STANFORD UNIVERSITY CONSENT FORM

Are you participating in any other research studies? _____ yes _____no

If so, please describe the other study or studies, including the title, purpose, a summary of the procedures you will experience, and contact information for the research staff:

PURPOSE OF RESEARCH

You are invited to participate in a research study of the microbes (microscopic living organisms) that inhabit the human body. These microbes are mostly bacteria, but also include single-celled, bacteria-like organisms called archaea, as well as viruses, yeasts, and more complex microscopic organisms. Different communities of microbes normally live in every accessible location on and in a healthy human body, including the skin, mouth, nose, vagina and gastrointestinal tract. Collectively, we refer to all the microbes that normally live on or in the human body as the 'native human microbiota'. Our study will mostly be concerned with bacterial part of the native human microbiota. Researchers and physicians are increasingly aware of how this native human microbiota influences human health, both positively and negatively. By collecting samples from these communities over time, we hope to learn how stable (or unstable) these communities are during normal conditions, and to learn how they are affected by disturbances such as a change in diet, a course of antibiotics, or a colonic cleansing (as if preparing for a colonoscopy). Alternatively, you may be asked for up to three fecal specimens prior to any perturbation, so that the feces can be incubated in a flask in the laboratory and studied for the changes in the microbial communities after the addition of compounds (such as arsenic) to the flask. Our research will contribute to a more detailed understanding of the normal activities of the native human microbiota, and may also help us to prevent or reverse harmful changes in the microbiota. We will use 4 primary methods to study the microbiota: 1) determine the identity and relative abundance of the various microbial types in a sample, 2) assess the relative abundance of the many different microbial genes carried by these microbes, 3) estimate the relative expression levels of these genes (how much each gene is being used), and 4) measure the concentrations of a wide range of different chemical compounds in the microbial habitat.

In addition to data about the microbiota, we will collect information from and about the participants in the study, so that we can relate the composition and activity of the microbial communities to the history, health and activities of their human hosts. Much of this information will be obtained from questionnaires that ask about the health, diet, activities, family status, and personal history of the study subjects; other information may come from routine physical examination of the subjects (e.g. height, weight, blood pressure, pulse, etc.). We will ask some participants for two additional sources of information about them (participants may decline). The first source is one or more blood samples which will be used for standard clinical tests (e.g.

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cholesterol levels), assessments of the immune system, and measurements of small molecules that reveal metabolic activity in both native microbes and the human host. The second additional source is genetic testing and gene expression profiling using human nucleic acids. Genetic testing (sometimes called DNA testing or genotyping) means determining which variants of certain genes are carried by the participants. The human genes that will be tested are ones known or suspected to be involved in microbial interactions with the host. The DNA for genotyping can be obtained from blood samples, saliva samples or cheek swabs. Gene expression profiling means determining which genes are actively being used by a particular population of cells. The RNA used for gene expression profiling needs to come from blood samples. The information we obtain from and about participants may be released publicly in an anonymous fashion, along with the data we obtain about the microbiota, so that other scientists can also look for relationships between the microbiota and various aspects of human activity, history and health.

You were selected as a possible subject in this study because you are a healthy adult who expressed an interest in participating. This research study will involve up to 200 healthy adults aged 18 or older, of either gender and any ethnicity or origin, who will be recruited from the U.S. (primarily from the San Francisco Bay Area). Individuals under 18 years of age, and women who are pregnant, or who intend to become pregnant, will not be enrolled, because ciprofloxacin, the antibiotic used in some study arms, is not recommended for these individuals. Furthermore, such individuals will not be enrolled in other arms of the study since pregnancy is known to have strong, specific effects on the gut microbiota that would complicate the interpretation of data from this study. Women who are breastfeeding will not be enrolled in study arms involving ciprofloxacin, but may be enrolled in other study arms. Individuals will not be enrolled if any of the following conditions are met: current diagnosis of a serious chronic health condition; any hospitalizations or antibiotic use within 6 months prior to the study; acute illness, immunization or international travel within 4 weeks prior to the study; routine use of any prescription medication except birth control, hormone replacement therapy, or medication that does not primarily affect the gut microbiota or GI tract; allergy or sensitivity to corn, potatoes or corn or potato products (for study arms involving diet supplementation); allergy, sensitivity, or history of adverse reaction to fluoroquinolone antibiotics (for study arms involving ciprofloxacin); inability or unwillingness to minimize exposure to sunlight or UV light during and shortly after taking ciprofloxacin (for study arms involving ciprofloxacin); allergy, sensitivity, or history of adverse reaction to polyethylene glycol (for study arms involving colon cleansing); planned international travel of more than 3 weeks during the study or to regions without modern sanitation. By agreeing to participate in this study, you indicate that you are a healthy adult and none of the exclusion criteria listed in this paragraph apply to you.

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Your participation in this study is entirely voluntary. Your decision whether or not to participate will not affect your medical care, since this study does not provide medical care and if you require care that is inconsistent with this study, your participation in the study will be withdrawn. If you wish to participate in this study, you must sign this consent form. If you decide to participate, you are free to withdraw your consent (including your authorization regarding the use and disclosure of your health information) and to discontinue participation at any time without prejudice to you or any effect on your medical care. If samples that you have provided for the study have already been stored in an anonymous fashion (so they can not be linked to you), and you decide to stop participating in the study, it will not be possible to stop further research on the samples you have already provided. If you decide to terminate your participation in this study, you should notify Les Dethlefsen at 541-207-5791 or dethlefs@stanford.edu.

