PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Serological surveillance of influenza in an English sentinel network: pilot study protocol
AUTHORS	de Lusignan, Simon; Borrow, Ray; Tripathy, Manasa; Linley, Ezra; Zambon, Maria; Hoschler, Katja; Ferreira, Filipa; Andrews, Nick; Yonova, Ivelina; Hriskova, Mariya; Rafi, Imran; Pebody, Richard

VERSION 1 – REVIEW

REVIEWER	Mohamed DILAI Institut Agronomique et Vétérinaire Hassan II, Morocco
REVIEW RETURNED	07-Oct-2018

GENERAL COMMENTS	Very Important study. I have only one comment regarding the serological assay. the HI assay has been demonstrated to have a large variation results between laboratories. It could be perfect to confirm the serological result with a second test; Single Radial Heamolysis (SRH) (with low variation results between laboratories).

REVIEWER	Oana Sandulescu Universitatea de Medicina si Farmacie Carol Davila, Infectious Diseases
REVIEW RETURNED	21-Nov-2018

GENERAL COMMENTS	This is an interesting study protocol regarding the serological surveillance of influenza in the UK. Overall, I found the protocol to be scientifically sound and I feel that the main objectives of the protocol can be achieved through the type of study proposed. To ensure the best accuracy in describing the study protocol for the general public and the journal's readership, I have a set of queries to the authors, which are listed below and which could help in clarifying certain aspects in the manuscript: GENERAL COMMENTS: - Minor spelling or spacing issues should be addressed in the manuscript (examples include "back-ground", "population- based", "This pilot test our ability", "allow us to extract and used health data", etc.). - The study's main focus is on influenza, and only testing for influenza is described in this study protocol. Therefore, it might be more appropriate to exclude "and other infections" from the title and from the manuscript (i.e., "and respiratory diseases" from the first paragraph in the Introduction section). INTRODUCTION: - Certain phrases in the Introduction section are too general and quite far from the study's main focus. For example, the phrase "At present, two serum samples have to be analysed in order to

	evaluate an increase of antibody between acute and convalescent samples" presents general knowledge regarding the use of serology for diagnostic purposes, but it doesn't have much to do with the study (I did not see a specification in the protocol that more than one sample per patient would be collected in this pilot study, or that testing will be used for diagnostic purposes). Another example would be referencing young children when only adults will be included in the pilot study: "However, when analysing immunologically naïve individuals, such as young children or a naïve population at the beginning of a pandemic". - There are multiple references throughout the manuscript regarding the potential for linking virology specimens and serology (i.e., "The network also collects virology specimens to detect influenza in season – allowing matching in individuals between swab report and serology.", "collecting serology data from a sentinel network and linking it to virology", "The result of that sample will be linked to that patient's pseudonymised record and any influenza virology swab data if patient participated in the nasopharyngeal swabbing project", etc.). Since so much emphasis is placed on this link, the protocol could be revised to allow patients with acute infection suggestive of ILI to be preferentially invited for study enrollment, and to create a subgroup of symptomatic patients (with information on age strata, sample size for such a subgroup, etc.). The only current reference to this in the manuscript is that "There will be no attempt to select patients for serology on the basis of whether they have had […] a virology specimen taken for influenza", and "This pilot is not targeting those who have had virology specimens." This suggests that this linking between virology specimens and serology, which is stressed as a main strength of the project, will not really be possible for most patients, as patients might only be, by chance, selected for serology if they are symptomatic for ILI at the time of presentation, which furthermore does not appear to be likely at all since the section on Population sampling specifies that "Patients, who attend their pilot sentinel network practice for routine blood test" will be invited to participate in the study. OBJECTIVES: - The study's objectives are quite general. While I do see how a pilot study can aim to clarify exactly what the specific objectives of a future, larger, study could be, I would recommend that the objectives be rephrased to explain how each objective could become measurable to a certain extent. For example, the last objective refers to: "evaluate the feasibility" and some measures of this evaluation are provided in the section on Statistical and modelling analysis. I would recommend the objective to specify such proxy instruments to be used for measuring feasibility. And this can also be applied to the other objectives. - The following phrase in the last objective does not really seem as feasible based on this pilot project: "make recommendations about how best to […] apply these approaches to other vaccine preventable infections". Influenza epidemiology is quite different from that of other vaccine-preventable diseases, and therefore, a seroepidemiology study for influenza would also need to be quite different from one for measles, for example, and would importantly need to be repeated yearly, or at least more often than one for measles, where one positive IgG can translate into prolonged, even life-long immunity, in the absence of the antigenic shifts and drifts that are characteristic for influenza viruses. STUDY DESIGN:

	- Despite the fact that the manuscript talks about "representativeness" of the study population, the section on Population sampling is quite elusive, as it does not explain exactly what type of sampling will be employed. One can infer that convenience-based sampling would be applied, but it would be best if this were clearly specified. Furthermore, the sampling reference frame should also be described, i.e., will this be the patient registry of the 5-6 practices? And what would this amount to? Any expected differences between the practices? Which is the study catchment area? Will the sample size differ between practices based on the size of the population base of the practice? - A verb is missing here: "Analysis carried out". - As someone working in infectious diseases, I am not comfortable with the term "opportunistic" used in the following phrase, and would therefore recommend rephrasing this sentence: "The SEU archive is an opportunistic collection". - A clearer justification for the proposed sample size should be offered, currently the only information given is that "The number of samples is based on a combined list size of 50,000 across the participating practices"; "target number of samples. (minimum 100, maximum 150 per age band)." Furthermore, there is also the question of representativeness. Should this range of 100-150 patients sampled be uniform throughout the different age bands? This should be taken into account when planning the study, and the number of patients included per age band should be calculated in order to ensure representativeness for something: either the general population in the catchment area, or the population attending the practices, etc. While it is indeed useful to prove that sampling can be performed throughout age bands, it might not be necessary or useful to have the same number of subjects sampled in the different age groups. - The title for Table 1 presents the following information: "This table represents the week in which sample collection would be complete, if all patients consented." It was my initial assumption that patients would be enrolled throughout the influenza season. However, upon revisiting the manuscript I did not find any indication of when exactly the project will be performed, and whether sampling will be continuous throughout the season. This information should be provided. The current statement in the manuscript is that "The project proposes to pilot a population-based seroprevalence survey, […] following the 2017/18 influenza season." which, combined with the data in Table 1 suggests that all samples will be collected in a short time span (a minimum of 1 week and a maximum of 5 estimated weeks). Therefore, I do not see how this project would respond to the aim of measuring seroincidence, as mentioned in both the Abstract and the Objectives section of the full manuscript. LABORATORY ANALYSIS: - Rephrasing is needed for: "The standard method for sending specimens to the laboratory is via a pathology request software". - How soon will samples be sent following collection? Any issues worth mentioning related to storage of samples before and during transport to PHE's SEU/VEU lab? - "samples will be […] stored in archive freezers". Further details regarding the type of freezers should be provided: -20? -80? - For the following phrase "Additionally, analysis using influenza neutralisation and/or Neuraminidase Antibody Inhibition assays in the format of the Enzyme-linked Lectin Assay may be considered to investigate the functionality of the antibody further." Further

	information should be provided here, to describe how cases would potentially be selected for this type of analysis. DATA EXTRACTION: - The following information is repetitive (with at least two other occurrences in the manuscript): “The RCGP RSC and PHE have worked together to provide state of the art National influenza surveillance for over 50 years” - For the following phrase: “Practices are computerised, and data entered into computerised medical record systems either as coded data, or free text. We will extract the coded data” further information should be provided regarding the amount of data that would be expected to have been entered as free text and would therefore be lost for analysis. STATISTICAL AND MODELLING ANALYSIS: - One of the elements to be evaluated is listed as: “Feasibility of extending the pilot approach to a wider number of practices and collection of additional sample types e.g. oral fluid ascertained”. Further details should be provided regarding what is meant by “oral fluid” here. - For the following phrase: “University of Surrey will report the extent to which we have samples from common households – this might provide information about shared infections/levels of immunity within households” this should also be discussed as a potential study limitation, as including a large proportion of people from the same household, who are expected to have had the same type of exposure, would lead to potential sampling bias. - Why would feedback “about the utility of the data provided” be provided to the patients? While it does make perfect sense to provide the practices with feedback on this, and to provide patients with the results of their serology test, informing them of the study progress would not need to be a standard procedure, it would suffice to have a phrase in their informed consent form stating who they can contact should they be interested in details on the study progress. ETHICS: - The manuscript specifies that “PHE has ethical approval (05/Q0505/45) for the collection and use of unlinked and anonymised residual serum samples in cross-sectional antibody prevalence studies”. However, since in this study a separate informed consent for a separate blood sample will need to be obtained, Ethics approval of this informed consent form would be needed, supplementary to the existing approvals. DISCUSSION: - One of the study’s strengths is presented as: “pilots a much lower cost method of establishing a serology bank”. Costs are not listed as part of this study’s objectives, and therefore a cost analysis cannot be the main strength of the project.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1 Comments

