Effects of exposure to per- and polyfluoroalkyl substances (PFAS) on innate and adaptive immune responses to tetanus-diphtheria (Td) vaccination among adults in a community-based panel study

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RESEARCH PROTOCOL TEMPLATE
(HRP-503a)

STUDY INFORMATION

- **Title of Project:**
  Effects of exposure to per- and polyfluoroalkyl substances (PFAS) on innate and adaptive immune responses to tetanus-diphtheria (Td) vaccination among adults in a community-based panel study

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1.0 Research Design

1.1 Purpose/Specific Aims

The purpose of the study is to investigate how exposure to Perfluorononanoic acid (PFNA) modifies immune responses to vaccination. We hypothesize that PFNA suppresses the innate immune response to vaccine stimulus, consequently leading to a decrease in antibody production. To assess relationships between exposure to PFAS and innate and acquired immune responses to a vaccine stimulus, we will use a combined approach involving omics technologies and traditional immunological assays. This study is a supplement to an existing protocol ("Human health effects of drinking water exposures to per- and poly-fluoroalkyl substances (PFAS): A multi-site cross-sectional study", Pro2020001052).

A. Objectives

1. Evaluate associations between PFAS levels, markers of innate immune activation, and changes in specific antibody levels.
2. Investigate the relationship between PFNA levels and modifications in immune specific transcriptomic and metabolomic responses to vaccination.

B. Hypotheses / Research Question(s)

We hypothesize that increasing PFNA exposure is associated with a suppressed innate immune, measured as pro-inflammatory cytokines and CRP, which leads to a diminished antibody response to vaccination. We aim to use transcriptomic and metabolomic pathway analysis to identify cellular responses that support this hypothesis.

1.2 Research Significance

Exposure to per- and polyfluoroalkyl substances (PFAS), recognized as "forever" chemicals due to their persistent nature, poses a significant threat to public health. With more than 98% of Americans having detectable levels of PFAS in their blood, these compounds have been linked to immune suppression by observations of increased infectious disease risks and diminished antibody response to vaccination, including routine tetanus-diphtheria vaccination in children [1-15]. Previous studies have demonstrated differences in the time course of production of specific IgG following tetanus-diphtheria (Td) vaccination in a small study of 12 adult participants over 7 time points in a 30-day period [6]. The proposed study will focus on exposure to Perfluorononanoic acid (PFNA) in Paulsboro, NJ, and add measurement of innate immune responses following the vaccine stimulus. The results of this study will increase knowledge about immunotoxicity resulting from PFAS exposure, which has been determined to be the critical effect for human health risk assessment by the U.S. EPA (Environmental Protection Agency) and EFSA (the European Food Safety Authority) [16,17]. The most recent scientific reviews of immunotoxic PFAS endpoints highlight the need for more relevant longitudinal human studies, and probing the immune response through vaccination has been a standard approach [18-20]. We will measure several markers of immune response in serum and oral fluid samples using standard immunoassays and targeted metabolomic analysis. An innovative aspect of our approach is the pilot testing of oral fluid collection as a less burdensome method, compared to venipuncture, for serial measurement of immune responses in epidemiological studies. Oral fluid (OF) is a biofluid that is a mixture of saliva and non-salivary components, which contains both innate and adaptive immune components, including specific immunoglobulins and cytokines [21-30]. OF has been shown to be a reliable biomatrix for detecting antibodies that represent serum concentrations, even though antibodies in this fluid are often found in lower concentrations when compared to serum. If found to be feasible and reliable in this study, this method will be useful in future community-based studies of immune response where repeated blood draws can often be a major barrier. Overall, this research project is crucial step in developing rational and effective measures to protect public health in the face of widespread PFAS exposure.

1.3 Research Design and Methods

This study is a pre-post clinical trial. We will assess the impact of PFAS immune responses to routine booster vaccination against Tetanus-Diphtheria (Td) over a 30-day period among 20 adults recruited from a community in New Jersey that had drinking water contaminated with PFAS, predominantly PFNA. Within the larger study, 775 adults had serum PFAS measurements between July 2021 and September 2023. ("Human health effects of drinking water exposures to per- and poly-fluoroalkyl substances (PFAS): A multi-site cross-sectional study", Pro2020001052). For this study, we will recruit participants from the participants in the larger study who agreed to be contacted for future studies.

