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Oral fluid collection by post for viral antibody testing

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Abstract

Background The objective of this study was to estimate the prevalence of hepatitis B virus exposure (HBV) in the population of the Republic of Ireland, by using oral fluid (saliva) collection by post for hepatitis B anti-core antibody (anti-HBc). This paper discusses the methodological approach used and the strategies that were adopted to improve response rates.

Methods The sampling frame used was the Register of Electors for Irish parliamentary elections. A multistage stratified cluster sample was taken, and a total of 962 households were selected nationally. A four-letter approach was employed for sample collection. Households received an initial letter outlining the purpose of the study. This was followed by a letter containing six swabs for oral fluid collection, along with easy-to-follow instructions. Non-respondents received two reminder letters, and were also telephoned where possible. A telephone helpline was provided. All testing was anonymous and unlinked.

Results The study achieved a good household response rate (60.4%), and more than 98% of the 1738 specimens received were suitable for testing. The prevalence of anti-HBc in the Irish population was estimated to be 0.51%. The observed design effect was 1.29.

Discussion From a review of the literature, this is the first study where a representative sample of a national population was asked to self-collect oral fluid samples and return these by post for serological testing. The technique may have many future applications in epidemiological research.

Keywords Hepatitis C antibodies, oral fluid samples, serological testing

Collecting oral fluid (saliva) samples by post for antibody marker testing can serve a variety of purposes in epidemiological research. The requirements are that the antibody be detectable in oral fluid, and that it is stable in unfrozen oral fluid for at least several days and preferably several weeks. The main advantage of oral fluid is that it is quicker and easier to obtain than blood, and that it can be self-collected. There is no risk of needle stick injury and it is painless. Patients can self-collect samples at home in their own time.

The technical feasibility of collecting oral fluid samples by post has already been demonstrated.¹⁻⁴ However, from a review of the medical literature using the Medline database, no study of oral fluid collection by post for serological testing involving a representative sample of a healthy national population has been published to date.

The objective of this study was to estimate the prevalence of exposure to hepatitis B virus (HBV) in the population of the Republic of Ireland, using oral fluid collection by post for hepatitis B anti-core antibody (anti-HBc). This paper discusses the methodological issues that arose, and the strategies that were adopted to improve response rates.

Materials and Methods

The sampling frame used was the Register of Electors for Irish parliamentary elections. This lists the names and addresses of all adults 18 years or older who are registered to vote in parliamentary elections.

A multistage stratified cluster design was used.⁵ The country was divided into six strata (urban and rural, and three levels of socioeconomic status), using a classification developed by the Small Area Health Research Unit, Trinity College, Dublin.⁶ Eighteen district electoral divisions (DED) were chosen with a probability proportional to size, three from each stratum. From these 18 DED, an equal number of households was selected at random.

Cluster sampling was used for two reasons. Firstly, the electoral register is a paper listing, consisting of 3444 separate listings of voters. Thus, obtaining all 3444 listings and taking a simple random sample would be very difficult. Secondly, taking a sample from only 18 DED allowed us to ascertain whether people written to had died or moved, by contacting the local postmasters. Thus, a more accurate net response rate could be calculated.

Sample size calculations were performed using the Epi Info software package.⁷ Our experimental hypothesis was that the prevalence of anti-HBc in the Irish population was 1% \pm 0.5%. This was based on extrapolating from published data on hepatitis B positivity in blood donors and antenatal women attending antenatal clinics.⁸ A response rate of 50% was also assumed, based on expected response rates for postal questionnaire studies.⁹

Due to the chosen study design, a design effect needed to be chosen. The design effect is the ratio of the variance of the estimate assuming a simple random sample design compared to the variance of the estimate under the actual multistage stratified random sample design used here. It is the multiplying factor by which a sample size calculation needs to be increased to take account of the study design used. In the absence of a known design effect, some authors have recommended that a design effect of two should be adopted for sample size calculations in stratified random sample studies.¹⁰ Accordingly, it was decided to assume a design effect of two.

