Study Protocol

Project Title: Randomized placebo controlled trial of vitamin D supplementation and normal dietary intake of vitamin D and changes in the gut microbiome and markers of colorectal cancer risk

Short Title: Vitamin D Microbiome Trial (VDMT)

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Confidential

Revision History

Protocol Version	Protocol Date	Summary of Changes	Consent Change?	Consent Date?
Version 1	Nov 23, 2021		New	
Version 2	Jan 21, 2022	Removed urine collection and extended study timeline and supplement consumption	Yes	
Version 3	Mar 3, 2022	Added study visit and GPAQ	Yes	

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1 Introduction

Increase in early onset colorectal cancer (EOCRC) has been dramatic since 2012, with a 46% increase among those under 50. Recent data indicate a strong risk factor for EOCRC is insufficient dietary intake of vitamin D, and vitamin D insufficiency among adults in the US is 42%, with those ages 20-39 with the highest prevalence. Evidence indicates that dietary and circulating vitamin D levels are regulators of immune homeostasis, in part by altering the gut microbiome. The human gut microbiome is the community of bacteria that reside within the human intestine, and their composition and function are strongly linked to CRC. Very little is known about the complex interaction of vitamin D with the microbiome over time in one individual, and its impact on markers of CRC risk. In order to produce robust analysis of these interactions, longitudinal samples with detailed dietary intake information from healthy human subjects consuming vitamin D or placebo are needed.

2 Background, Rationale, and Aims

2.1 Relationship between Dietary Intake and the Microbiome

Aim 1: *To elucidate the complex relationship between the diet and microbiome using longitudinal data and mathematical modeling*.

The complex relationship between dietary intake and the microbiome, and the potential health implications of human exposure to microbial metabolites, are only beginning to be understood.[1] It is well known that altered dietary intake can trigger rapid, although transient, changes in the composition of the microbiome in as little as 1 to 2 days.[2–4] The biggest factors in determining microbial response to diet are thought to include an individual's starting microbiome, long-term dietary habits, and environmental exposures.[5,6] However, there is some controversy over the extent to which long-term dietary patterns (i.e. vegan v. omnivore) influence the composition of the microbiome.[6]

We still do not have a good understanding of how small dietary differences from day-to-day impact the microbiome. Recently the application of machine learning to a large dataset of microbiome, diet, anthropometrics, and blood test data was successfully used to predict blood glucose response to variable meal compositions.[7] This finding suggests that personalized nutrition and optimal diets for health may be feasible in the future. A longitudinal dataset with accurately recorded dietary data and multiple samples over 12 weeks will provide valuable insight into the changes that occur at the individual level over time, while controlling for dietary trends and initial microbiome composition.

2.2 Vitamin D Intervention

Aim 2: *To identify shifts in the composition of the human microbiome and CRC risk factors specifically caused by the consumption of Vitamin D supplements.*

Vitamin D is one of 4 fat-soluble vitamins that are only obtained from dietary or supplementary intake, and which is necessary for healthy immune function, bone metabolism and electrolyte absorption.[8–11] Since the 1980's intake vitamin D containing foods, milk, eggs, fish and mushrooms has been declining in the United States.[12] Most concerning has been the sharp decline in consumption of cow's milk, which is a primary source of vitamin D, particularly among young females.[13] Unfortunately, there has also been a sharp rise in EOCRC since 2000, from a rate of 5.9 cases/100,000 to 8.5 in 2018 (SEER). Projections for 2030 suggest 23% of rectal and 11% colon cancer will be diagnosed in US adults under the age of 50.[14] Recently, however, a study using incident cases of EOCRC from the Nurses' Health Study II, demonstrated a 50% reduced risk of EOCRC from total dietary intake of vitamin D >/= 450 IU/day vs <300 IU/day (HR = 0.40, 95% CI, 0.26-0.93).[15]

