BIOMONITORING STUDY

Utah Statewide Investigation of Neonatal Blood Lead Levels Using Newborn Blood Spot Specimens

October 20, 2014

Prepared by the

Utah Department of Health
Division of Disease Control and Prevention
Bureau of Epidemiology
Environmental Epidemiology Program
# TABLE OF CONTENTS

ACKNOWLEDGMENT .................................................................................................................. 3
EXECUTIVE SUMMARY ............................................................................................................. 4
INTRODUCTION ........................................................................................................................... 5
DATA AND METHODS ................................................................................................................ 6
FINDINGS ....................................................................................................................................... 8
DISCUSSION .................................................................................................................................. 9
CONCLUSIONS AND RECOMMENDATIONS ........................................................................ 14
AUTHORSHIP, REVIEW AND CITATION .............................................................................. 15
CERTIFICATION .......................................................................................................................... 16
REFERENCES .............................................................................................................................. 17
TABLES AND FIGURES ............................................................................................................. 25
DEFINITIONS ............................................................................................................................... 28
DESCRIPTION OF LABORATORY METHODS ........................................................................ 31
ACKNOWLEDGMENT

This investigation is a biomonitoring demonstration project for the Utah Environmental Public Health Tracking Network (UEPHTN). The UEPHTN is funded by a grant from the Centers for Disease Control and Prevention (CDC), Environmental Public Health Tracking Branch. The current UEPHTN award numbers are U50CCU822437, 1U38EH000954, and 5U38EH000182 (UEPHTN 2012).
EXECUTIVE SUMMARY

Lead poisoning has been recognized as a serious threat to public health for many years. In Utah, health care providers screen children based on clinical history and symptoms. Pregnant women and newborns are not routinely screened. Elevated blood lead levels (EBLLs) can cause several adverse health effects including impaired neurological or hematological development.

This pilot project investigated the use of newborn blood spots as a sample media to conduct surveillance for blood lead in newborn children. Six thousand and sixty-eight (6,068) randomly selected blood spot cards from children born in Utah during the 2007, 2008, and 2009 birth cohorts were analyzed for blood lead levels (BLLs). From each card, a sample of the blood spot and a sample of a blood-free area of the card were digested and analyzed for lead using inductively coupled plasma mass spectrometry. Quality control samples were analyzed with every batch of blood spot samples to assure laboratory data quality. The difference between the amount of lead in the blood spot and the amount of lead in the blood-free area of the card was considered the child’s BLLs. After rejecting results for administrative recording errors or because the blood-free area of the card was contaminated with more lead than the blood spot (resulting in a negative value difference), the results for 5,590 Utah newborns were evaluated at the county level. The geometric mean for the state was 0.38 µg/dL (Maximum = 51.88 µg/dL). Twelve children were found to have EBLL ≥ 10 µg/dL. However, this finding is based on a testing methodology that has not been validated and the estimates of performance were less than ideal. The EEP does not recommend any change to current public health action or policy related to blood lead surveillance based on these results.

Biomonitoring is being explored nationally as a better way to understand the true exposure people experience which may contribute to adverse health effects. Before neonatal blood spots can be used as a routine screening media, additional work would need to be conducted to determine the sensitivity, specificity, and predictive value positive of this methodology. Prior to any decision leading to implementing this screening methodology as part of Utah’s current public health surveillance activities, consideration with respect to cost, alternative methods, and case follow-up should occur.
INTRODUCTION

**What is lead**: Lead is a toxic, heavy, bluish-gray metal that occurs naturally in the Earth’s crust. Because lead has a low melting point, is malleable, and is resistant to corrosion, lead has had a wide variety of uses for more than five-thousand years. Trace amounts of lead can be found in soils, water, and the air. The rapid increase in industrial and mechanical uses, mining activities, and fossil fuel uses over the last 60 years has led to more than a thousand-fold increase in lead concentrations found in the environment. Efforts have been in place for the last 30 years to reduce lead concentrations found in the environment (ATSDR 2007).

**Maternal and fetal exposure during pregnancy**: Lead poisoning has been recognized as a serious threat to public health for many years. Modern efforts to reduce environmental lead contamination by eliminating lead in gasoline and paint have helped reduce environmental exposure. Still, lead continues to be released as part of certain industrial and mining processes. Lead exposure can occur as part of one’s occupation, art-based hobbies, or home environment (ATSDR 2007). Lead exposure throughout one’s life can result in lead storage in the bones (McGowan 1996; Rastogi et al. 2007; Silbergeld et al. 1993). Pregnant women may experience concurrent environmental exposure to lead (Jarup 2003; Hinwood et al. 2013; Hossain and Triche 2007; Lee et al. 2005). In addition, lead stored in bone tissue may be released during pregnancy (Rastogi et al. 2007; Silbergeld 1991). Lead is able to cross the placental barrier, thus exposing the fetus (Caserta et al. 2013; Esteban-Vasallo et al. 2012, Garcia-Esquinas et al. 2013; Gomaa et al. 2002; Goyer 1990; Gundacker and Hengstschläger 2012; Al-Saleh et al. 2011). Maternal diet may influence fetal and neonatal exposure to maternal blood lead by increasing the bioavailability of lead in the blood (Hernandez-Avila et al. 2003; Lee et al. 2005; Schell et al. 2003).

**Rocky Mountain Biomonitoring Consortium (RMBC) and the Utah Environmental Public Health Tracking Network (UEPHTN) biomonitoring initiatives**: In 2001, the Centers for Disease Control and Prevention (CDC) established the National Biomonitoring Program within its Division of Laboratory Sciences (APHL 2009, CDC 2008). Concurrent with that action, CDC awarded pilot money for state laboratory biomonitoring to develop and propose biomonitoring demonstration projects. At that time, the Utah Public Health Laboratory (UPHL) joined with the state laboratories of Arizona, Colorado, Montana, New Mexico, and Wyoming to form the Rocky Mountain Biomonitoring Consortium (RMBC). The RMBC had a number of goals, one of which was to explore the ability for each state’s laboratory to specialize in some of the laboratory service requirements of the participating states and then provide those laboratory services to the other states. The consortium identified and proposed nine demonstration projects, one of which was to use neonatal blood spots to conduct heavy metal biomonitoring. In 2003, the RMBC became one of eight grantees to receive funding to implement their proposed demonstration projects (APHL 2009). With that support, the UPHL developed laboratory methodology and capacity to analyze neonatal blood spots for lead, mercury, and cadmium (Chaudhuri et al. 2009).

