1. PURPOSE

    1.1. To define the procedure to be used for completion of departmental REB applications.

2. SCOPE

    2.1. This procedure applies to those individuals in the Department of Laboratory Medicine and Pathology who are required to complete REB applications for human research purposes.

    2.2. This procedure is focused on retrospective research studies on de-identified archived samples of excess diagnostic material, i.e. human studies with "minimal risk" not requiring additional interventions beyond standard of care, and thus no patient consent.

3. RESPONSIBILITIES

    3.1. It is the applicant's responsibility to understand the requirements of the REMO system at the University of Alberta to appropriately complete the REB application.

    3.2. It is the Supervisor's responsibility to oversee and countersign applications from trainees (post-doctoral fellows, graduate and undergraduate students).

4. NOTES

    4.1. None

5. REQUIRED DOCUMENTS

    5.1. None

6. PROCEDURE

    6.1. Background Information

        6.1.1. To use REMO, you must have a valid CCID and password. Consult your supervisor if you require access to the University of Alberta system.

        6.1.2. Prior to starting the application on REMO, ensure that you have reviewed the appropriate User Guides at https://remo.ualberta.ca/REMO/Rooms/DisplayPages/LayoutInitial?Container=com.webridge.entity.Entity[OID[87A7858B8B79684D8D4E9D0A57E307C3]] and if necessary, the online tutorials – human health research itemized at https://remo.ualberta.ca/REMO/Rooms/DisplayPages/LayoutInitial?Container=com.webridge.entity.Entity[OID[CA3B5D25CF74124583004C5AB9380BB4]].

        6.1.3. If you require further REMO training, sign up for one of the training sessions listed on the REMO home page at

https://remo.ualberta.ca/REMO/Rooms/DisplayPages/LayoutInitial?Container=com.webridge.entity.Entity[OID[AC482809EC03C442A46F2C8EEC4D75D3]].

6.1.4. To start an application on REMO, go to https://remo.ualberta.ca/REMO/Rooms/DisplayPages/LayoutInitial?Container=com.webridge.entity.Entity[OID[AC482809EC03C442A46F2C8EEC4D75D3]].

6.1.5. Please use one of the REMO compatible browsers (Internet Explorer, Google Chrome or Firefox).

6.1.6. If this is the first time you are logging on, click on "REGISTRATION" under the title "To Self Register" on the REMO home page. Please do not ignore this page as it is necessary for you to review the first time logon information before an application may be created.

6.1.7. The first time logon page may be suppressed by a pop-up blocker. In this case you should click the pop-up message banner, allow pop-ups for this site, and establish REMO as a trusted site. Instructions on how to establish REMO as a trusted site are also available in the folder "Internet Tips" at https://remo-test.ualberta.ca/SANDBOX/Rooms/DisplayPages/LayoutInitial?Container=com.webridge.entity.Entity[OID[1BCDDFE4A45F8F4D9554734B38D41304]].

6.1.8. On the first page of the "REGISTRATION" section:

6.1.8.1. Ensure that your demographic information is correct

6.1.8.2. Select the correct "Department/Employer" from the drop down list

6.1.8.3. Describe your role or position at the University, Covenant Health, or Alberta Health Services including unit of affiliation; if you are a student/trainee investigator, describe your home unit and/or program of study

6.1.8.4. Check off any "Additional Human Research Roles"

6.1.8.5. Check "yes" or "no" for the question "Are you a student (i.e. Medical Resident, Post-Doctoral Fellow, Graduate or Undergraduate Student)?"

6.1.9. Click on "Register" at the bottom of the page.

6.1.10. If all areas have been completed successfully, the login page to REMO will appear. Enter your CCID username and password to go to your REMO home page.

6.2. Once you have registered and signed into the REMO website, begin your application by clicking on "Create HERO Study" on the left hand side of the page.

6.3. Complete all questions marked by a red asterisk (*) as well as all relevant questions to ensure that the REB has enough information to complete the review of the application.

6.4. The following information (steps 6.5 to 6.28) should be completed for REB applications from the Department of Laboratory Medicine and Pathology and suggested wording, where applicable, has been provided. REMO questions are in normal type, information to enter is in bold type.

6.5. 1.1 Study Identification:

- 1.0 * Short Study Title: **Restricted to 250 characters**
- 2.0 * Complete Study Title: **Can be exactly the same as Short Study Title**
- 3.0 * Select the appropriate Research Ethics Board: **Check "HREB Biomedical"**
- 4.0 * Is the proposed research: **Check "Funded" OR "Unfunded"** depending on whether actually $$ from any source have been allocated specifically to this project
- 5.0 * Name of Principal Investigator: **Select e.g. "Mengel Michael" from the dropdown list**
- 6.0 Investigator's Supervisor: **Leave this blank**
- 7.0 * Type of research/study: **Select "Faculty/Staff Research"**
- 8.0 Study Coordinators or Research Assistants: **Leave this blank**
- 9.0 Co-Investigators: People listed here can edit this application but do not receive HERO notifications unless they are added to the study email list.

  Click on "Add" and select the appropriate individuals from the dropdown list. Continue to click "Add" until all required individuals have been added.
- 10.0 Study Team (Co-investigators, supervising team, other study team members): People listed here cannot edit this application and do not receive HERO notifications:

  Click on "Add" and enter at least the first and last name of the appropriate individuals. Continue to click "Add" until all required individuals have been added.