A. Research Procedures

All study visits will take place in the local Rutgers study office space (541B Mantua Avenue, Paulsboro, NJ) used for the main study. At study visit 1, participants will then have their blood drawn (15 mL) by venipuncture by trained research staff. Total amount of blood collected at visit one will be 15 mL (1 tablespoon). Participants will self-administer their oral fluid collection using the Oracol sampler (https://www.malmed-oracol.co.uk/products/oracol/) by swabbing their gumline for 1-2 minutes under the supervision of research staff. After baseline sample collection, participants will be given a Td booster vaccination. The second, third, and fourth study visits will occur 24, 48, and 72 hours after the first visit to capture the innate immune response, while visits five, six, and seven will take place 7, 14, and 30 days after the first visit to capture the adaptive immune response. At study visits 2-7, participants will have venous blood drawn (15 mL each visit) and will collect their oral fluid using the Oracol sampler by swabbing their gumline for 1-2 minutes under the supervision of research staff.

Additionally, data collected about the subject collected in the main study (including serum PFAS levels, residential history, and history of Td vaccination) will be used in this study.

B. Data Points

Td specific antibody levels, cytokine levels, CRP, RNA transcripts and metabolites related to immune response and activation.

C. Study Duration

The study will take approximately one year. Individual participation will consist of 7 study visits over a 30-day period. The initial study visit should take less than 45 mins including the consent process and post-immunization observation period, while study visits 2-7 should take 15 minutes. Length of participation for each subject will be 30 days.

D. Endpoints-NA

1.4 Preliminary Data – NA

1.5 Sample Size Justification

Twenty subjects will be enrolled. Identifying exposures predicting immune and disease outcomes requires sufficient power to account for false positives due to multiple comparisons. The goal of 20 adult subjects for the vaccination study was selected after statistical power calculations using data from the modeled Kielson (2016) study were done. Assuming the Kielson et al. correlation of 0.761 between PFAS level and change in diphtheria antibody concentration, then 20 subjects we would have 98.5% power to detect that the correlation is different from zero. Thus, we should have adequate power to detect effect sizes similar to those reported by Kielson even with some attrition and participant drop out. We may see a larger correlation as we expect a larger spread in our distribution of PFAS, yielding greater power. However, even with a smaller correlation of 0.7, we would have 95% power.

1.6 Study Variables

A. Independent Variables, Interventions, or Predictor Variables
Intervention: Participants will be immunized with TENIVAC, which is FDA approved booster vaccine against Tetanus and Diphtheria. Td antibody levels will be measured to determine post-vaccination antibody status.

B. Dependent Variables or Outcome Measures
Tetanus Toxoid IgG, Diphtheria Toxoid IgG, Cytokines, and CRP concentrations in serum and oral fluid. RNA sequencing, and LC/GC-HRMS to identify transcripts and metabolites of immune response in serum.

1.7 Drugs/Devices/Biologics

A. Schedule and Administration
Each subject will receive a Td vaccination at the first study visit following consent and distribution of vaccine information sheet (VIS). TENIVAC (Tetanus and Diphtheria Toxoids Adsorbed) is an active immunization for the prevention of tetanus and diphtheria in persons 7 years of age and older. TENIVAC will be administered intramuscularly as recommended in the subject's deltoid muscle at a dose of 0.5 mL which contains 5 Lf of Tetanus Toxoid and 5 Lf of Diphtheria Toxoid. Other ingredients per 0.5 mL dose include 1.5 mg of aluminum phosphate (0.33 mg of aluminum) as the adjuvant and ≤5.0 mcg of residual formaldehyde. Vaccinations will be administered only by medically qualified personnel.

B. Drug/Device Accountability and Storage Methods
TENIVAC, purchased as either single dose vials or prepared syringes will be stored at the local study site in a refrigerator between 2° to 8°C (35° to 46°F) per manufacture recommendation and secured behind a locked door only accessible to study staff. Detailed logs for vaccine storage, administration, and refrigerator temperature will be kept. Just before use, the single-dose vial or syringe will be shaken well until a uniform, white, cloudy suspension results. Preparation, and disposal of TENIVAC will be done by trained study staff/research assistants. Syringes and/or vials will be disposed of in the proper biohazard sharps container.