DURATION OF STUDY INVOLVEMENT

This entire research study is expected to last for approximately 5 years. Your primary involvement in the study is expected to last between 25-36 weeks, depending on which arm of the study you are enrolled in. During most of the 25-36 weeks, we will request one set of microbiota samples per week. During some weeks, especially before, during and after any deliberate perturbations of the microbiota, we will request samples on a defined schedule that may include every day, every second day, or every third day. We may request that some participants continue to provide follow-up microbiota samples at less frequent intervals for up to two years after completing the study interval described above. Participation in this follow-up sampling is not required.

PROCEDURES

If you choose to participate, Dr. Relman and his research study staff may ask you to:

1) Fill out detailed questionnaires that ask you about your health, diet, activities, family status and personal history. Most questionnaires will have to be done once (e.g. listing the places where you've lived), but some questionnaires may be repeated several times (e.g. asking about recent intestinal symptoms such as indigestion or passing gas). The total time needed to complete all questionnaires during the study is estimated to be several hours. Some questions may address issues that are present in your medical records, but we will not ask to see your records. If you have not had a physical exam in the past year, if you can't remember the measurements, or if you think the values may have changed, we may ask you to participate in an exam that we arrange to collect data such as your height, weight, waist circumference, blood pressure, pulse rate and respiration rate. (Estimated time ¼ to ½ hour, exclusive of travel.) We will ask you to report to us any unusual changes that occur during the study interval in features that were recorded on the initial questionnaires (e.g. you start or

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stop eating categories of food such as meat or gluten, your exercise/activity level increases or decreases beyond its previous range, you change your residence).

2) Collect samples of your own microbiota. We will provide all the necessary materials, and show you how to use them. Most participants will be asked to provide only some, not all, of the following sample types (you will provide the same types of samples on every sampling day throughout the study): stool, urine, saliva (as for providing diagnostic clinical samples), dental plaque from designated tooth surfaces (collected using a sterile toothpick or small brush), swabs from designated areas of the skin, nose, mouth, throat and vagina. (Estimated time less than ¼ hour per sampling episode.) You will be asked to store your samples in your home freezer until transfer of the frozen samples to the laboratory is arranged; this may occur every 2-4 weeks for participants in the Palo Alto area and every 2-3 months for participants returning samples via commercial package shipment. We will provide small coolers and cold packs as necessary. During most weeks of the study, we will ask you to provide one set of samples per week (e.g. every Monday). During some weeks of the study, particularly before, during and after any deliberate disturbances, we will request samples on a fixed schedule that may be daily, every second day, or every third day. There may be a total of 40-70 sampling episodes over 25-36 weeks of the study, depending on what study arm you participate in.

3) Some arms of the study include one or two deliberate perturbations (discrete events which are known to affect the native microbiota in at least some body sites); there are three different types of perturbation in the study as a whole, although individual participants will experience zero, one or two perturbations depending on which arm of the study they participate in. (Please refer to the chart of procedure for different sample arms at the end of this section.) You will know at the start of the study what type or types of perturbations you are being asked to participate in, and when these are scheduled to occur. (The study staff will work with you to schedule the sampling and perturbation at times that are convenient for you and will provide us with the necessary samples.) The three perturbation types are: 1) Diet supplementation with resistant starch: adding 50 g of HiMaize starch or 40 g of unmodified potato starch (about the weight of an energy bar) to your diet every day for 5 days. Both HiMaize starch (from corn) and unmodified potato starch contain a high proportion of resistant starch which is not digested or absorbed in the small intestine; instead it is broken down by certain bacteria present in the colons of most people. Both forms are white, almost flavorless powders that can be mixed with water, juice, smoothies, cereal or other foods. (Minimal time requirement, minor disruption to normal routines.) 2) Short course of ciprofloxacin: taking one 500 mg tablet twice a day for 5 days. Ciprofloxacin is an antibiotic in the fluoroquinolone class; this dosage and duration might typically be prescribed for an uncomplicated urinary tract infection. (Minimal time requirement for actually taking the tablets, although some participants may spend time coping with common side effects such as nausea (5.2% of patients), diarrhea (2.3%), and vomiting (2%.)) 3) Colonic cleansing: drinking up to 16 glasses of Golytely solution (polyethylene glycol and electrolytes) in one

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day and following a clear liquid diet for that day. This is an invasive procedure because it will induce a large volume of diarrhea to empty the entire colon of partially digested food and fecal material; it is a typical preparation step taken the day prior to receiving a colonoscopy. Participants need to plan for a 6-8 hour period at home without other responsibilities.

4) Some participants will be asked to provide either a single blood sample or multiple blood samples over time; **providing blood samples is optional for participants.** This is an invasive procedure using a needle inserted into a vein in the arm, similar to the procedure for donating blood, although the maximum total volume of blood samples we would request is smaller than the typical blood donation volume. (Estimated time ¼ to ½ hour per sample, exclusive of travel.) The samples may be used for typical clinical tests (e.g. measuring levels of cholesterol and triglycerides), for assessing the activation status of the immune system, for measuring the concentration of many different small molecules that reflect both human and microbial metabolic activities, and/or for extracting nucleic acids for genetic testing and gene expression profiling (if permission is granted, see item 5.) The purpose of multiple blood samples is to look for changes in these measurements that may be related to changes in the microbiota. Between 16-30 mL of blood (1-2 tablespoons) will be withdrawn at a single time. Most participants who are asked to provide multiple samples will be asked for 8 samples over 6 weeks (8-16 tablespoons total) surrounding a perturbation event; in no case will participants be asked to provide more than 3 blood samples (3-6 tablespoons total) in any 1 week or more than 16 samples (16-32 tablespoons total) over any 10 week interval.

