Author’s response to reviews

Title: Eliciting parental support for the use of newborn blood spots for pediatric research

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Author’s response to reviews: see over
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Giulia Mangiameli
Executive Editor
BMC Medical Research Methodology

Dear Editor Mangiameli:

Enclosed is our revised manuscript titled “Eliciting parental support for the use of newborn blood spots for pediatric research” for consideration for publication as a research article in BMC Medical Research Methodology. We thank the three peer reviewers for their comments in helping to improve our manuscript. Below are point-by-point responses to the comments that were provided.

This manuscript has not been published except in abstract and poster form to the American College of Epidemiology Annual meeting, Silver Spring, MD, Sept 8, 2014. It is not being considered for publication elsewhere. All authors have approved the manuscript and have no conflicts of interest to declare.

Sincerely,

Edwina Yeung, PhD, ScM
Point-by-point responses are included below. Reviewer comments have been **bolded** for distinction.

**Reviewer:** William Siero

**Reviewer’s report:**

This paper reports on the parental consent for research use of routinely collected dried blood spots and explores the factors associated with consent and sample volume sufficiency. Dried blood spots and other routinely collected samples represent a significant cost effective opportunity for research. The paper is well written and deserving of publication pending the following minor revisions.

We thank Dr. Siero for his time on reviewing the manuscript and positive feedback on our work.

**Minor revisions:**

**Background:**

• **P5.67**, DBS can be used for genetic and epigenetic analyses. These should be listed as a possible utility to strengthen the importance of the paper.

We agree and have added a sentence on this in our opening introductory paragraph: “Advances have also made it possible for their use in genetic [2] and epigenetic analyses.[3]”

• **P5.74**, Countries and US states screen a varying number of disorders. Please clarify this wording “…conditions screened for, from 1 to 54 inclusive….” to outline this.

We have now reworded to, “Technologic advances have expanded the number of metabolic, endocrine and other conditions screened for, but vary by number in each U.S. State and across different countries.”

• **P5.76**, The researchers may wish to note that DBS are used internationally in many research projects.

We have now edited this sentence to say: “…in all 50 US states and elsewhere across the globe…”. And on Page 14 we added: “Many international research studies have also turned to using DBS due to its minimal invasiveness and feasibility without relying on trained phlebotomists.[37]”

• The researchers would be worthwhile noting research from Australia on written consent for use of Guthrie cards in research and that 93.5% of parents **WHOSE** newborns are screened (>99% of all births) consent for research use of their Guthrie card. J Paediatr Child Health. 2014 May;50(5):399-404. doi: 10.1111/jpc.12484. Epub 2013 Dec 23.

We thank the reviewer for this reference! We have added it into our discussion section (page 13): However, the acceptability of anonymous research remains high. A new consent protocol was introduced in Victoria, Australia which asked parents’ permission to use remaining newborn screening samples in de-identified health research.[31] For over 77,000 newborns, only 6.5% of parents declined the use of their child’s residual DBS in secondary research and another 1.4% did not respond.[31]

**Results:**

• Please report details of participants included (details included in figure 2) in results section rather than Methods.
We felt the number of households included in the Upstate KIDS study in total (n=5034) was better left in the methods section since that describes how the study was established and referenced our baseline study paper. However, we have now repeated this number and the details of participants included per figure 2 at the beginning of the results section.

- Please report the study attrition of the 8 month survey materials “Analyses were restricted to families who returned 8 month survey materials as they expressed continued response to the overall study and differences in factors by consent status identified in this group would not be a function of factors related to overall response rates.”

We have now clarified that out of the 5,034 households originally recruited (page 6) 3696 (73%) returned 8-month materials. “Analyses were restricted to families who returned 8-month survey materials (n=3696, 73%)...”

Discussion:
- The authors state the analyses are “restrict to families that were reached,” however, there is no evidence presented that those who did not respond to were not reached. The researchers should note that the lack of interaction with the families may be regarded as a weakness of the study and that a non-response may not necessarily represent a “no” for use of their DBS.

We apologize if the statement was unclear. The quoted statement above was in reference to the study by Linabery et al. Nevertheless, we now acknowledge (page13): However, one weakness of our study was that we did not actively reach out to parents with missing DBS consents to confirm their opinions of DBS use.

- There is little discussion of the limitations of the study and specifically the study sample. The study utilized in the analyses is a specialised one (infertility) and may or may not be broadly representative of other population based studies. The authors should outline what the total births over the study enrolment period were, original recruitment rate for the study was and report the attrition rate. The implications of these and other participant characteristics should on the generalizability of the findings should be discussed.

We mentioned that we had a predominantly white cohort and that the New York State (excluding NYC) experience may not be generalizable to all states. We have now added to this dialogue these additional points raised by the reviewer:

Our findings may also not be broadly generalizable to all population based studies due to low initial response rate for participating in the study (22%) [16] and the attrition (27%) experienced at the time of DBS consent.

