1958 Birth Cohort Biosample Strategy Guidelines
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Naveed Sattar and Paul Welsh
University of Glasgow

Helen Colhoun
University of Dundee

Susan Ring
University of Bristol
Objective of the scientific strategy guidelines

The 1958 Birth Cohort is a unique and powerful longitudinal epidemiological study, with tissue samples stored in biobanks, which will allow further biomarker and epidemiological work. Available tissue includes saliva, plasma and serum samples which are described in detail in Appendix 1. DNA and lymphoblastoid cell lines are also available from cohort members but are not covered by this document. Use of the samples is covered by Research Tissue Bank Ethical Approval (09/H1010/12) and requests to use the material are assessed by the Access Committee for CLS Cohorts (ACCC) (see http://www2.le.ac.uk/projects/birthcohort). The tissue samples are a finite resource and the ethical approval requires that requests to access the material is subject to peer review. The material was collected during the Biomedical Sweep in 2003 and is the remains of samples analysed at that time. There has been little interest in the samples until late 2012 when the ACCC started to receive requests to access the material.

The objective of this document is to facilitate access to the 1958 stored tissue samples so that they get the widest possible usage while ensuring that scientific rigour is applied in selecting proposals that will yield data which are i) reliable ii) epidemiologically or clinically informative iii) novel. As such, applications will be considered in light of the cohort design; successful proposal should maximise the epidemiological strengths of the cohort, whilst also recognising limitations of the biobank (in terms of blood draw protocols, processing, storage, and sample availability).

This document provides a framework for addressing and determining the scientific rationale for access issues for biomarker work. This document does not prescribe rigid criteria because it is impossible to predict the nature of access requests or long-term trends in scientific interest. This document, whilst not exhaustive, sets a framework for making relevant decisions, giving some relevant examples where appropriate. This document has been developed to reflect current best practice and will be reviewed regularly to ensure it remains in line with current guidelines. The strategy also needs to reflect current funder policy and the ACCC will consult/update funders if there are any proposed changes to the strategy.
1. Use of the samples should be specifically relevant to the 1958 study

Applications to use 1958 samples should clearly demonstrate that the proposed study will make use of longitudinal data and cannot be carried out in samples obtained from another source. All data generated from samples will be returned to the 1958 cohort and made available to other users. Samples will only be issued under the terms of a material transfer agreement which includes the statement:

“It is a condition of access to the samples that information obtained from the samples (including any derived data, for example, derived haplotypes or the results of bioassays) is submitted to the University of Bristol for inclusion in the central 1958BC database. All genotypes, and all bioassay results that are important enough to be used in a publication must be returned to the 1958BC database.”

Recipients will also be required to return or destroy any unused material at the end of the project as requested by the ACCC under the terms of the material transfer agreement.

2. Scientific strength of the proposal, and potential impact

Critically, one must always ask whether a particular biomarker to be measured will answer a relevant and meaningful question. Using longitudinal studies as a cross-sectional resource is rarely impactful (aside from Mendelian Randomisation studies). Further, use of longitudinal data to investigate associations (hazard ratios, or risk ratios) must be justified on the grounds of potential clinical (or social) relevance. Which questions are generally meaningful in biomarker studies?

i) Clinical questions which might change the guidelines for clinicians, or give a clear public health message. Examples could include:
   a. Disease diagnosis e.g. HbA1c for diabetes or LFTs for NAFLD
   b. Vitamin D status in pregnant women and BMD in their children

ii) Clinical or social questions which might risk stratify patients e.g.
   a. Does NT-proBNP add informative to existing CVD risk scores?
   b. Do novel biomarkers improve prediction of clinical or social outcomes beyond established predictors

iii) Disease pathogenesis. Observational studies tend to be poor in investigating causality, even where impressive multivariable adjustment models are built. Wherever possible, proposals of this nature should consider whether a robust approach to causal identification can be applied, for example including whether the DNA resource can be combined with the proposal to use a Mendelian randomisation approach (assuming valid genetic instrumental variables are known and measured):
a. Do natriuretic peptides protect against diabetes?

iv) **Stratifying patients for therapy** based on phenotypes. Does a particular biomarker predict better or worse response to particular therapies?

3. **Novelty of the scientific aims**

Often the proposals with the most obvious and immediate scientific rigour will be the least novel studies; several cohorts may have conducted similar studies before. As such the balance between a proposal’s strength (in terms of potential impact) and its novelty (which studies have measured the biomarker and related measures to outcomes before) is a key factor. If a proposal to measure a novel biomarker with little previous literature is interesting and potentially impactful, this must be considered in light of what is known regarding the biomarker (points below). Often, if a biomarker is particularly novel, a small pilot study may be useful prior to committing samples from the bioresource.

