Collecting saliva samples for DNA extraction from children and parents: findings from a pilot study using lay interviewers in the UK

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Abstract

In recent years there has been a substantial increase in the collection of biological data on social surveys. Biological data has hitherto been primarily collected by medically trained personnel in a clinic or laboratory setting or using specialist nurse interviewers in a home-visit setting. However, improvements in technology and the development of minimally or non- invasive data collection methods have made it increasingly feasible to collect bio-measures in a home setting using non-medically trained lay interviewers. In the field of genetic research, it has become increasingly common to collect DNA from saliva samples. This paper provides an account of a pilot study investigating the feasibility of collecting saliva samples for DNA extraction from mothers, fathers and children aged around 11 years old using lay interviewers on the UK Millennium Cohort Study. The pilot study was carried out in 2011 in five areas of the UK with one interviewer in each area. 45 families took part in the pilot and saliva samples were obtained from 73 per cent of mothers, 76 per cent of fathers and 74 per cent of children. We demonstrate that it is indeed viable to collect saliva samples for DNA extraction from children and parents using lay interviewers in a home setting, and provide practical suggestions about how the data collection process could be improved in order to achieve higher response rates and improved specimen quality. Our findings are relevant to other surveys planning to incorporate saliva sample collection for DNA extraction, particularly for those involving lay interviewers in a home setting.

Keywords

bio-measures, DNA, interviewer training, longitudinal, Millennium Cohort Study, Saliva samples

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University of Bristol, who processed and analysed the samples.

Introduction

In recent years there has been a substantial increase in the collection of biological data on social surveys. This has been driven by growing scientific interest in the interplay between social and biological factors in explaining human behaviour (Hobcraft, 2007; Kumari et al., 2006) and facilitated by technological developments and the development of minimally or non-invasive data collection methods which have greatly improved the feasibility of collecting such data, particularly in large-scale, population-based surveys using non-medically trained interviewers (Lindau and McDade, 2007).

In the field of genetic research, it has become increasingly common to collect DNA from saliva samples. Saliva sample collection is straightforward and non-invasive and does not require medically trained personnel; it can be easily carried out by lay interviewers or by respondents themselves. As the samples are stable at room temperature, they can be mailed and processed in batches, reducing postage and laboratory costs compared with other collection methods. As it is non-invasive, co-operation rates are often higher than for blood samples taken using venipuncture, which can only be carried out by phlebotomists or other medically trained personnel.

This paper provides an account of a small-scale pilot study investigating the feasibility of collecting saliva samples for DNA extraction using lay interviewers in a home setting. The pilot study formed part of the development work for the fifth wave of the Millennium Cohort Study (MCS), a large, nationally representative, multi-disciplinary longitudinal study of a cohort of children born in 2000-2001 who have been followed over time. It provides new insights about the practicalities of collecting saliva from using lay interviewers in a home setting.

Experience from other surveys

Many cross-sectional and longitudinal surveys internationally collect physical measurements such as height, weight, infant head circumference, waist, hip and arm measurements, grip strength and walking speed. Some examples include the Health Survey for England, English Longitudinal Study of Ageing (ELSA) and the US Health and Retirement Study (HRS). It is well-established that, with appropriate training, field interviewers are able to carry out these measurements in a home setting. The collection of medical measurements such as blood pressure, ECG, lung function and biological samples such as blood, saliva, urine, teeth and hair, has typically been carried out either by personnel trained to take blood in a research clinic setting, such as in the Avon Longitudinal Study of Parents and Children, or by nurse interviewers in a home-visit setting, as in the Health Survey for England, the UK National Diet and Nutrition Survey, the 1958 British Cohort Study (National Child Development Study) and *Understanding Society:* the UK Household Longitudinal Study (UKHLS).

Nurse or clinic visits are often used as follow-up to an interviewer visit. However, a major drawback of this approach is that there are usually high drop-off rates. Clemens, Given and

Purdon (2012) looked at the response rates to the follow-up nurse and clinic visits on a range of surveys carried out by the National Centre for Social Research, a UK survey research agency and showed that this ranged from 65 per cent (on UKHLS) to 86 per cent (on ELSA) of those who completed an interview. They also showed that follow-up clinic visits can suffer from even higher rates of drop-out. For example on the Diet and Nutrition Survey of Infants and Young children, only 44 per cent of those interviewed took part in a clinic visit. Another major drawback of clinic and nurse visits is that, compared with interviewer visits, they tend to be more expensive and can add significantly to survey costs, particularly if they are additional to the interviewer visit.

