The Gut Microbiome and Cognitive Function of Dementia Patients Living in Long-Term Care Facilities

A Major Qualifying Project
Submitted to the Faculty of Worcester Polytechnic Institute
in partial fulfillment of the requirements for the Degree in Bachelor of Science
in
Biology and Biotechnology
By

Allison O'Neill

Professor Jill Rulfs, Advisor

Date: April 25, 2019
Sponsoring Organization:
University of Massachusetts Medical Center

Abstract

A prominent theory about the etiology of Alzheimer's Disease (AD), called the inflammation hypothesis, points to inflammation from an infection as a causative agent of the biodegradation of the brain. To test this hypothesis, residents from long-term care facilities were cognitively assessed and their microbiomes were analyzed. With a small sample size, conclusive evidence was not obtained to support or reject this hypothesis, however the cognitive assessments used, including the NIH Toolbox, were analyzed. It was concluded that the NIH Toolbox is not an effective tool to track the progression of dementia at this time as it is not validated for participants over the age of 85, requires extensive time and equipment, and the results are difficult to interpret.

Acknowledgements

This project was made possible by many individuals who provided support, guidance, study materials, and resources throughout the process. It was a pleasure to be able to collaborate with all of these individuals in order to create a cohesive project.

First, I would like to thank my project advisor, Professor Jill Rulfs from the Biology and Biotechnology department of Worcester Polytechnic Institute, for guiding me through the MQP process. She was able to help me find a clinical research project which would fulfill both the WPI curriculum and my personal interests and career aspirations. Beyond finding the project, she also supported my work through weekly meetings to discuss planning and developing my project as well as reviewing my written work.

I would also like to thank the primary investigator of the study, Dr. John Haran from the Emergency Medicine Department of the University of Massachusetts Medical School. Dr. Haran welcomed me into his team as a volunteer and allowed me to investigate several of his clinical studies before committing to working on the long-term care facility microbiome study. He also allowed me to choose a topic of interest within the study as a whole to conduct my own project. Through Dr. Haran, I was given access to participants for the study as well as vital resources to the project, including sample collection materials, cognitive testing materials, and analytical software.

I would like to thank the research associates, Amanda Higgins and Protiva Dutta, from the Emergency Medicine Department of the University of Massachusetts Medical School, for helping me with the data collection and interpretation during the project. Protiva guided me through the early steps of the project, including the consent process and initial cognitive assessments. During this period, Protiva was very helpful in showing me the different areas from which I could choose a project topic. In the later stages of the project, Amanda was of great assistance with utilizing the NIH Toolbox to assess participants. Amanda also completed several chart reviews which made this project more complete. In the final stages of the project, Amanda was very helpful during the data analysis portion of the project, as she helped me to create and interpret all figures seen in the paper.

Lastly, the staff and participants from the long-term care facilities were a pleasure to work with. Without their willingness to participate in exams and collect samples for the study, there would be no project.

Table of Contents

1.0 Introduction

Dementia is one of the most commonly diagnosed conditions in the elderly population worldwide, with about 9.9 million new diagnoses each year. It is estimated that there are currently 47.5 million people living with a dementia diagnosis in the world. Dementia prevalence increases with age, as the risk of disease begins at the age of 60 and increases every year. The highest prevalence of dementia is in people over the age of 90, but there is a marked prevalence in people between the ages of 71 and 79 (Plassman *et al*., 2007).

Dementia is a general term for diseases which include a noticeable cognitive decline. There are several kinds of dementia, with Alzheimer's Disease, Diffuse Lewy Body dementia, frontotemporal dementia, and vascular dementia making up the majority of dementia diagnoses (Bolla, Filley, & Palmer, 2000). Alzheimer's Disease is the most common kind of dementia. Other, less common forms of dementia include Parkinson's dementia, Creutzfeldt-Jakob disease, and Wernicke-Korsakoff dementia. Each of these specific diseases has hallmark clinical and biological manifestations. In some patients, testing does not reveal conclusive evidence to diagnose a specific form of dementia, and thus a diagnosis of unspecified dementia is given.

One of the clinical signs of dementia is a cognitive decline which causes a decreased ability to function independently. Due to this decrease in functionality, many people with dementia require additional care, which can range from daily nursing visits to full-time monitoring. Many families opt to place loved ones with dementia into long-term care facilities to provide the latter with appropriate full-time care (Schulz *et al*., 2004). Studies have shown that dementia, depression, and anxiety are the most common psychiatric disorders among elders in long-term care facilities (Seitz, Purandare, & Conn, 2010).

Within the past 30 years, many research endeavors have changed the landscape for diagnosis, treatment and prevention of dementia and related diseases. Recent advances in technology and dementia research have shed light on the underlying pathophysiology of many kinds of dementia. These breakthroughs have allowed for more precise diagnoses and treatment options for dementia and the other specific cognitive diseases. The recent research into the prevalence of dementia has also highlighted some potential risk factors for the disease, which in turn allow for speculation into the mechanisms which lead to the cognitive decline. For example, major breakthroughs regarding vascular dementia have revealed the underlying cause of the disease which allows for identification of treatment options and preventative measures (Ritchie & Lovestone, 2002).

While these research endeavors have revealed great insights into disease mechanisms and treatment possibilities for some of the specific dementia diseases, such as vascular dementia, the most common form of dementia, Alzheimer’s Disease, is still one of the most confounding forms of dementia. There is evidence that Alzheimer's Disease could be caused by an infection causing an inflammatory immune response which spreads to the brain (van der Flier, W. M. & Scheltens, 2005).

The purpose of this clinical study is to explore the changes in the gut microbiome of people living in long-term care facilities in relation to continued cognitive decline in order to support the hypothesis that that Alzheimer' Disease result from neuroinflammation secondary to an infection in the gut.

2.0 Background

Before beginning to investigate the possible relationship between the gut microbiome and Alzheimer's Disease, it is important to fully grasp the current understanding of both topics. In this section, the significance of the gut microbiome will be explored with specific focus on the gut microbiome of the elderly. Additionally, the etiology, symptoms, diagnosis, and disease tracking of Alzheimer's Disease will be reviewed.

2.1 The Gut Microbiome

Our bodies are home to trillions of microorganisms, including bacteria, viruses, and fungi. These microbes outnumber our own human cells ten to one, and the combined genome of these microbes is two hundred times the number of genes in our human genome (Bäckhed, *et al*., 2005). We are not born with our specific microbiome, instead we form our unique microbiomes through our life experiences. The formation of a unique microbiome begins at birth, a relatively diverse gut microbiome has been observed as soon as after the first week of life, and an adult-like microbiome is fully developed by the end of the first year of life (Pflughoeft and Versalovic, 2011). Thus, the events of the first two years of life are very significant in the formation of the microbiome. Many variables have been identified as being very impactful on the overall microbiome. These factors include the genetic makeup, physiology, immune system, environment, pathobiology, lifestyle and diet of the host, as well as the other people the host may encounter (Turnbaugh, *et al.*, 2007).

Each region of the body contains a specific microbiome which differs greatly from that of the other regions. The main regions of the body which have complex, diverse microbiomes are the mouth, airways, skin, gut and vagina. Of all of these regions, the gut microbiome has been linked to many bodily functions and contributes to overall health (Proctor, 2011). For instance, recent studies have shown that specific gut bacteria can influence weight through promoting different metabolic mechanisms (Turnbaugh *et al*., 2006). Additionally, other studies have aimed to reveal a connection between the gut microbiome and insulin action in the body. These results could potentially associate the bacteria in the gut with one of the most common metabolic disease in the United States, diabetes mellitus (Serino *et al*., 2013).

2.1.1 The Gut-Brain Connection

The gut microbiome has been linked to other health factors outside of the gut. Just as the organs of the digestive and immune systems are able to communicate with the other organs and systems of the body, the gut microbes make use of these communication pathways as well. The Vagus nerve is the longest cranial nerve which extends from the brain down to the

abdomen and contains mostly parasympathetic nerve fibers. The Vagus nerve branches into the pharynx, larynx, esophagus, cardiac muscle, bronchioles, stomach, gallbladder, pancreas, and small intestine. The Vagus nerve is able to transmit motor impulses which affect speech, heart rate, bronchoconstriction, and digestion (Henry, 2002).

Through the Vagus nerve, the gut microbes are able to influence other parts of the body. The vagal nerve fibers connect to epithelial tissue lining the digestive organs; thus, the fibers do not directly contact the microbes. The microbes, however, can release chemicals which can be sensed by the vagal nerve fibers, and then transmitted to the rest of the body. Some gut bacteria have been shown to release neurotransmitters, such as dopamine, serotonin, and acetylcholine, which can directly interact with neurons in the brain. These communications between the brain and the gut microbes has been shown to be bidirectional, meaning that just as the microbes are able to send messages to the brain, the brain can also send messages to the gut microbes through the same pathway (Bonaz, Bazin, & Pellissier, 2018).

2.2 The Microbiome and Aging

One of the many factors attributed to a change in the microbiome is aging. There are extensive studies which show that the gut bacteria in particular change drastically during the aging process. This change is progressive and gradual, mimicking the aging process itself (O'Toole & Jeffery, 2015). There are obvious life changes that can occur during the aging process that can affect the microbiome, such decreased physical activity, drug treatments, and increased antibiotic use. Many studies point to the decrease in intestinal mobility as a key factor in age-related diseases because this change can allow for overgrowth of bacteria in the intestines. In a recent review article, Riaz *et al.* summarizes conditions which the composition of the gut microbiome has been shown to influence, such as Parkinson's disease, rheumatoid arthritis, *C. difficile* infections, bone loss, and metabolic diseases, which are all common in the geriatric population (Riaz Rajoka *et al*., 2018).

It is unclear whether these gut microbiome changes direct aging or are a byproduct of the aging process. Preliminary studies in *C. elegans* and *Drosophila* have supported the hypothesis that bacterial interactions with cells can both increase and decrease longevity. These studies, however, have not been completed to show a similar reaction in human cells (Heintz & Mair, 2014).

Additionally, gut microbes have been shown to be related to the progression of several diseases which are common among the elderly population, such as diabetes, fatty liver disease, osteoporosis and some cancers. All of these conditions can drastically affect the daily lives of the elderly patients (Shimizu, 2018). Another review article poses the possibility of using the microbiome as a strategy to alter the effects of specific diseases (Zapata & J Quagliarello, 2015).

2.2.1 Long-term Care Facilities and The Microbiome

Currently in the United States, about six percent of elderly adults are cared for in long-term care facilities. These older adults tend to have medical issues and dependency issues which exceed the family's ability to care for the older adult (Center for Disease Control, 2017). A

change in the living environment can affect the microbiome, and thus health. Living in these faculties, older adults live in close contact with many other people with different medical conditions and are subject to a diet which, in most cases, has low fiber and high fat contents (Haran, Bucci, Dutta, Ward, & McCormick, 2018). A previous study conducted by the University of Massachusetts Medical School showed that gut and fecal microbiomes of patients on the same floor were very similar compared to patients living on a different floor within the same facility. This study also showed a relationship between decreased diversity in the gut microbiome and overall frailty. Another finding of this study was that the microbiomes of the residents of long-term care facilities was relatively stable unless interrupted by antibiotics (Haran, Bucci, Dutta, Ward, & McCormick, 2018).

2.3 Alzheimer's Disease and Dementia

Dementia is a term used to describe a set of symptoms including impaired memory, poor judgement and reasoning skills, and decreased focus and attention. Dementia itself is not a specific disease. Alzheimer's Disease (AD) is the most common and well-known form of dementia, although other forms of dementia exist including frontotemporal dementia, Parkinson's dementia, and vascular dementia (Hill *et al*., 2014). All of these diseases are characterized by decreased cognitive function. As the most prominent form of dementia, Alzheimer's Disease has been studied greatly. Through these investigations it has been found that about thirty to fifty percent of cases have a genetic component, which includes specific genes which predispose individuals to AD (Breitner, Silverman, Mohs, & Davis, 1988). Accordingly, fifty to seventy percent of AD are sporadic, having no predisposition. Eight key characteristics of AD have been identified as follows: "uncontrolled oxidative stress, up-regulated pro-inflammatory signaling, changes in innate-immune signaling, progressive accumulation of lesions, synaptic signaling deficits, neurite and brain cell atrophy, altered gene expression patterns that are different from healthy brain aging, and progressive cognitive impairment and dementia" (Hill *et al*., 2014). These characteristics are all also seen as side effects of microbial infections. Through many recent studies examining the relationship between the microbiome and neurological function, the microbiome has been shown to have a profound impact on the central nervous system, cognition, and behavior (Cryan & O'Mahony, 2011).

2.3.3 Etiology of Alzheimer's Disease

Currently, there are three hypotheses about the etiopathogenesis of Alzheimer's Disease: the cholinergic hypothesis, the amyloid cascade hypothesis, and the inflammation hypothesis (Sochocka, Zwolińska, & Leszek, 2017). The cholinergic hypothesis of Alzheimer's Disease proposes that the neurotransmitter acetylcholine and degradation of associated neurons cause Alzheimer's Disease. Acetylcholine has been shown to be important in memory and other cognitive functions of the brain. However, degradation of cholinergic neurons can be seen in many patients without Alzheimer's Disease (Francis, Palmer, Snape, & Wilcock, 1999). The amyloid cascade hypothesis proposes that the amyloid plaques and subsequent neurofibrillary tangles cause neuronal cell death which can cause Alzheimer's Disease. This

hypothesis has been supported and rejected by several studies, as amyloid plaques and neurofibrillary tangles can be seen in patients without Alzheimer's Disease, and a mechanism for how the two clinical findings cause neuronal death is unknown (Reitz, 2012).

The inflammation hypothesis of Alzheimer's Disease proposes that a low-grade, chronic neuroinflammatory response induces apoptosis of cells in the brain by microglia and astrocytes and inhibits cellular repair mechanisms. Several pathogens have been identified as potential causes for neuroinflammation, including bacterial and viral species. The infection triggering the neuroinflammation could originate in almost any part of the body and spread to the brain through cytokines and other chemical messengers (Holmes *et al*., 2009).

2.3.2 Symptoms and Diagnosis

The symptoms of Alzheimer's Disease include impaired memory, difficulty concentrating, problems finishing routine tasks, confusion regarding time, visual and spatial difficulties, language problems, social withdrawal, and mood changes. These symptoms can be evaluated through cognitive tests, neuropsychological tests, and interviews with friends and family. With the onset of these symptoms, doctors generally perform a physical examination to rule out other conditions, and may conduct laboratory and brain-imaging tests to confirm the diagnosis (Dubois *et al*., 2007)

Historically, a large issue with diagnosing all forms of dementia was that the diagnosis was made too late and thus there were no treatment options. Studies have shown that many forms of dementia, including Alzheimer's Disease, begin years before the traditional symptoms begin. This time period is called the preclinical phase. If the disease is caught and diagnosed during this phase, interventions may be taken in order to slow the progression of the disease. (Sperling *et al*., 2011)

Additionally, in some cases, once a person shows the symptoms of Alzheimer’s Disease, a diagnosis may be given without thorough investigation. Without brain-imaging and other diagnostic tools, the diagnosis of Alzheimer's Disease may be incorrect and interfere with treatment. Additional research into prevention and treatment of Alzheimer's Disease has decreased the incidence of faulty diagnosis, as there is now an emphasis on diagnostic accuracy (Gaugler *et al*., 2013).

A large number of diagnoses of Alzheimer's Disease are determined post-mortem through autopsies of the brain. Two clinical signs of Alzheimer's Disease are the presence of amyloid plaques and neurofibrillary tangles in the neurons of the brain. While the presence of these two findings have been linked to Alzheimer's Disease, there is little evidence which supports that these findings are the direct cause of the symptoms of the disease (Guillozet, Weintraub, Mash, & Mesulam, 2003). These findings, however, have allowed for several diagnostic and treatment endeavors. By tracking the presence of the amyloid plaques and neurofibrillary tangles, the progression of the disease may also be able to be tracked in accordance (Guillozet, Weintraub, Mash, & Mesulam, 2003).

2.3.3 Cognitive Testing

Cognitive scales are useful tools for diagnosing and monitoring dementia as many people will show varying signs of cognitive decline at different points of time. These

assessments should be valid, reliable, and objective (Sheehan, 2012). Assessing the cognitive function of patients with Alzheimer's Disease and other forms of dementia is essential for creating and testing therapies. Some of the most common cognitive assessments are the Mini-Mental State Exam (MMSE), the Brief Interview for Mental Status (BIMS), and the Clinical Dementia Rating (CDR). The MMSE consists of 11 directed questions and gives the participant a score out of 30 points. Using the MMSE, a score of 24 points or greater is considered normal, and a score of less than 24 points is abnormal. A score of between 18 and 23 points indicates mild cognitive impairment, and a score of 17 points or less indicates severe cognitive impairment. Furthermore, a score of less than 21 points indicates an increased odds of dementia. The MMSE is recognized nationally as a valid cognitive exam (Tombaugh & McIntyre, 1992). The BIMS assessment uses direct, performance-based questions to analyze the recall and temporal orientation of a participant. Strict grading criteria allow for participants to be given a score out of 15 total points. On this scale, a score of seven or less is considered severely impaired, a score of between 8 and 12 is considered mildly impaired, and a score of more than 13 is considered cognitively intact (Saliba *et al*., 2012). The CDR is a five-point assessment which assesses six domains of cognitive function; memory, orientation, judgement and problem solving, community affairs, personal care, and home and hobbies. The CDR is completed after a semi-structured interview with the participant. The examiner gives the participant a score from 0 to 3 based on the given prompts with consideration only to cognitive decline. The scores for each domain are then entered into a tabulation software available online and a final score is given. A score of 0 represents normal cognitive function, a score of 0.5 represents very mild dementia, a score of 1 represents mild dementia, a score of 2 represents moderate dementia, and a score of 3 represents severe dementia (Hughes, Berg, Danziger, Coben, & Martin, 1982).

