IJADR International Journal of Alcohol and Drug Research

The Official Journal of the Kettil Bruun Society for Social and Epidemiological Research on Alcohol

doi: 10.7895/ijadr.v4i2.209 IJADR, 2015, 4(2), 131 – 137 ISSN: 1925-7066

Retrospective assessment of prenatal alcohol exposure by detection of phosphatidylethanol in stored dried blood spot cards: An objective method for determining prevalence rates of alcohol consumption during pregnancy

Aileen E. Baldwin, Joseph Jones, Mary Jones, Charles Plate, and Douglas Lewis

United States Drug Testing Laboratories, Inc. Des Plaines, IL United States

Abstract

Aims: To analyze the efficacy of screening banked newborn dried blood spots (DBS) for detection of phosphatidylethanol (PEth), a direct alcohol biomarker, with the purpose of performing a retrospective assessment of statewide prevalence rates of alcohol consumption in late pregnancy that results in risky prenatal alcohol exposure.

Design: Residual DBS samples collected for newborn screening and stored by a state department of public health were examined for concentrations of PEth. The prevalence of prenatal alcohol exposure, as determined by this direct alcohol biomarker, was compared to prevalence rates of alcohol consumption during pregnancy that have been derived from multiple state-based and national studies using maternal self-report surveys.

Setting: DBS cards representative of the general newborn population were collected from multiple hospitals across a single midwestern state.

Participants: Two hundred fifty anonymous newborn DBS collected for routine metabolic screening in a midwestern state were requested through the Virtual Repository of Dried Blood Spots.

Measures: Concentrations of PEth, a highly specific biomarker of alcohol consumption, were analyzed using a liquid chromatography–tandem mass spectrometry method validated by our laboratory.

Findings: Of 250 DBS examined, 4% were positive for PEth (PEth $\geq$ 8 ng/ml) which is indicative of exposure to maternal alcohol consumption during the last month of pregnancy.

Conclusions: Detection of PEth from newborn DBS cards can identify prenatal alcohol exposure and also be used for retrospective surveillance of alcohol consumption during the last three to four weeks of pregnancy, using specimens that are collected for routine metabolic screening and stored by many state health departments.

Prenatal alcohol exposure (PAE) is the leading preventable cause of birth defects. PAE produces an array of neurological, behavioral, and physical abnormalities collectively known as fetal alcohol spectrum disorders (FASD). Alcohol can interfere with normal prenatal development, with the extent of damage dependent on the quantity, frequency, and timing of exposure to alcohol during fetal development (May et al., 2013; Spagnolo, 1993). Prenatal exposure to binge-like levels of alcohol has been shown to be particularly harmful and result in more severe deficits (Bailey et al., 2004; Bonthius & West, 1990; Goodlett, Pearlman, & Lundahl, 1998; Goodlett, Peterson, Lundahl, & Pearlman, 1997). The most severely affected children, resulting from chronic exposure to high levels of alcohol are diagnosed with fetal alcohol syndrome (FAS), a disorder defined by growth deficiencies and specific facial and nervous system malformations. In the United States, FAS is estimated to occur in 2–7 per 1,000 births, while the prevalence of all levels within the continuum of FASD estimated to be as high as 2–5% of school-age children (May et al., 2009).

Estimates of the prevalence of alcohol consumption during pregnancy in the United States are derived from state and national surveys that rely on maternal self-report of drinking habits during pregnancy, and they range from 7–

Correspondence: Aileen E. Baldwin, 1700 South Mount Prospect Road Des Plaines, IL 60018. Telephone: (847) 375-0770; Fax: (847) 375-0775; E-mail: aileen.baldwin@usdtl.com
Financial support: This study was internally funded by United States Drug Testing Laboratories, Inc. (USDTL). USDTL is a commercial reference laboratory in the business of analyzing biological specimens for the presence of alcohol and drug biomarkers. This does not alter our adherence to all the journal's policies on sharing data and materials
Declaration of interest: Keywords: Prenatal Alcohol Exposure (PAE), Alcohol, Pregnancy, Prevalence, Phosphatidylethanol (PEth)

30%. The Behavioral Risk Factor Surveillance System (BRFSS) is a state-based survey conducted annually by the National Center for Chronic Disease Prevention and Health Promotion within the Centers for Disease Control and Prevention (CDC). BRFSS data collected from 2006 through 2010 indicate that 7.6% of pregnant women reported alcohol consumption during the 30 days prior to being interviewed and 1.4% reported binge drinking (Centers for Disease & Prevention, 2012).