In 2003, the Environmental Epidemiology Program (EEP) within the Utah Department of Health (UDOH) was awarded funding to start developing the Utah Environmental Public Health Tracking Network (UEPHTN) (UEPHTN 2013). In collaboration with the RMBC, the UEPHTN
acquired money to specifically conduct a biomonitoring demonstration project. The UEPHTN received supplemental funding to conduct neonatal blood spot monitoring in 2009 and again in 2012 and 2013. With that supplemental funding, the UEPHTN contracted with the UPHL to randomly select and test blood spots in the 2007, 2008, and 2009 birth cohorts. These cohorts were selected because they were available for testing.

**Report Objectives:** This report presents a statistical review of laboratory results provided by the UPHL to the EEP for newborn blood spot testing for lead. The first purpose of this review is to develop an understanding of the geographic distribution of risk associated with elevated blood lead levels (EBLLs) among the newborn population in Utah. A secondary purpose of this review is to consider the utility of blood spot biomonitoring for conducting prospective blood lead surveillance.

**Authority and Funding:** This study was conducted as part of the UDOH Executive Director’s responsibility to investigate public health concerns within Utah. The executive director delegates responsibility for environmental health investigations to the EEP. Biomonitoring, population, and geographic data for this investigation are collected, maintained, and made available by the UEPHTN. The UEPHTN also funds the SAS® and ArcGIS® analytical software application licenses that were used to conduct this investigation. The UEPHTN is funded by a grant from the CDC (UEPHTN 2013). Personnel time used to conduct this investigation was charged against state-funded Environmental Health Administrative funds.

**Institutional Review Board:** This investigation was reviewed and approved by the UDOH Institutional Review Board (IRB) on March 20, 2007 (IRB #151) for analysis of the 2007 birth cohort. An additional IRB approved an expansion of the project to include samples from the 2008 and 2009 birth cohorts. This IRB approval was issued on January 11, 2012 (IRB #330). The purpose of this investigation was to gain an understanding of the geographic distribution of blood lead levels (BLL) and to explore the feasibility of using blood spots as a convenience sample for blood cadmium surveillance. The study protocol presented to the IRB did not allow the UPHL or EEP to have identifiable data for the infants whose blood spots were used in this investigation, nor did the study protocol include procedures for informing caretakers of infants with EBLL about the laboratory findings. The use of blood spots for this kind of surveillance is new and information about the reliability of the results and the use of those results in guiding patient care and treatment is known.

**DATA AND METHODS**

**Study Design:** This investigation is a retrospective statistical review of biomonitoring results for neonatal BLL among Utah newborns. However, statistical reviews lack the power to link EBLL incidence to putative risk factors (Jekel et al. 1996; Mann 2003). A statistical review is a tool used by the EEP to better understand the health status of a population, identify priorities for public health action, and assess public health activities.

This investigation funded testing of randomly selected blood spot cards for the biomonitoring of infants born in Utah and submitted for testing to the UPHL. The UPHL randomly sampled 6,068
cards from the 2007, 2008, and 2009 birth year cohorts. These samples represent 3.70% of the total number (163,869) of children born in Utah during those years. This sample size is the result of funding and not based on the expected prevalence or a sample size and power calculation. The UPHL submitted test result data to the EEP for analysis. The EEP evaluated the quality of the results and analyzed the data by county of residence.

**Blood Spot Analysis:** A detailed description is contained in the “Description of Laboratory Methodology” later in this report (Chaudhuri et al. 2009). Briefly, two paper punches, called “dots” were taken; one from the blood spot area and one from the blood-free area of each blood spot specimen card. The blood-free area punches were used to determine the level of lead contamination on the paper.

Heavy metals from each dot were extracted using an acid digestion. The clarified extract was analyzed for lead by inductively coupled plasma mass spectrometry (ICP-MS).

**Vital Records Birth Data:** Vital records birth data were obtained from the Utah Department of Health, Office of Vital Records and Statistics. These data are standardized and made available through the UEPHTN (UEPHTN 2013). Vital birth records were used to quantify the total births occurring in Utah by county for the birth cohort years. Records of birth with maternal addresses outside of Utah or of undetermined sex were excluded from the tabulation of Utah children born during the 2007-2009 study period.

**Blood Spot Sampling Data:** Six thousand and sixty-eight (6,068) samples were randomly drawn from newborn blood spots on Utah children born during 2007, 2008, and 2009, and were analyzed for whole-blood lead. Data regarding the child’s sex and mother’s residential ZIP code were obtained from the New Born Screening Program, UDOH. When the UPHL received the cards, they were given an additional sample identification number specific to this project. The data provided to EEP were de-identified data that included only the project-specific sample identification number, and the child’s birth year, sex, and ZIP code information. Two thousand nine hundred and thirty-seven (2,937; 48.5%) were from female infants and 3,124 (51.5%) were from male infants. Seven samples did not have a sex identified with them. The analytical results for 6,032 samples were geo-referenced to the mother’s county of residency, using the ZIP code provided on the accession form. Thirty-six samples lacked the ZIP code and could not be geo-referenced to a Utah county. Five samples were missing the birth year data. The 42 cards missing the sex codes, valid Utah ZIP codes, or birth year data were excluded from the final analysis.

The infant’s BLL was calculated as the difference between the lead level measured in the laboratory for the blood spot minus the lead level for the paper blank from the same blood spot card. Six hundred and fifty-nine cards (11% of the cards) were tested multiple times as part of the laboratory quality control process or to confirm elevated results. The average difference between the lowest and highest calculated lead levels was 0.17 µg/dL (standard deviation = 2.42 µg/dL, maximum = 141.7 µg/dL).