The National Institute of Health (NIH) released a comprehensive neurobehavioral exam which measures motor, emotional, sensational, and cognitive abilities of the participants (NIH Toolbox). Participants between the ages of 3 and 85 years of age are asked to complete a set of assessments on an iPad. The assessments should take less than 30 minutes and provide an accurate report of the neurobehavioral function of the participant (Weintraub *et al.,* 2013).

3.0 Materials and Methods

In order to assess the microbiome and cognitive state of individuals living in long-term care facilities, the participants first gave their consent to be a part of the study and then cognitive exams were performed, stool samples were collected, and laboratory assays were conducted and analyzed.

3.1 Consent

Residents from three units of two long-term care facilities in Worcester were included in this study. The study was approved by the Institutional Review Board of the University of Massachusetts Medical School. Additionally, the facility managers and staff of the long-term care facilities agreed to aid in the data collection of the study. The inclusion criteria for the

studies specified that only English-speaking adults over 65 years of age living in a long-term care facility can participate in the study. Additionally, participants must be able to understand and give informed consent to be able to participate in the study, or have a legal health care proxy enacted, who can understand and give informed consent on their behalf. Lastly, the participants must be comfortable with the procedural protocol of the study. A preliminary chart review revealed that almost all residents in the units were candidates for the study. Those who were not candidates for the study were those who could not understand English in order to consent.

For both the cognitively-able residents and the health care proxies, the goal of the study and the expectation of participants was described in detail. During the consenting process, the cognitively-able residents or health care proxies signed a consent form and a release of health information form (See appendices A and B), which would allow the UMass Medical Center staff to have access to the medical and demographic information of the residents. Once enrolled, each participant was given an identification number. All rules set forth by HIPPA were followed. The privacy of all residents was upheld.

3.2 Sample and Data Collection

Once residents were enrolled in the study, the staff were instructed to begin collecting stool samples once every 2 weeks for 90 days. A freezer was supplied to each wing and was stored in a locked soiled utility room. The staff of the facility were supplied the collection materials and shown the proper technique for collecting the samples as well as labelling and recording the samples. The samples were transported from the facility to UMass Medical School in Biohazard coolers.

Chart reviews were performed following sample collection. Demographic and medical information was gathered following study guidelines (see appendices C and D). The BIMS score was taken from the chart review, as all of the facilities utilized it as a cognitive assessment (See appendix E). The initial cognitive status of the participants was assessed using the Mini-Mental State Exam (See appendices F and G). After the assessment, each participant was given a clinical dementia rating (CDR, See Appendix H). The information from these interviews was further investigated through chart reviews.

Further cognitive testing was done using assessments from the NIH Toolbox on an iPad. Five assessments from the NIH Toolbox inventory were used: a pattern sequence memory test, a vocabulary comprehension test, a picture comparison exam, a list sorting working memory exam and a dimensional change card sort exam (See Appendices I, J, K, L, and M). The NIH Toolbox uses the participant's age, highest level of education and handedness in order to tailor the questions of some assessments to the individual. All identifiable participant information was coded in the iPad. To access the participant information on the NIH Toolbox app on the iPad, a 4-digit password was required.

3.3 Analysis

The analysis of the stool samples was performed by the primary investigator of the study, Dr. John Haran and an off-site institution. All samples were stored at -80 C until the

analysis was performed. Sample processing and analysis was conducted as previously demonstrated in *Haran et al*. 2017. Interpretation of cognitive exam results was done through the REDcap and the Prism systems. Interpretation of microbiome sequencing was completed as in Haran *et al*. 2017.

4.0 Results

A total of 16 participants were included in this observational study. With this small sample size, it is not possible to draw conclusions from the data, however, further studies can show trends and associations between cognitive assessments, stages of cognitive decline, and the microbiome, as well as other medical conditions. Not all participants completed all cognitive assessments or gave stool samples to be analyzed for microbiome data.

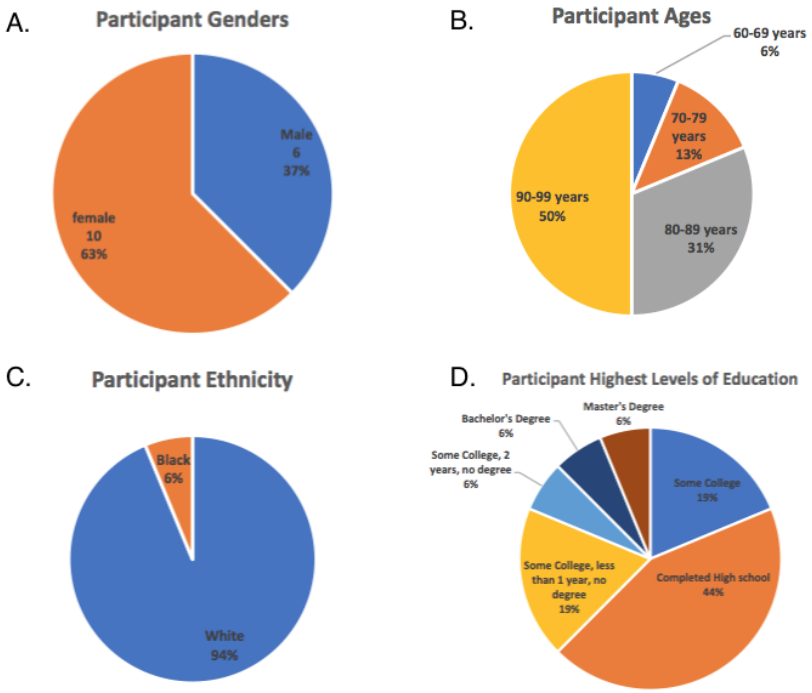


Figure 1: Participant Demographics

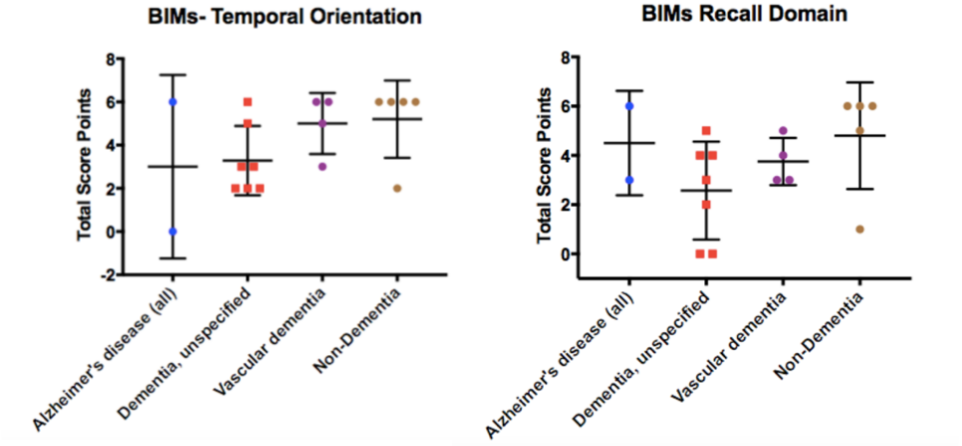
A. The genders of the participants based on the medical electronic record system. B. The age ranges of the participants. C. The ethnicities of the participants. D. The highest levels of education of the participants as recorded in the electronic medical record system.

While the small sample size does not allow for any conclusions to be drawn from the data, simply acquiring more participants will not solve the problem. This sample is not representative of the population and thus conclusions cannot be drawn from the data. As seen in figure 1A and 1B, the majority of the participants in the study were females between the ages of eighty and ninety-nine years of age. As seen in figure 1C, the participants were predominantly white and not

of Hispanic descent. There was, however, a range of highest levels of education from high school to master's degree. In order to establish a sample which is representative of the population, more men, people aged from sixty to seventy-nine years of age, and people with different ethnic backgrounds must be included in the study.

4.1 Cognitive Assessments

Each of the participants was assessed cognitively using the previously mentioned methods. The participants were grouped according to dementia diagnosis, forming four groups: participants diagnosed with Alzheimer's Disease, participants diagnosed with vascular dementia, participants diagnosed with unspecified dementia, and participants without a recorded dementia diagnosis. These diagnoses were identified through the electronic medical record system of the long-term care facility. Due to the small sample size, trends cannot be determined. The majority of participants were diagnosed with unspecified dementia. Only two participants were diagnosed with Alzheimer's Disease. The results obtained are presented in the following figures.



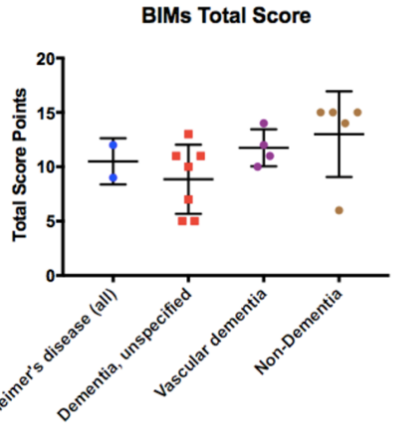


Figure 2: BIMs Scores

The total BIMS score and recall and orientation domains based on the information in the electronic medical system presented as groups of participants with diagnosed dementia, Alzheimer's Disease, Vascular Dementia, or unspecified, compared to participants with no recorded dementia diagnosis. The center bar represents the mean of the data points in the subgroups and the bars above and below represent the standard deviation from the mean.

As part of the procedures of the long-term care facility, a BIMS assessment is completed for each resident at least once every four months. The results of these assessments were recorded in the electronic medical system with the total scores and individual responses, as shown in Appendix E. Assessments were completed by long-term care facility staff and not repeated by study coordinators. The total score of the BIMS is reported as a number out of 15 total points. A total score of between 13 and 15 denotes a cognitively intact participant, a score of between 08 and 12 denotes moderate impairment, and a score of between 00 and 07 denotes severe impairment. Additionally, the two domains can be assessed separately, as the temporal orientation domain has a total of 7 possible points and the recall domain has a total of 8 possible points.

As can be seen in Figure 2, there is great variation of the scores in each of the groups of participants; one participant with a diagnosis of Alzheimer's Disease scored very highly on the BIMs while another scored very low, and similarly, several participants without a dementia diagnosis scored very well but another participant scored very low. In the future, adding more participants to the study could show a marked difference between the average scores for participants with a dementia diagnosis and for participants without a dementia diagnosis as well as between the different forms of dementia. Additionally, the BIMS score can be tracked long-term to show cognitive decline and track participants based on when the diagnosis was given.

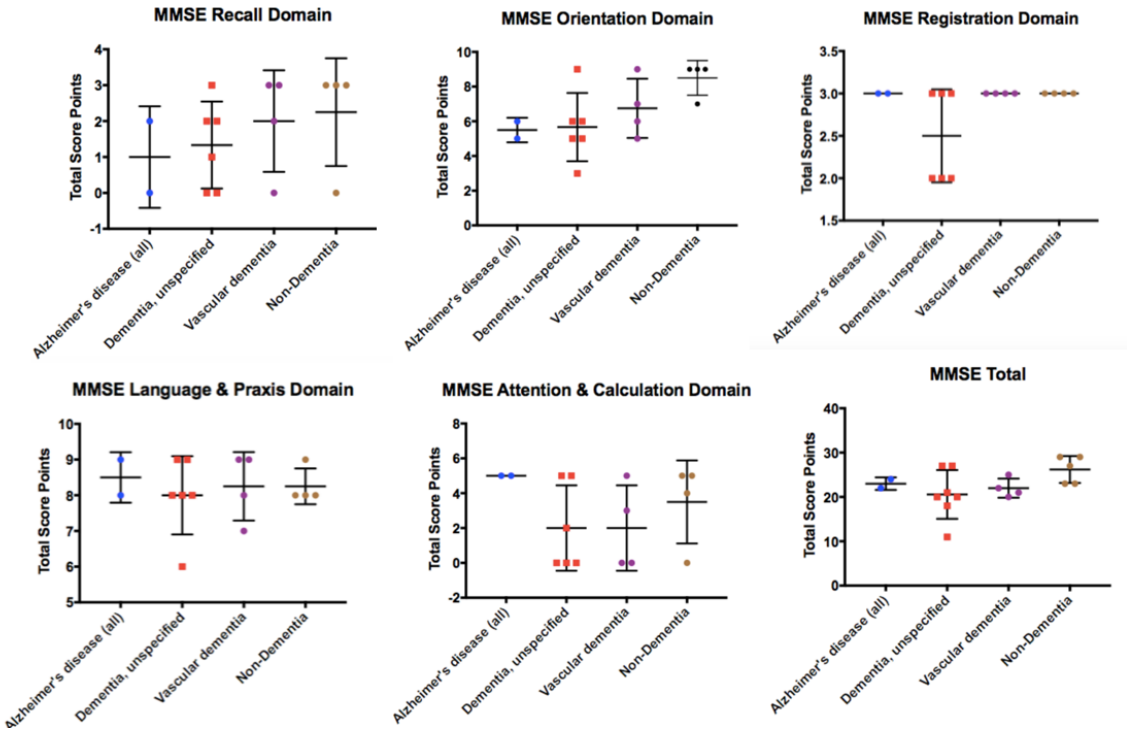


Figure 3: MMSE Scores

The total MMSE score and subdivided domains based on the information gained from a structured interview as groups of participants with diagnosed dementia, Alzheimer's Disease, Vascular Dementia, or unspecified, compared to participants with no recorded dementia diagnosis. The center bar represents the mean of the data points in the subgroups and the bars above and below represent the standard deviation from the mean.

Each participant was assessed using the MMSE during a structured interview with a study coordinator and scored according to the sheet in Appendix G. The MMSE gives a total score out of 30 which can be broken down into three main results; a score of more than 24 denotes normal cognitive status, a score of between 18 and 23 denotes mild cognitive impairment, and a score of less than 17 denotes severe cognitive impairment. Additionally, the scores can be assessed according to the domains for which the MMSE tests; the recall domain has a maximum point value of 3, the orientation domain has a maximum point value of 10, the registration domain has a maximum point value of 3, the language and praxis domain has a maximum point value of 9, and the attention and calculation domain has a maximum point value of 5. The assessment was completed once during the study time-frame. Similar to the BIMS assessment, the sample size is too small to be able to see trends within the four groups of participants. Most participants from all groups scored a total of three on the registration domain of the MMSE. Like the BIMS, the MMSE can be used to track cognitive decline and more specific changes to cognition as there are more domains tested.

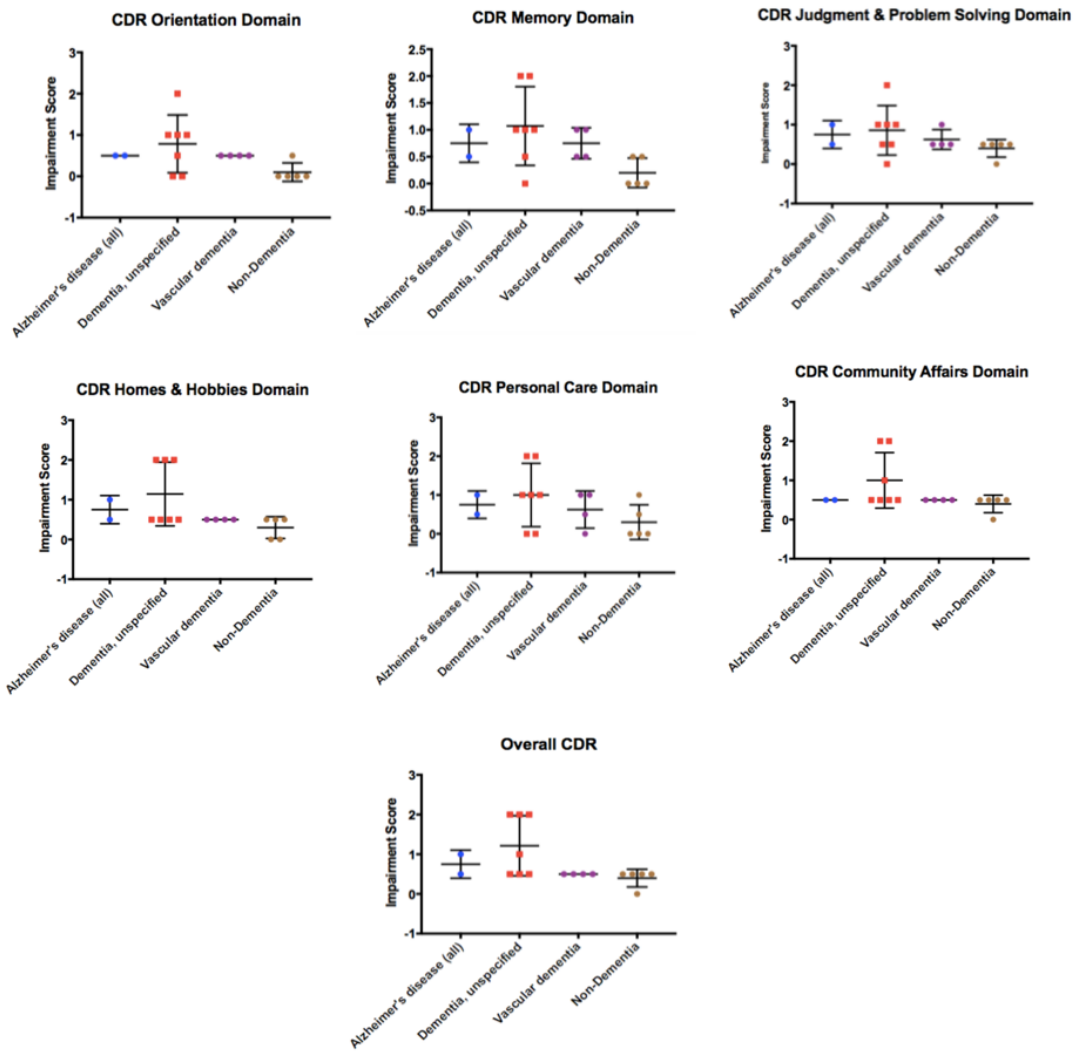


Figure 4: CDR Scores

The total CDR score and subdivided domains based on the information gained from a semi-structured interview as groups of participants with diagnosed dementia, Alzheimer's Disease, Vascular Dementia, or unspecified, compared to participants with no recorded dementia diagnosis. The center bar represents the mean of the data points in the subgroups and the bars above and below represent the standard deviation from the mean.

