Development of fecal collection protocol for calprotectin measurement in persons with inflammatory bowel disease

Marissa M. Baus

Medical College of Ohio

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FINAL APPROVAL OF SCHOLARLY PROJECT
Master of Science in Nursing

Development of Fecal Collection Protocol for Calprotectin Measurement in Persons with Inflammatory Bowel Disease

Submitted by

Marissa Baus

In partial fulfillment of the requirements for the degree of Master of Science in Nursing

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Development of Fecal Collection Protocol
For Calprotectin Measurement
In Persons with Inflammatory Bowel Disease
Marissa M. Baus
Medical College of Ohio
2005
DEDICATION

I dedicate this project, with utmost sincerity, to those few, special individuals who have inspired, upheld, encouraged, and carried me through this journey. To Aaron, my sole mate and my solid rock, through this entire process you have never swayed in your confidence or support for me. You have sacrificed hours of our life for my quest. You have been patient and exhibited the true essence of self-abandonment. Thank you. Dear parents, you are the reason I exist, the inspiration to fully live every moment, and the motive to progress. You have taught me the value of dedication and hard work. You are awe-inspiring in what you have accomplished, endured and sought after in life. You have lifted me up and filled me with your love and support through this process. Thank you. To Kevin and Barry, as siblings we all have chosen paths in life that allow us to grow, explore, advance, and search after contentment. As older brothers you have tread a steady and straight path for me to follow. Thank you for leading the way, safeguarding me and upholding me as I trail behind. This accomplishment is nothing in comparison to how much I have seen in each of you through this process. I am truly blessed and thank you. I could not have done this without each one of you. This quest ends here, with much gained, until another I pursuit.
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The promises of the academia are endless, but if not presented in a graspable manner, they emerge as unachievable and bewildering. Fortuitously, my experience with academia was guided by individuals who had a vision for their work and were able to convey that vision to me. The energy they emitted was stirring. They guided with patience, stamina and, when necessary, an overt gentleness. The scholarship, reward and satisfaction I received from this project would not have been possible without them. For the conveyed passion, support, and guidance, I am extremely grateful. In essence, Dr. Pocotte and Dr. Smolen, thank you.
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CHAPTER I

Introduction

Inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are both gastrointestinal disorders that have similar clinical manifestations. IBD and IBS are often mistakenly interchanged and misdiagnosed because of the similarities of their presenting manifestations (Thompson, 2001; Walsh, Grunert, Telford, & Otterson, 1995). It is vital that the confusion between IBD and IBS come to an end in order to allow for both IBD and IBS to be accurately diagnosed and managed medically. The following introduction will detail the similarities and differences between IBD and IBS, provide a table for comparison ease, and in addition, propose a method for, and substantiate the necessity of an objective differentiation of IBD and IBS.

IBD encompasses two incurable, chronic, idiopathic gastrointestinal (GI) disorders characterized by chronic inflammation of the bowel with periods of exacerbation and remission. The exact cause of IBD is unknown, but several theories correlate an interactive role between immune, genetic, and environmental factors. The dysregulated immune response is considered the primary factor in the pathogenesis of IBD (Ardizzone & Porro, 2002; Knigge, 2002). The prevalence of IBD is 100-200 per 100,000 in the general population, and approximately 10,000 new cases occur annually. IBD equally affects men and women and is typically present in those between the ages of 15 and 25 or the ages of 55 and 65 (Knigge).

The two major disorders of IBD are ulcerative colitis (UC) and Crohn's disease (CD). Although UC and CD represent two distinct disorders, they are both usually grouped together because of their similar clinical manifestations and symptoms, chronic
clinical course and unknown etiologies (Schwarz & Blanchard, 1991). While UC involves inflammation and ulcerations of almost exclusively the rectum and colonic lining (called the mucosa), it never invades the small intestine. CD differs from UC as it occurs in segments or patches along the entire GI tract penetrating all layers of the bowel. Nevertheless, both UC and CD involve inflammation and ulceration of the affected area resulting in diarrhea (possibly containing blood and mucous), abdominal cramping, vomiting, weight loss, anorexia, fever, elevated white blood cell count, anemia, and lethargy with structural changes of the GI tract (Bruno, 2004). In addition, individuals with UC or CD have the possibility to exhibit extra-colonic chronic inflammatory diseases of the skin, eyes, hepatobiliary system and joints, with arthritis and arthralgias being the most common extra-colonic manifestations (Bruno; Knigge, 2002).

IBS has clinical manifestations and symptoms somewhat similar to IBD, and differentiating between them can be arduous. IBS is a chronic disabling disorder of the lower GI tract. IBS affects more than 20% of the North American population (Ehrenpresis, 2005). IBS is mainly characterized by erratic bowel activity such as diarrhea (possibly containing mucous), constipation, or both, in alteration, along with abdominal pain, extreme abdominal tenderness, bloating, nausea, anorexia, and a feeling of urgency and incomplete evacuation. Abdominal symptoms are often relieved with evacuation (Burke & Lemone, 2004). Other extracolonic symptoms associated with IBS include lethargy, backache, and urinary symptoms (Gonsalkorale, Toner, & Whorwell, 2004). One important difference between IBD and IBS is that examination of the IBS individual’s GI tract reveals the absence of structural abnormalities (William, 1994). Changes in gastrointestinal motor function, enhanced perception of stimuli arising from
the gut wall, and psychosocial factors are thought to be major contributors for symptom generation in IBS (Barbara, De Giorgio, Stanghellini, Cremon, & Corinaldesi, 2002). Approximately 70 to 75% of IBS sufferers are female and 25 to 30% are male (Frank et al. 2002), accounting for the female dominance of 3:1 in the population. The majority of IBS clients are between the ages of 25 and 64 years.

Despite the similarities of their presenting symptoms, IBD and IBS are actually rather dissimilar diseases. Differential diagnosis of these two diseases is extremely important because their clinical prognoses and medical management are quite different. IBD is a much more severe disease and can become life threatening and require surgery in some cases, whereas IBS can often be treated with diet restrictions, stress reduction, and medication.
Table 1

The Different Characteristics and Clinical Manifestations of Inflammatory Bowel Disease (IBD) and Irritable Bowel Syndrome (IBS)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IBD</th>
<th>IBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence</td>
<td>100-200/100,00, 20% in general population</td>
<td>10-15% in general population</td>
</tr>
<tr>
<td>Age</td>
<td>15-65</td>
<td>25-64</td>
</tr>
<tr>
<td>Gender</td>
<td>About equal</td>
<td>Female: male = 3:1</td>
</tr>
<tr>
<td>Geography</td>
<td>Temperature Zones</td>
<td>Worldwide</td>
</tr>
<tr>
<td>Seek Health Care</td>
<td>All</td>
<td>About 30%</td>
</tr>
<tr>
<td>Need for surgery</td>
<td>Often</td>
<td>No</td>
</tr>
<tr>
<td>Physical Disability</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Social Inconvenience</td>
<td>Often</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Structural Change in Gut</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical Manifestations</th>
<th>IBD</th>
<th>IBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal Bleeding and Anemia</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Mucous in Stool</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Fever, Elevated White Blood Cells</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Malnutrition, Nausea, Vomiting</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Abdominal Pain, Discomfort, Cramping, Tenderness</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Erratic Bowel Activity, such as diarrhea, constipation or both, in alteration, along with bloating, urgency, or feeling of incomplete evacuation.</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Lethargy</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Complication: Skin, Hepatobiliary, Joint and Eyes specifically arthralgias and arthritis</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
Thus far, colonic visualization with flexible sigmoidoscopy or colonoscopy is the preferred tests for diagnosing IBD and IBS (Colletti, 2004). These tests are the most sensitive tests because the entire colon can be examined, revealing intestinal abnormalities that occur specifically in IBD, allowing a diagnostic distinction from IBS. The fundamental difference between IBD and IBS is that IBD has structural abnormalities and IBS does not.

A sigmoidoscopy or colonoscopy is an invasive and expensive diagnostic procedure. Therefore, the recent recognition of fecal calprotectin, a calcium and zinc binding anti-inflammatory protein found in high concentrations in inflammatory cells such as polymorphonuclear neutrophils, offers an attractive alternative as an index of intestinal inflammation and as a promising and useful non-invasive objective tool in the screening for IBD in clients with abdominal pain and diarrhea (Roseth, Aadland, Jahnsen, & Raknerud, 1997) Research has provided evidence that during intestinal inflammation, neutrophils infiltrate the mucosa, increasing fecal calprotectin concentrations in the gut. Clients with inflammation and structural abnormalities of the GI tract, such as in IBD (but not IBS), exhibit a significantly higher elevation of calprotectin concentrations (>50 mg/kg, and usually range between 200 – 20,000 mg/kg) in their stool than in the general population (Tibble, Sigthorsson, Bridger, Fagerhol, & Bjarnason, 2000; http://www.phical.com/Doc/Instructions.pdf). For those reasons, fecal calprotectin is a sensitive and specific marker for differentiating IBD from IBS, as well as a possible mechanism for monitoring disease activity and in documenting response to medical treatment.
It was noted in a study by Roseth, Aadland, & Grzyb, 2004 that there is a significant increase in the concentration of fecal calprotectin with increased disease activity as determined by histological (microscopic structure of the GI tract) criteria when compared to clients with low disease activity. Furthermore, Roseth et al. found that calprotectin levels were seen to be decidedly correlated to the degree of inflammation and subsequent disease activity, rather than the extension of the disease. Clinical indices, like the Crohn’s Disease Activity Index (CDAI), Harvey-Bradshaw “simple index” (SI), Perianal Disease Activity Index (PDAI), and Inflammatory Bowel Disease Questionnaire (IBDQ), have customarily been used as the best indicators of disease activity (Sostefni et al., 2003). The recent discovery and implementation of calprotectin as a monitor of disease activity, along with the above indices, is significant clinically, providing an objective scientific measurement of disease activity, in addition to the subjective indices.

Once IBD is eliminated as a diagnosis based on the absence of observable structural defects of the GI tract during a sigmoidoscopy or colonoscopy, IBS may be diagnosed using the Manning or Rome diagnostic criteria. The Manning and Rome diagnostic criteria are multiple symptom-based criteria defining the clinical syndrome of IBS and provide the framework to categorize the symptoms of IBS, and allow for its proper diagnosis. The Manning and Rome diagnostic criterion for IBS are solely subjective diagnostic tools. In the criteria of Manning, Thompson, Heaton, & Morris, 1978, p.653, the symptoms associated with IBS include relief of pain with bowel movements, looser and more frequent stools with onset of pain, passage of mucus, and a sense of incomplete evacuation. The Rome diagnostic criteria includes abdominal discomfort or pain for at least 12 weeks in the preceding 12 months (which need not be
consecutive) and two of the following three features: (1) relieved with defecation; (2) onset associated with a change in frequency of stools; and (3) onset associated with a change in form (appearance) of stool. Supportive features include abnormal stool frequency, consistency, abnormal passage of stool and bloating or abdominal distention. (Drossman, Corazziari, Talley, Thompson, & Whitehead, 2000; Shen & Soffer, 2003) (Appendix A).

Subsequent to the differentiation between IBD and IBS and the establishment of a definitive diagnosis, clients should be able to seek out proper medical management relevant to their specific diagnosis. Yet, another problem arises as research studies indicate that current therapeutic recommendations, i.e., conventional drugs and/or surgical therapies, are not reliably effective in alleviating the symptoms of IBD and IBS or restoring the IBD and IBS client’s quality of life (Lea et al., 2003). Meanwhile, the intensity and reoccurrences of the symptoms of IBD and IBS prevent many clients from actively living a “normal” life, interfering with their involvement in sports, personal relationships, and employment opportunities. Overall, these diseases impact every aspect of the client’s life, greatly decreasing the quality (Guthrie et al., 2002; Luscombe, 2000). As a result, it has been proposed that complementary, alternative or unconventional therapies and systems of care be implemented as treatment for both IBD and IBS. These therapies will be referred to as complementary care modalities for the purposes of this paper. The rate of use of complementary care modalities is rising in the United States, reportedly increasing from 33.8% in 1990 to 42.1% in 1997 (Eisenberg et al., 1998). To date, however, there are surprisingly few publications in the scientific literature that have thoroughly investigated the mechanisms of action of complementary care modalities and
their efficacy for sufferers of IBD and IBS. As will be shown in the literature review that follows, research has provided evidence that complementary care modalities, such as cognitive behavioral therapy, relaxation response meditation, hypnotherapy, stress management, education modification of health behaviors, and Gentle Touch, Reiki, Therapeutic Touch (TT), and Healing Touch (HT) are effective approaches. However, they have been underutilized in the treatment of IBD and IBS. Very little research provides evidence for the efficacy of complementary care modalities, especially Reiki, in the treatment of IBD and IBS. A future larger study, has been proposed to establish the reliability of complementary care modalities, specifically Reiki, as a nursing intervention for those suffering from IBD. Measurement of fecal calprotectin will serve as a biomarker of disease activity in control, sham-Reiki, or Reiki treatment groups. It is necessary to establish a fecal collection protocol for the future proposed study.

The remaining portion of Chapter I will address the specific problem related to the use of calprotectin as a tool for the differentiation of IBD and IBS and the difficulties surrounding conventional treatments for IBD and IBS. It will be followed by a brief discussion of Dorthea Orem’s self-care deficit of nursing theory (SCDNT) (2001), the theoretical framework guiding this project, also the purpose of this project, its significance, and a brief summary will be included.

Statement of the Problem

Symptoms of IBD and IBS are chronic, episodic, and most importantly, very similar to one another. Differential diagnosis of these two diseases is exceptionally challenging due to the fact that they have very similar clinical manifestations. Yet, a differential diagnosis is invaluable because it ensures that proper treatment will be
implemented for both IBD and IBS. As previously stated, calprotectin is a sensitive marker that can be used in the differentiation of IBD and IBS. In order for calprotectin to be employed, it is necessary to construct a Fecal Specimen Collection Protocol (FSCP) that can be utilized by sufferers of IBD and IBS during stool collection to prevent sampling errors that may taint laboratory results. Currently, in the literature, there are no protocols described that are used to guide the collection of stool samples for analysis and more specifically for use in a quantitative enzyme-linked immunoassay (ELISA) detecting concentrations of calprotectin. The development of a fecal specimen collection protocol is essential to initiate the use of calprotectin in the differentiation of IBD and IBS, as well as an instrument for monitoring changes in disease activity and document response to treatment.

Identification of Theoretical Framework

Orem’s (2001) Self Care Deficit Theory of Nursing (SCDTN) was utilized to provide the framework for this project. The theory of self-care deficit can be summarized as the simple relationship between self-care demand and self-care agency (Orem). Orem’s SCDTN is based on three general sub-theories that incorporate seven basic concepts. The sub-theories are (a) the theory of self-care, (b) the theory of self-care deficit and, (c) the theory of nursing systems. The basic concepts include self-care, self-care requisites, self-care demand, self-care agency, self-care deficit, nursing agency, and nursing systems. Orem’s model begins by focusing on each individual’s ability to perform self-care, defined as “the practice of activities that individuals initiate and perform on their own behalf in maintaining life, health, and well-being (Orem, 2001, p. 44).” The goal of self-care is the attainment of self-care requisites. The self-care
requisites are defined as the needs of people required for taking care of themselves at
different stages of their lives. These requisites or needs could arise from either
maturational changes, situational events, or other traumatic changes (Orem). The self-
care demand develops from a specific self-care requisite and is defined as "the particular
self-care requisites for individuals in relation to their conditions and circumstances
(Orem, 2001, p. 62).” Once the self-care demand is identified, the individual’s self-care
agency or their ability to meet the newly acquired self-care demand can be assessed. If
the self-care agency is inadequate to meet the self-care demand, the nursing agency
becomes involved. The nursing agency is defined as an educated individual, usually a
nurse, who is implemented to assist others in their therapeutic self-care demands and to
regulate the development of their self-care agency (Orem). The nursing agency functions
to help the client attain self-care requisites in three ways (a) wholly compensatory
interventions, (b) partly compensatory interventions, and (c) supportive educative
interventions this is the nursing system (Orem). The SCDTN framework was chosen as a
basis for this project based the applicability of its concepts to the project. Throughout
this paper these concepts will be broken down into applicable terms that will evolve into
the complex relationships that connect this project.

Orem’s SCDTN (2001) basic concepts are correlated in the present project in the
following manner. Sufferers of IBD and IBS may have difficulties performing the
entirety of their self-care due to their disorders and the chronic nature of their illnesses.
Both IBD and IBS symptoms can cause pain and fatigue and hinder the sufferer’s ability
to care for himself or herself and thus, decreases their overall quality of life (Guthrie et
al., 2002; Luscombe, 2000; Orem). It can then be stated that many IBD and IBS
individuals suffer inadequate self-care agency because of the sufferer's inability to care for themselves. The decrease quality of life (Guthrie et al.) is presumed to be influenced by inadequate self-care agency, which lends to development of self-care requisites, specifically in the area of elimination, food, activity and rest, social interaction, well-being, and normalcy. After identification of the above requisites, it is necessary to identify the IBD and IBS sufferer’s specific self-care demands and/or self-care limitations within the requisite areas. The primary self-care demand to be met in clients suffering from IBD and IBS is the reduction of the symptoms, leading to decreased pain, and subsequently, an improved quality of life.

Consequently, when a client’s self-care demands outweigh his or her ability to meet these demands, as may occur in clients suffering from IBD and IBS, a dependent care agent or nursing agency becomes involved to assist the client meet his or her self-care demands. When the IBD and an IBS client has a self-care demand that needs to be met, and their self-care agency is inadequate to meet the demand, the client is said to have a self-care deficit. When a self-care deficit occurs, the nurse, acting as the nursing agency, provides support, guidance and teaching to help the individual with the deficit acquire knowledge and skills to overcome the deficit.

To explain further, the employment of a dependent-care agency or nursing agency to reduce, with the hope to eventually eliminate, the client’s self-care deficit is a primary focus of this project. Initially, the goal of the nursing system is to engage the nursing agency's to assess the client’s self-care deficit and determine whether the client’s demand involves a partial, complete or supportive-educative intervention. Then, it is the nursing agencies’ responsibility to design an intervention that includes a deliberate action that the
dependent-care agency or the nursing agency will take to meet the client’s identified self-care deficit. Subsequently, the dependent-care agency or the nursing agency will temporarily meet the clients’ self-care demand and ultimately increase the client’s capabilities to meet his or her own self-care demand with the desire to reduce and eventually eliminate the self-care deficit.

In this project, the client’s possible lack of education, in regards to their altered state of health, is explored as the reason that individuals with IBD and IBS may not be meeting his or her self-care deficit. This lack of education will be addressed by the employment of a nursing agency with a supportive-educative intervention. This larger study will implement the Fecal Specimen Collection Protocol (FSCP) and a complementary care intervention known as Reiki, as the supportive-educative intervention, in an attempt to provide the IBD sufferer with support, guidance and teaching to help them acquire knowledge and skills to overcome their deficit.