Cards with a paper blank lead level higher than the blood spot lead level, resulting in a negative value calculated BLL, were not included in the final analysis. The lowest positive calculated BLL for cards with multiple tests was used for the final analysis. After exclusion of cards for
missing administrative data or for negative calculated BLLs, samples for 5,590 children with positive calculated BLLs were evaluated.

Data from two different funding cycles, the 2009 cycle and the 2012 cycle, were used for this pilot project. During the time between the cycles, changes occurred in UPHL staff and equipment used in this project. Before combining the data of the two funding cycles, it was necessary to ensure that there were no cycle-specific differences in the testing of samples and reporting of results that would invalidate the analytical results derived from combined data sets. The t-test was used to determine if the calculated blood lead for the 2009 and the 2012 funding cycles were done by the same laboratory testing and reporting methodology. The calculated BLLs were not normally distributed. The Anderson-Darling test \( A^2 = 1.125 \) is well above the 99% critical value \( = 1.092 \). Therefore, the log transformation of the calculated BLLs was used for the t-test. The variances of the data for the two funding cycles were not equal (funding cycle 2009 mean was 1.34 [for the log transformed calculated lead levels], standard deviation [SD] = 0.93; and funding cycle 2012 mean was 1.30 [SD = 0.82]; tests for the equality of variance = 1.29, degrees of freedom = 3,060, p-value for accepting the hypothesis of equal variance [the null-hypothesis] is <0.0001). The Satterthwaite t-test for unequal variance showed that the two sampling periods likely used the same testing and reporting methodology (t-value = 1.82, p-value = 0.07). It should be noted that this finding is weak, the p-value being just slightly above the 0.05 threshold. However, based on these results, the data from the sampling cycles were pooled for the final analysis.

The t-test was also used to determine whether there were differences in the calculated BLLs between female and male children. The mean of the log-transformed lead levels for females was 1.31 (SD = 0.88) and 1.34 (SD = 0.88) for males. The test for equality of variance (F = 1.00, degrees of freedom = 2,701, p-value = 0.97) suggests that the variance between female and male results were not statistically different. The pooled t-test suggested that there was no difference in the distribution of calculated BLLs for females and males (t-value = 1.09, degrees of freedom = 5,588, p-value of the null hypothesis = 0.27). Therefore, the results of female and male children were pooled for the final analysis.

Current standards, recommendations and guidelines: In 2012, the Centers for Disease Control and Prevention (CDC) Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) published a report (ACCLPP 2012) that forms the basis for the current child exposure guidelines. The ACCLPP recognized that any lead in the body can result in some harm. Based on current prevention strategies, the ACCLPP guidance is that children ages 1 to 5 years old are considered to have EBLLs when their BLL \( \geq 5 \) micrograms per deciliter (µg/dL) (ACCLPP 2012, 2013). In Utah, the current reportable threshold is \( \geq 10 \) µg/dL for all people (UAC 2013). These reference values apply to children. No reference value has been set for newborns (ACCLP 2012, 2013; Wengrovitz and Brown 2009).

Data Analysis: The geometric mean and highest computed lead level were used to summarize the data. The geometric mean is the preferred measure of central tendency, when the data are highly skewed toward and bounded by zero (0.0). The number of children born with lead levels above the current state threshold (\( \geq 10 \) µg/dL) for elevated blood lead was tabulated. The number of children with blood lead levels above the current CDC guidance (\( \geq 5 \) µg/dL) was tabulated
also. These two tabulations were used to estimate a total statewide burden of new born children with EBLLs.

**FINDINGS**

For the 5,590 Utah newborns included in the analysis for this investigation, the geometric mean BLL was 0.38 µg/dL (range = 0.00 to 51.88 µg/dL). These 5,590 represent approximately 3.4% of the births (163,869) in Utah between 2007 and 2009. The geometric mean and maximum BLLs tabulated by sex, birth year, and county are presented in Tables 1, 2, and 3, respectively. The county was determined by the maternal residential address at the time of birth. There is no available information about the residential tenure of the mothers.

Twelve (12) newborns were identified with BLLs ≥10 µg/dL and 29 newborns were identified with BLLs ≥5 µg/dL. Based on these counts, the rate of children with EBLLs would be approximately 2.15 children per 1,000 births using the current state standard, and approximately 5.19 children per 1,000 births will have BLLs above the new CDC guidelines (ACCLPP 2013). Utah has approximately 53,000 births per year (based on the number of births per year between 2007 and 2012) (UDOH 2013). These findings suggest that approximately 114 children could be born each year with EBLLs ≥ 10 µg/dL and 275 children could be born each year with BLLs ≥ 5 µg/dL. However, this testing methodology was not validated with a gold standard screening method, and no conclusions should be made that lead to a public health action or policy change, based on these results. In addition, the estimates of specificity and predictive value positive were low indicating that the methodology does not perform well.

County data are presented geographically in Figure 1 and Table 3. The distributions of samples taken represented between 1.8% (Duchesne County) and 22.3% (Grand County) of the children born during the project sampling period per county. The geometric mean ranged from 0.26 (Daggett County) to 0.71 (Kane County). Only six counties: Davis, Salt Lake, Sevier, Utah, Washington, and Weber had children born with EBLLs above the Utah threshold or the CDC guideline levels.