6.6. 1.2 Additional Approval

- 1.0 * Departmental Review: **Select "MH Laboratory Med & Pathology"**
- 2.0 Internal Review: **Leave this blank**

6.7. 1.3 Study Funding Information

- 1.0 * Type of Funding: Select the appropriate descriptors such as "Internal Funds (eg. Start-up funds, TLEF, Operational, etc)", "Grant (external)". **Select all that apply**.

  If OTHER, provide details: **Leave this blank**

- 2.0 * Indicate which office administers your award. *(It is the PI's responsibility to provide ethics approval notification to any office other than the ones listed below):*

  Select "University of Alberta - Research Services Office (RSO)"

- 3.0 * Funding Source
  - ♦ 3.1 Select all sources of funding from the list below: **Select the appropriate funding agencies; if not on the list, leave this blank and complete the next question**
  - ♦ 3.2 If not **available** in the list above, write the Sponsor/Agency name(s) in full (you may add multiple funding sources): **Insert the names of the funding agencies such as "Faculty of Medicine and Dentistry, start up funds" or "Division of Nephrology, UAH"**
- 4.0 * Indicate if this research sponsored or monitored by any of the following: **Select the appropriate entity or "Not applicable"**

  If applicable, indicate whether or not the FDA Investigational New Drug number or FDA Investigational Device Exception is required: *The researcher is responsible for ensuring that the study complies with the applicable US regulations. The REB must also meet particular review criteria and this application will likely receive full board review, regardless of level risk.* **Insert "Not applicable"**

6.8. 1.4 RSO Managed Funding

- 1.0 If your funds are managed by Research Services Office (RSO), select the Project ID and title from the list below to facilitate release of your study funds. *(Not available yet)*
- 2.0 * To connect your ethics application with your funding: provide all identifying information about the study funding – multiple rows allowed. For Project ID, enter a Funding ID provided by RSO/PeopleSoft Project ID *(for example, RES0005638, G018903401, C19900137, etc).* Enter the corresponding title for each Project ID.

  Complete the table with the appropriate Project ID, Project Title, Speed Code and Other Information as applicable if available, or leave blank if no funding is available

6.9. 1.5 Conflict of Interest

- 1.0 *Are any of the investigators or their immediate family receiving any personal remuneration (including investigator payments and recruitment incentives but excluding trainee remuneration or graduate student stipends) from the funding of this study that is not accounted for in the study budget? **Select "No"**
- 2.0 * Do any of investigators or their immediate family have any proprietary interests in the product under study or the outcome of the research including patents, trademarks, copyrights, and licensing agreements? **Select "No"**
- 3.0 Is there any compensation for this study that is affected by the study outcome? **Select "No"**

- 4.0 Do any of the investigators or their immediate family have equity interest in the sponsoring company? (This does not include Mutual Funds) **Select "No" or declare**
- 5.0 Do any of the investigators or their immediate family receive payments of other sorts, from this sponsor (i.e. grants, compensation in the form of equipment or supplies, retainers for ongoing consultation and honoraria)? **Select "No" or declare**
- 6.0 Are any of the investigators or their immediate family, members of the sponsor's Board of Directors, Scientific Advisory Panel or comparable body? **Select "No" or declare**
- 7.0 Do you have any other relationship, financial or non-financial, that, if not disclosed, could be construed as a conflict of interest? **Select "No" or declare**
- **If Yes is answered to any of the above questions, an explanation must be entered on this page of the REMO application and the REB may ask for a Conflict of Interest Declaration.**

6.10. 1.6 Research Locations and Other Approval

- 1.0 *List the locations of the proposed research, including recruitment activities. Provide name of institution or organization, town, or province as applicable. **Enter** "**University of Alberta Hospital (UAH)" or others if applicable**
- 2.0 *Indicate if the study will use or access facilities, programmes, resources, staff, students, specimens, patients or their records, at any of the sites affiliated with the following *(select all that apply):* **Select "Alberta Health Services Institutions and Facilities in the GREATER EDMONTON" or others if applicable**

  List all facilities or institutions as applicable: **Leave blank**
- 3.0 Multi-Institution Review
  - * 3.1 Has this **study** already received approval from another REB? **Select "No"**

    If "Yes" is selected, the following question will pop up:
  - 3.2 Indicate if the proposed research has already received ethics approval from other Research Ethics Board or institution. Choose all that apply: *(The University of Alberta has entered into formal reciprocity agreements with the REBs listed below. Because of this agreement, if you have <u>already</u> received approval from one of the REBs specified below. <u>Please UPLOAD the other REBs APPLICATION, APPROVAL and APPROVED CONSENT FORMS</u> to Section 7.1 (11.0). In doing this your study will be eligible for a delegated review instead of requiring full board review.)*

    Select the appropriate REB from the checklist (University of Calgary Conjoint REB, HREBA Cancer Committee, HREBA Clinical Trials Committee, HREBA Community Health Committee, University of British Columbia affiliated REB (UBC), University of Lethbridge (U of L), University of Saskatchewan REB, Other)

- **If "Other" is selected above, the following question pops up:**

  3.3 If OTHER, list the REB or Institution: **Click "Add" and insert the name of the REB or Institution**

- 4.0 Does this study involve pandemic or similar emergency health research? **usually Select "No" or "Yes" if applicable (e.g. ProvLab)**

  If YES, are you the lead investigator for this pandemic study? **Leave blank**

- 5.0 If this application is closely linked to research previously approved by one of the University of Alberta REBs or has already received ethics approval from an external ethics review board***(s)***, provide the HERO study number, REB name or other identifying information. Attach any external REB application and approval letter in Section 7.1.11 – Other Documents. **Insert the appropriate information in the text box such as "HERO Pro00034887 (Dr. Michael Mengel)"**