1. **Very Important study. I have only one comment regarding the serological assay. The HI assay has been demonstrated to have a large variation results between laboratories. It could be perfect to confirm the serological result with a second test; Single Radial Heamolysis (SRH) (with low variation results between laboratories).**

Authors' comments:

Many thanks, this is an interesting point! However, we have not currently established this assay in the laboratory and are therefore not planning to include it in the pilot study described here. We may consider this analysis in the future.

Reviewer 2 Comments

This is an interesting study protocol regarding the serological surveillance of influenza in the UK. Overall, I found the protocol to be scientifically sound and I feel that the main objectives of the protocol can be achieved through the type of study proposed. To ensure the best accuracy in describing the study protocol for the general public and the journal's readership, I have a set of
queries to the authors, which are listed below and which could help in clarifying certain aspects in the manuscript:

1. **Minor spelling or spacing issues should be addressed in the manuscript (examples include "back-ground", "population- based", "This pilot test our ability", "allow us to extract and**
   used health data", etc.).

Authors' comments:
Thank you for pointing this out. These have now been corrected in the manuscript as the following:

"Background"," population based", "This pilot tests our ability", "Practices who have agreed to be part of the RCGP RSC and have allowed us to extract and use health data for surveillance and research".

2. **The study's main focus is on influenza, and only testing for influenza is described in this study protocol. Therefore, it might be more appropriate to exclude "and other infections" from the title and from the manuscript (i.e., "and respiratory diseases" from the first paragraph in the Introduction section).**

Authors' comments:

Thank you for pointing this out. We have amended the title and excluded respiratory diseases from the first paragraph of the introduction as requested. Please see below:

Serological surveillance of influenza in an English sentinel network: pilot study protocol

This pilot study explores the potential to establish a serology bank, based on a sentinel network, and focussed on influenza.

INTRODUCTION:

3. **Certain phrases in the Introduction section are too general and quite far from the study's main focus. For example, the phrase "At present, two serum samples have to be analysed in order to evaluate an increase of antibody between acute and convalescent samples" presents general knowledge regarding the use of serology for diagnostic purposes, but it doesn't have much to do with the study (I did not see a specification in the protocol that more than one sample per patient would be collected in this pilot study, or that testing will be used for diagnostic purposes). Another example would be referencing young children when only adults will be included in the pilot study: "However, when analysing immunologically naïve individuals, such as young children or a naïve population at the beginning of a pandemic".**

Authors' comments:

This is very reasonable and improves the focus. We have removed the above statements from the protocol.

4. **There are multiple references throughout the manuscript regarding the potential for linking virology specimens and serology (i.e., "The network also collects virology specimens to detect influenza in season – allowing matching in individuals between swab report and serology.", "collecting serology data from a sentinel network and linking it to virology", "The result of that sample will be linked to that patient's pseudonymised record and any influenza virology swab data if patient participated in the nasopharyngeal swabbing project", etc.). Since so much emphasis is placed on this link, the protocol could be revised to allow patients with acute infection suggestive of ILI to be preferentially invited for study enrolment, and to create a subgroup of symptomatic patients (with information on age strata, sample size for such a subgroup, etc.). The only current reference to this in the manuscript is that "There will be no attempt to select patients for serology on the basis of whether they have had […] a virology specimen taken for influenza", and "This pilot is not targeting those who have had virology specimens." This suggests that this linking between virology specimens and serology, which is stressed as a main strength of the project, will not really be possible for most patients, as patients might only be, by chance, selected for serology if they are symptomatic for ILI at the time of presentation, which furthermore does not appear to be likely at all since the section on Population sampling specifies that "Patients, who attend their pilot sentinel network practice for routine blood test" will be invited to participate in the study.**

Authors' comments:

Thanks for this important observation. We have reduced the number of times we make reference to this to a single time in the discussion. For this study we are intending to sample opportunistically, only focussing on an even spread of age-groups, but this is an important point to flag for future studies. There would be merit in inviting as suggested. The earlier statements have been removed.