**DISCUSSION**

**Health effects of elevated blood lead levels in children**: Lead poisoning is one of the leading environmental causes of preventable child health problems today (ACCLPP 2012, 2013; Erickson and Thompson 2005; Norman and Bordley 1995). Lead affects virtually every organ system. Fetuses and young children are particularly vulnerable to the neurotoxic and other developmental effects of blood lead (Meyer et al. 2003). Blood lead exposure in the developing fetus before the completion of the blood-brain barrier may be particularly harmful to neurological development (Cleveland et al. 2008a). Lead acquired from previous exposure history can be stored in the mother’s bone mass, which then becomes available during pregnancy (Markowitz and Shen 2001). Lead crosses the placenta and can affect the developing fetus (Goyer 1990). Research has shown fetal and infant development is at risk for lead toxicity from
maternal lead stores (Gomaa et al. 2002; Hu et al. 2006; Jedrychowski et. al. 2008; Shen et. al. 1998). Although considerable progress has been made to reduce adult and childhood exposure to lead over the last 30 years, lead continues to be a common environmental health risk. The CDC recognized that no known safe threshold exists for blood lead (Wheeler 2013). Studies of children have demonstrated that blood lead concentrations as low as 2 µg/dL are inversely associated with cognitive and developmental deficiencies (Bellinger and Needleman 2003; Canfield et. al. 2003, 2004; Chiodo et al. 2004; Gilbert and Weiss 2006; Jusko et. al. 2008; Lanphear et. al. 2000, 2005; Needleman and Gatsonis 1990; Pocock et. al. 1994; Schwartz 1994; Surkan et. al. 2007; Tellez-Rojo et. al. 2006; Weitzman et. al. 2004). Adverse health effects that are associated with low-level BLLs in the fetus or child include:

<table>
<thead>
<tr>
<th>Fetal effects</th>
<th>Child effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Intrauterine growth retardation</td>
<td>• Inability to correctly form white blood cells</td>
</tr>
<tr>
<td>• Low-birth weight</td>
<td>• Poor mental development, reduced intelligence, and learning disabilities</td>
</tr>
<tr>
<td>• Preterm delivery</td>
<td>• Development of attention deficit disorder</td>
</tr>
<tr>
<td>• Restricted mental development</td>
<td>• Impaired hearing</td>
</tr>
<tr>
<td>• Congenital malformations</td>
<td>• Impaired motor development</td>
</tr>
</tbody>
</table>


**Economic effects of elevated blood lead levels in children**: For children older than one year of age, each 1 µg/dL increase in a child’s BLL has been shown to result in an intelligence quotient (IQ) loss of 0.46 points (Canfield et. al. 2003; Jusko et. al. 20008; Salkever 1995). Each IQ point lost represents a loss of 2.39% of a child’s potential lifetime earnings (Landrigan et. al. 2002). Earning potential varies depending on education level, occupational interests, sex, and economic health. The current average potential lifetime earning is $2.4 million (Julian 2012). Using that average, Utah will experience a total lifetime earnings loss of $56 million due to the number of children per birth cohort projected to be born with BLLs with 5 µg/dL or greater. This figure does not include the additional societal costs such as additional health care costs, costs due to increased crime, and family burden costs (Muennig 2009). It is not known if this same economic effect applies to newborn children with EBLL.

**Public Health Surveillance (Why do biomonitoring?)**: Health is one of the most important assets a human being is given. It permits each person to fully develop their capacities thus allowing them to enjoy the highest quality of life. The mission of public health is to promote and protect people’s health. Public health accomplishes its mission through ten essential public health services (Harrell and Baker 1994; IOM 1988). The first of these services is to “monitor health status to identify community health problems” (Stanbury et. al. 2012). Public health surveillance is the systematic, ongoing, population-based collection of data that leads to early detection and response to public health concerns (Choi 1998; Thacker et. al. 1996). This service helps public health officials and policymakers identify and assess communities with public health challenges; define public health priorities; develop and implement informed public health
policy; monitor and evaluate public health actions; discover knowledge about public health concerns; and guide public health outreach, education and intervention activities (Dicker 2002; Stanbury et al. 2012; Thacker 2000; Thacker et al. 2012). To conduct public health surveillance, environmental epidemiology collects data about environmental hazards, exposure, and adverse health outcomes (Malecki et al. 2008; Thacker et al. 1996).

Health outcomes (e.g., disease, disability, death, etc.) surveillance is the collection and registration of “cases.” Ascertainment of cases is dependent on the willingness and timing of people seeking medical assistance, and the capacity of health care to report conditions. This surveillance process has the advantage of involving the health care system which is much larger and has more direct contact with people than public health agencies. The cost of health care registries varies depending on the level of active versus passive surveillance and the degree of additional data collection through abstraction or other linkages that occur as part of the surveillance process. A drawback of health outcomes surveillance is that knowledge of cases is after the fact and tends to be curative rather than preventive focused (Aldrich and Griffith 1993; Thacker et al 1996).

Exposure surveillance, also called biomonitoring, is the monitoring of individual members of the population for the presence of an environmental agent or its subclinical or preclinical effects. Biomonitoring may occur in conjunction with health outcome surveillance (i.e., a child presenting with symptoms of EBLLs and is tested) or may occur by sampling otherwise healthy people (i.e., a child is tested as part of an active screening requirement) (Albertini et al. 2006; Angerer et al. 2007; Thacker et al. 1996). Sampling usually involves collection of biological specimens (blood, urine, milk, saliva, hair, adipose, or other tissues) during a health care event (i.e., a routine physical) or through soliciting volunteers (Farmer et al. 1996; Needham et al. 2007). Biomonitoring has proven to be more costly and more difficult than health outcome or hazards surveillance. Because of the cost and difficulty of obtaining samples, many investigations model exposure from hazards surveillance data rather than conducting exposure surveillance (Angerer et al. 2007). An advantage of biomonitoring is that these data provide a better understanding of exposure in the diseased and healthy populations. This concept is important in understanding thresholds (i.e., a dose-response curve) that contribute to the development, progression, and disposition of associated adverse health outcomes (Albertini et al. 2006; Angerer et al. 2007; Farmer et al. 1996, Needham et al. 2007).

One way to overcome sampling difficulties for biomonitoring is the use of samples collected for other purposes (Albertini et al. 2006). Blood spots, usually collected to look for a variety of adverse genetic conditions for newborns, are an efficient and un-intrusive method to conduct heavy metal surveillance among newborns (Olshan 2007).

**Pilot Biomonitoring Project:** One of the objectives for this project was to assess the feasibility of using blood spots as a mechanism for conducting public health surveillance for EBLLs in Utah residents (Olshan 2007).