6.11. 2.1 Study Objectives and Design

- 1.0 Date that you expect to start working with human participants. **Click the calendar icon and select the appropriate start date**
- 2.0 Date that you expect to finish working with human participants, in other words, you will no longer be in contact with the research participants, including data verification and reporting back to the group or community: **Click the calendar icon and select the appropriate start date**
- 3.0 * Provide a lay summary of your proposed research suitable for the general public *(restricted to 300 words)***.** If the PI is not affiliated with the University of Alberta, Alberta Health Services or Covenant Health, please include institutional affiliation. **Insert an appropriate summary that includes Background, Aims, Proposal and Benefits**

  See Appendix 1 for example wording

- 4.0 * Provide a description of your research proposal including study objectives, background, scope, methods, procedures, etc) *(restricted to 1000 words)***.** Footnotes and references are not required and best not included here. Research methods questions in Section 5 will prompt additional questions and information. **Insert an appropriate description that includes Background, Methods and Procedures, Study Objectives, Hypothesis, Scope and Study Design,**

  See Appendix 1 for example wording

- 5.0 Describe procedures, treatment, or activities that are above or in addition to standard practices in this study area *(eg. extra medical or health-related procedures, curriculum enhancements, extra follow-up, etc)***: Insert** "**No procedures or activities in addition to standard of practice are necessary"**
- 6.0 If the proposed research is above minimal risk and is not funded via a competitive peer review grant or industry-sponsored clinical trial, the REB will require evidence of scientific review. Provide information about the review process and its results if appropriate. **Insert "Not applicable"**
- For clinical research only, describe any sub-studies associated with this application. **Insert "Not applicable"**

6.12. 3.1 Risk Assessment

- 1.0 * Provide your assessment of the risks that may be associated with this research: **Select "Minimal Risk - research in which the probability and magnitude of possible harms implied by participation is no greater than those encountered by participants in those aspects of their everyday life that relate to the research (TCPS2)"**
- 2.0 * Select all that might apply: **Ensure that all boxes are set to "No"**
- 3.0 * Provide details of the risks and discomforts associated with the research, for instance, health cognitive or emotional factors, socio-economic status or physiological or health conditions: **Insert appropriate text**

  See Appendix 1 for example wording
- 4.0 * Describe how you will manage and minimize risks and discomforts, as well as mitigate harm: **Insert "Not applicable"**
- 5.0 * If your study has the potential to identify individuals that are upset, distressed, or disturbed, or individuals warranting medical attention, describe the arrangements made to try to assist these individuals. Explain if no arrangements have been made: **Insert "Not applicable"**

6.13. 3.2 Benefit Analysis

- 1.0 * Describe any potential benefits of the proposed research to the participants. If there are no benefits, state this explicitly: **Insert appropriate text**

  See Appendix 1 for example wording
- 2.0 * Describe the scientific and/or scholarly benefits of the proposed research: **Insert appropriate text**

  See Appendix 1 for example wording

- 3.0 Benefits/Risks Analysis: Describe the relationship of benefits to risk of participation in the research: **Insert appropriate text**

  See Appendix 1 for example wording

6.14. 4.1 Participant Information

- 1.0 * Who are you studying? Describe the population that will be included in this study. **Insert appropriate text**

  See Appendix 1 for example wording

- 2.0 * Describe the inclusion criteria for participants (e.g. age range, health status, gender, etc.). Justify the inclusion criteria (e.g. safety, uniformity, research methodology, statistical requirement, etc). **Insert appropriate text**

  See Appendix 1 for example wording

- 3.0 Describe and justify the exclusion criteria for participants: **Insert appropriate text**

  See Appendix 1 for example wording

- 4.0 * Will you be interacting with human subjects, will there be direct contact with human participants, for this study? **Check "No"**

  If NO, is this project a chart review or is a chart review part of this research project? **Check "Yes"**

- 5.0 Participants

  How many participants do you hope to recruit *(including controls, if applicable).* **Insert appropriate number in text box**

  Of these how many are controls, if applicable *(Possible answer: Half, Random, Unknown, or an estimate in numbers, etc).* **Insert appropriate number or range of numbers**

  If this is a multi-site study, for instance a clinical trial, how many participants *(including controls, if applicable)* are expected to be enrolled by all investigators at all sites in the entire study? **Insert appropriate number otherwise leave blank**

- 6.0 Justification for sample size: **Insert appropriate text**

  See Appendix 1 for example wording

- 7.0 Does the research specifically target aboriginal groups or communities? **Check "Yes" or "No" as applicable to the research**

6.15. 4.2 Chart Reviews

- 1.0 Estimate the number of records you will access and provide the start and end dates of the data pull *(e.g. We will review approximately 300 charts from December 2005 to November 2009.)* **Enter appropriate text as per above example**
- 2.0 How will you receive the data: **Select "Data is coded by the study team and a key is maintained separate from the main data"**
- 3.0 If a member of the study team is pulling the data, does the individual normally have access to the records, eg for clinical purposes? **Select "Yes"**
- 4.0 Will individual patient consent be sought or is a waiver of consent required? If requesting a waiver of consent, describe why it is not reasonable, feasible or practical to obtain consent. **Insert the appropriate text**

  See Appendix 1 for example wording

6.16. 4.3 Recruit Potential Participants to 4.8 Aboriginal People: **Leave these sections blank**