Recent studies have determined the impact of habitual vitamin D intake and vitamin D supplementation on the human microbiome, and demonstrated beneficial modifications in composition.[16] Specifically, 12 weeks of weekly oral dose of 50,000 IU vitamin D_3 in 80 women increased the *Bacteroides/Firmicutes* ratio and increased probiotic bacteria *Akkermansia* and *Bifidobacterium*.[16] It is yet unclear mechanistically how Vitamin D alters the composition and function of the microbiome, but several lines of evidence indicate a bi-directional interaction. Indirect effects of Vitamin D on the microbiota include systemic effects through the vitamin D receptor (VDR), which is expressed not only in the proximal colon but also functions as a transcription factor in a large number of genes that control immune response.[17,18] Likewise, specific bacteria influence VDR expression in host cells.[19,20]

In addition to vitamin D intake, the gut microbiome structure and function is also a significant risk factor for CRC development. Multiple studies demonstrate significant loss of diversity, and increases in pathogenic bacteria, *Fusobacterium nucleatum* and *Bacteroides fragilis,* are risk factors for CRC development and progression. Recent preliminary data using data from The Cancer Genome Atlas, demonstrated a distinct intra-tumor microbial composition in EOCRC vs late-onset, along with positive association between T regulator cells and inflammasome pathway.[21] Thus, it is possible that the vitamin D insufficiency or deficiency may be also affecting gut microbiome structure and function negatively, increasing EOCRC risk.

Although dietary vitamin D supplementation has been used in the clinical setting for decades, the effect of supplementary vitamin D consumption on the structure of the microbiome has not been studied in humans in fine scale or with concomitant adjustment for dietary intake. Understanding the interaction of vitamin D with the microbiome in humans could lead to important advancements in the understanding of how vitamin D together with diet impacts the microbiome composition, and ultimately, risk of EOCRC. This study has the potential to lay the ground work for an adjunctive therapy to manipulate the microbiome to reduce risk of EOCRC. This proposed study is designed to **evaluate the effect of vitamin D supplementation on the normal structure of the microbiome** and data **will not be used to diagnose, prevent, cure or treat disease**.

3 Experimental Design

Study participants will complete 16 days of active study involvement after providing informed consent on day 0, which will be conducted over a period of 12 weeks. There will be 4 in-person visits, including the consent visit where the participants will have contact with the researchers. Visit 1 will be the consent visit and participants who consent will receive training on how to collect stool samples, take supplements and dietary record keeping with an online diet record tool called ASA24. Visit 1 will include a fasting blood draw and body composition measurement. At visit 1 participants will receive stool collection kits for week 1 and their supply of dietary intervention supplements (gummies) as well as written instruction on how many gummies to take each day during the intervention period. They will also complete a food frequency questionnaire, the DHQIII online. At visit 2 participants will receive collection kits for week 2 and their supply of dietary intervention gummies as well as written instruction on how many gummies to take each day during the intervention period. At study visit 3, participants will complete a fasting blood draw, stool kit for Study day 16, and intervention gummies. At visit 4, this will conclude the study. Participants will complete a fasting blood draw, body composition measurement, dietary recall and mental health survey. The process for returning fecal samples is described below in Section 6.2.

3.1 Study Outline

Study Day 0 (Study Visit 1)

- Fasted blood draw
- Body composition
- DHQIII online
- Stool history
- Mental health questionnaires

Study Days 1-14

- Consume vitamin D or placebo as gummies for 14 days
- Consume normal diet and keep detailed dietary records using ASA24 (daily 24-hour recalls)
- Collect 1 stool sample per day for 14 days
- Study Visit 2/Study day 7 – delivery of 2nd week of stool collection kits

Study Day 15 (Study Visit 3)

- Fasted blood draw
- Provide vitamin D or placebo gummies to last through week 9
- Stool history
- Post self-perceived stress scale

Study Day 16 (Study Visit 4)