OBJECTIVES:

5. **The study's objectives are quite general. While I do see how a pilot study can aim to clarify exactly what the specific objectives of a future, larger, study could be, I would recommend that the objectives be rephrased to explain how each objective could become measurable to a certain extent. For example, the last objective refers to: "evaluate the feasibility" and some measures of this evaluation are provided in the section on Statistical and modelling analysis. I would recommend the objective to specify such proxy instruments to be used for measuring feasibility. And this can also be applied to the other objectives.**

Authors' comments:

We think this is a reasonable comment. Our objectives probably set out our approach to the project as a whole – well beyond this initial pilot. We have rephrased these and focussed on objectives to:

1. Recruit practices willing to take an additional sample for serology at the time of routine blood tests.
2. To collect at least 90 % of sample target from all age bands.
3. To send these samples to the serology analysis unit at Public Health England – receiving the samples sent
4. Linking the samples received, and their results, to the medical history in the medical record.

6. **The following phrase in the last objective does not really seem as feasible based on this pilot project: "make recommendations about how best to […] apply these approaches to other vaccine preventable infections". Influenza epidemiology is quite different from that**

of other vaccine-preventable diseases, and therefore, a seroepidemiology study for influenza would also need to be quite different from one for measles, for example, and would importantly need to be repeated yearly, or at least more often than one for measles, where one positive IgG can translate into prolonged, even life-long immunity, in the absence of the antigenic shifts and drifts that are characteristic for influenza viruses.

Authors' comments:

Thank you for your comment. This is really a long term aspiration of the project if successful. We have removed this statement to make our objectives more focussed.

STUDY DESIGN:

7. **Despite the fact that the manuscript talks about "representativeness" of the study population, the section on Population sampling is quite elusive, as it does not explain exactly what type of sampling will be employed. One can infer that convenience-based sampling would be applied, but it would be best if this were clearly specified. Furthermore, the sampling reference frame should also be described, i.e., will this be the patient registry of the 5-6 practices? And what would this amount to? Any expected differences between the practices? Which is the study catchment area? Will the sample size differ between practices based on the size of the population base of the practice?**

Authors' responses:

This is a very reasonable statement. We have sought to demonstrate we can collect across all age bands, in this pilot study. However, this is an important point to add. We have added to the **discussion** section as follows:

We set an arbitrary collection strategy across adult age-bands. However, this distribution is not representative of our practices populations' age distribution. Any full scale study would set out to represent the age-sex profile of the population and its geographical distribution.

8. **A verb is missing here: "Analysis carried out".**

Authors' comments:

Thank you for pointing this out. This has been corrected as follows:
Analysis will be carried out using the blood collected in RCGP RSC practices and sent to the Seroepidemiology Unit (SEU) archive.

9. **As someone working in infectious diseases, I am not comfortable with the term "opportunistic" used in the following phrase, and would therefore recommend rephrasing this sentence: "The SEU archive is an opportunistic collection".**

Authors' comments:

Thanks for this comment. In primary care "opportunistic" is used in this way – however we are happy to change the sentence as helpfully suggested. It is important we are readable by all audiences and it is likely this article / programme of work will be of greater interest to the infectious disease community.

10. **A clearer justification for the proposed sample size should be offered, currently the only information given is that "The number of samples is based on a combined list size of 50,000 across the participating practices"; "target number of samples. (minimum 100,**

maximum 150 per age band).” Furthermore, there is also the question of representativeness. Should this range of 100-150 patients sampled be uniform throughout the different age bands? This should be taken into account when planning the study, and the number of patients included per age band should be calculated in order to ensure representativeness for something: either the general population in the catchment area, or the population attending the practices, etc. While it is indeed useful to prove that sampling can be performed throughout age bands, it might not be necessary or useful to have the same number of subjects sampled in the different age groups.

Authors' response:

This is a pilot of a process of collection, sample delivery and data linkage. We accept there is scope to make our sampling more representative, as in the point about ages above. We have therefore added to the limitations section the following sentence, as this may be important for a more definitive study.