6.17. 5.1 Research Methods and Procedures

- 1.0 * This study will involve the following *(select all that apply)*
  The list only includes categories that trigger additional page(s) for an online application. For any other methods or procedures, please indicate and describe in your research proposal in the Study Summary, or provide in an attachment: **Select "Health and Biological Specimen Collection or Use of Previously Collected Specimens"**
- 2.0 * Is this study a Clinical trial? (Any investigation involving participants that evaluates the effects of one or more health-related interventions on health outcomes? **Select "No"**
- 3.0 If you are using any tests in this study diagnostically, indicate the member(s) of the study team who will administer the measures/instruments: **Leave blank**
- 4.0 If any test results could be interpreted diagnostically, how will these be reported back to the participants? **Insert appropriate text**

  See Appendix 1 for example wording

6.18. 5.2 Clinical Trial to 5.10 Food, Nutrition and Nutraceuticals Information: **Leave these sections blank**

6.19. 5.11 Health and Biological Specimen Collection

- 1.0 * Indicate health or biological specimen(s) that will be collected *(for example, body tissues or fluids, be specific)***: Insert appropriate text**

  See Appendix 1 for example wording

- 2.0 * This study will involve the following *(select all that apply)***: Check "Secondary analysis of sample previously collected for clinical or research purposes"**

- 3.0 Explain how the specimen will be collected. **Insert appropriate text**

  See Appendix 1 for example wording

- 4.0 Explain HOW the specimen will be stored: **Insert appropriate text**

  See Appendix 1 for example wording

  Explain HOW LONG the specimens will be stored: **Insert appropriate text**

  See Appendix 1 for example wording

- 5.0 Explain WHERE the specimens will be stored *(e.g. include information if the specimens will be sent out of the province)***:** : **Insert appropriate text**

  See Appendix 1 for example wording

- 6.0 Specify all intended uses of collected specimen: **Insert appropriate text, such as "Total RNA and / or miRNA extraction from FFPE blocks. Pathology review of glass slides."**

6.20. 5.12 Registries and Databases (including Biobanks): **Leave this section blank**

6.21. 5.13 Biohazard Safety

- 1.0 ONLY FOR AMENDMENT OR RENEWAL**:** If this application is for the amendment or renewal of a pre-existing clinical study: have new biohazards and/or manipulations been added to the research that were not identified in the original study protocol? For a new study, please select "Not Applicable." **Select "Not applicable" as stated for new study or select "Yes" or "No" from checklist if amendment / renewal as appropriate**

  If you selected NO, this amendment or renewal is exempt from requiring further review by the EHS Biosafety Division and the original biohazard approval remains valid. You do not need to respond to any of the other questions in this section.

If you selected YES, this amendment or renewal is considered new research - please respond to question 2.0.

- 2.0 Will your research involve the use of one or more of the following? Provide a response for each item. **If you are required to answer this question for an amendment / renewal, select "Human clinical specimens, including blood or other body fluids, or primary culture of human cells"**

6.22. 5.14 Radiation Safety: **Leave this section blank**

6.23. 6.1 Data Collection:

- 1.0 * Will the researcher or study team be able to identify any of the participants at any stage of the study? **Select "Yes"**

- 2.0 Will participants be recruited or their data be collected from Alberta Health Services or Covenant Health or data custodian as defined in the Alberta Health Information Act? **Select "Yes"**

- 3.0 Primary/raw data collected will be *(check all that apply)***: Select Directly identifying information - the information identifies a specific individual through direct identifiers (e.g. name, social insurance number, personal health number, etc.)**

- 4.0 If this study involves secondary use of data, list all original sources: **Insert appropriate text, such as "Original sources are: pathology laboratory information systems, electronic and paper patient charts"**

- 5.0 In research where total anonymity and confidentiality is sought but cannot be guaranteed *(eg. where participants talk in a group)* how will confidentiality be achieved? **Insert "Not applicable"**

6.24. 6.2 Data Identifiers

- 1.0 * Personal Identifiers: will you be collecting - at any time during the study, including recruitment - any of the following *(check all that apply)***: Select "Surname and First Name, Full Date of Birth and Other". In "Other" insert, for e.g. "date or surgery, date of transplantation, date of transplant failure if applicable, date of biopsy" as applicable**

- 2.0 Will you be collecting - at any time of the study, including recruitment of participants - any of the following *(check all that apply)***: Select "Health Care Number, Other". In "Other" insert "pathology accession number" as applicable**

- 3.0 * If you are collecting any of the above, provide a comprehensive rationale to explain why it is necessary to collect this information: **Insert appropriate text, for e.g. "The rationale is to be able to link the histopathology diagnosis to clinical and laboratory data**

related to a specific biopsy/sample and follow-up."