Please circle and initial one of the two statements:

_____ I am willing and interested in providing blood samples.

_____ I am not interested in providing blood samples.

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5) Some participants will be asked to participate in genetic testing and/or gene expression profiling. Genetic testing will involve determining which human gene variants are carried by the participant for particular genes known or suspected to be involved in microbial interactions with the host. It will be done by obtaining the sequence of nucleotides in defined, short sections of the participant's DNA; the DNA will be obtained from blood samples (see item 4), saliva samples or cheek swabs. Gene expression profiling will involve assessing which human genes are active in a sample of cells. It will be done by obtaining the nucleotide sequences of RNA molecules from blood samples (see item 4). **Participation in genetic testing or gene expression profiling is optional for participants.**

Please circle and initial one of the two statements:

_____ I am willing and interested in undergoing genetic testing and/or expression profiling.

_____ I am not interested in undergoing genetic testing or expression profiling.

6) Some participants experiencing a ciprofloxacin perturbation will be asked to take part in physiological testing, including a lactate threshold test, and a test for aerobic capacity ($\dot{V}O_2$peak test). Such testing will be conducted on the Stanford University campus, and will occur three times before and after the perturbation. **Physiological testing is optional for participants.** Certified personnel will administer the testing. The lactate threshold test is a non-maximal effort exercise test on a stationary bicycle or treadmill. Blood lactate (finger or earlobe pinprick) will be measured at regular intervals to until lactate threshold is detected. The purpose of the $\dot{V}O_2$peak test is to determine maximum exercise capacity, and participants will be required to either pedal a stationary exercise bicycle or run on a treadmill at progressively harder workloads until they become fatigued and decide to stop. Oxygen consumption will be measured using a mouthpiece. Heart rate may be monitored throughout the tests using a chest strap. At any time during these tests, the participant can request to stop the test. The lactate threshold and $\dot{V}O_2$peak tests may be performed at the same time, and will take approximately 45-60 minutes in total, including a warm-up and cool-down period.

Please circle and initial one of the two statements:

_____ I am willing and interested in physiological testing.

_____ I am not interested in physiological testing.

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7) If you are taking ciprofloxacin as a deliberate perturbation of the microbiota, monitor yourself for any serious or potentially serious side effects of ciprofloxacin using the information we provide, responding immediately as appropriate. **Notify us promptly (Les Dethlefsen, 541-207-5791, dethlefs@stanford.edu) of any serious or potentially serious side effects of ciprofloxacin that you experience or suspect, and immediately stop taking ciprofloxacin.** Potentially serious side effects are any that involve the musculoskeletal system (such as pain in tendons, ligaments, muscles or joints), the central nervous system (such as agitation or confusion), the peripheral nervous system (such as shooting pain or extreme sensitivity), or skin (such as itching or severe sunburn). Also, while you are taking ciprofloxacin and for several days thereafter, minimize your exposure to sunlight and ultraviolet light, since some people experience heightened sensitivity to sunburn while taking ciprofloxacin. (Normal outdoor activities wearing sun protection would be ok; deliberate sunbathing or tanning, or more than a short time in the sun at high-UV locations such as the beach, desert or high elevation would not.)

For some of the analyses that we will perform, your samples may be sent to collaborating researchers outside of Stanford. The samples will be identified by non-revealing codes; your identity will not be revealed to research collaborators outside of Stanford.

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There are several arms of the study that differ from each other in duration, in how many microbial perturbations are experienced (if any), and in which microbial perturbations are used. In addition, some participants will undertake optional procedures, such as providing blood samples or participating in genetic testing. Participants are not assigned randomly to study arms; when joining the study participants will decide with study staff which arm to join, based on participant desires and the needs of the study. Particular study arms may no longer be available to new participants once a sufficient number of participants have already joined that arm. The charts that follow give examples of sampling schedules for study arms with 0, 1 or 2 perturbations. The exact schedule of sampling and study procedures is somewhat flexible.

Example of typical timing of procedures for Study Arm 1 (zero perturbations)

The chart below shows the timing of 8 blood samples. Some participants may be asked to provide only a single blood sample instead of 8, and participants are not required to provide blood samples. Slight variations in the timing of sampling may occur.

Week 1: Microbiota samples on 5 days: MTWThF

Weeks 2-15: Microbiota samples on 1 day: M

Week 16: Microbiota samples on 1 day: M, blood sample on 1 day: M

Week 17: Microbiota samples on 5 days: MTWThF, blood sample on 1 day: M

Week 18: Microbiota samples on 7 days: MTWThFSSu, blood samples on 2 days: MTh

Week 19: Microbiota samples on 5 days: MTWThF, blood samples on 2 days: MTh

Week 20: Microbiota samples on 3 days: MWF, blood sample on 1 day: M

Week 21: Microbiota samples on 1 day: M, blood sample on 1 day, M

Weeks 22-35: Microbiota samples on 1 day: M

Week 36: Microbiota samples on 5 days: MTWThF

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Example of typical timing of procedures for Study Arm 2 (one perturbation, shown as ciprofloxacin)

The chart below shows the timing of 8 blood samples. Some participants may be asked to provide only a single blood sample instead of 8, and participants are not required to provide blood samples. Slight variations in the timing of sampling may occur. The sample schedule shows a perturbation with ciprofloxacin; if the perturbation were to be colonic cleansing instead, it would occur around the end of week 12 or during week 13.