The National Survey on Drug Use and Health (NSDUH) is administered by the Substance Abuse and Mental Health Services Administration (SAMHSA). Combined results from the 2011 and 2012 NSDUH surveys found that 8.5% of pregnant women reported alcohol use, 2.7% reported binge drinking, and 0.3% reported heavy drinking ("Results from the 2012 National Survey on Drug Use and Health: Summary of National Findings," 2013).

The Pregnancy Risk Assessment Monitoring System (PRAMS) is a joint surveillance project between the CDC and state health departments to collect state-specific data on maternal behavior before, during, and after pregnancy. Data collected from the 29 states that participated in PRAMS between 2006 and 2010 indicate that an average of 7.0% of pregnant women reported having any alcoholic drinks during the last three months of their pregnancy (CDC, 2006-2010).

The National Birth Defects Prevention Study (NBDPS) is a multi-state, CDC-sponsored case-control study of potential risk factors and causes of birth defects. Based on NBDPS data collected from October 1997 through December 2002, 30.3% of all women reported drinking alcohol at some point during pregnancy, and 8.3% reported binge drinking (Ethen et al., 2009).

Each of these surveys has strengths and weaknesses in terms of its ability to subjectively assess maternal alcohol consumption during pregnancy. The percentage of women who drank alcohol at any time during pregnancy reported in the NBDPS study (30.3%) was almost four times higher than rates reported by the 2011 BRFSS report (7.6%) and more than three times higher than rates reported by the 2011 NSDUH report (8.5%), suggesting that differences in study methodology impact estimates. Another limitation is that women with severe alcohol use problems may be less likely to participate in these studies, with recollection bias and under-reporting of alcohol potentially occurring due to embarrassment and fear of stigmatization.

Alcohol biomarkers can be used as objective measurements for monitoring maternal alcohol consumption and PAE. Phosphatidylethanol (PEth) is a direct biomarker of alcohol metabolism that has generated interest in the field of alcoholism research in recent years, as it has been shown to be a highly sensitive and specific indicator of alcohol use and misuse. PEth is a unique phospholipid formed only in the presence of ethanol from phosphatidylcholine, present within membranes of red blood cells, through a metabolic process catalyzed by phospholipase D (Alling, Gustavsson, & Anggard, 1983; Gustavsson & Alling, 1987; Mueller, Fleming, LeMahieu, Lybrand, & Barry, 1988). Human red blood cells do not have the enzymatic machinery to efficiently degrade PEth, causing the accumulation of PEth in the cellular membranes following exposure to ethanol (Aradottir, Moller, & Alling, 2004). The natural decomposition of PEth results in a slow elimination, with a half-life of approximately 4 days in adults and PEth detectable in blood for up to 28 days following the last drinking episode (Aradottir, Asanovska, Gjerss, Hansson, & Alling, 2006; Aradottir et al., 2004; Hansson, Caron, Johnson, Gustavsson, & Alling, 1997; Hartmann et al., 2007; Varga, Hansson, Lundqvist, & Alling, 1998; Wurst et al., 2010). A number of studies have examined the use of PEth as a sensitive and specific marker of heavy episodic drinking, with some evidence for the correlation between alcohol intake and concentration of PEth in blood (Aradottir et al., 2006; Nalesso et al., 2011; Stewart, Law, Randall, & Newman, 2010; Wurst et al., 2010). A recent study by Stewart et al. (2010) using a more sensitive liquid chromatography–tandem mass spectrometry assay found that PEth was also a sensitive indicator of moderate to heavy alcohol consumption, with PEth being detectable in 93% of subjects consuming an average of two or more drinks per day. Measurement of PEth in dried blood spots (DBS) is as reliable as detection in whole blood samples and provides a more convenient technique for collection, transport, and storage (Faller et al., 2011). Additionally, post-collection synthesis of PEth does not occur within the filter paper matrix of DBS cards, removing the possibility of false-positive results (Jones, 2011). Our laboratory previously developed and validated a highly sensitive liquid chromatography–tandem mass spectrometry system for the extraction and detection of PEth from DBS samples to facilitate detection of PAE (Jones, 2011). Bakhireva et al. (2014) recently published results from a prospective cohort of 60 pregnant women and newborn pairs that reported detection of newborn PEth in DBS demonstrated 100% specificity and newborn PEth screening could accurately detect PAE with a higher sensitivity than other currently used direct and indirect alcohol metabolites. Their previous analysis of PEth in newborn DBS samples has also demonstrated the feasibility of collecting an additional DBS card during routine newborn screening for measurement of PEth as an indicator of PAE, a practice that also provides overall cost savings compared to either meconium fatty acid ethyl esters (FAEE) analysis or analysis of PEth from blood specimens obtained by phlebotomy (Bakhireva et al., 2013). To date, no studies have examined the feasibility of measuring PEth from stored DBS specimens, which are routinely collected for newborn screening. Screening of stored, residual DBS specimens would provide a new method for retrospective assessment of alcohol consumption during late pregnancy while also producing objective prevalence estimates of PAE in a general newborn population.