- Provide remainder vitamin D or placebo gummies to last through week 12
- Provide stool collection kit for Study day 16

Study Day 16 (Study Visit 5)

- Fasted blood draw
- Stool history
- ASA24 dietary record
- Body composition
- Collect final stool sample
- Post self-perceived stress scale
- Global Physical Activity Questionnaire (GPAQ)

3.2 Visit/Intervention Schedule

Study Visit Number	1							2								3	4	5
Study day (0-15)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Week of study	1	2						2							3			12
Decide if you want to participate	✓																	
Study training	✓																	
Study checkup								✓								✓	✓	✓
Complete questionnaires	✓															✓	✓	✓
Measure body composition	✓															✓		✓
Collect stool		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓
Record diet		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓
Take dietary supplement		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Fasting blood draw	✓															✓		✓

4 Statistical Design and Microbiome Analysis

This longitudinal study will allow for the use of each subject as their own control. We will perform testing for associations between diet, taxa, groups of bacterial taxa (and their functional capacities) with metadata parameters in different combinations. We will test the null hypotheses that there are no associations between dietary intake variability and microbiome composition within and between subjects, and between dietary intervention groups. Finally, we will test the null hypothesis that vitamin D has no effect on the microbiome.

Primary Outcome: Microbiome composition and the relationship with dietary and supplemental intake of vitamin D and serum vitamin D levels

Secondary Outcomes: Circulation inflammatory markers (e.g. TNFα, IL-8, CRP); markers of gut permeability (e.g. zonulin, intestinal fatty acid binding protein, soluble CD14, lipopolysaccharide and lipopolysaccharide-binding protein); glucose and insulin; and other nutritional and microbial metabolites.

4.1 Sample Size

We will enroll 40 adults in this study. The adults will be randomized, with 20 individuals per study arm, to receive either vitamin D (4000 IU daily) or placebo during the intervention period. To control for initial vitamin D (25(OH) D3) levels at baseline (including those with hypovitaminosis), subjects will be randomized using 1:1 matching based on vitamin provided from blood samples at study visit 1. Study PI will be blinded to subject randomization. We will obtain 15 stool samples and 15 24-hour dietary recalls from each individual, for a total of 561 stool samples and 561 dietary records. We will obtain pre- and post- intervention blood samples from each individual for a total of 81 blood samples. We performed a power analysis using vitamin D as the primary exposure, and the top 10 dominant microbial taxa as the primary outcome. Using monte carlo simulation of microbial taxa distributed according to a lognormal distribution, and a non-parametric Mann-Whitney U test for significant differentiation, we found that this sample size is sufficient to detect a change of ≥ 1.24 standard deviations in any one of the dominant taxa. This analysis accounted for multiple hypothesis testing using Bonferroni correction.

5 Participant Selection

5.1 Inclusion Criteria

- Adult 18 years or older

Exclusion Criteria

- Women who are currently pregnant or breastfeeding
- Use of antibiotics within the last 2 weeks
- Use of supplementary vitamin D within the last month
- Self-reported, pre-existing history of inflammatory bowel disease, heart disease or diabetes
- Students under 18
- Tanning/sun exposure > 60 min at a time in last 4 weeks
- No phenobarbital, carbamazepine, spironolactone chronic therapy; or steroid use within the last 2 weeks
- Severe allergy to ingredients found in supplements

It might be possible that some students enrolled in BIO 1125 may be under the age of 18, however, these students will be excluded from study enrollment as we are only studying the effects on adults as part of this research.

5.2 Recruitment

Participants will be recruited from the Baylor University and the surrounding communities. This proposed study is planned to provide data for use in a undergraduate research engagement course (BIO 1125) at Baylor University. Citizen science is a growing movement that increases the involvement of everyday people and students in the collection and analysis of data in collaboration with professional scientists. Healthy adult participants will be recruited for this study from the University and surrounding community, as well as from students who express interest in taking this citizen science undergraduate research engagement course. Subjects will not be required to take the course to participate in the study, and students will not be required to participate in the study to take the course.