Week 1: Microbiota samples on 5 days: MTWThF

Weeks 2-10: Microbiota samples on 1 day: M

Week 11: Microbiota samples on 1 day: M, blood sample on 1 day: M

Week 12: Microbiota samples on 5 days: MTWThF, blood sample on 1 day: M

Week 13: Microbiota samples on 7 days: MTWThFSSu, blood samples on 2 days: MTh, ciprofloxacin taken on 5 days: MTWThF

Week 14: Microbiota samples on 5 days: MTWThF, blood samples on 2 days: MTh

Week 15: Microbiota samples on 3 days: MWF, blood sample on 1 day: M

Week 16: Microbiota samples on 1 day: M, blood sample on 1 day, M

Weeks 17-24: Microbiota samples on 1 day: M

Week 25: Microbiota samples on 5 days: MTWThF

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Example of typical timing of procedures for Study Arm 3 (two perturbations, shown as resistant starch and colonic cleansing, could also be resistant starch and ciprofloxacin)

The chart below shows a single blood sample. If a participant in arm 3 provides 8 blood samples instead, they would be spread over 6 weeks surrounding one of the perturbations, as shown for arms 1 and 2. Slight variations in the timing of sampling may occur. For most participants in this arm, the first perturbation during week 11 will be dietary supplementation with resistant starch, and the second perturbation during week 22 will be either colonic cleansing or ciprofloxacin.

Weeks 1-9: Microbiota samples on 1 day: M

Weeks 10: Microbiota samples on 5 days: MTWThF

Week 11: Microbiota samples on 7 days: MTWThFSSu, blood sample on 1 day: M, resistant starch given on 5 days MTWThF

Week 12: Microbiota samples on 5 days: MTWThF

Week 13: Microbiota samples on 3 days: MWF

Weeks 14-20: Microbiota samples on 1 day: M

Week 21: Microbiota samples on 5 days: WThFSSu, colonic cleansing on Su

Week 22: Microbiota samples on 5 days: MTWThF

Week 23: Microbiota samples on 3 days: MWF

Weeks 24-34: Microbiota samples on 1 day: M

WOMEN OF CHILDBEARING POTENTIAL

If you are a woman who is able to become pregnant and you are enrolled in an arm of the study including ciprofloxacin exposure, it is expected that you will use an effective method of birth control to prevent exposing a fetus to a potentially dangerous agent with unknown risk. **If you are pregnant, or if you intend to become pregnant during the study interval, you may not participate in this study.** You understand that if you are pregnant, or if you become pregnant and you take ciprofloxacin, your fetus may be exposed to ciprofloxacin which is not recommended for individuals under 18 years of age. The reason for this recommendation is that ciprofloxacin may adversely affect the cartilage layers that are essential to normal bone growth. If you are breastfeeding, you may enroll only in arms of the study that do not involve ciprofloxacin exposure to avoid exposing your child to ciprofloxacin via breast milk. To confirm to the extent medically possible that you are not pregnant while taking ciprofloxacin, you agree to begin the course of ciprofloxacin shortly after the onset of a menstrual period. If you become pregnant during the study, notify Les Dethlefsen at 541-207-5791, or dethlefs@stanford.edu.

SAMPLE BANKING FOR FUTURE RESEARCH

We request permission to obtain and store microbiota, blood and nucleic acid samples from you in order to have the capacity to analyze them repeatedly, with multiple methods. In addition to the intended research questions addressed by the purposes and procedures of the current study, unanticipated research questions may arise which could also be addressed using microbiota and other samples collected for this study. The reuse of existing sample collections, when appropriate, may speed the progress of research. For example, evidence may emerge suggesting that a newly-described bacterial gene found only in some types of colonic bacteria has a specific effect on human health. Stool samples collected for this study may be an appropriate and readily-available resource for investigating the normal range in abundance of these bacterial types and this bacterial gene in healthy adults, which will help researchers to investigate the new hypothesis. There are several things you should know before allowing your samples to be stored for future research:

1. Anonymity: Any samples that you provide will be stored in freezers under a unique code for security and confidentiality. The code will be associated with anonymous patient data about you (the answers you provide on questionnaires, and data obtained from other samples you provide). The samples you provide and anonymous patient data about you may be shared with other investigators in the future. There will be separate files (both paper and electronic) that link your sample codes with your name and other public identifiers (information which could be used to identify you); **only the study personnel of the current study will have access to the files with your name and public identifiers**. Your identity (name and other public identifiers) will not be shared with other investigators. The files that link your identity

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to your sample codes and anonymous patient data will be destroyed after the conclusion of the current study. After that time, it will not be possible for you to withdraw your consent for research to continue on the samples you have provided, because it will not be possible to link those samples to you.

2. Right to Withdraw. You have the right to refuse to allow your microbiota or blood samples to be studied now or saved for future study. (If you do not want your samples to be studied now, you should not participate in the current study.) If you decide to participate, you may later decide to withdraw from this study at any time, and may also ask that we stop conducting research on samples you have already provided. However, once your samples are stored anonymously (after your identity is no longer linked to the sample codes), it will not be possible to withdraw your consent to continue studying the samples you have provided.
3. Completion of Research. Microbiota samples will be analyzed by one or more methods to describe the composition and/or activity of microbial populations. Blood samples will be analyzed by one or more methods to analyze aspects of host health and immune activity. Nucleic acid samples (extracted from blood samples, saliva samples or cheek swabs) will be analyzed to determine which human gene variants are present, and which human genes are being expressed. Remaining material will be stored after the completion of the current study to permit additional analyses in the future. The samples will be stored anonymously once the files linking participant identity to the sample codes have been destroyed after the conclusion of the current study.
4. Follow Up Contacts. Investigators in this study may try to re-contact you in the future.
5. Use in Commercial Development of Products. Any of your samples which are used in research may result in new products, tests or discoveries. In some instances, these may have potential commercial value and may be developed and owned by the Investigators, Stanford University and/or others. However, donors of samples do not retain any property rights to the materials. Therefore, you would not share in any financial benefits from these products, tests or discoveries.