The CDR was completed by a study coordinator after other cognitive assessments were completed and scored according to the chart in Appendix H. The CDR records a rating out of 3 total points in 6 different domains and then computes an overall score. Each of the domains is scored based on the participant's ability to complete certain tasks, influenced solely by their cognitive capabilities. An online CDR tabulator weights the domains and computes a total score out of 3. A score of 0 indicated no dementia, a score of 0.5 indicates questionable dementia, a score of 1 indicates mild dementia, a score of 2 indicated moderate dementia and a score of 3 indicates severe dementia. As with the other cognitive assessments, there is great variation within

the assessments. Subsequent CDR assessments can readily track the progression of cognitive decline as one score relates to each of the six domains.

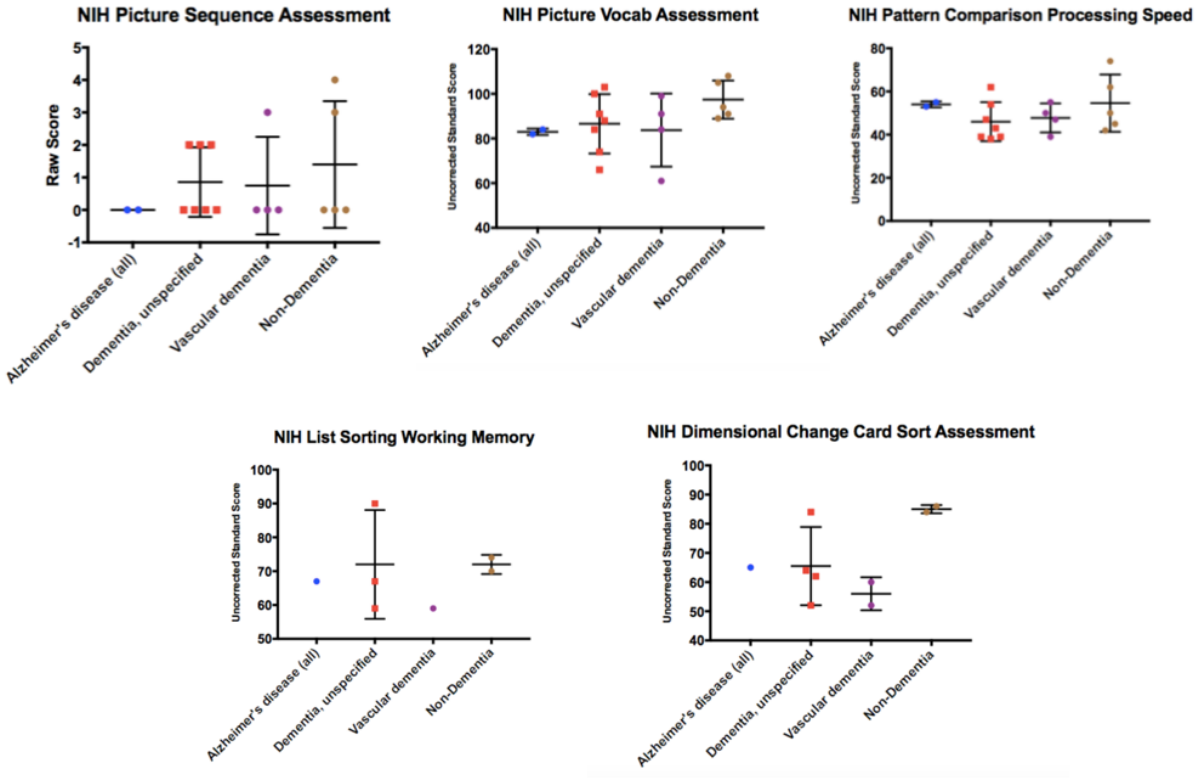


Figure 5: NIH Toolbox Scores

The cognitive scores obtained from the assessments of the NIH Toolbox. Scores are reported as the uncorrected standard score or the raw score. The scores are presented as groups of participants with diagnosed dementia, Alzheimer's Disease, Vascular Dementia, or unspecified, compared to participants with no recorded dementia diagnosis. The center bar represents the mean of the data points in the subgroups and the bars above and below represent the standard deviation from the mean.

Each of the participants was asked to complete five cognitive assessments from the NIH Toolbox. Each assessment had specific rules and directions as outline in appendices I, J, K, L, and M. Many participants did not complete all five assessments due to time restrictions, participant refusal, or inability to complete the tasks of the assessments due to medical conditions. The scores for the NIH Toolbox are reported as either the raw score for the picture sequence memory test or as the uncorrected standard score for the other tests, because many of the participants are older than the validated age of the NIH Toolbox, and therefore their results cannot be related to either the population as whole or others of their age. Though in the future, using the NIH Toolbox may allow for comparison between the participants and the population as a whole and other with similar features, right now the results can only be compared to the other participants in the study. Additionally, for the picture sequence memory test, any participant over the age of 85 received a score of 0 regardless of their performance on the assessment.

4.2 Comparison of Results

While all of these assessments were used to identify and define the cognitive decline of the participants in the study, the results of the CDR, BIMS, and MMSE can all present the results in terms of dementia progression with similar scoring systems. The CDR simply gives a number which relates to the cognitive abilities of the participant. A score of zero denotes no dementia, 0.5 denotes very mild dementia, 1 denotes mild dementia, 2 denotes moderate dementia, and 3 denotes severe dementia. For the purposes of comparison, the CDR scores of less than 1 represent mild dementia to cognitively intact.

The BIMS assessment gives a total score out of 15 points. A score of between 13 and 15 denotes cognitively intact participants, a score of between 8 and 12 denotes moderate cognitive impairment, and a score of less than 7 denotes severe cognitive impairment. Accordingly, the three categories of cognitive status, intact, moderate impairment, and severe impairment, can be given a score of 1, 2, and 3 respectively in order to be compared to the results of the CDR, which can be seen in Figure 6.

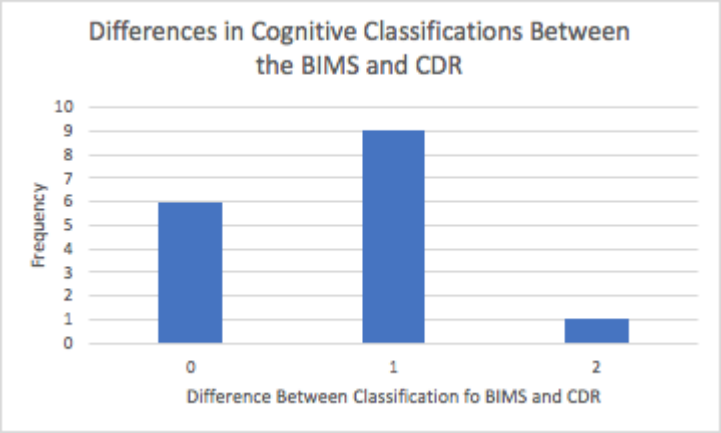


Figure 6: Differences in Cognitive Classifications Between the BIMS and CDR

The differences between the classifications of cognitive abilities of the participants, as either cognitively intact, mildly impaired, or severely impaired. The assessments were used on all participants and the results of the CDR were compared to the results of the BIMS. The x-axis shows the change in the scores from the BIMS to the CDR. 0 refers to no change in classification, 1 refers to an addition of 1 to the score from the BIMS, 2 refers to an addition of 2 to the score of the BIMS.

When comparing the results of the BIMS to the results of the CDR, the same classification was given 37.5% of the time. Mostly, the CDR over-classified the participant's dementia states, as 62.5% of the cases, the CDR score was either one or two points greater than the BIMS score.

The results of the MMSE can be compared similarly. The MMSE assessment gives a total score out of 30. A score of greater than 24 denotes cognitive intact participants, a score of between 18 and 23 denotes mild cognitive impairment, and a score of less than 17 denotes severe cognitive impairment. Just as with the BIMS, these results, intact, mild impairment, and

severe impairment, can be given a score of 1, 2, and 3 respectively in order to be compared to the results of the CDR and BIMS. The comparison of the MMSE to the BIMS and CDR can be seen in Figures 7 and 8.

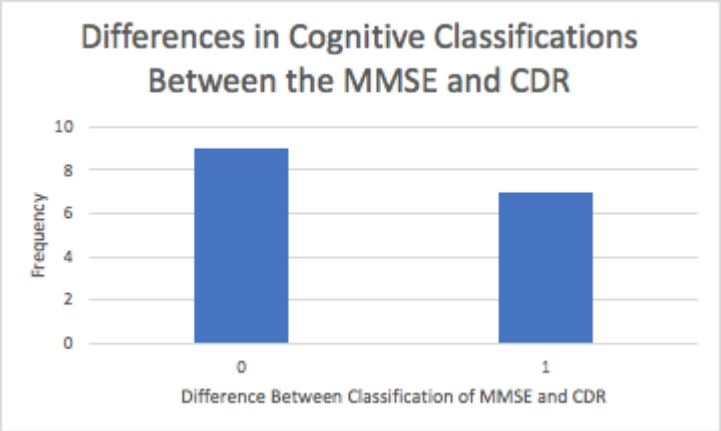


Figure 7

The differences between the classifications of cognitive abilities of the participants, as either cognitively intact, mildly impaired, or severely impaired. The assessments were used on all participants and the results of the CDR were compared to the results of the MMSE. The x-axis shows the change in the scores from the MMSE to the CDR. 0 refers to no change in classification, and 1 refers to an addition of 1 to the score from the MMSE.

When comparing the MMSE and the CDR, the classifications of the two assessments were the same 56.25% of the time. The other 43.47% of the cases, the CDR score was one greater than the score of the MMSE. Overall, the CDR seems to give classifications of dementia more readily than the BIMS or MMSE.

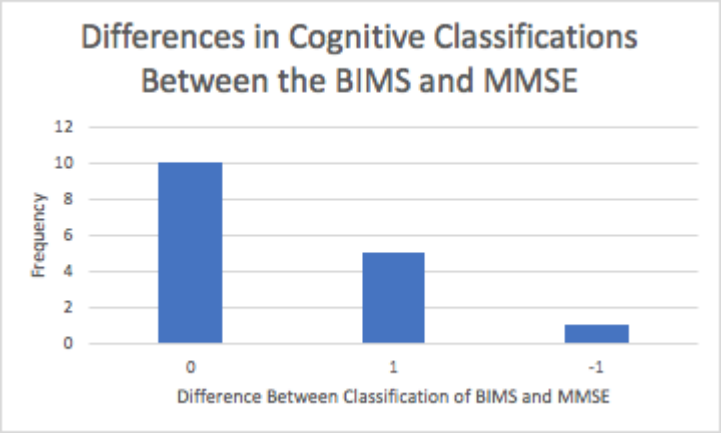


Figure 8

The differences between the classifications of cognitive abilities of the participants, as either cognitively intact, mildly impaired, or severely impaired. The assessments were used on all participants and the results of the MMSE were compared to the results of the BIMS. The x-axis shows the change in the scores from the BIMS to the MMSE. 0 refers to no change in classification, and 1 refers to an addition of 1 to the score from the BIMS, and -1 refers to a subtraction of 1 from the score from the BIMS.

When comparing the BIMS and MMSE, the scores of the two assessments were the same 62.5% of the time. Otherwise, the MMSE usually gave a score of one greater than that of the BIMS, but on one occasion gave a score of one less than the BIMS score.

4.3 Cognition and the Microbiome

The dementia diagnoses from the BIMS, MMSE, and the raw score from the Picture Sequence Memory Test of the NIH Toolbox were combined with the principal components of the microbiome composition and plotted on PCA plots as shown in Figure 9. As with the results from the cognitive scores, the sample size was too small to draw concrete conclusions about the relationship between the dementia diagnosis and the composition of the microbiome.

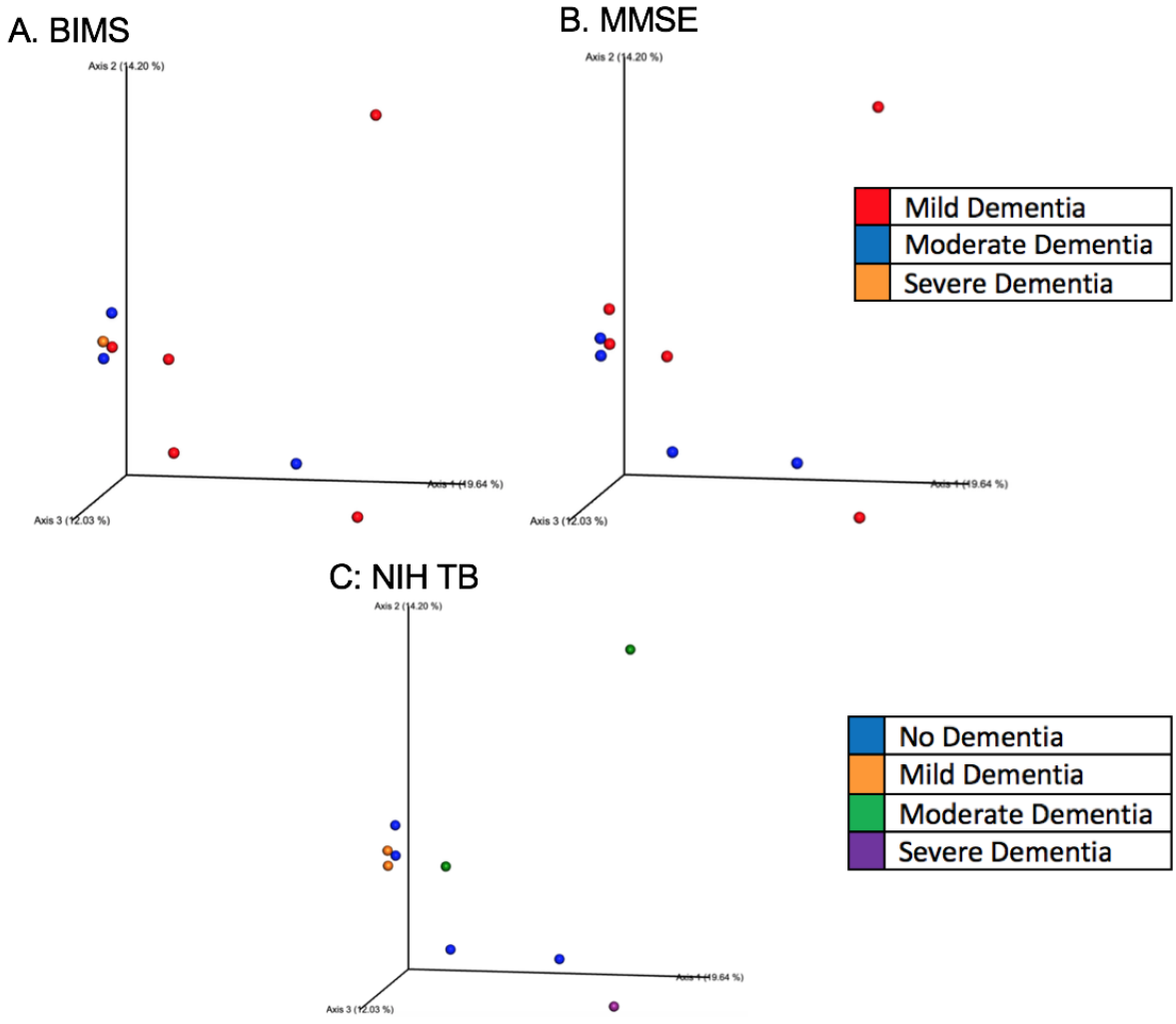


Figure 9: PCA Jaccard-Type Beta Diversity Plot of Dementia Diagnoses from Different Assessments

The principal components of the analysis of the microbiome composition plotted against 3 difference axes 19.64%, 14.20%, 12.03%, colored coded by dementia diagnosis from the A. BIMS assessment, B. MMSE assessment and C. the Raw Score from the Picture Sequence Memory Test of the NIH Toolbox.