The value of a public health surveillance system can be assessed by understanding the usability of the information derived through surveillance and the reliability and efficacy of the surveillance methodology. The usefulness of the information can be evaluated by understanding
the scientific basis, relevance, and ability to be translated into public health actions or policy (Malecki et al. 2008). Analytical soundness is usually measured by statistical comparisons of the surveillance methodology with a gold standard in terms of sensitivity, specificity, and predictive value positive (German 2000).

The UPHL used commercially prepared venous blood containing a standardized concentration of lead (i.e., standard reference material) to spike blank blood spot papers that were included with each batch of newborn blood spots to assure quality of the laboratory analyses. Because the UPHL used commercially available standard reference material that were spiked on to filter paper and included those spiked filter papers with known lead levels as part of the quality control, there is certainty about the ability of the UPHL to accurately and consistently test blood spots (Chaudhuri et al. 2009). In addition, the UPHL successfully participated in the Wisconsin State Laboratory of Hygiene proficiency testing program for filter paper blood lead testing during the duration of this study, further demonstrating the utility and accuracy of the laboratory analytical method.

Validation of the blood spot results using paired venous blood samples from the infants would be necessary to assess the epidemiologic performance measures (specificity, sensitivity and predictive values) used in evaluating screening methodology. Because this project relied on the availability of stored blood spots, this was not possible. Therefore the true specificity and sensitivity of the blood spot testing methodology cannot be determined. The UPHL has a policy that when an elevated lead value is detected, the sample is automatically confirmed by analysis of a replicate punch from the same blood spot. In this study, 11% of the cards (659 of 6,088 cards) were reanalyzed to confirm high lead concentrations. By categorizing the relationship of the lowest and highest positive test value with respect to whether the child represented by the card had an EBLL (≥10 µg/dL), the specificity was estimated to be 78% and the predictive value positive was estimated to be 45%. The sensitivity could not be estimated using this approach (German 2000). The mean difference between the lowest and highest test for the cards was 1.4 µg/dL (maximum difference = 141.2 µg/dL, standard deviation = 6.9 µg/dL).

Typically, a surveillance system would be founded on one or more surveillance objectives. Surveillance objectives include quantifying and characterizing the magnitude of public health concerns in a population at risk; obtaining increased understanding of the epidemiology of a public health concern; empowering sound preventive or mitigating public health actions and policies; and evaluating those actions or policies. Justification of conducting surveillance requires balancing the costs, the collection of personal information, and the legal concerns against needs and strengths of the surveillance objectives. An effective surveillance system typically links laboratory analysis with additional data (Sneider and Stroup 2000; Stanbury et al. 2012; Teutsch 2000; Thacker et al. 1996; Thacker and Birkhead 2002).

This report presents data that quantifies the magnitude of elevated blood lead in newborn children. However, there is insufficient data to fully characterize that health concern or understand the epidemiology involved with respect to probable risk factors. To be useful, these data would have to be linked to other data collected about the mother and her pregnancy. For example, it would be important to know what the historical and current exposures to lead are for the mother, and whether any of the current exposures can be mitigated.
Currently, there is no guidance on the testing and treatment of infants that are younger than age 9 months for blood lead (ACCLP 2012, 2013; Wengrovitz and Brown 2009). However, infants and newborns are increasingly recognized as an at-risk population (Archer et al. 2012; Gardella 2001; Rastogi et al. 2007). There are few treatment options for newborns with EBLLs. Those options, such as chelation (when the BLL \( \geq 45 \, \mu g/dL \)), result in other health concerns and need to be used selectively. Treatment of newborns with EBLLs will not reverse any damage that has already occurred (Cleveland et al. 2008a). However, the identification of high risk populations of women of childbearing age through public health surveillance can guide early health care decisions about prenatal testing and counseling (Hossain and Triche 2007). Simple interventions, such as better hand washing behaviors, and calcium and iron supplementation, may reduce the mother’s BLLs and thus the fetal exposure (Cleveland et al. 2008b; Rastogi et al. 2007). The findings of this investigation suggest that as many as 114 children could be born each year with EBLLs (\( \geq 10 \, \mu g/dL \)). However, this finding is based on a testing methodology that has not been validated and does not appear to have good specificity and predictive power. The EEP promotes screening for women of childbearing age for blood lead (Hossain and Triche 2007). However, these results do not indicate a need to change the adult screening policy. The geometric mean BLL found in this investigation was 0.38 \( \mu g/dL \) which is higher than the national geometric mean BLL of 1.66 \( \mu g/dL \) (CDC 2014).

Currently, adult screening in Utah is conducted when mandated because of high risk occupational requirement, or when there are clinical manifestations suggesting adult blood lead toxicity.

This pilot project used de-identified data to examine the efficacy of using blood spots as a means of conducting noninvasive blood lead screening among newly born children in Utah. The UPHL charged EEP $30 per test for the lead testing for this pilot project. If this approach became part of the routine services provided by the UPHL, and a larger volume of testing was performed annually, it is anticipated the cost per sample for testing would be reduced.

Because the children were not identified, the results for the 12 children with EBLLs were not reported to the health care provider or guardians of those children.

**Methodology Limitations**: The public often wants public health investigations to determine whether health risks can be linked to a putative environmental concern. The methods used in this investigation do not have the capability to definitively link the findings of elevated neonatal blood lead exposure risk to any inherent or external risk factors including environmental exposures. This kind of investigation is sometimes referred to as a “snapshot,” and presents data about the health status of newly born children in Utah during the 2007-2009 time period. A concern with “snapshot” investigations is that the data in this report may be used to generate inferences leading to public health policy or action based on this single assessment. The environmental risks associated with those counties where significant results were found should be conducted before any change to public health policy or program actions are made regarding lead poisoning in children (Meliker and Sloan 2011).
An investigation that uses population-based summary data rather than individual-level data is called an ecologic study by epidemiologists. This surveillance project is an ecologic study. An interpretation error commonly associated with ecologic studies is to apply population-level risk findings to the individual. This kind of interpretation error is called an “ecologic fallacy.” For example, this study found the state risk for elevated blood lead among neonates was 2.15 per 1,000 births. This risk metric should not be applied to individuals. An individual may have no risk or a risk several times higher than the overall state risk (Greenland 2001; Greenland and Robins 1994; Morgenstern 1982, 1995; Rockhill 2005).