Please circle and initial one of the two statements:

_____ I consent to my samples being saved for future research.

_____ I do not consent to my samples being saved for future research.
The samples will be used only for the current study.

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LEARNING OF RESULTS FROM MICROBIAL ANALYSES AND GENETIC TESTING

Microbial Analyses

Given the current state of medical and scientific knowledge and the fact that you are currently healthy, we do not anticipate that our analysis of the microbial communities in your samples will have any bearing on your current medical care. For people without disease, there are not yet recognized standards for what constitutes a 'safe' or 'healthy' microbiota. A number of correlations have been reported between various aspects of the microbiota and factors related to diet, health, and medical history. Some of the known effects of diet on human health have been suggested (with varying degrees of supporting evidence) to be partially or entirely caused by microbial processes. However, it must be expected that some of the published correlations and theories linking the microbiota and health will prove to be valid only in limited contexts, or not at all. Furthermore, even if a particular change in the microbiota were known to be desirable, our ability to bring about that change may be limited.

Despite the uncertain state of our current knowledge, some individuals promote particular theories and treatments that address symptoms or issues related to (or claimed to be related to) the microbiota. There is a genuine, although unmeasurable risk that someone with a microbiota-related health concern will pursue a course of action that is ineffectual or even harmful. To minimize this risk, participants who want to learn of the results we obtain from analysis of their own microbiota samples will receive that information during an individual conference with a member of the study staff. Participants will be told what has been learned from their own samples, and how their results compare to those of other healthy adults, in the context of our current scientific understanding of the microbiota. Participants will be encouraged to ask questions, particularly if they are considering taking steps to influence their microbiota. However, the study staff will not be (and are not qualified to be) providing individualized health or medical advice to participants; they can only guide participants to published scientific evidence that is relevant to their concerns. Participants who feel the need for individual advice are encouraged to speak with their usual health care provider.

Please circle and initial one of these two statements:

_____ I wish to be told of the results of the microbial analysis of my samples, at an individual conference with a member of the study staff as described above.

_____ I do not wish to be told the results of the microbial analysis of my samples.

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Genetic Testing

As part of the analysis on your samples, we may do genetic testing. Genetic research is research that studies genes, including gene characteristics and gene versions that are transmitted by parents to children. Genetic research may include looking at information, such as personal appearance and biochemistry, gene sequences, genetic landmarks, individual and family medical histories, reactions to medications and responses to treatment. Genetic research raises certain questions about informing you of any results. Possible risks of knowing results include: anxiety; other psychological distress; and the possibility of insurance and job discrimination. A possible risk of not knowing includes being unaware of the need for treatment. These risks can change depending on the results of the research and whether there is a treatment or cure for a particular disease.

Sometimes patients have been required to furnish information from genetic testing for health insurance, life insurance, and/or a job. A Federal law, the Genetic Information Nondiscrimination Act of 2008 (GINA), generally makes it illegal for health insurance companies, group health plans, and employers with 15 or more employees to discriminate against you based on your genetic information.

The genetic testing we intend to do involves determining the sequence of genes known or suspected to be involved with microbial interactions with the host. In some cases, gene variants that we might detect have a statistical association with the risk of particular diseases, but in many cases the gene variants we will investigate have no known association with any disease. Many human gene variants that are known to influence the risk for prominent diseases (such as breast cancer and Alzheimer's disease) will not be examined in this study, since there is no reason to suspect microbial involvement in these cases. The genetic testing of your samples in this study will be used for research purposes only, and you will not be told the results of the tests.

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SUBJECT'S RESPONSIBILITIES

If you decide to participate in this study, you should:

- Follow the instructions of the Protocol Director and study staff.
- Complete the questionnaires.
- Provide microbiota samples as directed, according to the sampling schedule, using the materials we provide.
- Keep appointments for transferring samples to study personnel. Keep appointments for a physical exam and/or to provide blood samples, if you have agreed to do so. If you must miss an appointment, contact study personnel to reschedule as soon as possible.
- Follow the instructions for participating in the deliberate perturbation events at the scheduled times, if you are enrolled in a study arm with perturbations. Keep any associated materials stored safely, for your use only.
- Keep the Protocol Director or research staff updated about changes to your contact information.
- Tell the Protocol Director or research staff about changes during the study interval to features that are covered in the questionnaires.
- Tell the Protocol Director or research staff if you believe you might be pregnant.
- Ask questions as you think of them.
- Tell the Protocol Director or research staff if you change your mind about staying in the study.

While participating in this research study, you should not take part in any other research project without approval from all of the Protocol Directors. This is to protect you from possible injury arising from such things as extra blood drawing, extra x-rays, the possible interaction(s) of research drugs, or other similar hazards.

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WITHDRAWAL FROM STUDY

If you first agree to participate and then you change your mind, you are free to withdraw your consent and discontinue your participation at any time. Your decision will not affect your ability to receive medical care, and except for any monetary compensation associated with parts of the study you do not complete, you will not lose any benefits to which you would otherwise be entitled. If you decide to withdraw your consent to participate in this study, you should notify Dr. David Relman at (650) 852-3302

If you withdraw from the study for any reason,

- Please arrange for the return any unused sample collection materials or other materials from the study.
- Please let us know whether you also want us to stop using any samples you have already given us that can be identified as having come from you.
- Please arrange for transfer of any remaining samples stored in your freezer (if you are willing to have us continue studying any existing samples).

The Protocol Director may also withdraw you from the study without your consent for one or more of the following reasons:

- Failure to follow the instructions of the Protocol Director and/or study staff.
- You develop a condition (e.g., pregnancy) or need medical treatment that conflicts with the purposes or procedures of this study.
- The Protocol Director decides that continuing your participation could be harmful to you.
- The study is cancelled.
- Other administrative reasons.
- Unanticipated circumstances.