We set an arbitrary collection strategy across adult age-bands. However, this distribution is not representative of our practices populations' age distribution.

11. **The title for Table 1 presents the following information: “This table represents the week in which sample collection would be complete, if all patients consented.” It was my initial assumption that patients would be enrolled throughout the influenza season. However, upon revisiting the manuscript I did not find any indication of when exactly the project will be performed, and whether sampling will be continuous throughout the season. This information should be provided. The current statement in the manuscript is that “The project proposes to pilot a population-based seroprevalence survey, […] following the 2017/18 influenza season.” which, combined with the data in Table 1 suggests that all samples will be collected in a short time span (a minimum of 1 week and a maximum of 5 estimated weeks). Therefore, I do not see how this project would respond to the aim of measuring seroincidence, as mentioned in both the Abstract and the Objectives section of the full manuscript.**

Authors' comments:

Like many projects we regrettably have not been able to work to our intended timescale. We have continued sample collection through the current influenza season and will continue collection until the target has been reached.

LABORATORY ANALYSIS:

12. **Rephrasing is needed for: “The standard method for sending specimens to the laboratory is via a pathology request software”.**

Authors' comments:

Thank you. This has now been rephrased as follows:
General Practices use a pathology request software, such as the ICE system to send biological specimens to their local laboratory for testing.

13. **How soon will samples be sent following collection? Any issues worth mentioning related to storage of samples before and during transport to PHE's SEU/VEU lab?**

Authors' comments:

Thank you for your comment. Serum is extracted from blood samples received at the PHE SEU laboratory. This serum is stored in -80° C freezers and will be transported across to Colindale in a single batch once collection is complete. Samples will be sent using first class post so would take 1-2 days.

14. **“samples will be […] stored in archive freezers”. Further details regarding the type of freezers should be provided: -20? -80?**

Authors' comments:

Thank you for spotting this. Samples will be stored in -80° C serum archive freezers in SEU/VEU. This has also been added to the protocol.

> **15. For the following phrase "Additionally, analysis using influenza neutralisation and/or Neuraminidase Antibody Inhibition assays in the format of the Enzyme-linked Lectin Assay may be considered to investigate the functionality of the antibody further." Further information should be provided here, to describe how cases would potentially be selected for this type of analysis.**

Authors' comments:

Thank you for your comment. The samples will be sent from the archive in one batch to the analysing laboratory. Transport will be initiated promptly after completed collection of the targeted number of specimens.

The analysis will be performed by Haemagglutination Inhibition assays as a minimum – this analysis will be performed using representative vaccine and circulating strains. Where sample volume permits, additional analysis using influenza neutralisation and/or Neuraminidase Antibody Inhibition assays in the format of the Enzyme-linked Lectin Assay may be considered to investigate the functionality of the antibody further.

We have added the following to the Methods section:
Where sample volumes after completion of the HAI permit, additional analysis using influenza neutralisation and/or Neuraminidase Antibody Inhibition assays in the format of the Enzyme-linked Lectin Assay may be considered to investigate the functionality of the antibody further.

DATA EXTRACTION:

> **16. The following information is repetitive (with at least two other occurrences in the manuscript): "The RCGP RSC and PHE have worked together to provide state of the art National influenza surveillance for over 50 years"**

Authors' comments:

Thanks for spotting this repetitive statement we have kept the one in Introduction as this seems the more logical place to say this.

> **17. For the following phrase: "Practices are computerised, and data entered into computerised medical record systems either as coded data, or free text. We will extract the coded data" further information should be provided regarding the amount of data that would be expected to have been entered as free text and would therefore be lost for analysis.**

Authors' comments:

Thank you for spotting this. Practices have been asked to code 43L.. into patient record when a patient consents to providing a sample for Serology. Once this sample is received and processed by SEU, pseudonymised sample information is sent to the research team. The research team would then match the pseudonymised information sent by the lab, to the pseudonymised data extracted from the practice which would include this code.

We have added the following to the methods section to make this clearer:

Practices will code 43L. (Sample serology) into patient record when a patient consents to providing a sample for Serology. We will extract the coded data, and our results will be based on this element of the record. We will extract all coded data, pseudonymising as close to sources as possible.

STATISTICAL AND MODELLING ANALYSIS:

18. One of the elements to be evaluated is listed as: "Feasibility of extending the pilot approach to a wider number of practices and collection of additional sample types e.g. oral fluid ascertained". Further details should be provided regarding what is meant by "oral fluid" here.