4. **Biomarker characteristics; pre-analytical variables**

Given the scarce nature of the bioresource, pre-analytical considerations as to whether a biomarker can be measured to give reliable results in the 1958 tissue samples are a key consideration (specific details for each sample type are provided in Appendix 1):

i) **Sample processing:** The 1958 blood samples were sent by post. The time spent with serum/plasma in contact with cells will have a significant impact on some biomarkers, but not others. Platelets release inflammatory factors, cells metabolise others, and the time spent at room temperature may adversely affect labile proteins. As such it should be noted that UK biobank have investigated pre-analytical characteristics of several of the more common biomarkers:

a. Glucose requires fast separation and assay to be conducted on first thaw.

b. **C-reactive protein (CRP)** is extremely robust to pre-analytical variables.

c. Limited existing data suggest metabolomics analysis may not be appropriate in samples not rapidly separated or at least within 24 hours

Given this, proposals must make it clear, with robust data to support the proposal, that the biomarkers to be measured will be reliably measured using the 1958 samples. This could be demonstrated with a pilot study, or published data, showing that sample processing time has no impact on the biomarker, or at least has a highly predictable effect (Passing-Blok regression, Bland-Altman plots etc). Pilots are always helpful before committing considerable time and money on novel biomarkers.
ii) Freeze-thaw: The EDTA samples have not been previously thawed, whereas the citrate has. Many immunoassays, which measure based on antigenic structure rather than protein activity, are very robust to freeze thaw. This is likely to be the case for most biomarkers that are relatively unaffected by the sample processing time. Nonetheless, in supporting a proposal, data on the impact of freeze-thaw on a biomarker would be useful. In order to maximise use of the resource, it should be considered whether a previously thawed aliquot would be more appropriate to use (where possible) for a biomarker known to be robust to freeze-thaw.

iii) Sample type: There is more EDTA available than serum or citrate. The remaining serum aliquot is therefore important. Therefore, biomarkers which can be measured on EDTA should be in order to save the scarce serum resource for outstanding proposals. Very few non-haematological biomarkers are routinely measured in citrated plasma samples.

iv) Sample stability: All blood samples are stored at ≤-70°C, so this issue is of limited relevance for biomarkers in the 1958 study.

5. Assay test platform

Assays should, where possible, be carried out using gold standard automated methods. In order of preference;

i) On an automated clinical chemistry/immunoassay platform in an accredited NHS laboratory, or a lab that participates in external quality assurance schemes for that assay

ii) On an automated platform in a laboratory using manufacturer recommended or internal quality control material

iii) Using single-plex assays such as ELISAs

iv) Using multiplex immunoassays

This list is intended as broad guidance, and there will be other potential assay methodologies. The gold standard for measuring vitamin D (25OHD2 and D3) is liquid chromatography tandem mass spectroscopy. Many aspects of this assay can be automated and carried out in NHS labs.

There is a broad trend towards use of multiplex assays to make optimal use of bioresources in epidemiology. Our own experience suggests that this technology should be used with caution. We have experience with Luminex (magnetic beads), Randox (bio-chips) and MSD (Multi-spot ELISA with electrochemiluminscence reporter) platforms. We have found:

i) Extra information comes at the cost of vastly reduced sensitivity and precision (higher CVs).
ii) Luminex beads system is rather sub-optimal for human blood samples; the beads tend to clog together making the assay method difficult/impossible to carry out within manufacturer recommended tolerances.

iii) The assay panel in multiplex assays are often of limited incremental value. Assaying C Reactive Protein (CRP) and Interleukin 6 (IL-6) in a study may be useful, but the incremental value of a dozen other cytokines may be limited or lack cost benefit, particularly when a majority are below the limit of sensitivity, or have limited or uncertain biological relevance. NB: multiplex assays often lead to reduced sensitivity for some tests and tend to lower CVs. Furthermore, where assay perform better e.g. MSD platform, there may be issues with respect to external

iv) Comparisons of data since some assays give results which are not externally comparable to values obtained by gold-standard methodologies, thereby required a conversion or “fiddle” factor.

Given the above, any proposal should be able to demonstrate that the assay they propose is sensitive enough to detect a signal (<20% CV as absolute and more desirable <10%) in a majority of the samples (commensurate with the aims). Ideally the platform/manufacturer used should be established in the literature to maximise the potential impact of the results, and minimise potential referee criticisms.