5.3 Protections for the Use of Students as Research Subjects

Analysis of the proposed research study data will take place as part of a newly redesigned course at Baylor University offered through the Biology Department, and some of the participants will be recruited from the pool of students who express interest in taking this class. De-identified data generated from the research study will be used by the students in the course to learn about computational biology and the microbiome. Identifying metadata such as gender, race, and age will not be shared with the class. Leigh Greathouse, this study's principal investigator (PI) and adjunct course instructor, has extensive experience and involvement with other human microbiome projects.

Importantly, participation in this research study and course will present a unique learning opportunity for students to be involved in citizen science. We believe that the students will be highly invested in learning about data analysis techniques and methodology in part because they will be analyzing their own data. We also believe that the act of participating in the research study is an excellent opportunity for the students in the class who are considering careers in biomedical research and medicine.

Our study will include a dietary intervention, students will analyze the complete de-identified dataset, and we plan use the data generated for publication.

Study participates with be provided a mental health resource sheet should they need to seek professional help at any time during the study.

To prevent coercion of the study research participants, the following protections are in place:

1. Students who express interest in the course during the recruitment period will be able to register regardless of their intent to participate in the study. Prior to the start of class, students will be informed that the class will use data generated from this study. They will be offered the opportunity to participate in study as well as a copy of the consent form. They will be asked to provide informed consent for the research study before the beginning of the course.
    a. Students who do not consent to take part in the research study or who are excluded from participating in the research study will still be given permission to take the class.
    b. Study participation will be open to all members of the University community and public who are not interested or eligible to take the course.
2. Gathering of informed consent will be completed by a study staff member and not the study PI/course instructor.
3. The study PI/course instructor will be blinded to the identity of the participants in the study until completion of the course to prevent any bias in grading.
    a. Students will not be graded for their participation in the research study and their completion of the study will not affect their success in the course.
4. Students will not be compensated for their participation in the research study and will receive no direct benefits from participation.
5. The course will be elective.

All data generated from the research study will be de-identified before use in the class. Individuals who choose to participate in the research study can elect to receive a personalized report about their microbiome composition and dietary intake. This report will be generated at the end of the course after final grades have been distributed to keep all potentially identifying information private throughout the duration of the course. If a student completes the research study and then drops the course they may still elect to receive their personalized report. Standard Baylor University policies for dropping courses will still apply to the academic portion of the course.

6 Study Procedures

6.1 Demographics and Anthropometrics

At visit 1, basic demographic data will be collected including age, gender, race/ethnicity, and medication use. Participant weight will be measured to the nearest kg and height will be measured to the nearest cm at using an electronic scale and wall mounted tape-measure. Body composition will be measured using the InBody device, which is a bioelectrical impedance device that measures percent body fat and lean muscle, and has been used by Dr. Greathouse in other IRB approved studies at Baylor University. For students and study participants who have mental or physical issues where this would not be appropriate, such as a history of eating disorders, the option to just provide self-reported height and weight will be offered.

6.2 Fecal Sample Collection

Fecal collection will be completed by self-sampling. Self-sampling has proven to be highly successful based on Dr. Dan Knights' American Gut Project collaboration utilizing self-sampling of fecal material from 7,000 individuals.[22] The participant will be trained on the self-sampling procedure after providing consent at visit 1. Briefly, the participant will collect a small quantity of fecal material using a provided collection kit once per day during each day of the study that they produce a stool. Participants will be instructed on proper sample storage until the sample is returned at a study visit.