Authors' comments:

Thanks for spotting this. Although alternatives were discussed early in the protocol development, we will be looking at the feasibility to extend this study to a larger number of practices. We have deleted the reference to oral fluid ascertained from the Methods and Analysis section.

19. For the following phrase: "University of Surrey will report the extent to which we have samples from common households – this might provide information about shared infections/levels of immunity within households" this should also be discussed as a potential study limitation, as including a large proportion of people from the same household, who are expected to have had the same type of exposure, would lead to potential sampling bias.

Authors' comments:

This is an important statement and possible limitation, but also a potential strength for example where household or within nursing home transmission takes place.

We have added the following to the strengths and limitations section of the discussion:

Collection from the same household may results in selection bias as it is likely that they will have had similar exposures, however it is also a possible strength of the network in trying to understand more about transmission within households or communal establishments, such as old peoples' homes.

20. Why would feedback "about the utility of the data provided" be provided to the patients? While it does make perfect sense to provide the practices with feedback on this, and to provide patients with the results of their serology test, informing them of the study progress would not need to be a standard procedure, it would suffice to have a phrase in their informed consent form stating who they can contact should they be interested in details on the study progress.

Authors' comments:

Thank you for pointing this out. This has now been removed from the protocol.

ETHICS:

21. The manuscript specifies that "PHE has ethical approval (05/Q0505/45) for the collection and use of unlinked and anonymised residual serum samples in cross-sectional antibody prevalence studies". However, since in this study a separate informed consent for a separate blood sample will need to be obtained, Ethics approval of this informed consent form would be needed, supplementary to the existing approvals.

Authors' comments:

Thank you for spotting this. We have added the following to ethics and dissemination:

Ethical approval was granted by the Proportionate Review Sub- Committee of the London – Camden & Kings Cross on 6 February 2018. This study received approval from Health Research Authority on 7 February 2018.

DISCUSSION

22. One of the study's strengths is presented as: "pilots a much lower cost method of establishing a serology bank". Costs are not listed as part of this study's objectives, and therefore a cost analysis cannot be the main strength of the project.

Authors' comments:

The reviewer is correct that we have no formal cost assessment so reasonable this is removed as a strength. However, we have made the following observation in the discussion.

Detailed serology surveys are also conducted, but these are extremely expensive.

VERSION 2 – REVIEW

REVIEWER	Oana Sandulescu Universitatea de Medicina si Farmacie Carol Davila, Infectious Diseases
REVIEW RETURNED	29-Dec-2018

GENERAL COMMENTS	I thank the authors for revising the manuscript and addressing all my previous comments. I now only have two minor queries related to the abstract, described below: - While the Discussion section now mentions the limitation related to representativeness, the abstract still states that the aim of the study is: "To pilot a method for provision of nationally representative serum samples" (different from the aim described in the full manuscript, which is: "To pilot a mechanism to undertake population-based surveys that collect serological specimens") - Why is the term "residual blood samples" used for the samples to be collected specifically for this study in the following phrase: "Approximately 100-150 residual blood samples will be collected from each of the following age-bands"?

VERSION 2 – AUTHOR RESPONSE

Reviewer 2 Comments

I thank the authors for revising the manuscript and addressing all my previous comments.

1. **While the Discussion section now mentions the limitation related to representativeness, the abstract still states that the aim of the study is: "To pilot a method for provision of nationally representative serum samples" (different from the aim described in the full manuscript, which is: "To pilot a mechanism to undertake population-based surveys that collect serological specimens")**

Authors' comments:
Thank you for pointing this out. These have now been corrected in the manuscript as the following:

"To pilot a mechanism to undertake population based surveys that collect serological specimens and associated patient data to measure sero-positivity and seroincidence due to seasonal influenza, and create a population based serology bank."

2. **Why is the term "residual blood samples" used for the samples to be collected specifically for this study in the following phrase: "Approximately 100-150 residual blood samples will be collected from each of the following age-bands"?**

Authors' comments: Thank you for pointing this out. These have now been corrected in the manuscript as the following:

"Approximately 100-150 blood samples will be collected from each of the following age-bands - 18-29, 30-39, 40-49, 50-59, 60-69, 70+ years."