

Guidelines for use of the Nurses' Health Study Blood Bank: External collaborators.

A. Submitting a Proposal to the Advisory Committee.

1. Any investigator wishing to develop a collaboration with the Nurses' Health Study (NHS) Research Group to use the NHS blood bank should first send a two-page description of the proposed analyses ("letter of intent") to Dr. Graham Colditz, Principal Investigator. If a project is judged feasible (given NHS biomarker and database resources), of substantial scientific interest, and is not currently under consideration by an NHS Investigator, the investigator will be invited to submit a detailed proposal to the NHS Advisory Committee. The format of the letter of intent and full proposal are described in detail below.

2. Letter of intent. The letter of intent should briefly outline the hypothesis being proposed, its significance, the reason for proposing use of NHS samples, the proposed laboratory assays, and any required covariate data. Letters of intent can be submitted at any time throughout the year. Within approximately 14 days, the applicant will be notified whether submission of a full grant proposal would be appropriate.

The reasons for proposing use of the Nurses Health Study (NHS) archive, rather than another data source, must be clearly described. Although the NHS archive is a unique resource, it is also finite. Therefore, the archive will be used only for analyses where other, less precious, blood collections cannot provide adequate or similar information. The assessment of markers of disease prognosis will generally not be considered an appropriate use of the NHS archive. In addition, proposals to evaluate highly speculative hypotheses are not considered appropriate and will not be approved by the Advisory Committee. Finally, laboratory analyses which are either already funded or have been proposed by NHS investigators will not be considered for approval by the Advisory Committee.

3. Study proposal. Full study proposals will be reviewed by the Advisory Committee three times per year. Submission deadlines are February 15, June 15, and October 15. The proposal's format should be similar to an NIH grant (i.e., specific aims, background and significance, preliminary studies and methods) but should be no longer than 10 pages in length. Before the Advisory Committee gives final approval, the following issues must be addressed to confirm that a particular association can be reasonably evaluated using NHS blood samples.

- (a) Stability of the biomarker for 24-48 hours in whole blood,
Blood samples from NHS participants were received in the NHS Laboratory as whole blood. Once received, they were centrifuged and aliquotted into plasma, buffy coat, and RBC components and archived in liquid nitrogen freezers. All samples were processed on the day they were received. The vast majority of blood samples were received and processed within 24-30 hours of collection; less than 3 percent were received more than 48 hours after collection. The majority of samples were kept cool (with a chill pack) during transport to our laboratory.
- (b) Appropriateness of using samples collected in sodium heparin tubes,
NHS samples were collected using sodium heparin as the anticoagulant. The laboratory needs to confirm that these tubes are routinely accepted for the analysis

of interest, otherwise a pilot study will need to be conducted to establish that sodium heparin will not interfere with assay performance.

(c) Laboratory assay to be used,

All assays must be conducted using the best available technology to insure that the appropriate parameter is assayed, the plasma volume required is minimized, and the assay reproducibility is maximized. The definition of "acceptable" plasma volume will be determined on a study-by-study basis and will depend in large part on the importance/priority of the study hypothesis. In the proposal, the applicant should be clear in describing the various assay methods currently available and their rationale for using the specific assay being proposed.

(d) Reproducibility of the laboratory assay,

The laboratory conducting the analyses must be able to conduct the assay with a high degree of precision (i.e., low coefficient of variation or high reliability coefficient). This information must be obtained through a blinded evaluation of the laboratory. Unfortunately, coefficients of variation provided by laboratory investigators are not sufficient, as, in our experience, these data do not always reflect the true magnitude of laboratory error. The evaluation must be recent and, if at all possible, should have been performed by the same technician who will be conducting the study analyses.

(e) Range of the biomarker in the NHS cohort

For many biomarkers of interest knowledge of a usual range in an adult population will be sufficient (e.g., plasma antioxidant levels); in this instance, the usual range and how this range was determined (i.e., in what population) should be briefly described. However, for other assays, where the range may vary substantially by population (e.g., plasma levels of DDE/PCBs), a pilot study to determine levels observed in the NHS may need to be conducted prior to receiving final approval for conducting a project. (The NHS has extra plasma samples from several hundred participants that might be available to address this issue).