The primary objective of this study was to determine if measurement of PEth from de-identified, stored DBS cards from a state repository would be a feasible method for undertaking population-based surveillance studies of PAE and alcohol consumption during the last three to four weeks of pregnancy. The stability of PEth in DBS in room-temperature conditions for up to 12 months was first

examined to determine whether this common storage condition would impact PEth concentration. The prevalence rate of prenatal exposure to alcohol on a statewide scale was then analyzed using detection of PEth as an objective measurement of PAE.

Methods

Study Design

The study protocol was reviewed and approved by the Western International Review Board (WIRB study number 1135236), and the research was conducted in accord with prevailing ethical principles. Following IRB approval, a request for 250 de-identified residual DBS specimens from the general population within a single midwestern state was submitted through the Newborn Screening Translational Research Network (NBSTRN) Virtual Repository of Dried Blood Spots, which is a centralized, web-based system that maintains information about stored specimens available from participating states. A written request and study protocol were reviewed and approved by the state Advisory Committee. An application for a research agreement with the Department of Public Health and a study protocol were then submitted to the state Research and Ethics Review Committee for consideration. Upon study approval, the state Newborn Screening Laboratory released 250 de-identified DBS specimens to United States Drug Testing Laboratories, Inc. (USDTL). The DBS specimens had been collected from multiple hospitals across the state in March 2012 and stored at −80°C for 12 months. The de-identified DBS specimens were removed from the freezer on April 2, 2013, and shipped to USDTL without any identifiable information.

To investigate the stability of PEth in DBS samples following storage at room temperature, authentic DBS were collected from the Quality Assurance Laboratory at USDTL. DBS specimens were stored at room temperature in sealed containers for up to 12 months. PEth concentrations were determined from at least four samples at each time point, with the concentration determined on day zero used as the initial comparative value (100%). PEth values are reported as a mean percentage of those initial concentrations.

Analytical Method

Analysis and measurement of a major molecular form of PEth, PEth 16:0/18:1, was conducted using liquid chromatography–tandem mass spectrometry (LC/MS/MS) following a previously described method (Jones, 2011). Briefly, three 3.1-mm punches from stored DBS specimens were extracted in 1.0 ml methanol containing a deuterated analog of PEth 16:0/18:1 as an internal standard, evaporated and reconstituted into mobile phase A (20% 2 mM ammonium acetate: 58% acetonitrile: 22% isopropanol). The specimens were analyzed by LC/MS/MS using Leap Technologies PAL HTS XT autosampler along with a Shimadzu Prominence LC system that consists of a DGU-20A5 degasser, a LC-20AD XR binary pump, and a CTO-20AC column compartment. Separation was achieved using an Agilent Poroshell 120 EC C-8 column with an AB SCIEX 5500 tandem mass spectrometer detector using electro-spray ionization in the negative mode. Using this LC/MS/MS method, the limit of detection is 2.0 ng/ml and the limit of quantitation is 8.0 ng/ml (Jones, 2011).

Results

Stability of PEth in Stored DBS Cards

Many newborn screening programs store residual DBS samples for secondary uses after completion of routine newborn screenings. These include laboratory quality assurance and test validation, research and evaluation purposes related to newborn screening programs, test refinement and result verification, and in some instances for new test development, population surveillance and public health research. Storage conditions for the DBS cards are determined by state laboratories depending on the purpose and length of storage required by that state, with storage conditions that vary from ambient temperature to −80°C.

Stability of PEth in whole blood can be maintained for at least 14 months at −80°C, while PEth in DBS samples has been shown to maintain stability when stored at either room temperature or frozen at −20°C for 30 days (Faller et al., 2012; Helander & Zheng, 2009). Multiple states store residual specimens at room temperature; hence we analyzed the stability of PEth on DBS cards when stored for up to 12 months at room temperature to determine whether this storage condition would impact the concentration of PEth detected.

