste disposal. Annually, 50 tons of mercury are emitted into the environment. There is significant risk of toxicity to individuals who reside close to heavy mercury-industrialized areas such as the south Asian countries, where coal factories are abundant (1).

ii. *Standard Reference Levels*

Due to the various risks of mercury exposure, many health organizations across the world have determined reference levels for individuals to monitor their mercuric intake. According to the World Health Organization, reference levels fall within the range of 0.7 to 2 μg methylmercury per kilogram body weight (μg/kg body weight) per week for organic mercury exposure (2). In regards to inorganic mercury (mercuric chloride) and methylmercury, the United States Environmental Protection Agency (EPA) has established reference doses of 0.3 μg/kg body weight/day and 0.1 μg/kg body weight/day, respectively (8). These reference levels have been established for the various sources of exposure to mercury poisoning.

In many human populations, fish consumption is one of the most frequent forms of exposure to mercury. Therefore, the US Food and Drug Administration (FDA) recommends individuals to not exceed consumption of methylmercury in both finned fish and shellfish of 1 mg methylmercury/kg (9).

iii. Exposure to Dental Amalgams

One of the more frequent sources of mercury is the exposure to dental amalgams, more commonly known as silver fillings, which are common in individuals with extensive dental fillings made of amalgam. With a 50 percent mercury composition (1 g Hg per filling), dental amalgams are responsible for at least 60-95 percent of mercury deposits in human tissues (7). Exposure to dental amalgams is extremely common, as those affected range from young children to the elderly. With each of these individuals committing to an average of two visits a year for approximately one hour, the amount of amalgam exposure can become noticeable. In addition to patients, the individuals that are most commonly affected by the exposure to amalgams are dental professionals, especially dental assistants (6). The mercury released from amalgam fillings accumulates in and is excreted from the kidneys. Quantification of total body mercury burden is most accurately obtained from urine mercury levels, as due to the fact that both inorganic and elemental mercury are primarily found in urine, as opposed to other common bodily fluids such as blood and saliva (10).

iv. Economic Impact on Health

With the dental profession being held in high regard, it is important to analyze the mercury content of amalgams, as they have been known to excrete mercuric vapor. There are many alternative options to treat tooth decay, and these include resin composite, glass ionomer, resin ionomer, porcelain, and gold alloys. However, many individuals in need of care are significantly underprivileged, and their dental plans only cover amalgam fillings and restorations. Due to this economic situation, these individuals are thus exposed to the possibility of elemental toxicity and poisoning, with various adversities that could possibly follow. Extreme cases include psychiatric disturbance, paresthesia, paralysis, breathing difficulty, mercuric pigmentation, and more (11). Furthermore, many of these individuals work in factory and industrial environments where mercury exposure is high in all three forms of mercury (1,3).

Methylmercury is known to adversely affect neurological development in fetuses, infants and children. Those affected by mercury display deficits in memory, fine motor control, language, and attention. Elemental and inorganic mercury exposure through vapors results in

more visual symptoms which include mood swings, nervousness, insomnia, and neuromuscular deficiencies. Extremely high exposures can result in respiratory failure and death (11). In terms of dental amalgams, long term evidence of mercury effects remains inconclusive.

v. ***Hypothesis***

Because dental amalgams are a potential source of mercury in humans, we hypothesize that increased numbers of amalgam fillings will lead to increased levels of mercury in the urine. In other words, if an individual has a preponderance of amalgam fillings and restorations as opposed to an alternative material, then his or her urine will contain a greater concentration of total mercury in μg/L, due to a direct correlation between number of amalgams and mercury concentration. This hypothesis will be tested through the development of a direct mercury analysis assay.

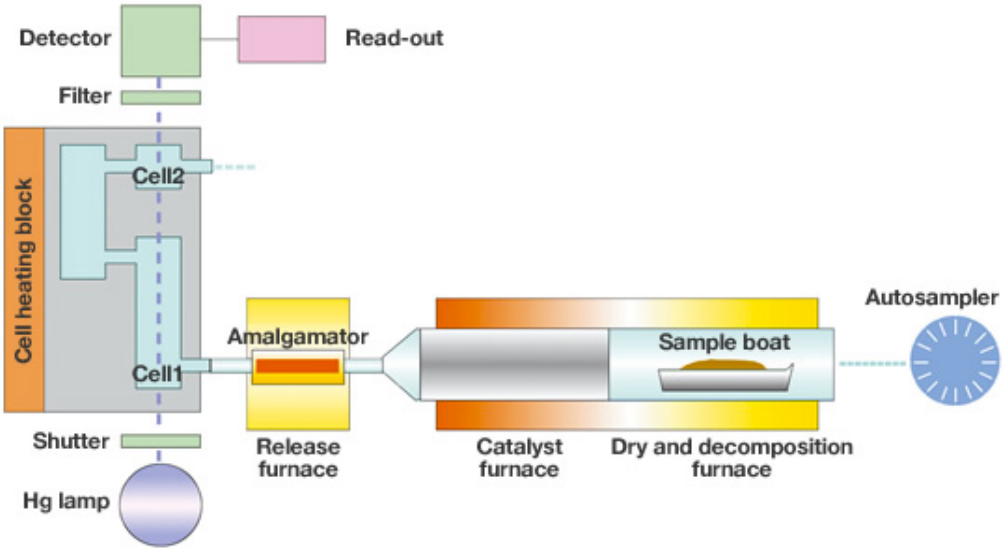
CHAPTER 2. ASSAY DEVELOPMENT & METHODS

i. Overall Assay Variety

Scientific research has explored various samples for mercury content through more traditional and common techniques such as mass spectrometry and cold vapor atomic absorption. However, these methods are quite taxing in terms of both cost and time. The instruments required for the more traditional and complex methods are much more expensive than direct analysis, and in addition to the expenses, sample preparation and digestion significantly increase assay length by more than double the amount of time. Therefore, the procedure of direct mercury analysis with the use of a DMA-80 Direct Mercury Analyzer (Milestone Scientific) is crucial in reducing both cost and test time while testing for the mercury content of dried urine samples of a diverse population with a varied number of amalgam fillings.

ii. DMA Assay Principles of Operation

Direct Mercury Analysis with utilization of the Milestone Scientific DMA-80 Direct Mercury Analyzer is run through the combination of amalgamation and thermal decomposition through combustion techniques. Punches of dried urine are placed directly into the nickel boats which are then put through a drying and thermal decomposition phase at approximately 600 degrees Celsius. Oxygen flow then carries products of the decomposition phase to a catalyst tube, where all of the mercury is converted to its elemental form. The elemental mercury then enters and is trapped in the gold amalgamation tube, which is then heated so that the trapped mercury is released to the atomic absorption spectrophotometer. Here, an absorption reading is taken and the total mercury concentration in a sample is determined.



http://www.milestonesci.com/direct-mercury-analyzer/dma80-principles.html

Figure 2.1. *DMA-80 Schematic displaying analyzer components and principles of operation. Basic principles include thermal decomposition, amalgamation, and reduction of all Hg forms to elemental Hg.*

iii. Materials & Methods

▸ *General Assay*

Materials Required

- 1000 ppm mercury (Hg) stock (Perkin Elmer)
- DI H2O
- 37% HCl stock solution (Sigma-Aldrich)
- 69% HNO3 stock solution (Fruka)
- 15 mL Polypropylene Screw Cap Tubes (Perkin Elmer)
- 50 mL Polypropylene Screw Cap Tubes (Perkin Elmer)
- 8 mL Polypropylene Tubes (Perkin Elmer)
- 10 quartz boats (Milestone Scientific)
- 120 nickel boats (Milestone Scientific)
- Dried Filter Strips (PerkinElmer - Ahlstrom 226)
- DMA-80 Direct Mercury Analyzer (Milestone Scientific)
- Oxygen tank (Industrial Grade)
- Keyboard
- DMA-80 Terminal Screen (Milestone Scientific)
- DMA-80 Flowmeter (Milestone Scientific)