The points in close proximity to each other indicate similarities between the composition of the samples. In all three plots, there is a cluster of four points which are all close in proximity, indicating similar microbiome compositions, but these points have different dementia diagnoses ranging from no dementia to severe dementia depending on the assessment. There are two points which are very distant from the others, which indicates very limited similarities between the microbiome composition.

The similarities in the composition of the microbiome could be due to the fact that many of the participants of the study live in close proximity on the same wing of the long-term care facility. It has been shown in previous studies that the compositions of microbiomes of long-term care facility residents tends to be similar among those living in the same wing (Haran, Bucci, Dutta, Ward, & McCormick, 2018). Additionally, many of the residents took antibiotics during the course of the study, which could account for the extreme outliers seen in Figure 9. In order to draw conclusions about the composition of the microbiome and dementia status, a much larger sample size including residents from more facilities.

4.4 Future Evidence

The largest drawback of this study was the small and unrepresentative sample which did not allow for any conclusions to be drawn. Looking forward, one goal of the study is to include more participants with a more representative sample. Additionally, performing cognitive assessments on the participants on a set schedule will allow for more accurate tracking of cognitive decline and relate the decline to the microbiome. Ideally, the stool sample and the cognitive assessments would be collected and completed within a week of each other, in order to accurately relate the two.

When looking at the results, it is hypothesized that there will be a difference in either diversity or presence of certain strains of bacteria in the microbiomes of the participants with Alzheimer’s Disease compared to the participants with other forms of dementia or without a diagnosis of dementia. These results could be seen in a PCA plot similar to the plots seen in Figure 9. In order to say that the composition of the microbiome and the dementia diagnoses are related, the plot would show clusters of points with similar compositions with the same dementia diagnosis. With the information from the PCA plots, the similar microbiomes could be compared to identify the strains the samples have in common. These strains could then be further studied for their possible influence on cognitive status.

Additionally, within the group of participants with Alzheimer's Disease, the changes in the gut microbiome could also be tracked with any changes in the cognitive function. Either the similarities in the microbiome composition or the changes related to cognitive status could allow for more investigation into specific microbial influences on Alzheimer’s Disease and potentially support the inflammatory hypothesis of Alzheimer’s Disease.

5.0 Discussion

5.1 Cognitive Testing

While drawing concrete conclusions about the use of the different cognitive exams to track cognitive decline in Alzheimer's and dementia patients is difficult using the results from this study, there are still meaningful observations about the different exams which can be noted. The most striking differences between the exams were the ease of administration, the reactions of the participants, the depth of information gained, and the ease of interpretation of the results.

5.1.1 Ease of Administration

First, the ease of administration of the different exams was variable. The BIMS assessment is the easiest to preform and score as there were only three questions with very detailed instructions for scoring. The CDR was also fairly easy to perform as there weren't specific questions that needed to be asked, rather through a semi-structured interview the study coordinator was able to gain insight into the participant's cognitive ability. While it was easy to complete the interview, the scoring system was more difficult and up to the discretion of the examiner to decide the points based on loose guidelines. The MMSE was easy to administer as there were set questions and a straightforward scoring guide. The questions on the MMSE were more extensive than the questions on the BIMS and required additional equipment, such as scrap paper, a pen, and possibly a clipboard. The exams on the NIH Toolbox were by far the most difficult to administer as there was extensive equipment, set-up, and directions for each exam. The exams of the NIH Toolbox were also the longest of the cognitive exams given with each of the five exams taking between seven and twenty minutes to complete.

5.1.2 Participant Reactions

Performing the cognitive exams with the participants could be challenging depending on the reactions of the participants. Explaining the purpose of the questionnaires at times had participants feeling pressured to do well and caused anxiety. The CDR causes the least amount of stress on the participants since through the semi-structured interview, there were not strict correct or incorrect answers. During the other exams, however, the participants understood that they were being assessed. The BIMS assessment was completed by the facility staff and therefore the participants reactions could not be gauged. During the MMSE, some participants who did poorly on the beginning question seemed to be distressed and the drawing activity was a bother to others. The NIH Toolbox was not well received by the participants as the population was not familiar with the use of iPads and the exams were very skill-based.

5.1.3 Results of Examinations

The examinations also differed in the amount of information which could be obtained and the ease of interpretation of the results. The BIMS, MMSE, and CDR all had simple results which could be interpreted readily. Each of these exams came with a score interpretation guide which explained the meanings of different scores. The NIH Toolbox did not come with such a score, and instead reported scores for different exams with different measurements, such as the uncorrected standard score, corrected standard score, raw score, and T score, which were all explained in a lengthy interpretation guide. While the BIMS, MMSE, and CDR can all be readily interpreted with limited training, interpreting the results of the NIH Toolbox exams requires far more effort and training.

As can be seen in Figures 2, 3, 4 the BIMS, MMSE, and CDR are comprised of different domains which correspond to different cognitive abilities. For example, the BIMS score combines two cognitive domains, recall and orientation. The MMSE is comprised of five cognitive domains, and the CDR is comprised of six cognitive domains. With sequential assessments, the cognitive decline of participants can be tracked and categorized depending on the scores within the different domains, which can be very useful. The different assessments of the NIH Toolbox also test the different elements of cognition but tracking the scores for the different domains is more difficult as many of the assessments combine two domains and the domains are not readily available.

5.1.4 The NIH Toolbox as a Dementia-Tracking Assessment

While the BIMS, MMSE, and CDR were all designed to assess the cognitive status of dementia participants, the NIH Toolbox was designed to provide a concise and validated neurobehavioral exam for people between the ages of 3 and 85. Only 5 of the 16 participants of this study were 85 years of age or younger, and thus had validated scores. For some of the exams, mostly the pattern sequence memory exam, no data could be assessed for participants over the age of 85. When asked, the NIH Toolbox representative explained that verification of the NIH Toolbox for participants over 85 is being conducted. The iPad used during this study was not included in the verified tools to conduct the assessments. The screen size of the iPad was too small. Several participants verbalized that seeing the pictures or words on the iPad was difficult.

Many participants were reluctant to use the iPad as they did not have much experience with similar technology. Accordingly, the participants required assistance from administrators to complete the exams. For instance, the examiner would have to press the buttons on the iPad for participants who were unable to. Two of the 5 assessments, the pattern comparison exam and the dimensional change card sort exams, indicate that the examiner cannot assist the participant as reaction time was a factor in the analysis. For the best results, the exams should be completed during the same sitting. For the population of the study, the assessments were broken into time periods. Still, many participants requested to stop after one of the assessments. Exams were thus given on multiple occasions spread out over time periods ranging from weeks to months. The order in which the exams were given fluctuated as some exams caused more stress than others.

Additionally, many of the participants of the study could not be assessed using the NIH Toolbox for a variety of reasons. The participants of the study living on the specified dementia unit of the facility were not cognitively able to understand the directions of the exams. Other participants were nonverbal and thus unable to complete exam which require speaking, mostly the dimensional change card sort exam. Physical incapacities including arthritis and post-stroke weakness prevented other participants from completing some exams as they were unable to work with the iPad screen. In some cases, such as the picture comparison test, if a participant was unable to press the screen, the assessment would not be able to be performed at all because reaction time was monitored by the assessment. Thus, conditions other than just cognition dramatically affected the results of the exams. With the other exams, other conditions could affect the results, but not to as great of an extent.

5.2 Cognition and the Microbiome

Only a small portion of the participants of the study provided stool samples for analysis of the microbiome. There are notable factors from the collection which can affect the results of the analysis and may need to be addressed at a later time, such as the confounding factors of the study and the sample collection.

5.2.1 Confounding Factors

While the small sample size prevents any conclusions from being drawn from the data in the study, there are other factors from the microbiome analysis which may affect the results as well. Of the participants in the study, 14 of the sixteen reside in the same long-term care facility, on two separate wings. Two other participants reside in another facility on the same wing. There is evidence that microbiomes of individuals in the same environment, inferring the same wing of the same facility, are very similar. Having participants from three different wings introduces a confounding factor. Including more participants from the same wings as these participants could negate this as the microbiomes of the individuals from the different wings could be compared.

As part of the initial chart review, the amount of times the participants were administered antibiotics during the past year was recorded. Since antibiotics can have a drastic effect of the composition of the microbiome, it was important to track the administration of antibiotics to study participants. During the study, two of the participants were given antibiotics. One participant was on antibiotics immediately before the stool sample was collected, which could affect the diversity and composition of the microbiome. While it is important to track the administration of antibiotics, it cannot be controlled in the study as the participants must be treated for any illnesses they may have during the study. In the future, any participants that are given antibiotics during the study should have additional samples collected following the antibiotics to allow sufficient time for the microbiome to reconfigure.

There are additional human factors, other than taking antibiotics, which can affect the results of the study. The diets and activities of the participants were not regulated during this study. These differences among the participants introduce other confounding variables into the study. In the future, these confounding variables will still be present as there are limited ways to account for the varying activity levels and diets of the participants.

5.2.2 Sample Collection

The frequency of sample collection was not strictly enforced. Although samples were intended to be collected once every 2 weeks for 90 days, staff at the facility did not prioritize sample collection and thus samples were collected infrequently. Dates and times of samples were collected and stored. Accordingly, the samples were not taken on the day that the assessments were conducted, and sometimes sample collection and cognitive assessments were several weeks apart.

In the future, the cognitive assessments and stool samples should be coordinated so that an accurate snapshot of the participant's cognitive status and microbiome composition can be analyzed. The samples should also be consistently spaced to be able to track the change in cognition over time and relate it to a disease course. The accuracy of the sample collection will also allow for tracking environmental and health effects on the microbiome and cognition.

6.0 Recommendations

In order to obtain results which could support or reject the inflammation hypothesis of Alzheimer's Disease, changes to several aspects of the study should be made. First, many more participants must be recruited to be a part of the study to ensure that there is a representative population. For this to be possible, the study must expand into many long-term care facilities to ensure a diverse population. Also, more participants with a diagnosis of Alzheimer's Disease must be included for the results to be able to relate back to the disease.

With the representative population, the sample collection must be standardized. While a collection schedule was created, the staff of the facilities did not follow through with collection of stool samples. In order to obtain meaningful results, the samples must be collected on a regular basis. Additionally, the cognitive assessments should follow the sample collection in order to provide an accurate correlation between the cognitive status and microbiome. A reward system for the staff of the facilities could be enacted in order to motivate the staff to collect samples when indicated.

Similar to the sample collection, the cognitive assessments should be standardized as well. Using eight cognitive tests was time consuming for both the study coordinators and participants. In order to complete the assessments within a timeframe close to sample collection, all cognitive assessments cannot be conducted. For this study, using the MMSE would be sufficient in order to track the cognitive decline of the patients. The MMSE is easy and quick to administer to participants, allows for tracking of six cognitive domains, and is not repetitive with the procedures of the facilities.

While this project did not result in concrete evidence to support to reject the inflammation hypothesis of AD, insights from the project can be utilized in the future to continue this important work.

Citations

Bäckhed, Fredrik, et al. “Host-Bacterial Mutualism in the Human Intestine.” *Science*, American Association for the Advancement of Science, 25 Mar. 2005, science.sciencemag.org/content/307/5717/1915.

Bolla, L. R., C. M. Filley, and R. M. Palmer. "Dementia DDx. Office Diagnosis of the Four Major Types of Dementia." *Geriatrics (Basel, Switzerland)* 55.1 (2000): 6. *PubMed.* Web.

Bonaz, B., Bazin, T., & Pellissier, S. (2018). The Vagus nerve at the interface of the microbiota-gut-brain axis. *Frontiers in Neuroscience, 12*, 49. doi:10.3389/fnins.2018.00049

Breitner, J. C.,S., Silverman, J. M., Mohs, R. C., & Davis, K. L. (1988). Familial aggregation in Alzheimer’s disease. *Neurology, 38*(2), 207. doi:10.1212/WNL.38.2.207

Carabotti, M., Scirocco, A., Maselli, M. A., & Severi, C. (2015). The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Annals of Gastroenterology : Quarterly Publication of the Hellenic Society of Gastroenterology*, *28*(2), 203–209.

Center for Disease Control (2017). National Center for Health Statistics: Nursing Home Care. Retrieved from https://www.cdc.gov/nchs/fastats/nursing-home-care.htm

Cryan, J. F., & O’Mahony, S. M. (2011). The microbiome-gut-brain axis: From bowel to behavior. *Neurogastroenterology & Motility, 23*(3), 187-192. doi:10.1111/j.1365-2982.2010.01664.x

David A Relman; The human microbiome: ecosystem resilience and health, *Nutrition Reviews*, Volume 70, Issue suppl_1, 1 August 2012, Pages S2–S9, https://doi.org/10.1111/j.1753-4887.2012.00489.x

Dubois, B., Feldman, H. H., Jacova, C., DeKosky, S. T., Barberger-Gateau, P., Cummings, J., . . . Scheltens, P. (2007). Research criteria for the diagnosis of Alzheimer's Disease: Revising the NINCDS–ADRDA criteria. *The Lancet Neurology, 6*(8), 734-746. doi://doi.org/10.1016/S1474-4422(07)70178-3

Francis, P. T., Palmer, A. M., Snape, M., & Wilcock, G. K. (1999). The cholinergic hypothesis of Alzheimer's Disease: A review of progress. *J Neurol Neurosurg Psychiatry, 66*(2), 137. doi:10.1136/jnnp.66.2.137

Gaugler, J. E., Ascher-Svanum, H., Roth, D. L., Fafowora, T., Siderowf, A., & Beach, T. G. (2013). Characteristics of patients misdiagnosed with Alzheimer's Disease and their medication

Gershon, R. C. (2013). Cognition assessment using the NIH Toolbox. *Neurology, 80*(11), S54. doi:10.1212/WNL.0b013e3182872ded

Guillozet, A. L., Weintraub, S., Mash, D. C., & Mesulam, M. M. (2003). Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. *Archives of Neurology, 60*(5), 729-736. doi:10.1001/archneur.60.5.729

Haran, J. P., Bucci, V., Dutta, P., Ward, D., & McCormick, B. (2018). The nursing home elder microbiome stability and associations with age, frailty, nutrition and physical location. *Journal of Medical Microbiology, 67*(1), 40-51. doi://doi.org/10.1099/jmm.0.000640

Heintz, C., & Mair, W. (2014). *You are what you host: Microbiome modulation of the aging process* doi://doi.org/10.1016/j.cell.2014.01.025

Henry, T. R. (2002). Therapeutic mechanisms of Vagus nerve stimulation. *Neurology, 59*(6), S3. doi:10.1212/WNL.59.6_suppl_4.S3

Hill, J. M., Clement, C., Pogue, A. I., Bhattacharjee, S., Zhao, Y., & Lukiw, W. J. (2014). Pathogenic microbes, the microbiome, and Alzheimer's Disease (AD). *Frontiers in Aging Neuroscience, 6*, 127. Retrieved from

https://www.frontiersin.org/article/10.3389/fnagi.2014.00127
Holmes, C., Cunningham, C., Zotova, E., Woolford, J., Dean, C., Kerr, S., . . . Perry, V. H. (2009). Systemic inflammation and disease progression in alzheimer disease. *Neurology, 73*(10), 768. doi:10.1212/WNL.0b013e3181b6bb95
Hughes, C. P., Berg, L., Danziger, W., Coben, L. A., & Martin, R.L. (1982). A new clinical scale for the staging of dementia. *British Journal of Psychiatry, 140* (6), 566-572. doi:10.1192/bjp.140.6.566
Kelley S.T., and Gilbert, J.A.. "Studying the Microbiology of the Indoor Environment. *Genome Biology*, BioMed Central, 28 Feb. 2013, genomebiology.biomedcentral.com/articles/10.1186/gb-2013-14-2-202.
Meadow, J. F., Altrichter, A. E., Bateman, A. C., Stenson, J., Brown, G., Green, J. L., & Bohannan, B. J. M. (2015). Humans differ in their personal microbial cloud. *PeerJ*, *3*, e1258. http://doi.org/10.7717/peerj.1258
O'Toole, P. W., & Jeffery, I. B. (2015). Gut microbiota and aging. *Science, 350*(6265), 1214-1215. doi:10.1126/science.aac8469
Pflughoeft, K. J., & Versalovic, J. (2011). Human microbiome in health and disease. *Annu. Rev. Pathol. Mech. Dis., 7*, 99-122.
Plassman, B. L., et al. "Prevalence of Dementia in the United States: The Aging, Demographics, and Memory Study." *Neuroepidemiology* 29.1-2 (2007): 125-32. Web.
Proctor, L. (2011). The human microbiome project in 2011 and beyond. *Cell Host & Microbe, 10*(4), 287-291. doi://doi.org/10.1016/j.chom.2011.10.001
Reitz, C. (2012). Alzheimer's Disease and the amyloid cascade hypothesis: A critical review. *International Journal of Alzheimer's Disease, 2012*, 369808. doi:10.1155/2012/369808
Riaz Rajoka, M. S., Zhao, H., Li, N., Lu, Y., Lian, Z., Shao, D., . . . Shi, J. (2018). Origination, change, and modulation of geriatric disease-related gut microbiota during life. *Applied Microbiology and Biotechnology, 102*(19), 8275-8289. doi:10.1007/s00253-018-9264-2
Ritchie, Karen, and Simon Lovestone. "The Dementias." *The Lancet* 360.9347 (2002): 1759-66. Web.
Saliba, D., Buchanan, J., Edelen, M. O., Streim, J., Ouslander, J., Berlowitz, D., & Chodosh, J. (2012). MDS 3.0: Brief interview for mental status. *Journal of the American Medical Directors Association, 13*(7), 611-617. doi://doi.org/10.1016/j.jamda.2012.06.004
Salyards, Jade. Microbiomes Germ Clouds and the Future of DNA Jurisprudence. Pittsburgh Journal of Technology Law and Policy, [S.l.], v. 16, n. 1, p. 58-75, apr. 2016. ISSN 2164-800X. Available at: <https://tlp.law.pitt.edu/ojs/index.php/tlp/article/view/178>. Date accessed: 13 sep. 2018. doi:https://doi.org/10.5195/tlp.2015.178.
Serino, M., Fernández-Real, J. M., Fuentes, E. G., Queipo-Ortuño, M., Moreno-Navarrete, J., Sánchez, Á, . . . Tinahones, F. (2013). The gut microbiota profile is associated with insulin action in humans. *Acta Diabetologica, 50*(5), 753-761. doi:10.1007/s00592-012-0410-5
Schulz, R., et al. "Long-Term Care Placement of Dementia Patients and Caregiver Health and Well-Being." *JAMA* 292.8 (2004): 961-7. Web.
Seitz, Dallas, Nitin Purandare, and David Conn. "Prevalence of Psychiatric Disorders among Older Adults in Long-Term Care Homes: A Systematic Review." *International Psychogeriatrics* 22.7 (2010): 1025-39. *Cambridge Core; Cambridge University Press.* Web.
Sheehan, B. (2012). Assessment scales in dementia. *Therapeutic Advances in Neurological Disorders*, *5*(6), 349–358. http://doi.org/10.1177/1756285612455733
Shimizu, Y. (2018). Gut microbiota in common elderly diseases affecting activities of daily living. *World Journal of Gastroenterology, 24*(42), 4750-4758. doi:10.3748/wjg.v24.i42.4750
Sochocka, M., Zwolińska, K., & Leszek, J. (2017). The infectious etiology of Alzheimer's disease. *Current Neuropharmacology, 15*(7), 996-1009.