No significant decrease of PEth concentration was seen in DBS stored at room temperature for three months, with 96.3% of the initial PEth concentration detectable after 90 days (Figure 1). PEth was relatively stable after storage at room temperature for six months, with only 18.2% of the initially measured concentration of PEth lost after this storage period. After nine and 12 months of storage at room temperature, 68.8% and 65.9% of the initial PEth concentrations were detected, respectively. Despite the significant decrease in PEth levels, for each of the specimens tested after storage for nine or 12 months, PEth concentrations remained detectable above the limit of quantitation (PEth ≥ 8 ng/ml), the minimum concentration of PEth that can be reliably and reproducibly detected using our assay.

Prevalence of PAE Determined by PEth Detection in DBS From Newborns

Of 250 DBS cards analyzed, 4% of the specimens were above the lower limit of quantitation for PEth (PEth ≥ 8 ng/ml) (Figure 2), with concentrations of PEth ranging from 8.2–30.1 ng/ml, and a mean PEth concentration of 17.6 ng/ml. Based on these findings, 4% of the newborn samples screened positive for PEth, indicating that exposure to maternal alcohol consumption occurred during the last month of pregnancy.

Figure 1

Percent of initial PEth concentrations following storage of dried blood spot cards at room temperature for 3, 6, 9, and 12 months

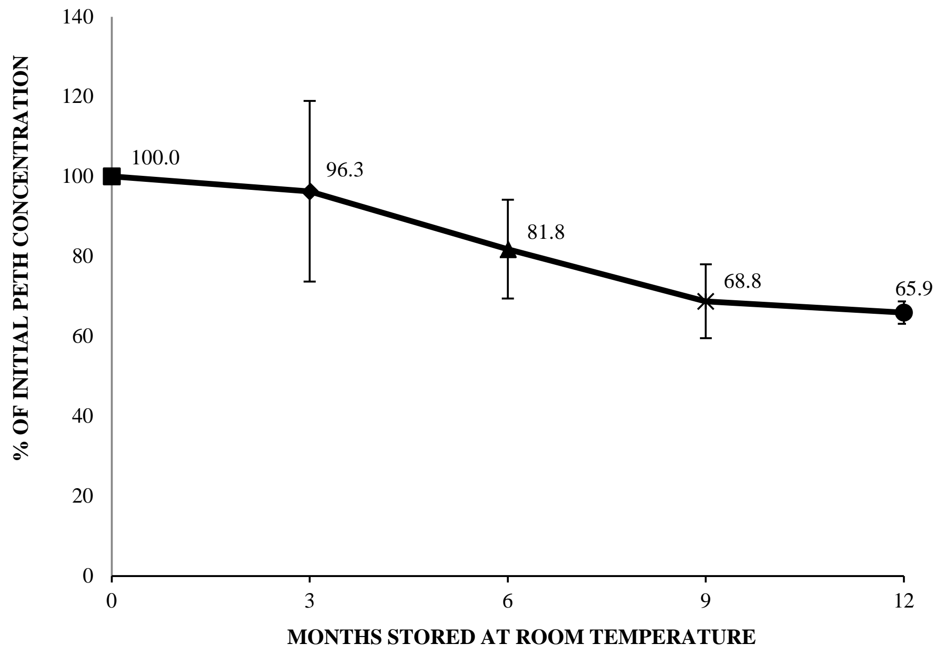
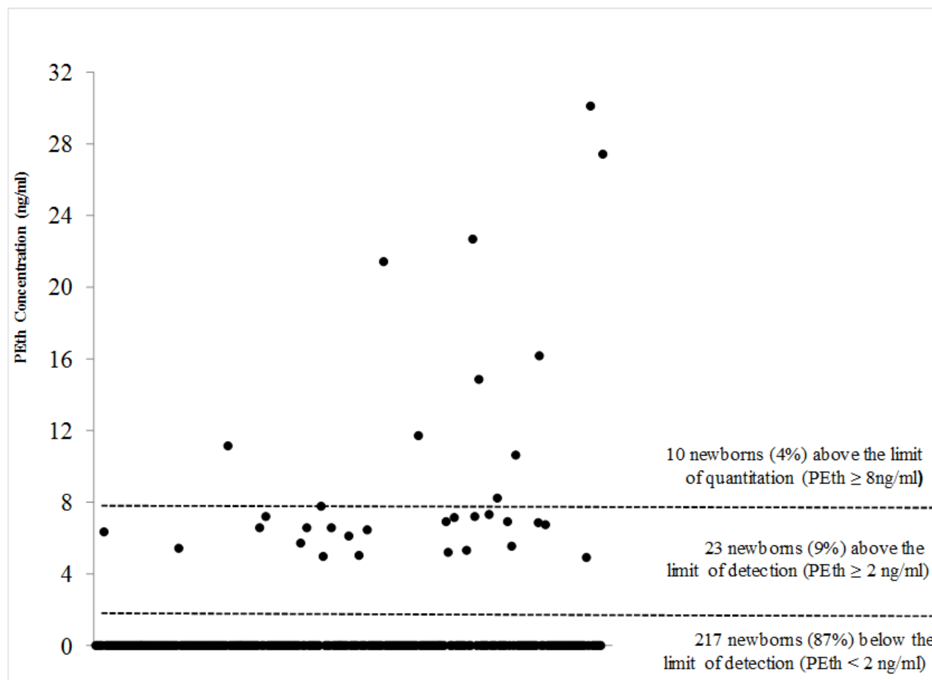


