PROTOCOL TITLE:
Intravesical Lactobacillus to Reduce Urinary Symptoms after Spinal Cord Injury

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1.0 Study Summary

Study Title	Intravesical Lactobacillus to Reduce Urinary Symptoms after Spinal Cord Injury
Study Design	For efficiency in achieving the specific aims of the study within the funding period, enrollment and data collection for Specific Aims 1 and 2 will occur simultaneously throughout the study period. We anticipate that most subjects participating in Aim 2 will be recruited from Aim 1.
Primary Objective	The objectives of the proposed research among this population are: 1) to define clinically meaningful change (i.e. differentiating states of health and illness) with respect to urinary symptoms, urine inflammation, cultivable bacteria, and the urine ecosystem; and 2) to determine the optimal intravesical *Lactobacillus RhamnosusGG*® (LGG®) dose to be used to reduce urinary symptoms in a future clinical trial.
Secondary Objective(s)	
Research Intervention(s)/ Investigational Agent(s)	*Specific Aim 2 (SA2) only: Lactobacillus RhamnosusGG®*
IND/IDE #	16306
Study Population	Spinal Cord Injury (SCI), Multiple Sclerosis (MS), or Spina Bifida (SB)
Sample Size	SA2: 182 (68 local and 114 nationally)
Study Duration for individual participants	SA 1: at least 2 weeks until 2 non-symptomatic urine samples are collected. SA 2: 5 days till LGG® instillation has occurred (duration depends on when participants experiencing urinary symptoms, and which dosage group they are in.)
Study Specific Abbreviations/ Definitions	AAPMR- American Academy of Physical Medicine and Rehabilitation ACRM- American Congress of Rehabilitation Medicine AHRQ- Agency for Healthcare Research and Quality ANOVA- Analysis of Variance ASCIP- Academy of Spinal Cord Injury Professionals, Inc ASIA- American Spinal Injury Association ASVs- Amplicon sequence variant CFU- Colony-forming units CITI- Collaborative Institutional Training Initiative CNMC- Children's National Medical Center DNA- Deoxyribonucleic Acid EAU- European-based Guidelines on Urological Infections

	FDA- Food and Drug Administration HIPAA- Health Insurance Portability and Accountability Act IDSA- Infectious Diseases Society of America IND- Investigational new drug ISCoS- International Spinal Cord Society LGG®-Lactobacillus Rhamnosus NB- Neurogenic Bladder NGS- Next generation sequencing NIDRR- National Institute for Disability, Independent Living, and Rehabilitation Research NIH- National Institute of Health NINDS CDE- National Institute of Neurological Disorders and Stroke Common data points NRH- National Rehabilitation Hospital NRN- National Rehabilitation Network OTUs- Operational Taxonomic units PCORI- Patient Centered Outcomes Research Institute QCP- Qualitative clinical phenotype REDCap- Research Electronic Data capture ROC- receiver operating characteristic rRNA- Ribosomal ribonucleic acid SCI- Spinal Cord Injury USQNB-IC -Urinary Symptom Questionnaire for people with Neurogenic Bladder/Intermittent Catheterization uNGAL- urinary neutrophil gelatinase-associated lipocalin UTC- Urinary tract colonization UTI- Urinary Tract Infection uWBC- Urinary White blood cells WBC- White blood cells

2.0 Objectives*

2.1 Describe the purpose, specific aims, or objectives.

SA2. Estimate the effect of intravesical LGG® dose on urinary symptoms (primary outcome), uNGAL, uWBC, nitrites, cultivable bacteria, and the urine microbiome (secondary outcomes). The effect (clinically meaningful change) is defined both in terms of the amount (20% or more) and the timing (within 48 hours of completing the intervention).

2.2 State the hypotheses to be tested.

SA2:

H2a. There will be a greater number of treatment successes, defined as at least 20% reduction in urinary symptoms within 48 hours of completion of intervention, in the "High" dose compared with the "Low" LGG® groups.
H2b. "High" dose intravesical LGG® will result in greater reduction in post-intervention uNGAL and uWBC levels, and a greater number of absent nitrites, than "Low" dose intravesical LGG®.
H2c. "High" dose intravesical LGG® will result in fewer amounts (species, CFUs) of cultivable bacteria from urine as compared to "Low" dose.
H2d. *Exploratory*: Composition of the urine microbiome, as determined by next generation sequencing (NGS), will differ within-person between "symptomatic" (pre- LGG® instillation) and after instillation of LGG®; these within-person results will be more pronounced (larger effect size) for those in the "High" dose group relative to those in the "Low" dose group.

3.0 Background*

3.1 Describe the relevant prior experience and gaps in current knowledge.

Urinary tract infection (UTI) is the most common outpatient infection world-wide, and for people with spinal cord injury (SCI) and neurogenic bladder (NB), it is the most common infection, secondary condition, cause for emergency room visits, and infectious cause of hospitalization. Despite its prevalence, attempts to ameliorate UTI among people with SCI are stymied by long-standing diagnostic challenges which arise from evidence gaps around "gold standard" diagnostic tests (urinalysis and urine culture) that have lower sensitivity and specificity for UTI in this population. A high prevalence of chronic inflammation leading to persistence of white blood cells (WBC) in the urine confounds the utility of WBC count, pyuria, and leukocyte esterase as biomarkers for UTI; nitrites in urine indicate the presence of only specific (but not all) organisms, many of which are present to a greater extent in the urine of people with SCI; and people with SCI have a high prevalence of asymptomatic bacteriuria. These physiologic changes render the gold standard diagnostic tests less useful for identifying UTI in persons with SCI.

3.2 Describe any relevant preliminary data.

Preliminary Data: Given these shortcomings in the evidence, there is a dire need to address gaps in knowledge around UTI. This *first requires* advancement of evidence around: 1) urinary symptom reporting and understanding, 2) bladder inflammation, and 3) the urine ecosystem as potential sources of additional, informative, objective outcomes. ***Until advancements occur in each of these areas to allow for an evidence-based consensus on which urinary symptoms do and do not represent UTI, how much inflammation does or does not represent a UTI, and therefore what is and what is not a UTI, we cannot advance interventions to positively impact the prevalent problems of urinary symptoms and UTI***. To that end, our work to date and preliminary data have focused heavily on urinary symptoms and the urine ecosystem:

1. We have developed and validated the Urinary Symptom Questionnaire for people with Neurogenic Bladder-Intermittent Catheterization (USQNB-IC).
2. Utilizing NGS techniques, our team was first to compare and contrast the urine microbiome of healthy controls without NB with those of people with SCI and NB; in so doing we trans formatively challenged clinical dogma around healthy urine by showing that healthy urine is not sterile, and in fact, a healthy urine ecosystem exists
3. This preliminary data (3-4) led us to obtain approval from the FDA for an IND to use intravesical *Lactobacillus RhamnosusGG®* (LGG®) *(*IND #16306) in preparation for the next phase of research.
4. We conducted a first-in-human trial of intravesical LGG® (1 or 2 doses in a 30-hour period). In that 18-month project, 80% of participants were retained to completion, justifying our recruitment and retention estimates in the proposed trial; intravesical LGG® was found to be safe and well-tolerated. Our manuscript showed 74 (77%) adults and 6 (86%) of children completed the study. 64 of these instilled *LGG*® for a total of 357 instillations (range 1-41 per person). There were 59 Serious Adverse Events, 44% (26/59) of which were categorized as infectious genitourinary. There was no statistical relationship between Serious adverse events and use or dose of the intervention. (manuscript accepted for publication by Therapeutic Advances in Urology; final results submitted to national conference for 2019).
5. Preliminary efficacy results from this trial (manuscript in progress) indicate a significant ($p<.05$) drop in all endorsed USQNB-IC symptoms during the LGG® intervention phase compared with pre-LGG® intervention.

3.3 Provide the scientific or scholarly background for, rationale for, and significance of the research based on the existing literature and how will it add to existing knowledge.

Among contemporary guidelines for diagnosis of UTI in adults and children who require urinary catheters due to NB are: the US-based IDSA Guidelines for diagnosing catheter-associated UTI (regardless of type of catheter or presence/absence of neuropathic

bladder)[3]; the European-based EAU Guidelines on Urological Infections (2017); and a systematic review by Madden-Fuentes et al. (2013) used for children with NB due to spina bifida (who primarily utilize intermittent catheterization). The 2017 EAU Guidelines largely rely on the 2009 IDSA Guidelines, so diagnostic criteria are identical for both. Both the IDSA and EAU guidelines require *at least one* symptom indicative of UTI, including: new onset or worsening fever, rigors, altered mental status, malaise, lethargy, flank pain, costovertebral angle tenderness, acute hematuria, pelvic discomfort, dysuria, urgent or frequent urination, suprapubic pain or tenderness, increased spasticity, autonomic dysreflexia, and sense of unease. In contrast, Madden-Fuentes et al. (2013) require *at least two symptoms* (from the list of fever>38°C; abdominal pain; new back pain; new or worse incontinence; pain with catheterization or urination; and malodorous/cloudy urine) – of these, only "fever" matches the IDSA/EAU symptom list. Further, the IDSA and EAU specifically state that "cloudy or malodorous urine" is ***not*** *considered a symptom of catheter-associated UTI*, whereas Madden-Fuentes does allow consideration of malodorous/cloudy urine, however at least one additional symptom is required to meet the symptom criterion of UTI. In addition, Madden-Fuentes specify ≥10 WBC/hpf and $\geq 10^5$ colony-forming units (CFU)/ml of a single organism on culture whereas the IDSA and EAU guidelines state that pyuria "should not be interpreted as an indication to initiate antimicrobials." Further, none of these guidelines is specific to the adult population with SCI, although populations studied in the systematic review by Madden-Fuentes are, arguably, the most closely related to adult SCI. Ronco et al (2011) attempted to address the problem using receiver operating characteristic (ROC) curves to determine optimal colony forming units (CFU) and white blood cell (WBC) cut points to improve diagnostic criteria for UTI in male patients with SCI using intermittent catheterization. They found that clinical signs correlated poorly with objective measures of urine CFU and WBC, and that "there are no satisfactory CFU and WBC thresholds" for UTI diagnosis. As a result, UTI diagnosis in people with SCI is largely subjective and based on clinical experience (since diagnostic tests are not better than chance).

4.0 Study Endpoints*

4.1 Describe the primary and secondary study endpoints.

SPECIFIC AIM 2

We will obtain what Intravesical LGG® dose (group: high or low), USQNB-IC, urine collection for urinalysis, culture, NGAL and next generation sequencing as described above in addition to patient satisfaction questions.

4.2 Describe any primary or secondary safety endpoints.

Secondary safety endpoints are reviewing adverse events (AEs) reported weekly to determine if any AEs were related to instilling *Lactobacillus GG*

5.0 Study Intervention/Investigational Agent

1.1 Description: *Describe the study intervention and/or investigational agent (e.g., drug, device) that is being evaluated.*

Intervention Materials: Instillation of intravesical LGG® requires the following supplies: sterile gloves, new catheter, LGG® capsules, 0.9% normal saline, sterile cup, 60cc syringe, and bed pad (chuck). As we've successfully done to date, subjects will be provided with (either face-to-face exchange or sent by mail) kits with materials for 2 ("Low" dose) or 4 ("High" dose) instillations. In addition, a participant letter will be provided for each participant to give to their care provider to inform them of their participation in the study, what the intervention is and how to contact us if they have any questions or concerns about the study.

Intervention: LGG® (Culturelle Probiotic with *Lactobacillus RhamnosusGG*®, shown to produce the largest amount of antibacterial substances against pathogenic bacteria) will be used.[3]For the LGG® instillation, participants will be instructed to mix the contents of 1 LGG® capsule into 45 cc sterile 0.9% saline. After mixing, participants will draw up the 45cc liquid LGG® mixture into a 60cc syringe and instill via the intermittent catheter after the last catheterization prior to going to bed. Participants will receive 2 or 4 LGG® capsules (depending on randomization group) for the instillation intervention. Two additional capsules will be provided to each group – one of these additional capsules capsule will be used for training and one capsule will be a back-up in the event a capsule breaks during the preparation process. Therefore, subjects will receive 4 (low dose) or 6 (high dose) LGG capsules in their kits but will only instill 2 or 4 LGG capsules as instructed using the self-management protocol for probiotic (SMP-PRO). Any unused capsules will be returned to the study coordinator at the end of the participant's participation.
Subjects will remain in the study for up to 29 months, with participation ending after one completed intervention (either 2 or 4 doses) and post-intervention assessments are complete. If urinary symptoms do not occur warranting instillation during the ensuing 29 months, participants will be asked to return any remaining capsules. The Project Coordinator or RA will dispense all LGG® and count (and collect, if necessary) any unused capsules to maintain counts. Participants will be instructed to complete the USQNB-IC weekly until study completion.

Self-Management Protocol: The Self-Management Protocol using Probiotics (SMP-Pro) will be used to direct participants if and when to initiate intravesical LGG® instillations. The SMP-Pro will be utilized for those participants randomized to the "Low" dose group; a modified SMP-Pro will be used for those participants randomized to "High" dose group.

5.1 Drug/Device Handling: If the research involves drugs or device, describe your plans to store, handle, and administer those drugs or devices so that they will be used only on subjects and be used only by authorized investigators.