6. Assay test characteristics

This is a practical consideration, once a strong scientific case for a biomarker has been made in a proposal. An automated assay will have a dead volume (often ~200uL). For all assays the volume of sample consumed by the assay should also be considered in light of the potential impact of the study. If an EDTA sample has been previously thawed, the repeated use of this sample for other assays should be considered. If the volume remaining is too small for an automated assay it may remain sufficient for use in an ELISA assay by manual pipetting by a technician.

Often, multiple tests can be run on the same sample in automated platforms thereby maximising efficiency.

7. Global Discovery Versus Specific Hypothesis

All the above refers to specific tests of hypotheses; an alternative approach would be to reserve part of the resource for a more global discovery approach; specifically, it would be of interest across a wide range of disease states and phenotypes to acquire as much data as possible on the lipidome,
proteome and metabolome from high dimensional methods. For consideration might be mass spectroscopy (often semi-quantitative) and Nuclear magnetic resonance (NMR) based methods for quantitation of many small molecular weight metabolites and some peptides and proteins. Most experts in the field suggest if sufficient volume is available, the best approach for metabolomics is a combination of mass spectroscopy and NMR. Also for consideration are antibody-based arrays for high dimensional protein quantitation. Other methods to consider include proximal ligation assays for proteins, NMR based methods and mass spectroscopy methods for molecular species lipid analyses etc. Also one might consider serum micro RNAs worth detecting and quantifying.

Many of these approaches require relatively little volume (e.g. at least 600 serum metabolites can be detected and quantified with 120 ul, whereas other Mass spectroscopy platforms can yield potentially more than 1000 metabolites on 20 ul serum). However what is also true is that for many of the available platforms there is a surprising dearth of good data on the within person repeatability over short periods of time, the test re-test repeatability, pre-analytic effects on sensitivity and specificity and so on i.e. basic QC. For protein arrays etc, sensitivities may be particularly important to check since for some specific measurements high sensitivity single-plex ELISAs are employed (e.g. IL-6 in cohort studies) since conventional assays (and potentially arrays) cannot reliably pick up such low levels. Therefore before committing such a precious resource to any of these platforms careful consideration and possibly some pilot studies with less valuable samples are to be recommended. Furthermore, for some of these techniques, the statistical analyses can be very complex and in some cases, the best bioinformatics approach to analyse data, in particular data generated from mass spectroscopy, remains unclear. Finally, in all cases, whilst new techniques allow discovery science, the linkage of any measurements to pre-defined outcomes or to answer specific questions on disease pathology will help focus analyses.
SUMMARY

The 1958 tissue samples are a valuable resource but there are limitations regarding their suitability for some assays due to the sample processing history. Recommendations for ACCC for approving use of the samples are:

- Scientific strength of the proposal must justify use of 1958 cohort samples.
- Evidence must be provided to show methodology is appropriate given the processing history of the samples. Eg. Evidence from published literature or pilot data generated on samples processed in a similar manner.
- The assay test platform should have proven quality assurance measures in place.
- The methodology should include measures to ensure the quality of any remaining sample is not jeopardised and can be used in further assays which can be used on freeze thawed samples.
- At least one aliquot of each sample type should be reserved for future global discovery projects.

ACRONYMS

ACCC  Access Committee for CLS Cohorts
CRP  C-reactive protein
EDTA  Ethylenediaminetetraacetic acid
ELISA  Enzyme-linked immunosorbent assay
HbA1c  glycosylated hemoglobin \((\text{hemoglobin A1c})\)
IL-6  Interleukin 6
MSD  Meso Scale Discovery
NAFLD  Non-alcoholic fatty liver disease
NMR  Nuclear magnetic resonance
NT-proBNP  N-terminal prohormone of brain natriuretic peptide
### APPENDIX 1 - 1958 Birth Cohort Biological Sample Information

Table 1 – Summary of Samples collected and assays included in biomedical sweep from September 2002 to March 2004