1.8 Specimen Collection

A. Primary Specimen Collection

- **Types of Specimens**: Blood and oral fluid samples. At each visit, blood samples (15 mL) will be collected using standard venipuncture performed by a phlebotomist. Oracol swabs will be used to harvest oral fluid rich with GCF (and will be self-collected by the participant under the direction and supervision of research staff by gently swabbing their gumline for 2 minutes. In total there will be 280 specimens collected for this study (7 blood samples + 7 saliva samples per subject x 20 subjects). All specimen collections will happen at the local study site, 541B Mantua Avenue, Paulsboro, NJ.
- **Annotation**: The blood and oral fluid samples will be labeled by a study ID and visit number. The same study ID will be used for samples/data collected in the main study as in the supplemental study.
- **Transport**: The blood and oral fluid samples will be collected at the Paulsboro Study site which is also approved by REHS for blood and saliva processing.
- **Processing**: The research assistants will process and aliquot the blood and oral fluid.
- **Storage**: The specimens will be stored in the -80^{o}C freezer at the lab then transported to Rutgers EOHSI or shipped to the fee for service analytical lab (Comprehensive Laboratory for Untargeted Exposome Science (CLUES), Gangarosa Department of Environmental Health at Emory University) by a research assistant with IATA training.
- **Disposition**: Any excess specimens will be retained by Dr. Laumbach in a -80˚C freezer at EOHSI (Room 232). Access to the freezer room is card controlled.

B. Secondary Specimen Collection - NA

1.9 Data Collection

A. Primary Data Collection

- **Location**: Subjects will be screened at the local study site in Paulsboro, either in person or over the phone.
- **Process of Data Collection**: Research assistants will do the screening.
- **Timing and Frequency**: Data collection will take place at screening, and at each visit.
- **Procedures for Audio/Visual Recording**: - NA
- **Study Instruments**: A screening questionnaire developed for this study will be used.
- **Ethnographic Studies, Interviews, Or Observation**: - NA
- **Subject Identifiers**: Subject's contact information (name, email, mailing address, and phone number) will be collected to schedule appointments. All documents will be coded by study ID and visit number entered directly into the REDCap database.

B. Secondary Data Collection-NA

1.10 Timetable/Schedule of Events

2.0 Project Management

2.1 Research Staff and Qualifications

Robert Laumbach MD, MPH, CIH Associate Professor, Environmental and Occupational Health, Rutgers School of Public Health. Dr. is a physician-scientist who is board-certified in Family Medicine and in Occupational and Environmental Medicine. He conducts clinical and epidemiological research on the health effects of environmental contaminants, including air and water pollutants, and has had federally funded field projects to study the role of cytokines, including IL-6 and IL-10, in inflammation and acute phase responses to exogenous agents, including interactions with psychosocial stress. Dr. Laumbach is the Principal Investigator for this study and CDC funded PFAS Multi-Site Study Paulsboro study site.

Pamela Ohman Strickland, PhD, MS Associate Professor of Biostatistics at the Rutgers School of Public Health. Dr. Ohman Strickland serves as the Director of the Biostatistics, Epidemiology and Research Design Core for the New Jersey Alliance for Clinical and Translational Science (NJ ACTS) as well as Faculty Director for the Rutgers University Biostatistics and Epidemiology Services center and Director of the Biostatistics Facility Core of the Center for Environmental Exposure and Disease (an NIEHS Center) at Rutgers. Dr. Ohman Strickland has collaborated in a wide variety of epidemiological studies of acute and chronic effects of air pollution, on biomarkers of inflammation, oxidative stress, etc., using methods such as generalized linear models, categorical data analysis, factor analysis, path analysis, etc. Dr. Ohman Strickland will serve as a Co-Investigator for this study, providing essential epidemiological and statistical support.
Kathy Black, PhD, MPH, Senior Research Associate, Rutgers Environmental and Occupational Health Sciences Institute. Dr. Black will act as the study coordinator and will be responsible for the training of the study research assistants. Dr. Black has over 20 years of experience coordinating environmental health studies.

Alicia Legard and Adriana De Resende, Clinical Research Coordinators, Rutgers Environmental and Occupational Health Sciences Institute. Alicia and Adriana studies have extensive experience in managing and performing laboratory procedures, including data analysis, for environmental

contaminant studies and clinical trials. For the current project, they will assist with overseeing the clinical trial as needed.