- The control of the drugs or devices used in this protocol will be accomplished by following an established, approved, organizational SOP (e.g., Research Pharmacy SOP for the Control of Investigational Drugs, etc.., Please reference that SOP in this section.

Study drug will be stored and dispensed by the MHRI Pharmacy located at the Medstar Washington Hospital Center. Study medication will be released to the Research Coordinator upon presentation of a prescription signed by one of the authorized study personnel.

The MHRI Research Pharmacy operates in compliance with all federal, state, and local regulations concerning the receipt, storage, handling, and dispensing of study medications.

5.2 If the drug is investigational (has an IND) or the device has an IDE or a claim of abbreviated IDE (non-significant risk device), include the following information:

- *Identify the holder of the IND/IDE/Abbreviated IDE.*

  Suzanne Groah, MD, MSPH

- *Explain procedures followed to comply with sponsor requirements for FDA regulated research for the following:*

	Applicable to:		
FDA Regulation	***IND Studies***	***IDE studies***	***Abbreviated IDE studies***
21 CFR 11	***X***	***X***	
21 CFR 54	***X***	***X***	
21 CFR 210	***X***		
21 CFR 211	***X***		
21 CFR 312	***X***		
21 CFR 812		***X***	***X***
21 CFR 820		***X***	

All FDA regulations applicable to this study are followed, and records are kept in study coordinator's office. Annual reports are submitted to FDA, and other reporting to FDA will occur throughout the year as needed.

6.0 Procedures Involved*

6.1 Describe and explain the study design.

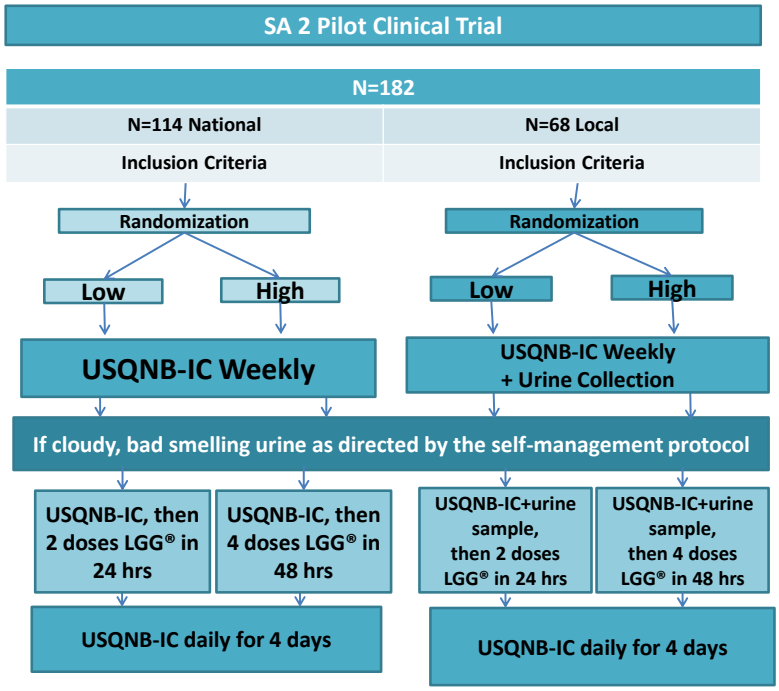
For efficiency in achieving the specific aims of the study within the funding period, enrollment and data collection for Specific Aim 2 will occur simultaneously, continuing throughout the study period. We anticipate that most (but not necessarily all) subjects

participating in Aim 2 will be recruited from Aim 1. Analyses will occur as collection of all data from Specific Aims 1 and 2 is ongoing, to the extent possible, so that all Specific Aims and objectives can be achieved within the 36-month period.

6.2 Provide a description of all research procedures being performed and when they are performed, including procedures being performed to monitor subjects for safety or minimize risks.

SA2:

Design: Prospective, randomized pilot intervention clinical trial.
Target Enrollment: 182 subjects total at least 1-year post-SCI.
Setting: National sample (N=114 intervention only, no urine collection) and local Washington, DC metropolitan area (N=68 intervention + urine collection).
Controls: This is a 2-arm dose-finding pilot clinical trial, as such there is no control group.
Recruitment: We conservatively estimate that 40% will not develop symptoms and use the intervention. Therefore, we will recruit 182 participants national database, the Neurogenic Bladder Research Group and United Spinal Association, 114 of whom will be recruited will only do the intervention and USQNB-IC to record urinary symptoms and 68 locally will participate in the intervention, USQNB-IC to record urinary symptoms and have urine sampling. We have successfully achieved this level of recruitment and retainment in our prior work See figure, right.



Inclusion Criteria 1) age≥18 years; 2) SCI, MS, or SB at least 1-year duration; 3) neurogenic bladder; 4) utilizing intermittent catheterization for bladder management; 5) a history of 2 or more episodes of urinary symptoms in the past year; and 6) community dwelling.

Exclusion Criteria: 1) known genitourinary pathology beyond neuropathic bladder (i.e., vesicoureteral reflux, bladder or kidney stones, etc.); 2) use of prophylactic antibiotics; 3) instillation of intravesical agents to reduce UTI (i.e., gentamycin); 4) immunodeficiency; 5) any antibiotics within the past 2 weeks; 6) psychologic or psychiatric conditions influencing the ability to follow instructions; and 7) participation in another study in which results would be confounded.

Informed Consent: Will be obtained by CITI and HIPAA certified PI, Project Coordinator or Research Associate.

Data Collection: All data will be collected through REDCap forms.

Initial Assessment: Participants will complete the International SCI Lower Urinary Tract Function Basic Data Set [NINDS CDE], and NINDS SCI CDE (medical history, prior/concomitant medications [per NINDS CDE].

Randomization: Randomization will be achieved using a two-stage covariate adaptive procedure and will be completed separately depending on whether s/he is local (N=40, intervention+USQNB-IC+urine sampling) with a 40% attrition/non-use rate, requiring randomization of N=68) or national (N=68, intervention +USQNB-IC only, with a 40% attrition/non-use rate, requiring randomization of n=114). The Project Coordinator will maintain two lists of randomization codes (to be prepared *a priori* by the Project Co-PI Participants in the two groups (local, national) will be randomized 1:1 to either receive "Low" or "High" dose LGG®.

Intervention Training: Our completed training protocol and manual on instillation of intravesical LGG® developed through our PCORI-supported pilot safety trial will serve as the training protocol and manual for those randomized to the "Low" dose group. A second manual will be developed with LGG® number of instillations and duration modified for the "High" dose participants. Each subject will be given a manual (hard copy or electronic, per subject preference) and will participate in a 1-on-1 training session with our Consumer Expert.