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Early Morning Saliva (Sarstedt salivettes)</th>
<th>Late morning Saliva (Sarstedt salivettes)</th>
<th>Citrated Plasma Residue</th>
<th>Plain serum residue</th>
<th>EDTA Plasma (citrate-phosphate-dextrose-adrenaline)</th>
<th>CPDA Plasma (citrate-phosphate-dextrose-adrenaline)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maximum number of aliquots remaining</strong></td>
<td>1 (varying volumes)</td>
<td>1 (varying volumes)</td>
<td>1 (varying volumes)</td>
<td>1 (varying volumes)</td>
<td>Up to 6 x 500 µl * + 1 varying volume</td>
<td>Up to 6 x 500 µl ** + 1 varying volume</td>
</tr>
<tr>
<td><strong>Number of cases with at least 1 500µl sample remaining</strong></td>
<td>6618</td>
<td>6618</td>
<td>7597</td>
<td>6400</td>
<td>8063</td>
<td>7848</td>
</tr>
<tr>
<td><strong>Processing protocol</strong></td>
<td>Transported by post at ambient temp. Frozen - at -80 °C in temporary storage, Shipped at ambient temperature to Germany for analysis. Refrozen on arrival. No information regarding how samples were shipped back but currently stored at -80 °C.</td>
<td>Transported by post at ambient temp. Frozen - at -80 °C in temporary storage, Shipped at ambient temperature to Germany for analysis. Refrozen on arrival. No information regarding how samples were shipped back but currently stored at -80 °C.</td>
<td>Shipped by post at ambient temp. 0.5ml of whole blood removed for analysis of glycosylated haemoglobin, Remainder centrifuged, aliquots frozen at -70 °C, transported frozen to Glasgow Royal Infirmary for analysis. Residue retained at -80 °C</td>
<td>Shipped by post at ambient temp. Centrifuged and the supernatant serum used for analysis in Newcastle. Residue retained at -80 °C</td>
<td>Shipped by post at ambient temp. Centrifuged and supernatant plasma stored in 0.5ml individually barcoded aliquots at -80 °C.</td>
<td>Specific blood tube for production of lymphoblastoid cell lines. Shipped to Bristol by post at ambient temp. Centrifuged and plasma removed. Peripheral blood lymphocytes separated on a Ficoll gradient and cryopreserved for subsequent transformation into immortalised cell cultures. The supernatant</td>
</tr>
</tbody>
</table>
plasma was sent to St George’s Hospital Medical School (SGHMS) for aliquoting into 0.5ml individually barcoded tubes which were frozen at -80°C for long-term storage.

<table>
<thead>
<tr>
<th>Processing Location</th>
<th>St George’s Hospital Medical School then Germany</th>
<th>St George’s Hospital Medical School then Germany</th>
<th>Royal Victoria Infirmary, Newcastle</th>
<th>Royal Victoria Infirmary, Newcastle</th>
<th>St George’s Hospital Medical School</th>
<th>ALSPAC, University of Bristol then St George’s</th>
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</thead>
<tbody>
<tr>
<td>Days from taking sample to arrival in lab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Time to reach ALSPAC</td>
</tr>
<tr>
<td></td>
<td>1 day 18.9%</td>
<td>2 days 47.1%</td>
<td>3 days 24.2%</td>
<td>4 days 7.2%</td>
<td>5 days 1.5%</td>
<td>&gt;5 days 1.0%</td>
</tr>
<tr>
<td></td>
<td>1 day 18.9%</td>
<td>2 days 47.1%</td>
<td>3 days 24.2%</td>
<td>4 days 7.2%</td>
<td>5 days 1.5%</td>
<td>&gt;5 days 1.0%</td>
</tr>
<tr>
<td>Existing assays</td>
<td>Cortisol</td>
<td>Cortisol</td>
<td>glycosylated haemoglobin fibrinogen, tissue plasminogen activator, von Willebrand factor, C-reactive protein.</td>
<td>triglycerides, total and HDL cholesterol, total and allergen-specific immunoglobulin E, insulin-like growth factor 1</td>
<td>DNA</td>
<td>Lymphoblastoid cell lines</td>
</tr>
<tr>
<td>Current location</td>
<td>ALSPAC</td>
<td>UK Biobank</td>
<td>ALSPAC</td>
<td>UK Biobank</td>
<td>ALSPAC and UK Biobank</td>
<td>ALSPAC and UK Biobank</td>
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* See table 2 for more details

** See table 3 for more details
Table 2 – EDTA Plasma - Further details of number of 500 µl aliquots

<table>
<thead>
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<th>Number of 500µl aliquots available</th>
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<tr>
<td>6</td>
<td>5110</td>
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<tr>
<td>5</td>
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<tr>
<td>4</td>
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<tr>
<td>3</td>
<td>122</td>
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<tr>
<td>2</td>
<td>54</td>
</tr>
<tr>
<td>1</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 3 – CPDA Plasma - Further details of number of 500 µl aliquots

<table>
<thead>
<tr>
<th>number of 500µl aliquots available</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>7137</td>
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<tr>
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<td>2</td>
<td>65</td>
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<td>1</td>
<td>59</td>
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