- 4.0 If identifying information will be removed at some point, when and how will this be done? **Insert appropriate text**

  See Appendix 1 for example wording

- 5.0 * Specify what identifiable information will be RETAINED once data collection is complete, and explain why retention is necessary. Include the retention of master lists that link participant identifiers with de-identified data: **Insert appropriate text**

  See Appendix 1 for example wording

- 6.0 If applicable, describe your plans to link the data in this study with data associated with other studies (e.g within a data repository) or with data belonging to another organization: **Insert appropriate text, such as "The molecular expression results will be partially linked to other existing research databases in the area of native and transplant kidney diseases, e.g. to data under existing ethics protocols by Dr. M. Mengel (Pro00034887) and Dr. P. Campbell (Pro00003083)."**

6.25. 6.3 Data Confidentiality and Privacy

- 1.0 * How will confidentiality of the data be maintained? Describe how the identity of participants will be protected both during and after research. **Insert appropriate text**

  See Appendix 1 for example wording

- 2.0 How will the principal investigator ensure that all study personnel are aware of their responsibilities concerning participants' privacy and the confidentiality of their information? **Insert appropriate text, such as "All involved study personnel are also involved in patient care under AHS and thus underwent privacy training in that area. Any non-healthcare related personnel will be sent for privacy protection training courses if not previously completed."**

- 3.0 External Data Access

  - * 3.1 Will identifiable data be transferred or made available to persons or agencies outside the research team? **Select "No"**
  - 3.2 If YES, describe in detail what identifiable information will be released, to whom, why they need access, and under what conditions? What safeguards will be used to protect the identity of subjects and the privacy of their data. **Insert "Not applicable"**

- 3.3 Provide details if identifiable data will be leaving the institution, province, or country *(eg. member of research team is located in another institution or country, etc.)*. **Insert "Not applicable"**

6.26. 6.4 Data Storage, Retention, and Disposal

- 1.0 * Describe how research data will be stored, e.g. digital files, hard copies, audio recordings, other. Specify the physical location and how it will be secured to protect confidentiality and privacy. *(For example, study documents must be kept in a locked filing cabinet and computer files are encrypted, etc. Write N/A if not applicable to your research)* **Insert appropriate text**

  See Appendix 1 for example wording

- 2.0 * University policy requires that you keep your data for a minimum of 5 years following completion of the study but there is no limit on data retention. Specify any plans for future use of the data. If the data will become part of a data repository or if this study involves the creation of a research database or registry for future research use, please provide details. *(Write N/A if not applicable to your research)* **Insert appropriate text, such as "The data may be used in an anonymous, de-identified way as a reference data set for developing a consensus molecular diagnostic standard."**

- 3.0 If you plan to destroy your data, describe when and how this will be done? Indicate your plans for the destruction of the identifiers at the earliest opportunity consistent with the conduct of the research and/or clinical needs: **Insert appropriate text, such as "At this point there are no plans to destroy the data."**

6.27. 7.1 Documentation: **Leave all sections blank except for Study Budget. Upload the Study Budget under Question 11.0 - Other by first clicking the "Add" button. When the dialog box appears, type in an appropriate name (e.g. "Study Budget") for the file. Click the "Browse" button to locate the file and select it, then click "OK" to add the file. If uploading more than one file, click the "OK and Add Another" button and continue as above.**

6.28. When all documents are uploaded at the 7.1 Documentation page, click on "Continue" to go to the final page. Click on "exit" to return to the study workspace.

(NOTE: At this point, the application information is complete – for submitting the application to REMO press "Submit Study" on the left in the Main Menu.

Appendix 1 Example Wording

2.1 Study Objectives and Design Summary – for question 3.0:

Background: The kidneys are targets for antibody-mediated injury in numerous autoimmune diseases such as systemic lupus erythematosus (SLE), cryoglobulinemia, and anti-neutrophil cytoplasmatic antibody (ANCA)-associated small vessel vasculitis. This process of native kidney injury is similar to that of antibody mediated rejection (ABMR) in transplanted kidneys. Powerful immunosuppressive drugs with significant side-effects are usually needed to treat these autoimmune diseases. Histopathology of small biopsy specimens taken from the affected kidney is the current diagnostic gold standard, but is of limited accuracy in detecting and measuring these disease processes. Thus, patients are often over-treated or under-treated. We want to improve the diagnostic precision by translating and validating more accurate molecular diagnostics as part of routine pathology assessment, so that patients can be managed more effectively.

Aims:
1. To assess and refine the diagnostic value of molecular signatures described in the literature as being associated with native kidney injury response;
2. To validate these molecular signatures as diagnostic tests;
3. To assess the reproducibility of the related laboratory technologies and platforms in a multicenter diagnostic setting;

Proposal: Based on data in the literature and from our previous research, we have generated a molecular biomarker signature for antibody-mediated kidney tissue injury. This has been developed and validated in kidney transplants as the role model and we foresee the potential to translate its utility to antibody-mediated diseases in native kidneys. Using recently-available technologies that allow rapid and cost-effective gene expression assessment, we propose to test this molecular signature in routinely-processed native kidney biopsies with antibody-mediated autoimmune diseases in relation to histological criteria and clinical outcomes.

Benefits: Translating recent molecular discoveries, into routine pathology patient care offers enormous potential to significantly improving diagnostic accuracy in native kidney diseases. More precise diagnoses will allow more specific and effective therapy and therefore improve patient care.

2.1 Study Objectives and Design Summary – for question 4.0:

Background: Many diseases are currently diagnosed by histopathology. Despite its strengths, histopathology has limitations including a reliance on subjective assessment of paraffin sections and arbitrary classifications of lesions. Further, diagnosis by histopathology frequently lacks direct relationship to function or biology, and is poorly reproducible. Therapeutic decisions are compromised by these limitations of precision in diagnosis, with under- or over-treatment as the consequence.

Kidneys are well suited for validating new molecular diagnostics in the areas of inflammatory/immune (non-cancer) diseases because they have abundant biopsies, standardized consensus histopathology scoring systems, and extensive clinical and outcome data. The histological classification systems for native kidney diseases suffer from arbitrary rules and subjectivity in application. This leads to limited reproducibility and significant risk for misclassification with consequent suboptimal treatment of individual patients. *Therefore, the ability to more precisely stage native kidney diseases, particularly those related to antibody mediated injury, will allow for improved patient care by better guiding personalized therapy*.