Intervention Materials: Instillation of intravesical LGG® requires the following supplies: sterile gloves, new catheter, LGG® capsules, 0.9% normal saline, sterile cup, 60cc syringe, and bed pad (chuck). As we've successfully done to date, subjects will be provided with (either face-to-face exchange or sent by mail) kits with materials for 2 ("Low" dose) or 4 ("High" dose) instillations.

Intervention: LGG® (Culturelle Probiotic with *Lactobacillus RhamnosusGG*®, shown to produce the largest amount of antibacterial substances against pathogenic bacteria) will be used.[3] For the LGG® instillation, participants will be instructed to mix the contents of 1 LGG® capsule into 45 cc sterile 0.9% saline.[4,5] After mixing, participants will draw up the 45cc liquid LGG® mixture into a 60cc syringe and instill via the intermittent catheter after the last catheterization prior to going to bed. Participants will receive 2 or 4 LGG® capsules (depending on randomization group) and 2 additional capsules (1 used for training and 1 that will be extra if a capsule breaks during the preparation process). Therefore, subjects will receive 4 (low dose) or 6 (high dose) LGG capsules in their kits but will only instill 2 or 4 LGG as instructed using the self-management protocol for probiotic (SMP-PRO). Subjects will remain in the study for up to 29 months, with participation ending after one completed intervention (either 2 or 4 doses) and post-intervention assessments are complete. If urinary symptoms do not occur warranting instillation during the ensuing 29 months, participants will be asked to return any

remaining capsules. The Project Coordinator or RA will dispense all LGG® and count (and collect, if necessary) any unused capsules to maintain counts. Participants will be instructed to complete the USQNB-IC weekly until study completion.

Self-Management Protocol: The Self-Management Protocol using Probiotics (SMP-Pro) will be used to direct participants if and when to initiate intravesical LGG® instillations. The SMP-Pro will be utilized for those participants randomized to the "Low" dose group; a modified SMP-Pro will be used for those participants randomized to "High" dose group.

6.3 Describe:

- *Procedures performed to lessen the probability or magnitude of risks.*

Each participant will have 1 on 1 training about the instillation process to ensure and avoid any risks during the study. Research staff will always be available if any concerns ever arise during the study, and each participant will have several ways (phone, email) of contacting the study team

- *All drugs and devices used in the research and the purpose of their use, and their regulatory approval status.*
    - Culturelle *Lactobacillus rhamnosus GG* will be instilled intravesically to decrease urinary symptoms. FDA approval under IND16306 effective date April 9, 2019.
- *The source records that will be used to collect data about subjects. (Attach all surveys, scripts, and data collection forms.)*
    - REDCAP will be used for all data collection

6.4 What data will be collected during the study and how that data will be obtained.

Data will be collected including: Survey or questionnaires, urine sample data, adverse events (printed and electronic) and Lactobacillus drug related data. This will be recorded using REDCAP and excel protected documents on a locked computer. This will be obtained from the participant by a research coordinator or from the lab processing the urine.

6.5 If there are plans for long-term follow-up (once all research related procedures are complete), what data will be collected during this period.

No long-term follow-up will occur

6.6 For Humanitarian Use Device (HUD) uses provide a description of the device, a summary of how you propose to use the device, including a description of any screening procedures, the HUD procedure, and any patient follow-up visits, tests or procedures.

N/A

7.0 Data and Specimen Banking*

7.1 If data or specimens will be banked for future use, describe where the specimens will be stored, how long they will be stored, how the specimens will be accessed, and who will have access to the specimens.

- If participants consent to it, a small amount of urine that will be saved and stored at Children's National Medical Center (CNMC) for up to 10 years. This sample could be used for potential future research on urinary symptoms. Only study approved staff will have access to the specimens.

7.2 List the data to be stored or associated with each specimen.

- Protocol ID, participant ID number and date collected

7.3 Describe the procedures to release data or specimens, including: the process to request a release, approvals required for release, who can obtain data or specimens, and the data to be provided with specimens.

- All participants will consent to having some of the specimen frozen and kept for up to 10 years. Everyone who obtain data regarding the specimen has to be approved study staff. De-identified data may be obtained by seeking approval from study PI.

8.0 Sharing of Results with Subjects*

8.1 Describe whether results (study results or individual subject results, such as results of investigational diagnostic tests, genetic tests, or incidental findings) will be shared with subjects or others (e.g., the subject's primary care physicians) and if so, describe how the results will be shared.

Results of the study will be shared with participants once results are available upon study completion.

9.0 Study Timelines*

9.1 Describe:

- *The duration of an individual subject's participation in the study.*

SA2: Up to 29 months or when low or high dose of Lactobacillus instillation has occurred.

- *The duration anticipated to enroll all study subjects.*

2- 2 1/2 years

- *The estimated date for the investigators to complete this study (complete primary analyses)*

8/14/2022

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10.0 Inclusion and Exclusion Criteria*

10.1 Describe how individuals will be screened for eligibility.

Screening will occur over the phone or in person at MedStar NRH to determine eligibility.

10.2 Describe the criteria that define who will be included or excluded in your final study sample.

SA2: **Inclusion Criteria** 1) age≥18 years; 2) SCI, MS or SB at least 1-year duration; 3) neurogenic bladder; 4) utilizing intermittent catheterization for bladder management; 5) a history of 2 or more episodes of urinary symptoms in the past year; and 6) community dwelling.
Exclusion Criteria: 1) known genitourinary pathology beyond neuropathic bladder (i.e., vesicoureteral reflux, bladder or kidney stones, etc.); 2) use of prophylactic antibiotics; 3) instillation of intravesical agents to reduce UTI (i.e., gentamycin); 4) immunodeficiency; 5) any antibiotics within the past 2 weeks; 6) psychologic or psychiatric conditions influencing the ability to follow instructions; and 7) participation in another study in which results would be confounded.

10.3 Indicate specifically whether you will include or exclude each of the following special populations: (You may not include members of the above populations as subjects in your research unless you indicate this in your inclusion criteria.)

- Adults unable to consent- No
- Individuals who are not yet adults (infants, children, teenagers)- No
- Pregnant women- No
- Prisoners- No

11.0 Vulnerable Populations*

11.1 If the research involves individuals who are vulnerable to coercion or undue influence, describe additional safeguards included to protect their rights and welfare.

- *If the research involves pregnant women, review "CHECKLIST: Pregnant Women (HRP-412)" to ensure that you have provided sufficient information.*
- *If the research involves neonates of uncertain viability or non-viable neonates, review "CHECKLIST: Neonates (HRP-413)" or "HRP-414 – CHECKLIST: Neonates of Uncertain Viability (HRP-414)" to ensure that you have provided sufficient information.*
- *If the research involves prisoners, review "CHECKLIST: Prisoners (HRP-415)" to ensure that you have provided sufficient information.*
- *If the research involves persons who have not attained the legal age for consent to treatments or procedures involved in the research ("children"), review the "CHECKLIST: Children (HRP-416)" to ensure that you have provided sufficient information.*
- *If the research involves cognitively impaired adults, review "CHECKLIST: Cognitively Impaired Adults (HRP-417)" to ensure that you have provided sufficient information.*

12.0 Local Number of Subjects

12.1 Indicate the total number of subjects to be accrued locally.

SA2: 68

12.2 If applicable, distinguish between the number of subjects who are expected to be enrolled and screened, and the number of subjects needed to complete the research procedures (i.e., numbers of subjects excluding screen failures.)