The use of Orem’s SCVTN (2001) provides a structure and a framework appropriate to the purpose of this research project. A schematic model represented in Figure 1 also depicts the relationship.
Figure 1. Theoretical Framework of Study

Theory of Self-Care

Identification of Self-Care Requisites

Identification of Self-Care Demands

Theory of Self-Care Deficit

Wholly Compensatory

Theory of Nursing Systems → Partially Compensatory

Nursing Agency or Dependent-Care Agent Intervention

Supportive-educative

Deliberate Action

Development of Self-Care Agency

Development of Fecal Specimen Collection Protocol

Reduced Symptoms and Increased Quality of Life in Individuals with IBD

Reiki Intervention

Statement of Purpose

The purpose of this scholarly project is to develop a protocol for the collection of fecal specimens from individuals suffering from inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). In establishing a method of fecal collection without actual implementation, this protocol will assist MCO SON faculty members in a future, larger study to guide the collection of fecal specimens from individuals who are suffering from IBD. The future study will use the collected stool samples to assess fecal calprotectin concentrations in the stool, prior to and subsequent to, Reiki intervention (a complementary care modality), as a method for monitoring changes in disease activity in response to the intervention.

Significance

Research has shown that concentrations of the neutrophil granulocyte derived protein calprotectin are elevated and notably higher in the feces of sufferers of IBD (Fagerhol, 2002). The detection of significantly elevated calprotectin concentration using the Elisa calprotectin test has shown to have 100% sensitivity and 97% specificity for differentiating IBD and IBS (Tibble et al., 2000). The utilization of the Elisa calprotectin test may not only eliminate or reduce the use of sigmoidoscopy or colonoscopy, an invasive and expensive diagnostic procedure, but will provide a sensitive and specific marker for differentiating IBD from IBS. The significance of this project is that there is minimal information on a standardized protocol for the collection of stool that will be utilized in the Elisa test in assessing for calprotectin concentrations. In order to utilize calprotectin as a differential marker, the fecal specimen collection protocol must be
developed to set a quality control standard, eliminating sampling errors that may blight laboratory results.

Another significant rationale for pursuing this project is based on the need for a means to monitor disease activity and document response to medical treatment. Traditionally, clinical indices like the Crohn’s disease activity index (CDAI), Harvey-Bradshaw “simple index” (SI), Perianal Disease Activity Index (PDAI), and Inflammatory Bowel Disease Questionnaire (IBDQ), have been used as the primary indicators of disease activity (Sostefni et al., 2003). Research has made known the sensitivity and specificity of Elisa calprotectin test in predicting IBD disease activity is 90% and 83%, respectively (Sostegni et al.). Combining the use of these indices with the calprotectin assay could provide more accurate and objective measures to monitor disease activity.

In addition, this protocol will be employed as a tool for the collection of fecal specimens from individuals who are suffering from IBD in a much larger study. The proposed larger study will use the collected stool samples to assess fecal calprotectin concentrations in the stool, prior to and subsequent to Reiki intervention (a complementary care modality) as an instrument for monitoring changes in disease activity in response to the intervention. The larger study optimistically will provide evidence on the reliability of calprotectin as a monitor for disease activity, as well as, make available new research on the efficacy of complementary care modalities in treating the symptoms of IBD.

Finally, impaired quality of life and psychological distress are common in sufferers of IBD and IBS. The symptoms severity and unpredictability can be extremely
troublesome and inhibit individual’s social involvement, not to mention impair individual’s coping mechanism to stressors, leading to uncontrolled anxiety and depression (Guthrie et al., 2002; Luscombe, 2000). The socioeconomic impact of IBD and IBS is also considerable and often drains healthcare resources (Gonsalkorale et al., 2004). The unveiling of a successful treatment for IBD and IBS would be significant for those suffering from the incapacitating disease, as well as, those caring for these clients. In nursing practice, it is useful to be acquainted with the efficacy of the various complementary care interventions in treating IBD and IBS, especially if pharmacological interventions are not very effective (Lea et al., 2003) and considering that IBD affects approximately 20% and IBS approximately 10 to 15% of the general population. It is the nurses’ responsibility, when acting as a client’s nursing agency, to develop interventions that are going to promote self-care for clients with IBD and IBS. The nursing agency must be knowledgeable, but the research must be available to present the knowledge. Upon the implementation of the Fecal Specimen Collection Protocol (FSCP) and the launch of the large study, the efficacy of complementary care intervention will hopefully be validated and implemented in nursing practice. Nurses can expand their practice to include complementary care interventions with the intention of providing better care and assisting sufferers of IBD and IBS to care for themselves.

Summary

In summary, Chapter I contained an introduction that detailed the similarities and differences between IBD and IBS, proposed a method for, and substantiated the necessity of an objective differentiation of IBD and IBS. It then followed with a synopsis of Orem’s SCDTN (2001) used as a theoretical framework (Figure 1) to support this project.
along with rationale for selecting Orem’s theoretical framework. The overall statement of the problem, statement of the purpose, and significance was then discussed. Chapter II will further define and describe the nursing theoretical framework and specifically correlate it to this project and present relevant literature.
CHAPTER II

Literature Review

Introduction

With the aim of developing a comprehensive literature review, establishing a premise for this project and ultimately developing the Fecal Specimen Collection Protocol (FSCP), the literature was extensively searched. The search revealed an absence of literature on standardized protocol for the collection of stool and, for that reason, none was reviewed. This lack of applicable literature and nursing research made a definitive point that this project is extremely significant.

Since there has been no research accomplished on protocol development for collection fecal specimens, two articles were reviewed on general protocol development. These articles provided information on protocol development and evaluation. The articles will commence the literature review. The remaining literature review will comprise the responses of both IBD and IBS to numerous complementary care modalities.

The rationale behind the remaining content of this literature review is multifaceted. First, as formerly stated, inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are physiologically very different diseases, however, they share many of the same symptoms and respond in a similar manner to complementary care interventions (Thompson, 2001; Lea et al., 2003). Therefore, a review of literature covering the responses of both IBD and IBS to numerous complementary care modalities is provided. Second, it has been shown that IBD and IBS sufferers have increased levels of anxiety, stress, and pain, as well as, a decreased ability to relax lending to an overall
lower perception of quality of life (Smolen & Topp, 2001). Thus, the literature review will establish the past success of complementary care modalities in treating anxiety, pain, and stress, as well as their capacity to increase an individual’s ability to relax and, hence, hopefully increase individual’s perceptions of their quality of life. Therefore, the overall purpose of this literature review was to ascertain information on general protocol development and evidence of the success of complementary care modalities in treating the symptoms of both IBD and IBS, as well as other associated symptoms. This evidence will then be used to validate the importance of the development of a Fecal Specimen Collection Protocol (FSCP) that will be utilized to guide stool collection, enabling calprotectin concentrations to be measured and exploited as a tool in the differentiation of IBD from IBS, as well as a possible mechanism for monitoring disease activity and, in documenting disease response to the use of Reiki, as a complementary care modality, in a larger future study.

This chapter begins with a description of how Dorothea Orem’s (2001) Self-Care Deficit Theory of Nursing (SCDTN) was used in this project as the framework to provide organization and context for the correlation between self-care deficits, nursing interventions and decreased pain and an increased quality of life in individuals with inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). The association of this framework is represented in a schematic model found in Figure 1. Then, as stated above, the remainder of this chapter contains an extensive literature review, presenting past research on protocol development and the efficacy of complementary care modalities. Initially, the literature review will divulge research specific to protocol development and then progress to the success of complementary care modalities in
treating the symptoms associated with IBD and IBS, in addition to their ability to ease anxiety and stress correlated to these diseases. The literature review will then expound further to represent previous research on the value of complementary care modalities in ameliorating anxiety, stress, and pain, as well as, increasing relaxation in the general healthy population and for sufferers of other ailments such as AIDS/HIV and musculoskeletal disorders.

The information contained within this literature review is organized and presented in the following manner. It will begin with the general protocol review, then proceed onward through each of the complementary care intervention under investigation. The eight complementary care interventions are Cognitive Behavioral Therapy (CBT), Relaxation Response Mediation, Hypnotherapy, Stress and Contingency Managements, Education Modification of Health Behaviors, Gentle Touch, Reiki, Therapeutic Touch (TT), and Healing Touch (HT). The ensuing literature consists of research that covers a number of elective, non-conventional therapies referred to as Complementary and Alternative Medicine (CAM) merged into a solitary study.

*Nursing Theoretical Framework*

As previously stated, Orem’s (2001) SCDTN was employed to provide the framework for this project. Orem’s SCDTN is based on three general sub-theories that incorporate seven basic concepts. The sub-theories are (a) the theory of self-care, (b) the theory of self-care deficit and, (c) the theory of nursing systems. The basic concepts include self-care, self-care requisites, self-care demand, self-care agency, self-care deficit, nursing systems, and nursing agency.
Orem’s (2001) theory of self-care is based on the fact that every individual has personal care requirements that must be met on a daily basis to enable their own functioning and development (Orem). These personal care requirements are referred to as the individual's self-care needs and taking action to meet these requirements is imparted as performing self-care. Self-care is a regulatory function or deliberate action in which individuals meet their self-care requisites ensuring a supply of material needed for continued life, for growth and development, and for maintenance of human integrity.

Self-care requisites are expressions of actions that must be performed by or for individuals to ensure normal human functioning and development (Orem, 2001). They define the purpose for individuals engaging in self-care. There are eight universal self-care requisites listed by Orem. These requisites include an adequacy and a balance of air, water, food, elimination, activity and rest, solitude and social interaction, hazards, and normalcy. In most circumstances, individuals are capable of meeting the above requisites by performing the necessary self-care actions. However, capabilities do not always exist and just because they do exist, that does not equate to deliberate nursing actions being taken. Therefore, in such circumstances, appropriate nursing judgments emerge that assess the adequacy of the self-care agency and determine self-care demands.

Self-care demands are present when there are identifying unmet self-care requisites. These demands are essentially plans for self-care action through which identified unmet self-care requisites can be met by the individual, a dependent care agency or nursing agency. In order to meet these demands, an individual’s capabilities must be adequate to perform the self-care. The “complex acquired capability to meet one’s continuing requirements for care of self” (Orem, 2001) is referred to as the
individual’s self-care agency. The self-care agency is affected by an individual’s basic conditioning factors. The basic conditioning factors include (a) age, (b) gender, (c) developmental state, (d) health state, (e) socio-cultural orientation, (f) health care system factors, (g) family system factors, (h) pattern of living, (i) environmental factors, and (j) resource availability and adequacy (Orem). In the circumstance that self-care demands are present and the self-care agency is inadequate to meet these demands, a self-care deficit develops.

The evolution into the theory of self-care deficit simply implies, as stated above, that self-care demands are present and the self-care agency is inadequate to meet these demands. The presence of a self-care deficit requires the involvement of a nursing agency to assist in meeting the individuals’ self-care demands and in exonerating the evolved self-care deficit. The involvement of a nursing agency leads into the development of the theory of nursing systems.

The theory of nursing systems describes the nurse as the nursing agency, assessing the client’s self-care deficit, developing interventions and providing nursing care to “legitimate clients whose self-care agency or dependent-care agency and because of their own or their dependent’s health states, is not adequate or will become inadequate for knowing or meeting their own or their dependent’s self-care demands” (Orem, 2001). The ultimate goal of the nursing system is to increase an individual’s capabilities to meet a need (Orem).

It is the nursing responsibility to acquire the knowledge of the degree of the self-care deficit to determine if the self-care deficits identified requires a complete, partial or supportive-educative intervention. A complete self-care deficit means that the individual
has no capabilities to meet their self-care demands. A partial self-care deficit means
limited capabilities to meet self-care demands and a supportive-educative intervention
entails providing support and education to help the individual meet their self-care
demands. Once the extent and cause of the self-care deficit is established, nursing
interventions can be implemented to assist individuals in meeting their self-care demands. Nursing interventions being executed to temporarily aid individuals and to promote the
development of self-care agency is the root of the theory of nursing systems.

The purpose of this present project is to develop a Fecal Specimen Collection
Protocol (FSCP). The employment of this FSCP in a proposed larger, future study, with
the complementary care intervention, Reiki, are considered to be partial and supportive-educative interventions. In this future study, Reiki is the deliberate action to be taken by
the dependent-care agency or nursing agency to temporarily meet the IBD client’s
demand. Entailed in the intervention is the supportive-educative aspect that provides
clients with the means to collect their own stool specimen by providing educational
instruction using the FSCP. Subsequent to proper stool collection, IBD clients can be
assessed for the effectiveness of Reiki in meeting their self-care demand and attenuate the
debilitating effects of the evolved self-care deficit. Client’s will also become educated in
Reiki intervention and will eventually perform their own Reiki intervention, meeting their
self-care demand or need. The demand, as stated previously, is the reduction of
symptoms in clients suffering from IBD leading to decreased pain and an improved
quality of life and developing their self-care agency (Figure 1).
Review of Literature

Protocol Availability, Development and Evaluation

A protocol is considered to be a vehicle by which clinical practice is conducted, variations in clinical practice are reduced, quality care is enhanced, sampling errors are lessened, and cost of care is contained. The above criteria can be fulfilled if the proposed protocol represents a consensus on the most appropriate and accurate method to perform care or a specific task and is utilized as a tool to guide practice (Zellman, Fair, Hoube, & Wong, 2002). Two research articles that examine the availability, applicability, utilization and development of protocols were found to be applicable to this project. No research articles were located that specifically discussed protocols for collecting fecal samples.

The purpose of the research performed by Zellman et al. (2002), was to describe key elements of a set of hospital prenatal substance exposure protocols and to relate variations in protocol content to the state legislative environment and hospital characteristics. In order to determine hospital prenatal substance exposure protocols, Zellman et al. sent out surveys to 806 hospitals that were likely to deal with prenatal substance exposure. Along with the survey, the investigators requested nurse managers and hospital administrators with responsibility for prenatal care to provide their hospital's prenatal substance exposure protocol. Of the 806 hospitals surveyed, 510 (63%) completed and returned the survey. Of that 510, 166 (33%) reported that their hospital had a prenatal substance exposure protocol. Overall, a total of 87, actual examples of prenatal substance exposure protocols, were received. The surveys and protocol examples were reviewed, coded and analyzed. The resulting data provided evidence that
one third ($n = 27$) of the protocols lacked any goal or statement of purpose, 28% failed to present their contents clearly, and overall protocol differed greatly between the responding hospitals. Zellman et al. then concluded that more attention needs to be devoted to the development of prenatal substance exposure protocols as their lack of clarity precludes most from meeting protocol development goals such as encouraging standardized care.

Similar to Zellman et al. (2002) research design, Paech, Pavy, Kristensen, & Wojnar-Horton (1997) also utilized questionnaires to ascertain information from a convenience sample about protocol implementation and efficacy. Paech et al. were exclusively assessing for proper management of post-operative nausea and vomiting, using their designed protocol. Paech et al. developed the protocol from extensive research and post-operative observation. The article intricately details the steps involved in their protocol development. After a year of the protocol being implemented in post-operative clinical practice Paech et al. designed and initiated the present study to assess the use, efficacy and acceptance of the protocol by the medical staff and a specific hospital. Questionnaires were distributed to 50 ward nursing staff with a 94% return, and thirty were distributed to medical staff and 57% were returned. After the questionnaires were reviewed, Paech et al. concluded that the implementation of their management protocol had been well-accepted by staff, appeared to have reduced delay in client treatment and improved client care, and had significantly reduced staff workload.
Therapies Adjunct to the Medical Management of IBD and IBS

Cognitive Behavioral Therapy

John Winston Bush, PhD, from the New York Institute for Cognitive and Behavioral Therapies (CBT), explains CBT treatments in two stages, which are the cognitive stage and the behavioral stage. The cognitive stage of the therapy helps individuals understand how certain thinking patterns cause ill symptoms and a distorted picture of life, making them feel anxious, depressed or angry as well as act irrationally without good reason. Whereas, the behavioral stage of the therapy helps individuals weaken the connections between troublesome thoughts and the habitual reactions to them. Some of the reactions include fear, depression or rage, and self-defeating or self-damaging behavior. Collectively, these two stages of the therapy will teach individuals how to calm their mind and body, causing them to feel better, think more clearly, and make better decisions (Bush, 2004).

The four article reviews that ensue will each assess different facets of Cognitive Behavioral Therapy (CBT) to determine its effectiveness in treating symptoms of IBS. The articles suggest that CBT is correlated to changes in the physiological symptoms of IBS by treating the underlying psychosocial behaviors and the deep-seated mental status of the clients.

In the study by Tkachuk, Graff, Martin, & Berstein (2003) the effectiveness of cognitive behavioral group therapy (CBGT) on psychological distress, quality of life and GI symptoms was compared with home based symptoms monitoring with weekly telephone contact (SMTC). Tkachuk et al. hypothesized that following CBGT, participants would experience a significant decrease in GI symptoms and an increase in
psychological functioning and health related quality of life, in comparison to participants in the SMTC. The study involved the participation of one hundred and four specialist-referred participants, eighteen years or older, meeting the Rome I criteria for IBS and having experienced symptoms of IBS for longer than three months. Ninety-six percent of the participants in the study were women which, is expected due to the fact that 60 to 65% of those that suffer from IBS are women. Participants enrolled in the study were assessed for psychiatric co-morbidity using the structured clinical interview. Participants diagnosed with organic mental disorder were excluded from the study. Medical treatments, including pharmacotherapeutics, were maintained throughout the duration of the study. Participants were randomly enrolled in either the CBGT or SMTC. Those enrolled in CBGT participated in ten 90 minute group sessions over nine weeks. In comparison, the control group was contacted weekly by telephone over the same nine weeks. Participants in CBGT and SMTC were tested prior to treatment, after treatment, and 3 months following termination of treatment, using a battery of self-reporting measures. The self-reporting measures included (a) Beck Depression Inventory-II, (b) State-Trait Anxiety Inventory-Trait Scale, (c) Cognitive Scale for Functioning Bowel Disease, (d) Assertiveness Questionnaire, and (e) Medical Outcome Short Form-36 Health Survey. After analysis of the data, it was found that the hypothesis was supported by the research. Participants receiving CBGT had a significant reduction ($p = .016$) in GI symptoms and a significant increase ($p = .004$) in psychological functioning and health related quality of life in comparison to the control group.

