RESEARCH PLAN

A) Rationale

Approximately seventy-four percent of Americans are living with digestive symptoms like diarrhea, gas, bloating, and abdominal pain. Intestinal issues can indicate the possibility of more serious conditions in the future. In patients with Inflammatory Bowel Disease (IBD) such as Crohn's Colitis, Gastrointestinal (GI) tract cancers, or Ulcerative Colitis (UC), chronic inflammation puts the individual at a major risk for gastrointestinal malignancies most commonly, the terminal ileum or the perianal region. Crohn's disease (CD) and ulcerative colitis (UC) are conditions that cause inflammation in the GI tract. Inflammatory Bowel Diseases including Crohn's Disease and Ulcerative Colitis are both characterized by chronic relapsing intestinal inflammation. CD and UC are usually characterized by damage created to the epithelial barrier separating the intestinal lumen and the mucosal immune system.

Those individuals that are afflicted with these diseases are at a higher risk of developing cancers associated with the GI tract. Since there is currently no cure for Crohn's disease, its poses a great burden on those that suffer from the disease. Considering that the disease is usually diagnosed at a young age, IBD can put a significant financial burden on the individual as well as their family

B) Research Question

The hypothesis presented in this paper is that if an assay for bacterial swarming can be optimized, then patients can be diagnosed with IBD using a quicker, cheaper, and non-invasive method. Furthermore, if the swarming bacteria could be injected out of a patient and preserved in its state, it can be given to a patient in large quantities to possibly treat IBD, Crohn's, and Ulcerative Colitis.

The aim of this research is to provide clinics with a reproducible assay in which they can avoid extensive surgeries as well diagnose many intestinal diseases with a simple stool sample. As the conditions are optimized, resources, time, and money are saved changing medicine for the better.

C) Methods/Procedures

Many swarming assay factors are going to be tested in this experiment in order to optimize the plate assay. The factors being included: Vortexing, centrifuging, pipetting, PBS Dilutions, dry time, varied volumes of the plate, varied agar concentrations, and varied volumes of the inoculation. Human fecal samples were obtained in 2015 (under protocol IRB# 2009-446 and 2015-4465) and since have been preserved in -80°C freezers. The standard assay will consist of: preparation of a 0.5% agar Lysogenic Broth 20 mL plate (100ml H20, 1g Tryptone, 0.5 g Yeast Extract, 0.5g NaCl, 0.5g agar), removal of frozen samples from -80 degree Celsius freezer, thawing on ice, inoculating 5 microliter spot on 0.5% agar LB plates, drying of plates under biological hood, and placement in standard incubator.

I. The vortexing of the samples will be done through a standard vortex shaker. The samples will be removed from the freezer, thawed, and vortexed for varied amounts of time: 5 seconds, 30 seconds, and 60 seconds. After vortexing the samples, they will be inoculated in 5 μl spots.

II. When centrifuging the samples, they will be placed in a table top microcentrifuge for one minute. When they are removed, plates will be inoculated with either the supernatant or the pellet.

III. The pipetted samples will be pumped with a micropipette for various values. The number of pumps include 5 pumps, 25 pumps, and 50 pumps. After pumping the sample, the plates will be inoculated with 5 microliter spots.

IV. PBS Dilutions will be conducted using a phosphate buffered saline solution. The values will include ½, ¼, and ⅛ dilutions. The dilutions will be created by placing 5 µl of PBS into an aloquote with 5 µl of the samples. 5 µl will be taken from this mixture and placed into another 5 µl of the sample to create the ¼ dilution. Lastly, 5 µl will be taken from this mixture and placed into 5 µl of the sample to create a ⅛ dilution.

V. The two different dry times used will be either before or after the plates are inoculated. In one experiment, the plates will be dried under a standard biological hood before the plates are inoculated while in another experiment, the plates will be dried after they are inoculated under a biological hood.

VI. Varied volumes of the plate include 15 mL, 20 mL, 25 mL, and 30 mL. The plates will be poured with varied volumes of LB Agar and dried.

VII. The different agar concentrations being used in this experiment are 0.4%, 0.5%, 0.6%, and 0.7%. The LB Agar will be made with different percentages of agar following the standard directions of preparation, only altering the grams of agar.

VIII. There will be 3 different volumes of inoculation used including 2.5 µl, 5 µl, and 7.5 µl. Plates will be spotted with one of these values using a micropipette.

IX. Areas of the swarming phenotype will be measured using Jmap software and analyzed using GraphPad Prism software.

D) Bibliography

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ADDENDUM

No changes have been made to the research plan throughout this experiment.