To avoid room-temperature storage bias, students and members of the University community who participate in the study will be asked to drop off samples daily at one of two on-campus locations. Stools samples will be stored at -80 within 24 hours of drop off. Participants who do not visit the University campus each day will be given FedEx stamped addressed envelopes and asked to drop samples in the mail daily. **The FedEx labels will be for 24 hour delivery using special type B labels intended for use with biospecimens. Further, these stool collection kits and methods are the same methods used with the American Gut Project kit and study (N=15,096), which conducted extensive temperature bias controls (McDonald et al. *mSystems,* 2018).**

We anticipate that not all participants will pass a stool each day. Participants who do not pass a stool each day, will be asked to record their dietary intake normally for that day, but to skip the stool collection and to return an empty collection kit for the day(s) they do not pass a stool.

6.3 Dietary Record Keeping

The DHQIII utilized for the study will be the version that takes account of the subject's dietary history one year prior (with portion size) to study entry; the online tool DHQIII was created by the National Cancer Institute. The daily dietary questionnaire will be based on a 24-hour dietary recall. Participants will record everything they eat and drink each day during the study. Each participant will undergo training to complete dietary records at visit 1 after providing consent. Participant training will include encouragement to actually measure foods eaten using measuring spoons and cups whenever possible. Participants will record their intake using 24-hour dietary recalls with an online tool (Automated Self-Administered 24-Hour Recall [ASA24]-2016. Bethesda, MD: National Cancer Institute). The ASA24 is based on the United States Department of Agriculture's (USDA) Automated Multiple-Pass Method (AMPM), which has been validated. The ASA24 system provides secure, password-protected diet entry for participants and de-identified, password-protected data access for researchers. The system does not ask for or store any personal identifying information. Dietary records will be analyzed for nutrient content using the ASA24-generated output which is based on the USDA Food and Nutrient Database for Dietary Studies (FNDDS). From the DHQIII, the Healthy Eating Index (HEI-2016) will be calculated, which is used to determine of the overall diet quality. The HEI is calculated based on 12 components which each have an individually assigned score to give a maximum score of 100. Those individuals having a score above 80 are considered to have a healthy diet,

whereas those below 50 are considered to have a poor dietary intake, and do not meet 75% of the recommended dietary allowances.

6.4 Dietary Supplement Intervention

The recommended dose of vitamin D for adults is 600 IU/day and has an upper limit of 4000 IU/day, with clinical intervention to replete deficiency being 50,000I IU/day. We have set our dose at the upper daily limit, established by the Institute of Medicine, since this study is not mean to replete a clinical deficiency. The supplement will be provided as Nordic Naturals vitamin D3 gummies, which have been certified by a third party with Good Manufacturing Procedures. For these gummies, 4 gummies will provide 4000 IU of vitamin D3. Subjects will consume 4 gummies at the largest meal of the day for 12 weeks. Subjects who are assigned to the placebo group will be provided with placebo gummies, Yum Earth Organic Vegan Fruit Snacks, which have the same composition as the supplement but no vitamin D3. Both will be put in bottles without manufacture labeling so as to disguise the vitamin D from placebo. If for any reason we are not able to get these placebo products, we have the ability to print 3D molds and make our own placebo gummies, which we have done before in our food lab. See Table 1 for list of ingredients.

Table 1. Ingredients list of vitamin D3 and placebo supplements

	Vitamin D	Placebo
Ingredients	vitamin D (cholecalciferol)	Organic rice syrup
	Organic tapioca syrup	Organic cane sugar
	Organic cane sugar	Pectin
	purified water	Citric Acid
	pectin	Ascorbic Acid
	citric acid	Natural flavors colored with organic concentrate (Apple, carrot, pumpkin,blackcurrent)
	furamic acid	Organic sunflower oil
	natural flavor	Organic carnauba wax
	sodium citrate dihydrate	
	fruit and vegetable juice (color)	
Allergens	Gluten Free	Vegan
	Contains milk derivatives	Gluten Free
	Non-GMO	Non-GMO

6.4 Mental Health Assessment

Mental and physical health: Mental health will be measured with several instruments: First, is the College Undergraduate Stress Scale, which assesses life stressors similar to the Holmes Rahe scale, but adapted for college students (Renner & Mackin, 1998). In addition, the Medical Outcomes Study Questionnaire Short Form 36 Health Survey (SF-36; Ware & Gandek, 1998)

will be used to assess general health. This scale is highly reliable with estimates of both physical and mental health typically above 0.90.