(f) Stability of the biomarker over time (i.e., how well does a single measure reflect long-term blood levels),

In the NHS cohort overall, only one blood sample per participant has been collected. Thus data must be available which indicate that assay of a single blood sample will provide a sufficiently integrated measure of longer term exposure (generally the exposure of interest with chronic diseases) that an association between the biomarker and disease could reasonably be detected, if indeed one exists. An example of an assay that would not be appropriate would be luteinizing hormone; its pulsatile secretion results in such large peaks and valleys that a single sample will provide an extremely misclassified exposure measure. If these data are not already available, applicants should consider conducting a pilot study to assess stability over a minimum of a 4-week period.

5. It is anticipated that Advisory Committee decisions will be made four to eight weeks of proposal submission. The Advisory Committee will decide to accept, accept pending revisions,

or reject a proposal. For either of the latter two outcomes, a summary of the reasons for the Advisory Committee decision will be provided. An "accept pending revisions" will be given if the proposal has considerable scientific merit, yet one or more issues need to be addressed before the project can proceed. Arrangements will be made to provide an expedited review of a revised proposal, which addresses the concerns of the Advisory Committee.

For proposals that will require the development of funding outside the proposing organization, the approval process described above must be factored into the timing of any grant application. The Advisory Committee and NHS Investigators cannot take responsibility for missed deadlines.

B. Conducting Studies Using the NHS Archive.

1. The exact nature and scope of the project must be described in a written collaborative agreement and signed by the external collaborator, the primary NHS investigator, and a representative from each investigators institution. Use of biomarker data (or other covariate data) from the NHS cohort is limited to the defined, specific project for which the Advisory Committee approval was obtained. If further research or analytic activities develop from the original project, the external collaborator must obtain appropriate approval for such activities. In signing the collaborative agreement, external collaborators also will be confirming that they have read these guidelines ("Guidelines for use of the Nurses' Health Study Blood Bank") and both understand and agree to comply with them.

2. Since no funds have been allocated to manage the development of these outside collaborative arrangements, other than those associated with the Advisory Committee, all costs must be borne by the collaborating outside investigators institution. Unless the initial development and review of the proposal requires substantial data exploration to determine feasibility, it is not anticipated that this cost would exceed \$5000/proposal. The actual cost will be based on the time required of an NHS Investigator and programmer to determine approximate case and control numbers that might be considered appropriate for the proposed analyses and related exposure distributions.

3. Outside collaborators must provide a draft of any grant proposal (e.g., NIH grant) to the collaborating NHS investigator at least two months prior to the application due date. This will allow the NHS investigator an opportunity to provide feedback, and will provide time to obtain any additional data (e.g., other exposure distributions) that will maximize the probability of funding for the proposal. In keeping with policies of the Brigham and Women's Hospital, the final grant proposal must be reviewed by the Associate Co-Director of the Channing Laboratory (Graham A. Colditz, MD, DrPH) at least 10 business days before submission. Failure to meet this deadline will result in delay of submission. This institutional policy also is followed by all NHS investigators and cannot be circumvented. The primary NHS investigator will provide a letter of support to the external investigator to be included in the application indicating Nurses' Health Study interest in collaborating on the proposed study.

4. Study costs

(a) External collaborators must provide funds to cover the cost of retrieving, aliquotting

and shipping of specimens, receiving and cataloguing of returned specimens, data entry of results and for additional freezer space (necessitated by the aliquotting of samples). Funds also must be provided for the initial programming needed to identify case and control samples.

(b) In addition to the cost of the laboratory analysis of case-control samples, funds must be available to conduct (a) a test of laboratory reproducibility immediately prior to submitting any study samples if the previous assessment occurred 6 or more months in the past and (b) for quality control specimens to be analyzed along with the study samples (in approximately a 1:10 ratio).

(c) The cost of all pilot studies required to determine the feasibility and validity of the proposed project may be assumed by the potential external collaborator.

(d) At least one Nurses' Health Study investigator may be included as a co-investigator (with appropriate time commitment) on any grant proposal where use of NHS specimens is proposed. The level of effort will vary according to the size and complexity of the project but will usually be in the range from 5% to 10% FTE per year.

(e) To insure integrity of the Nurses' Health Study data, it is the policy of the NHS that no data leave the Channing Laboratory. Secondly, because of the complexity of the database and the NHS Investigators' knowledge of the strengths as well as the limitations of these data, substantial input is required of NHS Investigators to insure both valid and maximal use of the available data. For these reasons, a data analysis center (NDAC) is being created to provide data analyses for all outside collaborators. The outside collaborator in conjunction with the primary NHS investigator will draw up analysis plans; these plans will be given to the NDAC statistician who will oversee all analyses. To cover the costs of needed complex programming and data management, each study must include 5% FTE statistician time and 20% FTE programmer time.