- DMA-80 USB Key (Milestone Scientific)
- Catalyst Tube (Milestone Scientific)
- Amalgamator Tube (Milestone Scientific)
- Cooking flour
- DBS puncher (PerkinElmer)
- 6-mm “large” punch
- 96-well Sized Punch Catcher
- Sample Organization Tray
- Laboratory grade tape
- Cleaning Brush
- Seronorm Trace Elements Urine Controls Level I and II (Sero)
- ClinChek Trace Elements Urine Controls Level I and II (ClinChek)
- Bio-Rad Lyphocheck Quantitative Urine Control Level I (Bio-Rad)
- ZRT Laboratory software application, Lab Assistant

Reagent Preparation

Materials Required

- DI H2O
- 37% HCl stock solution (Sigma-Aldrich)
- 69% HNO3 stock solution (Fruka)
- 50 mL Polypropylene Screw Cap Tubes (Perkin Elmer)
- Micropipettes - 1 mL, 10 mL, 200 uL

Preparing 3.7% HCl

1. Obtain a 50 mL polypropylene tube and add 45 mL DI H2O
2. With extreme caution, add 5 mL 37% HCl stock solution to the 50 mL polypropylene tube
3. Fasten the cap to the tube and vortex until internal contents are thoroughly uniform
4. Label the tube and store at room temperature

Preparing 10% HNO3

1. Obtain a 50 mL polypropylene tube and add 42.76 mL DI H2O
2. With extreme caution, add 7.24 mL 69% HNO3 stock solution to the 50 mL polypropylene tube
3. Fasten the cap to the tube and vortex until internal contents are thoroughly uniform
4. Label the tube and store at room temperature

Standards, Calibrators, Controls

Materials Required

- 1000 ppm mercury (Hg) stock (PerkinElmer)
- DI H2O
- 3.7% HCl
- Seronorm Trace Elements Urine Controls Level I and II (Sero)
- ClinChek Trace Elements Urine Controls Level I and II (ClinChek)
- Bio-Rad Lyphocheck Quantitative Urine Control Level I (Bio-Rad)
- 1 mL pipette
- Dried Filter Strips (PerkinElmer-Ahlstrom 226)
- Laboratory Tape
- 15 mL Polypropylene Screw Cap Tubes (PerkinElmer)
- 8 mL Polypropylene Tubes (PerkinElmer)

Preparing High Mercury Standard (100 ug/L)

1. Obtain a 15 mL polypropylene tube and add 100 uL of 1000 ppm mercury stock solution by PerkinElmer to 9.9 mL 3.7% HCl
2. Vortex the contents of the tube to create Solution A.
3. Obtain an additional 15 mL polypropylene tube with 9.9 mL 3.7% HCl and add 100 uL of Solution A.
4. Properly label the solutions and prepare fresh standards for each time they are needed (typically on a weekly basis)

Preparing Solutions for Standard Curve

1. Obtain 6 polypropylene tubes (8 mL) and label them 0 ug/L (blank), 0.3906 ug/L, 1.5625 ug/L, 6.25 ug/L, 25 ug/L, and 100 ug/L.
2. Dispense 4 mL 100 µg/L high mercury standard with a 10 mL autopipette into the 100 ug/L tube.
3. Dispense 3 mL 3.7% HCl into each of the remaining tubes (0 ug/L (blank), 0.3906 ug/L, 1.5625 ug/L, 6.25 ug/L, 25 ug/L).
4. Transfer 1 mL 100 ug/L solution into the tube marked 25 ug/L tube and vortex.
5. Transfer 1 mL 25 ug/L solution into the tube marked 6.25 ug/L and vortex.
6. Transfer 1 mL 6.25 ug/L solution into the tube marked 1.5625 ug/L and vortex.
7. Transfer 1 mL 1.5625 ug/L solution into the tube marked 0.3906 ug/L tube and vortex.
8. Do not transfer any additional solution into the 0 ug/L blank with 3.7% HCl.

Creating Dry Mercury Standards

1. Transfer 1 mL of each mercury standard, calibrator and control created above to individual filter strips.
2. Place filter strips with tape on lab bench and let dry for at least four hours.
3. Store at -20 degrees Celsius in sealed bags until needed.

Setting up the DMA-80 Direct Mercury Analyzer

Materials Required

- DMA-80 Direct Mercury Analyzer (Milestone Scientific)
- Oxygen tank (Industrial Grade)
- Keyboard
- DMA-80 Terminal Screen (Milestone Scientific)
- DMA-80 Flowmeter (Milestone Scientific)
- DMA-80 USB Key (Milestone Scientific)
- USB Barcode Scanner

Oxygen Connection

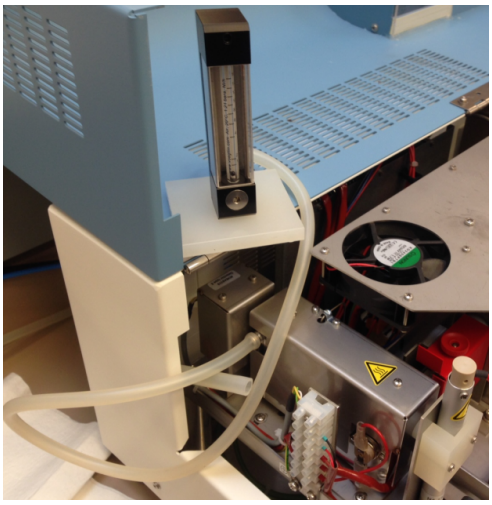
1. Connect the analyzer directly to an industrial grade oxygen tank.
2. Set the oxygen tank output pressure to 70 psi.

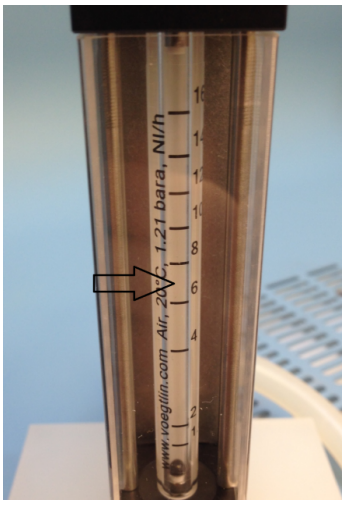
Activating the Analyzer

1. Switch the on/off switch to “on”. This will then initialize the machine and turn on the connected computer.

2. Open the top component of the analyzer and disconnect the exhaust tubing.

3. Connect the DMA-80 flow meter to the analyzer and set flow between 6 and 8 on the flow gauge. Utilize the adjustment knob on the back side of the analyzer to correct flow if it fluctuates.

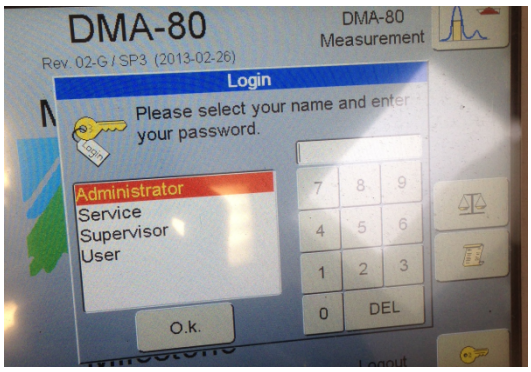


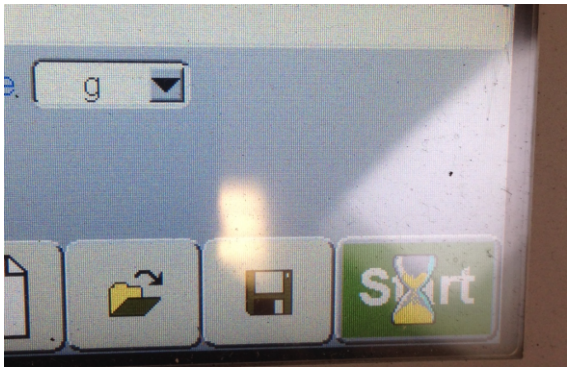


4. Re-connect the exhaust tubing once flow is corrected and set.

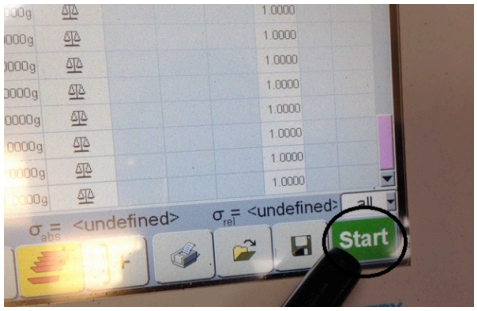
Logging in to Analyzer Portal

1. Connect the DMA-80 USB Key, Keyboard and USB Barcode Scanner to the computer.
2. Use passcode to login as "Administrator"; the analyzer will now be in standby mode (hourglass on green "Start" button).