With regards to the difference in participant characteristics, our baseline paper as referenced [16] (Buck Louis et al. Paediatr Perinat Epidemiol 2014; 28(3): 191-202.) already published the comparisons and identified what many other studies have found, that non-Hispanic whites and higher SES mothers were more likely to participate. We have now noted these observations in the methods section in reference to the original sampling (page 7):

We previously compared the characteristics of non-participants (n=13,490) against participants finding that non-Hispanic white race and higher socioeconomic status (i.e., education, private insurance) to be associated with participation.[16]

Discretionary Revisions and general comments:
Methods:
• If data is available, in addition to the education, other measures of socio-economic status should be included.

Unfortunately, we do not currently have other measures apart from education, insurance status and infertility treatment (which is a strong SES factor). In the future, we hope to link the data with publically available GIS information on SES characteristics for where the participants live (e.g. poverty levels, urban vs rural).

Results:
• As the study is arguing that DBS are feasible for population-based research it would be informative to know how representative the Upstate KIDS study is.

As mentioned above, we already published these comparisons in our baseline paper and have now highlighted our findings on page 7.

• The researchers report the relationship between consent and nulliparous women. It would be interesting to see if there was a relationship between parity and consent rate.

We are unsure what the reviewer means by this comment. The parous women were more likely to consent (as they would be the opposite of the nulliparous group).

Discussion:
• While technically a response rate of 62% is a majority. Such a low response rate from already engaged participants is perhaps not reassuring. Such a response rate may be due to not attempting the consent either over the phone or in a face-to-face manner. The authors should consider discussing the role of approach type (mail only) on response rate.

We agree that face-to-face contact might have resulted in higher response. We have also added this observation. However, written consent was required by the IRB.
Reviewer: William Funk

Reviewer’s report:
This manuscript evaluates the utility and feasibility of retrieving archived dried blood spot (DBS) samples to support epidemiological research. The paper is very timely given increasing interest in using DBS for assessing environmental risk factors for chronic diseases and cancers. Because newborn DBS are collected by all states in the US, and because they capture early life exposures that occur during critical periods of human development, archived DBS collected for routine newborn screening represents a valuable resource. In addition, recent improvements in analytical methods now permit quantification of toxicant in very small volumes of blood, which has led to the development of many new assays for assessing exposures in DBS that were not possible previously. Consequently, evaluating the challenges and logistics associated with retrieving archived newborn DBS samples is highly important. The study was well designed, and the paper is well written. However, I do have suggestions for minor revisions.

We thank Dr. Funk for his review of the manuscript and the useful feedback. We hope to have addressed his concerns.

1. The paper discusses two distinct challenges associated with using archived newborn DBS samples in epidemiological research. The first is the feasibility of getting the samples (i.e., parental consent), and the second is what can be measured once the samples are obtained. While the paper is largely focused on the first of these challenges, it is noted that a panel of targeted analytes were measured in the samples, including cytokines and environmental chemicals. However, results from these analyses are not presented.

We thank the reviewer for the insight. We recently published a paper (Yeung et al. Paediatr Perinat Epidemiol. 2015 Jul;29(4):317-25.) regarding the Obesity panel of DBS adipokines in relation to birth size measures in Upstate KIDS. We have now added this recent publication (which had not been accepted prior to our initial manuscript submission) on page 7, stating: More specifically, DBS adipokines as measured by Upstate KIDS was associated with birth size and gestational age in similar fashion as results from cord blood measures by other studies.[26]

2. On line 116 the authors state that they “aimed to assess these biomarkers of in utero infection or placental transfer of environmental chemicals in relation to adverse birth outcomes and early childhood development”. This statement is misleading since they did not present any biomarker results, nor did they evaluate any health endpoints.

We tried to clarify and revised this sentence to emphasize that this was the rationale for collecting the samples rather than the current aims of the paper: We asked for permission to assess these biomarkers of in utero infection or placental transfer of environmental chemicals for future work in assessing their relation to adverse birth outcomes[17,18] and early childhood development.[19-21]

3. While the paper did a very nice job of evaluating the feasibility of obtaining archived DBS samples, caution is needed not overstate what is being reported in the current study.

We were unsure which specific section this comment refers. We have now added limitations of our work regarding generalizability from the previous reviewer. We would be happy to revise the specific section(s) that the reviewer found to be overstated.

4. It was noted that most of the retrieved samples had adequate blood to perform the laboratory analyses. However, it would have been useful to report more information on the quality of the
samples. For example, what was the estimated blood volume remaining in the archived samples? Was there evidence of smearing, serum rings, blood applied on top of blood, and other sample quality issues that can affect lab results?