Figure 2

Concentration of PEth measured in residual dried blood spot specimens from a midwestern state



Discussion

Analysis of Stored DBS for PEth Detection in Newborns

Storage of DBS cards at room temperature is a suitable environment for maintaining relative PEth stability for storage periods of up to six months. The stability of PEth decreases after six months at room temperature, with about 34% of the initially measured concentration being lost after 12 months. Regardless of this significant reduction in PEth concentration, the stability of PEth on DBS cards at room temperature is still a significantly more stable matrix than FAEEs in meconium, another commonly used specimen for detection of PAE. Storage of meconium for 24 hours at room temperature was found to result in a loss of 86% of the total initial FAEE concentration (Moore, Jones, Lewis, & Buchi, 2003). For shipment and storage purposes, DBS cards are an easier, more cost-effective, and stable specimen for analysis of PAE.

Storage of DBS cards at −20°C has been shown to substantially improve the stability of PEth and improve the detection of PEth in stored samples versus storage at room temperature for 30 days (Faller et al., 2012). It is conceivable that long-term storage at −20°C would be beneficial for the stability of PEth. Based on data from the NBSTRN, 18 states currently store DBS specimens at −20°C, seven states maintain DBS specimens between 4–8°C, and 16 states use room-temperature storage conditions. Other recent studies examining the stability of markers of metabolic and genetic disorders have demonstrated that DBS storage conditions with lower temperature and humidity are essential for maintaining sample integrity (Adam, Chafin, & De Jesus, 2013; Adam et al., 2011).

Prevalence Estimates Based on Surveys versus Objective Measurement

Results from this study indicate that 4% of the newborn DBS specimens analyzed were positive for detection of PEth (PEth ≥ 8 ng/ml), which is indicative of exposure to maternal alcohol consumption during the previous month. An additional twenty-three newborn DBS specimens with PEth concentrations above the limit of detection (PEth ≥ 2 ng/ml) could be reliably distinguished from analytical noise but failed to reach the limit of quantitation necessary to be considered above a positive cut-off using the current assay (Figure 2). It is feasible that with further improvements to the analytical sensitivity of this assay through changes in the procedure and/or analytical platform, the limit of quantitation could decrease and samples between 2–8 ng/ml would be considered positive for PEth, thereby substantially increasing the percentage of newborns detected for exposure to alcohol. However, further studies are necessary to examine the correlation of reported alcohol intake and PEth concentrations in the mother and newborn at birth to determine if detection of low levels of PEth (2–8 ng/ml) is reflective of very light alcohol use and higher levels of PEth (PEth ≥ 8 ng/ml) are more reflective of moderate to heavy alcohol consumption.

The elimination rate of PEth in newborns has not been analyzed; the half-life of four days for PEth and the window of detection for PAE are based on data from studies on adult alcoholics. A single study conducted among social drinkers found a slower elimination of PEth in the test subjects, with a reported half-life of PEth ranging from 4.5–12.0 days (Gnann, Weinmann, & Thierauf, 2012). However, the two test subjects in this study for whom a half-life of more than 10 days was observed had measurable PEth at baseline. The remaining eight subjects who were PEth negative at baseline eliminated PEth with a half-life ranging from 4–9 days. Further studies are necessary to examine the elimination rate of PEth in newborns to determine whether the window of detection for PAE exceeds the four-week window that has been observed among adult alcoholic subjects.