Similar to the study by Tkachuk et al. (2003), Taylor, Read, & Hills (2004) tested the effectiveness of group therapy on relieving symptoms of IBS, but combined it with
hypnotherapy to test the summative effect of the two. Taylor et al. also differed by proposing the use of a standard protocol to circumvent the therapist interaction in effecting the results. The overall purpose of the study by Taylor et al. was to assess the feasibility and short-term effectiveness of CBT and hypnotherapy on groups of IBS sufferers when using a standard protocol, circumventing the experimenter effect. Therapists involved in the delivery of the protocol were required to participate in a 2-day training session and two follow-up sessions to ensure they understood and followed the set of rules. Taylor et al. questioned if professionally led group CBT and hypnotherapy are feasible options for the treatment of IBS. To answer this question, 158 participants (120 females and 38 males), 18 or older, and diagnosed with IBS from a medical practitioner, were recruited for this study. Participants suffering from an organic mental disorder were excluded. The therapeutic intervention consisted of 16 weekly group sessions of CBT and hypnotherapy, each lasting 3 hours. Two series of tests were given to each participant pre and post treatment. The different tests included (a) Quality of Life Questionnaire, (b) GI Symptoms Rating Scale, (c) Prevention of Enjoyment of Life Scale, and (d) Semi-Structured Qualitative Questionnaire. After data analysis, it was concluded that combined CBT and hypnotherapy are effective treatments for IBS and are significant at the $p < .001$ level, when delivered by trained therapists using a standard protocol.

In contrast, the article written by Greene & Blanchard (1994) tested the effectiveness of intense individual cognitive therapy (CT), rather than group therapy, in treating IBS. It was proposed by Greene & Blanchard that the effectiveness of intense individual CT for alleviating symptoms of IBS is directly related to the participant’s
ability to adapt to stressors that exacerbate IBS symptoms. Specifically, in this study, the thought is that anxiety disorder and its potentially distorted and maladaptive cognitions may underlie IBS (Latimer, 1983). It was therefore hypothesized, that intense individual CT, that directly addresses the anxiety problems of clients with IBS, could be advantageous in alleviating the symptoms of IBS. The study by Greene & Blanchard included a small sample size \( (n = 20) \) white adult clients (15 females and 5 males), 18 or older, and meeting the Latimer’s (1983) criteria for IBS with symptoms for over 3 months. Participants were referred by personal physician or self-referred on the basis of local media coverage. Exclusion from the study was based on individuals meeting the criteria for an organic mental disorder after a structured interview and/or having received previous CT. The participants were randomly assigned to either CT or a control group. Pre-tests, post-tests and 3-month follow-up tests were completed by each participant. The tests included (a) Autonomic Thoughts Questionnaire, (b) Beck Depression Inventory, (c) State-Trait Anxiety Inventory, (d) Dysfunctional Attitudes Scale, and (e) GI Symptoms Diary. After the analysis of the above tests that there is a significance \( (p < 0.05) \) difference in the efficacy of treatment between symptoms monitoring and CT. Intense individual CT is a significantly \( (p < 0.05) \) more effective treatment for relieving symptoms of IBS, especially abdominal pain, constipation and diarrhea.

Corresponding to the above study by Green & Blanchard (1994), Payne & Blanchard (1995) were very impressed by the remarkable results of the study and desired to assess whether the high level of success achieved by Green & Blanchard could be replicated using different therapists. In comparison to Green & Blanchard, Payne & Blanchard wanted to develop a condition that accounted for and controlled therapist
attention and client expectations of benefit to determine whether the latter accounted for the significant improvements in GI symptoms. Therefore, Payne & Blanchard wanted to assess whether cognitive changes were, in fact, occurring to a greater extent in cognitive therapy (CT) than other conditions and to what extent cognitive changes reduced GI symptoms. Their study involved individuals ($n=43$) who were diagnose with IBS and met Rome I criteria. The participants were randomly assigned to one of three experimental conditions: (a) cognitive therapy group; (b) support group (SG) and; (c) symptoms monitoring waiting control list group. The treatment for participants involved in CT consisted of approximately 600 minutes of ten individual sessions, whereas the SG 600 minutes were completed during eight group sessions. Participants in the monitoring group simply monitored their symptoms in a GI diary for 8 weeks and then were given the opportunity to enter the SG. Participants in all three groups completed a battery of psychosocial tests pre and post treatment and at a 3 month follow-up assessment. The tests included (a) Beck Depression Inventory, (b) State Trait Anxiety Inventory, Dysfunctional Attitude Scale, (c) Automatic Thoughts Questionnaire, and (d) Hassles Scale. Along with the above tests, participants were also responsible to record daily symptoms in a GI diary. The overall analytical conclusion provided data that revealed that the results with CT, as correlated to Green & Blanchard (1994) study, were replicable and that CT was superior to the other control groups in significantly ($p < .05$) reducing GI symptoms of abdominal pain, constipation, diarrhea, bloating, belching and nausea.

Comparable to the study by Greene & Blanchard (1994), Boyce, Talley, Balaam, Koloski, & Truman (2003) performed a comparison study attempting to provide data that
CBT is a more effective treatment for IBS than relaxation therapy and standard care alone. The study recruited 105 participants (78 women and 27 men), 18 or older, from a gastroenterology hospital and through newspaper advertisement. In order to participate in the study, individuals had to meet the Rome I criteria for IBS. Exclusions for the study included individuals with (a) psychotic illnesses, (b) history of alcoholism, (c) current psychological treatment, and (d) use of pharmacologics. The study randomly placed participants into one of three groups: (a) the control; (b) Cognitive Behavioral Therapy; or (c) relaxation training. After randomization, participants completed eight sessions of treatment, once a week, over an 8 week period. Data were collected pre-treatment, mid-treatment, post-treatment and at 26 weeks and 52 weeks following cessation of treatment. The instruments used to collect the data included (a) Bowel Symptom Severity Scale, (b) Hospital Anxiety and Depression Scale, (c) Medical Outcomes Study Short Form-36, (d) Locus of Control of Behavior, and (e) Automatic Thoughts Questionnaire. Data analysis provided information to prove that, significant ($p < 0.05$) improvements over time in the symptoms of IBS, anxiety and depression, and functioning, occurred in all the participants regardless of what treatment they received. This study then suggested that CBT does not have a significant ($p < 0.05$) advantage over relaxation therapy or routine clinical care in treating the symptoms of IBS.

*Relax Response Meditation*

The relaxation response is a physical state of deep rest that changes the physical and emotional responses to stress resulting in decreased metabolism, decreased heart rate, decreased blood pressure, and decreased rate of breathing, as well as slower brain waves (Benson & Klipper, 2000). The Relaxation Response is a simple technique, represents a
form of meditation which has been practiced in the religious arena for many years. Once learned the practice takes 10 to 20 minutes a day and can relieve the stress and tension that stands between you and a healthier life. The technique was developed by Herbert Benson, M.D. at Harvard Medical School, tested extensively and written up in his book entitled, "The Relaxation Response" (Benson & Klipper).

Despite the fact that IBS is commonly thought of as a stress related disorder, very few studies have been done to test relaxation training on relieving the symptoms of IBS. Therefore, Keefer & Blanchard (2001 and 2002) thought it would be justified to further study the effectiveness of relax response meditation (RRM) on IBS. They wrote the two articles reviewed in this section, one testing the short term effectiveness of RRM (Keefer & Blanchard, 2001) and the other a follow-up study (Keefer & Blanchard, 2002) testing IBS symptom reduction over the long term.

The initial study by Keefer & Blanchard (2001) tested the effectiveness of Benson’s (2000) RRM program in relieving the symptoms of IBS on 13 participants (9 females and 4 males), over the age of 17, recruited through local gastroenterologist and by newspaper advertisement. All participants were experiencing gastrointestinal distress at least three days per week and had received a diagnosis of IBS from their physician. Participants were excluded if they were Bipolar I or II, schizophrenic, or other psychoses, or if they were suicidal. Keefer & Blanchard hypothesized that (a) participants receiving treatment would experience a reduction in abdominal pain, diarrhea, and constipation, (b) after receiving treatment, all participants would exhibit a significant decrease in the individual GI symptoms that characterize IBS, and (c) treatment gains would be maintained at three month follow-up. Participants were then matched into pairs and
randomly assigned to either a six week meditation condition or a symptoms monitoring condition. Those assigned to the meditation condition received six, 30 minute, weekly treatment sessions. Participants in the monitoring group monitored their symptoms for six weeks and then were crossed over into the treatment group. Participants were taught, prior to treatment, how to monitor their GI symptoms in daily diaries. These diaries, along with two questionnaires designed by Keefer & Blanchard were the basis for the data collection. Participants completed the questionnaires pre and post treatment and at a 3 month follow-up. Keefer & Blanchard found that RRM was superior to the control ($p = 0.04$). They also discovered, following the RRM treatments, participant’s IBS symptoms of belching ($p = 0.02$) and flatulence ($p = 0.03$) were significantly reduced and were maintained at the 3 month follow-up ($p < 0.05$).

The follow-up study, conducted by Keefer & Blanchard (2002) was proposed to determine whether the effects of RRM on IBS symptoms reduction were maintained over the long-term. It was hypothesized by Keefer & Blanchard that, with the continued practice of the meditation technique and the incorporation of relaxation into one’s lifestyle over a one year period, it would allow clients to maintain their treatment gains and/or continue to improve. Ten of the original thirteen participants were involved in the follow-up study. Participants monitored their IBS symptoms for one week in the original GI symptoms diary and completed a brief follow-up questionnaire. The data were collected and compiled to show that RRM is a useful technique for treating symptoms of IBS, especially over the long-term.
Hypnotherapy

Hypnotherapy (also called hypnosis) comes from the Greek word hypnos, meaning sleep. Hypnosis was coined circa 1840 by Dr James Braid, a Scottish physician. Hypnotherapy distinctively attempts to address the client’s subconscious mind while, the client is in a deep relaxed state. Hypnotherapists utilize analytical techniques, attempting to uncover problems deemed to lie in a client’s past or in the client’s current life. At the same time, the hypnotherapist accesses the client’s inner resources to effect beneficial changes by stimulating the innate healing capacity of the client’s own body (http://www.general-hypnotherapy-register.com/HypnotherapyExplained.htm).

In previous research, hypnotherapy has been shown to effectively improve the quality of life and overall wellbeing in clients with IBS. However, a diminutive amount of research has been done to test the effectiveness of hypnotherapy on the extracolonic symptoms related to IBS (i.e., nausea, lethargy, backache and urinary symptoms). Gonsalkorale, Houghton & Whorwell (2002) believed that more research needed to be done. Therefore, they designed a study to provide sound data on the effectiveness of hypnotherapy in treating the extracolonic symptoms of IBS. Gonsalkorale et al. hypothesized that clients who undergo hypnotherapy to treat IBS will show marked improvement to bowel and extracolonic symptoms, quality of life, anxiety and depression. Their study included 250 clients (200 women and 50 males), over the age of 19, meeting the Rome I criteria for IBS, and having experienced symptoms for over two years. Treatment was only offered to clients attending gastroenterology clinics and whose symptoms had not responded to conventional treatments. The treatment included 12 sessions of hypnotherapy over a 3 month period and required at home practice
sessions between meetings. At the beginning and at the end of the treatment, participants completed a number of questionnaires. The questionnaires included (a) IBS Questionnaire, (b) Quality of Life Questionnaire, (c) Extracolonic Symptoms Questionnaire, and (d) Hospital Anxiety and Depression Scale. The values from these studies were calculated, and the results supported the hypothesis. IBS symptoms of abdominal pain, bloating and bowel habit disturbances, together with all extracolonic symptoms, were significantly reduced \((p < 0.01)\) after hypnotherapy and were considered to interfere with life far less than before. This decreased interference in life was evident by a marked improvement in quality of life and anxiety and depression scores.

The above study by Gonsalkorale et al. (2002) is one of many studies conducted by Dr. P.J. Whorwell and his counterparts over the years. Dr. P.J. Whorwell is a forefather in the utilization of hypnotherapy in the treatment of IBS in the United Kingdom. Galovski & Blanchard (1998) realized Whorwell’s advancements in the field of hypnotherapy in the United Kingdom and systematically replicated Whorwell, Prior, & Faragher’s (1984) study to investigate it’s applicability to the U.S. population. Galovski & Blanchard hypothesized that hypnotherapy would be effective in the treatment of IBS in the U.S. population. The sample for Galovski & Blanchard’s study included 9 women and 3 men \((n = 12)\), ranging from the ages 23-58, who had previously been diagnosed with IBS by a physician or gastroenterologist, and had been experiencing symptoms for over 6 months. The twelve subjects were split into two groups of six and randomly assigned to either a 6-week symptoms monitoring control condition or to the hypnotherapy treatment condition. The treatment condition consisted of 12 half-hour hypnotherapy sessions, weekly. Participants were also given tapes of sessions and asked
to practice auto-hypnosis on a daily basis. Two weeks prior to and at the conclusion of the sessions, participants were asked to complete two psychological tests, the Beck Depression Inventory and the State-Trait Anxiety Inventory. Throughout the duration of the treatment, all subjects also completed a GI symptoms diary. In addition, a 2-month follow-up was arranged and participants were asked to report their overall improvement per a visual analogue scale. After much examination and calculation of the test results, it was evident at a significance level of $p < .05$, that hypnotherapy was effective in the treatment of IBS in the U.S. population and remained effective at the two-month follow-up.

Quite the opposite of Gonsalkorale et al. (2002) and Galovski & Blanchard (1998), Forbes, MacAuley, & Chiotakou-Faliakou (2000) conducted a study that tested the effectiveness of autohypnosis in compared to individual hypnotherapy. Forbes et al. concern was that the lack of compliance with individual hypnotherapy was due to the considerable amount of time and money invested from the client in the therapy. Therefore, they felt that autohypnosis would be an adequate solution if effective. Forbes et al. hypothesized that autohypnosis would be just as effective as traditional hypnosis in relieving the symptoms of IBS. Fifty-two clients (37 women and 15 men), who had failed to respond to conventional treatment, meeting Manning et al. (1978) and Rome criteria for IBS, and who had been experiencing symptoms of IBS for 6 months or longer, were enrolled in the study. Participants were randomly assigned to either six sessions of individual hypnotherapy or autohypnosis. Participants completed a series of questionnaires before and after treatments, along with daily entries in a GI symptoms diary. The questionnaires included (a) Quality of Life Scale Short Form-36, (b) General
Health Questionnaire Short Form-28, and (c) Hospital Anxiety and Depression Scale.
The study concluded that autohypnosis and individual hypnosis are both valuable in relieving symptoms of IBS, but the results for individual hypnotherapy were numerically better than those for the auto hypnotherapy. Therefore, autohypnosis is less effective than individual hypnotherapy in relieving the symptoms of IBS.

The preceding studies all provide significant evidence about the effectiveness of hypnotherapy in the treatment of IBS, but they failed to establish any premise for the mechanism of its effectiveness. Therefore, Palsson, Turner, Johnson, Burnett, & Whitehead (2002) developed a study that would not only test the success of hypnotherapy in treating IBS but also investigate the physiological and psychological mechanisms behind its success. The four hypotheses Palsson et al. proposed were (a) hypnosis treatment reduces visceral pain sensitivity, (b) hypnosis improves IBS symptoms by relaxing intestinal smooth muscle, (c) hypnosis treatment reduces physiological arousal, and (d) the improvement in symptoms is mediated by reduction in the psychological trait somatization. The sample for their study consisted of 15 women and 3 men ($n = 18$) with a mean age of 38, who were diagnosed by a physician and met the Rome I criteria for IBS and had been having symptoms for more than a year. Participants who were currently taking medications for the treatment of IBS were excluded from the study. The study was conducted without a control group. Each participant received seven 45 minute sessions of one-on-one hypnotherapy for 12 weeks. The therapist conducted the sessions using verbatim script developed by Palsson et al. Participants were given hypnosis tapes and were instructed to practice hypnosis daily at home. Preceding the initiation of treatment and two weeks after cessation of treatment, participants were required to
maintain a 14 day GI symptoms diary, as well as, complete a Symptoms-Checklist-90-Revised (SCL-90R), the Beck Depression Inventory, Barostat assessment of muscle tone and rectal pain threshold, and the Stress-related Physical Symptoms Inventory. After extensive statistical analysis, the overall study concluded that there is a high response rate to hypnotherapy in participants with IBS. In regards to each of their above stated hypothesis, Palsson et al. found that (a) average rectal pain thresholds did not change significantly \( (p = .996) \), (b) smooth muscle tone remained unchanged as compared to baseline, and there was (c) no significant physiological change \( (p > .05) \) in heart rate, blood pressure, finger temperature, forehead electromyographic (EMG). However, there was a significant change \( (p = 0.01) \) in stress reactivity in skin conduction, and (d) there was a significant reduction \( (p < 0.05) \) in the SCL-90R scales for somatization, anxiety, and total number of psychological symptoms endorsed, nevertheless depression score had not significant change. Despite the already stated results, the most significant finding related to the mechanism behind hypnosis was the effect it had on normalizing stool consistency and on reducing abdominal pain. Thus, the findings suggest that hypnosis may improve IBS symptoms, primarily by altering the participant’s focus of attention and/or by changing his/her belief about the meaning of sensation arising from the GI tract. These cognitive changes persisted for 10 months, in the participants, as conveyed in a global rating scale of systems status.

Analogous to Palsson et al. (2002) first hypothesis that hypnosis treatment will reduce visceral pain sensitivity, Lea et al. (2003) study assessed the effect of hypnotherapy on visceral sensitivity, specifically rectal sensitivity, using a distention technique in hypersensitive, hyposensitive and normal sensitive IBS clients. Lea et al.
hypothesized that there would be a change in visceral sensitivity and a symptomatic improvement in clients undergoing the hypnotherapy treatments. Twenty-three individuals, diagnosed with IBS and meeting the Rome I criteria, and 17 healthy individuals, participated in the study. Individuals were excluded from the study if they drank alcohol and/or smoked excessively. Twelve-weeks of 1 hour, weekly sessions was the treatment protocol for all participants. It was also requested of participants that they practice their hypnosis skills on a daily basis using a provided audio-tape. Rectal sensitivity data were collected using participant’s sensory and motor response to distention of the rectum before and after the hypnotherapy treatment. Symptomatology, was recorded using a visual analogue scale, while anxiety and depression levels were assessed with the Hospital Anxiety and Depression Scale. The conclusion that was drawn following data analysis was that hypnotherapy significantly \( p = .04 \) improves abnormal sensory perception in irritable bowel syndrome and correlates with a reduction in abdominal pain.