For SA2 we anticipate a 40% dropout rate/not ending up using *Lactobacillus GG.* Locally we will enroll N=68 participants but need to complete N=40, and nationally we will recruit N=114 but need N=68 to complete research goal.

13.0 Recruitment Methods

13.1 Describe when, where, and how potential subjects will be recruited.

Human subjects for this research project will be recruited through the MedStar NRH database, electronic medical records and the 60+ MedStar NRH outpatient sites along with non-MedStar SCI rehabilitation facilities in the Washington-Baltimore area. IRB-approved flyers will be posted in all MedStar NRH sites. Recruitment will also occur through social media and spinal cord injury related advocacy organizations. Once someone has been identified as a potential candidate for this study by Center staff, s/he will be screened by study personnel to determine eligibility.

13.2 Describe the source of subjects.

MedStar NRH database and the 60+ MedStar NRH outpatient sites, non-MedStar SCI rehabilitation facilities in the Washington-Baltimore area, social media sites, and spinal cord injury related advocacy organizations will be used to recruit subjects for this study.

13.3 Describe the methods that will be used to identify potential subjects.

First, the above sources will be used to recruit subjects. Once subjects have contacted someone on the research staff or research staff has contacted a potential subject, we will use of the Inclusion/Exclusion form to identify if they are a potential subject for this study (Aim 1 or 2). Once the potential subject has been determined to fit the inclusion/exclusion, research staff will;

Phone screening: Research staff will explain the study and go over all main points of the consent form. Afterwards staff will answer any questions the potential participant may have. Research staff will then either email the informed consent or send in regular mail for the potential participant to read over, contact us with any questions and sign the consent form and send back to us. Once the person obtaining the consent has signed the document, a copy of the signed informed consent form will be sent back to the participant for their records.

In person: Research staff will explain the study and go over all main points of the consent form. After that staff will answer any questions the potential participant may have. The potential participant is then given the option to read through and sign the consent form while at MedStar NRH or bring it home to read and sign the document. They will then bring the informed consent back to research staff for them to sign the consent and give the participant a copy of the signed consent form for their records.

13.4 Describe materials that will be used to recruit subjects. (Attach copies of these documents with the application. For advertisements, attach the final copy of printed advertisements. When advertisements are taped for broadcast, attach the final audio/video tape. You may

submit the wording of the advertisement prior to taping to preclude re-taping because of inappropriate wording, provided the IRB reviews the final audio/video tape.)

Flyers will be used to post on social media and around the hospital to recruit

13.5 Describe the amount and timing of any payments to subjects.

Payments can be received as cash (in-person), check, or Tango eGift card.

Each participant will be paid:

- SA 1: Each participant will receive $15 per urine sample (AIM 1).
- SA 2: Stipends will not be provided to participants as this may incentivize participants to utilize the Lactobacillus intervention

14.0 Withdrawal of Subjects*

14.1 Describe anticipated circumstances under which subjects will be withdrawn from the research without their consent.

- *Not adhering to the protocol*
- Changes in participant health that will exclude them from the study.

14.2 Describe any procedures for orderly termination.

- Each participant will be contacted by phone to address why the PI determined they need to be withdrawn from the study

14.3 Describe procedures that will be followed when subjects withdraw from the research, including partial withdrawal from procedures with continued data collection.

- Study staff will contact participant to collect reason for subject withdrawal. A note signed by PI will be added in subject folder, including withdrawn date and reason will be entered into REDCap

15.0 Risks to Subjects*

15.1 List the reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related the subjects' participation in the research. Include as may be useful for the IRB's consideration, a description of the probability, magnitude, duration, and reversibility

of the risks. Consider physical, psychological, social, legal, and economic risks.

Participation in any research may violate human rights, cause loss of confidentiality, and reveal protected health information. Other potential risks or side effects include: discomfort from catherization (SA2) and instillation of intravesical Lactobacillus (SA2), emotional discomfort from answering survey questions (SA2), and urinary tract infections (SA2).

15.2 If applicable, indicate which procedures may have risks to the subjects that are currently unforeseeable.

Though determined as safe & tolerable from a previously funded study, instillation of intravesical Lactobacillus (SA2) may have unforeseeable risks that are undetermined.

15.3 If applicable, indicate which procedures may have risks to an embryo or fetus should the subject be or become pregnant. -

15.4 N/A

15.5 If applicable, describe risks to others who are not subjects. N/A

15.6

16.0 Potential Benefits to Subjects*

16.1 Describe the potential benefits that individual subjects may experience from taking part in the research. Include as may be useful for the IRB's consideration, the probability, magnitude, and duration of the potential benefits.

In SA2, potential benefits may include: self-management of urinary symptoms with intravesical Lactobacillus Rhamnosus GG, fewer medical visits and reduced exposure to antimicrobials.

16.2 Indicate if there is no direct benefit. Do not include benefits to society or others.

17.0 Data Management* and Confidentiality

17.1 Describe the data analysis plan, including any statistical procedures or power analysis.

SA2 Analysis:

H2a. There will be a greater number of treatment successes, defined as at least 20% reduction in urinary symptoms within 48 hours of completion of the intervention, in the "High" dose compared with the "Low" dose LGG® groups. The number of items endorsed on the USQNB-IC at the time that the SMP-Pro directs participants to begin the intervention will be used to set each participant's target for "treatment success" irrespective of their treatment group. The number of items endorsed on the *last* (of the four) USQNB-IC that are completed after instillation of the LGG®, i.e., on the day s/he completes the satisfaction survey) will be compared to this target. If the difference represents at least a 20% reduction compared with the first USQNB-IC (pre-instillation), then that treatment is counted as a "success"; if it is 0-19.99%, treatment will be counted as a "failure". We will estimate the proportions of successes in each arm and compare these by chi-square or logistic regression modeling to analyze the proportion of each arm achieving success. Candidate covariates are age, sex, and time since injury. We will use a two-step process to assess each covariate for univariate association (independent samples t-tests or chi squared, as appropriate) with: a) the dichotomous outcome ($p<0.10$ constitutes evidence of an association with the outcome to warrant further consideration), *and* b) treatment group assignment ($p<0.15$ constitutes sufficient evidence of an association with predictor to warrant inclusion in the final model). We estimate a 30% likelihood of "success" in the "Low" dose arm, an 80% chance of being male, and a 50% chance of being randomized to the low dose arm.