In addition to the results presented here, a recent study examining 201 de-identified DBS cards collected at the University of New Mexico Hospital found that 15.5% of DBS cards were above the lower limit of quantitation for PEth (PEth ≥ 8ng/ml) and 6.5% were above a 20 ng/ml cutoff concentration (Bakhireva et al., 2012). While this sampling was drawn from a single hospital rather than from a general statewide population, these findings further support our conclusion that previous estimates of risky drinking during pregnancy and prevalence of PAE do not accurately reflect the scope of this public health issue. Analysis of larger populations from multiple states is necessary to determine if prevalence rates of PAE between 4.0–15.5% are consistent in other populations in the United States.

Further Considerations for Future Studies Screening Residual DBS for PAE

Newborn screening DBS samples are collected from nearly all of the more than four million babies born each year in the United States. In addition to testing for more than 30 specific hereditary and congenital disorders, residual DBS are also an extremely valuable resource for conducting basic health-related epidemiological research. Because of the significant number of ethical considerations in regards to the retention and use of residual DBS, the Secretary's Advisory Committee on Heritable Diseases in Newborns and Children (SACHDNC) published a briefing paper that encourages states to develop policies and procedures to address the retention of DBS specimens (Therrell et al., 2011). Among the recommendations in this report, the SACHDNC recommends that state newborn screening programs develop a policy (that has been reviewed by a legal authority) to specify who can access, use, and distribute DBS specimens; assure that parents are aware of newborn screening activities and educate them about the retention and use of specimens; facilitate a national dialogue about policies for the retention and use of residual newborn screening specimens, including consent processes; and explore the feasibility of establishing a voluntary national repository of DBS specimens in which families may choose to participate. States that maintain a biorepository of stored residual DBS samples for research

purposes, such as the current study, have an extensive review process for researchers requesting residual specimens to ensure that DBS are used in a secure, anonymous, and privacy-protected manner and that all ethical considerations and concerns are properly addressed. The ethical considerations and clinical utility for storage of identifiable DBS cards and whether these stored DBS cards could be used to confirm PAE at a later time in the child's life have not been analyzed. Results from the current study, however, demonstrate that DBS cards stored at room temperature can be analyzed for PEth up to 12 months after birth to confirm PAE and potentially much longer after birth if stored at −80°C.

Conclusions

Determining objective prevalence rates of PAE is necessary to identify vulnerable populations; target prevention and treatment resources; and evaluate the strengths and limitations of various prevention, intervention, and treatment strategies. Conducting multiple statewide population-based surveillance studies on the prevalence of PAE would also benefit newborn screening testing programs by examining the potential benefits of expanding newborn screening to include the identification of exposure to dangerous environmental teratogens (such as alcohol) that can cause birth defects. This study demonstrates that PEth analysis of DBS cards can be used for both retrospective surveillance studies of PAE and alcohol consumption during the final three to four weeks of pregnancy using DBS specimens collected for routine newborn screening.

References

Adam, B. W., Chafin, D. L., & De Jesus, V. R. (2013). Stabilities of hemoglobins A and S in dried blood spots stored under controlled conditions. *Clinical Biochemistry*, *46*(12), 1089–1092. doi:10.1016/j.clinbiochem.2013.05.043

Adam, B. W., Hall, E. M., Sternberg, M., Lim, T. H., Flores, S. R., O'Brien, S., . . . Hannon, W. H. (2011). The stability of markers in dried-blood spots for recommended newborn screening disorders in the United States. *Clinical Biochemistry*, *44*(17–18), 1445–1450. doi:10.1016/j.clinbiochem.2011.09.010

Alling, C., Gustavsson, L., & Anggard, E. (1983). An abnormal phospholipid in rat organs after ethanol treatment. *FEBS Letters*, *152*(1), 24–28.

Aradottir, S., Asanovska, G., Gjerss, S., Hansson, P., & Alling, C. (2006). Phosphatidylethanol (PEth) concentrations in blood are correlated to reported alcohol intake in alcohol-dependent patients. *Alcohol and Alcoholism*, *41*(4), 431–437. doi:10.1093/alcalc/agl027

Aradottir, S., Moller, K., & Alling, C. (2004). Phosphatidylethanol formation and degradation in human and rat blood. *Alcohol and Alcoholism*, *39*(1), 8–13.