(f) The arrangement for payments will be through formal subcontracts with the Brigham and Women's Hospital in which full overhead as approved by NIH will be considered a direct cost to the proposing institution cost base.

5. Human Subjects considerations

(a) All projects must receive approval from the Brigham and Women's Human Subjects Committee prior to implementation.

(b) As analyses of genetic susceptibility to disease are associated with complex ethical considerations, a full discussion of the ethical implications of these analyses must be part of the initial proposal. The NHS investigators and/or the Advisory Board would normally consult with the NHS Ethical Advisory Committee prior to seeking approval from the Brigham and Women's Human Subjects Committee. Investigators should be aware that analyses, which identify women at very high risk of disease, are particularly problematic in this regard.

6. Before any aliquotting of samples is begun, the programs used to generate cases and

controls must be carefully reviewed and signed off on by an NHS epidemiologist and NDAC statistician in addition to the study programmer and the external collaborating investigator. Importantly, the sign off must be by a NHS investigator who understands how the cases and controls are being defined, is familiar with NHS variable definitions, and can understand the code generated by the programmer. Laboratory assay of the wrong cases or controls, because of errors in their initial identification, can be very expensive and would waste a precious resource.

7. To the extent possible, all analyses will be conducted as a single batch with appropriate masked QC samples added to the batch. If, as is frequently the case, a large number of samples are being assayed in a study, the precision of the assay must be monitored on an ongoing basis using masked QC samples. Results from these QC samples must be reported on a batch-by-batch basis to the NHS investigator who will be responsible for monitoring reproducibility.

8. A proposed timeline for completion of aliquotting projects should be discussed prior to submission of any grant. All projects need to be completed within the constraints of the current NHS system. Although additional staff may be hired if they are needed consistently, it is not possible to substantially increase (and then decrease) staffing levels for any single project. NHS facilities do not allow for such staffing changes and it is not possible to adequately train new technicians in a sufficiently short period of time to allow such changes. At the beginning of a project, external collaborators should review with the NHS a proposed schedule for project completion and may contact the Blood Study Project Director to discuss study progress.

9. The external collaborator must agree to keep the NHS investigators updated on the progress of the study by providing either a written or verbal report at least every 6 months. Failure to adhere to a reasonable progress schedule (as assessed by the Advisory Committee) could lead to termination of the collaborative relationship with no further data tables or additional analyses provided.

10. Any plasma, DNA, or RBC sample remaining after the completion of the approved laboratory assays must be returned promptly to the NHS sample archive.

C. Data Analysis and Publication Issues.

1. The external collaborating investigator should forward all laboratory results to the Nurses' Health Study. All primary data sets of laboratory results will be maintained on the NHS SUN computer.

2. All data analyses will be conducted on the NHS SUN computer (see section B.3.d above). The most efficient way for these analyses to be accomplished will be for the outside investigator and the collaborating NHS investigator to agree on the analysis plan in advance (to whatever extent possible). Once the laboratory assays are complete and results sent to the NHS, the external collaborating investigator will provide to the NDAC statistician a set of data analysis requests and a series of empty tables that indicate how the results are to be presented. The NHS data analysis center (NDAC) will proceed to complete the analyses and return the completed tables to the collaborating investigator. In completing the analysis plan, the NHS investigator also will work as needed with the NDAC statistician in supervising the NHS programmer assigned to the project.

3. At least one member of the NHS Investigative team may be a coauthor on any manuscript resulting from this collaboration and, as such, will need to sign-off on any manuscript prior to its submission for publication. This will take the form of a brief note indicating review and approval of the final manuscript by the NHS Investigator; this note will be attached to the manuscript when sent for Channing Review. All manuscripts must be submitted for review to the Channing Laboratory and the Department of Medicine at the Brigham and Women's Hospital ("Channing Review"). This additional review also is required of all NHS investigators. External investigators should plan on the entire process taking at least 4 weeks (and longer if there are issues to be resolved concerning analysis or interpretation of the data). Any initial presentation of these data at meetings also must receive sign-off from the designated NHS collaborating investigator(s).

4. Any dispute regarding data interpretation may be brought to the Advisory Committee for consideration. Where appropriate, the Advisory Committee will seek additional consultation from independent experts. Since the Advisory Committee meets as a group only once per year, considerable delay in coming to a resolution could occur. Therefore, it behooves all collaborating investigators to work closely with the designated NHS investigator in resolving any dispute. Final decisions rest with Dr. Colditz, the NHS Principal Investigator, and must be justified with the Advisory Committee.