Increasingly, therefore, lay interviewers in countries including the UK, Germany and the US are beginning to carry out medical measurements, such as blood pressure and collect biological samples using non-invasive methods, such as dried blood spots and saliva; typically yielding compliance rates over 90% (Clemens, Given and Purdon, 2012; McFall, Conolly and Burton, 2012; Schonlau et al., 2010; Jaszczak, Lundeen and Smith, 2009; Erickson and Mierzwa, 2012; Guyer and Ofstedal, 2012). ALSPAC and NCDS in the UK and the Wisconsin Longitudinal Study in the US have also included self-administered saliva sample collection which is mailed back by respondents.

To our knowledge, however, the only example of a major longitudinal study which has included the collection of saliva samples for DNA from children in a home setting is the Fragile Families and Child Well-Being Study in the US. Field interviewers collected saliva samples from both children and their mothers during the home visit for the age 9 follow-up wave of data collection, and achieved collection rates of 86 per cent of children and 80 per cent of biological mothers who took part overall. .

The Millennium Cohort Study proposed to collect saliva for DNA extraction from children and both their biological parents, where co-resident, at the age 11 survey. There are particular benefits from collecting DNA from parents as well as children in terms of exploring genetic inheritance and in epigenetic research, which the study aimed to exploit by seeking funding to provide DNA from saliva as a resource to the research community.

Design and methods

Survey context

The MCS is a birth cohort study following over 19,000 UK children born in 2000/1. The data collection for the study takes place in the home and involves face-to-face interviews with multiple informants in each family. Four waves of the study have been carried out so far at 9 months (2001/2), age 3 (2003-4), age 5 (2006) and age 7 (2008). The fifth wave (age 11) took place during 2012, and development work began in 2010. The data collection for the study is competitively tendered and sub-contracted to a suitably experienced fieldwork agency. For wave 5, the contracted agency was Ipsos MORI.

The home visits for the age 11 survey consist of an interview with the main carer of the child (usually the mother) and partner (usually the father); direct assessments of the children's cognitive function; measurements of height, weight and body-fat, and a paper self-completion questionnaire for the children. Previous waves of the survey have included biological samples: oral fluid via mouth swabs (but not for the purposes of DNA extraction) at age 3; and shed milk teeth for environmental lead exposure at age 7.

The development work for the age 11 survey included an initial pilot of all survey elements, consents and materials. The feasibility of saliva collection was tested in this first pilot, building on prior development work. The sample for the pilot was recruited from five areas in the UK representing a diversity of types of region. Five interviewers worked on the pilot, one in each area. None of the interviewers had prior experience of saliva sample collection.

In total, interviews were carried out with 45 families (including one set of twins). Quotas were set to ensure a cross-section of different children and family types, and the fieldwork period was three weeks. None of the families had taken part in the MCS previously. Table 1 shows the achieved quotas.

Table 1: MCS Pilot sample profile

Demography	Sub-group	Number of interviews achieved
Gender	Boys	26
	Girls	20
Social grade of parent[1]	AB (highest)	11
	C1	9
	C2	9
	DE (lowest)	16
Ethnicity of child	Black or other minority ethnic	5 (3 in London)
Family composition	Single parent household	16

Data collection procedures

The fieldwork procedures for the saliva sample collection were adapted from those used successfully on ALSPAC and Fragile Families. All of the protocols were reviewed and approved by a medical research ethics committee. Interviewers were required to attend a three-day, face-to-face briefing session, where they received training on all elements of the survey, including the requirements for collecting the saliva samples.

Gaining informed consent and securing co-operation

Interviewers were required to gain informed consent, from both parents and children, for the saliva sample collection. Parents were required to give written consent for the collection of their own

sample and one parent was asked to give written consent for the collection of the sample from the child. Children were also required to consent verbally to the sample collection. Information leaflets were developed for both parents and children explaining the saliva sample collection. The content of these leaflets, and our approach to interviewer training, was informed by pre-pilot qualitative interviews and focus groups carried out with parents and children exploring the acceptability of, and concerns surrounding the request to collect a sample of saliva for DNA extraction. Given their age and the fact that parental consent was also required, for the child leaflet, the explanation of why the saliva sample was being collected was kept quite basic, with more focus on the practicalities involved. Copies of the leaflets are in the Appendix.