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POSSIBLE RISKS, DISCOMFORTS, AND INCONVENIENCES

There are risks, discomforts, and inconveniences associated with any research study. These deserve careful thought. You should talk with the Protocol Director if you have any questions. Risks you should be aware of include:

1) Risks associated with sampling:

Self-collection of stool samples involves a small increased risk of contaminating the skin with feces compared to normal toilet practices; however, normal hand-washing minimizes the likelihood of adverse outcomes. Some participants will be asked to collect a subset of their samples in sample tubes that already contain several milliliters (less than a teaspoon) of a preservative solution that is considered a skin, eye and respiratory irritant. Spills or splashes of this solution could result in irritation; rinsing the affected body parts with water and wiping up the spilled material for disposal in the trash are expected to be adequate responses. Self-collection of microbiota samples from sensitive body sites (e.g. mouth, nose, throat, vagina) involves a small additional risk of minor injury or irritation beyond those of normal hygiene practices.

2) Risks associated with knowledge of the microbiota:

Participants may feel more anxious about potential risks to their health, if they choose to be told of the results from the analysis of their microbiota, and they learn of reported associations between characteristics of their own microbiota and adverse health outcomes. There is an unknown risk that participants anxious about the potential health effects of the microbiota will pursue unproven or harmful methods to attempt to influence their own microbiota.

3) Risk of side effects of ciprofloxacin (for study arms involving ciprofloxacin):

Rare, serious side effects: severe allergic reactions; mental symptoms such as seizures, confusion, agitation or depression; peripheral nerve symptoms such as burning or shooting pain or extreme sensitivity to stimuli; musculoskeletal symptoms such as weakness, spasms, muscle or joint pain or tendon rupture; liver damage; skin burning, itching, rash or blisters following exposure to sunlight or UV light. **Do not continue to take ciprofloxacin if you experience these symptoms**.

Potentially serious side effects: Because a high proportion of the rare, serious adverse effects of fluoroquinolones involving the central and peripheral nervous systems and the musculoskeletal system occurred with continued antibiotic use after less serious adverse symptoms had been experienced, we consider even less serious symptoms involving these body systems to be potentially serious. These symptoms include headaches, dizziness, lightheadedness, insomnia,

tingling, numbness or prickling sensations, changes in sensory perception such as ringing in the ears or altered taste or smell, and soreness in muscles, tendons or joints. In the vast majority of cases, these side effects disappear rapidly once the drug is stopped, with no further complications. As with other medications, severe allergic reactions can be preceded by less serious allergic symptoms such as a skin rash or facial swelling. **Do not continue to take ciprofloxacin if you experience these symptoms**.

Pseudomembranous colitis is another potentially serious side effect that has been reported with nearly all antibiotics, including ciprofloxacin. **If you experience any bloody diarrhea at all, or watery diarrhea that lasts more than 24 hours, stop taking ciprofloxacin.**

Common ciprofloxacin side effects: nausea (5.2% of patients), diarrhea (2.3%), vomiting (2.0%), abdominal pain/discomfort (1.7%), headache (1.2%), rash (1.1%).

Less common (<1%) side effects: sensitivity to sunlight/UV light (tendency to sunburn). Other less common side effects are listed above as potentially serious side effects.

4) Risk of developing ciprofloxacin-resistant microbes:

As with all antibiotics, microbes that are exposed to ciprofloxacin can experience genetic change causing them to become resistant to ciprofloxacin. The normal microbial populations of the human body contain opportunistic pathogens; that is, organisms that usually do not cause disease, but that can cause disease if introduced to a vulnerable location or if the body's defenses are impaired. If you were to develop an opportunistic infection, and if the infecting organism were already resistant to ciprofloxacin, the spectrum of effective antibiotics to treat your infection might be reduced. Cross resistance between ciprofloxacin (Cipro) and older antibiotics in the quinolone/fluoroquinolone class is not unusual. However, newer antibiotics of this class are available, for which high level cross resistance with ciprofloxacin is generally not observed. However, once they are exposed to one of the newer fluoroquinolones, a ciprofloxacin-resistant microbe may develop resistance to it more rapidly than would a ciprofloxacin-sensitive microbe. Cross resistance between ciprofloxacin and antibiotics of other classes (such as tetracyclines, macrolides, cephalosporins, or the penicillins) is generally observed only at low to moderate levels of resistance. In contrast to the types of resistance found for many other types of antibiotics, high level resistance to ciprofloxacin is unlikely to be transferred from one organism to another. Hence, it is unlikely that ciprofloxacin resistance will be transferred from a member of the normal microbial populations of the human body to a pathogenic organism that enters the body after ciprofloxacin use is discontinued.

5) Risk of side effects of Golytely (for study arms involving colonic cleansing):

Reported effects of Golytely (R) oral solution are primarily related to its intended effect of temporarily causing a large volume of diarrhea. According to the prescribing information, "nausea, abdominal fullness and bloating are the most common adverse reactions (occurring in up to 50% of patients) to administration of GoLYTELY. Abdominal cramps, vomiting and anal irritation occur less frequently. These adverse reactions are transient and subside rapidly.

6) Risks associated with providing blood samples (for participants who agree to do so):

Participants may experience pain or bruising associated with the needle puncture to collect a blood sample, and may briefly feel faint or dizzy.

There is a slight risk of an infection due to the blood draw.