3. The analyzer will warm up for approximately 15 minutes. Once warmed up, the green "Start" button will no longer have an hourglass symbol.



▸ *Utilizing the DMA-80 Direct Mercury Analyzer - Method*

Materials Required

- DMA-80 Direct Mercury Analyzer (Milestone Scientific)
- DMA-80 Terminal Screen (Milestone Scientific)
- DMA-80 USB Key (Milestone Scientific)
- Keyboard

Method Selection

1. The assay utilizes two separate methods, one for cleaning and one for sample analysis:

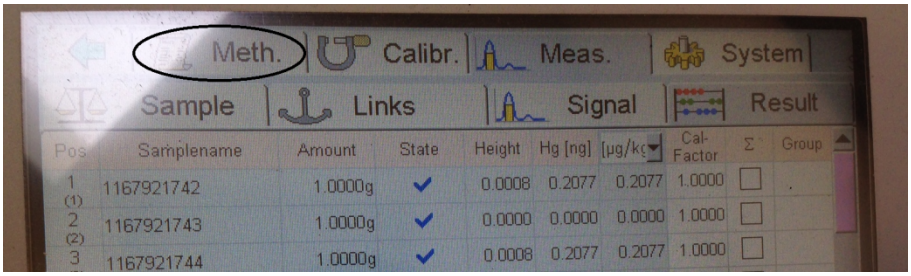
Table 2.1. *Method Specifications for the Direct Mercury Analyzer*

Method	Max Start Temp. (T)	Purge Time (P)	Amalgamator Time (H)	Signal Recording Time (R)
CleanProcedure.m80	350°C	60 seconds	12 seconds	30 seconds
DriedUrine.m80	250°C	60 seconds	12 seconds	30 seconds

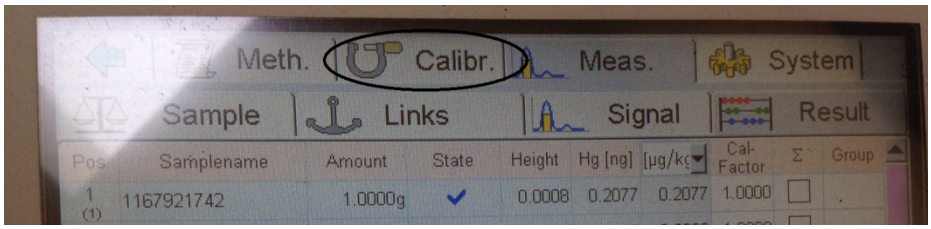
2. Under the “Method” tab, select the desired method out of the two preset methods.

Cleaning Sample Boats

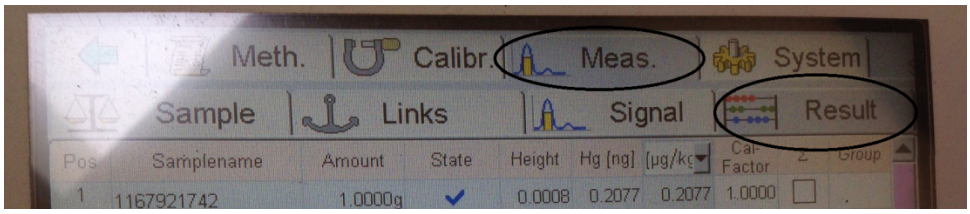
1. Clean all residues from each individual sample boat with a wire brush to avoid sample contamination.
2. Load boats into 40 position auto-sampler on the DMA-80 Mercury Analyzer, starting at position 1.
3. Select “CleanProcedure.m80” under the “Method” tab.



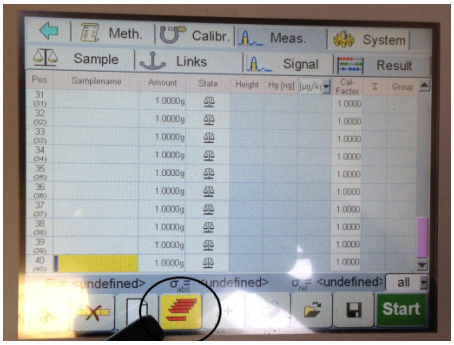
4. Under the “Calibration” tab, select the most recent calibration file.



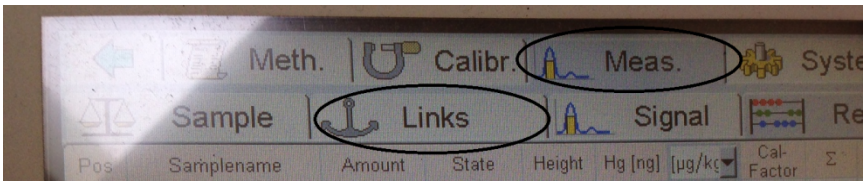
5. In the “Result” section of the “Measurements” tab, create a new sample; be sure to save any previous work.



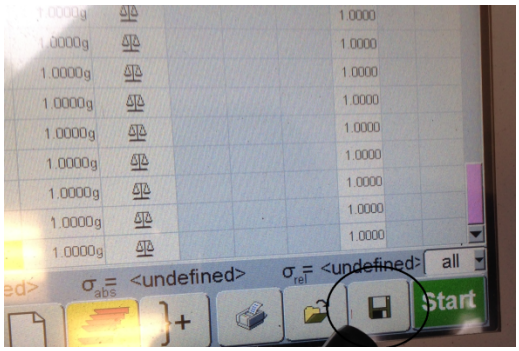
6. Select the multi-sample icon.



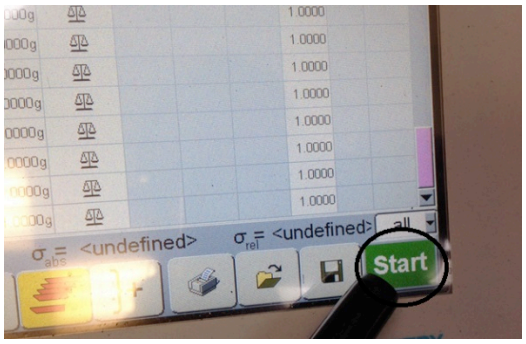
7. Add boat positions according to number. Set default weight of all boats to 1.000 g.
8. Click on the “Links” tab to ensure that the desired calibration file and method are being utilized.



9. Save the current run under the name “Boat Clean” and continue to use the same file for each clean (unless excess residue results).



10. Click on the green “Start” button.



11. Once the clean procedure is complete, re-save the file and select “okay” when asked to overwrite.

Creating New Calibration File

1. Select “DriedUrine.m80” under the “Method” tab.
2. Create a new calibration under the “Calibration” tab. Save the calibration with the desired file name.
3. Select the multi-sample icon.
4. Calibrations consist of 14 samples. Add the required positions to the measurement list. Change the “State” to “C” for calibration.