Interviewers were instructed to give these leaflets to parents and children in advance of the survey visit and to encourage the respondents to read them prior to their visit. At the visit, interviewers had to ensure that the respondents had read and understood the letters and leaflets, and that they had explained the procedure clearly before they attempted to carry it out. In addition, the interviewer had to establish whether or not the co-resident parents were the natural parent of the study child, as only biological resident parents were eligible for the saliva sample collection.

As consent to collect a saliva sample from the child could only be given by someone with legal parental responsibility, and not all parents have this, interviewers therefore had to ascertain who had legal responsibility prior to collecting consent. Natural mothers, natural fathers who are married to the natural mother and adoptive parents all automatically have legal parental responsibility. But for other parents e.g. step-parents and cohabiting natural fathers, it was necessary for the interviewer to ask a series of questions to ascertain whether they had legal parental responsibility.

After consent had been obtained from a parent with legal parental responsibility to collect a sample from the child, the next step was for the interviewer to gain the child's consent. The child retained the right to refuse, even if a parent had consented for them to give a sample. The process for gaining consent from the child was to ensure that they read their leaflet, and that they understood that they didn't have to provide a sample if they did not want to. The child was not required to sign anything, but the interviewer had to sign a form to confirm the child's informed consent.

Collection of saliva samples

Samples were collected using an Oragene 500 DNA self-collection kit made by DNA Genotek based in Ontario, Canada. This kit has been widely used on other surveys. Respondents were instructed, both in the saliva collection leaflet, and by the interviewers, not to eat, drink, smoke, or chew gum in the 30 minutes prior to providing the sample.

Respondents were asked to spit into a funnel until the amount of liquid saliva reached the fill line marked on the side of the tube (about 5 ml). On average, this should take about 5 minutes. The tube was then given back to the interviewer, who then had to hold the tube upright and close the lid by firmly pushing the lid until they heard a loud click. The preservative liquid in the lid would then be released into the tube. The interviewer then mixed this with the saliva by replacing the funnel from the top of the tube with a small cap and shaking the capped tube for five seconds.

Sending samples back to the laboratory

Interviewers were asked to write the time and date that the sample was collected on the side of the tube and to attach the relevant barcode label. They were provided with a set of barcode labels for each family, colour coded for different respondents (pink for the natural mother, blue for the natural father and yellow for the child). The barcode consisted of a nine digit family serial number and a letter to identify the person. No other identifying information was recorded on the sample. Interviewers also had to enter the family serial number and person ID number onto a despatch note. The despatch note was for the laboratory to cross check the samples against it as they arrived.

Interviewers were instructed to place the tubes into individual plastic bags containing absorbent material (to contain leakages) and place both the tubes and the completed despatch note into a padded envelope. The packaging was compliant with the appropriate UN regulation (UN3373) on shipping of biological substances and the envelope was labelled 'exempt human specimen'. The interviewers were asked to return all of the samples that they had collected to the laboratory twice a week. There was no requirement to send a complete set of samples from a family together.

Reconciliation of consent forms and samples

It was essential that only samples for which consent had been obtained for collection were processed in the laboratory. Although consent was recorded electronically by the interviewers, this needed to be cross-checked against the hard-copy of the paper consent forms. Ipsos MORI booked in the consent forms on a spreadsheet detailing, for each interviewed family, whether the child, mother and/or father had provided a sample. A visual check was carried out to confirm that the names on the consent forms were the same as those recorded electronically as eligible for their own collection and able to give consent on behalf of the child.

When the samples arrived at the laboratory, the serial numbers were recorded, and this list was sent at regular intervals to the Ipsos MORI Field department who chased up any interviewers who had yet to send back a consent form for samples that had been received by the lab or where the consent form but not the sample had been received. At the end of fieldwork, Ipsos MORI sent a full list to the laboratory of all valid consents received. Any samples for which consent had not been obtained would have had to have been discarded, although this did not actually occur.