Power: With N=54 required in each treatment/dosing arm (20 local and providing urine + 34 national and not providing urine in each dosing arm), and with sex as an example covariate, we will have 80% power to detect an OR as small as 4.2 with α =0.05. Without covariates, our sample size has 80% power with α =0.05 to detect a difference in proportions as close as 30% in the "Low", and 56.5% in the "High" dose group. We will use descriptive statistics to summarize patient satisfaction with the interventions at both doses; we will not use inference tests on satisfaction data. The total sample (108) needed for this level of power and effect size must be augmented to accommodate loss to follow up as well as the potential for participants to experience no symptoms (and therefore, no need to use the intervention) for the full 29 months of observation; we conservatively estimate the total "attrition" to be 40%. Given this, we will enroll N=91 in each treatment/dosing arm (34 local and providing urine + 57 national and not providing urine in each dosing arm).

H2b. "High" dose intravesical LGG® will result in greater reduction in post-intervention uNGAL and uWBC levels, and a greater number of absent nitrites, than "Low" dose intravesical LGG®. before and after their instillations, we will compare before and Inference tests are not powered for these secondary outcomes. In our subsample of 40 local participants who will contribute urine after levels (uNGAL by simple subtraction; uWBC and nitrites by comparison of level (0 = no change; +1=increase by one level (from 0 to 1); -1 = decrease by one level; or +/-2 (for uWBC if change is 2 *or more* levels in either direction). T-test (uNGAL) and chi square tests will be used to compare changes in each outcome by treatment arm; Bonferroni controls for multiple (3) comparisons will be used to control family wise error rates. We plan for 20 contributors per treatment arm to support conclusions in H2b, H2c, and H2d; this sample size is not related to power, but instead maximizes our capacity to collect and analyze this data over the funding period.

H2c. "High" dose intravesical LGG® will result in fewer (species, CFU) cultivable bacteria from urine as compared to "Low" dose. Inference tests are not powered for this secondary outcome. We will compare the average #species and CFU for each species individually and create a "number of species" for each participant based on their results before and after instillation. We will use the Holm method[6] to control for multiple comparisons (of unknown numbers of culture result comparisons per person), and then explore patterns that may emerge based on person or dose arm of which species tend to be reduced or most affected. Results will be useful for hypothesis generation and will be described fully.

H2d. Exploratory: Composition of the urine microbiome, as determined by next generation sequencing, will differ within-person between "symptomatic" (pre-LGG® instillation) and after instillation of LGG®. These within-person results will be more pronounced (larger effect sizes) for those in the "High" dose group relative to those in the "Low" dose group. Inference tests are not powered for these secondary outcomes. In our subsample of local participants who will contribute urine before and after their instillations, separately by dose group (high/low), we will compare the before and after levels of alpha diversity (estimated by Unifrac indices) within person using permutational multivariate analysis of variance (adonis) as implemented in the vegan R package.[7] Significance will be determined as an event that occurs in less than 5% of 10,000 permutations. Bonferroni or Benjamini-Hochberg FDR multiple test correction methods will be applied. All analyses will be performed separately by treatment arm in mothur, QIIME and RStudio (RStudioTeam 2015). Determination of whether the effect size is larger for the high than low dose participants will be determined qualitatively and without an inference test.

Data analysis of SA2:

H3a: Beta diversity values, and algorithmic clustering of amplicon sequence variants (ASVs), from next generation sequencing (NGS) will each be less accurate than our integrative model (with quantitative and qualitative clinical data) at detecting clinically meaningful and interpretable symptom clusters *across* people with urinary symptoms (i.e., in SA2).

H3a: Beta diversity values (method 1) will be computed based on the ASVs for individuals who are symptomatic but fall into different QCPs. These values will be compared by ANOVA (if there are >3 QCPs) or t-test (if there are two QCPs). We will use multi stage heterogeneous ensemble (meta-) learning for the clustering of ASVs (method 2), accomplished with the R package EnsemblePCReg. This program integrates results from seven different machine learning approaches, including random forests gradient boosting machines, K-nearest neighbors, and Bayesian additive regression trees. Accuracy of the ASV clustering will be determined based on whether, and if so which, QCPs are recovered and if individuals are "correctly" classified together, if they have the same QCP or if there are interpretable organism "signatures" that characterize QCPs. Our model (method 3), trained on "ideal", known, simulated data will incorporate a realistic collection of qualitative and quantitative inputs, and will yield a prediction rule

that we will test against the *a priori* QCPs. This network will be used to classify individuals based on all variables (symptoms, urinalysis, urine culture, and sequencing). Accuracy of the prediction rule will be defined based on % correct identification of QCP. These three methods (beta diversity, clustered ASVs, and our model outputs) will be compared quantitatively on their "accuracy" (overall % correct) and qualitatively on the nature of misclassifications, e.g., characterizing all individuals with the majority QCP may optimize overall % correct, while missing 100% of a phenotype that is relatively rare.

H3c: Algorithmic combinations of ASVs from NGS *with* standard clinical assessments will be less accurate than our integrative model (with quantitative and qualitative clinical data) at detecting clinically meaningful and interpretable: i) change from baseline (within person); and, ii) symptom clusters (across person).

We will apply multi-stage heterogeneous ensemble (meta-) learning for the clustering of ASVs plus standard clinical assessments with the R package EnsemblePCReg, and compare this purely computational approach (without theoretical, clinical, or patient perspectives) to our integrative network's prediction rule. These two approaches will be applied to the change from baseline data (H3b) and to the symptom cluster data (H3a). Accuracy of the purely computational clustering will follow determinations laid out for H3a and H3b; in this hypothesis we are testing whether adding all of the quantitative information in a data-driven way (i.e., excluding qualitative inputs) will improve performance of detecting symptom clusters (H3a) or changes from baseline (H3b). The inputs and results for our network from H3a and H3b will be re-used for H3c.

Power for SA3 (all hypotheses): No inference tests are planned so no power calculations are relevant.

17.2 Describe the steps that will be taken to secure the data (e.g., training, authorization of access, password protection, encryption, physical controls, certificates of confidentiality, and separation of identifiers and data) during storage, use, and transmission.

Confidentiality will be maintained by ensuring that no identifying features are observable in data from the research studies. Subjects will be identified in data files by an alphanumeric study code only so that no individual identification can be made, and all hardcopy data will be stored in locked offices or cabinets to which only the study PI and coordinator will have a key. The alphanumeric code list will be kept on a password protected computer. These procedures will be effective in maintaining confidentiality. Data will be maintained for a period of ten (10) years, after which digital files will be deleted and paper files will be shredded and discarded.