7) Risks associated with physiological testing (for participants who agree to do so):

The exercise associated with physiological testing procedures may result in episodes of transient light- headedness, fainting, abnormal blood pressure, chest discomfort, nausea or fatigue. Discomforts include exercising at a moderate to high level of exertion and from wearing a mask. VO2 and lactate assessments do not require the participants to exert themselves greater than their regular training demands.

8) Unforeseen risks:

Although the deliberate microbial perturbations and other procedures in the protocol are well-studied and have been used extensively, there may be unknown risks associated with them, or other aspects of this study.

Discomforts and inconveniences you should be aware of are:

- Inconvenience of collecting and storing microbiota samples at your home, and arranging their transport to the laboratory.
- Discomfort and inconvenience of experiencing a large volume of diarrhea on the day of colonic cleaning (for study arms involving colonic cleansing).
- Potential discomfort and inconvenience of experiencing side effects of ciprofloxacin (for study arms involving ciprofloxacin).
- Potential discomfort and inconvenience of experiencing side effects of resistant starch supplementation, primarily increased flatulence (for study arms involving resistant starch).

- Inconvenience of minimizing exposure to sunlight/UV light while taking ciprofloxacin and shortly thereafter (for study arms involving ciprofloxacin).
- Inconvenience of adding resistant starch to your regular diet (for study arms involving resistant starch).
- Inconvenience of traveling to the laboratory (or another location) to provide blood samples, physiological testing, or have a physical exam (for participants who agree to do so).

POTENTIAL BENEFITS

The most important anticipated benefits of this study are general benefits to society of our improved understanding of the human microbiota, not specific benefits of any sort to the participants in the study. **We cannot and do not guarantee or promise that you will receive any benefits from this study.**

ALTERNATIVES

The alternative to participating in this study is not to participate.

PARTICIPANT'S RIGHTS

You should not feel obligated to agree to participate. Your questions should be answered clearly and to your satisfaction.

If you decide not to participate, tell the Protocol Director. Your medical care will not be affected.

You will be told of any important new information that is learned during the course of this research study, which might affect your willingness to continue participation in this study.

CONFIDENTIALITY

Your identity will be kept as confidential as possible as required by law. Except as required by law or as authorized by you, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier. Your name, address and social security number (but no other information about you) will be used by the Palo Alto Veterans Institute for Research (PAVIR), the organization administering the funding for this study, to comply with accounting and tax requirements associated with providing you compensation for your participation in the study. We have obtained a Certificate of Confidentiality for this project,

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which allows researchers to legally refuse to disclose your identity in many situations (see explanation below).

Your research records may be disclosed to researchers beyond those involved in the current study, including researchers outside of Stanford; however, in this case, your records will be identified only by a unique, non-revealing code so that your identity is not disclosed. Information linking your identity to the code will be kept in a secure location and access limited to research study personnel of the current study.

The results of this research study may be presented at scientific or medical meetings or published in scientific journals, including information about you derived from questionnaires (e.g. diet, health) or from samples (e.g. composition of the microbiota, human gene variants). However, your identity will not be disclosed**.**

Patient information may be provided to Federal and other regulatory agencies as required. The Food and Drug Administration (FDA), for example, may inspect research records and learn your identity if this study falls within its jurisdiction.

Certificate of Confidentiality

To help us protect your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health. The researchers can use this Certificate to legally refuse to disclose information that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings, for example, if there is a court subpoena. The researchers will use the Certificate to resist any demands for information that would identify you, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of Federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

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USE AND DISCLOSURE OF PROTECTED HEALTH INFORMATION

Authorization To Use Your Health Information For Research Purposes

Because information about you and your health is personal and private, it generally cannot be used in this research study without your written authorization. If you sign this form, it will provide that authorization. The form is intended to inform you about how your health information will be used or disclosed in the study. Your information will only be used in accordance with this authorization form and the informed consent form and as required or allowed by law. Please read it carefully before signing it.

What is the purpose of this research study and how will my health information be utilized in the study?
We hope to learn about the stability (or instability) of the native human microbiota under normal conditions, to learn how those communities are affected by perturbations such as a change in diet, a course of antibiotics, or a colonic cleansing, and to look for relationships between the human microbiota and human health. Your health information will be used along with information about the microbiota from your samples to look for such relationships. Your health information may be disclosed anonymously to other researchers, at scientific or medical conferences, and in scientific publications.

Do I have to sign this authorization form?
You do not have to sign this authorization form. But if you do not, you will not be able to participate in this research study. Signing the form is not a condition for receiving any medical care outside the study.

If I sign, can I revoke it or withdraw from the research later?
If you decide to participate, you are free to withdraw your authorization regarding the use and disclosure of your health information (and to discontinue any other participation in the study) at any time. After any revocation, your health information will no longer be used or disclosed in the study, except to the extent that the law allows us to continue using your information (e.g., necessary to

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maintain integrity of research). If you wish to revoke your authorization for the research use or disclosure of your health information in this study, you must write to: Dr. David Relman at VAPAHCS 154T, 3801 Miranda Ave., Palo Alto, CA 94304.

What Information Will Be Obtained, Used, or Disclosed?

Your health information related to this study, may be used or disclosed in connection with this research study, including, but not limited to: Information about the composition and activity of the microbial communities in your samples will be obtained and recorded. Information about the identified and unidentified chemical compounds present in your samples will be obtained and recorded. Information about clinical tests on your blood samples and your immune activity will be obtained and recorded (if you agree to provide blood samples). Information about which gene variants you possess at certain locations will be obtained and recorded (if you agree to genetic testing). Your answers to questions about your health, diet, activities, family status, and personal history will be recorded. The questions we will ask include your ethnic background, where you have lived, who has lived with you, when, where and how long you have traveled, what exposures you have had to animals, soil, and water, what health issues and medical treatments you have had, your current and past diet and use of alcohol and tobacco, your current and past height, weight, waist circumference, blood pressure, pulse rate and respiration rate, and your current overall and gut-health related quality of life.