5. Once a calibration point is complete, the “State” will switch from a pink to a black “C”.
6. Sample calibration list:

Table 2.2*. Example Calibration Specifications for Direct Mercury Analysis Method*

Position	Sample Name	Amount	State	Height	Hg [ng]	[µg/kg]	Cal. Factor
1 (1)	Air Blank 1	1.000 g					1.000
2 (2)	Air Blank 2	1.000 g					1.000
3 (3)	0 µg/L	1.000 g	C		0	0	1.000
4 (4)	0 µg/L	1.000 g	C		0	0	1.000
5 (5)	0.3906 µg/L	1.000 g	C		0.3906	0.3906	1.000
6 (6)	0.3906 µg/L	1.000 g	C		0.3906	0.3906	1.000
7 (7)	1.5625 µg/L	1.000 g	C		1.5625	1.5625	1.000
8 (8)	1.5625 µg/L	1.000 g	C		1.5625	1.5625	1.000
9 (9)	6.25 µg/L	1.000 g	C		6.25	6.25	1.000
10 (10)	6.25 µg/L	1.000 g	C		6.25	6.25	1.000
11 (11)	25 µg/L	1.000 g	C		25	25	1.000
12 (12)	25 µg/L	1.000 g	C		25	25	1.000
13 (13)	100 µg/L	1.000 g	C		100	100	1.000
14 (14)	100 µg/L	1.000 g	C		100	100	1.000

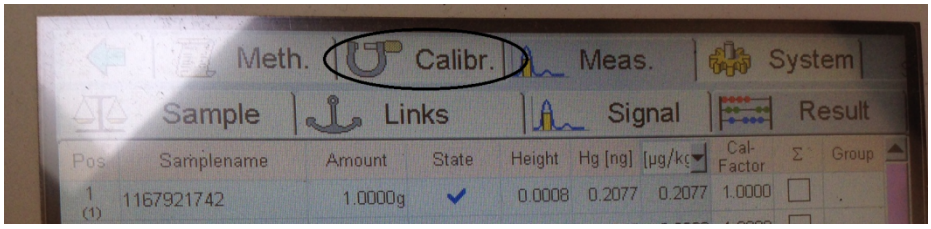
Preparing and Executing Calibration Sample Run

1. Place 12 clean boats in positions 3-14 of the auto-sampler. Positions 1 and 2 are for air blanks.
2. Punch 6 punches (6mm holes) of the required standard into a small-sized punch tray. Keep separate punch trays for each calibration standard.
3. Place punches into their respective boats and save the calibration file.
4. Press the green “Start” icon. Results will display under the “Calibration” tab.

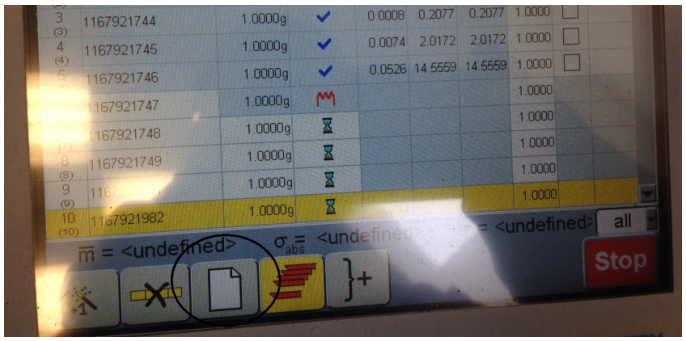
5. Once complete, save results **immediately** to avoid data loss. Results will switch from red to blue in color once saved.
6. Select "square" under the "Approximation" drop down list. The R^2 value is most ideal when it is approximately 1.00.
7. Save the calibration run in both the "Results" tab and the "Measurements" tab.

Preparing and Executing Mercury Sample Run

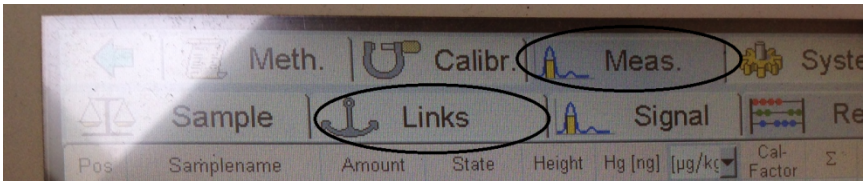
1. Ensure that DMA-80 Mercury Analyzer is on, "Administrator" is logged in, and desired calibration files are previously saved.
2. Select "DriedUrine.m80" under the "Method" tab.
3. Select the desired calibration curve under the "Calibration" tab.



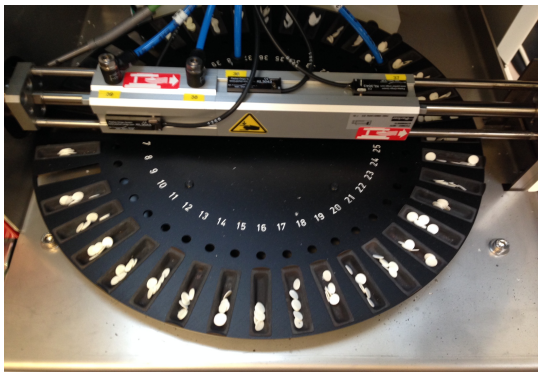
4. Create a new sample list by selecting the "Blank Sheet" icon.



5. Select the multi-sample icon.
6. Enter the required number of samples to be run by selecting the "add samples" icon.
7. Enter 1.000 g as a default weight for all samples.
8. Save the sample run under a desired file name.
9. Ensure that the desired calibration file and correct method is selected under the "Links" tab.



10. Load the required amount of previously cleaned sample boats onto the auto-sampler.
11. Punch 6 punches (6mm holes) of the required dry samples, calibrators, controls, and filter paper blanks into small-sized punch trays. Keep separate punch trays for each entity.
12. Place the punches into their respective boats and ensure that the boats are fully positioned into the auto-sampler.



13. Select the green "Start" button to start the sample run. Once complete, save the results under a desired file name.

iv. Determining Correction Factor

▸ *Correction Principle*

Due to daily instrumental variation in the assay protocol, final readings of mercury concentration need to be adjusted by a correction factor for each sample run. This correction factor is determined by running a set of controls with pre-determined mercury concentrations (for each sample run) and analyzing the variation between the observed experimental results and the expected results.

▸ *Correction Methods and Controls*

Controls Required

- Seronorm Trace Elements Urine Controls Level I and II (Sero)
- ClinChek Trace Elements Urine Controls Level I and II (ClinChek)
- Bio-Rad 376 Lyphocheck Quantitative Urine Control Level I (Bio-Rad)

Standard Mercury Concentrations

- Seronorm Level I: 0.036 µg/L
- Seronorm Level II: 39.8 µg/L
- ClinChek Level I: 2.48 µg/L
- ClinChek Level II: 18.1 µg/L
- Bio-Rad 376 Level I: 13.8 µg/L

Adjustment Procedure

1. Created a grading sheet on Microsoft Excel as seen below:

Mercury Correction Factor

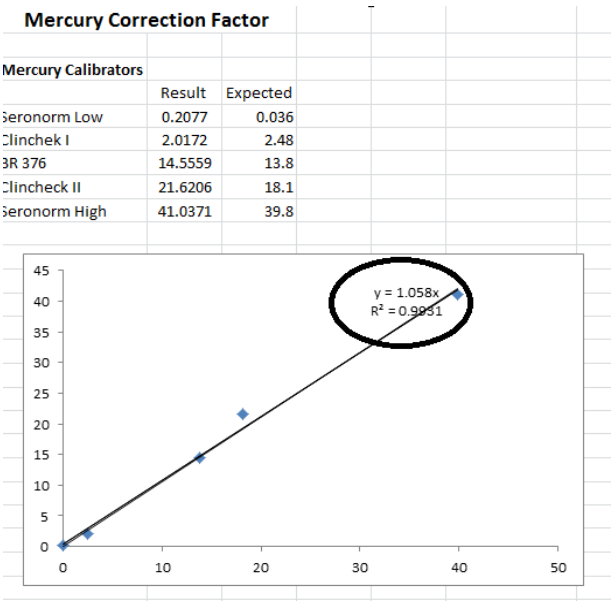
Mercury Calibrators

	Result	Expected
Seronorm Low		0.036
Clinchek I		2.48
BR 376		13.8
Clincheck II		18.1
Seronorm High		39.8

Mercury Control Results

		Mercur Expected
	- 2SD	0.32
A	Mean	0.40
	+ 2SD	0.47
	- 2SD	0.59
B	Mean	0.96
	+ 2SD	1.33