Depression and anxiety: will be assessed with the Hospital Anxiety and Depression Scale (Stern, 2014). This scale has been validated in patient populations and general community settings. It is one of the National Institute for Health and Care Excellence (NICE) recommended tools for diagnosis of depression and anxiety.

Perceived Stress Scale: The Perceived Stress Scale(PSS) is a validated scale that measures the degree to which respondents find their lives "unpredictable, uncontrollable, and overloading." Reliability of this scale has been established to be greater than 0.86. As well content, predictive, and concurrent validity have been established for this measurement tool in adolescents and adults (Cohen S, Kamarck T, & Mermelstein R. 1983; Yarcheski A, & Mahon N. 1999)

Alcohol and drug use: We will use the World Health Organization's tool for screening alcohol, tobacco & drug use (Newcombe, Humeniuk & Ali, 2005). This screening instrument is used to assess use of a range of substances including tobacco, stimulants, depressants, hallucinogenics, and opioids.

All of these scales have been used by Dr. Greathouse in previous studies approved by the Baylor University IRB for use in studies with University students.

6.5 Physical Activity

Physical activity will be assessed using the Global Physical Activity Questionnaire (GPAQ), which was designed by the World Health Organization. The GPAQ is a valid measure of moderate to vigorous activity among healthy adults (Cleland et al. 2014). The GPAQ consists of 16 questions that estimate an individual's level of physical activity in 3 domains (work, transport and leisure time). We will used this tool to measure average regular levels of physical activity in our participants.

6.6 Blood Draw

Blood will be drawn by a licensed phlebotomist from a peripheral arm vein at the designated visits through standard sterile methods. At each blood draw, ~15mL of blood of blood will be collected. Participants will be asked to fast for at least 8 hours prior to the blood draw. Participants will remain in a seated position throughout the procedure and be observed for light-headedness, bleeding, and bruising before, during, and after the procedure. If a participant experiences light-headedness they will be given the option to recline or lay down and continue to be monitored until the light-headedness resolves. If the phlebotomist is unable to successfully locate a vein after three attempts, the blood draw will not be completed.

6.7 Follow-up

Subjects enrolled study may elect to be contacted by telephone, text, or email daily during the data collection period as a reminder to collect a sample, complete a dietary record, and/or to check on their progress.

6.8 Microbiome Analysis

We will use RNA gene sequencing or 16S gene amplicon sequencing or shotgun DNA sequencing as well as novel methods in development relying on sequencing a subset of the fecal microbial RNA or DNA to accurately characterize taxonomic composition of the microbiome.

7 Participant Withdrawal

Participants may withdraw voluntarily at any time.

Participants may be withdrawn from the study by the PI for the following reasons:

- The participant is diagnosed with a disease that may affect their ability to pass feces.
- The study is stopped.

If a subject withdraws from the study, no further data will be collected on the subject. The data that have been collected to that point will continue to be used unless the subjects notifies the PI.

8 Assessment of Safety

8.1 Safety of biological sample collection

Collecting fecal samples is a low risk procedure. Fecal collection kits will contain gloves for participants to wear while collecting their own samples when the chance of contact with stool is highest. The sampling kit contains ammonium sulfate, and participants will be instructed not to ingest this substance. Participants will be instructed to wash their hands thoroughly after completing each stool collection.

Fasting blood draws a generally considered minimal risk procedures. There is a small risk of pain and discomfort during the blood draw and it is possible that bruising at the site of the draw will occur.