doi:10.2174/1570159X15666170313122937
Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., . . . Phelps, C. H. (2011). Toward defining the preclinical stages of Alzheimer's Disease: Recommendations from the national institute on aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's Disease. *Alzheimer's & Dementia, 7*(3), 280-292. doi://doi.org/10.1016/j.jalz.2011.03.003
"The Microbiome." *Obesity Prevention Source*, 24 Aug. 2017, www.hsph.harvard.edu/nutritionsource/microbiome/.
Tombaugh, T. N., & McIntyre, N. J. (1992). The mini-mental state examination: A comprehensive review. *Journal of the American Geriatrics Society, 40*(9), 922-935. doi:10.1111/j.1532-5415.1992.tb01992.x
Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C., Knight, R., & Gordon, J. I. (2007). The human microbiome project: exploring the microbial part of ourselves in a changing world. *Nature*, *449*(7164), 804–810. http://doi.org/10.1038/nature06244
Van der Flier, W. M., and P. Scheltens. "Epidemiology and Risk Factors of Dementia." *J Neurol Neurosurg Psychiatry* 76 (2005): v2. Web.
Weintraub, S., Dikmen, S. S., Heaton, R. K., Tulsky, D. S., Zelazo, P. D., Bauer, P. J., . . .
Zapata, H., & J Quagliarello, V. (2015). *The microbiota and microbiome in aging: Potential implications in health and age-related diseases* doi:10.1111/jgs.13310

Appendices

A. Study Consent Form

UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL

COMMITTEE FOR THE PROTECTION OF HUMAN SUBJECTS IN RESEARCH

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Title of research study: **The Elderly Gut Microbiome among Long-Term Care Facility Patients and the Risk of *Clostridium difficile* Carriage and Infection**

Investigator: **John P. Haran, M.D.**

Sponsor: **Department of Emergency Medicine and the Center for Microbiome Research**

Why are you being invited to take part in a research study?

You are being asked to participate because you have been living in a long-term care facility and we are interested in studying your intestinal microbiome profile.

What should you know about a research study?

Your participation is entirely voluntary.

You may decide not to take part or decide to quit the study at any time, without any changes in the quality of the health care you receive.

You will be told about any new information or changes in the study that might affect your willingness to participate.

You are free to ask all the questions you want before deciding if you want to be in this study.

Why are we doing this research?

The main purpose of this study is to describe the changes that occur to a patient as they transition care to a long-term care facility compared to the microbiome profile present among patient already living within this setting. The microbiome is a collection of the bacteria, fungi, and viruses that normally live in your intestines. Diarrhea is a known complication of many antibiotics that are used to treat bacterial infections. Sometimes this diarrhea is caused by the bacteria *Clostridium difficile*. Additionally some people carry *Clostridium difficile* in their intestines without any diarrhea. *Clostridium difficile* infection is becoming an increasing problem in the United States and people living in long-term care facilities are at the greatest risk of this disease. We are trying to understand how the healthy bacteria that live in our intestines change as someone moves into a long-term care facility and how it relates to *Clostridium difficile* present in their stool. We hope to identify specific factors among this bacterial community in which we can intervene to stop *Clostridium difficile* from either colonizing the intestine or causing diarrheal disease.

How long will the research last?

We expect that you will be in this research study for a total of 90 days.

How many people will be studied?

We expect about 300 people will be in this research study.

What happens if I say yes, I want to be in this research?

If you agree to participate in this research study you will be asked to sign this consent form. After you sign, the research assistant will interview you to review your medical history, allergies, and past experiences on antibiotics. This should take only about 5 minutes. You will then be given information sheets about this study and copies of the signed consent.

As part of this study we will be collecting stool samples at 4 different time points over a total of 90 days. We will ask you to try to produce the first stool sample at your convenience after agreeing to this study. After this there will be 3 other time points occurring monthly in which to collect the additional stool samples, those being at study days 30 (+/- 1), 60 (+/- 1), and 90 (+/- 1) after entering this study. We will also ask that if you experience any diarrheal symptoms that you collect one additional stool sample within this window of diarrhea. With 4 total samples being collected we will use the long-term care facility staff, nurses and aids, to help you collect the samples and will be reminding you of the samples collection the day before a scheduled collection time.

You will be given all the equipment to cleanly collect and store the stool sample. We will ask that upon collection of any stool sample time point you place it into the sample collection cup, seal the cup and place it in the storage bag. You will then need to place this bag and sample into a freezer. The facility and our study staff will help you in this process. We then ask that you or your nurse then call the research pager number at 508-387-1794. A member of the research team will arrange to pickup of the sample in less than 24 hours. We will collect and hold onto your stool samples indefinitely, meaning we do not plan on destroying these samples and will use them in future studies on the bacteria in your gut. No data about your genes will be looked at as part of this study. We are only investigating the genes of the bacteria that live in your intestines.

A research assistant will contact you on sample collection days to remind you to collect the stool sample or they will remind you in person if you are still within the hospital. At the end of the study we will perform a study interview to collect final information about your experience. This will last less than five minutes.

What are the risks of being in this study?

The only risk of being in this study is a loss of your personal information. This is very unlikely to happen, and we will do everything to make sure that your information is protected.

What are my responsibilities if I take part in this research?

If you take part in the research, it is important for your safety that you:

- Follow the directions of the study doctor and research staff.
- Tell your other health care providers that you are in a research study.
- Tell your study doctor and staff about all medications you are taking (prescription and over the counter) and all of your health issues.
- Call the study doctor or staff at (508) 421-5527 if you have any questions.

Will being in this study help me in any way?

There is no direct benefit to you from being in this study. However, your participation may help reduce the spread of *Clostridium difficile* in the future as a result of knowledge gained from the research.

Will being in this study cost me any money?

There will be no additional cost to you from being in this research study. Any costs for the standard treatment of your condition will be billed to you or your health insurance.

What happens to information about me?

Efforts will be made to limit access to your personal information, including research study and medical records, to people who have a need to review this information. We cannot promise complete secrecy. The UMMS Institutional Review Board (the committee that reviews, approves, and monitors research on human subjects) and other representatives of UMMS may need to review records of individual subjects. As a result, they may see your name, but they are bound by rules of confidentiality not to reveal your identity to others. Your identity will remain confidential in any study results that are made public.

What happens if I am injured because I took part in this research?

The University of Massachusetts Medical School does not provide funds for the treatment of research-related injury. If you are injured as a result of your participation in this study, treatment will be provided. You or your insurance carrier will be expected to pay the costs of this treatment. No additional financial compensation for injury or lost wages is available.

You do not give up any of your legal rights by signing this form.

Will I be given any money or other compensation for being in this study?

You will not be compensated for your involvement in this study. We do not have enough funds to make a compensation possible.

What happens if I do not want to be in this research?

If you decide not to take part in the research, it will not affect your usual care and it will not be held against you.

What happens if I say yes, but I change my mind later?

You are free to leave the study at any time. There are no penalties and you do not lose any benefits to which you are otherwise entitled. Data that has already been used will remain part of the study database and may not be removed in order to maintain the integrity of the research. However, any identifiable information will be destroyed so that no one can tell the data belonged to you.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, you can talk to the research team by either paging the research pager at 508-387-1794 and a representative from the research team will be back in contact with you within 24 hours or you may call the primary investigator, John P. Haran, MD, at (508) 421-5527.

This research has been reviewed and approved by an Institutional Review Board. You may talk to them at (508) 856-4261 or irb@umassmed.edu for any of the following:

- Your questions, concerns, or complaints are not being answered by the research team.
- You cannot reach the research team.
- You want to talk to someone besides the research team.
- You have questions about your rights as a research subject.
- You want to get information or provide input about this research.

Signature Block for Capable Adult

Your signature documents your permission to take part in this research.

Signature of subject	Date
Printed name of subject	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	

Signature Block for Adult Unable to Consent

Your signature documents your permission for the named subject to take part in this research.

Printed name of subject	
Signature of legally authorized representative	Date
Printed name of legally authorized representative	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	

Assent

- ❑ Obtained
- ❑ Not obtained because the capability of the subject is so limited that the subject cannot reasonably be consulted.

My signature below documents that the information in the consent document and any other written information was accurately explained to, and apparently understood by, the subject, and that consent was freely given by the subject.

Signature of witness to consent process	Date

Printed name of person witnessing consent process

B. Authorization for Medical Records

Subject Name (print) _____ MR# _____

UMass Memorial Medical Center
AUTHORIZATION TO DISCLOSE
PROTECTED HEALTH INFORMATION FOR RESEARCH PURPOSES

The privacy law, Health Insurance Portability & Accountability Act (HIPAA), protects my individual identifiable health information (Protected Health Information, or PHI). The privacy law requires me to sign an authorization (or agreement) in order for researchers to be able to use or disclose my protected health information for research purposes in the study entitled:
The Elderly Gut Microbiome among Long-Term Care Facility Patients and the Risk of *Clostridium difficile* Carriage and Infection

I authorize UMass Memorial Medical Center to disclose my protected health information to:

- UMass Medical School including the researcher John Patrick Haran, M.D. his research staff
- Federal and State authorities that oversee research
- Sponsor of research, Department of Emergency Medicine

Protected Health Information (PHI) that may be disclosed includes all boxes below marked with an "X", and PHI which is listed in the sections titled "Other" below.

General Records	
☐ Cardiac Studies (Heart)	☒ Laboratory Reports
☒ Consultations	☒ Office/Clinic Notes
☒ Discharge Summaries	☒ Operative/Procedure Reports
☐ EEG/EMG/Sleep Studies	☒ Pathology Reports
☒ Emergency Service Records	☐ Problem List
☒ Home Health Records	☐ Pulmonary Studies (Lung/Respiratory)
☐ Hospice Records	☒ Radiology (X-ray/CAT/MRI/Ultrasound/Nuclear)
☐ Immunization Records	☐ Rehabilitation Notes (PT/OT/Speech)
Other (Specify): Medication Orders, Medication History, Hospitalization Records	
Statutorily Protected Records	
☐ Abortion	☐ Domestic Violence Counseling
☐ Alcohol/Drug Abuse	☐ HIV/AIDS Test Results/Treatment
☐ Psychiatric Health	☐ Sexually Transmitted Diseases
☐ Sexual Assault Counseling	
Other (Specify):	

Subject Name (print) _____ MR# _____

My protected health information will be disclosed as listed above for the following reasons:

- The goal of this research is to see how the intestinal microbiome of patients living in long-term care facilities is affected by the environment of the facility. We are specifically interested in changes that lead to increased risks for *Clostridium difficile* colonization and infection. *Clostridium difficile* diarrhea is a know complication of many antibiotics that are used to treat infections and happens more frequently in patients that live in a long-term care facility setting. How the normal bacterial in your gut changes in response to living in these facilities and any exposure to antibiotics is not well known. The purpose of the disclosure is to be able to conduct this research.

I do not have to sign this Authorization. If I decide not to sign the Authorization:

- It will not affect my treatment, payment or enrollment in any health plans, or affect my eligibility for benefits.
- I will not be allowed to participate in the research study.

If I sign the Authorization, I understand that:

- I have the right to withdraw, or revoke the Authorization.
- If I revoke the Authorization, I will send a **written** letter to: **John P. Haran, M.D., Department of Emergency Medicine, 55 Lake Avenue North, Worcester, MA 01655** to inform him of my decision.
- If I revoke this Authorization, researchers may only use the protected health information **already** collected for this research study.
- If I revoke this Authorization my protected health information may still be used and disclosed should I have an adverse event (a bad effect).
- If I change my mind and withdraw the Authorization, I will not be allowed to continue to participate in the study.
- Any disclosure carries the potential for re-disclosure. Once UMass Memorial Medical Center releases my protected health information, it may no longer be protected by the HIPAA privacy rule.
- The entities receiving my protected health information will use it as described in the Consent Document for this study.
- I may not be allowed to review some of the research-related information in my medical record until after the study is completed. When the study is over, I will have the right to access the information again.
- I will receive a signed copy of this authorization for my personal records.

This Authorization does not have an expiration date.

If I have questions about the research study, I should contact: **John P. Haran, M.D. at (508) 421-5527.**

Subject Name (print) _____ MR# _____

If I have not already received a copy of the Privacy Notice, I may request one. If I have any questions or concerns about my privacy rights, I should contact the UMass Memorial Medical Center Privacy Officer at the phone number 508-334-5551.

I HAVE READ AND UNDERSTAND THE ABOVE STATEMENTS AND AUTHORIZE THE DISCLOSURE OF THE INFORMATION REQUESTED ABOVE

Signature of Subject	Date
Subject Name (Printed	

Use boxes below if parent or legal representative is signing for research subject

Subject's Legal Representative Signature	Relationship	Date

Print Name of Legal Representative

Please explain Representative Relationship to Subject and include a description of Representative's Authority to act on behalf of Subject:

_____

_____

_____

Person obtaining HIPAA Authorization	Date

NOTE TO PI:

Forward the original signed authorization to:

Health Information Management – Room HB 354
UMass Memorial Medical Center
55 Lake Avenue North
Worcester, MA 01655

Give a copy of the signed authorization to the research subject, and keep a copy for the study files.