2. The slope of the line created by plotting expected mercury results on the x-axis versus experimental results on the y-axis will then be utilized to correct the mercury sample run results. The results will be multiplied by the slope as the correction factor. An example of a mercury adjustment is seen below:



Mercury Correction Factor

Mercury Calibrators

	Result	Expected
Seronorm Low	0.2077	0.036
Clinchek I	2.0172	2.48
BR 376	14.5559	13.8
Clincheck II	21.6206	18.1
Seronorm High	41.0371	39.8

CHAPTER 3. URINE COLLECTION TEST

To test our original hypothesis that increased numbers of amalgam fillings will lead to increased levels of mercury in the urine, we designed a study that involved collecting urine from healthy individuals with varying numbers of amalgam fillings. The characteristics of our study population were as follows:

i. Age Range

Study participants varied in age, ranging from as young as nineteen years old to as old as sixty-seven years old. The median age of participants was forty-three years old. The range allowed for collection of a diverse population.

ii. Health Status

Study participants were all healthy individuals with no major health issues. This was to prevent the data from being affected by anything other than dental amalgams.

iii. Number of Amalgam Fillings

Participants were required to indicate the number of amalgam fillings they had. The number of fillings ranged from zero fillings to sixteen fillings.

iv. Protocol Approval

The urine collection test was conducted under approval by ZRT Laboratory's official confidentiality and consent form. Participants read through the procedure thoroughly and signed before urine collection.

v. Collection Times

Participants were instructed to collect approximately 50 mL of urine at four different times during the day: morning (A), early afternoon (B), evening (C), and bedtime (D). The collection at the different times allowed for the consideration of food consumption and any environmental exposure that may affect mercury content analysis.

vi. Sample Procedure

Liquid samples from all participants were dried onto filter paper, punched as 6 mm punches, and analyzed with the DMA-80 Direct Mercury Analyzer.

DATA & RESULTS

The urine collection experiment was conducted to generate data that displays the capabilities of the direct mercury analysis assay as an efficient and effective method of mercury detection, as well as to indicate the effects of dental amalgams upon an individual's urine mercury content. The data obtained in the study supported these initial motives, demonstrating that the direct mercury analysis assay accurately and efficiently reported measurements of urine mercury in individuals with amalgam fillings.

i. Overview of Collection Study

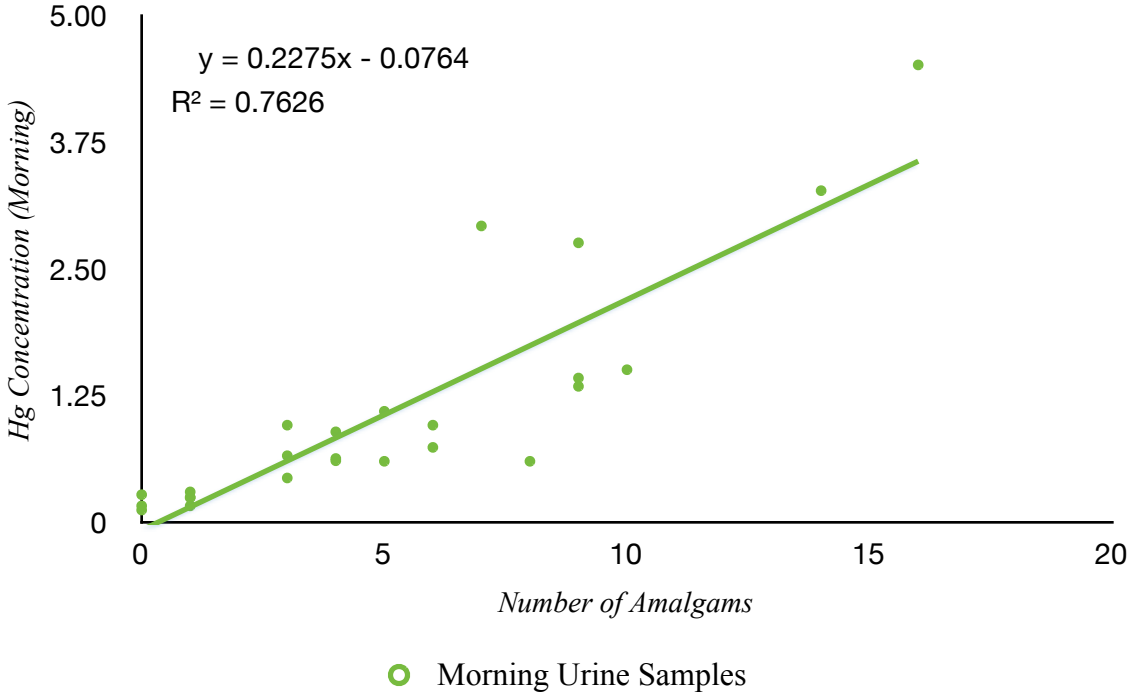
Following initial linearity confirmation in regards to controls, mercury content was tested for study participants. Average values ranged from 0.1559 µg/L to 3.7549 µg/L, with a mean concentration of 1.0040 µg/L and a median value of 0.6160 µg/L (Table 3.1). The morning collection results displayed a linear relationship between number of amalgams and mercury concentration with an R-squared value of 0.7626 (Figure 3.1A). Linearity was also visually present in the afternoon samples, with an R-squared value of 0.6624 (Figure 3.1B). The evening samples displayed greater variation than both the morning and afternoon samples, resulting in an R-squared value of 0.5543 (Figure 3.1C). The bedtime collections returned greater linearity with an R-squared value of 0.7087 (Figure 3.1D). Finally, the average concentrations that accounted for total mercury concentration throughout the day displayed the most linear relationship, with an R-squared value of 0.9167 (Figure 3.1E).

Table 3.1 *Mercury Concentrations of Individuals with Varying Number of Amalgams*