Methods and Procedures: Since the completion of the human genome project and advent of high-throughput genomics platforms, numerous discovery studies have described the molecular phenotype of various diseases. However, only a few diagnostic molecular tests based on these tremendous discoveries have been translated into routine clinical use.

Using microarrays ('gene chips') for whole genome expression analysis, comprehensive gene lists have been published in relation to standard histopathology lesions in human kidney biopsies. These large-scale, genome-wide studies have shown that the molecular phenotype of certain disease states is highly stereotyped, i.e. hundreds of molecules change their expression profile in a coordinated fashion. Therefore, only a few 'top' molecules will very likely suffice to assess the molecular phenotype in the tissue. For example, Sis et al. described the increased expression of 117 endothelial cell-associated transcripts in antibody-mediated allograft rejection (ABMR) and Hidalgo et al. recently described sets of transcripts associated with the presence of donor-specific anti-HLA Antibodies (DSA) as the causative agent for ABMR. In addition, the Reed laboratory demonstrated the potential diagnostic utility of mammalian target of rapamycin (mTOR) effectors, phosphorylated 70 S6-kinase, and phosphorylated S6 ribosomal protein expression in heart allograft biopsies with ABMR. Based on the above discoveries that endothelial, NK cell, and certain inflammatory transcripts are primarily associated with the presence of antibody-mediated tissue pathology, we generated a literature-selected list of 14 genes based on ROC curve analysis for predicting antibody mediated tissue injury. We hypothesize that quantification of this gene set in native kidneys with crescentic and proliferative glomerulonephritis will provide clinically-relevant diagnostic and prognostic information.

The NanoString nCounter Analysis System, a high-throughput gene expression platform that, in contrast to other high-throughput gene expression platforms, works on archival formalin-fixed, paraffin embedded (FFPE) diagnostic tissue samples, recently became available. Comparison of the NanoString nCounter gene expression system with microarrays and TaqMan PCR has shown it to be more sensitive than microarrays and similar in sensitivity to real-time PCR. A recent Nature Medicine paper reported successful gene expression profiling of FFPE archival tissues of breast cancer samples. As a result, in September 2013, the FDA approved the Prosigna Breast Cancer Prognostic Gene Signature Assay, which utilizes the NanoString nCounter platform, as standard-of-care.

Therefore, we think that the NanoString molecular profiling system will enable translation of molecular signatures from the discovery stage to routine clinical practice and patient care, allowing for increasingly

personalized diagnostics and therapeutics. Robust performance assessment of novel molecular diagnostics would typically require prospective study design. The unique ability of the NanoString system to function reliably in FFPE archival material, however, allows us to perform robust validation studies in a retrospective setting. This represents a true *'game changer'* for molecular diagnostics and opens the avenue for direct translation of recent 'omics' discoveries into routine diagnostics. We have access to the NanoString nCounter platform at the Pathology Translational Core of the Department of Laboratory Medicine and Pathology at the University of Alberta.

Study Objectives:

1. To compare the expression of molecular injury signatures in biopsies from patients with crescentic glomerulonephritis in the setting of ANCA-associated small vessel vasculitis with:
    a. biopsies with antibody-mediated glomerulonephritis but without crescent formation;
    b. biopsies with proliferative immune complex glomerulonephritis (e.g. IgA, post-infectious, membranous glomerulonephritis) but without crescent formation;
    c. biopsies without crescentic or proliferative glomerulonephritis (e.g. minimal change, hypertension, diabetes).
2. To correlate the gene set expression data with histological consensus classification scores, response to treatment, organ function, and outcome.
3. To conduct technical replication experiments on the NanoString platform to assess the reproducibility of the assay.
4. To conduct a retrospective multicenter study as validation for the single center results.

We hypothesize that quantitative molecular assessment of refined sets of transcripts associated with specific inflammatory, immune, and/or injury related disease processes in kidneys can provide relevant diagnostic and disease-staging information in native kidney biopsies with crescentic and proliferative glomerulonephritis processed as standard-of-care, i.e. formalin fixed and paraffin embedded.

Scope and Study Design:

We will conduct a retrospective single center study to address the first three objectives. From the archives of the University of Alberta Hospital, Division of Anatomical Pathology, we will recruit 100-150 renal biopsy specimens from patients with crescentic glomerulonephritis in the setting of ANCA-associated small vessel vasculitis. Biopsies will be identified through the natural language search function in our laboratory information system. Corresponding treatment and clinical outcome data will be retrieved from electronic medical records. Data will be deindentified after linking to gene expression results but prior to analysis. Analogously, 30-50 biopsies meeting the criteria for each of the three previously-described comparison groups will also be recruited.

For the multicenter validation study, we will utilize the existing network of the European Vasculitis Study Group through our collaborator Dr. Ingeborg Bajema in Leiden, Holland. Archived, formalin-fixed, paraffin-embedded, clinically well-annotated specimens procured during the large European multicenter vasculitis studies will be recruited according to the study groups described above.

3.1 Risk Assessment – for question 3.0

Only excess ('left-over') diagnostic material from patients for which biopsies or surgery were previously performed as standard-of-care will be approached for the project. Furthermore, all diagnostic procedures based on these tissue specimens will have been completed, i.e. only specimens will be included for which pathology reports have been signed out with a final diagnoses as part of routine patient care. Only excess diagnostic tissue derivates, i.e. paraffin sections will be used for the proposed studies. There is therefore no additional risk to patients since no invasive procurement of additional research material will be done.