**CONCLUSIONS AND RECOMMENDATIONS**

This report provides a description and findings of a pilot surveillance project using newborn blood spots as a possible sampling media to conduct surveillance for heavy metal exposure. EBLLs in the developing fetus or newborn child can cause a number of adverse health effects, including harm to neurological and hematological development. There is no level of blood lead that is considered safe. Currently, BLLs $\geq 10$ $\mu$g/dL in children in Utah are considered elevated enough to warrant investigation (UAC 2013). This study found that approximately 2.15 newborns per 1,000 live births or 114 children per year (where the 2007-2012 average birth year cohort is 53,000 births) may have EBLLs at birth. However, this estimation is based on a testing methodology that has not been validated and statistical indications of performance were less than ideal. While the EEP promotes routine screening for women of childbearing age for lead exposure, based on these results the EEP does not recommend a policy change to current practice.

Before neonatal blood spots can be used as a routine screening media, additional work would need to be conducted to determine and improve the sensitivity, specificity, and predictive value positive of the testing methodology. BLL results using newborn blood spot samples would need to be validated using blood from the same newborn. In addition, prior to any decision on whether to or how to implement this testing methodology as part of Utah’s routine public health surveillance activities, consideration with respect to the need for routine blood lead surveillance in newborns, cost, alternative methods, and case follow-up should occur.
AUTHORSHIP, REVIEW AND CITATION

This report was prepared by:

Sam LeFevre
Environmental Epidemiology Program
Bureau of Epidemiology
Utah Department of Health

Mail: PO Box 142104, Salt Lake City, Utah 84114-2104
Street: 288 North 1460 West, Salt Lake City, Utah 84116
Phone: (801) 538-6191
Fax: (801) 538-6564
Email: slefevre@utah.gov

Contributors:

Sanwat N Chadhuri, PhD, Scientific Advisor, Chemical and Environmental Laboratory, Utah Public Health Laboratories
Jason Barnes, Chemist
Merril Chipman, Chemist
Robyn Atkinson-Dunn, PhD, Director, Utah Public Health Laboratories
Kim Hart, MS, LCGC, Manager, Newborn Screening Program
B Gregory Williams, MPH, CPM, Manager, Utah Environmental Public Health Tracking Network

Certifying Reviewers:

Allyn K Nakashima, MD, State Epidemiologist
Cristie Chesler, Director, Bureau of Epidemiology
Wu Xu, PhD, Director, Center for Health Data and Informatics

Recommended Citation:

CERTIFICATION

This report titled “Utah Statewide Investigation of Neonatal Blood Lead Levels Using Newborn Blood Spot Specimens” was prepared by the Environmental Epidemiology Program, Utah Department of Health. This report describes the findings of a pilot surveillance project using newborn blood spots as a medium for conducting blood lead surveillance. Editorial and technical review was completed by UDOH certifying reviewers and program partners.

Approved by:

Cristie Chesler
Director, Bureau of Epidemiology
Utah Department of Health

Allyn K Nakashima, MD
State Epidemiologist
Utah Department of Health

Wu Xu, PhD
Director, Center for Health Data
Utah Department of Health
REFERENCES

Web links for citations of government or organizational websites may wrap onto multiple lines.


# TABLES AND FIGURES


<table>
<thead>
<tr>
<th>Sex</th>
<th>Total Births Statewide</th>
<th>Samples Tested</th>
<th>Geometric Mean Blood Lead Level µg/dL</th>
<th>Highest Observed Blood Lead Level µg/dL</th>
<th>Number Tests with Blood Lead Levels Greater than 10 µg/dL</th>
<th>Number of Tests with Blood Lead Levels Greater than 5 µg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>79,813</td>
<td>2,702</td>
<td>0.37</td>
<td>28.47</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Male</td>
<td>84,056</td>
<td>2,888</td>
<td>0.38</td>
<td>51.88</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Both</td>
<td>163,869</td>
<td>5,590</td>
<td>0.38</td>
<td>51.88</td>
<td>12</td>
<td>29</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Year</th>
<th>Total Births Statewide</th>
<th>Samples Tested</th>
<th>Geometric Mean Blood Lead Level µg/dL</th>
<th>Highest Observed Blood Lead Level µg/dL</th>
<th>Number Tests with Blood Lead Levels Greater than 10 µg/dL</th>
<th>Number of Tests with Blood Lead Levels Greater than 5 µg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>54,773</td>
<td>2,256</td>
<td>0.40</td>
<td>51.88</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>2008</td>
<td>55,346</td>
<td>805</td>
<td>0.33</td>
<td>4.37</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2009</td>
<td>53,750</td>
<td>2,529</td>
<td>0.37</td>
<td>12.55</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>
FIGURE 1. Geometric mean blood lead levels by county in Utah among children born between 2007 and 2009. “C” is the number of newborn children tested in each county. “GM” is the geometric mean blood lead level in µg/dL. These findings are based on maternal residential address at the time of birth.
TABLE 3. Summary of Utah neonatal blood lead levels by county for children born between 2007 and 2009. These data are based on the maternal residential address at the time of birth.