C. Study Enrollment Data

Study Identification Number _____

Cover Sheet for Resident Enrollment

1. How many months/years have you been at this facility? _____

2. Do you have a roommate? Y/N

a. If so how long have you been roommates?_____

b. Is the roommate enrolled? Y/N and if so roommates ID number_____

3. What floor is the resident on and what is their room number? Floor_____Room#_____

4. Over the past year when was the patient placed on antibiotics

Date	Reason/Infection	Antibiotic Name/s	Duration

5. Over the past year when was the resident hospitalized

Date	Reason	Antibiotics Given	Duration

D. Comprehensive Assessment

Patient Study Identification Number _____ Date _____

Long-Term Care Comprehensive Geriatric Assessment Form

WNL = Within Normal Limits ASST = Assisted
IND = Independent DEP = Dependent

Cr Cl: _____

Chief lifelong occupation:_____ Education: (years)_____

Infection Control
- ☐ MRSA
- ☐ VRE
- ☐ Flu shot given
- ☐ Pneumococcal vaccine given
- ☐ TB test done
- ☐ Tetanus/Diphteria

Cognitive Status
- ☐ WNL
- ☐ Dementia
- ☐ Other

MMSE _____

Emotional
- ☐ WNL
- ☐ Depression
- ☐ Other
- ☐ ↓Mood
- ☐ Anxiety
- ☐ Hall/Del

Behaviours
- ☐ Verbal Non-aggressive
- ☐ Verbal Aggressive
- ☐ Physical Non-aggressive
- ☐ Physical Aggressive

Communication:

Speech
- ☐ WNL
- ☐ Impaired

Hearing
- ☐ WNL
- ☐ Impaired

Vision
- ☐ WNL
- ☐ Impaired

Strength
- ☐ WNL ☐ Weak

Upper: PROXIMAL DISTAL R L
Lower: PROXIMAL DISTAL R L

Footcare needed ☐ Y ☐ N

Dental care needed ☐ Y ☐ N

Skin Integrity Issues ☐ Y ☐ N

Legal NoK:_____

Advanced Directives ☐ Y ☐ N

Code Status
- ☐ Do Not Attempt to Resuscitate
- ☐ Do Not Hospitalize
- ☐ Resuscitate

Mobility	Transfers	IND ASST Dep
	Walking Aid	IND Slow ASST Dep
Balance	Balance	WNL Impaired
	Falls	N Y Frequency
Elimination	Bowel	CONSTIP CONT INCONT
	Bladder	CATHETER CONT INCONT
Nutrition	Weight	STABLE LOSS GAIN
	Appetite	WNL FAIR POOR
ADLs	Feeding	IND ASST Dep
	Bathing	IND ASST Dep
	Dressing	IND ASST Dep
	Toileting	IND ASST Dep

Marital Status
- ☐ Married
- ☐ Divorced
- ☐ Widowed
- ☐ Single

Family Stress
- ☐ None
- ☐ Low
- ☐ Moderate
- ☐ High

Problems/Past history	Med adjust req. Associated Medication
1. _____	❍ _____
2. _____	❍ _____
3. _____	❍ _____
4. _____	❍ _____
5. _____	❍ _____
6. _____	❍ _____
7. _____	❍ _____
8. _____	❍ _____
9. _____	❍ _____
10. _____	❍ _____
11. _____	❍ _____
12. _____	❍ _____
13. _____	❍ _____
14. _____	❍ _____
15. _____	❍ _____

Current Frailty Score

Scale

1. Mildly frail ☐ 2. Moderately frail ☐ 3. Severely frail ☐ 4. Very severely ill ☐ 5. Terminally ill ☐

Research Assistant Name_____

Research Assistant Signature_____

E. Brief Interview for Mental Status (BIMS) Mental Status

Resident Name _____ Identification # _____ Date _____

Brief Interview for Mental Status (BIMS)

Repetition of Three Words

Ask resident: *"I am going to say three words for you to remember. Please repeat the words after I have said all three. The words are:* ***sock, blue*** *and* ***bed****. Now tell me the three words."*

Number of words repeated after first attempt:

☐ **0. None** ☐ **1. One** ☐ **2. Two** ☐ **3. Three**

After the resident's first attempt, repeat the words using cues *("sock, something to wear; blue, a color; bed, a piece of furniture")*. You may repeat the words up to two more times.

Temporal Orientation (orientation to month, year and day)

Ask resident: *"Please tell me what year it is right now."*

Able to report correct year

- ☐ **0. Missed by > 5 years**, or no answer
- ☐ **1. Missed by 2-5 years**
- ☐ **2. Missed by 1 year**
- ☐ **3. Correct**

Ask resident: *"What month are we in right now?"*

Able to report correct month

- ☐ **0. Missed by > 1 month**, or no answer
- ☐ **1. Missed by 6 days to one month**
- ☐ **2. Accurate within 5 days**

Ask resident: *"What day of the week is today?"*

Able to report correct day of the week

- ☐ **0. Incorrect**, or no answer
- ☐ **1. Correct**

Recall

Ask resident: *"Let's go back to the earlier question. What were the three words that I asked you to repeat?"* If unable to remember a word, give cue *("something to wear," "a color," "a piece of furniture")* for that word.

Able to recall "sock"	☐ **0. No - could not recall**	☐ **1. Yes, after cueing ("something to wear")**	☐ **2. Yes, no cue required**
Able to recall "blue"	☐ **0. No - could not recall**	☐ **1. Yes, after cueing ("a color")**	☐ **2. Yes, no cue required**
Able to recall "bed"	☐ **0. No - could not recall**	☐ **1. Yes, after cueing ("a piece of furniture")**	☐ **2. Yes, no cue required**

Summary Score

Add scores for each question and fill in total score (00-15).
Enter 99 if the resident was unable to complete the interview. _____

Abbreviated Instructions for Conducting the BIMS

Intent: To determine the individual's attention, orientation and ability to register and recall new information.

Please note: For more in-depth instructions for completing the BIMS, please refer to Chapter 3: MDS Items Section C: Cognitive Patterns.

SHOULD THE BRIEF INTERVIEW FOR MENTAL STATUS BE CONDUCTED?

- The interview should be attempted if the individual is at least sometimes understood verbally or in writing, and if an interpreter is needed and one is available.
- The interview should not be attempted if the individual is rarely/never understood or an interpreter is needed but not available.

BASIC INTERVIEW INSTRUCTIONS FOR BIMS:

1. Interview any individual not excluded as indicated above.
2. Conduct the interview in a private setting if at all possible.
3. Be sure the individual can hear you. An individual with a hearing impairment should be tested using their usual communication devices/techniques as applicable.
4. Sit so that the individual can see your face.
5. Give an introduction.
   Suggested language: *"I would like to ask you some questions. We ask everyone these same questions. This will help us to provide you with better recommendations. Some of the questions may seem very easy, while others may be more difficult."*
6. If the individual expresses concern that you are testing his or her memory, he or she may be more comfortable if you reply: *"We ask these questions of everyone so we can make sure that we can meet your needs."*

Coding Tips:

- Nonsensical responses should be coded as zero.[1] A nonsensical response is any response that is unrelated, incomprehensible, or incoherent; and is not informative with respect to the item being rated.
- Refusal to answer a specific question is coded as "0."

Use Code 99 if:

- the individual chooses not to participate, or
- 4 or more items were coded 0 because the individual chose not to answer or gave a nonsensical response.

[1] Nonsensical response means any response that is unrelated, incomprehensible, or incoherent; it is not informative with respect to the item being rated.

Repetition of Three Words

This section determines if the individual is able to actively engage in a verbal interaction. Inability to repeat three words on the first attempt may indicate a hearing impairment, a language barrier or inattention.

Interview Instructions:

1. Say to the individual; *"I am going to say three words for you to remember. Please repeat the words after I have said all three."*
2. Immediately after presenting the three words, say to the individual: *"Now tell me the three words."*

Repetition of Three Words
Ask the individual: *"I am going to say three words for you to remember. Please repeat the words after I have said all three. The words are: sock, blue and bed. Now tell me the three words."* **Number of words repeated after first attempt** **0. None** **1. One** **2. Two** **3. Three** After the individual's first attempt, repeat the words using cues *("sock, something to wear; blue a color; bed, a piece of furniture")*. You may repeat the words up to two more times.

Coding Instructions:

1. Record the maximum number of words that the individual correctly repeated on the first attempt. This will be any number between 0 and 3.
2. The words may be recalled in any order and in any context.

Important: After scoring, and in preparation for **Recall,** repeat the three words, this time using category cues: "*sock, something to wear; blue, a color; bed; a piece of furniture*."[2] You may repeat these words and the corresponding cues up to two more times.

[2] Category cue means a phrase that puts a word in context to help with learning and to serve as a hint that helps prompt the individual. For example, the category cue for sock is "something to wear."

Temporal Orientation (Orientation to Year, Month and Day)

Temporal orientation means the ability to place oneself in correct time.[3] For the BIMS, it is the ability to indicate the correct date in current surroundings.

Interview Instructions:

1. Ask the individual each of the 3 questions separately.
2. Allow the individual up to 30 seconds for each answer and do not provide clues.
3. If the individual specifically asks for clues (e.g. *"is it bingo day?"*) respond by saying, *"I need to know if you can answer this question without any help from me."*
4. In some cases, you may need to write the individual's response in the margin and go back later to count how many years, months or days were missed. Do your best to keep focused on the interaction with the individual, not adding or subtracting.

Temporal Orientation (orientation to year, month, and day)
Ask the individual: *"Please tell me what year it is right now."* **A. Able to report correct year** **0. Missed by more than 5 years or no answer.** **1. Missed by 2-5 years** **2. Missed by 1 year** **3. Correct**
Ask the individual: *"What month are we in right now?"* **B. Able to report correct month** **0. Missed by more than 1 month or no answer.** **1. Missed by 6 days to 1 month** **2. Accurate within 5 days.**
Ask the individual: *"What day of the week is today?* **C. Able to report correct day of the week** **0. Incorrect or no answer** **2. Correct**

Coding Instructions:

Code as indicated in the corresponding boxes.

[3] Temporal orientation means the ability to place oneself in correct time. For the BIMS it is the ability to indicate the correct date in current surroundings.

Recall

Individuals with cognitive impairment can be helped to recall if provided clues. Providing memory cues can help maximize the individual's function and decrease frustrations for those individuals who respond.

Interview Instructions:

1. Ask the individual the following: *"Let's get back to an earlier question. What were those three words that I asked you to repeat?"*
2. Allow up to 5 seconds for spontaneous recall of each word.
3. For any word that is not correctly recalled after 5 seconds, provide a category cue (*something to wear, a color, a piece of furniture*). Give a cue for each word separately.
4. Category cues should be used only after the individual is unable to recall one or more of the three words.
5. Allow up to 5 seconds after category cueing for each missed word to be recalled.

Recall
Ask individual: *"Let's go back to an earlier question: What were those three words that I asked you to repeat?"* If unable to remember a word, give cue (*something to wear; a color; a piece of furniture*) for that word.
A. Able to recall "sock" 0. **No** – could not recall 1. **Yes, after cueing** ("*something to wear*") **2. Yes, no cue required**
B. Able to recall "blue" 0. **No** – could not recall 1. **Yes, after cueing** ("*a color*") **2. Yes, no cue required**
C. Able to recall "bed" 0. **No** – could not recall 1. **Yes, after cueing** ("*a piece of furniture*") **2. Yes, no cue required**

Coding Instructions:

1. Code as indicated in the corresponding boxes.
2. If on the first try (without cueing), the individual names multiple items in a category, one of which is correct, they should be coded as correct for that item
3. If however, the interviewer gives the individual the cue and the individual then names multiple items in that category, the item is coded as *"could not recall,"* even if the correct item was in the list.

TOTAL SCORE

Enter the total score as a two-digit number. The total possible BIMS score ranges from 00 to 15.

13 – 15: cognitively intact
08 - 12: moderately impaired
00 – 07: severe impairment

F. Mini Mental State Examination

Mini-Mental State Examination (MMSE)

Patient's Name: _____ Date: _____

***Instructions:* Ask the questions in the order listed. Score one point for each correct response within each question or activity.**

Maximum Score	Patient's Score	Questions
5		"What is the year? Season? Date? Day of the week? Month?"
5		"Where are we now: State? County? Town/city? Hospital? Floor?"
3		The examiner names three unrelated objects clearly and slowly, then asks the patient to name all three of them. The patient's response is used for scoring. The examiner repeats them until patient learns all of them, if possible. Number of trials: _____
5		"I would like you to count backward from 100 by sevens." (93, 86, 79, 72, 65,) Stop after five answers. Alternative: "Spell WORLD backwards." (D-L-R-O-W)
3		"Earlier I told you the names of three things. Can you tell me what those were?"
2		Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them.
1		"Repeat the phrase: 'No ifs, ands, or buts.'"
3		"Take the paper in your right hand, fold it in half, and put it on the floor." (The examiner gives the patient a piece of blank paper.)
1		"Please read this and do what it says." (Written instruction is "Close your eyes.")
1		"Make up and write a sentence about anything." (This sentence must contain a noun and a verb.)
1		"Please copy this picture." (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.) 
30		TOTAL

(Adapted from Rovner & Folstein, 1987)

G. Scoring and Interpreting the Mini-Mental State Examination

Instructions for administration and scoring of the MMSE

Orientation (10 points):

- Ask for the date. Then specifically ask for parts omitted (e.g., "Can you also tell me what season it is?"). One point for each correct answer.
- Ask in turn, "Can you tell me the name of this hospital (town, county, etc.)?" One point for each correct answer.

Registration (3 points):

- Say the names of three unrelated objects clearly and slowly, allowing approximately one second for each. After you have said all three, ask the patient to repeat them. The number of objects the patient names correctly upon the first repetition determines the score (0-3). If the patient does not repeat all three objects the first time, continue saying the names until the patient is able to repeat all three items, up to six trials. Record the number of trials it takes for the patient to learn the words. If the patient does not eventually learn all three, recall cannot be meaningfully tested.
- After completing this task, tell the patient, "Try to remember the words, as I will ask for them in a little while."

Attention and Calculation (5 points):

- Ask the patient to begin with 100 and count backward by sevens. Stop after five subtractions (93, 86, 79, 72, 65). Score the total number of correct answers.
- If the patient cannot or will not perform the subtraction task, ask the patient to spell the word "world" backwards. The score is the number of letters in correct order (e.g., dlrow=5, dlorw=3).

Recall (3 points):

- Ask the patient if he or she can recall the three words you previously asked him or her to remember. Score the total number of correct answers (0-3).

Language and Praxis (9 points):

- Naming: Show the patient a wrist watch and ask the patient what it is. Repeat with a pencil. Score one point for each correct naming (0-2).
- Repetition: Ask the patient to repeat the sentence after you ("No ifs, ands, or buts."). Allow only one trial. Score 0 or 1.
- 3-Stage Command: Give the patient a piece of blank paper and say, "Take this paper in your right hand, fold it in half, and put it on the floor." Score one point for each part of the command correctly executed.
- Reading: On a blank piece of paper print the sentence, "Close your eyes," in letters large enough for the patient to see clearly. Ask the patient to read the sentence and do what it says. Score one point only if the patient actually closes his or her eyes. This is not a test of memory, so you may prompt the patient to "do what it says" after the patient reads the sentence.
- Writing: Give the patient a blank piece of paper and ask him or her to write a sentence for you. Do not dictate a sentence; it should be written spontaneously. The sentence must contain a subject and a verb and make sense. Correct grammar and punctuation are not necessary.
- Copying: Show the patient the picture of two intersecting pentagons and ask the patient to copy the figure exactly as it is. All ten angles must be present and two must intersect to score one point. Ignore tremor and rotation.

(Folstein, Folstein & McHugh, 1975)

Source: www.medicine.uiowa.edu/igec/tools/cognitive/MMSE.pdf

Provided by NHCQF, 0106-410

H. Clinical Dementia Rating

CDR™ Scoring Table

Subject Initials _____

CLINICAL DEMENTIA RATING (CDR™):	0	0.5	1	2	3

	Impairment				
	None 0	Questionable 0.5	Mild 1	Moderate 2	Severe 3
Memory	No memory loss or slight inconsistent forgetfulness	Consistent slight forgetfulness; partial recollection of events; "benign" forgetfulness	Moderate memory loss; more marked for recent events; defect interferes with everyday activities	Severe memory loss; only highly learned material retained; new material rapidly lost	Severe memory loss; only fragments remain
Orientation	Fully oriented	Fully oriented except for slight difficulty with time relationships	Moderate difficulty with time relationships; oriented for place at examination; may have geographic disorientation elsewhere	Severe difficulty with time relationships; usually disoriented to time, often to place	Oriented to person only
Judgment & Problem Solving	Solves everyday problems & handles business & financial affairs well; judgment good in relation to past performance	Slight impairment in solving problems, similarities, and differences	Moderate difficulty in handling problems, similarities, and differences; social judgment usually maintained	Severely impaired in handling problems, similarities, and differences; social judgment usually impaired	Unable to make judgments or solve problems
Community Affairs	Independent function at usual level in job, shopping, volunteer and social groups	Slight impairment in these activities	Unable to function independently at these activities although may still be engaged in some; appears normal to casual inspection	No pretense of independent function outside home Appears well enough to be taken to functions outside a family home	Appears too ill to be taken to functions outside a family home
Home and Hobbies	Life at home, hobbies, and intellectual interests well maintained	Life at home, hobbies, and intellectual interests slightly impaired	Mild but definite impairment of function at home; more difficult chores abandoned; more complicated hobbies and interests abandoned	Only simple chores preserved; very restricted interests, poorly maintained	No significant function in home
Personal Care	Fully capable of self-care		Needs prompting	Requires assistance in dressing, hygiene, keeping of personal effects	Requires much help with personal care; frequent incontinence

Score only as decline from previous usual level due to cognitive loss, not impairment due to other factors.

f:\institut\cultadap\project\2925\etude2925\final-versions\cdr\cdroriq.doc-30/05/2006

I. NIH Toolbox Pattern Sequence Memory Test

C.2.6.4 Picture Sequence Memory Test Instructions Age 8+

If the participant has difficulty dragging the pictures across the screen, during the demonstration sequence, practice sequences, or the actual test sequences, he/she may point and the examiner will move the pictures in the way the participant indicates. The examiner should ensure the participant completely understands how the process will work and can **say something like the following:** You point to the picture you want to move, then point to the gray box where you want to move it. I'll move it there for you.

The test begins with a Title Screen: NIH Toolbox PSM 8+ Form A, B or C. At the bottom of the screen is a button ***Touch and Hold Here to Continue***. Touch and hold that button to begin the test.

On the next screen, read the text: **Now we are going to play a memory game, but first I am going to teach you how to move the pictures on this screen.**

When finished reading, touch the button at the bottom of the screen that says ***Touch and Hold Here to Continue***.

The next screen begins the three-step demonstration sequence. If the steps are not followed, the Touch and Hold Here to Continue button will not be active.

Step 1: Moving Pictures from the Yellow Box to the Gray Boxes
Read the text above the yellow box on the screen and demonstrate the actions described.
Say: **Here you see some pictures in a yellow box. I want to show you how to move the pictures to the gray boxes. You can move pictures from this yellow box to the gray boxes, like this. Touch on the picture in the yellow box; then drag the picture you touched to the gray box where you want to move it. Now you try with this picture.**

After the participant demonstrates understanding of this step, touch the button at the bottom of the screen that says ***Touch and Hold Here to Continue***.