8.2 Safety of dietary supplement intervention

Vitamin D occurs naturally in low amounts in foods, including cow's milk, fish, eggs and mushrooms. Most commercially available vitamin D supplements are made from processing sheep wool to extract lanolin. This product is then saponified and refined using solvent washes. This crude cholesterol is then subjected to a series of chemical reaction to produce 7-dehydrocholestrol, which is then exposed to UV light to form vitamin D3. The upper limit of

vitamin D intake as set forth by the Institute of Medicine and USDA is 4000 IU/day for adults over 19. Safety testing for various doses of vitamin D has been previously conducted in healthy adults at 3 doses, 400, 4000, and 10,000 IU/day, with 4000 IU dose produce milder hypercalcemia in 3% of subjects (N=373) ages 50-70.[23] Thus, this does has been deemed safe in adults for long-term intake.

9 Potential Benefits

Subjects will not personally benefit from giving specimens. The results are not expected to be clinically meaningful to the study participants. The research will benefit the larger research community by enabling the development of better methods to relate dietary intake to the microbiome.

Participants may request an electronic information packet with their individual results via email. Results will include the composition of their gut (most abundant taxa) as well as other relevant individual markers and summary information of dietary intake. To maintain confidentiality, personalized information packets will be sent (at the completion of the course associated with this study) by the study PI. Also included in this informational packet will be a comparison of that individual's microbial community to those of other study participants, in de-identified aggregate. This may include a graphical representation of ecological distance to other patients, comparison diversity scores, and other summary statistics. This will be accompanied by a disclaimer that this information should be used for research purposes only and is not a reliable clinical test for any purpose. Participants will be notified via email upon completion of the study of this information's availability upon request.

10 Study Timeline/Duration

Recruitment for the study will begin during January 2022 with the data collection completed by December 2022.

11 Data Management

11.1 Data Collection

De-identified participant data will be entered into a secure database, stored on a password-protected server with full disk encryption, behind both Baylor University secure firewall. DHQIII, and 24-hour dietary recall data collected using ASA24 are secured at the hosting site using industry standard security controls, including firewalls and encryption. All data entered into the DHQIII and ASA24 system at the Respondent's computer are encrypted by the internet browser (e.g., Internet Explorer, Firefox) before they are transmitted to DHQIII or ASA24's servers using Secure Socket Layer (SSL) Technology.

11.2 Monitoring Plan

This is a minimal risk trial. Data will be monitored by the PI. Sample acquisition carries minimal to no known medical risk to the subjects. The nature of risk for accessing private records will be minimal to none, since all data collected will be de-identified. Possible invasion of privacy may occur as other participants may observe the fecal specimen being dropped off. Interviews will be conducted in private rooms or sectioned-off areas in order to maintain privacy of our participants. Participant data will be de-identified. As a result of the de-identification process, individuals participating will be assigned a unique identifier number. This unique identifier will be used to link de-identified samples and data collected from the interview (data listed in the Data Collection section). Study coordinators will have access to the link during the data collection period. The study PI will only have access to the link to the unique identifier after the course completion to protect the identity of student study participants during the course. The identifiers will be maintained in a database on a secure computer protected with password access and full-disk encryption. If a breach in confidentiality occurs, we will report the breach to the Institutional Review Board (IRB) and the HIPPA Privacy Officer as per institutional policy.

12 Administrative Considerations

12.1 Research Ethics Approval

The study will be conducted in accordance with the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP), the Declaration of Helsinki, and the appropriate regulatory requirement(s). Baylor University IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the participants. The study will only be conducted at sites where IRB approval has been obtained. The protocol, informed consent, written information given to the patients, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files will be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

12.2 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts

should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

12.3 Declaration of Interests

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) will have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. The following situations include conflicts of interest: when substantial changes in business or financial interests occur, when an activity that presents a potential conflict of interest is anticipated, or when submitting an application for research support or technology transfer, submitting research protocols to the IRB, or receiving financial contributions. All Baylor University investigators will follow the University conflict of interest policy.

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