<table>
<thead>
<tr>
<th>County</th>
<th>Total Births Statewide</th>
<th>Samples Tested</th>
<th>Geometric Mean Blood Lead Level µg/dL</th>
<th>Highest Observed Blood Lead Level µg/dL</th>
<th>Number Tests with Blood Lead Levels Greater than 10 µg/dL</th>
<th>Number of Tests with Blood Lead Levels Greater than 5 µg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaver</td>
<td>383</td>
<td>13</td>
<td>0.35</td>
<td>1.16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Box Elder</td>
<td>2,915</td>
<td>118</td>
<td>0.36</td>
<td>2.65</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cache</td>
<td>7,417</td>
<td>262</td>
<td>0.36</td>
<td>4.61</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carbon</td>
<td>967</td>
<td>45</td>
<td>0.40</td>
<td>2.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Daggett</td>
<td>35</td>
<td>2</td>
<td>0.26</td>
<td>0.62</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Davis</td>
<td>18,367</td>
<td>597</td>
<td>0.33</td>
<td>11.55</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Duchesne</td>
<td>982</td>
<td>18</td>
<td>0.58</td>
<td>1.71</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Emery</td>
<td>576</td>
<td>17</td>
<td>0.30</td>
<td>1.23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Garfield</td>
<td>188</td>
<td>10</td>
<td>0.51</td>
<td>1.08</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Grand</td>
<td>314</td>
<td>70</td>
<td>0.69</td>
<td>3.39</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Iron</td>
<td>2,842</td>
<td>85</td>
<td>0.37</td>
<td>3.17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Juab</td>
<td>701</td>
<td>24</td>
<td>0.46</td>
<td>1.45</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kane</td>
<td>247</td>
<td>11</td>
<td>0.71</td>
<td>2.19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Millard</td>
<td>634</td>
<td>17</td>
<td>0.38</td>
<td>2.21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Morgan</td>
<td>554</td>
<td>19</td>
<td>0.32</td>
<td>0.68</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Piute</td>
<td>67</td>
<td>7</td>
<td>0.42</td>
<td>0.77</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rich</td>
<td>106</td>
<td>7</td>
<td>0.36</td>
<td>0.61</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Salt Lake</td>
<td>57,681</td>
<td>1,868</td>
<td>0.41</td>
<td>15.46</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>San Juan</td>
<td>540</td>
<td>28</td>
<td>0.53</td>
<td>1.75</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sanpete</td>
<td>1,181</td>
<td>29</td>
<td>0.44</td>
<td>3.64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sevier</td>
<td>1,057</td>
<td>33</td>
<td>0.42</td>
<td>47.27</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Summit</td>
<td>1,591</td>
<td>55</td>
<td>0.43</td>
<td>4.84</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tooele</td>
<td>3,198</td>
<td>85</td>
<td>0.40</td>
<td>1.84</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Uintah</td>
<td>2,293</td>
<td>83</td>
<td>0.43</td>
<td>2.05</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Utah</td>
<td>36,918</td>
<td>1,319</td>
<td>0.34</td>
<td>51.88</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Wasatch</td>
<td>1,312</td>
<td>33</td>
<td>0.37</td>
<td>1.75</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Washington</td>
<td>7,846</td>
<td>271</td>
<td>0.34</td>
<td>12.23</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Wayne</td>
<td>108</td>
<td>8</td>
<td>0.36</td>
<td>3.14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Weber</td>
<td>12,849</td>
<td>456</td>
<td>0.38</td>
<td>29.04</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
DEFINITIONS

**ACCLPP**: Advisory Committee on Childhood Lead Poisoning Prevention. A committee established within the U.S. Department of Health and Human Services (DHHS) to advise and guide the secretary of DHHS and the director of CDC on the scientific knowledge, technical developments and practical implementation for childhood lead poisoning prevention. For more information see: http://www.cdc.gov/nceh/lead/acclpp/acclpp_main.htm.

**ArcGIS**: A computer application that provides mapping and spatial analysis of spatially referenced data. ArcGIS is a product developed and available through ESRI. For more information see: http://www.esri.com or http://www.arcgis.com.

**Biomonitoring**: A way of measuring which substances humans have been exposed to and the level of exposure to those compounds, through analysis of body fluids (e.g., saliva, urine, or blood, etc.) or tissues (e.g., epithelial cells obtained by swabbing the mouth, or hair, or nail clippings, etc.) for those compounds or metabolites of those compounds.

**Blood Spots**: Drops of blood placed on a filter card and dried. Blood spot cards are prepared for infants by sampling the blood obtained by a heel stick.

**BLL**: Blood lead level. The amount of lead in the blood, quantified as micrograms of lead per deciliter of blood (µg/dL). In Utah, blood lead levels are considered elevated if there is 10 or more µg of lead per dL of blood (≥10 µg/dL).

**CDC**: Centers for Disease Control and Prevention. A federal agency within the U.S. Department of Health and Human Services responsible for investigating disease trends and causalities, and promoting best disease prevention practices. For more information see: http://www.cdc.gov/.

**EBLL**: Elevated blood lead levels. Blood lead levels at or exceeding 10 or more µg of lead per dL of blood (≥10 µg/dL). This is an action level established by Utah Administrative Code R386-703 Injury Reporting Rule. See: http://www.rules.utah.gov/publicat/code/r386/r386-703.htm.

**EEP**: Environmental Epidemiology Program. A program within the Bureau of Epidemiology, Division of Disease Control and Prevention, UDOH. The EEP was established in 1996 and is responsible for investigating diseases related to the environment. The program has two sections.
One section conducts surveillance and data management activities, including managing the UEPHTN. The other section conducts health hazards risk assessment, including cancer investigations. The program is staffed by personnel with experience and expertise in environmental epidemiology, environmental sciences, toxicology, statistics, public health informatics and geomatics, and health education. For more information see: http://health.utah.gov/enviroepi/.

**ESRI**: ESRI (formally known as Environmental Systems Research Institute) is a leading developer and supplier of GIS software and geographically referenced data. ESRI is headquartered in Redlands, California. The EEP uses the ArcGIS software application developed by ESRI. For more information see: http://www.esri.com.

**Geometric mean**: A type of average or measurement of central tendency that uses the products of a set of numbers rather than the summation. The geometric mean of a data set \( \{a_1, a_2, \ldots, a_n\} \) is given by \( (a_1a_2 \cdots a_n)^{1/n} \). The geometric mean is used to describe the average of blood lead levels because the distribution of values in the data set is skewed towards zero, and no value can be less than zero.

**GIS**: Geographic Information Systems. A GIS includes computer software and geographically referenced data. The EEP uses ArcGIS as the computer software and obtains data from ESRI or AGRC.