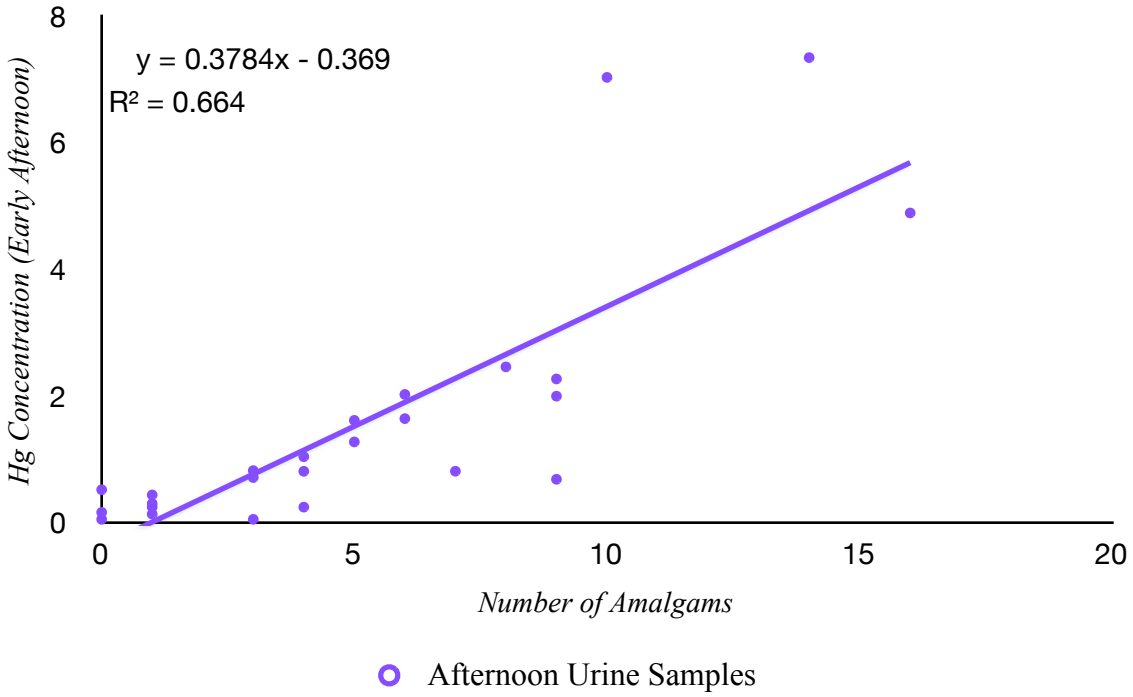
Participant	*Amalgams*	*A*	*B*	*C*	*D*	*Average*
1	3	0.9589	0.0527	0.1373	0.5201	0.41725
2	1	0.1628	0.2453	0.3827	0.3397	0.282625
3	1	0.2453	0.3003	0.1452	0.2453	0.234025
4	0	0.1215	0.1628	0.1628	0.1899	0.15925
5	9	2.7583	1.9970	0.8219	0.9514	1.63215
6	0	0.2728	0.0527	0.1353	0.1628	0.1559
7	10	1.5060	7.0309	0.7122	1.8059	2.76375
8	4	0.6299	1.0411	0.3553	0.4377	0.616
9	4	0.6077	0.2453	0.6299	0.7524	0.558825
10	6	0.7396	2.0242	0.3827	0.8767	1.0058
11	0	0.1628	0.5201	0.2453	0.2453	0.293375
12	5	0.6024	1.6152	0.0802	0.6573	0.738775
13	8	0.6024	2.4595	1.3353	0.8767	1.318475
14	16	4.5139	4.8899	4.4377	1.1779	3.75485
15	9	1.3420	0.6848	1.9299	1.7430	1.424925
16	6	0.9589	1.6425	0.8020	0.9863	1.097425
17	5	1.0958	1.2748	0.2728	0.2728	0.72905
18	14	3.2731	7.3429	0.7945	1.6844	3.273725
19	9	1.4240	2.2692	1.0684	1.4458	1.55185
20	4	0.8931	0.8109	0.3553	0.1628	0.555525
21	7	2.9248	0.8121	0.2728	0.7122	1.180475
22	3	0.4377	0.7122	0.1353	0.5201	0.451325
23	1	0.3003	0.1353	0.1353	0.2453	0.20405
24	1	0.2453	0.4377	0.1353	0.1795	0.24945
25	3	0.6573	0.8219	0.1903	0.1353	0.4512

**Mercury Concentrations (µg/L) of study participants taken at four different collection times: morning (A), afternoon (B), evening (C), bedtime (D). Amalgam numbers ranged from 0 fillings to 16 fillings.*

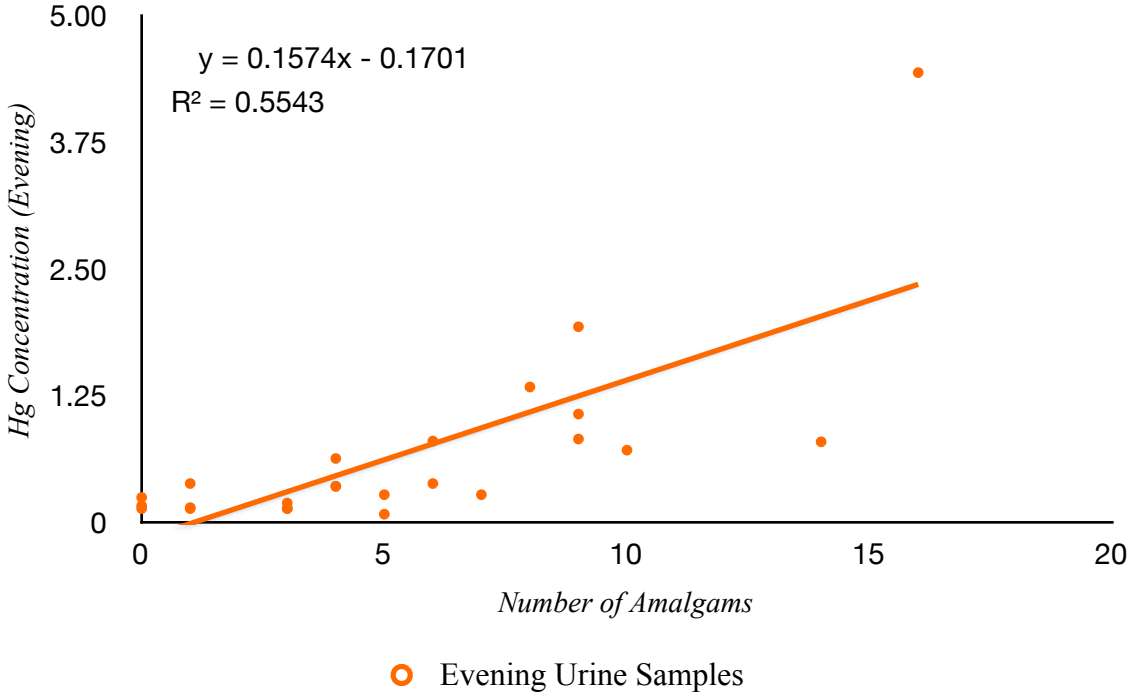
A. Morning Hg Concentration vs. Number of Amalgams



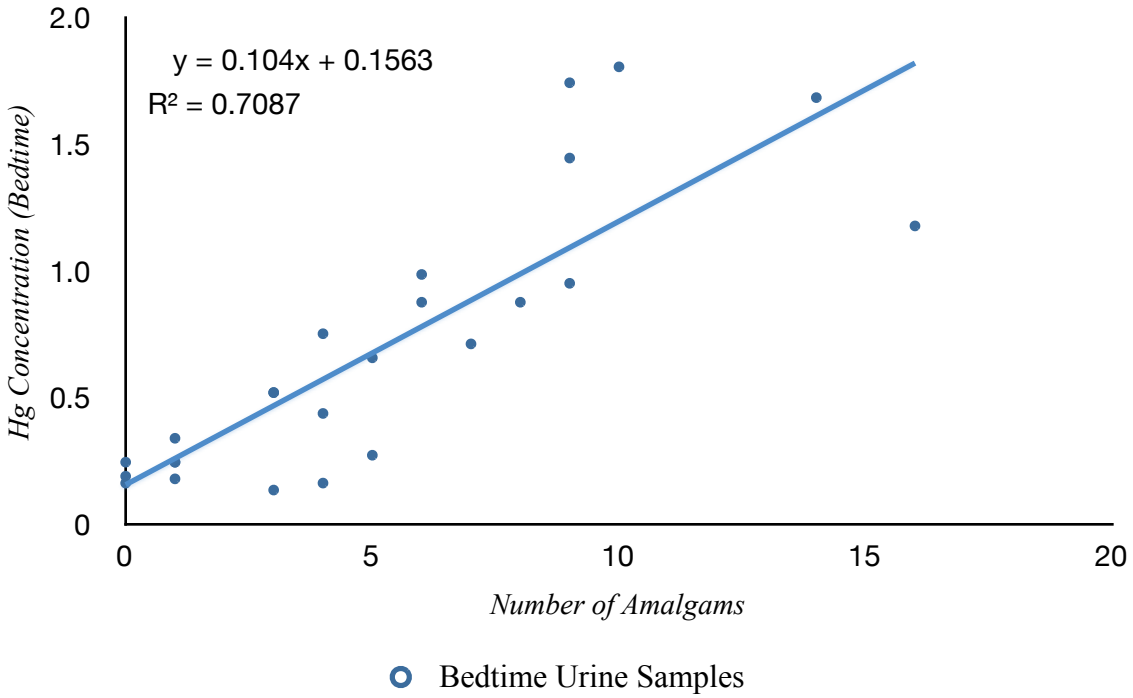
B. Afternoon Hg Concentration vs. Number of Amalgams