Who May Use or Disclose the Information?

The following parties are authorized to use and/or disclose your health information in connection with this research study:

- The Protocol Director (Dr. David Relman)
- The Stanford University Administrative Panel on Human Subjects in Medical Research and any other unit of Stanford University as necessary.
- The project coordinator (Les Dethlefsen)

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Who May Receive / Use the Information?
The parties listed in the preceding paragraph may disclose your health information to the following persons and organizations for their use in connection with this research study:

- The Office for Human Research Protections in the U.S. Department of Health and Human Services
- National Institutes of Health
- Palo Alto Institute for Research and Education (the grant administrator) will have records of your participation in the study, but not of any health information obtained during the study.
- Research staff

Your information may be re-disclosed if the recipients described above are not required by law to protect the privacy of the information.

When will my authorization expire?
Your authorization for the use and/or disclosure of your health information will continue until January 1, 2025, or when the research project ends, whichever is earlier.

Will access to my medical record be limited during the study?
To maintain the integrity of this research study, you may not have access to any health information developed as part of this study until it is completed. At that point, you would have access to such health information if it was used to make a medical or billing decision about you (e.g., if included in your official medical record).

_____ _____
Signature of Participant Date

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FINANCIAL CONSIDERATIONS

Payment
You are eligible to receive compensation for participating in this study. The amount of compensation depends on which arm of the study you are involved with. $200 will be paid for each diet or colon cleanout perturbation you participate in, along with the associated sampling. $300 will be paid for each antibiotic perturbation you participate in, along with the associated sampling. $200 will be paid for participation in study arms without a deliberate perturbation. In addition, you will be paid $30 for participating in physiological testing, $20 for each scheduled blood sample you provide, and $10 for each scheduled set of follow-up microbiota samples you provide for up to 2 years after the primary study interval. Payments may only be made to U.S. citizens, legal resident aliens, and those who have a work eligible visa. You will need to provide your social security number to receive payment.

Costs
If you participate in this study, there may be additional costs to you. These include the personal time it will take to come to all of the study visits. The study will pay for those services, supplies, procedures, and care associated with this study that are not a part of your routine medical care. If you would like to review the list of such covered services, supplies, procedures and care, please tell us now or at any time during the study. Participation in this study is not a substitute for health insurance. You and/or your health insurance must pay for those services, supplies, procedures, and care that you require during this study for routine medical care. You will be responsible for any co-payments and/or deductibles as required by your insurance.

Sponsor
Financial support will be provided by the NIH.

COMPENSATION FOR RESEARCH-RELATED INJURY

All forms of medical diagnosis and treatment -- whether routine or experimental -- involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study. If such complications arise, the Protocol Director and the research study staff will assist you in obtaining appropriate medical treatment. In the event that you have an injury or illness that is directly caused by your participation in this study, reimbursement for all related costs of care first will be sought from your insurer, managed care plan, or other benefits program. **You will be responsible for any associated co-payments or deductibles as required by your insurance.** If costs of care related to such an injury are not covered by your insurer, managed care plan or other benefits program, you may be responsible for these costs. If you are unable to pay for such costs, the Protocol Director will assist you in applying for supplemental benefits and explain how to apply for patient financial assistance from the hospital.

You do not waive any liability rights for personal injury by signing this form.

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CONTACT INFORMATION

- Appointment Contact: If you need to change your appointments, please contact Dr. Les Dethlefsen at 541-207-5791, or dethlefs@stanford.edu.
- Questions, Concerns, or Complaints: If you have any **questions, concerns or complaints about this research study**, its procedures, risks and benefits, or alternative courses of treatment, you should ask the Protocol Director. You may contact Dr. David Relman at (650) 852-3308.
- Injury Contact: If you feel you have been **hurt by being a part of this study**, please contact the Protocol Director, Dr. David Relman, at (650) 852-3308.
- Alternate Contact: If you cannot reach the Protocol Director, please call the research team at (650) 493-5000, ext 63163.
- Independent of the Research Team Contact: If you are not satisfied with the manner in which this study is being conducted, or if you have any concerns, complaints, or general questions about the research or your rights as a research study subject, please contact the Stanford Institutional Review Board (IRB) to speak to an informed individual who is independent of the research team at (650)-723-5244 or toll free at 1-866-680-2906. Or write the Stanford IRB, Stanford University, 3000 El Camino Real, Fibe Palo Alto Square, 4th Floor, Palo Alto, CA 94306.

EXPERIMENTAL SUBJECT'S BILL OF RIGHTS

As a research participant you have the following rights. These rights include but are not limited to the participant's right to:

- be informed of the nature and purpose of the experiment;
- be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized;
- be given a description of any attendant discomforts and risks reasonably to be expected;
- be given an explanation of any benefits to the subject reasonably to be expected, if applicable;
- be given a disclosure of any appropriate alternatives, drugs or devices that might be advantageous to the subject, their relative risks and benefits;
- be informed of the avenues of medical treatment, if any available to the subject after the experiment if complications should rise;
- be given an opportunity to ask questions concerning the experiment or the

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procedures involved;

- be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation without prejudice;
- be given a copy of the signed and dated consent form;
- and be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

May we contact you about future studies that may be of interest to you?

Yes_____ No_____

Signing your name means you agree to be in this study and that you were given a copy of this consent form.

_____
Printed Name of Subject

_____ _____
Signature of Subject Date

_____ _____
Signature of Person Obtaining Consent Date

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