To protect against adverse events associated with administration subjects will be provided a copy of their signed consent form, which will contain emergency contact numbers for the physician responsible for their medical care. Throughout the study we will monitor participant reactions to the intervention, and if they appear to not want or dislike a treatment, as manifested in increased displeasure or in the rejection of the treatment in gestures or words, the study Investigators will withdraw the intervention. Both patients and caregivers will be able to discontinue participation at any point during the study.

While we do not anticipate adverse events, we will advise the subject to seek medical attention if we feel it is necessary due to any potential adverse effect or inter-current illness. Adverse experiences that are both serious, unexpected and potentially related to study drug will be reported the IRB within 24-48 hours of being notified by the investigator by telephone to MedStar Health Research Institute's Office of Research Integrity (ORI). Additionally, the study clinician (SG at MedStar NRH and AS at TIRR) will complete an MHRI Serious Adverse Event Report (at MedStar NRH), or TIRR's IRB Serious Adverse Event Report form and submit electronically.

17.3 Describe any procedures that will be used for quality control of collected data.

- Study team will conduct monthly quality assurance checks per quality assurance SOP

17.4 Describe how data or specimens will be handled study-wide:

- *What information will be included in that data or associated with the specimens?*

Subject ID and collection date will be the only information included on the specimens

- *Where and how data or specimens will be stored?*
  - Frozen samples will be stored at Children's National Medical Center (CNMC)
  - Naming convention: Date, Subject ID, sample # MM/DD/YYYY DOD
- *How long the data or specimens will be stored?*
  - Up to 10 years if study participants consent to it. If not, until study analyses are completed
- *Who will have access to the data or specimens?*
  - Study approved staff
- *Who is responsible for receipt or transmission of the data or specimens?*
  - *Study staff at MedStar NRH and CNMC*
- *How data or specimens will be transported?*

Data will be on locked computer or in a participant folder in a locked drawer in a locked office. The specimens will be transported using a cooler to ensure specimens are chilled and transported to the lab at CNMC immediately following collection.

18.0 Provisions to Monitor the Data to Ensure the Safety of Subjects*

This section is required when research involves more than Minimal Risk to subjects.

18.1 Describe:

- *The plan to periodically evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe. The plan might include establishing a data monitoring committee and a plan for reporting data monitoring committee findings to the IRB and the sponsor.*

A Data Safety Monitoring Board (DSMB) has been established. The DSMB will meet two times a year (or as requested by the DSMB). Each report will consist of five sections, including: 1) recruitment, 2) adverse effects of the investigational product (i.e. the intravesicular Lactobacillus instillation), and 3) data accrual and quality. The recruitment section will include counts for screening (eligible v. ineligible), enrollment (consented v. declined), and retention (completion of one episode of exposure of intervention vs. withdrawal or lost-to-follow-up). For reporting purposes, subgroups will include standard demographics (i.e. gender, race, and ethnicity). The adverse effects section will report all adverse outcomes that appear to be associated with the investigational product, and will include specific event details, relevant tests and laboratory results, relevant histories (including pre-existing and co-existing medical conditions), use of concomitant medications, and investigational product dose, frequency,, timing of instillation, manufacturer's lot number, and manufacturer's expiration date (as appropriate for completion of the MedWatch FDA Form 3500A).

In addition to DSMB we will have an independent research monitor. The research monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO.

If an SAE is related to the study drug and is reported, an emergency DSMB meeting will occur within 48 hrs of event reported to the study team.

- *What data are reviewed, including safety data, untoward events, and efficacy data.*
    - Reported Adverse Events will be reviewed weekly, If an SAE is reported by phone or email it will be reviewed by study staff immediately

- *How the safety information will be collected (e.g., with case report forms, at study visits, by telephone calls with participants).*
    - If an Adverse Event is reported through their weekly USQNB-IC completion, the study coordinator will follow-up with participant to gather more information and then discuss with study PI. If adverse event is reported by email or phone, we will contact participant as described above
- *The frequency of data collection, including when safety data collection starts.*
    - Study related data will be collected weekly through USQNB-IC. Participants also have the option to phone/email if they are experiencing any AEs. All study participants are directed to call study team immediately if experiencing a SAE related to study drug.
- *Who will review the data?*
    - Study team including PI
- *The frequency or periodicity of review of cumulative data.*
    - Completion will be reviewed weekly
- *The statistical tests for analyzing the safety data to determine whether harm is occurring.*
    - Based on participant reporting of adverse events. DSMB will meet 2 times a year or more frequently if needed. DSMB will also be available for emergency meeting as needed
- *Any conditions that trigger an immediate suspension of the research.*
    - Serious Adverse Events caused by *Lactobacillus GG.*

19.0 Provisions to Protect the Privacy Interests of Subjects

19.1 Describe the steps that will be taken to protect subjects' privacy interests. "Privacy interest" refers to a person's desire to place limits on whom they interact or whom they provide personal information.

- Research will be performed following review and approval of MedStar Institutional Review Boards (IRB). The MedStar IRBs are registered under a Federal Wide Assurance granted to MedStar Health Research Institute (MHRI) by the Office for Human Research Protections of the U.S. Department of Health and Human Services. The FWA number is FWA00000504. Under this Assurance, the MedStar IRBs carry out their duties in accordance and compliance with

mandates set by the Department of Health and Human Services, the Food and Drug Administration, federal sponsors, state regulations, and other principles and norms.

- MHRI's IRB oversees MedStar NRH.
- Guidelines for securing data involving PHI will be adhered to in our study protocols.
- Continuing and final reports to the IRB will be approved by Dr. Groah.

Confidentiality will be maintained by ensuring that no individually-identifying features are observable in data to be collected or generated. Subjects will be identified in data files by an alphanumeric study code only so that no individual identification can be made, and all hardcopy data will be stored in locked offices or cabinets to which only the study PI and coordinator will have a key. The alphanumeric code list will be kept on a password protected computer. These procedures will be effective in maintaining confidentiality. Data will be maintained for a period of ten (10) years, after which digital files will be deleted and paper files will be shredded and discarded.

19.2 Describe what steps you will take to make the subjects feel at ease with the research situation in terms of the questions being asked and the procedures being performed. "At ease" does not refer to physical discomfort, but the sense of intrusiveness a subject might experience in response to questions, examinations, and procedures.

To protect against adverse events and to make the subjects feel at ease, we will be provide a copy of their signed consent form, which will contain emergency contact numbers for the physician responsible for their medical care. Both study participants and clinicians will be able to discontinue participation at any point during the study.

While we do not anticipate adverse events, we will advise the subject to seek medical attention if we feel it is necessary due to any potential adverse effect or inter-current illness. Adverse experiences that are both serious and unexpected will be reported the IRB within 24-48 hours of being notified by the investigator by telephone to MedStar Health Research Institute's Office of Research Integrity (ORI). Additionally, the study clinician (SG, as relevant) will complete an MHRI Serious Adverse Event Report form and submit electronically.