Findings

Gaining informed consent and securing co-operation

The interviewers were fairly successful at gaining informed consent, and securing co-operation from respondents, and achieved high rates of sample collection. As illustrated in Table 2, samples were collected and processed from 73% of mothers, 76% of eligible fathers, and 74% of children.

Table 2: Saliva sample collection response rates

Saliva Sample			

	Number interviewed	Number collected	% of respondents providing sample
Children	46	34	74
Mothers	45	33	73
Fathers	25	19	76

Although the majority of respondents were happy to participate in this element of the survey, the co-operation rates for this element were not as high as in other similar surveys such as Fragile Families. However, we felt that these were relatively good co-operation rates for a pilot study involving newly recruited families. We would expect co-operation rates to be higher amongst the study members themselves, as they have a pre-existing commitment to and experience of the study, including provision of samples and a range of consents. In addition, we would expect that at the main stage some of the barriers to participation that we identified in the pilot study would have been addressed, leading to higher co-operation rates.

The main reasons for non-participation were worries about what would be done with the samples, or dislike of the actual process involved. Some parents and children refused because they did not like the idea of spitting into a tube. This was particularly true for some of the children who, when faced with the tube, found the actual process too 'yucky' to complete. One or two commented that they would be happy to give a sample via another means, such as a swab. Some found the idea embarrassing, especially in front of a stranger. In some cases, interviewers found that some people were more willing to provide the sample if they could go away and produce it on their own without others watching.

In addition, a number of parents refused because they were worried and uncertain about the uses that the data would be put to. Some were concerned that data might end up on police or government databases and be used to check up on them, or to identify them in relation to criminal activities. Although the leaflet did contain assurances regarding this, a small number of parents refused point blank for these reasons; their views were so strong and fixed, that interviewers felt that no amount of further information or persuasion techniques were likely to be effective, because they had a fundamental mistrust of the whole concept of DNA databases and data security. One parent queried whether any reassurances about security given now would still hold in future, for example, if the law changed.

In terms of administering the consent procedures, none of the interviewers had difficulty with defining legal parental responsibility. Although none of the respondents asked for further information about the laboratory where the data would be stored or how the data would be linked, interviewers felt that should this have arisen they would not have been sufficiently informed to

respond confidently.

Collection of saliva samples

Interviewers reported that the saliva samples took between 5 and 10 minutes per sample to complete. However, it is worth noting that some interviewers found it difficult to record timings accurately, particularly in cases where not all saliva samples were taken at the same time.

A number of children reported finding it hard to spit into the tube and found the process a bit awkward. Parents were generally happy with the process but some also found it difficult to produce enough saliva (especially just after they had been interviewed). Having said that, all respondents who agreed to give saliva completed the donation.

The order in which saliva samples were taken varied from interviewer to interviewer and from household to household. Most interviewers found it helpful to introduce or reintroduce this element of the survey later in the appointment once a rapport had been established. This resulted in some initial refusals being converted into successful outcomes. This highlights the importance of flexibility in timing.

In terms of the equipment, interviewers did not report any significant problems with the testing kit. However, in some cases saliva was present on the tubes when the interviewers were handling the samples. As a result, the majority of interviewers felt that disposable gloves should have been provided. Another issue raised was that interviewers were unable to write on the space provided on the tubes. As this information was captured in the despatch note, this did not have a significant impact on the pilot survey.

Reconciliation of consent forms and samples

In total, 86 saliva samples were collected, and all were received by the laboratory. A reconciliation process was carried out four times during the three week fieldwork period. This involved the laboratory sending Ipsos MORI the serial numbers of the samples they had received to date. Ipsos MORI field staff then cross-checked this list with the consent forms received and updated the sheet to confirm that consent had been received for each individual sample. The sheet was then returned to the laboratory.

During the final check it came to light that, although Ipsos MORI had received consent forms for 86 samples, one interviewer had clearly got confused when using the barcode labels and whilst the correct label was stuck onto the saliva sample, an incorrect label (for another respondent) was used on the consent form.

The majority of interviewers did understand which bar-code labels to use, and the colour coding system, to distinguish samples from children, mothers and fathers, worked well.