Step 2: Moving Pictures from the Gray Boxes to the Yellow Box
Read the text above the yellow box on the screen and demonstrate the actions described.

Say: **You can also move pictures from the gray boxes back to the yellow box, like this. Touch the picture you want to move. Then drag that picture to the yellow box. Now you move the other picture back to the yellow box.**

After the participant demonstrates understanding of this step, touch the button at the bottom of the screen that says ***Touch and Hold Here to Continue***.

Step 3: Moving Pictures between the Gray Boxes

Read the text above the yellow box on the screen and demonstrate the actions described.

Say: **You can also move the pictures from one gray box to another, like this. First, you touch the picture in one gray box. Then, drag that picture to the gray box where you want the picture to go. Now you try it; move the picture from here to here.**

After the participant demonstrates understanding of this step, touch the button at the bottom of the screen that says ***Touch and Hold Here to Continue***.

Introduction to the Practice Items

When finished reading, touch the button at the bottom of the screen that says ***Touch and Hold Here to Continue***.

Test Item (Trial1): As the pictures are presented, an audio recording will say appropriate labels:

Form A: Play in the park	Form B: Go to the fair	Form C: Work on the farm
1. Fly a kite	• Get a hot dog	• Put the cow in the barn
2. Play in the sand	• Go for a pony ride	• Drive the tractor
3. Go down the slide	• Pet the sheep	• Feed the pig
4. Pull the wagon	• Win a dog show	• Load the cart
5. Swing on the monkey bars	• Watch the rodeo	• Put the carrots in the basket
6. Lay out the blanket	• Catch a pig	• Pick the fruit
7. Play catch	• Judge the cake	• Fix the fence
8. Catch a butterfly	• See a puppet theater	• Cut the sheep's wool
9. Kick the ball	• Take a picture of a clown	• Collect the honey
10. Take the baby for a walk	• Look at the babies	• Shovel out the barn
11. Draw on the sidewalk	• Go on a ride	• Peel the corn
12. Pet the dog	• Get some tickets	• Chop some wood
13. Feed the ducks	• Get some ice cream	• Collect the eggs
14. Push the swing	• Play a game	• Put the blanket on the horse
15. Smell a flower	• Milk a cow	• Plant the tomatoes

After the pictures are presented and as the pictures are scrambled, an audio recording will say: **That's how to Play in the Park** (Form A); **Go to the Fair** (Form B); **Work on the Farm** (Form C). **Now, you move the pictures in the same way you saw them on the screen.**

When all pictures have been placed in gray boxes, a pop-up will appear on the screen, **"Are you finished?"** Ask this of the participant. If participant says YES, touch and hold the YES button. If the participant says NO, touch any of the pictures and the pop-up will disappear. If no picture is moved out of a gray box, the pop-up will continue to be on the screen. The pop-up will close if a picture is moved back to the yellow box and will reappear each time all pictures have been placed in gray boxes.

Read the screen: **Now we're going to do the same pictures with some more pictures added. Everyone is asked to do this more than once.** When finished reading, touch the button at the bottom of the screen that says ***Touch and Hold Here to Continue.***

J. NIH Toolbox Picture Vocabulary Test

C.2.1.1 NIH Toolbox Picture Vocabulary Test Instructions Age 3+

The task is introduced with the Title Screen: NIH Toolbox PVT 3+. At the bottom of the screen is a button ***Touch and Hold Here to Continue***. Touch and hold that button to begin the test.

The next screen is slightly different for participants ages 3-6 and those ages 7+. The two screens are printed below.

Ages 3-6:

Say: **Let's look at some pictures. You'll hear a word and see four pictures on the screen. Touch the picture that means the same as the word that was said. Some words will be easy and some will be harder. Answer as best as you can. If you need to hear a word again, I can play it again.**

If you think you made a mistake and want to change your last answer, I can bring back that word and pictures. Are you ready to play?

When participant is ready, touch button that says ***Touch and Hold Here to Continue***.

Ages 7+:

Say: **You are going to be asked the meaning of some words. For each item, you will hear a word and see four pictures on the screen. Touch the picture that you think best matches the meaning of the word that was said. If you are not sure, make your best guess. If you need to hear the word again, touch the button that has a picture of an EAR, also called the PLAY AGAIN button. After you touch a picture, you will hear a new word and see more pictures.**

You will keep hearing words and touching pictures until you are done. If you want to change your last answer, touch the button with the HAND that says GO BACK. The pictures you just saw will reappear and you will hear the word again. Touch your choice, and then more words and pictures will appear. Tell me when you are ready to start.

When participant is ready, touch button that says ***Touch and Hold Here to Continue***.

Practice item 1:

An audio recording says: **Let's try one for practice: Banana. Touch the picture of Banana.**

If correct, the audio recording says: **That's right!** The program will automatically go to the next practice item.

If incorrect, the Banana picture will light up and an audio recording will say: **This is a banana. Let's try again.**

The examiner should allow the participant up to three chances to answer this practice item correctly.

After three unsuccessful attempts by the participant, the examiner should touch the picture of the banana and say: **This is a banana.** The next practice item will appear automatically.

Practice item 2:

An audio recording says: **Let's try another one: Spoon. Touch the picture of Spoon.**

If correct, an audio recording says: **That's right!**

If incorrect, the Spoon picture will light up and an audio recording will say: **This is a spoon. Let's try again.**

The examiner should allow the participant up to three chances to answer this practice item correctly.

After three unsuccessful attempts by the participant, the examiner should touch the picture of the spoon and say: **This is a spoon.**

The introduction to the test items will appear automatically.

Test items

The introductory screen to the test items is slightly different for participants ages 3-6 and those ages 7+. The two screens are printed below.

Ages 3-6:

Read: **Now, let's try some more. Remember, you will hear a voice say a word and then you will see four pictures. One of the pictures will show what the word means. Touch that picture and you will hear a new word and see four more pictures. Any questions?**

When participant is ready, touch button that says ***Touch and Hold Here to Continue***.

Ages 7+:

Read: **Now, you're going to do some more. Remember, you will hear a word and then you will see four pictures. One of the pictures will show what the word means. Touch that picture and you will hear a new word and see four more pictures. Again, touch the picture that shows what the word means. If you don't know, make your best guess. Any questions?**

When participant is ready, touch button that says ***Touch and Hold Here to Continue***.

An audio recording will introduce each word with the instructions: **Touch** (for participants ages 3-6) and just the **Word** (for participants ages 7+). The items will continue in this format until the test is completed.

> Remember: If a participant has difficulty touching the screen, he/she may point and the examiner can touch the screen. In this case, the examiner should say something like the following to the participant: **You can point to your choice and then I will touch the screen for you**. If the participant says that he/she does not understand the word after several repetitions, the examiner may say the word ONCE MORE.

K. NIH Toolbox Pattern Comparison Speed Processing Test

C.2.5.2 Pattern Comparison Processing Speed Test Instructions Ages 7+

The task is introduced with the Title Screen: NIH Toolbox PC 7+. At the bottom of the screen is a button ***Touch and Hold Here to Continue***. Touch and hold that button to begin the test.

Handedness: If this was not entered during registration, the iPad app will not allow this test to be administered. Instead, the examiner will need to ask a set of questions to ascertain handedness and to enter it in the participant registration screen. Please refer to Appendix 2 for details.

Once handedness is entered in the participant registration screen, it will automatically update the instructions on the test so that "RIGHT" or "LEFT" is displayed where appropriate

An audio recording says:

On each screen, you are going to see two pictures. Sometimes the two pictures are the same and sometimes the two pictures are NOT the same. If the pictures look the same, touch the 'Yes' button. If they do NOT look the same, touch the NO button. If you make a mistake during the practice, you will hear a message to try again. Use your _____ (dominant hand from handedness, audio will say "right" or "left") index finger to respond. Let's try some for practice.

When the audio recording is complete, **Touch and hold here to continue** appears on the screen. Touch and hold that button to begin the administration.

Practice Items:

There are 6 practice items. The first practice item is introduced with an audio recording asking: **Are these the same? Remember, touch YES if they look alike or are the same and touch NO if they do not look alike and are NOT the same.**

For the other five practice items, an audio recording asks: **Are these the same?**

If the participant answers correctly, an audio recording says: ***That's right!*** The next item will appear.

If the participant answers incorrectly, an audio recording explains why the answer is wrong. The correct answer will flash; item will stay on screen. If participant chooses correctly, an audio recording will say: ***That's right!*** and the next item will appear.

If the participant chooses incorrectly another time, the program automatically moves to the next practice item after repeating the explanation above.

Practice item	Correct response	Incorrect response
1	Yes: both yellow flowers	No
2	No: a flower with stem & top of a flower	Yes
3	No: one present & many presents	Yes
4	Yes: both single presents	No
5	No: purple flower & red flower	Yes
6	Yes: both red flowers	No

NOTE: If a participant makes an error a second time after being corrected on two different practice items, the test will discontinue.

Test Items:

L. NIH Toolbox List Sorting Working Memory Test

C.2.3 NIH Toolbox List Sorting Working Memory Test

This task assesses working memory and requires the participant to recall and sequence different visually and orally presented stimuli. Pictures of different foods and animals are displayed with both an accompanying audio recording and written text that name the item. The participant is asked to say the items back to the examiner in size order from smallest to largest.

Equipment and materials needed:

- iPad
- Bluetooth wireless keyboard
- NIH Toolbox List Sorting Working Memory Examiner Answer Sheet

Detailed information on equipment and materials needed for all tests can be found in Appendix 4.

Setting up Bluetooth Wireless Keyboard:

- Go to the Settings app of the iPad and make sure Bluetooth is turned on.
- Make sure the keyboard is in discovery mode by pressing the round button at the top right end of the keyboard and the small green light to the left of the button begins flashing.
- Select the Keyboard when it appears on the My Devices list on the iPad.
- When a pop up window appears on the iPad screen, follow the instructions and enter the required code on the keyboard.

  The usual keyboard on the iPad will not work when the external keyboard is working. To turn off the external keyboard, press the round button at the top right end of the keyboard, move at least 15 meters from the iPad with the keyboard, or touch the "information" button ⓘ on the iPad and touch "forget this device."

Using the Keyboard

Once the participant answers (gives the list of objects), the examiner types **1** if the response (list) is correct or **0** if the response (list) is incorrect.

For the practice items, after choosing **1** or **0**, the examiner presses the spacebar to move on to the next item (list of objects). There is feedback presented by the prerecorded voice and repeated trials for errors.

For the test items, after the examiner chooses **1** or **0**, the question: **Are you ready?** appears on the screen. The examiner presses the spacebar to move on to the next item (list of objects) after the participant answers the question affirmatively. The examiner can change an entered response (1 or 0) prior to pressing the spacebar. Once the spacebar is pressed, the examiner can not go back to the previous item.

Ctrl + Shift on the Keyboard is equivalent to the gesture used to STOP an administration on the iPad. Like the iPad, when Ctrl + Shift is entered on the keyboard, a login screen will pop up and ask for the password of the user who started the assessment. As described earlier, three options will appear: resume, skip, and stop.

There are two different conditions: 1-List and 2-List.

In the 1-List condition, participants are required to order a series of objects (*either* food or animals) in size order from smallest to largest. The test begins with a two object item. The participant must succeed at one of the first two object items to continue. (See the discontinue rules below).

In the 2-List condition, participants are presented *both* food and animals and are first asked to say the food objects in size order, and then the animal objects in size order.

In each condition, participants ages 7-85 have two practice items; both items are presented in a "flashing" mode.

Because the participant answers orally, this measure cannot be self-administered. An examiner must record on a wireless keyboard whether each response is correct; specifically, after the participant answers (gives the list of objects), the examiner types **1** if the response (list) is correct or **0** if the response (list) is incorrect.

For the practice items, after choosing **1** or **0**, the examiner presses the **spacebar** to move on to the next item (list of objects). There is feedback presented by a prerecorded voice and there are repeated trials for errors.

For the test items, after the examiner chooses **1** or **0**, the question: **Are you ready?** appears on the screen. Once confirming that the participant is ready and attending, the examiner presses the **spacebar** to move on to the next item (list of objects).

For the examiner, there is a code at the bottom right side of the iPad screen to indicate which item is being administered. The examiner should use this code to look up the correct response on the NIH Toolbox List Sorting Working Memory Examiner Answer Sheet, and then score the item using the keyboard (**1** or **0** and pressing the spacebar).

Introduction:

For an item to be marked correct, the participant must name all the stimuli in the correct order without any intrusions. A participant may change his/her response. It is also acceptable for a participant to give a synonym for either a food item or an animal. For example, in English, responses such as these should be marked as correct: puppy for dog, bunny for rabbit, and lamb for sheep.

Several discontinue rules are embedded in the software:

- If a participant does not complete a practice item correctly, the test itself is not administered.
- If a participant has incorrect responses on both parts of a test item, the test condition being administered (1-List or 2-List) is discontinued.
- If the participant has a score of 1 point as the sum of the two sets of 2-stimulus items (*pig-mouse* and *bird-cow*; *banana-watermelon* and *apple-blueberry*) *and* a score of 0 points on the set of 3-stimulus items (*pumpkin-strawberry-banana* and *dog-horse*-rabbit) in the 1-list condition, the test is discontinued and the 2-list condition is *not* administered.
- If the participant has a score of 0 points as the sum of the two sets of 2-stimulus items (*pig-mouse* and *bird-cow*; *banana-watermelon* and *apple-blueberry*), the test is discontinued and the 2-list condition is *not* administered.

Throughout the test, the examiner may need to remind the participant: **It is important to pay attention to the size of the objects on the screen** (not what they may have experienced) **when putting things in size order from smallest to biggest.**
If necessary, examiners can provide short breaks between items. It is also acceptable during the 2-List condition to remind participants of the rules (e.g., tell me the foods first, then the animals).

C.2.3.2 NIH Toolbox List Sorting Working Memory Test Instructions Age 7+

Before beginning the test, the wireless keyboard should be paired by Bluetooth with the iPad, if this has not already been done. While administering this measure, the examiner needs to be able to view the iPad and have the keyboard and the NIH Toolbox List Sorting Working Memory Test Examiner Answer Sheet easily accessible for scoring participant responses.

The task is introduced with the Title Screen: NIH Toolbox LS 7+. To check the Bluetooth connection, the examiner is asked at the bottom of the page to: ***Press Spacebar to Continue.*** Touching the **spacebar** begins the test. If the spacebar does not work, try repairing the keyboard or changing the batteries in the keyboard.

> **REVIEW:** All the 1-List practice items have the same format. First, the examiner asks for the names of the pictures in size order from smallest to largest. If the participant answers incorrectly, there are two more opportunities to get a correct answer. The first comes after a verbal explanation and a repeat of the question; the second comes after replaying the item on the screen, followed by a verbal explanation and then repeating the question. If, after three opportunities, the participant continues to answer incorrectly, the test will be discontinued and no more items will be presented. Both practice items are detailed below.

1-List Condition

Read the screen: **You are going to see some pictures one at a time on the screen. After each set of pictures, you will see a blank screen. When you see the blank screen, I want you to tell me the names of the pictures in size order from smallest to biggest. For example, if I show you a motorcycle, a bus, and a car, you would say: motorcycle, car, bus.**

From now on, you will hear most of the instructions from the iPad. Do you have any questions?

Let's practice. Press **Spacebar** to continue.

1-List Practice Item 1:

Trial 1: After the pictures are presented and the screen is blank, an audio recording says: **Now say the animals in size order.**

Correct Response: If participant says DOG, HORSE, press **1** and then press **spacebar**. An audio recording says: **That's right.** When it appears on the screen, ask: **Are you ready?** When the participant answers affirmatively, press the **spacebar** and 1-List Practice Item 2 will be presented.

Incorrect Response: If participant does not say DOG, HORSE, press **0** and then press **spacebar.** An audio recording says: **Let's try that again.**

Trial 2 (if Trial 1 was incorrect): An audio recording continues: **You saw a DOG and a HORSE; the DOG is smaller than the HORSE. Now say the animals in size order.**

Correct Response: If participant says DOG, HORSE, press **1** and then press **spacebar**. An audio recording says: **That's right.** When it appears on the screen, ask: **Are you ready?** When the participant answers affirmatively, press the **spacebar** and 1-List Practice Item 2 will be presented.

Incorrect Response: If participant does not say DOG, HORSE, press **0** and then press **spacebar**. An audio recording says: **Let's try that one more time.**

Trial 3 (if Trial 2 was incorrect): After the pictures are presented one more time and the screen is blank, an audio recording says: **You saw a DOG and a HORSE; the DOG is smaller than the HORSE. Now say the smaller animal and then the bigger animal.**

Correct Response: If participant says DOG, HORSE, press **1** and then press **spacebar**. An audio recording says: **That's right.** When it appears on the screen, ask: **Are you ready?** When the participant answers affirmatively, press the **spacebar** and 1-List Practice Item 2 will be presented.

If participant answers the question *incorrectly* after 3 trials, press **0 and then press spacebar**. **Test will be discontinued.**

1-List Practice Item 2*:*

Trial 1: After the pictures are presented and the screen is blank, an audio recording says: **Now say the animals in size order, starting with the smallest animal.**

Correct Response: If participant says RABBIT, SHEEP, ELEPHANT, press **1** and then press **spacebar**. An audio recording says: **That's right.** When it appears on the screen, ask: **Are you ready?** When the participant answers affirmatively, press the **spacebar** and the 1-List introduction screen will be presented.