The purpose of the study by Gonsalkorale, Toner, & Whorwell (2004) is interrelated to the findings from the earlier study by Palsson et al. (2002). It was revealed by Palsson et al. that hypnotherapy is correlated to a cognitive change in individuals with IBS. Gonsalkorale et al. were also proposing in their study that impaired quality of life and psychological distress in individuals with IBS may be associated with unhelpful cognitions. Therefore, they hypothesized that cognitive change would occur in a positive direction following hypnosis, resulting in improved quality of life and decrease GI symptoms in individuals with IBS. The study by Gonsalkorale et al. had a sample size of 78 clients, 62 women and 16 males, ages 17 to 69. Participants for this study were
referred from gastroenterology clinics and hospitals having met the Rome I criteria for IBS and having had symptoms for over two years and being unresponsive to previous conventional treatments. All of the participants attended twelve sessions of one-on one hypnotherapy over a three month period. Pre and post tests given to each of the participants included a validated IBS questionnaire recording IBS symptoms, extracolonic features and quality of life measures, the Hospital Anxiety and Depression Scale, and the Cognitive Scale for Function Bowel Disorder (FBD). The results for this study supported the previously stated hypothesis and showed at a significance level of < 0.01 that cognitions positively improved with hypnotherapy and that this improvement correlated with improvements in symptoms.

**Stress and Contingency Management**

Studies have shown that the symptoms of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are correlated to stress. It has been implied in previous studies that stress is an added insult to sufferers of IBS and IBD. It is difficult to refer to it as an antecedent, but a relationship has been shown to exist. It is undetermined if stress initiates the development and recurrence of these conditions or if living with these conditions creates more stress, which aggravates the symptoms and leads to a vicious cycle of symptom exacerbation. Either way, the illness related stress manifests itself in the individuals operational thought and impacts their quality of life. Thus, it is a logical assumption that behavioral therapy, such as stress and contingency management, will be effective in treating IBS and IBD and improving individual’s quality of life.

Fernandez, Perez, Amigo, & Linares (1998) believed this to be the case so they purposed a study to explore the superiority of behavioral therapy, specifically stress
management and contingency management, over conventional medical treatment and placebo condition. They also wanted to identify the most efficient treatment and the clinical or behavioral variable which would make one treatment more appropriate than another. The hypothesis was that behavioral interventions will have superior improvement rates over medical treatments. Fernandez et al. chose a study group ($n = 90$) who had been diagnosed with IBS by meeting Manning et al. (1978) criteria, had been suffering from it for over a year and did not respond to medical treatment. The study resembled the Solomon’s four group design. It involved two experimental groups the stress management group and the contingency management group. As well as, two control groups, the medical treatment group and the placebo group. Participants were randomly assigned to one of the four groups and took part in 12 weekly sessions of approximately one hour each. Prior to and throughout the duration of the treatment, participants kept an IBS symptoms diary that was used for the data analysis. A twelve-month follow-up was done via telephone conversation with the participants to determine the long-term effectiveness of the treatment. It was concluded, following data analysis, that IBS consists mainly of the acquisition of maladjusted behavior, and the techniques of behavioral modification allow the disappearance of diminution of the client’s chronic illness behavior. Anxiety and stress control techniques are appropriate in clients whose symptoms are aggravated under these conditions. Stress and contingency management, as forms of behavioral modification, are superior to conventional medical treatment in treating IBS.

A study by Milne, Joachim, & Niedhardt (1986) also wanted to investigate the beneficial effects of stress management in the same manner as Fernandez et al. (1998).
However, Milne et al. were interested in whether practicing stress management techniques would decrease disease activity and promote psychosocial functioning in individuals with IBD, rather than in individuals with IBS. The study was conducted as a randomized control trial. The study sample ($n = 80$) had all been diagnosed with ulcerative colitis of Crohn’s disease by means of endoscopic or sigmoidoscopic examination and were under active medical supervision. All individuals involved in the study completed a baseline assessment interview as well as a follow-up assessment at 4, 8 and 12 months. The assessments included three questionnaires, the Crohn’s Disease Activity Index (CDAI), the Inflammatory Bowel Disease Stress Index, and the Individual Stress Assessment Questionnaire (ISA). The individuals assigned to the treatment group attended six 3-hour classes in two individual groups of 20. In conclusion of the study, the 4, 8 and 12 month follow-up scores indicated a significant ($p < 0.05$) decrease in scores on the CDAI and the IBD stress index, indicating a decrease in disease activity. In contrast, the control group at all three follow-ups had no significant change in scores from the baseline. Therefore, this study has shown that stress management techniques may have a significant therapeutic benefit for IBD clients.

Not dissimilar to the work done by Milne et al. (1986), Garcia-Vega & Fernandez-Rodriquez (2004) prepared a study that would determine whether stress management and self-directed stress management, compared to conventional medical treatment, would provide a greater decrease in disease activity and enhance psychosocial functioning specifically in clients diagnosed by a gastroenterologist, with a non-active stage of Crohn’s disease (CD). The individuals ($n = 45$) involved in this study were pre-assessed by a gastroenterologist and then randomly assigned to one of the two treatment
groups (stress management \( n = 15 \), self-directed stress management \( n = 15 \)) or the control group (conventional medical treatment, \( n = 15 \)). The subjects underwent eight individual sessions specific to each condition. Upon completion of each treatment, session participants filled in a CD symptoms diary. Subsequent to the completion, the treatment individuals received a 6 and 12 month followed-up assessment by a gastroenterologist. Data from the pre and post assessment, as well as, from the CD symptom diaries, was analyzed, and it was concluded, at a significance level of \(< .10\), that stress management and self-directed self management are significantly more effective in reducing the symptomolgy related to disease activity and enhance psychosocial functioning, due to this symptom reduction.

**Education Modification of Health Behaviors**

It has been found, through observation, that when given an explanation and suggestions on how to manage their symptoms, as well as, provided with support, most clients with inflammatory bowel disease (IBD) irritable bowel syndrome (IBS) and develop coping mechanisms and experience, to a lesser degree, the exacerbation of these diseases and improved health related quality of life (HRQoL). Yet, prior to this study, not much has been done in the way of research to test to what degree client education and support counseling can improve the course of IBS and IBD. Colwell, Prather, Phillips, & Zinsmeister (1998) believed that education can be directed to helping clients with IBS manage their symptoms holistically through lifestyle changes in the areas of diet, stress management, and exercise. Hence, they designed a study to provide data that supported their belief. The purpose of their study was to determine what effect an outclient IBS educational class had on the short and long term outcomes of clients’ health-promoting
behaviors and symptoms and to estimate the association between health-promoting behaviors and symptoms. Fifty-two white adults, diagnosed with IBS, were referred to the study by their physicians. The average duration of symptoms for the participants was 8.4 years. The inventions involved participants attending a three hour IBS structured and planned group class, one time. Clients were educated on healthy lifestyle modification in the areas of diet, stress management and exercise. Prior to treatment and at one month and three month follow-ups, clients completed a Health Promoting Lifestyle Profile and Bowel Disease Questionnaire. Upon completion of the study and evaluation of the data, it was found that significant improvements ($p < 0.01$) were noted in all the symptoms associated with IBS. It was then concluded that a structured IBS education class for clients with IBS improves symptoms and some health promoting behaviors. The study was unable to provide a direct relationship between behaviors and symptoms.

In a very different attempt to educate and provide support, Smith et al. (2002) devised a nurses-led counseling study hypothesizing that counseling directed toward physical and psychological morbidity, in clients suffering from IBD, will improve their HRQoL. Three different sample groups were selected for this study. A large sample size of one-hundred IBD clients was recruited from a gastroenterology clinic. The IBD sample was diagnosed based on a symptoms history and confirmed with sigmoidoscopy, rectal biopsy and endoscopic features. A second sample, composed of fifty volunteers without a history of disease, was recruited as the healthy control group. The last sample group, consisting of 28 clients with psoriatic arthritis, was compiled as the disease control group. The treatment included a specific nursing led counseling package and was only given to half of the inflammatory bowel disease group, per random assignment.
Assessment procedure prior to and at 6 and 12 months after treatment included (a) Short-Form 36, (b) Anxiety and Depression Questionnaire, (c) HRQoL Questionnaire, (d) Styles, and (e) Strategies Questionnaire and Life Events Inventory. The control groups were only assessed prior to treatment and did not receive any intervention. The data analysis concluded ($p < 0.05$) that psychosocial morbidity can be improved, however, physical morbidity cannot by stipulation of nursing led counseling.

**Gentle Touch**

The two literature reviews that follow reveal evidence on a new therapeutic modality entitled healing by gentle touch. Healing by gentle touch is defined as a “non-invasive intervention that involves the gentle placing of hands on various parts of the body with the intention of promoting healing and which is complementary to medical treatment” (Weze, Leathard, Grange, Tiplady, & Stevens, 2005, p. 5). Therefore, Weze, Leathard, & Gretchen (2004) and Weze, Leathard, Grange, Tiplady, & Stevens, (2005) developed, implemented and published two studies that assessed the effectiveness of healing by gentle touch. The two studies are reviewed in this section. Both of the studies implicated the participation of Clare Weze and Helen L. Leathard as primary investigators. The initial study, which was published by Weze et al. (2004), attempted to assess the effectiveness of gentle touch on a small population of individuals who were suffering exclusively from musculoskeletal disorders. The second study, by Weze et al. (2005), was expanded in an attempt to evaluate the effectiveness of gentle touch, to include a larger sample size and a wide variety of ailments.

The initial study, developed by Weze et al. (2004), as stated previously, intended to assess the effectiveness and safety of healing by gentle touch on clients suffering from
various musculoskeletal disorders including back pain/injury, joint pain/injury, osteoarthritis, and rheumatoid arthritis. This uncontrolled, preliminary evaluation of healing by gentle touch was carried out at The Centre for Complementary Care (CCC) in Eskdale, Cumbria. All clients that had attended the centre between 1995 and 2001 were invited to participate in the study. Seventy-six clients, of which, 45 were female, 27 were male and 4 undisclosed, volunteered to participate in the study, completing a course of four one hour treatments, and contributed data to the study. The data were gathered from the 76 participants in the form of pre- and post-treatment questionnaires. The questionnaires incorporated the EuroQoL (EQ-5D), a generic state of health measure and visual analogue scales (VAS), which assesses for subjective rating of physical impairments (pain, disability, immobility, sleep disturbances, and the ability to carry out usual activity), as well as, psychological impairments (stress, panic, fear, anger, relaxation, coping ability and depression/anxiety). Pre- and post-test scores were analyzed with the Wilcoxon signed ranks tests, and the results showed VAS scores for stress, pain and disability were statistically significantly ($p < 0.001$) reduced post-treatment. Improvements in the individuals’ ability to cope, relaxation level and sleep patterns were also improved, but not significantly. The EuroQoL indicated a significant ($p < 0.001$) shift toward less severe problems with mobility, pain, usual activity and anxiety or depression. The study found no adverse effects resulting from the treatment. It was then concluded by Weze et al. that the findings indicated that healing by gentle touch is a safe and effective adjunct to conventional medical treatment, with the potential to ameliorate some of the more stressful aspects of musculoskeletal disorders.
Concurrent with the above initial study Weze et al. (2005) were spearheading another larger expanded study. This study was also intended to evaluate the effectiveness and safety of healing by gentle touch in clients attending The Centre for Complementary Care (CCC) in Eskdale, Cumbria. This expanded study continued with the same method as the previous study. Once again, all clients attending the CCC between 1995 and 2001 were invited to participate in this study. The main difference was that the 2005 study was not limited to those suffering from musculoskeletal disorders. This study was extended to subjects with a wide range of ailments. Mental health ailments were the most prevalent problems, followed by musculoskeletal conditions, cancer, myalgic encephalomyelitis, and gastro-intestinal disorders. Treatment protocol was much the same as in the initial study. Volunteers were required to complete four one hour treatment sessions within 4-6 weeks and answer pre- and post-treatment questionnaires (en suited with the EQ-5D and the VAS) after their fourth session. Three hundred clients, 194 females, 100 males and 6 undisclosed, completed the study and the questionnaires contributing the necessary data to be analyzed. Wilcoxon signed ranks tests were used to analyze the data and showed statistically significant ($p < 0.0004$) improvements in both psychological and physical functioning, particularly in stress reduction, pain relief, and increased relaxation and ability to cope. General health ratings also significantly ($p < 0.0004$) increased between study entry and end of treatment. Weze et al. then were able to draw the same conclusion as Weze et al. (2004), which is that gentle healing is safe, effective and associated with measurable improvements in the clinical features. Weze et al. also concluded that healing by gentle touch is a useful
treatment for a wide range of ailments and, in general, well-being in a substantial population of subjects.

Reiki

Reiki is another complementary care modality that has recently increased in popularity but whose medicinal value is highly under researched in the scientific literature (Mansour, Beuche, Laing, Leis, & Nurse, 1999). Reiki, according to Nield-Anderson & Ameling (2000), is the healing art of exploiting the universal energy (Chi or Ki) or life force to provide healing through passionate touch. This passionate touch is performed by the gentle lying of hands in a series of positions, on the individuals’ body, by the practitioner, in order to reconnect the individual to Universal Energy connecting to the body’s innate power of healing to promote self-healing. The significant growth in Reiki use, coupled with the anecdotal evidence of its efficacy in the literature, indicates a need for further scientific research to assure the safety and usefulness of this important complementary care modality in treating ailments (Miles & True, 2003). The two article reviews portrayed below represent current research on the effectiveness of Reiki treatment that is relevant to the present project.

Wardell & Engebretson (2001) realized that despite the popularity of touch therapies, theoretical understanding of the mechanisms of effect is not well developed, and there is limited research measuring biological outcomes. Therefore, they designed a study with the intent to test a framework of relaxation or stress reduction as a mechanism of touch therapy, exclusively 30 minutes Reiki touch therapy. Wardell & Engebretson planned to assess the effectiveness of the Reiki therapy on promoting relaxation and reducing anxiety by monitoring select psycho-social, physiological and biological
measures related to stress-reduction response, including the (a) State Trait Anxiety Inventory (STAI), (b) blood pressure, (c) galvanic skin response (GSR), (d) muscle tension, (e) skin temperature, and (f) salivary IgA and cortisol, before, during and immediately after the Reiki session. A sample of 23 essentially healthy subjects, 18 female and 5 males were conveniently selected to participate in the study. All participants received a 30 minute session of Reiki therapy. The results indicated that, when comparing before and after measures, anxiety was significantly \( p = 0.02 \) reduced, salivary IgA levels rose significantly \( p = 0.03 \), and there was a significant \( p < 0.01 \) drop in systolic blood pressure. However, salivary cortisol levels were not significantly different, and a slight increase in skin temperature and a slight decrease in muscle tension during the treatment did not equate statistical significance. Wardell & Engebretson came to the conclusion that the findings from this study suggest that Reiki therapy, correlated to both biochemical and physiological changes in the direction of anxiety reduction and relaxation promotion, warrant further research to explore these effects.

Analogous to the study by Wardell & Engebretson (2001), the preliminary study designed by Miles (2003) assessed the effectiveness of Reiki on pain and anxiety using the State Trait Anxiety Inventory (STAI). In spite of this similarity, this study did not involve the evaluation of biological correlates of Reiki. It, however, entailed a small educational program, teaching individuals with HIV/AIDS first degree Reiki, in a small inner city hospital. Miles hoped that the Reiki intervention would reduce the HIV/AIDS related pain and anxiety and hypothesized that those individuals who participated in the Reiki treatments would have reduced pain and anxiety following the treatments. The 30 participants involved in the study were referred by a physician and had HIV/AIDS related
emotional disturbances, ranging from anxiety and depression to mild psychosis. The pain reported by participants included peripheral neuropathy, gastrointestinal distress, myalgias, and headaches. In order to assess anxiety and pain levels pre-and post-treatment, participants were asked to fill out the State Trait Anxiety Inventory (STAI) and Visual Analog Scale (VAS) questionnaires. Clients were assessed on the 3rd or 4th day, following 20 minutes of Reiki treatment, to determine whether there were changes in anxiety and pain levels. After evaluation of the program and analysis of the questionnaires, it was concluded that there was a decrease in reported pain and feelings of anxiety. Miles then concluded that, not only does this study provide data on the effectiveness of Reiki treatment in alleviating anxiety and pain in HIV/AIDS clients, but it also justifies the need for designing a formal study on the efficacy of Reiki in treating pain and anxiety.

*Therapeutic Touch*

Meehan (1998) explained therapeutic touch (TT) as a form of energy work that seeks to correct bodily energy imbalances by affecting an invisible human energy field that surrounds the body. It is believed by Meehan that TT practitioners restore balance by assessing the client's energy field, clearing it, and transferring their energy to the client. This transfer of energy to facilitate healing can only take place if the human is calm, at peace, in state of openness, and having a conscious intent to express compassion without personal attachment. The majority of research on TT, to date, provides circumstantial, subjective evidence supporting its effectiveness. The research study and quality improvement study reviewed below will expand the research further to include scientific basis for TT. The first research study explores the scientific rationale for the
effectiveness of TT by not only assessing the psychosocial and physical, but specific biological measures as well. The second study is a quality improvement study that serves to represent the largest published sample size ($n = 605$) ever assessing scientifically to evaluate TT in an in-client, hospital-based setting.

The specific aim of the study by Olson et al. (1997) was to evaluate the effectiveness of therapeutic touch in reducing the adverse immunological effects of stress in a sample of highly stressed students, specifically healthy medical and nursing students who were taking professional board examinations. The long-term goals of this study were to develop methods by which a variety of stress-reduction techniques can be tested for efficacy. It was proposed by Olson et al. that the T-lymphocyte function (CD25) and immunoglobulin levels be assessed before and after the completion of TT treatments to serve as objective biological markers of stress levels. Olson et al. hypothesized that, through this objective assessment of these biological markers, it would be evident that students who underwent TT intervention would have higher levels of immunocompetence than those who did not receive the TT intervention. Specifically, Olson et al. made three detailed hypothesis related to the student immunocompetence post TT intervention. First, it was hypothesized that students who are highly anxious before professional board examinations and who undergo therapeutic touch, will have less decrease in levels of IgG, IgA, IgM, and the subclasses of IgG than highly anxious students who do not have therapeutic touch. Secondly, students who are highly anxious before professional board examinations and who undergo therapeutic touch, will have greater CD25 response to mitogens than students who do not have therapeutic touch. Finally, students who are highly anxious before professional board examinations and who undergo therapeutic
touch, will have greater response to Haemophilus vaccine than those who do not have therapeutic touch. The data were analyzed by using $t$ tests on mean differences between experimental and control groups. The results indicated that subjects who received TT and subjects who did not, had significantly ($p = 0.05$) different levels of IgA and IgM. However, the CD25 and IgG levels differed slightly in the between the two groups, but the differences were not statistically significant. Therefore, the first hypothesis was partially supported and the second was not. In regards to the third hypothesis, it was determined that no difference existed pre- and post- treatment in titers of antibodies to Haemophilus influenzae, allowing the third hypothesis to also be rejected. It was then deduced that this pilot study provided evidence to show that further study of TT, as an intervention that may be useful in reducing the adverse immunologic consequences of anxiety related to stress in otherwise healthy students, is necessary. Change in immune function related to anxiety and the relief of anxiety can be measured.