C. Evening Hg Concentration vs. Number of Amalgams



D. Bedtime Hg Concentration vs. Number of Amalgams



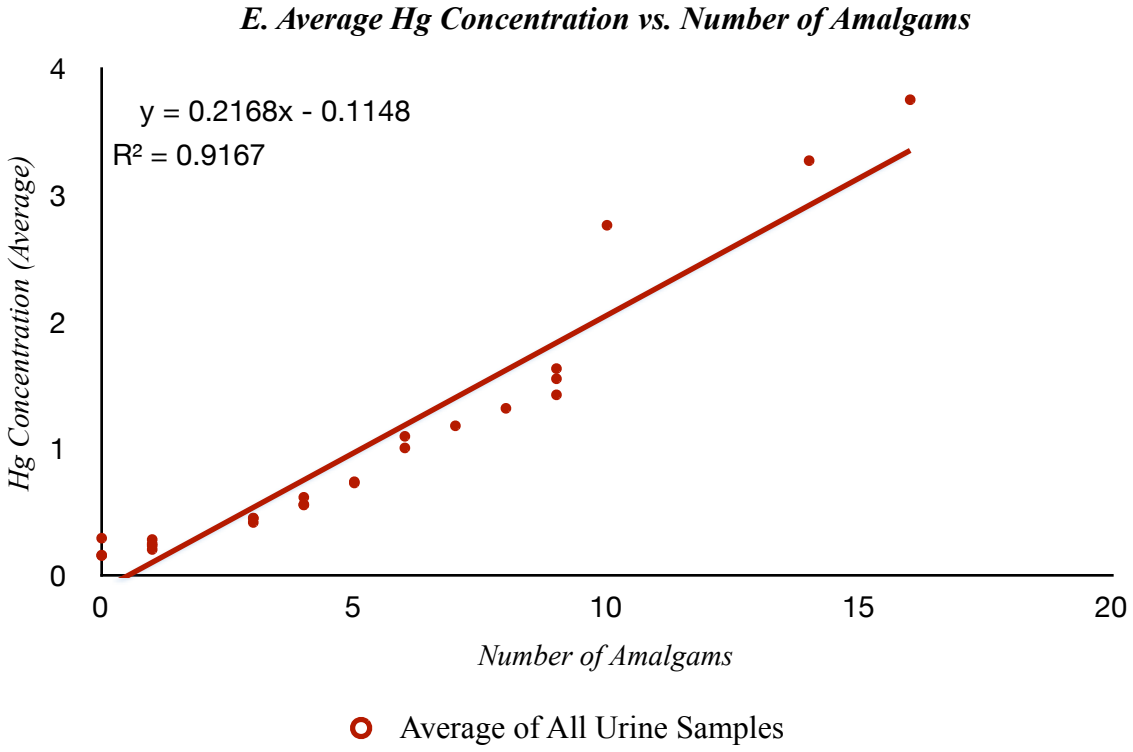


Figure 3.1. *Correlation between Hg concentration (µg/L) and number of amalgams in the morning (A), afternoon (B), evening (C), bedtime (D), and on average throughout the full day (E). All correlations utilize corrected Hg concentration values.*

ii. Assay Validation Results

‣ Intra-assay Precision

Intra-assay precision was determined to assess the amount of variation in a sample's reported concentration within the same assay run. Intra-assay precision was obtained by selecting six random non-study samples that covered a wide reference range. Each sample was analyzed twenty times each within the same run. Results can be seen below.

Table 3.2. *Values of Intra-Assay Precision for the Dried Urine Mercury Assay*

Dry Urinary Mercury		
N=20	***Mean Concentration (ug/L)***	***Coefficient of Variation (C.V. %)***
Sample # 1	0.21	74.11%
Sample # 2	3.19	12.16%
Sample # 3	1.40	4.58%
Sample # 4	0.96	8.83%
Sample # 5	0.39	24.37%
Sample # 6	5.28	5.68%

**Samples used were non-study samples that consisted of urine samples that were collected in a previous laboratory study. Mercury values for these samples were already known, allowing for comparison during the intra-assay procedure. Variation is apparent at levels below 0.5 µg/L, as this is the minimum detection limit of the DMA-80 Direct Mercury Analyzer for dried urine.*

‣ Inter-assay Precision

Inter-assay precision testing was conducted to determine the amount of variation in a sample's reported concentration between different assay runs. Inter-assay precision was determined by selecting six random non-study samples that covered a wide reference range. Each sample was analyzed over a one month period in from a storage state of room temperature, refrigerated, and frozen. Results can be seen below. N=8 for all samples.

Table 3.2. *Values of Inter-Assay Precision for the Dried Urine Mercury Assay*

Dry Urinary Mercury			
N=8	***Sample State***	***Concentration (ug/L)***	***Coefficient of Variation (% CV)***
Sample #1	Room Temp	0.25	25.9
	Refrigerated	0.26	34.68
	Frozen	0.27	22.54
Sample #2	Room Temp	4.4	40.01
	Refrigerated	3.21	11.85
	Frozen	3.22	9.11
Sample #3	Room Temp	1.51	8.34
	Refrigerated	1.57	6.17
	Frozen	1.52	6.16
Sample #4	Room Temp	0.9	10.82
	Refrigerated	0.89	11.13
	Frozen	1.01	12.83
Sample #5	Room Temp	0.4	23.72
	Refrigerated	0.74	126.51
	Frozen	0.4	26.45
Sample #6	Room Temp	5.25	16.23
	Refrigerated	5.08	6.32
	Frozen	4.95	7.63

**Samples used were non-study samples that consisted of urine samples that were collected in a previous laboratory study. Mercury values for these samples were already known, allowing for comparison during the inter-assay procedure. Variation is apparent at levels below 0.5 µg/L, as this is the minimum detection limit of the DMA-80 Direct Mercury Analyzer for dried urine.*

▸ Assay Linearity

Assay linearity was measured to check whether the direct mercury analyzer could detect changes in mercury concentration of samples with various dilutions. Six samples of known Mercury levels were diluted to different ratios and compared to an expected recovery to determine linearity. 3.7% hydrochloric acid was used as the diluent.

Table 3.3. *Values of Linearity for the Dried Urine Mercury Assay*

Dried Urinary Mercury Linearity						
Sample 1						
	Neat	1:2	1:4	1:8	1:16	1:32
Result (µg/L)	2.47	0.91	0.50	0.31	0.21	0.16
Expected (µg/L)	2.47	1.24	0.62	0.31	0.15	0.08
%Recovery	100	73.90	81.24	100.03	132.87	206.68
Sample 2						
	Neat	1:2	1:4	1:8	1:16	1:32
Result (µg/L)	1.12	0.52	0.39	0.34	0.16	0.16
Expected (µg/L)	1.12	0.56	0.28	0.14	0.07	0.04
%Recovery	100	93.67	138.43	244.25	227.91	455.82
Sample 3						
	Neat	1:2	1:4	1:8	1:16	1:32
Result (µg/L)	0.71	0.39	0.50	0.24	0.30	0.21
Expected (µg/L)	0.71	0.36	0.18	0.09	0.04	0.02
%Recovery	100	109.19	282.62	269.59	667.87	924.47
Sample 4						
	Neat	1:2	1:4	1:8	1:16	1:32
Result (µg/L)	3.46	1.74	0.82	0.46	0.43	0.43
Expected (µg/L)	3.46	1.73	0.87	0.43	0.22	0.11
%Recovery	100	100.34	94.94	105.45	200.32	400.65
Sample 5						
	Neat	1:2	1:4	1:8	1:16	1:32
Result (µg/L)	9.35	3.69	2.10	1.21	0.73	0.55
Expected (µg/L)	9.35	4.68	2.34	1.17	0.58	0.29
%Recovery	100	78.87	89.95	103.51	124.91	187.32
Sample 6						
	Neat	1:2	1:4	1:8	1:16	1:32
Result (µg/L)	2.58	1.16	0.68	0.46	0.29	0.30
Expected (µg/L)	2.58	1.29	0.65	0.32	0.16	0.08
%Recovery	100	90.24	106.08	141.42	177.35	367.59

**Samples used were non-study samples that consisted of urine samples that were collected in a previous laboratory study. Mercury values for these samples were already known, allowing for comparison during the inter-assay procedure.*