Fatima Haynes, MS, PhD Candidate in Exposure Science at Rutgers School of Graduate Studies. Fatima has experience working in molecular toxicology laboratories and on other community health research studies. She will serve as a research assistant responsible for participant screening and consent, data collection, and analysis. This study will serve as her dissertation project.

Alanna O'Neil, BS, PhD Candidate in Exposure Science at Rutgers School of Graduate Studies. She has a BS in Environmental Science from Juniata College. From July 2021 to present day, Alanna has worked at the Paulsboro PFAS Health Study. Among her duties included enrolling participants and administering questionnaires. Alanna will provide general research support as needed.

Tonya Kidd and Nancy Victor will provide general research support and assist with participant screening and consent. Both Tonya and Nancy are residents of Gloucester County and also work at the Paulsboro PFAS Health Study site.

2.2 Research Staff Training

Only staff working on the main study will also work on this supplement. The staff have been trained by Dr. Laumbach in study procedures.

2.3 Other Resources - NA

2.3 Research Sites

All data and specimen collection will take place at the local study office space (541B Mantua Avenue, Paulsboro, NJ) including recruitment.
De-identified samples will be analyzed at Rutgers-EOHSI (170 Frelinghuysen Road, Piscataway, NJ 08854) and at the Comprehensive Laboratory for Untargeted Exposome Science (CLUES), Gangarosa Department of Environmental Health at Emory University (1518 Clifton Rd, Atlanta, GA 30322) for metabolomic analysis on a fee-for service basis.

3.0 Multi-Center Research - NA

4.0 Subject Considerations

4.1 Subject Selection and Enrollment Considerations

A. Method to Identify Potential Subjects
Adults who enrolled in the main study who consented to be contacted for future studies will be asked to participate in this study. We will recruit participants from the first and fifth quintiles of PFNA serum concentrations to maximize contrast in exposure, balanced recruitment from the first and fifth quintiles of exposure will be randomized.

B. Recruitment Details
The study will recruit only main study participants. Previous subjects, who completed their main study visit and consented to be contacted for future studies, will be called by a research assistant, and asked if they are interested in participating in a supplemental study. If so, an appointment will be made for a study visit.

C. Subject Screening
Subjects are screened for the main study. Adults who participate in the main study will be eligible for this supplemental study.

- **Inclusion Criteria**
  Adults (18 years of age and older), weigh at least 110 pounds, and who participated in the main study may be eligible to participate in this supplemental study.
- **Exclusion Criteria**
  - Pregnancy
  - History of difficult blood draws
  - History of adverse reaction to prior vaccinations
  - Currently taking immune suppressants
  - Recent dental surgery or dental procedure within 4 weeks of starting study
  - Had a Td booster in the past 10 years

D. Privacy Protections
Prospective subjects are identified by their participation in the main study. Identifiers for the main study are stored in the study REDCap database. Only the study team has access to the database.

4.2 Obtaining Identifiable Information About Non-Subjects - NA

4.3 Number of Subjects

A. Total Number of Subjects
20 subjects will be enrolled.

B. Total Number of Subjects If Multicenter Study - NA

C. Feasibility
The main study recruited 775 adults and for the small number of subjects in this study, we expect to be able to complete recruiting and testing in a timely manner.

4.4 Consent Procedures

A. Consent Process

- **Location of Consent Process**
  Consent will take place in the local study office in Paulsboro.
- **Ongoing Consent**
  Study activities will be discussed with the subject at the start of each visit. All questions will be answered.
- **Individual Roles for Researchers Involved in Consent**
  Screening and consenting may be done by the coordinator or a research assistant.
- **Consent Discussion Duration**
  We expect the consent process to take approximately 10 minutes. The study visit will take place immediately after the consent.
- **Coercion or Undue Influence**
  Only main study adult volunteers will be asked to participate in the supplemental study. The consent form will clarify that participation in the supplemental study is not required.
  Subject Understanding
  The key study elements will be specifically mentioned during the consent discussion and all questions will be answered.
- **Protecting Privacy**
  Consent discussions will take place in a private office prior to participation. No additional information about the subject will be collected until after consent is obtained.