The following has now been added to page 9:

We punched areas of the DBS that had no obvious signs of double spotting in that DBSs were reasonably symmetrical and the blood spot area saturated the spot based on the redness of the opposite side of the Guthrie card. Harvested DBSs had no obvious signs of smearing or a plasma ring. The amount of eluted sample remaining was usually minimal to none for most analytes. Each DBS circle was equivalent to approximately 50 ul of newborn blood. The 3.2 mm punches for biomarker analyses were equivalent to approximately 3.275 µl with 30 µl buffer per punch.

**Overall this paper is of high quality and represents an important contribution to the field.**

We thank the reviewer for his enthusiasm for our work.
Reviewer: Peter Burgard

Reviewer's report:

The manuscript 'Eliciting parental support for the use of newborn blood spots for pediatric research' by Edwina Yeung et al. addresses two important questions. First, how population based data concerning epidemiological data can be retrieved from DBS can be used for other purposes than newborn screening, and second, the attitude (possible consent) towards exploitation of available DBS specimen. From a methodological point of view the study is well designed, particularly the report of absolute risk reduction in addition to odds ratios deserves appreciation. The items of the STROBE statement to evaluate observational studies are well addressed and document the accuracy of the approach. Both questions have technical as well as ethical facets, which are dealt with in a paradigmatic way. The manuscript is well drafted, however, I would like to give some comments how the message could be improved.

We thank Dr. Burgard for his review and for the useful feedback on our paper.

- Minor Essential Revisions

1. Although the sample size if large, it can be regarded as a selected sample drawn from families which have already shown that they are willing to participate in research, i.e. the KIDS study, or have had some previous – possibly beneficial - contact with the medical system, e.g. a stay of their child in a NICU. This should be discussed as a possible bias.

   We had mentioned that “Consent may differ... if parents were not part of an ongoing cohort study.” We have now elaborated with the points made above (page 15): “As participants were self-selected into the parent study, they may have had a more positive view of health research or might have also wanted additional contact with the medical system which could bias them towards consenting.”

2. The generalizability question raised by the authors themselves needs more discussion. First, I wonder whether the fact that consent rates support the findings from another study (ref. #34) can be regarded as an argument for generalizability. Second, the authors stress that fact that the consent has been asked explicitly for the measurement of cytokines and environmental chemicals, and should be extended to a discussion of the use for other research questions, particularly for pilot studies for other potential disorder to be tested for newborn screening.

   We have now also added some limitations raised by another reviewer regarding generalizability. Our findings may not be broadly generalizable to all population based studies due to low initial response rate for participating in the study (22%) [16] and the attrition (27%) experienced at the time of DBS consent.

   To clarify, the reference to #34 was less for generalizability which we already acknowledged earlier in that paragraph to be limited to NYS but to demonstrate similar feasibility.

   We have edited to read: Consent may differ if we had not explicitly excluded genetic or pharmacologic tests on the consent forms, described pilot tests for detecting new disorders as part of newborn screening, or if parents were not part of an ongoing cohort study.

3. Page 8: It should be explained why the technical aspects of the pulling and investigation of sample cards is important. The authors report that a total of 800 hours or six months were necessary for a second tier analysis, what is a considerable amount of time.
We have now added the rationale for publishing this information to our concluding paragraph: We presented the technical efforts used for such an undertaking so that future researchers can plan for the necessary resources required.

4. The ethical aspects of the approach deserve some more discussion, e.g. referring the pertinent publication by Ross, Lainie Friedman. "Mandatory versus voluntary consent for newborn screening?." Kennedy Institute of Ethics Journal 20.4 (2010): 299-328.

We added this reference but viewed that elaborating further on the ethical debate is outside the scope of our work as we did not ask specific questions of parents about their ethical views on the issue.

Policies on using DBS vary widely and multiple international organizations wrestle with their ethical use.[11,28-31] Debate continues whether all uses (even when de-identified) should require parental consent.[31]

5. A reference to the PCORI project also could increase the impact of the study, emphasizing the interest of patients and the general population in research strategies (e.g. Selby, Joseph V., Laura Forsythe, and Harold C. Sox. "Stakeholder-Driven Comparative Effectiveness Research: An Update From PCORI." JAMA (2015): 2235-2236.)

We agree and have incorporated this reference to PCORI in our discussion (page 14): Given the importance of involving stakeholders in research, as exemplified by the establishment of the Patient-Centered Outcomes Research Institute (PCORI) [34], such collaborations will likely increase in the future. Bringing together parents, departments of health and research institutions could assist with establishing neonatal research priorities.

References # 43 and 44 are identical, and one should be discarded.

We apologize for the duplicates. We are unsure what happened and have added more references and checked that none are duplicated.