Contrasting to the above study, Newshan and Schuller-Civitella (2003) were concerned that there were no formal evaluations of in client, hospital based therapeutic touch (TT) found in the literature. They discovered multiple reports on the implementation of hospital based TT, as well as, informal discussions on the effectiveness of the treatment in reducing anxiety, stress, pain, while promoting relaxation and wound healing. Therefore, Newshan & Schuller-Civitella proposed a quality improvement study to scientifically evaluate TT in an in client, hospital based setting. Their study represented the largest published sample size ($n = 605$) ever assessing the effects of TT to date. In order to standardize the treatment protocol, their study designated that all clients received treatment from practitioners who were trained in
the Krieger-Kunz Method of TT. The majority (62%) of the clients received one TT treatment. Each treatment was maintained for varying lengths of time, depending on the need of the individual client. Clients were assessed by the practitioner, pre-and post-treatment, with the TT performance improvement tool, which included a discomfort response change and outcome response scale. These tools were intended to compare the client perceived comfort level before and after treatment, in addition to, the clients stated and observed physiological response. Clients were also requested to fill out a Client Satisfaction Survey after their second treatment. Clients were also asked to complete a client satisfaction survey following the completion of 2 or more TT treatments. Both the TT performance improvement tool and the client satisfaction survey were designed specifically for this study. Succeeding data collection and analysis, this study suggested that TT, when provided in the clinical setting, is successful in decreases anxiety, and pain while promoting comfort, calmness and a sense of well being as in the hospital.

Newshan & Schuller-Civitella surmised that TT is safe and effective when used with hospitalized clients.

**Healing Touch**

Healing Touch (HT) is an energy-based approach to health and healing. Wilkinson et al. (2002) defines HT as a holistic therapy that purports to work within the human energy system to clear blockages and restore balance in the body. This non-invasive technique utilized the hands to clear, energize, and balance the human and environmental energy fields, thus affecting physical, emotional, mental, and spiritual health and healing. It is based on a heart-centered caring relationship in which the practitioner and client come together energetically to facilitate the client's health and
healing. (http://www.healingtouch.net/ccht.shtml). The article reviewed below researched the clinical effectiveness of Healing Touch (HT) on biological and psychosocial measures.

The study by Wilkinson et al. (2002) had two primary objectives; (1) to determine the clinical effectiveness of Healing Touch (HT) on variables assumed to be related to health enhancement; and (2) to determine whether practitioner training level moderates treatment effectiveness. In order to obtain the above objective, Wilkinson et al. sampled twenty-two clients, 19 women and 3 men, without any specified differentiating diagnostic conditions, who had never experienced HT. These twenty-two clients were assigned to one of three treatment conditions: (a) no treatment; (b) Healing Touch only; and (c) Healing Touch plus music and guided imagery. Each of the clients was given the option to experience the HT in their practitioner's offices or their own home. The effect of HT on three quantitative variables was measured pre- and post-treatment. The three quantitative variables included secretory immunoglobulin A (sIgA) concentrations, self-reports of stress levels, and client perceptions of health enhancement. Each client was also requested to complete a qualitative questionnaire about their individual perceived effects of HT. Prior to the onset of the study, Wilkinson et al. hypothesized that clients participating in the treatment conditions would (a) have an increased secretory immunoglobulin A (sIgA) concentrations in saliva, (b) report their stress rating as lowered and (c) perceive an enhancement in health. All of the above hypotheses were supported after data analysis was completed. The data provided evidence that clients experienced a positive slgA change and reported a statistically significant ($p < 0.0003$) reduction of stress level. Additionally, a perceived enhancement of health was reported
by 13 of 22 clients (59%) after both HT conditions. Themes of relaxation, connection, and enhanced awareness were identified in the qualitative analysis of the HT experience. Pain relief was reported by 6 of 11 clients (55%) experiencing pain. It was then concluded that the data supports the clinical effectiveness of HT in health enhancement, specifically for raising sIgA concentrations, lowering stress perceptions and relieving pain.

Complementary and Alternative Medicine (CAM)

Complementary and alternative medicine (sometimes dubbed unconventional, non-conventional, unproven, irregular) is defined by National Center for Complementary and Alternative Medicine (NCCAM) as a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine or medicine that is practiced by medical doctors or doctors of osteopathy and by their allied health professionals (http://nccam.nih.gov/health/whatiscam/#1). Negligible amount of factual scientific evidence exists regarding a few CAM therapies, but for most, there are essential questions that need to be answered through more extensive scientific studies. Hilsden, Verhoef, Best, & Pocobelli (2003) sought out to address some of the key questions in regards to CAM therapies in the study to follow. Complementary and Alternative Medicine (CAM) was exercised by Hilsden et al. to comprise a number of elective, non-conventional therapies including acidophilus, massage, flax seed, meditation, naturopathy, tai chi, salmon oil, chiropractic, gluten free diet, imagery, aloe vera, garlic, homeopathy, and gottschall diet.
In their study, Hilsden et al. (2003) sought to determine the prevalence of the use and perceived outcomes of Complementary and Alternative Medicine (CAM) in a large, diverse inflammatory bowel disease (IBD) population. Therefore, in order to determine the prevalence of use of CAM, it was proposed to survey members of the Crohn’s and Colitis Foundation of Canada by mail, providing the study with a large, geographically diverse sample. The list of members included 4688 individuals, of which 2847 were actually sampled. In all, Hilsden et al. discovered that the CAM use was highly prevalent due to the fact that 43% of the sample was using at least one type of CAM, of whom 55% were using CAM specifically to treat their IBD. Of the above 55% who were currently using CAM for their IBD, 60% reported a significant ($p < 0.001$) increase in energy level, sense of well being, and sense of control over the disease, with a decrease in stress level and IBD symptoms, after the CAM intervention. It was then apparent that the use of CAM is very common and is generally perceived by users to be beneficial.

**Summary**

In conclusion, the two studies initially reviewed provided pertinent data on the availability, applicability, utilization and development of protocols. The remaining studies bestowed significant data supporting the usefulness of complementary care modalities, i.e., Cognitive Behavioral Therapy (CBT), Relaxation Response Mediation, Hypnotherapy, Stress and Contingency Managements, Education Modification of Health Behaviors, Gentle Touch, Reiki, Therapeutic Touch (TT), and Healing Touch (HT), in treating the symptoms of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). In addition to providing evidence that the complementary care modalities are effective in alleviating the predominate symptoms of IBD and IBS, the
associated symptoms of IBD and IBS, such as, reducing anxiety, stress, and pain, while increasing relaxation are also improved. Therefore, the reviewed research provides justification for the development of the Fecal Specimen Collection Protocol (FSCP), which will be utilized to guide stool collection to obtain calprotectin concentrations. The established FSCP will ensure an accurate measurement of calprotectin, which will be utilized as a tool in the differentiation of IBD from IBS, as well as a monitor of IBD activity, in response to Reiki treatment.
CHAPTER III

METHOD

Introduction

It is important to point out that the procedures described in this chapter are not truly Experimental Methods that are intended to lead to collection and analysis of data. In other words, this is not a research study. Rather, this chapter is primarily a narrative of the thought process of how one actually finds an appropriate method for use in a future study. According to the faculty mentor Susan L. Pocotte, Ph.D., when one is setting up a series of methods for use in a future research study, it is routine to informally query peers as to usual and customary (i.e., already known) methods and processes. This informal query is not data collection per se. Therefore, the rigor of Institutional Review Board (IRB) procedures does not apply.

The purpose of this scholarly project is to assist MCO SON faculty members in setting up a method of fecal collection without actual implementation, while at the same time learning about the theory of research process. It is intended that the fecal samples will, in turn, be analyzed for the biomarker calprotectin. There is a commercial kit available for the assay of fecal calprotectin (Appendix D). However, the kit instructions do not specify how to collect the feces from the human. Due to the fact that the collection of human feces in a health care setting is so basic, formal protocols are not easily found in the literature. All articles pertaining to human feces referenced in this paper allude to feces collected but without specification of how. The reason for this is human fecal collection is assumed to be common knowledge. However, the MCO SON faculty members who are the principle investigators on the future larger study were not
certain of this common knowledge. Therefore, it was necessary to simply ask health care professionals how to collect human feces.

The requirements of the SON scholarly project for the MSN student include a manuscript outcome. Therefore, the technical terminology of research process will be used in this chapter as a framework with which to narrate the informal query regarding how to collect human feces.

The purpose of this project, as previously stated, is to assist MCO SON faculty members in setting up a method of fecal collection without actual implementation. This was accomplished in this project through the development of a protocol for the collection of fecal specimens from individuals suffering from IBD and IBS. The developed protocol is referred to as the Fecal Specimen Collection Protocol (FSCP) and will act as a future guide in the collection of fecal specimens and aid in ensuring the prevention of sampling errors that may transpire and taint laboratory results. It is imperative that sampling errors are avoided, because it is proposed that the collected fecal specimens be analyzed to detect calprotectin concentration, that could then be utilized as a sensitive and specific marker for differentiating IBD from IBS in future studies. The devised FSCP is also essential, given as it is to be used in the near future, in a large study, to guide the collection of fecal specimens from individuals who are suffering specifically from IBD and undergoing a Reiki intervention. The proposed larger study will use the collected stool samples to assess calprotectin concentrations in the stool, prior to and subsequent to Reiki intervention, as an instrument for monitoring changes in disease activity in response to the intervention.
This larger study has been proposed to scientifically assess the effects of Reiki treatment on individual’s disease activity by measuring the calcium-binding protein, calprotectin, which has been proven to be secreted from neutrophils into fecal matter during exacerbation of IBD. Calprotectin is extremely stable in feces. Research has confirmed that the measuring of fecal calprotectin is an effective, noninvasive measure of disease activity. The concentration will provide an indication of the effects of Reiki on disease activity before and after treatment. Therefore, the constructed FSCP is vital in that it will help prevent sampling errors and can be implemented by sufferers of IBD to guide the collection of stool for analysis. Properly collected stool will allow the specimen to be used to differentiate IBD from IBS and enable the monitoring of changes in disease activity in response to Reiki intervention.

This chapter will further describe the process for the development of the protocol for the collection of fecal specimens. The description will be based on the framework of language and terms that describe research processes even though this is not an actual act of research. The research design, a description of the subjects informally queried, a narrative of the process used to query professionals, and a description of how information will be compiled and analyzed will be discussed.

Design

The process of determining appropriate experimental methods for use in a future larger research study (e.g. fecal specimen collection protocol) fits the criteria for a descriptive study design. This form of design typically uses data derived from surveys, case studies, observational strategies or more qualitative methods for gathering the information to draw informed conclusions and recommendations of the study without the
manipulation of any variables. The purpose of descriptive studies was defined by Burns & Grove (2001) as a way of developing theory, justifying current practice, making judgments, or determining what others are doing in similar situations.

Procedure

Development of the Fecal Specimen Collection Protocol (FSCP) (Appendix C) involved two phases. This first phase was primarily a narrative of the thought process of how one actually ascertains an appropriate method for stool collection that will be used in a future study. It also included the information gathered from both an informal query of health care professionals already known to the student and review of the literature as to the usual and customary (i.e., already known) methods and processes. This information served as the foundation for the proposed guidelines that will be utilized to direct fecal collection and will serve to give surety that sampling errors are prevented. Phase two consisted of compiling a list of supplies utilized in the collection of feces from the health care professionals’ relevant answers to questions from the Fecal Collection Questionnaire (FCQ) (Appendix B) and from referencing clinical manuals and catalogs. The list is referred to as the Fecal Specimen Collection Supply List (FSCSL), of laboratory instruments and materials essential to the collection and transportation of fecal matter. The completion of both phases drew to a close the development of the FSCP.

In this process, the investigator generated a list of questions that served as a guide to the informal inquiry process. The list of questions served as a means for the investigator to organize his/her fact-finding. The questions are targeted to fecal human collection methods. Some questions required the inquiry of health care professionals, while others were answered via literature. Specifically, the instruction literature for the
fecal Calprotectin assay kit will be used as a primary source (Appendix D). For purposes of manuscript presentation, the list of questions were put into a format of a fifteen open and close ended question instrument, referred to as the Fecal Collection Questionnaire (FCQ) (Appendix B). The FCQ is formatted to acquire information on what techniques and materials health care professionals are currently using to collect fecal specimens in the clinical and laboratory settings. Additionally, it was determined what materials are given to clients, how clients are instructed to self-collect specimens in their home, and what instructions they are given in order to transport material to the laboratory. This information was then analyzed, compiled and utilized to cultivate the FSCP and the FSCSL.

*Setting/Target Population*

The process of inquiry included conversations with various health care professionals (*n* = 9) known to the investigator who are employed in a hospital setting. These conversations, whether they were in person or by telephone, were conducted in quiet private settings away from other workers and clients.

*Subjects*

The subjects included a convenient sample of nine volunteers from three local hospitals. Three of the volunteers are Registered Nurses (two are Emergency Room Nurses and one is an Ostomy and Incontinent Nurse), two are Licensed Practical Nurses, three are laboratory technicians and one is an orderly. Inclusion criteria for participants included individuals who volunteered to answer the questions and have been or are currently involved in the collection, storage or analysis of fecal samples. There were no
race or gender requirements. Exclusion criteria included anyone who has not been involved in feces collection, storage or analysis.

Sample

The sample consisted of nine individuals who volunteered to participate in the project in response to verbal request by the investigators. The sample size was small because this project was a fact-finding process regarding common knowledge as opposed to implementation and/or validation of a protocol. As no quantitative statistical analysis was required, a prior power of analysis was not conducted to determine appropriate sample size.

Protection of human rights

When one is setting up a series of methods for use in a future research study, it is routine to informally query peers as to usual and customary (i.e., already known) methods and processes. This informal query is not data collection per se. It is not necessary to obtain approval by the Internal Review Board in order to conduct the present informal inquiry and its documentation as a scholarly project manuscript. The process did not involve the actual collection of fecal samples, which would necessitate direct client interaction. The entirety of this inquiry was conducted through informal conversation. The Fecal Collection Questionnaire (FCQ) (Appendix B) was not shown to the health professionals. Rather, it served as an organizational tool for the investigator. The investigators transcribed all of the volunteer’s verbal responses in the form of scripts. The volunteers were not asked to put any of their answers in writing. Neither health professional identification nor demographic information was requested. Participants were requested to volunteer their job description in order to ascertain their involvement
with fecal specimen collection, storage or analysis. The health professional engaged in
conversation maintained their right to cease conversation at any time or not answer the
investigator’s questions.

Materials

In facilitating the development of stage one, which is the actual FSCP, the
investigators not only used the information gathered from the verbal interviews, or FSCQ
(Appendix B), they also referenced a number of laboratory handbooks and manuals as a
means of obtaining additional information on formerly established protocols and
instruments, which have been used in the collection of feces. The Fecal Collection
Questionnaire (FCQ) (Appendix B) provided the primary means to explore the range of
approaches and instruments clinical and laboratory professionals have used and are using
to collect stool for analysis.

The resources that were utilized for the second phase of the scholarly project
consisted of the volunteers’ answers to questions from the FCQ, as well as, the use of
clinical catalogs that provided retail product information that assisted with the
compilation of a list of essential materials necessary for fecal collection. The catalogs
and that were referenced and utilized to determine the most economical and viable tools
necessary for stool were the Fisher Scientific International Catalog, Horizon Medical
Technologies Catalog and Allegiance SP Laboratory Products and Services, collection.

Data Collection

The informal inquiries were conducted when the investigator met with the
volunteers individually at convenient, mutually agreed upon locations or via telephone
conference. Volunteers were initially contacted via telephone to arrange the meeting
time and place or the telephone conference time. These meetings, whether they were in person or by telephone, were conducted in quiet, private settings, conducive to the answering of the questions outlined in the FCQ (Appendix B).

The data collected in the first phase began by conducting the informal inquires and transcribing the volunteers’ verbal responses to the FCQ (Appendix B) in script format. The interviews were transcribed verbatim to ensure accuracy. A review of the acquired information and interview transcriptions provided the investigators with the pertinent data necessary to construct the protocol, which includes a list of the necessary steps for fecal collection. This phase also consisted of the consultation of laboratory manuals and handbooks to amass information on previously established protocol for the collection of fecal matter. During phase two and the making of the compilation list of supplies also entailed reviewing interview transcriptions, as well as, gathering data from laboratory catalogs that retail fecal collection materials.

Assumptions

The investigators held several assumptions going into and throughout the protocol development process. First of all, when initiating the project the investigators assumed that existing information on clinical and laboratory techniques existed, which has been proven effective in obtaining fecal specimen, with little or no sampling error. Secondly, the investigators also assumed that the volunteers provided accurate answers to the FCQ on accounts of techniques, procedures, and instruments and materials used to collect fecal matter. Thirdly, the investigators assumed that their understanding and transcription of the volunteers’ verbal responses is accurate and precisely similar to the volunteers. Finally, it is implicated in this project that the development of the Fecal Specimen
Collection Protocol (FSCP) (Appendix C) and the accompanied educational resources are supportive-educative nursing agency interventions. The establishment of the FSCP permitted fecal matter to be analyzed for the presence of the biomarker calprotectin. This biomarker will possibly allow for early detection of inflammatory bowel disease (IBD). Optimistically, this early disease detection will occur prior to intense symptom manifestation and exacerbation. Then, the assumption is made that clients can receive pre-emptive support and education, possibly allowing for the initiation of the proper nursing interventions and the reduction of their self-care deficits, before exacerbation of the disease occurs.

Limitations

It is possible to note only a few limitations to this scholarly project. First of all, the investigators involved in this project were inexperienced in the informal inquiry process and therefore, may have hindered the volunteer from conveying all pertinent information. Second, due to the fact that time was limited and sample size was small, the accuracy of the information that was acquired may be skewed.