B. Waiver or <u>Alteration</u> of Consent <u>Process - NA</u>

C. Documentation of Consent

- **Documenting Consent**
  The consent form will be stored in REDCap. Both the participant and study investigator will sign the consent form. A copy of the signed consent form will be emailed to the participant.

- **Waiver of Documentation of Consent (i.e., will not obtain subject's signature) - NA**

4.5 Special Consent Populations - NA

A. Enrolling Minors-Subjects Who Are Not Yet Adults - NA
B. Enrolling Wards of the State - NA
C. Enrolling Non-English-Speaking Subjects - NA
D. Enrolling Adults Lacking Decision-Making Capacity (Surrogate Consent) - NA
E. Special Consent Considerations - NA

4.6 Economic Burden and/or Compensation for Subjects

A. Expenses - NA
The subject is not expected to incur any expenses.

B. Compensation/Incentives
Subjects will be paid $50 for each study visit (up to $350 total).

C Compensation Documentation
Compensation will be documented in REDCap. Subjects will also be asked to sign the subject voucher for cash payments.

4.7 Risks of Harm/Potential for Benefits to Subjects

A. Description of Risks of Harm to Subjects

- **Reasonably Foreseeable Risks of Harm**
  Td Vaccination: Pain, redness, or swelling where the shot was given, mild fever, headache, feeling tired, and nausea, vomiting, diarrhea, or stomachache sometimes happen after Td vaccination. People sometimes faint after medical procedures, including vaccination. As with any medicine, there is a very remote chance of a vaccine causing a severe allergic reaction, other serious injury, or death.

  Venipuncture: Slight pain, some bleeding, or bruising may occur when blood is drawn. A total of 105 ml of blood (15 ml per study visit over a 30-day period) will be required for the planned assays.

  Oral fluid collection: When swabbing the gumline, there may be minor bleeding if done too roughly.

- **Risk of Harm from an Intervention on a Subject with an Existing Condition**
  The study will not enroll subjects with existing conditions related to adverse vaccination responses.

- **Other Foreseeable Risks of Harm**
  There is a risk of possible loss of confidentiality, but no sensitive information is collected in the study.

- **Observation and Sensitive Information – NA**

B. Procedures which Risk Harm to Embryo, Fetus, and/or Pregnant Subjects -NA
C. Risks of Harm to Non-Subjects -NA
D. Assessment of Social Behavior Considerations - NA
E. Minimizing Risks of Harm

All subjects will be screened prior to scheduling. Subjects with a history of adverse reactions to vaccination and difficulty with blood draws are excluded from the study out of an abundance of caution. All female subjects will receive a urine pregnancy test prior to vaccination. Subjects will be informed in the consent document that they will be given the results of the pregnancy test. Although Tetanus-Diphtheria is a routine vaccination, subjects will be provided with a vaccine information sheet (VIS) before vaccination to review all information, including risks associated with the specific vaccine type. The physician PI will be onsite to administer the vaccine and respond to any immediate reactions. Following vaccination, subjects will be visually monitored by medical personnel for at least 15 minutes. In case of anaphylactic or acute hypersensitivity reactions, Epinephrine hydrochloride solution (1:1,000) or other appropriate agents and equipment will be available for immediate use, and 911 will be called. In the case of any adverse event, the event will be documented, and a report will be sent to Vaccine Adverse Event Reporting System (VAERS) and IRB.

- **Certificate of Confidentiality - NA**
- **Provisions to Protect the Privacy Interests of Subjects**
  All data and specimen will be coded by a Study ID. All identifiable information will be stored in REDCap and accessible only to the study team.

F. Potential Direct Benefits to Subjects

Subjects will be boosted against Tetanus and Diphtheria.

5.0 Special Considerations

5.1 Health Insurance Portability and Accountability Act (HIPAA) - NA

5.2 Family Educational Rights and Privacy Act (FERPA) - NA

5.3 Code of Federal Regulations Title 45 Part 46 (Vulnerable Populations) - NA

5.4 General Data Protection Regulation (GDPR) - NA

5.5 NJ Access to Medical Research Act (Surrogate Consent) - NA

6.0 Data Management Plan

6.1 Data Analysis

Means, standard deviations, percentiles and histograms will summarize the distributions of outcome variables. Based on Kielsen (2016), we expect exponential rise in plasma antibody concentrations from 4 to 10 days. Thus, in the primary analysis, we will examine the linear increase in log of antibody concentrations from 4 to 10 days and whether that increase is modified by exposure to pfas through an interaction term between time and PFAS (log-transformed). Integration of exposure, metabolic response, and immune response has the potential to provide key insight into mechanisms underlying

exposure mediated immune suppression following Td vaccine. To examine the assumption that there's a linear increase from 4 to 10 days, linear models with cubic splines relative to time since immunization will assess goodness of fit across different strata of PFAS exposures. If the time of increase varies substantially from the 4 to 10-day assumption, then the initial linear models will be adjusted accordingly.