Based on the above assumptions, at a power of 80% and with a 95% CI, a sample size of 2640 was required. Since the average Irish household size is 3.2 people,¹¹ a sample of 825 households was required. It was assumed that approximately 10% of those listed on the electoral register would have moved elsewhere or died, based on the findings of a national health promotion study that also used the Irish electoral register.¹² Thus, it was decided to sample 900 households in total, or an extra five households per DED.

However, in some DED, a larger than expected number of people listed had either moved elsewhere or died. A small additional top-up sample was required in 10 of the 18 DED, where, in five or more households, the individual contacted had died or moved. Thus the final number of households sampled was 962. This was to ensure that roughly the same number of households were sampled in each DED.

Sample collection took place between November 1998 and January 1999. A four-phase approach was used for sample collection. Households received an initial letter outlining the purpose of the study. This was followed by a letter containing six swabs for oral fluid collection, along with easy-to-follow instructions. Non-respondents received two reminder letters, and were also telephoned where possible. A second set of swabs was sent out with the second reminder, as providing a second copy of the study instrument has been found to improve response rates in postal surveys.⁹

The respondents were asked to collect oral fluid from each household member, and to mark the age and sex on each sample container. All household members were asked to take part. These swabs were then posted to the Virus Reference Laboratory in Dublin. No name or address appeared on the samples. Respondents were also asked to return a postcard separately to the author in the North Eastern Health Board. This contained the respondents' names and addresses, indicating that they had participated. This was for the purpose of targeting non-respondents.

The use of a four-phase approach was based on the need to achieve as high a response rate as possible, but on the other hand not to antagonize people with multiple additional mail shots. The mailings were spaced at 7-day intervals to allow time for response. They were sent out midweek, so that they would arrive just before the weekend. Thus, recipients would have more time to take the samples, and the other household members would be more likely to be at home.

All letters were written on University College Dublin (UCD) notepaper, as the Virus Reference Laboratory is associated with the university. The association of studies with universities has been shown to improve response rates.¹³ A telephone helpline was provided to help people who had difficulty with the instructions on how to take an oral fluid specimen.

Much attention was paid to emphasizing the anonymous and unlinked nature of the study. This was mentioned twice in each of the four letters. The study received widespread publicity in the national newspapers. Copies of the newspaper articles were sent out with the first and second reminder letters, to lend further credence to the study.

A free prize draw for £200 was offered to encourage participation. This has been demonstrated to improve response rates in postal surveys.¹ Free postal addresses were used for both the samples and the postcards. It was emphasized in all correspondence that a postage stamp was not necessary for returning samples/postcards.

The statistical analysis used the svy package from Stata.¹⁴ This uses the complex survey data analysis methods described by Eltinge¹⁵ to estimate the correct variance for such studies. These take account of the non-independence of the cluster sample and the complex structure of the sampling.

Results

The net response rate among households was 60.4% (491/812), taking account of 150 households where the adult to whom the correspondence was addressed had moved elsewhere, or in a small number of cases had died. The response rate varied across the six different strata of the study. The lowest response rate was seen in urban low socioeconomic households (46.6%) and the highest response rate was seen in rural high socioeconomic households (66.4%). The age and sex profile of the respondent population closely matched the age and sex profile of the Irish population. The cumulative response rate by the phase of the study is shown in Table 1+.

Table 1 Cumulative response rate by phase of study

Phase of study	Cumulative response
Phases 1 and 2 (Initial/swabs letter)	31.1%
Phase 3 (Reminder 1 letter)	47.2%
Phase 4 (Reminder 2 letter)	60.4%

An attempt was made to telephone non-respondents, at the same time as the second reminder letter stage. Only 120 out of 429 (27.9%) non-respondents were successfully contacted. This was due to lack of telephone ownership, a large number of ex-directory telephone numbers and people not being at home when telephoned. The cumulative response rate improved from 47.2% to 60.4% following the second reminder letter and phone calls.