Bailey, B. N., Delaney-Black, V., Covington, C. Y., Ager, J., Janisse, J., Hannigan, J. H., & Sokol, R. J. (2004). Prenatal exposure to binge drinking and cognitive and behavioral outcomes at age 7 years. *American Journal of Obstetrics and Gynecology*, *191*(3), 1037–1043. doi:10.1016/j.ajog.2004.05.048

Bakhireva, L. N., Leeman, L., Savich, R. D., Cano, S., Gutierrez, H., Savage, D. D., & Rayburn, W. F. (2014). The validity of phosphatidylethanol in dried blood spots of newborns for the identification of prenatal alcohol exposure. *Alcoholism: Clinical and Experimental Research*, *38*(4), 1078–1085. doi:10.1111/acer.12349

Bakhireva, L. N., Savich, R. D., Raisch, D. W., Cano, S., Annett, R. D., Leeman, L., . . . Savage, D. D. (2013). The feasibility and cost of neonatal screening for prenatal alcohol exposure by measuring phosphatidylethanol in dried blood spots. *Alcoholism: Clinical and Experimental Research*, *37*(6), 1008–1015. doi:10.1111/acer.12045

Bakhireva, L. N., Savich, R. D., Cano, S., Goff, C., Annett, R. D., Rayburn, D. D., Savage, D. D. (2012). Validity and feasibility of neonatal screening for prenatal alcohol exposure by measuring phosphatidylethanol in dried blood spots of a newborn. [Abstract]. *Alcoholism: Clinical and Experimental Research, 36(*Suppl. 1), 43A.

Bonthius, D. J., & West, J. R. (1990). Alcohol-induced neuronal loss in developing rats: Increased brain damage with binge exposure. *Alcoholism: Clinical and Experimental Research*, *14*(1), 107–118.

Centers for Disease Control and Prevention. (2006–2010). Pregnancy Risk Assessment Monitoring System (PRAMS): Pregnancy Risk Assessment Monitoring System Online Data for Epidemiologic Research (CPONDER), Data for All States - 2006–2010; Alcohol Use. http://libguides.lib.msu.edu/c.php?g=96245&p=626239

Centers for Disease Control and Prevention. (2012). Alcohol use and binge drinking among women of childbearing age—United States, 2006–2010. *Morbidity and Mortality Weekly Report*, *61*(28), 534–538.

Ethen, M. K., Ramadhani, T. A., Scheuerle, A. E., Canfield, M. A., Wyszynski, D. F., Druschel, C. M., . . . National Birth Defects Prevention Study (2009). Alcohol consumption by women before and during pregnancy. *Maternal and Child Health Journal*, *13*(2), 274–285. doi:10.1007/s10995-008-0328-2

Faller, A., Richter, B., Kluge, M., Koenig, P., Seitz, H. K., & Skopp, G. (2012). Stability of phosphatidylethanol species in spiked and authentic whole blood and matching dried blood spots. *International Journal of Legal Medicine*, *127*(3), 603–610.

Faller, A., Richter, B., Kluge, M., Koenig, P., Seitz, H. K., Thierauf, A., . . . Skopp, G. (2011). LC-MS/MS analysis of phosphatidylethanol in dried blood spots versus conventional blood specimens. *Analytical and Bioanalytical Chemistry*, *401*(4), 1163–1166. doi:10.1007/s00216-011-5221-y

Gnann, H., Weinmann, W., & Thierauf, A. (2012). Formation of phosphatidylethanol and its subsequent elimination during an extensive drinking experiment over 5 days. *Alcoholism: Clinical and Experimental*

Research, *36*(9), 1507–1511. doi:10.1111/j.1530-0277.2012.01768.x

Goodlett, C. R., Pearlman, A. D., & Lundahl, K. R. (1998). Binge neonatal alcohol intubations induce dose-dependent loss of Purkinje cells. *Neurotoxicology and Teratology*, *20*(3), 285–292.

Goodlett, C. R., Peterson, S. D., Lundahl, K. R., & Pearlman, A. D. (1997). Binge-like alcohol exposure of neonatal rats via intragastric intubation induces both Purkinje cell loss and cortical astrogliosis. *Alcoholism: Clinical and Experimental Research*, *21*(6), 1010–1017.

Gustavsson, L., & Alling, C. (1987). Formation of phosphatidylethanol in rat brain by phospholipase D. *Biochemical and Biophysical Research Communications*, *142*(3), 958–963.