To investigate associations between RNA-seq and metabolomics data with PFAS exposure levels, differential analysis will identify genes and metabolites with altered expression/abundance across exposure groups. Pathway analysis will assess biological relevance by examining enrichment of gene sets and metabolic pathways. Correlation analysis will evaluate the relationship between individual gene expression/metabolite levels and PFAS exposure. Longitudinal analysis will assess temporal changes in both RNA-seq and metabolomics data following vaccination. Analyses will be adjusted for confounders, corrected for multiple testing, and assume stable PFAS concentrations over time. This comprehensive approach will elucidate the dynamic relationship between molecular profiles and PFAS exposure, advancing our understanding of PFAS toxicity and its impact on human health. For analyzing the oral fluid samples, as a feasibility/proof of concept study, no power calculations have been done. Accuracy will be assessed by comparison to ELISA reference methods by paired t-test and correlation analysis to examine overall bias and reliability. The Bland Altman plot will assess bias and homogeneity of error relative to ELISA across the range of values. Mean, standard deviations, and coefficients of variation will be calculated to benchmark the method for reference in future studies.

6.2 Data Security

All study data will be stored in REDCap is a secure, password-protected web-based application designed to support data capture for research studies. A unique study participant ID will be assigned in REDCap at the time of screening. Only study staff will be authorized to have access to the project. Only deidentified data will be exported for analysis. De-identification of data will consist of removal of all personal identifiers from analytic files. Investigators will keep the data, including links to personal identifiers, until study closure.

6.3 Data and Safety Monitoring

A. Data/Safety Monitoring Plan

Our plan for data and safety monitoring involves assessing adverse events related to vaccination and potential unexpected reactions or interactions. We will continue to monitor and document adverse events following vaccination, including both immediate and delayed reactions at study visits. Additionally, we will pay close attention to safety parameters specific to the vaccine being administered, as outlined in its FDA approval documentation, such as known adverse reactions, contraindications, and warnings. Evaluation will occur quarterly throughout the duration of the study. The responsibility for data review will primarily lie with the principal investigator, in collaboration with a designated safety monitoring team comprising of study staff. The data review process will commence at the initiation of the study. Safety information will be collected at each study visit.

B. Data/Safety Monitoring Board Details

We do not plan to establish a separate data monitoring committee for this study. Instead, safety data will be regularly reviewed by the principal investigator and the safety monitoring team. Findings from these reviews will be reported to the Institutional Review Board (IRB) and sponsor on a quarterly basis. Immediate suspension of the research will be triggered the occurrence of a single serious adverse event associated with the vaccine.

6.4 Reporting Results

A. Individual Subjects' Results
No, individual results will not be provided to participants as the research does not involve studying anything of clinical relevance.

B. Aggregate Results
When the study is completed, aggregate results will be posted on the EOHSI website and will be presented at community meetings.

C. Professional Reporting
Results will be presented at professional conferences and published in peer-reviewed journals.

D. Clinical Trials Registration, Results Reporting and Consent Posting
The study is a clinical trial and will be registered on clinicaltrials.gov. As required, the consent form and results will be posted.

6.5 Secondary Use of the Data- NA

7.0 Research Repositories – Specimens and/or Data

Excess serum and oral fluid specimens may be stored by Dr. Laumbach. The de-identified data collected in the study will be linked to the specimens by study ID and will not be distributed to others. The specimens will be stored in -80°C freezers at EOHSI Room 232. Freezer temperatures are monitored remotely, and notifications are sent in cases of malfunction. Access to the freezer room is controlled by key card.

8.0 Approvals/Authorizations

IBC approval for saliva and blood processing.

9.0 Bibliography

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