Data Analysis

The transcribed verbal responses to the Fecal Collection Questionnaire (FCQ) (Appendix B) served as the data set for analysis. Individual verbal responses to the open and closed ended questions will be scrutinized and compared with other volunteers’ verbal responses to assess for patterns and trends that may have emerged throughout the interviews. The accumulated information was then pulled together to develop the Fecal Specimen Collection Protocol (Appendix C), Fecal Specimen Collection Brochure (FSCB) (Appendix E), and Fecal Specimen Collection Supply List.
Summary

In conclusion, the overall aim of this scholarly project was to design a legitimate Fecal Specimen Collection Protocol (FSCP) and Fecal Specimen Collection Supply List (FSCSL) for the collection of fecal specimens from individuals suffering from IBD and IBS. The formulation of these protocols was necessary because the protocols will be utilized in the near future for a large study, and expectantly, this study will promote the employment of the protocols as a guide for the collection of fecal specimens. In addition to, it will expectantly serve as a guide by which, clinical practice is conducted, variations in clinical practice are reduced, quality care is enhanced, and sampling errors are lessened. In summary, this chapter described the method, research design, subjects, interview materials, collection and analysis of data that was used to achieve the development of the FSCP and the FSCSL.
CHAPTER IV

Results

Introduction

The content of this chapter describes the rudimentary demographic information obtained from the participants, the findings, and a delineation of the steps taken in the developmental process of the Fecal Specimen Collection Protocol (FSCP) (Appendix C). The answers to the informal inquiries of health care professionals, about stool collection, utilizing the Fecal Collection Questionnaire (FCQ) (Appendix B), were put in writing to ascertain repeated themes in the health care professional’s responses. This confirmation, in writing, of the narrated account of the informal discussion with health care professionals, as per the FCQ, will be presented below as the project’s findings. The transcribed informal inquires will serve as this project’s formidable set of data. This chapter will also divulge the final, noted transcript of the FSCP that is proposed to be utilized in clinical practice to guide the collection of feces. It is also important to note that the development of the Fecal Specimen Collection Protocol (FSCP) evolved in two phases. The first phase being that of the FCQ responses, literature review and the subsequent FSCP, whereas, the second phase basically consisted of a compiled list of materials, referred to as the Fecal Specimen Collection Supply List (FSCSL), and a photograph reference (Figure 2) of laboratory instruments and materials, ascertained from the FCQ responses and review of laboratory clinical catalogs. This chapter will describe both phases in detail.
Health Care Participants Demographics

Demographic information was collected from health care participants at the beginning of each discussion, via a verbal request from the investigator. This demographic information was merely utilized as a means of reassurance for the investigators on the participant’s involvement in fecal specimen collection, validating their contribution to the project. Table 2 provides the health care participants demographic information, including job description and education level.
Table 2

Demographic Characteristics of Health Care Providers (n = 9)

<table>
<thead>
<tr>
<th>Job Description and Education Level of Participants</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orderly</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Laboratory Technician</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td>Licensed Practical Nurse (LPN)</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>Bachelor of Science in Nursing Registered Nurse (BSN-RN)</td>
<td></td>
</tr>
<tr>
<td>Emergency Room Nurse</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Ostomy and Incontinent Nurse</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Masters of Science in Nursing Registered Nurse (MSN-RN)</td>
<td></td>
</tr>
<tr>
<td>Emergency Room Nurse</td>
<td>1 (11.1)</td>
</tr>
</tbody>
</table>
The health care participant’s demographics for the present project consisted of a convenient sample of nine volunteers from three local hospitals. The nine participants consisted of three Registered Nurses (33.3%), two being Emergency Room Nurses (22.2%) and one being an Ostomy and Incontinent Nurse (11.1%), two Licensed Practical Nurses (22.2%), three Laboratory Technicians (33.3%) and one Orderly (11.1%). The two Emergency Room Nurses had obtained her Masters of Science in Nursing, while the other Emergency Room Nurse and the Ostomy and Incontinent Nurse were Bachelor of Science prepared Registered Nurses. No other demographic information was obtained. All of the participant’s careers required them to be frequently involved in the collection, storage or analysis of fecal samples, thus meeting inclusion criteria. All of the participants volunteered to be involved in the project per verbal request from the investigators. All nine participants (100%) were involved in an informal discussion with the investigator and data were collected using the FCQ.

Findings

Development of the FSCP involved two phases. The first phase included the information gathered from both an informal query of health care professionals, utilizing the FCQ, and a review of the literature regarding the usual and customary methods and processes of fecal specimen collection. Phase two consisted of compiling a list of supplies utilized in the collection of feces from the health care professional’s relevant answers to questions from the FCQ (Appendix B) and from referencing laboratory clinical catalogs. The completion of both phases brought to a close the development of the FSCP.
Phase 1

In the first phase, the Fecal Specimen Collection Protocol (FSCP) was established using the investigator’s findings from the informal inquiries using the FCQ (Appendix B) and from the review of the literature. The responses to the FCQ from the health care professionals, and the information gathered from the literature will be narrated below. While reading through the FCQ inquiry recount, it is necessary to bring to mind the fact that the FCQ was designed so as to organize the investigator’s thoughts and was not structured as a means of data collection.

FCQ Question #1: Is there any protocol or official procedure that is utilized for fecal specimen collection in the hospital and at home?

All nine (100%) of the health care professionals questioned unanimously agreed that no protocol or official procedure is utilized in the clinical setting or taught to clients to guide the collection of feces. This finding was backed up in the literature due to the fact that the literature contained insufficient information about fecal specimen collection and a nonexistence of protocols or guidelines on the actual execution of fecal collection.

FCQ Question #2: Could you please detail for me the protocol or official procedure that is utilized by client for self-fecal specimen collection in the hospital and at home and that is utilized by health professional for fecal specimen collection in the hospital?

In the event that any of the participants would have answered yes to question one, they would have been asked to proceed with a detailed description of the exercised protocol or official procedure. It is evidenced above that 100% of the participants (n = 9) answered no to question one, hence 100% of the participants (n = 9) also answered no to
question two. The literature considered for question one was also pertinent here and did not depict any protocol or official procedures.

*FCQ Question #3:* What equipment do you recommend to use for collection and transportation in the hospital and at home?

The majority (88.9%) of the health care participants questioned answered this question with an assortment of answers. The amassed list of the assorted answers includes (a) a sterile container, (b) a specimen container or a sterile cup, (c) a toilet hat or a hat, (d) a bed pan or a sanitary pan, (e) a tongue blade, (f) a tongue depressor or a spatula, (g) a pair of gloves, (h) a fecal specimen container and hat combination, (i) old Tupperware, (j) old butter dishes or old cottage cheese containers, and (k) a biohazard bag. One participant (11.1%) replied that they were unsure of what equipment was used in stool collection. The literature simply implicated the use of a sterile specimen container in the collection and transportation of any laboratory specimen without specificity to the container’s contents, i.e., fecal matter, urine, blood. The clinical catalogs referenced designated specific sections for urinalysis and fecal testing. These sections did include the above items mentioned by the health care professionals, printed in distinctly different clinical terminology. Often, the clinical catalogs failed to differentiate between urine collection devices and stool collection devices, lending to the assumption that most equipment can be utilized for both urine and stool specimen collection. No containers were allocated specifically for use in gathering stool specimens for calprotectin analysis.
a. Can I see examples of the equipment?

Seven (77.8%) of the health care participants answered yes to this question and actually had specific examples available at the moment of discussion, to exhibit, allowing visualization and photographs to be taken. One (11.1%) participant did not have access to the proper collection equipment and one (11.1%) discussion was via telephone, and therefore, this question was inapplicable.

b. Can you give me specific brand names and catalog numbers for the above equipment?

The specific brand names and catalog number for the majority of the equipment was easily gained from investigating the equipment’s packaging and inscriptions. Since seven (77.8%) of the health care participants had the equipment readily available to examine, following question 3a, this information was obtained through exploration as stated above. The two (22.2%) remaining participants referred me to search laboratory catalogs and the internet for the equipment due to their inability to recollect the exact brand names at the moment of discussion. The laboratory clinical catalogs and the internet were referenced in addition to the information obtained from the collection examples presented by the participants and provided additional current products brand names and catalog numbers.

c. Do you have any extra equipment samples that I may have?

This question, in all its simplicity, perplexed a number of the participants, primarily due to their fear of crossing undefined boundaries as an employee at their specific facility. Two of the participants (22.2%) responded that they did not have any extra equipment available to give out. Three (33%) of the participants, promptly and
complacently, offered the equipment that had been exhibited as examples during the discussion of question 3a and b. Two (22.2%) of the participants needed to further check with a higher authority into the facility’s capacity to distribute free samples. One (11.1%) of the participants referred me to check with central supply in the basement of the facility or with the laboratory manager. One (11.1%) discussion was via telephone, and therefore, this question was inapplicable.

d. Do you have any extra equipment catalogs that I may have?

The majority of the participants \( n = 6, 66.7\% \) stated that equipment catalogs were not available on the units. Two (22.2%) of the participants provided me with further direction by means of forwarding me to check with central supply and purchasing in the basement of the facility or with the laboratory manager.

**FCQ Question #4:** Are there specific instructions given to clients prior to collection that guide collection in the hospital and at home? What are they?

In regards to specific instructions, no official formatted instructions were discovered to be given. Four (44.4%) of the participants stated that they gave the client no specific instruction. Five of the participants (55.6%) deemed it necessary to provide the clients with some instruction, which was apparently perceived as significant to that specific participant. The individual client instructions provided by the health care professionals included (a) Avoid urinating in the same cup as the stool. (b) Use sanitary pan. (c) Instruct client to place hat in toilet and to not void in the stool sample. (d) Instruct client to defecate into sanitary pan. Then use tongue blades to obtain specimen samples from two different sections of the feces if formed. If watery, just pour from sanitary pan into sterile specimen container. The client may also hold a sterile specimen
container to rectal area and go directly into cup. (e) Place fecal specimen container and hat combination, defecate in container, close container and bring to laboratory or doctor’s office.

a. Are there any handouts given to the client prior to collection to guide collection?

When the health care professionals were questioned about available handouts to assist clients in stool collection, nine (100%) replied that no handouts were available. The availability of visual aides in the any form was searched for in the literature, and none was found to be available.

b. Is the client instructed to void before defecating?

The participant’s response to this question was nearly divided straight down the middle. Four (44.4%) of the health care participants informed the client to void into the toilet before defecating whereas five (55.6%) of the participants did not. No preference or logic was provided in the literature to support either means of collection, with or without voiding.

c. Is the client able to extract the specimen for a bed pan, ostomy bag, or toilet?

The answers to this question, interestingly enough, could have fallen into what one would consider to be the apathetic category. As long as a specimen was obtained, 77.8% (n = 7) of the health care participants were not concerned how or from where the specimen was obtained. Two (22.2%) of the participants were unsure if the specimen was still viable if collected in any of the manners suggested in the question. The
literature did not address any tainting effects of various different locations for specimen retrieval.

d. Does it need to be a clean catch?

Any notion that fecal specimen collection is accomplished by means of clean catch was denounced in question 4c. This is determined based on the knowledge that a clean catch denotes catching the specimen in a clean container rather than retrieving it from various locations. Therefore, it was not surprising that 77.8% \((n = 7)\) of the health care participants stated that the specimen did not have to be a clean catch, 22.2% \((n = 2)\) of the participants were uncertain and one \((11.1\%)\) alleged that it was test dependent. Once again, the literature did not draw any conclusions in this interest.

e. Is the client instructed in any skin preparation prior to collection?

It was a collective decision by all nine \((100\%)\) of the health care professionals questioned that no special instruction on skin preparation prior to defecation was provided to the clients. A number of the health care participants tossed around the idea of washing the anal area prior to defecating. Nevertheless, none had ever instructed the clients in such a method. After deliberating for a moment, many of the participants concluded that washing the anal area would be suggestive of proper collective technique even though it was not implemented in clinical practice.

f. Is the client given any dietary restrictions prior to collection?

Seven \((77.8\%)\) of the participants gave no dietary restriction to participants prior to stool collection. Of these seven participants, some avowed to the fact that, depending on the test, they would examine the specimen and ask the clients about what foods they had consumed the day of or the day before the specimen was collected. One \((11.1\%)\)
participant affirmed instructing all clients not to consume any red food for two days prior to defecating, regardless of the test. One (11.1%) participant was undecided about any dietary restrictions.

\( g \). Is the client given any prescription medication restrictions prior to collection? If so, how long prior to collection should medication ingestion have ceased?

The answer to this question is similar to that of the preceding question 4f, and the proceeding question 4h, based on comparable content and stimulating similar thought analyses of the questions. Hence, seven (77.8%) of the participants gave no prescription medication restrictions prior to collection. One (11.1%) participant was undecided, even as one (11.1%) participant thought it was test dependant.

\( h \). Is the client given any over-the-counter medication restrictions prior to collection? If so, how long prior to collection should medication ingestion have ceased?

As previously stated, the answers to this question are the same as the last. Seven (77.8%) of the participants gave no over-the-counter medication restrictions prior to collection. One (11.1%) participant was undecided, while one (11.1%) participant though it was test dependant.

**FCQ Question #5:** Do any laboratory tests need to be run prior to specimen collection?

This question was hoped to be addressed specifically by the laboratory technicians due to their particular knowledge and expertise. Interestingly, two of the five (55.6%) no answers received to this question were that of the laboratory technicians. The remaining laboratory technician and a nurse (22.2%) questioned believed it was test
dependent. Two (22.2%) participants alluded to having no idea about laboratory analysis.

FCQ Question #6: Do any physiological symptoms affect enzyme levels that need to be screened for prior to collection?

An excess in the diversity of thoughts between the participants were demonstrated by the responses to this question. In the end, it is evident that none of the participants, if truth be told, are certain of the answer to this question. Many of the health care participants (n = 7, 77.8%) were unsure and made reference to the physician’s involvement in the area of physiological symptom assessment. One (11.1%) participant specifically stated, “It is the doctor’s assessment of the situation that determines the sequence of collection. I just assure that all symptoms are noted.” One (11.1%) participant was only concerned if the client was febrile, and if so, the collection is to be postponed.

FCQ Question #7: What amount of specimen is necessary for testing?

The answer to this question was discovered in the literature and was specifically, relevant to fecal calprotectin analysis. The literature, specifically the commercial kit instructions that are available for the assay of fecal calprotectin (Appendix D), stated that that approximately five grams, which is equal the weight of five paperclips or five ml, of stool is necessary for enzyme analysis. Due to the availability of the exact amount of specimen required for analysis, the participant’s answers were interesting, taken note of, and listed below. The answers included (a) A cup full, (b) approximately 2 ounces, (c) approximately 4 Tablespoons or 2 ounces, and (d) a pellet size or approximately 1 gram.
FCQ Question #8: Does stool consistency affect collection?

When asked about the consistency of the stool, the majority ($n = 6, 66.7\%$) of the participants queried were unconcerned with consistency. Three (33.3\%) of the participants stated concern over stool consistency as a determinant of ease of collection or difficulty but not as an indicator of the ability to collect.

FCQ Question #9: Is the specimen viable if stool is bloody?

The greater portion ($n = 6, 66.7\%$) of the participants did not consider the collection of bloody stool to be an obstruction to the analytical laboratory process. One (11.1\%) participant claimed to be devoid of the appropriate knowledge to answer this question, and two (22.2\%) participants firmly believed it was dependent on the test.

FCQ Question #10: Is the specimen viable if combined with urine?

The popular answer to this question was an astounding yes, with 88.9\% ($n = 8$) of the participants sure of the fact that urine contaminated the specimen. One (11.1\%) of the participants opposed the group with a belief that urine contamination did not affect the viability of the fecal specimen.

FCQ Question #11: How does the specimen need to be labeled in the hospital and at home?

Every one of the participants ($n = 9, 100\%$) questioned contributed to this question with a plethora of responses. Luckily, all of the responses recommended similar content for labeling. A compiled list of the suggested information for labeling the specimen includes (a) client’s full name, (b) date of birth, (c) client’s hospital number, (d) specimen type, (e) reason for test, (f) time of collection, (g) date collected, (h) present date, and (i) doctor.
**FCQ Question #12:** Is there a necessary time span for collection?

As far as time span of collection was concerned, two (22.2%) of the participants involved said no time span was dictated, three (33.3%) participants were not aware of any time span that was predetermined, and four (44.4%) of the participants had additional knowledge to impart. The additional knowledge consisted of the belief that stool should be collected (1) once a day (2) dependent on doctors instruction but usually once a day for 2-3 days (3) dependent on the client’s presenting symptoms and (4) specifically from the first bowel movement of the day, usually once a day.

**FCQ Question #13:** How much time can lapse between collection and testing?

Six (66.7%) of the nine participant’s responses were indeterminate as to the exact amount of time that is able to lapse between collection and testing. One (11.1%) participant was under the impression that twenty-four hours following collection was the cutoff time frame for specimen analysis. Two (22.2%) participants believed it was test dependent. The literature did not actually state a specific time frame for laboratory analysis subsequent to collection. The literature simply stated that fecal calprotectin is extremely stable in the feces for prolonged periods of time, before degradation (Roseth et al., 1997).

**FCQ Question #14:** How should the specimen be delivered to the lab if collected at home?

Information on specimen transportation was received from seven (77.8%) of the participants. Many of the same answers were recurring, such as (a) in a brown paper bag, (b) in a plastic Biohazard bag, (c) in any kind of bag they have around their home, and (d) in any plastic bag.
**FCQ Question #15:** How should the specimen be stored after collection if collected at home?

Quite a few of the participants ($n = 6, 66.7\%$) inquired of, believed that the specimen could be stored at either room temperature or in the refrigerator, which is approximately 40 degrees Fahrenheit, until transported to laboratory. One of these individuals went to great lengths to make it known that they understood that the specimen could only remain at room temperature for 24 hours, then it needed to be refrigerated. Three (33.3\%) of the participants admitted to a lack of knowledge about the method used for specimen storage. The literature provided the information to answer this question accurately. The literature states that the specimen should be hand delivered to the laboratory within four days of collection. In the event that the specimen cannot be delivered to the lab in four days, the specimen can be stored at room temperature for up to seven days. After seven days, the samples must be refrigerated at 2 degree Fahrenheit or -22 degrees Celsius. Temperatures should never exceed 30 degrees Celsius (Appendix D).

It was then determined, by the investigators, after analysis of the above listed health care professional responses to the FCQ, that a stepwise approach to stool collection is the most concise, efficient and straightforward approach to educate individuals on stool collection and, in actual fact, gather feces. The investigators also determined from the findings that, if the clients are properly educated on in-home stool collection and given the proper collection devices, in-home collection has the potential to be just as sanitary and effectual in preventing sampling errors as in hospital collection with the assistance of hospital personal. The client’s compliance, comfort levels and
erratic bowel habits were also taken into consideration, resulting in the in-home collection proposition. Therefore, all aspects of the FSCP will evolve around the procedures and processes clients will use in their home to collect stool samples. The FSCP will also detail in what mode clients are to transport the collected to stool samples to the laboratory or designated analysis location.