In cases where all excess diagnostic material is utilized for RNA extraction in this research and where additional diagnostic procedures might be required afterwards, remaining unstained slides as part of the standard diagnostic slide panel will be available in the archives of the Division of Anatomical Pathology at the University of Alberta Hospital.

3.2 Benefit Analysis – for question 1.0

The information obtained from this study may improve diagnosis and thus patient care. The ultimate goal of this research is to improve patient care through more precise diagnoses of disease processes. However, no immediate or future health benefit is guaranteed from participating in this study; nor will study patients benefit financially through claims to any products developed in future years as a result of any findings from this study.

3.2 Benefit Analysis – for question 2.0

Translating recent molecular discoveries into routine pathology patient care offers enormous potential to significantly improve diagnostic accuracy in native kidney diseases. More precise diagnosis will allow for more specific and effective therapy in individual patients and therefore improve patient care. The findings from this study will be published in peer-reviewed literature and presented to international nephropathology groups that represent the consensus process for diagnostic standards in kidney pathology.

3.2 Benefit Analysis – for question 3.0

Given that there is essentially no additional risk to the patient and only potential benefits, the balance is shifted towards obtaining benefits.

4.1 Participant Information – for question 1.0

Only excess ('left-over') diagnostic material from patients for which biopsies or surgery were previously performed as standard-of-care will be approached for the project. FFPE specimens of native kidney biopsies will be recruited from the University of Alberta Hospital archives at the Division of Anatomical Pathology. Only samples with residual diagnostic biopsy material available after all standard-of-care diagnostic procedures have been completed will be used. Samples will be selected according to original diagnoses and availability of residual FFPE material after completion of standard-of-care diagnostic work-up.

4.1 Participant Information – for question 2.0

Patients (adult or pediatric) who have undergone standard-of-care histological pathology work-up for FFPE native kidney biopsy or nephrectomy at the University of Alberta Hospital, with the following histological findings:

a. crescentic glomerulonephritis in the setting of antibody mediated small vessel vasculitis (e.g. ANCA-associated, SLE and others);
b. antibody-mediated glomerulonephritis without evidence of crescent formation;
c. proliferative immune complex glomerulonephritis (e.g. IgA, post infectious, membranous glomerulonephritis) without evidence of crescent formation;
d. native kidney disease without crescentic or proliferative glomerulonephritis (e.g. minimal change, hypertension, diabetes).

Archival FFPE biopsies for this study will also be recruited from external collaborators. These will be subject to IRB approval from the relevant center and the transfer of materials will be handled through appropriate material transfer agreements.

4.1 Participant Information – for question 3.0

Samples with no residual FFPE material will be excluded.

4.1 Participant Information – for question 6.0

Per year, approximately 300-350 native kidney biopsies are histologically assessed at the University of Alberta Hospital. Samples will be selected according to original diagnoses and availability of residual FFPE material after completed diagnostic work-up as part of standard-of-care. The proposed sample sizes are necessary to achieve sufficient statistical power for class comparisons of gene expression between different native

4.2 Chart Reviews – for question 4.0

Since the research will be performed retrospectively on derivatives (FFPE blocks) of excess diagnostic tissue specimens after all patient care activities have been completed, we request to waive consent.

5.1 Research Methods and Procedures – for question 4.0

The research results can potentially be used diagnostically after multicenter validation, but will not be reported back to the individual participants during the period of this study.

5.11 Health and Biological Specimen Collection – for question 1.0

No additional specimens will be collected. Only existing archival FFPE specimens (blocks and slides) will be used.

5.11 Health and Biological Specimen Collection – for question 3.0

From the archives of the University of Alberta Hospital, Division of Anatomical Pathology we will recruit 100-150 renal biopsy specimens from patients with crescentic glomerulonephritis in the setting of ANCA associated small vessel vasculitis. Analogously, 30-50 biopsies meeting the criteria for each of the three previously-described comparison groups will also be recruited. Biopsies will be identified through the natural language search function in our laboratory information system. A retrospective time frame of ten years will be used because this is the period of time for which FFPE specimens are archived on site at the UAH. Biopsy samples for routine diagnostic purposes were processed at the Department of Laboratory Medicine and Pathology, University of Alberta Hospital, Alberta Health Services following established routine processes for formalin-fixation, paraffin-embedding, cutting and staining as part of the standard-of-care diagnostic process.

After final sign-out of the respective biopsy report by the local pathologist, all stained routine paraffin slides will be collected by the PI for central pathology re-review. An extended 'research read' of histology lesions will be performed, i.e. a more detailed documentation of histological features will be directly entered into the database. All stained slides received will be returned to the Department of Laboratory Medicine and Pathology after research pathology review.

In parallel, the corresponding FFPE blocks of included biopsies will be retrieved from the archives of the Division of Anatomical Pathology at the UAH by trained laboratory staff working for the Translational Pathology Research Core at the Department of Laboratory Medicine and Pathology. These pathology core staff members are certified laboratory technologists fully familiar with the AHS divisional SOPs for FFPE block retrieval and refiling.

After retrieving the FFPE blocks, the certified staff will transport the specimens to the departmental Translational Pathology Core lab at 3-012 Li Ka Shing Research Center on university campus. At the pathology core laboratory, the FFPE blocks will be worked-up for RNA extraction. The pathology core lab

is an area with restricted access and the door to the lab is always locked with only immediate staff and investigators having access.