CHAPTER 4. DISCUSSION

i. Assay Analysis

The development of the direct mercury analysis assay for dried urine mercury and the results of the sample urine collection test supported the original hypothesis that if an individual contains a larger amount of amalgam fillings, he or she will produce urine with a greater mercury concentration. However, the concentrations that were measured in the urine collection test mostly fell within the standard reference range of 0.7 µg/kg to 2 µg/kg, some even below this range. Three individuals in the study exceeded the standard reference range of mercury, but not by a dangerously significant margin. Thus, although the original hypothesis is supported by the study data, there does not seem to be a significant health threat posed by dental amalgams. This argument can also be fortified by the age and health status of the study participants whose levels exceeded the World Health Organization's standard reference range for mercury. All three of these individuals had ten or more amalgam fillings, were over the age of forty, and were all free of any health issues. Their normal health status suggests that many other individuals have been leading healthy lives even with fillings made of an amalgam material, as previous research shows that it is a less costly option and provides more durability than other dental materials such as composite or resin [12].

ii. Clinical Testing Potential

By demonstrating a linear correlation between number of amalgams and average mercury concentration, the assay indicated that a wide range of different sample mediums can be analyzed by the direct mercury analysis method. Although no significant health threat was posed by the results of the urine collection test, the development of an assay that uses dried sample strips in direct mercury analysis notably increases the clinical potential of mercury sample testing. Many methods for testing liquid or frozen urine mercury samples through cold-vapor atomic absorption spectrophotometry are currently available. However, cold-vapor atomic absorption requires both sample digestion and sample extraction, which elongates the analytical testing process [16]. Moreover, the transportation and storage of liquid samples is both expensive and requires extensive care [13]. Due to the complications of circulating liquid samples, it becomes difficult

to test mercury in more internationally remote areas, where both fish consumption and occupational interactions contribute to mercury exposure [14]. As compared to a liquid sample, dried urine samples on filter paper strips are easy to transport due to their smaller size and weight. More importantly, a dried sample stored in a plastic bag with desiccant stays stable for approximately one month at a wider temperature range than liquid samples [15]. Within the laboratory, dried urine samples are much more time efficient in terms of processing, as they can be directly punched into sample boats of the DMA-80 Direct Mercury Analyzer without any sample digestion or sample extraction. This prevents researchers from having to spend time to dry individual samples for at least four hours and allows for a larger number of total samples to be analyzed each day. Additionally, only a small portion of the filter strip is required to be punched out for analysis, which leaves the remaining portion of the sample filter strip for storage. This provides researchers with a sample backup in case of future re-testing.

iii. Limitations

A limitation of the direct dried urine mercury analysis is the amount of sample collection that is required. Sample collections from multiple times during the day are necessary to obtain a correct indication of an individual's true urine mercury concentration. Due to variation between one individual's samples from different times, an average mercury concentration is best suited for analysis. The direct mercury analysis assay in discussion determines the mean mercury concentration of each individual patient, thus accounting for any variation produced by food consumption or occupational exposure throughout the day.

iv. Further Research Opportunities

The rapidly growing phenomenon of disease prevention as opposed to treatment has called for detailed analysis of various components and hormones in the human body. Mercury analysis has been vital in assessing bodily symptoms including memory loss, hair deterioration, nausea, and more life-threatening issues such as a breach of the blood-brain barrier [17]. The determination of these conditions has inspired researchers to explore even more opportunities to help individuals through sample testing internationally. With the development of a more cost-

friendly assay such as the direct mercury analysis in discussion, researchers can collect data from areas of interest. These include south-Asian regions in which coal mining factories, fish, and seafood consumption are widely abundant, and in neonatal hospital settings with a large number of pregnant women. This is due to the fact that fish consumption during pregnancy has been known to have varying effects on the birth of a child [18]. The efficiency of the dried urine mercury assay can contribute to building a more clear understanding of the mercury concentrations in these individuals' bodies, which can then further enable researchers to relay the information to medical professionals for future treatment.

REFERENCES

1. Wang, Qichao, Wenguo Shen, and Zhuangwei Ma. "Estimation of Mercury Emission from Coal Combustion in China." *Environmental Science & Technology*: 2711-713.

2. World Health Organization (WHO) (2008): Guidance for Identifying Populations at Risk from Mercury Exposure, 2008.

3. Ritchie, K A, E B Macdonald, R. Hammersley, J M O'neil, D A Mcgowan, I M Dale, and K. Wesnes. "A Pilot Study of the Effect of Low Level Exposure to Mercury on the Health of Dental Surgeons." *Occupational and Environmental Medicine* (1995): 813-17.

4. Lynch, D.p. "Mercury Vapour Levels in Dental Practices and Body Mercury Levels of Dentists and Controls." *Yearbook of Dentistry*: 206-09.

5. McFarland, R.B. and H. Reigel. 1978. Chronic mercury poisoning from a single brief exposure. J. Occup. Med. 20(8): 532-534.

6. Bansal, Mohit, Nidhi Gupta, and Shelja Vashisth. "Knowledge, Awareness and Practices of Dental Care Waste Management among Private Dental Practitioners in Tricity (Chandigarh, Panchkula and Mohali)." *Journal of International Society of Preventive and Community Dentistry*: 72.

7. Freitas, J. F. "A Survey of the Elemental Composition of Alloy for Dental Amalgam." *Australian Dental Journal*: 17-25.

8. Rice, Deborah C., Rita Schoeny, and Kate Mahaffey. "Methods and Rationale for Derivation of a Reference Dose for Methylmercury by the U.S. EPA." *Risk Analysis*: 107-15.

9. Mahaffey, Kathryn R., Robert P. Clickner, and Catherine C. Bodurow. "Blood Organic Mercury and Dietary Mercury Intake: National Health and Nutrition Examination Survey, 1999 and 2000." *Environmental Health Perspectives* (2003): 562-70.

10. Vahter, M. "Longitudinal Study of Methylmercury and Inorganic Mercury in Blood and Urine of Pregnant and Lactating Women, as Well as in Umbilical Cord Blood." *Environmental Research*: 186-94.

11. Bernhoft, Robin A. "Mercury Toxicity and Treatment: A Review of the Literature." *Journal of Environmental and Public Health*: 1-10.

12. Bernardo, M., H. Luis, M. D. Martin, B. G. Leroux, T. Rue, J. Leitao, and T. A. Derouen. "Survival and Reasons for Failure of Amalgam versus Composite Posterior Restorations

Placed in a Randomized Clinical Trial." *Journal of the American Dental Association* (2007): 775-83.

13. Schickling, C., and J. A. C. Broekaert. "Determination of Mercury Species in Gas Condensates by On-line Coupled High-performance Liquid Chromatography and Cold-vapor Atomic Absorption Spectrometry."*Applied Organometallic Chemistry*: 29-36.

14. Friedli, Hans R. "Mercury in the Atmosphere around Japan, Korea, and China as Observed during the 2001 ACE-Asia Field Campaign: Measurements, Distributions, Sources, and Implications." *Journal of Geophysical Research*.

15. Zava, Theodore T., Sonia Kapur, and David T. Zava. "Iodine and Creatinine Testing in Urine Dried on Filter Paper." *Analytica Chimica Acta*: 64-69.

16. Pourreza, N., and K. Ghanemi. "Determination of Mercury in Water and Fish Samples by Cold Vapor Atomic Absorption Spectrometry after Solid Phase Extraction on Agar Modified with 2-mercaptobenzimidazole."Journal of Hazardous Materials: 982-87.

17. Aschner, M., and J. Aschner. "Mercury Neurotoxicity: Mechanisms Of Blood-brain Barrier Transport." *Neuroscience & Biobehavioral Reviews*: 169-76.

18. Ramon, R., F. Ballester, X. Aguinagalde, A. Amurrio, J. Vioque, M. Lacasana, M. Rebagliato, M. Murcia, and C. Iniguez. "Fish Consumption during Pregnancy, Prenatal Mercury Exposure, and Anthropometric Measures at Birth in a Prospective Mother-infant Cohort Study in Spain." *American Journal of Clinical Nutrition* (2009): 1047-055.