Hansson, P., Caron, M., Johnson, G., Gustavsson, L., & Alling, C. (1997). Blood phosphatidylethanol as a marker of alcohol abuse: Levels in alcoholic males during withdrawal. *Alcoholism: Clinical and Experimental Research*, *21*(1), 108–110.

Hartmann, S., Aradottir, S., Graf, M., Wiesbeck, G., Lesch, O., Ramskogler, K., . . . Wurst, F. M. (2007). Phosphatidylethanol as a sensitive and specific biomarker: Comparison with gamma-glutamyl transpeptidase, mean corpuscular volume and carbohydrate-deficient transferrin. *Addiction Biology*, *12*(1), 81–84. doi:10.1111/j.1369-1600.2006.00040.x

Helander, A., & Zheng, Y. (2009). Molecular species of the alcohol biomarker phosphatidylethanol in human blood measured by LC-MS. *Clinical Chemistry*, *55*(7), 1395–1405. doi:10.1373/clinchem.2008.120923

Jones, J., Jones, M., Plate, C., Lewis, D. (2011). The detection of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol in human dried blood spots. *Analytical Methods*, *3*(5), 1101–1106.

May, P. A., Blankenship, J., Marais, A. S., Gossage, J. P., Kalberg, W. O., Joubert, B., . . . Seedat, S. (2013). Maternal alcohol consumption producing fetal alcohol spectrum disorders (FASD): Quantity, frequency, and timing of drinking. *Drug and Alcohol Dependence*, *133*(2), 502–512. doi:10.1016/j.drugalcdep.2013.07.013

May, P. A., Gossage, J. P., Kalberg, W. O., Robinson, L. K., Buckley, D., Manning, M., & Hoyme, H. E. (2009). Prevalence and epidemiologic characteristics of FASD from various research methods with an emphasis on recent in-school studies. *Developmental Disabilities Research Review*, *15*(3), 176–192. doi:10.1002/ddrr.68

Moore, C., Jones, J., Lewis, D., & Buchi, K. (2003). Prevalence of fatty acid ethyl esters in meconium specimens. *Clinical Chemistry*, *49*(1), 133–136.

Mueller, G. C., Fleming, M. F., LeMahieu, M. A., Lybrand, G. S., & Barry, K. J. (1988). Synthesis of phosphatidylethanol—A potential marker for adult males at risk for alcoholism. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(24), 9778–9782.

Nalesso, A., Viel, G., Cecchetto, G., Mioni, D., Pessa, G., Favretto, D., & Ferrara, S. D. (2011). Quantitative profiling of phosphatidylethanol molecular species in human blood by liquid chromatography high resolution mass spectrometry. *Journal of Chromatography A*, *1218*(46), 8423–8431. doi:10.1016/j.chroma.2011.09.068

Spagnolo, A. (1993). Teratogenesis of alcohol. *Annali dell Istituto Superiore di Sanita*, *29*(1), 89–96.

Stewart, S. H., Law, T. L., Randall, P. K., & Newman, R. (2010). Phosphatidylethanol and alcohol consumption in reproductive age women. *Alcoholism: Clinical and Experimental Research*, *34*(3), 488–492. doi:10.1111/j.1530-0277.2009.01113.x

Substance Abuse and Mental Health Services Administration. (2013). *Results from the 2012 national survey on drug use and health: Summary of national findings* (NSDUH Series H-46, HHS Publication No. (SMA) 13-4795). Rockville, MD: Author.

Therrell, B. L., Jr., Hannon, W. H., Bailey, D. B., Jr., Goldman, E. B., Monaco, J., Norgaard-Pedersen, B., . . . Howell, R. R. (2011). Committee report: Considerations and recommendations for national guidance regarding the retention and use of residual dried blood spot specimens after newborn screening. *Genetics in Medicine*, *13*(7), 621–624.

Varga, A., Hansson, P., Lundqvist, C., & Alling, C. (1998). Phosphatidylethanol in blood as a marker of ethanol consumption in healthy volunteers: Comparison with other markers. *Alcoholism: Clinical and Experimental Research*, *22*(8), 1832–1837.

Wurst, F. M., Thon, N., Aradottir, S., Hartmann, S., Wiesbeck, G. A., Lesch, O., . . . Alling, C. (2010). Phosphatidylethanol: Normalization during detoxification, gender aspects and correlation with other biomarkers and self-reports. *Addiction Biology*, *15*(1), 88–95. doi:10.1111/j.1369-1600.2009.00185.x