Phase 2

Phase two consisted of compiling a list, referred to as the Fecal Specimen Collection Supply List (FSCSL) (Table 3), and a photograph reference (Figure 2) of laboratory instruments and materials essential to the collection and transportation of fecal matter. The compiled FSCSL is revealed presently in Table 3 and the photograph reference illustrations in Figure 2.
Table 3

_Fecal Specimen Collection Supply List (FSCSL)_

<table>
<thead>
<tr>
<th>Supplies</th>
<th>Catalog or Website</th>
<th>Catalog or Purchase #</th>
<th>Prices</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fisherbrand Commode Specimen Collection System.</td>
<td>Fisher Catalog 2005/06 <a href="https://www1.fishersci.com/Coupon?gid=180758&amp;cid=1333">https://www1.fishersci.com/Coupon?gid=180758&amp;cid=1333</a></td>
<td>02-544-208</td>
<td>$166.84 + S&amp;H (Case of 60)</td>
</tr>
<tr>
<td>2. Aplicare Three Castile Soap Towelettes</td>
<td><a href="http://www.aplicare.com/familydetail.asp?ID=2100&amp;Cat1=Kit%20Component&amp;p=kit">http://www.aplicare.com/familydetail.asp?ID=2100&amp;Cat1=Kit%20Component&amp;p=kit</a></td>
<td>T-3012-1S</td>
<td>$118.73 + S&amp;H (Case of 1000)</td>
</tr>
<tr>
<td>3. Fisherbrand Specimen Bags with Biohazard Label</td>
<td>Fisher Catalog 2005/06 <a href="https://www1.fishersci.com/Coupon?gid=32742&amp;cid=1333">https://www1.fishersci.com/Coupon?gid=32742&amp;cid=1333</a></td>
<td>01-800-01</td>
<td>$133.74 + S&amp;H (Case of 1000)</td>
</tr>
</tbody>
</table>
Figure 2. Illustrations of Fecal Collection Supply List (FCSL)

Illustration 1
Commode Specimen Collection System

Illustration 2
Powder-Free Vinyl Exam Gloves

Illustration 3
Aplicare Three Castile Soap Towelettes

Illustration 4
Self-Adhesive Lab Labels

Illustration 5
Specimen Bag with Biohazard Label

Name:
DOB:
Study ID#:
Time:
Date:
The health care professional’s relevant answers, to questions from the Fecal Collection Questionnaire (FCQ) (Appendix B), pertaining to supplies utilized in the collection of feces, were reviewed, and a list was compiled. The investigators determined which supplies to incorporate into the Fecal Specimen Collection Supply List (FSCSL) (Table 3) and the FSCP Stepwise Approach to In-Home Specimen Collection (Appendix C) based on their reoccurrence to answers from the FCQ, uncomplicated nature of use, and effectiveness in collection. Two local hospital laboratories were visited and their laboratory clinical catalogs were referenced, along with websites obtained from the catalogs, to finalize the list. Prices and catalog numbers were included for use in the larger proposed study.

It was determined by the investigators that the origination of the Fecal Specimen Collection Protocol (FSCP) (Appendix C) will commence with clients visiting the laboratory, medical clinic, hospital or other facility, where by which the stool analysis will be conducted, or contact their treatment provider as a means to obtain the verbal education, written information and essential collection equipment proposed in the FSCP.

During the verbal education portion of the preliminary client contact, prior to actual stool collection, investigators have established five major points that need to be emphasized. First, clients are instructed to always collect stool from the first bowel movement of the day. This is suggested as an attempt to guarantee that the stool contents represent the full load of enzymes in the stool. Secondly, clients are informed that only a small amount of stool, approximately 5g (1 gram is equal the weight of one paperclip) or 5 ml, is necessary to perform the analysis. Therefore, clients are told to attempt defecation and stool collection even if they don’t feel that they will have a large bowel
movement. Thirdly, clients are instructed to urinate prior to the initiation of stool collection. If urine is combined with the stool sample it will taint the specimen and it will not be viable for analysis. Fourthly, clients are reminded to label the specimen with their name, date of birth, study identification number, time and date collected. If the specimen is devoid of any of the above listed labeling information, the specimen is discarded and not used for analysis. Finally, clients are instructed to store the specimen at room temperature or 72 degrees Fahrenheit, (22 degrees Celsius) and hand deliver the specimen to the lab within four day of collection to allow for a precise analysis in a timely manner. If a situation should arise that the specimen cannot be delivered to the lab in four days, clients should contact the laboratory, medical clinic, hospital or other facility where by which the stool analysis will be conducted or contact their treatment provider and inform them of the situation. The specimen can be stored at room temperature for up to seven days. After seven days the samples must be refrigerated at 2 degree Fahrenheit or -22 degrees Celsius. Temperatures should never exceed 30 degrees Celsius.

Succeeding the verbal educational intervention, clients will be given the proposed written information, including the designed Fecal Specimen Collection Brochure (FSCB) (Appendix E) which will detail the Fecal Specimen Collection Protocol (FSCP) (Appendix C) in a stepwise manner and the essential collection equipment that is delineated in the Fecal Specimen Collection Supply List (FSCSL) (Table 3). The proposed information will be utilized as a tool to guide them at home through the stepwise approach to stool collection. Furthermore, the FSCB incorporated the equipment pictures from Figure 2, with the aim to allow clients that learn through
visualization to better understand and implement the protocol. The essential collection equipment will be the actual materials the clients use to collect and transport the fecal specimens. Finally, the investigators propose that the medical staff or treatment provider at the client’s laboratory, medical clinic, hospital or other facility will read through the FSCB with the client, explaining each of the steps in the protocol, as well as, demonstrating how to make use of each of the pieces of the collection equipment, addressing any of the client’s questions or concerns.

The stepwise approach to in-home stool collection, as outlined in the FSCP Stepwise Approach to In-Home Specimen Collection (Appendix C), is transliterated below. To begin with and prior to the actual collection of the fecal matter, clients are instructed to prepare the blank white specimen label that is to be placed on the specimen container following collection. In order to properly prepare the blank, white specimen label, clients are instructed to use a permanent black marker to write their name, date of birth, study identification number, and the approximated time and date the stool was collected on the blank, white specimen label. Clients are then instructed to set the label aside for later use. Also, prior to stool collection, clients are instructed to prepare the biohazard labeled specimen transportation bag by unzipping the bag and setting it aside. Next, clients are instructed, if the urge exists, to void urinary bladder contents in to the toilet prior to stool collection and flush the voided urine down the toilet. Clients are instructed to void prior to defecation to prevent accidental urine leakage into the stool specimen, contaminating the specimen. At this point, clients are ready to prepare the Commode Specimen Collection System for collection. Clients are first instructed to unsnap the plastic lid from the internal specimen container and place it aside. Then, lift
the toilet seat and place the Commode Specimen Collection System on the toilet rim. After ensuring that the collection system is securely in place on the toilet rim, clients are instructed to put the toilet seat down on top of the Commode Specimen Collection System. After the Commode Specimen Collection System is opened and securely in place, clients are instructed to take hold of the Aplicare Three Castile Soap Towelettes package and open it. The Aplicare package contains three towlettes that will be used to cleanse the anal area. Clients are told to remove one towlette at a time and use each of the towlettes individually to gently wash the anal area from the front to back in a single stroke. Clients are informed to avoid scrubbing the anal area back and forth so not to introduce bacteria from the rectal area into the vaginal and urinary area. Defecation and the act of collecting the stool specimen can now begin. In order to obtain the stool specimen, clients are now instructed to sit on the toilet and defecate into the specimen collection system’s internal specimen container. Clients are informed that approximately five grams, which is equal the weight of five paperclips or five ml, of stool is necessary for enzyme analysis. For that reason, clients are told to attempt defecation and stool collection even if they don’t feel that they will have a large bowel movement. Next, clients are instructed to cleanse the anal area with toilet paper after defecating and place the toilet paper in toilet or waste basket but not in the internal specimen container. The stool collection is now concluded with the client washing their hands.

The clients are now instructed on how to prepare the specimen for transportation to the laboratory. Following defecation and hand washing, clients are instructed to put on a pair of the provided, vinyl free exam gloves. This is a septic technique attempting to prevent the spread of bacteria from avoiding direct contact with the feces. Clients are
instructed to pick up the lid that was removed and set aside in step one from the internal specimen container and snap it securely back on the internal specimen container. Having the lid securely on the internal specimen container prevents spilling of the fecal specimen. Now, clients are instructed to lift the toilet set and remove the entire Commode Specimen Collection System. In order to obtain the internal specimen container from the Commode Specimen Collection System, clients are informed to snap down forcefully on the white plastic supportive frame surrounding the internal specimen container. Once the frame is removed, it can be discarded. At this point, clients need to take the white specimen label containing their name, date of birth, study identification number, and the approximated time and date the stool was collected, stick it on the external side of the internal specimen container, and set the specimen container down. Clients are then instructed to remove one glove and use the un-gloved hand to hold open the biohazard specimen transportation bag. This helps to ensure that the outside of the biohazard specimen transportation bag is not contaminated with feces. Clients are now instructed to pick up the labeled internal specimen container in the hand that is still gloved and place the container into the biohazard specimen transportation bag. Clients are informed to not attempt to zip close the biohazard specimen transportation bag at this time, but simply to set the bag aside. Clients are then instructed to remove the Powder-Free Vinyl Exam Gloves and promptly dispose of them. Next, clients are told to wash their hands. Now, they can pick up the biohazard specimen transportation bag and zip it closed, making certain it is tightly closed before transporting specimen to lab. The specimen should then be hand delivered to the laboratory within four days of collection. Clients are informed that, in the event that a situation should arise that the specimen
cannot be delivered to the lab in four days, they should contact the laboratory, medical clinic, hospital or other facility by which the stool analysis will be conducted, or contact their treatment provider and inform them of the situation. The specimen can be stored at room temperature for up to seven days. After seven days, the samples must be refrigerated at 2 degree Fahrenheit or -22 degrees Celsius. Temperatures should never exceed 30 degrees Celsius.

Summary

The information gathered from the health care professionals, through informal discussions, using the Fecal Collection Questionnaire (FCQ) (Appendix B) along with the review of the literature and clinical catalogs, was scrutinized and the findings were compiled in this project. The findings indicated a definite need for the development of a protocol for stool collection. For instance, when questioned about an existent protocol in their facility for fecal specimen collection and when asked to outline a detailed description of the protocol or official procedure that is utilized for fecal specimen collection, nine (100%) of the health care professional questioned responded that no protocol was in existence, and they, therefore, could not detail the protocol utilized to guide stool collection in their facility. These findings, along with the absence of any relevant literature pertaining to fecal specimen collection, were used to support and create the developed Fecal Specimen Collection Protocol (FSCP) (Appendix C).

Chapter IV, also originally, presented several documents that were uniquely transpired for this project and as a result of the project’s findings. To briefly review, the first document that was developed was the Fecal Collection Questionnaire (FCQ) (Appendix B), which was simply a generated list of questions that served as a guide to
the informal inquiry process, formatted into a fifteen open and close ended question instrument. The second document was designed to present the demographic information of the health care professionals (Table 2) that participated in this scholarly project. The third document is the actual Fecal Specimen Collection Protocol (FSCP) Stepwise Approach to In-Home Specimen Collection (Appendix C). The fourth document was the Fecal Specimen Collection Supply List (FSCSL) (Table 3), that is merely a list of laboratory instruments and materials essential to the collection and transportation of the fecal specimen. Lastly, the Fecal Specimen Collection Brochure (FSCB) (Appendix E) was the fifth document drawn up to provide clients with a handout to guide in-home collection. The FSCB incorporates the entirety of the FSCP Stepwise Approach to In-Home Specimen Collection (Appendix C), along with the figures from Illustration 1. This chapter has provided a detailed description of the demographic information of the questioned health care providers. The finding from the inquiries utilizing the Fecal Collection Questionnaire (FCQ) and the literature and clinical catalogs reviews. Also, both phases in the development of the Fecal Specimen Collection Protocol (FSCP) were outlined, along with the related documents. Finally, the refined transcription of the FSCP that is proposed to be utilized in clinical practice to guide the collection of feces was presented. The significant and applicability of the above presented findings will be discussed further in the next chapter.
Chapter V
Discussion

It is important to again remark upon the fact that symptoms of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are chronic, annoying, episodic, and at times, debilitating. Overall, the manifested symptoms of these diseases impact every aspect of the sufferer’s life, greatly decreasing the quality (Guthrie et al., 2002; Luscombe, 2000). In addition, collectively, IBD and IBS present with very similar clinical manifestations, making differentiation difficult. The unveiling of a successful means of differentiating and treating IBD and IBS would be significant for those suffering from the incapacitating disease, as well as, those caring for these clients.

Findings

This scholarly project proposed a method to differentiate IBD and IBS, as well as, assist in the launching of a much larger study. The method proposed for the differentiation of IBD from IBS, and the initiation of the larger study, entailed a detailed method for feces collection in the form of a protocol. The developed protocol was never actually implemented during this project to collect fecal specimens. It is, however, merely a proposition for future practice. Again, it must be taken into account that protocols are detailed instructions regarding the most appropriate and accurate method to perform a specific task and are utilized as a tool to guide practice. Zellman et al. (2002) recommended protocol use in circumstances where a task undertaking necessitates quality assurance in order to provide instructions that enhance skills and ensure exactitude of practice. Currently, the literature provides no official methods or protocols utilized to guide the collection of stool samples for analysis. Therefore, developing a
protocol was exactly what was necessary to achieve the mission of this scholarly project. The development of an exhaustive protocol on fecal collection was initiated by means of amassing information by simple inquiry of health care professionals about stool collection, and the summative value of this information was then used to develop the protocol. The resulting protocol was officially designated as the Fecal Specimen Collection Protocol (FSCP) (Appendix C). The proposed FSCP can be applied, in the future, by sufferers of IBD and IBS, during stool collection, to prevent sampling errors that may taint laboratory results. The significance of a properly collected stool sample is that, following collection, the samples can be analyzed for the biomarker calprotectin, which is secreted in the stool of IBD sufferers but not those suffering form IBS. This serves as the differentiation in the two diseases. This FSCP is also momentous because it will be executed in the proposed future, larger study, to guide the collection of fecal specimens from individuals who are suffering distinctly from IBD. The future study will analyze the collected stool samples to determine concentrations of the biomarker calprotectin in the stool, prior to and subsequent to, a complementary care treatment, Reiki, as a way to monitor changes in disease activity. This larger study will hopefully validate the efficacy of complementary care intervention as a treatment option for IBD, and they will then be implemented in nursing practice with the intention of providing better care and assisting sufferers of IBD to care for themselves.

Implications for Nursing Theory

Orem’s (2001) Self Care Deficit Theory of Nursing was utilized to provide the framework for this project. The theory of self-care deficit can be summarized as the simple relationship between self-care demand and self-care agency (Orem). Basically, if
an individual is perceived to no longer have the ability to care for oneself, they are said to have developed a self-care deficit and therefore, their self-care demands are not being met. This failure to meet self-care demands implicates a deficient self-care agency and the need for a nursing agency intervention.

The application of Orem’s basic concepts to the findings of this project is projected in the following manner. Sufferers of IBD and IBS may have difficulties performing the entirety of their self-care due to the chronic nature of their illnesses. This inability to care for oneself can lead to an overall decrease in the quality of life (Guthrie et al., 2002; Luscombe, 2000; Orem, 2001). Therefore, with the possible failure of IBD and IBS sufferers to meet their self-care demands, a self-care deficit evolves, and the nursing agency intervention is required. This nursing agency intervention is necessary to facilitate IBD and IBS sufferers in improving their self-care, and subsequently, their quality of life.

The premise for the nursing agency intervention has been established above. However, in order for the nursing agency to know what are the most effective and appropriate means of intervention, the nurse must have a way of differentiating IBD and IBS, while keeping in mind that they are treated quite differently. It is also necessary to have a means to monitor changes in disease activity as a result of the nursing intervention and confirm the effectiveness of the nursing intervention. The present project made available a Fecal Specimen Collection Protocol (FSCP) (Appendix C) that is proposed to address both the need for disease differentiation and intervention monitoring. The proposed FSCP is to be implemented by sufferers of IBD and IBS following education from the nursing agency. Nurses will educate sufferers of IBD and IBS on how to
implement the FSCP during stool collection in order to prevent sampling errors that may taint laboratory results. If education is suitable and the implementation of the FSCP is followed with precision, the properly collected stool samples can be analyzed. This is significant because it has been proposed that the fecal specimens be analyzed for the biomarker calprotectin which is secreted in the stool of IBD sufferers but not those suffering from IBS. This will lead to the differentiation of IBD and IBS. It is also proposed that the biomarker calprotectin be used as a monitor of changes in disease activity in sufferers of IBD, subsequent to intervention.

The use of this provided Fecal Specimen Collection Protocol (FSCP) (Appendix C) by sufferers of IBD to guide stool collection for analysis and as a method of monitoring changes in disease activity, subsequent to intervention, is particularly notable. This is due to the fact that a larger future study has been proposed to utilize the protocol to do just that. The future study will analyze the collected stool samples to determine concentrations of the biomarker calprotectin in the stool, prior to and subsequent to, intervention. The intervention that is proposed in this larger study is Reiki. Reiki, which is a complementary care intervention, is necessary specifically because pharmacological interventions have not been shown to be effective in treating symptoms of IBD or in restoring the IBD client’s quality of life (Lea et al., 2003). It is anticipated that this larger study will assist in confirming the efficacy of complementary care interventions as a treatment option for IBD, and they will then be employed by nursing in clinical practice with the intention of imparting better care and assisting sufferers of IBD to care for themselves. It is useful for nurses to be acquainted with the efficacy of the various complementary care interventions in treating illnesses like IBD and IBS due to the fact
that the rate of use of complementary care modalities is rising in the United States, reportedly increasing from 33.8% in 1990 to 42.1% in 1997 (Eisenberg et al., 1998). The nurse has a responsibility, when acting as a client’s nursing agency, to promote self-care for client’s specific needs that are not being met by traditional means. This is often the case with sufferers of IBD and IBS. The nursing agency is responsible to be knowledgeable about new and alternative methods of intervention, such as Reiki, but the research must be available to attain the knowledge.

Implications for Nursing Practice, Education and Future Research

The findings of this scholarly project and the subsequent developed Fecal Specimen Collection Protocol (FSCP) (Appendix C) and Fecal Specimen Collection Brochure (FSCB) (Appendix E) have overt implications to nursing practice, education and further research. In relation to nursing practice and education, it will be necessary to correlate the two, for the purposes of this project. This connection between nursing practice and education is easily established based on the premise that education is an essential element of nursing practice. Identification of a client’s needs and providing the necessary supportive-educative care occurs throughout the scope of nursing practice. With this being said, client education is a primary focus of nursing practice. Client education begins at the moment of interaction with each client, providing them with an overabundance of educational information from an explanation of step-by-step procedures to medication administration. It is desired that, during this education interaction, nurses have the ability to provide the client with education materials to facilitate their learning process. It is extremely important for nurses to have these educational resources, i.e., brochures, pamphlets, booklets, etc., available to them. With
available resources nurse can contribute to a client’s ability to understand and implement the necessary care action. During the information gathering stage of this project, it was discovered that, when the health care professionals were questioned about available materials to assist clients in stool collection, 100% ($n = 9$) replied that none were available. Based on this fact, it was deemed necessary to develop a brochure that will provide clients with education materials, assisting in their retention of the information, enhancing their ability to properly collect stool specimen and aid in ensuring that sampling errors are prevented. The official brochure that was developed is referred to as the Fecal Specimen Collection Brochure (FSCB) (Appendix E) and is composed of the FSCP Stepwise Approach to In-Home Specimen Collection (Appendix C), and the figures from Illustration 1. The FSCB will not only assist the client, but it will allow nurses to perform their nursing tasks more effectively, contributing to the enhancement of their nursing practice.