When the samples arrived at the laboratory, sample identifiers from the tubes obtained from the mothers and children were scanned successfully using a bar-code scanner but those of the fathers (blue background) could not be scanned and needed to be logged manually.

After recording the total sample volume, the DNA was extracted following the DNA genotek

protocol for manual purification of DNA using the prepIT•L2P DNA extraction kit. The DNA was quantified in duplicate using an automated robotic system (Tecan UK) and the Invitrogen Quant-IT Pico Green dsDNA assay kit (Life Technologies, Paisley UK)

Quality of the samples

The total yields obtained from the samples are shown in Table 3. Over 81% of samples gave yields of at least 20 *µ*g, sufficient DNA for a range of genetic studies. However there was a large range of variation in the amount obtained from different individuals. To some extent this is reflected in the initial size of the saliva sample, as shown in Table 4, with smaller samples giving a lower yield.

Table 3: Total DNA yield

	Number collected	**Mean total yield (*µ*g)**	**Std dev**	**Range (*µ*g)**	**% with total yield > 20 *µ*g**
Children	34*	58.81	38.2	0.03-238.2	82.9%
Mothers	33	119.6	103.6	0.1-390.4	81.8%
Fathers	19	99.6	77.1	4.0 – 254.7	89.5%

* includes one set of twins

Table 4: DNA yield related to sample volume

	Number collected	**Mean total yield (*µ*g)**	**Std dev**	**Range (*µ*g)**	**Mean yield per ml of sample**
≤2ml	13	31.2	83.3	0.03 -306.9	15.6
2.1 to 3ml	39	98.3	86.6	0.1-390.4	105.2
3.1 to 4ml	28	97.8	69.0	21.0 – 258.1	52.1

4.1 to 5ml	6	141.3	45.9	79.1 – 290.8	45.9

The smallest samples (≤2ml) had a much lower yield per ml of sample which indicated that DNA extraction was not as efficient from these samples. Since the majority of these small volume samples were collected by one interviewer on one particular day, it suggests that the collection instructions had not been followed properly. Upon investigation, it was found that one interviewer had shaken the sample before taking off the funnel and putting on the cap. It is likely that 2ml of saliva were collected in this instance, but that it effectively spilled. Samples collected by the same interviewer at a later date were of the correct volume and gave higher yields of DNA, reflecting the fact that they had received further training on the correct use of the kits. Table 5 shows total DNA yields when samples collected incorrectly are excluded from the data and is a better indication of the yields that can be obtained. In this case over 92% of samples yielded more than 20 μg of DNA.

Table 5: Total DNA yield excluding samples collected incorrectly

	Number collected	**Mean total yield (μg)**	**Std dev**	**Range (μg)**	**% with total yield > 20 μg**
Children	28*	61.4	35.6	12.0-171.6	92.9%
Mothers	27	142.9	99.9	0.1-390.4	92.6%
Fathers	11	110.7	73.8	21.0 – 254.7	100%

* includes one set of twins

At the analysis stage it was discovered that some samples were turbid and discoloured suggesting that the instructions regarding not eating, drinking, smoking or chewing gum for 30 minutes before providing the sample were not consistently adhered to. This can result in lower quality DNA.

Conclusions

Our findings show that it is clearly feasible to get good quality, analytical samples of saliva from both children and their parents, using simple kits administered by lay interviewers in a household setting on a population-based survey in the UK.

In terms of maximising response to the saliva sample collection, interviewers fed back to us that provision of greater clarity and further information about the following would provide respondents with reassurance and potentially encourage participation:

- That the data will not be put on the "police DNA database"
- That it will be held in its own separate database that would always be kept separate from any other database, and never linked with data that was not generated via the MCS study itself (with families' permission).
- How data would be used (as well as information about how it would not be used), and in particular giving specific examples of socially beneficial uses of the data explained clearly and in simple, non-technical language.
- Clarification about what would happen if there was a change in the law regarding access to data. For example, would it be appropriate to provide reassurance that families would be re-contacted to re-consent if changes to the law resulted in changes in the purposes to which data could be used?

The information leaflet could easily be revised to cover these areas.

In common with other surveys, we found that respondents did not raise concerns that the sample collection was carried out by lay interviewers, rather than medically-qualified personnel. This is not surprising given the non-invasive nature of saliva sample collection. Similarly, although the interviewers had no prior experience of collecting saliva samples, they were all willing and able to do this.