**ICP-MS**: Inductively coupled plasma mass spectrometry. This is a laboratory methodology that is able to separate and quantify the amount of atoms or compounds present in a solution based on the mass of those atoms or compounds.

**RMBC**: Rocky Mountain Biomonitoring Consortium. The RMBC was a CDC-funded collaboration of the state laboratories of Arizona, Colorado, Montana, New Mexico, Utah, and Wyoming. The New Mexico Scientific Laboratory served as the grant coordinating laboratory and distributed funds from CDC to the other states within the consortium. Each state laboratory developed one or more biomonitoring analytical capabilities that could serve the needs of all of the states biomonitoring needs. Utah developed methodology for blood spot testing for heavy metals.

**SAS**: SAS (originally from “Statistical Analysis System”) is a globally recognized system of integrated computer software products provided by SAS Institute Inc. The SAS system includes a large variety of data manipulation and statistical analysis processes. The EEP uses the desktop version 9.2. For more information see: http://www.sas.com.

**UAC**: Utah Administrative Code. Administrative codes are rules promulgated by state agencies within the state executive branch of government, authorized by state law, to describe requirements and procedures for governmental operations. See: http://www.rules.utah.gov.
**UDOH**: Utah Department of Health. The UDOH is one of the executive agencies within Utah state government. The UDOH strives to improve health in Utah by promoting healthy lifestyles, evidence-based interventions, creating healthy and safe communities, and eliminating health disparities. The EEP is a program within the UDOH. For more information, see: http://health.utah.gov/.

**UEPHTN**: Utah Environmental Public Health Tracking Network. The UEPHTN is a data warehouse that contains health outcomes, environmental, and supporting data. For more information see: http://epht.health.utah.gov/epht-view/.

**µg/dL**: Micrograms per deci-liter. A microgram is one millionth of a gram. A deci-liter is one tenth of a liter.

**UPHL**: Utah Public Health Laboratory. A part of the Unified State Laboratories. The UPHL provides laboratory support to other state agencies and to the public. For more information, see: http://health.utah.gov/lab/index.html.
DESCRIPTION OF THE LABORATORY METHODOLOGY

Preparation of Samples and Internal Blanks: Filter paper punches, each ¼ inch (6.35 mm) in diameter, were punched from cards containing newborn dried blood spots directly into 15 mL polypropylene tubes (VWR International Inc., San Diego, California). Two sets of dots (in duplicate) were punched. The first set was comprised of two dots from the card adjacent to the newborn’s blood sample spots and is defined as the internal blank. The internal blank dots were assumed to have been exposed to the same environmental conditions as the actual blood samples and hence are utilized to assess extraneous environmental contamination from the hospital, contamination during transit to the laboratory, storage contamination, and contamination during laboratory handling. The second set of dots was comprised of two dots punched directly from the dried blood spots. An empty 15 mL polypropylene tube from the same lot as the other tubes was utilized as a control tube. This “blank” tube was filled with the same extraction solution as the tubes containing the actual samples and carried throughout the entire extraction procedure to assess contamination from the actual procedure.

The dots were extracted with a 2% hydrochloric acid solution (GFS Chemicals®, Columbus, Ohio) containing 0.05% 2-mercaptoethanol (Fluka, Milwaukee, Wisconsin), 0.001% l-cysteine (Fluka, Milwaukee, Wisconsin), and 10 µg/L iridium and rhodium (Spex Industries Inc., Edison, New Jersey). The latter two elements served as internal standards. 1.5 mL of the extraction solution was added to each tube and then vortexed for 15 minutes. The tubes were then allowed to stand overnight (about 16-18 hours), then vortexed for another 15 minutes, and lastly, centrifuged for 5 minutes at 5,000 RPM in an Eppendorf 5804 centrifuge (Brinkman Instruments, Inc., Westbury New York). The tubes were then placed into the autosampler of the inductive coupled plasma mass spectrometer (ICP-MS) for analysis.

Quality Control and Quality Assurance: With each batch of blood spot samples (typically ten spots), the following quality control samples were analyzed at a minimum: a set of calibration standards; a blank control (negative) to assure the system is free of contamination from previous analyses; a positive control using commercially prepared standard reference material (SRM) to assess accuracy; and a set of duplicate samples to assess precision. The SRM samples were prepared from a freeze-dried human whole blood toxicology control (level 1, lot number 9081) purchased from Utak Laboratories, Inc. (Valencia, California). The material had a known, verified mean lead concentration and an expected analytical range. The material was reconstituted by adding 3 mL of 18 mega ohm water with a volumetric pipette. The mixture was gently swirled for 5-10 minutes then allowed to stand for 1 hour for equilibration and subsequent warming to room temperature. The reconstituted blood was then spotted drop-wise with a Pasture pipette onto S&S (Keene, New Hampshire) 903, lot W011 filter paper. The blood was added until the dotted, printed circle was filled, which corresponds to a total blood volume of about 75 µL (CLSI 2007). This has also been calibrated during the present study. The spotted filter paper cards were dried for several hours then placed in TearZone Safeguard Specimen bags, stored under refrigerated conditions, and analyzed in the same manner as patient samples.
The calibration curve was constructed using aqueous-based samples and calculated using ordinary linear regression methods. Weighting was not used and the intercepts were not forced through the origin.

Additional quality assurance was demonstrated by the UPHL’s participation in the Wisconsin State Laboratory of Hygiene proficiency test program for filter paper blood analysis during the duration of this study. Every testing event was passed.

**Analysis**: The samples were analyzed utilizing ICP-MS. An Elan DRC II ICP-MS machine (PerkinElmer, Shelton, Connecticut) equipped with a Meinhard nebulizer and a quartz cyclonic spray chamber was used to make these readings. The dynamic reaction cell (DRC) was not utilized for this work. Arithmetic isobaric correction equations were utilized and two replicate readings were taken for each m/z.

For a more detailed description of the methods briefly described here, see Chaudhuri et al. 2009.