The telephone helpline received a total of 37 telephone calls. No caller to the helpline sought assistance on how to take the oral fluid samples, because perhaps the instructions were very clear. Most callers phoned to ask for more oral fluid swabs, to question whether individuals should be included or excluded, or to ask about the relevance of previous hepatitis A.

A total of 1738 specimens were received. These were first tested for total immunoglobulin G (IgG), to ensure that a sufficient quantity of oral fluid had been collected by the respondent. In all, 1714 out of 1738 specimens (98.6%) were positive for IgG, and thus were suitable for anti-HBc testing. Of the remaining 24 samples, 21 were negative for IgG and 3 had an insufficient oral fluid sample for further testing.

Five out of the 1714 samples tested were positive on combined anti-HBc IgG/IgM testing and on anti-HBc IgG testing alone. Thus, the crude prevalence of anti-HBc in the study population was 0.29% (95% CI : 0.04–0.55%).

Using the svy statistical package from Stata,¹⁴ and taking account of the multistage stratified random sampling design employed, the estimated prevalence of anti-HBc in the population of the Republic of Ireland was 0.51% (95% CI : 0–1.18%). The design effect for the multistage stratified random design used was 1.29, which was less than the design effect of 2.0 anticipated in the sample size calculations.

Discussion

To our knowledge this is the first nation-wide study of serological markers using oral fluid collection. A good household response rate of 60.4% was achieved, which compares favourably with postal questionnaire surveys.¹³

The oral fluid anti-HBc test used in this study has been demonstrated to have a sensitivity of 82% and a specificity of greater than 99% in unrefrigerated samples.¹⁶ Thus, it is a reliable test for estimating present or past hepatitis B infection.

Nearly all of the specimens received (98.6%) were suitable for testing, which indicates that the public did not have problems with sample collection. Although the estimated population prevalence figure of 0.51% had wide CI, the findings still indicate that Ireland is a very low prevalence country.

We accept that higher risk groups such as intravenous drug users or people with learning disability may not have been included in the sample, either because they were not registered to vote and thus would not appear on the electoral register, or alternatively because they chose not to take part. However, the prevalence of hepatitis B infection in these groups has already been documented,^{17,18} and the purpose of this study was to estimate the prevalence of infection in the general population.

The only similar published study involving oral fluid collection by post in a large healthy population was a Swiss study using postal oral fluid testing.¹ This achieved a response rate of 50.0%, in a population of 3000 university staff and students. However, they were testing for cotinine (a nicotine metabolite), which may be less controversial than testing for hepatitis B markers.

Much smaller scale studies have also been published, involving measurement of theophylline levels,³ thiocyanate levels,² and cortisol levels⁴ in mailed oral fluid specimens. These studies have demonstrated that biological specimens are stable in unrefrigerated oral fluid for extended periods, that oral fluid collection by post is feasible, and that there is a good correlation between oral fluid testing and serum testing. Other researchers have demonstrated that oral fluid testing for hepatitis B and HIV antibodies is of comparable sensitivity and specificity to serum testing.^{19,20} However, the author has not identified a published study where oral fluid collection by post was used for *serology* studies in a *national* population.

Oral fluid testing by postal collection is a novel means of conducting population epidemiological studies for serological markers. A variety of factors may have contributed to the good response rate achieved. These included the use of reminder letters and telephone calls, emphasizing the anonymous and unlinked nature of the study, the use of Freepost addresses, the provision of a telephone helpline and the timely issuing of press releases.

In conclusion, oral fluid collection by post for epidemiological purposes is easily understood by the public. They are willing to co-operate with such studies and are able to provide suitable samples. The methodology may have many future epidemiological applications.

KEY MESSAGES

- Collecting saliva samples by post is a novel means of conducting population-based epidemiological studies.
- This is the first nation-wide study of a serological marker using oral fluid collection.
- The response rate among households in the Republic of Ireland was 60.4% and the prevalence of hepatitis B anti-core antibodies was estimated at 0.51%.
- The methodology of self-collection was easily understood and the public was able to provide suitable samples in close to 100%.

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