However, it became clear during the pilot that a considerable amount of time needs to be spent during the interviewer training to ensure that interviewers follow the procedures accurately and feel adequately equipped to deal with any questions that might be raised by respondents in a confident manner. The pilot debrief provided a great deal of information in terms of the areas that interviewers were most frequently asked about: future training could be designed to cover these areas in more detail. Additionally, some interviewers felt that, given the nature of the research, they may not always have the specialist knowledge required to answer questions accurately and therefore suggested that further information and answers to more detailed questions could be provided on a website.

As we had only five interviewers on our pilot study, we did not examine interviewer effects on cooperation rates, but there is evidence from other surveys that there can be significant interviewer variation in consent rates for bio-measures (e.g. Korbmacher and Kreiger, 2012). Although this is in part due to differences in respondent characteristics between interviewer assignments, it is likely that interviewer attitudes to the sample collection also play a part. In this context, it is important that interviewer training addresses any negative attitudes or inaccurate perceptions about bio-measure collection among interviewers in order that they are better able to persuade reluctant participants.

Parents and children were generally happy with the collection process, and interviewers reported few problems with the collection kit itself, leading us to conclude that both the kit and the method of sample collection can be recommended for similar studies. In terms of the process of collection itself, we would recommend ensuring that interviewers are aware of the potential for respondent embarrassment being a barrier to participation, and the benefit of facilitating privacy in this context. We also think that it's important to provide interviewers with gloves or some other way of sanitising should saliva be present on the tubes when handed back and emphasise at the briefings when to shake the tubes (as doing this prematurely led to fluid leaking from the tubes in some cases). We would also recommend that interviewers are trained to remind respondents about the requirement not to eat, drink, smoke or chew gum for 30 minutes prior to the sample collection, as the analysis of the samples carried out by the lab showed that this had not always been observed.

The despatch and reconciliation processes used at the pilot enabled us to progress chase interviewers, reconcile consent forms and to successfully identify who the samples belonged to. However, it is important to bear in mind the small sample size for the pilot. The process, which involved the exchange of spreadsheets between Ipsos MORI field department and the laboratory, may be more difficult to replicate on a much larger scale, involving hundreds of interviewers and thousands of samples. Given the importance of accurate real-time specimen tracking, developing a database which can be shared between the field department and the laboratory, and that identifies problems in an automated way, may be worth investigating. Some surveys involving multiple specimens being shipped to multiple laboratories have taken this approach (e.g. Jaszczack, O'Doherty and McPhillips, 2012). However, the costs and benefits of this would depend on the survey context and would need to be carefully considered.

The analysis by the lab and the booking-in processes also revealed that some interviewers had not been following the procedures for sample collection and dispatch correctly. The importance of these processes for ensuring that high quality samples are collected and that the identification and reconciliation of samples can be carried out accurately indicated that further interviewer training would be beneficial. It is becoming increasingly common for surveys to adopt formal interviewer accreditation procedures for the collection of bio-measures, and we would recommend that this approach is taken on surveys which include saliva sample collection.

Overall, we felt that the pilot survey clearly demonstrated that the collection of saliva samples on the MCS was feasible, and could be expected to provide high quality data. Although a number of areas for improvement were identified, this was expected and demonstrates the value of pre-testing new data collection elements, even where they have been successfully incorporated on other surveys. Saliva sample collection was not included on the main stage of the fifth wave of the MCS as funding was not secured. However, it remains an aim for future sweeps, and these findings can be used to inform the development of fieldwork procedures. Our findings are also relevant to other surveys planning to incorporate saliva sample collection for DNA extraction, particularly for those involving lay interviewers in a home setting.

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[1] Social grade is a system of social classification based on occupation that is widely used in market research. It is derived from the occupation of the head on the household. AB refers to those in higher or intermediate managerial, administrative or professional occupations. C1 refers to those in supervisory or clerical and junior managerial, administrative or professional occupations. C2 refers to skilled manual workers, and D includes semi and unskilled manual workers. Those in social grade E are casual or the lowest grade workers, pensioners and others who depend on the welfare state for their income.

Appendix- Information Leaflets

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