Incorrect Response: If participant does not say RABBIT, SHEEP, ELEPHANT, press **0** and then press **spacebar**. An audio recording says: **Let's try that again.**

Trial 2 (if Trial 1 was incorrect): An audio recording continues: **You saw a RABBIT, SHEEP and ELEPHANT; the RABBIT is smaller than the SHEEP and the SHEEP is smaller than the ELEPHANT. The RABBIT is the smallest animal, the SHEEP is the next biggest animal, and the ELEPHANT is the biggest animal. Now, say the animals in size order, starting with the smallest animal.**

Correct Response: If participant says RABBIT, SHEEP, ELEPHANT, press **1** and then press **spacebar**. An audio recording says: **That's right.** When it appears on the screen, ask: **Are you ready?** When the participant answers affirmatively, press the **spacebar** and the 1-List introduction screen will be presented.

Incorrect Response: If participant does not say RABBIT, SHEEP, ELEPHANT, press **0** and then press **spacebar**. An audio recording says: **Let's try that once more.**

Trial 3 (if Trial 2 was incorrect): After the pictures are presented again and the screen is blank, an audio recording says: **You saw a RABBIT, SHEEP and ELEPHANT; the RABBIT is smaller than the SHEEP and the SHEEP is smaller than the ELEPHANT. The RABBIT is the smallest animal, the SHEEP is the next biggest animal, and the ELEPHANT is the biggest animal. Now, say the animals in size order, starting with the smallest animal.**

Correct Response: If participant says RABBIT, SHEEP, ELEPHANT, press **1** and then press **spacebar**. An audio recording says: **That's right.** When it appears on the screen, ask: **Are you ready?** When the participant answers affirmatively, press the **spacebar** and presentation of the 1-List introduction screen will be presented.

If participant answers the question *incorrectly*, press **0 and then press spacebar**. **Test will be discontinued.**

The 1-list practice items are summarized in the table below:

P1	**Presentation: Horse Dog (Flashing)**		
	Question	***Response***	***Next step***
Q1	Smaller first, then Bigger	If Dog Horse, press 1; If Horse Dog, press 0	If 1, goes to P2; If 0, goes to Q2
Q2	Smaller first, then Bigger	If Dog Horse, press 1; If Horse Dog, press 0	If 1, goes to P2; If 0, goes to Q3
Q3	Smaller first, then Bigger	If Dog Horse, press 1; If Horse Dog, press 0	If 1, goes to P2; If 0, test discontinued

P2	Presentation: Elephant Rabbit Sheep (flashing)		
	Question	**Response**	**Next step**
Q1	Animals in size order, starting with the smallest	If Rabbit, Sheep, Elephant, press 1; If any other order, press 0	If 1, goes to 1-list test items; If 0, goes to Q2
Q2	Animals in size order, starting with the smallest	If Rabbit, Sheep, Elephant, press 1; If any other order, press 0	If 1, goes to 1-list test items; If 0, goes to Q3
Q3	Animals in size order, starting with the smallest	If Rabbit, Sheep, Elephant, press 1; If any other order, press 0	If 1, goes to 1-list test items; If 0, test discontinued

1-List Test Items:

Say: **Let's look at some more pictures. Remember, after you see the pictures you will see a blank screen. Once you see this blank screen, I want you to tell me what you just saw in size order from smallest to biggest. It is important to pay attention to the size of the object on the screen when putting things in size order from smallest to biggest. Are you ready?**

Press the **spacebar** to continue and the presentation of the 1-List test items will begin.

As noted earlier, after the examiner chooses **1** or **0**, the question: **Are you ready?** appears on the screen. Once confirming that the participant is ready and attending, the examiner presses the **spacebar** to move on to the next item (list of objects).

For the examiner, there is a code at the bottom right side of the iPad screen to indicate which item is being administered. The examiner should use this code to look up the correct response on the NIH Toolbox List Sorting Working Memory Test Examiner Answer Sheet, and then score the item using the keyboard (1 or 0).

2-List Condition

> **REVIEW:** The 2-List practice items have the same format. First, the examiner asks for the food from smallest to largest and then for the animals from smallest to largest. If the answer is correct, the examiner moves on to the next question. If the participant answers incorrectly, there are two more opportunities to get a correct answer. The first opportunity comes after a verbal explanation and a repeat of the question; the second comes after replaying the item on the screen, followed by a verbal explanation and then a repeat of the question. If after a total of three opportunities, the participant continues to answer incorrectly, the test will be discontinued and no more items will be presented. The practice items are detailed below.

Say: **We're going to look at more pictures. This time, you will see both food and animals in a set of pictures. I'd like you to tell me the food first, and then the animals, in size order from smallest to biggest. It is important to pay attention to the size of the object on the screen when putting things in size order from smallest to biggest. Let's start by looking at some more pictures together.** Press **Spacebar** to continue.

After each answer, the examiner should indicate whether it was correct (**1**) or incorrect (**0**) on the keyboard. Then the question **Are you ready?** appears on the screen. *When the participant answers affirmatively, the examiner should press the* ***spacebar*** *to continue.*

2-List Practice Item 1:

Trial 1: After the pictures are presented and the screen is blank, an audio recording says: **Say the food, then the animal.**

Correct Response: If participant says BANANA, BEAR, press **1** and then press **spacebar**. An audio recording says: **That's right.** When it appears on the screen, ask: **Are you ready?** When the participant answers affirmatively, press the **spacebar** and 2-List Practice Item 2 will be presented.

Incorrect Response: If participant does not say BANANA, BEAR, press **0** and then press **spacebar**. An audio recording says: **Let's try that again.**

Trial 2 (if Trial 1 was incorrect): An audio recording continues: **The BEAR is an animal; the BANANA is a food. Now say the food first and then the animal.**

Correct Response: If participant says BANANA, BEAR, press **1** and then press **spacebar**. An audio recording says: **That's right.** When it appears on the screen, ask: **Are you ready?** When the participant answers affirmatively, press the **spacebar** and 2-List Practice Item 2 will be presented.

Incorrect Response: If participant does not say BANANA, BEAR, press **0** and then press **spacebar**. An audio recording says: **Let's try that once more.**

Trial 3 (if Trial 2 was incorrect): After the pictures are presented and the screen is blank, an audio recording says: **The BEAR is an animal; the BANANA is a food. Now say the food first and then the animal.** If participant says BANANA, BEAR, press **1** and then press **spacebar**. An audio recording says: **That's right.** When it appears on the screen, ask: **Are you ready?** When the participant answers affirmatively, press the **spacebar** and 2-List Practice Item 2 will be presented.

If participant answers the question *incorrectly*, press **0 and then press spacebar**. **Test will be discontinued.**

2-List Practice Item 2:

Trial 1: After the pictures are presented and the screen is blank, an audio recording says: **Say the food in size order from smallest to biggest and then say the animals in size order from smallest to biggest.**

Correct Response: If participant says PINEAPPLE, FROG, TIGER, press **1** and then press **spacebar**. An audio recording says: **That's right.** When it appears on the screen, ask: **Are you ready?** When the participant answers affirmatively, press the **spacebar** and the 2-list introduction screen will be presented.

Incorrect Response: If participant does not say PINEAPPLE, FROG, TIGER, press **0** and then press **spacebar**. An audio recording says: **Let's try that again.**

Trial 2 (if Trial 1 was incorrect): An audio recording continues: **You saw a frog, a pineapple, and a tiger. The pineapple is a food; the frog is the smallest animal, and the tiger is the biggest animal. Now, say the food in size order starting with the smallest food, and then the animals in size order, from smallest to biggest.**

Correct Response**:** If participant says PINEAPPLE, FROG, TIGER, press **1** and then press **spacebar**. An audio recording says: **That's right.** When it appears on the screen, ask: **Are you ready?** When the participant answers affirmatively, press the **spacebar** and the 2-list introduction screen will be presented.

Incorrect Response: If participant does not say PINEAPPLE, FROG, TIGER, press **0** and an audio recording says: **Let's try that one more time.**

Trial 3 (if Trial 2 was incorrect): After the pictures are presented and the screen is blank, an audio recording says: **You saw a frog, a pineapple, and a tiger. The pineapple is a food; the frog is the smallest animal, and the tiger is the biggest animal. Now, say the food in size order starting with the smallest food, and then the animals in size order, from smallest to biggest.**

Correct Response: If participant says PINEAPPLE, FROG, TIGER, press **1** and then press **spacebar**. An audio recording says: **That's right.** When it appears on the screen, ask: **Are you ready?** When the participant answers affirmatively, press the **spacebar** and presentation of the 2-list introduction screen will be presented.

If participant answers the question *incorrectly*, press **0 and then press spacebar**. **Test will be discontinued.**

The 2-list practice items are summarized in the table below:

P1	**Presentation: Bear Banana (Flashing)**		
	Question	***Response***	***Next step***
Q1	Food first, then Animal	If Banana Bear, press 1; If Bear Banana, press 0	If 1, goes to P2; If 0, goes to Q2
Q2	Food first, then Animal	If Banana Bear, press 1; If Bear Banana, press 0	If 1, goes to P2; If 0, goes to Q3
Q3	Food first, then Animal	If Banana Bear, press 1; If Bear Banana, press 0	If 1, goes to P2; If 0, test discontinued
P2	**Presentation: Frog Pineapple Tiger (flashing)**		
	Question	***Response***	***Next step***
Q1	Food first, then Animals in size order, starting with the smallest	If Pineapple Frog Tiger, press 1; If any other order, press 0	If 1, goes to 2-list test items; If 0, goes to Q2
Q2	Food first, then Animals in size order, starting with the smallest	If Pineapple Frog Tiger, press 1; If any other order, press 0	If 1, goes to 2-list test items; If 0, goes to Q3
Q3	Food first, then Animals in size order, starting with the smallest	If Pineapple Frog Tiger, press 1; If any other order, press 0	If 1, goes to 2-list test items; If 0, test discontinued

2-List Test Items

Read: **Let's look at some more pictures. Remember, after you see the pictures, you will see a blank screen. Once you see this blank screen, tell me the foods first in size order from smallest to biggest, then the animals, in size order from smallest to biggest. Are you ready?** Press the **spacebar** to continue and the presentation of the 2-List test items will begin.

As before, after the examiner chooses **1** or **0**, the question: **Are you ready?** appears on the screen. Once confirming that the participant is ready and attending, the examiner presses the **spacebar** to move on to the next item (list of objects).

NOTE There is a code at the bottom right side of the iPad screen to indicate which item is being administered. The examiner should use this code to look up the correct response on the NIH Toolbox List Sorting Working Memory Test Examiner Answer Sheet, and then score the item using the keyboard (1 or 0).

M.NIH Toolbox Dimensional Change Card Sort Test

C.2.4 NIH Toolbox Dimensional Change Card Sort Test (DCCS)

The NIH Toolbox Dimensional Change Card Sort Test is a measure of cognitive flexibility and attention. Two target pictures are presented that vary along two dimensions (e.g., shape and color). Participants are asked to match a series of bivalent test pictures (e.g., yellow balls and blue trucks) to the target pictures, first according to one dimension (e.g., color) and then, after a number of trials, according to the other dimension (e.g., shape).

The relevant dimension for sorting is indicated by a cue word (e.g., "shape" or "color") that appears on the screen for all participants and that, for young children ages 3-11, is also spoken by a prerecorded audio file.

Equipment and materials needed:

1. iPad
2. Home base

Detailed information on equipment and materials needed for all tests can be found in Appendix 4.

Practice items use white and brown colors and a Rabbit and Sailboat as shapes. Test items use blue and yellow colors and a Ball and Truck as shapes.

All instructions are on the iPad screen: The examiner reads them to and/or with the participant and points out the relevant aspects of the stimuli on the screen. The next screen appears when either the examiner or participant makes a choice.

There are two versions of this measure for ages 3-7. The first is the standard NIH Toolbox DCCS test for ages 3-7. The second is an experimental version (with "developmental extension," or DEXT) that was designed to extend the range of assessment downward, for those participants who have difficulty understanding the standard task. Both versions yield a score for the standard measure, and the DEXT version provides simple raw scores and percentages for the experimental items.

Standard administration:

Practice items

Participants of all ages are given four practice trials with each dimension – color and shape. If the participant responds incorrectly, an audio recording prompts him/her to choose the correct image. Similarly, a separate audio file plays each time the participant gets a practice item correct.

Rules:

- Participants must get at least three out of four practice trials correct to advance to the practice trials for the next dimension and then to the test trials.
- If a participant of any age gets fewer than three out of four practice trials correct, he/she will complete up to two more sets of four practice trials, with the same cutoff to advance to the test trials.
- If a participant of any age does not meet the cutoff, the task will automatically discontinue.

If the participant does not respond after five seconds, the examiner should prompt him/her, **saying:** Choose one of the pictures.

Test Trials

The examiner should not prompt the participant to respond during the test trials. If the participant does not respond after ten seconds, the program will automatically advance to the next test trial.

C.2.4.4 Dimensional Change Card Sort Test Instructions Ages 12+

This table outlines the item content read by as well as the actions for the examiner.

	iPad screen written content	Examiner (E) Actions
Title Screen	NIH Toolbox DCCS 12+	E touches and holds button to continue
Home Base Intro	We're going to play a matching game with colors and shapes. But first we are going to learn about Home Base. This is your Home Base. Put your finger on the Home Base and wait for the next picture.	E reads screen and points to Home Base; then touches and holds button to continue.

	iPad screen written content	Examiner (E) Actions
SHAPE intro	We'll play the SHAPE game first. In the SHAPE game, choose the picture that's the same SHAPE as the picture in the middle of the screen. If it's a BOAT, choose this picture.	E points to BOAT, then demonstrates use of button.
	If it's a RABBIT, choose that picture.	E points to RABBIT, then touches and holds button to continue.
Transition	Now you try. Keep your eyes on the star. ★ Answer as fast as you can without making mistakes. If you make a mistake, just keep going! Put your finger back on Home Base after you answer.	E reads screen, then touches and holds button to continue.
Shape Practice set 1	4 items sorted by shape	
More practice, if less than 3 out of 4 correct on set 1	Let's practice that some more. In the SHAPE game, choose the picture that's the same SHAPE as the picture in the middle of the screen. If it's a BOAT, choose this picture.	E chooses BOAT.
	If it's a RABBIT, choose that picture.	E chooses RABBIT.
Shape Practice set 2	4 items sorted by shape	
More practice, if less than 3 out of 4 correct on set 2	Let's practice that some more. In the SHAPE game, choose the picture that's the same SHAPE as the picture in the middle of the screen. If it's a BOAT, choose this picture.	E chooses BOAT.
	If it's a RABBIT, choose that picture.	E chooses RABBIT.
Shape Practice set 3	4 items sorted by shape	
Test ends, if less than 3 out of 4 correct on set 3		
COLOR intro	We can also match by COLOR. In the COLOR game, choose the picture that's the same COLOR as the picture in the middle of the screen. If it's BROWN, choose this picture.	E points to, then chooses, BROWN picture.
	If it's WHITE, choose that picture.	E points to, then chooses, WHITE picture.
Transition	Now you try. Keep your eyes on the star. ★ Answer as fast as you can without making mistakes. If you make a mistake, just keep going! Put your finger back on Home Base after you answer.	E reads screen, then touches and holds button to continue.
Color Practice set 1	4 items sorted by color	
More practice, if less than 3 out of 4 correct on set 1	Let's practice some more. In the COLOR game, choose the picture that's the same COLOR as the picture in the middle of the screen. If it's WHITE, choose this picture.	E chooses WHITE picture.
	If it's BROWN, choose that picture.	E chooses BROWN picture.
Transition	Now you try. Keep your eyes on the star. ★ Answer as fast as you can without making mistakes. If you make a mistake, just keep going! Put your finger back on Home Base after you answer.	E reads screen, then touches and holds button to continue.
Color Practice set 2	4 items sorted by color	

	iPad screen written content	Examiner (E) Actions
More practice, if less than 3 out of 4 correct on set 2	Let's practice some more. In the COLOR game, choose the picture that's the same COLOR as the picture in the middle of the screen. If it's WHITE, choose this picture.	E chooses WHITE picture.
	If it's BROWN, choose that picture.	E chooses BROWN picture.
Transition	Now you try. Keep your eyes on the star. ★ Answer as fast as you can without making mistakes. If you make a mistake, just keep going! Put your finger back on Home Base after you answer.	E reads screen, then touches and holds button to continue.
Color Practice set 3	4 items sorted by color	
Test ends, if less than 3 out of 4 correct on set 3		
Test item intro	Now we're going to play both games together. Remember, if you see the word SHAPE, you choose the picture that is the same SHAPE as the picture in the middle of the screen. If you see the word COLOR, you choose the picture that is the same COLOR as the picture in the middle of the screen. Remember, put your finger back on Home Base after you answer.	E reads screen, then touches and holds button to continue.
Transition	Now you try. Keep your eyes on the star. ★ Answer as fast as you can without making mistakes. If you make a mistake, just keep going. Put your finger back on Home Base after you answer.	E reads screen, then touches and holds button to continue.
Test items	30 mixed items	