All personal identifiable patient information will be removed once the archival FFPE samples are transported to the Pathology Core lab and patient/sample data has been entered into the database. At this point, every patient will be assigned a unique identifier (code number). In order to link the specimen data with related clinical and follow-up data for each patient, the code number will be entered into a separate log file, together with the patient name. Only the study coordinator and PI will have access to this log file, which is on a password protected IronKey drive always kept locked safely in the PI's office.

5.11 Health and Biological Specimen Collection – for question 4.0

HOW specimen stored:

The specimens (slides and blocks) are stored in the Pathology Research Core lab 3rd floor Li Ka Shing Research Center (room 3-012). The 3rd floor Li Ka Shing is protected by restricted access with code cards. Furthermore, the room in which the FFPE blocks are stored is permanently locked and only immediate core lab staff and investigators have access. In the locked room, the FFPE blocks are stored in a locked block filing cabinet.

HOW LONG specimen stored:

The glass slides for central pathology research review will be returned immediately after the research read is completed. The FFPE blocks will be stored at the Pathology Core lab 3rd floor Li Ka Shing for the time it takes to extract sufficient RNA.

If material is left-over in the FFPE block after RNA extraction, the certified lab technologist will return the blocks to the archives in the Division of Anatomical Pathology UAH.

In cases where no residual material is left in the FFPE blocks after RNA extraction, the 'empty' FFPE block will also be returned by the certified lab technologist to the archives in the Division of Anatomical Pathology UAH in order to assure completeness of the block archives. In rare cases where additional diagnostic stains are required, remaining routinely performed unstained slides will be available in the Division of Anatomical Pathology as part of the routine diagnostic slide panel.

6.2 Data Identifiers – for question 4.0

For success of the project, relating gene expression data from a biopsy to the corresponding clinical, laboratory and follow-up/outcome data is crucial.

In collaboration with the clinical sub-investigators on this study protocol, primary and raw patient data will be retrieved from various sources (hard copies and electronic patient medical charts, laboratory information systems) by a study clerk or fellow supervised by the PI and the subinvestigators or the

investigators themselves. All personal identifiable patient information will be removed once the archival samples are transported to the research lab and patient/sample data is entered into a database. At this point, every patient will be assigned a unique identifier (code number). In order to collect future follow-up data for each patient, the code number will be entered in a separate log file, together with the patient name and health care number. Only the study coordinator and PI will have access to this log file, which is on a password-protected IronKey drive always kept locked safely in the PI's office.

Patient follow-up and updates with identifiable information will be done by the investigators and/or supervised study clerk or fellow through review of patient charts and databases after regular patient visits as a part of standard clinical care. Clinical, pathology, and expression data, as well as follow-up data, will be entered, linked, and stored in an anonymous fashion in a separate database operated on servers protected behind University of Alberta firewalls. Data extracts from the database will be done via the unique code number. No patient sensitive information will be stored in the database.

The co-investigators, lab researchers, lab assistants, fellows, and other lab personnel will not be able to link patient/sample study identifier or the stored data with any information disclosing the identity of the patient. These individuals will work with the database extracted data in an anonymous fashion only. Furthermore, there will be no disclosure of confidential information to any third parties (e.g. external collaborators, industrial partners, study sponsors). No identification or subject names will circulate within internally or externally published documents.

6.2 Data Identifiers – for question 5.0

Once data collection is completed, no identifiable information will be retained (see above) in the main research database. Identifiable information is stored on a separate, password-protected IronKey drive which is locked in a separate location.

6.3 Data Confidentiality and Privacy – for question 1.0

All personal identifiable patient information will be removed once the archival samples are transported to the research lab and patient/sample data has been entered into the database. At this point, every patient will be assigned a unique identifier (code number). In order to collect future follow-up data for each patient, the code number will be entered in a separate log file, together with the patient name and health record number. Only the study coordinator and PI will have access to this log file, which is on a password-protected IronKey drive always kept locked safely in the PI's office.

Patient follow-up and updates with identifiable information will be done by the investigators and/or a supervised study clerk or fellow. The research lab is in an area with restricted access to the floor and in a room which is permanently locked and to which only the PI, sub-investigators, and directly supervised research personnel have access.

Clinical, pathology, and expression data, as well as follow-up data, will be entered and stored in an anonymous fashion in a separate database operated on servers protected behind University of Alberta

firewalls. Data extracts from the database will be done via the unique code number. No patient sensitive information will be stored in the database.

The co-investigators, lab researchers, lab assistants, and other lab personnel will not be able to link patient/sample study identifier or the stored data with any information disclosing the identity of the patient. These individuals will work with the database extracted data in an anonymous fashion only. Furthermore, there will be no disclosure of confidential information to any third parties (e.g. external collaborators, industrial partners, study sponsors). No identification or subject names will circulate within internally or externally published documents.

6.4 Data Storage, Retention, and Disposal – for question 1.0

To be able to collect future follow-up data for each patient, the code number will be entered into a log file, together with the patient name and health record number. Only the study coordinator and PI will have access to this log file, which is on a password-protected IronKey drive always kept locked safely in the PI's office (3rd floor Li Ka Shing Center). The research offices and lab are located in an area with restricted access to the floor and in separate rooms that are permanently locked and to which only the PI, sub-investigators, and directly supervised research personnel have access.

Clinical, pathology, and expression data, as well as follow-up data, will be entered and stored in an anonymous fashion, i.e. linked through the study specific code number in a separate database operated on servers protected behind University of Alberta firewalls. Data extracts from the database will be done via the code number. No patient sensitive information will be stored in the database.