19.3 Indicate how the research team is permitted to access any sources of information about the subjects.

All project staff that have contact with data, will pass web-based IRB and HIPAA training through the MedStar Research Institute's Office of Research Integrity.

20.0 Compensation for Research-Related Injury

20.1 If the research involves more than Minimal Risk to subjects, describe the available compensation in the event of research related injury

- No compensation is available.

20.2 Provide a copy of contract language, if any, relevant to compensation for research-related injury.

- N/a

21.0 Economic Burden to Subjects

21.1 Describe any costs that subjects may be responsible for because of participation in the research.

21.2 N/A

22.0 Consent Process

22.1 Indicate whether you will you be obtaining consent, and if so describe:

- *Where will the consent process take place?*

The consent process will either be in person or over the phone. If consent is not in-person, a consent will be mailed or e-mailed to the participant. If in-person, the subject will do the initial assessment after consent

Phone screening: Research staff will explain the study and go over all main points of the consent form. Afterwards, staff will answer any questions the potential participant may have. Research staff will then either email the informed consent or send in regular mail for the potential participant to read over, contact us with any questions and sign the consent form and send back to us. Once the person obtaining the consent has signed the document, a copy of the signed informed consent form will be sent back to the participant for their records.

In person: Research staff will explain the study and go over all main points of the consent form. Afterwards, staff will answer any questions the potential participant may have. The potential participant is then given the option to read through and sign the consent form while at MedStar NRH or bring it home to read and sign the document. They will then bring the informed consent back to research staff. Research staff will sign the consent and give the participant a copy of the signed consent form for their records.

- *Any waiting period available between informing the prospective subject and obtaining the consent.*

SA 2 does not require a waiting period if inclusion criteria is not current met but can be (i.e currently on antibiotics). Upon study enrollment participants will fill

out urinary symptoms weekly until symptoms occur that an instillation is required for the intervention.

- *Any process to ensure ongoing consent.*

Weekly check-ins with participants will ensure ongoing consent and compliance with the study until SA 1 and SA 2 are completed.

- *Whether you will be following "SOP: Informed Consent Process for Research (HRP-090).*

Yes, we will follow HRP-090

- *" If not, describe:*
  - *The role of the individuals listed in the application as being involved in the consent process.*
  - *The time that will be devoted to the consent discussion.*
  - *Steps that will be taken to minimize the possibility of coercion or undue influence.*
  - *Steps that will be taken to ensure the subjects' understanding.*

Non-English-Speaking Subjects- N/A

Waiver or Alteration of Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception)- N/A

Subjects who are not yet adults (infants, children, teenagers)- N/A

Cognitively Impaired Adults—N/A

Adults Unable to Consent- N/A

Adults Unable to Consent

- *For HUD uses provide a description of how the patient will be informed of the potential risks and benefits of the HUD and any procedures associated with its use.*

23.0 Process to Document Consent in Writing

23.1 Describe whether you will be following "SOP: Written Documentation of Consent (HRP-091)." If not, describe whether and how consent of the subject will be documented in writing.

Yes, SOP: Written Documentation of Consent will be followed.

24.0 Setting

24.1 Describe the sites or locations where your research team will conduct the research.

- *Identify where your research team will identify and recruit potential subjects.*

At MedStar NRH, over the phone, or at participants household.

- *Identify where research procedures will be performed.*

At MedStar NRH, over the phone, or at participants household.

- *Describe the composition and involvement of any community advisory board.* - N/A
- *For research conducted outside of the organization and its affiliates describe:*
  - *Site-specific regulations or customs affecting the research for research outside the organization.*
  - *Local scientific and ethical review structure outside the organization.* - N/A

25.0 Resources Available

25.1 Describe the resources available to conduct the research: For example, as appropriate:

- *Justify the feasibility of recruiting the required number of suitable subjects within the agreed recruitment period. For example, how many potential subjects do you have access to? What percentage of those potential subjects do you need to recruit?*

MedStar NRH Outpatient Network has 60+ locations, including one at MedStar NRH, to provide physician care and outpatient therapy in Washington DC, Maryland, Virginia, and Delaware. Over 400,000 patients are seen annually across the MedStar NRH Outpatient Network.

We have access to MedStar databases with 300+ individuals with spinal cord injury, and we will work with nationwide spinal cord injury organization

- *Describe the time that you will devote to conducting and completing the research.*
  - We have several members on the study team that will devote significant time on this study as budgeted to ensure study completion.
- *Describe your facilities.*

MedStar NRH has over 12,000 square feet of dedicated laboratory space in its Research Division. This area comprises of offices, workshop, development, four clinical research rooms fully accessible for participant testing

Children's National Medical Center is within walking distance from MedStar NRH on the same medical campus.,

The investigators and personnel in CRI have ample laboratory space in addition to 15,000 square feet of space for the Sheikh Zayed Institute for Pediatric Surgical Innovation.

Equipment: For the proposed study, the preliminary processing steps happen at a research facility wet-bench workspace because they require access to equipment and materials that facilitate sterile handling conditions and sterile processing techniques. This includes a natural gas pump and Bunsen burner, a vacuum pump and collection flask, containers for sterile serological pipettes and sterile Pasteur pipettes, containers for sterile PBS wash solution, and spray bottles for 70% Ethanol and 10% Clorox/Bleach solutions. Access to an autoclave, vortex, centrifuge, and -80 ºC ultra-cold freezer, is also required for equipment preparation, specimen processing, and long-term, pre-metagenomics specimen storage. The key to this preliminary specimen processing is separating bacterial cells from the urine. This separation is done with a centrifuge. We use a Beckman Coulter Allegra X-14R centrifuge. It has a rotor that is capable of spinning our 50mL native urine acquisition tubes at a force of 5000g (for 20 minutes, at a temperature of 4 ºC), which are conditions that are necessary to obtain the bacterial cells.

- *Describe the availability of medical or psychological resources that subjects might need as a result of an anticipated consequences of the human research.*
    - N/a
- *Describe your process to ensure that all persons assisting with the research are adequately informed about the protocol, the research procedures, and their duties and functions.*
    - We review the study protocol in detail with all study staff and asks questions to ensure they understand the protocol and the procedures involved. A log is also completed and signed off by study staff regarding duties and functions.

26.0 Multi-Site Research*

University of Pittsburgh (Pitt) will complete primary microbiome sequencing and assist in microbiome data analysis. Loyola University in Chicago, IL will complete secondary microbiome data analysis. Georgetown University (GU) will assist with all aspects of data analysis for this study. Appropriate agreements will be put in place with all institutions prior to sharing de-identified data.