Finally, in regard to further research, this project’s findings and the developed documents are essential to assist in the launch of the large study, which will make available a new knowledge base that can be implicated in nursing practice. This newly attained knowledge base will optimistically grant nurses the option to expand their practice to include complementary care interventions with the intention of providing better client care and specifically assisting sufferers of IBD and IBS to care for themselves.

**Summary**

This chapter further discussed the findings of this scholarly project as they were related to the purpose, significance and theoretical framework. Orem’s (2001) Self Care
Deficit Theory of Nursing (SCDTN) was intertwined with the findings to establish implications for nursing theory. Finally, implications for nursing practice, in combination with nursing education and further research, were also imparted.
References


Stress-Induced Immunosuppression and Therapeutic Touch. *Alternative Therapies, 3*(2), 68 - 75.


Hypnosis treatment for severe irritable bowel syndrome. *Digestive Diseases and Sciences 47*(11), 2605-2614.


Appendix A

Rome and Manning Diagnostic Criteria for Irritable Bowel Syndrome (IBS) \(^2\)\(^3\)

I. Rome II Criteria
The diagnostic criteria of IBS always presumes the absence of a structural or biochemical explanation for the symptoms. IBS can be diagnosed based on at least 12 weeks, which need not be consecutive, in the preceding 12 months of abdominal discomfort or pain that has two out of three features:
1. Relieved with defecation; and/or
2. Onset associated with a change in frequency of stool; and/or
3. Onset associated with a change in form (appearance) of stool.

Symptoms that Cumulatively Support the Diagnosis of IBS:
Abnormal stool frequency (may be defined as greater than 3 bowel movements per day and less than 3 bowel movements per week);
Abnormal stool form (lumpy/hard or loose/watery stool);
Abnormal stool passage (straining, urgency, or feeling of incomplete evacuation);
Passage of mucus;
Bloating or feeling of abdominal distension.

Supportive Symptoms of IBS:
1. Fewer than three bowel movements a week.
2. More than three bowel movements a day.
3. Hard or lumpy stools.
4. Loose or watery stools.
5. Straining during a bowel movement.
6. Urgency.
8. Passing mucus during a bowel movement.
9. Abdominal fullness, bloating, or swelling.

Diarrhea-predominant: 1 or more of 2, 4, 6 and none of 1, 3, or 5; or: 2 or more of 2, 4, or 6 and one of 1 or 5.
Constipation-predominant: 1 or more of 1, 3, 5 and none of 2, 4, or 6; or: 2 or more of 1, 3, or 5 and one of 2, 4 or 6.

Appendix A, con't.

II. Manning Criteria
Looser bowel movements with the onset of abdominal pain.
More frequent bowel movements with the onset of abdominal pain.
Pain relieved with bowel movements.
Abdominal distention.
Mucus with bowel movements.
Sensation of incomplete evacuation after a bowel movement.

3Note. Adapted from "Towards positive diagnosis of the irritable bowel syndrome,"
Appendix B

Fecal Collection Questionnaire (FCQ)

<table>
<thead>
<tr>
<th>1.</th>
<th>Is there any protocol or official procedure that is utilized for fecal specimen collection in the hospital and at home?</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Could you please detail for me the protocol or official procedure that is utilized by client for self-fecal-specimen-collection in the hospital and at home and that is utilized by health professionals for fecal specimen collection in the hospital?</td>
</tr>
<tr>
<td>3.</td>
<td>What equipment do you recommend to use for collection and transportation in the hospital and at home?</td>
</tr>
<tr>
<td></td>
<td>a. Can I see examples of the equipment?</td>
</tr>
<tr>
<td></td>
<td>b. Can you give me specific brand names and catalog numbers for the above equipment?</td>
</tr>
<tr>
<td></td>
<td>c. Do you have any extra equipment samples that I may have? If samples aren’t available, ask to photograph the equipment.</td>
</tr>
<tr>
<td></td>
<td>d. Do you have any extra equipment catalogs that I may have?</td>
</tr>
<tr>
<td>4.</td>
<td>Are there specific instructions given to clients prior to collection that guide collection in the hospital and at home? What are they?</td>
</tr>
<tr>
<td></td>
<td>a. Are there any handouts given to the client prior to collection to guide collection?</td>
</tr>
<tr>
<td></td>
<td>b. Is the client instructed to void before defecating?</td>
</tr>
<tr>
<td></td>
<td>c. Is the client able to extract the specimen for a bed pan, ostomy bag, or toilet?</td>
</tr>
<tr>
<td></td>
<td>d. Does it need to be a clean catch?</td>
</tr>
<tr>
<td></td>
<td>e. Is the client instructed in any skin preparation prior to collection?</td>
</tr>
<tr>
<td></td>
<td>f. Is the client given any dietary restrictions prior to collection?</td>
</tr>
<tr>
<td></td>
<td>g. Is the client given any prescription medication restrictions prior to collection? If so, how long prior to collection should medication ingestion have ceased?</td>
</tr>
<tr>
<td></td>
<td>h. Is the client given any over-the-counter medication restrictions prior to collection? If so, how long prior to collection should medication ingestion have ceased?</td>
</tr>
<tr>
<td>5.</td>
<td>Do any laboratory tests need to be run prior to specimen collection?</td>
</tr>
<tr>
<td>6.</td>
<td>Do any physiological symptoms affect enzyme levels that need to be screened for prior to collection? (Fever, nausea, vomiting etc.)</td>
</tr>
<tr>
<td>7.</td>
<td>What amount of specimen is necessary for testing?</td>
</tr>
<tr>
<td>8.</td>
<td>Does stool consistency affect collection?</td>
</tr>
<tr>
<td>9.</td>
<td>Is the specimen viable if stool is bloody?</td>
</tr>
<tr>
<td>10.</td>
<td>Is the specimen viable if combined with urine?</td>
</tr>
<tr>
<td>11.</td>
<td>How does the specimen need to be labeled in the hospital and at home?</td>
</tr>
<tr>
<td>12.</td>
<td>Is there a necessary time span for collection? (Once a day, week, month, in the morning, noon or night etc.)</td>
</tr>
<tr>
<td>13.</td>
<td>How much time can lapse between collection and testing?</td>
</tr>
<tr>
<td>14.</td>
<td>How should the specimen be delivered to the lab if collected at home?</td>
</tr>
<tr>
<td>15.</td>
<td>How should the specimen be stored after collection if collected at home?</td>
</tr>
</tbody>
</table>
Appendix C

Fecal Specimen Collection Protocol (FSCP)

Stepwise Approach to In-Home Specimen Collection

1. Prepare the blank white specimen label. Write on the label with a permanent black marker a name, date of birth, study identification number, and the approximated time and date the stool was collected. Set aside. Unzip the biohazard labels specimen transportation bag. Set aside.

2. If needed, void urinary bladder contents in toilet. Do not defecate. Retain all fecal matter in bowels. Flush the urine down the toilet. Voiding into fecal specimen will contaminate specimen.

3. Unsnap the lid from the internal specimen container of the Commode Specimen Collection System and place to side. Lift toilet seat and place the Commode Specimen Collection System on the toilet rim. Ensure the collection system is secure on the rim. Put the toilet seat down.

4. Take the Aplicare Castile Soap Towelettes package and open it removing one towlette at a time. Use the towlette to gently wash only the anal area from the front to back in a single stroke. Do not scrub the anal area back and forth. Repeat this with all three towlette one at a time.

5. Sit on the toilet and defecate into the specimen collection systems’ internal specimen container. Be sure to not void in the internal specimen container during defecation. Approximately 5g (1 gram is equal the weight of one paperclip) or 5 ml of stool is necessary.

6. Use toilet paper to cleanse the anal area after defecation and place toilet paper in toilet or waste basket. Do not put toilet paper in the internal specimen container.

7. Wash hands.


9. Pick up the lid that was removed in step one from the internal specimen container and snap it securely back on the internal specimen container.

10. Lift the toilet set and remove the entire Commode Specimen Collection System.

11. Remove the white plastic supportive frame from the internal specimen container by snapping it downward forcefully and discard the supportive frame.

12. Place specimen label on the outside of the internal specimen container. Set the internal specimen container aside.

13. Remove one glove and use the un-gloved hand to hold open the biohazard specimen transportation bag. Pick up the labeled internal specimen container in the gloved hand and place the container into the biohazard specimen transportation bag. Set the biohazard specimen transportation bag with specimen aside.

14. Remove the Powder-Free Vinyl Exam Gloves and dispose of them.

15. Wash Hands.

16. Zip closed the biohazard specimen transportation bag. Ensure it is closed before transporting specimen to lab.

17. The specimen should be hand delivered to the laboratory within four days of collection. The specimen can then be stored at room temperature or 72 degrees Fahrenheit, (22 degrees Celsius) for up to seven days. After seven day the samples must be refrigerated at 2 degree Fahrenheit of -22 degrees Celsius. Temperatures should never exceed 30 degrees Celsius.
Appendix D

Fecal Calprotectin Commercial Kit Contents

Appendix E

Fecal Specimen Collection Brochure (FSCB)
Abstract

The purpose of this scholarly project, using Orem’s (2001) self-care deficit nursing theory, was to develop a Fecal Specimen Collection Protocol (FSCP) that will be utilized, in a larger study, by individuals with inflammatory bowel disease (IBD). The first phase of the project included the gathering of information by both an informal query of health care professionals and a review of the literature, regarding the usual and customary methods and processes of fecal specimen collection. The second phase consisted of compiling a list of supplies, referred to by the health care professionals during the informal discussions that are utilized in the collection and transportation of feces, and from referencing clinical manuals and catalogs. The result was the developed FSCP.
PhiCal Test

A method for measurement of calprotectin in stool samples as a marker of organic disease in the intestine

For In Vitro Diagnostic Use

Intended use
The PhiCal test is a quantitative method for determination of calprotectin in stool samples and can be used as an aid in identifying patients with organic disease of the small intestine or large bowel. Functional disorders like irritable bowel disease, do not give increased fecal calprotectin concentrations.

Background
Various types of organic disease in the gastrointestinal tract cause damage to the intestinal lining (mucosa layer). Such damage may vary from increased permeability of the mucosa to inflammation and ulcerations. The bowel content is rich in bacteria and other microorganisms releasing substances which may be toxic or chemotactic, i.e. they stimulate leukocytes, in particular polymorphonuclear granulocytes (PMN) to migrate into the gut lumen where they release their contents including antimicrobial substances like calprotectin. This protein constitutes about 60% of total proteins in the cytoplasm of PMN and can reliably be estimated in small, random stool samples even after storage for seven days at ambient temperature. The concentration of calprotectin in stools reflects the number of PMNs migrating into the gut lumen. Calprotectin is a calcium and zinc binding protein produced by PMNs, monocytes and squamous epithelial cells except those in normal skin. After binding calcium it can resist degradation by leukocytic and bacterial enzymes. By competing with different enzymes for limited local amounts of zinc, calprotectin may inhibit many zinc dependent enzymes and thereby kill microorganisms or animal and human cells in culture. Different types of disease, for instance bacterial infections, rheumatoid arthritis or cancer can lead to activation of PMNs and increased levels of calprotectin in plasma, cerebrospinal fluid, synovial fluid or urine.

It is of special importance that the concentration of calprotectin in feces is correlated with the number of PMNs migrating into the gut lumen and that it can be detected reliably even in small (less than one gram) random stool samples. Furthermore, organic diseases of the bowel give a strong fecal calprotectin signal, i.e. elevations are often five to several thousand times the upper reference in healthy individuals indicating intestinal inflammation.

Patients with organic or functional abdominal disorders may have similar symptoms, and clinical examination alone may not be sufficient to give a specific diagnosis. Since further diagnostic procedures may be complex, expensive or expose the patient to pain, ionising radiation or other risks, there is a need for a simple non-invasive, inexpensive and objective method which can help in selecting patients for additional examination, for instance endoscopy. The latter normally requires general anaesthesia in children. Many studies have shown that PhiCal test can serve this purpose. Since abdominal symptoms are common both in children and adults, a negative PhiCal test can save many endoscopies and thereby also money.

Inflammatory bowel disease (IBD), i.e. ulcerative colitis and Crohn's disease may appear from early childhood to late adulthood, and the diagnosis is often delayed due to vague symptoms or reluctance to perform endoscopy and biopsy. The PhiCal test can with high probability rule out non-inflammatory disorders on the one side, and contribute to an earlier diagnosis of IBD on the other side since the test is regularly positive in active IBD. Furthermore, the PhiCal test can aid in the diagnosis of IBD relapse in patients who have been in remission.

Since calprotectin is very stable in stools, patients can collect small fecal samples at home and send to the laboratory by ordinary mail. Samples can also be frozen and sent or analysed later.

Principle of the test
The PhiCal test is based upon preparation of an extract of about 0.1 gram feces mixed with about 5 ml of extraction buffer in a closed tube for 30 minutes. After centrifugation, a sample from the supernatant is tested by an enzyme immuno assay specific for calprotectin. The immuno assay implies that samples and standards are incubated in separate microtiter wells coated with polyclonal antibodies against calprotectin. After incubation and washing of the wells, bound calprotectin (which has many antigenic sites) is allowed to react with immunoaffinity purified enzyme labelled anti-calprotectin. Thus the amount of enzyme bound is roughly proportional to the amount of calprotectin in the sample or standard, which can be determined by incubation with a substrate for the enzyme.

Detection limits
The analytical sensitivity of PhiCal test is 6.25 ng/ml, which corresponds to 15.6 mg calprotectin/kg faeces at a sample dilution of 1:2500.

Materials provided with the kit
1. Microassay Plate, 12 strips, 8 wells per strip, coated with polyclonal rabbit antibodies specific for calprotectin. The plate is stored in a sealed bag with desiccant.
2. Enzyme conjugated antibody, 15 ml alkaline phosphatase labelled, immunoaffinity purified IgG antibodies (from rabbit) against calprotectin in a buffer solution with Proclin 300 as a preservative.
4. Washing solution, 50 mL 20X concentrate, to be diluted with distilled water.
5. Sample diluent solution, 20 mL 10X concentrate to be diluted with distilled water.
6. Extraction solution, 50 mL 5X concentrate, to be diluted with distilled water. This concentrated solution is irritating to eyes and skin.
7. Standards, 5 vials with 1 ml calprotectin solution at known concentrations (6.25, 12.5, 25, 50 and 100 ng/ml). The value of each standard is printed on the vial label.
8. Control 1, one vial containing 1.0 ml. Ready to use. Do not dilute. The range of values is printed on the vial label and is expressed as ng/ml.
   Control 2, one vial containing 1.0 ml. Ready to use. Do not dilute. The range of values is printed on the vial label and is expressed as ng/ml.

Materials required but not provided

Faeces sample collection
1. Sample collection tube
2. Transport container

Faeces preparation
1. Disposable, breakable sterile inoculation loops
2. Disposable polystyrene screw cap tubes, 14 ml
3. Eppendorf tubes (1 - 1.5 ml)
4. Sensitive digital scale (40-150 mg)
5. Vortex mixer
6. Shaker
7. Microcentrifuge (10000g)
8. Freezer (-20°C)

Equipment for ELISA measurements
1. Multi-channel pipette, 50-200 µl
2. ELISA plate washer
3. ELISA plate reader (filter 405 nm)
4. Distilled water
5. Stop solution (NaOH 1M)

Precautions and warnings
1. For in vitro use only.
2. Reagents, samples and microtiter strips should be allowed to reach room temperature (20-25°C) before starting the test.
3. Wear gloves when doing the test.
4. Materials of human origin used in this kit have been tested and confirmed negative for HBsAg and anti-HIV I and II and anti-HCV antibodies. However, they should be treated as a potential biohazard, and shall be handled and disposed of according to local laboratory legislation.
5. Warning: do not interchange components from the different kit batches. Satisfactory performance of the test is guaranteed only when components from the same batch of Calprest are used.
6. Kits should not be used beyond their expiry date
7. Avoid mixing caps between reagent vials
8. Keep the bottom surface of wells clean and avoid scratching.
9. The substrate reagent contains sodium azide as preservative at concentrations lower than 0.1 % (w/w). Sodium azide is highly toxic to aquatic organisms. Avoid to empty into drains.
10. Substrate reagent should be pale yellow. The substrate is light sensitive. Store in the dark and shake before use.
11. Unused microplate strips should be re-sealed air tight in the foil bag with desiccant and stored at 2-8°C.
12. Insufficient washing of the ELISA plate can lead to erroneous values of Calprotectin due to incomplete removal of reagents. Routine maintenance of aspiration/wash system is strongly recommended.
13. When handling extraction buffer, in case of contact with eyes rinse immediately with plenty of water and seek medical advice.
14. When handling extraction buffer, wear suitable protective clothing.
15. All reagents, except for the substrate and the concentrated washing solution, contain Proclin 300 as a preservative agent below the allowed limits.

Reagent preparation

Extraction buffer
Dilute concentrated Extraction solution by adding 1 part (50 ml) of it to 4 parts (200 ml) of freshly distilled water to obtain 250 ml working solution. Mix well.

Diluent buffer
Dilute concentrated Dilution solution by adding 1 part (20 ml) of it to 9 parts (180 ml) of distilled water to obtain 200 ml working solution. Mix thoroughly.

Washing solution
Prepare the washing solution by diluting the content of the whole vial (50 ml) with distilled water to a final volume of 1000 ml.

Reagent stability (unsealed reagents)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage conditions</th>
<th>Storage time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjugate</td>
<td>2-8 °C</td>
<td>1 month</td>
</tr>
<tr>
<td>Substrate</td>
<td>2-8 °C</td>
<td>3 months</td>
</tr>
<tr>
<td>Standards</td>
<td>2-8 °C</td>
<td>1 month</td>
</tr>
<tr>
<td>Controls</td>
<td>2-8 °C</td>
<td>1 month</td>
</tr>
</tbody>
</table>

Stability of working solutions

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage conditions</th>
<th>Storage time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washing solution</td>
<td>20-25 °C</td>
<td>7 days</td>
</tr>
<tr>
<td>Extraction solution</td>
<td>2-8 °C</td>
<td>3 months</td>
</tr>
<tr>
<td>Dilution liquid</td>
<td>2-8 °C</td>
<td>7 days</td>
</tr>
</tbody>
</table>

1. The expiry date is printed on all component labels.
2. Avoid exposure to high temperature, direct sunlight or extreme humidity.
3. Unused microtiter strips resealed air tight in the plastic bag with the drying pad inside can be stored for one month.
Faeces sample collection and preparation

1. Collect approx. 1-5 g stool and place it in a suitable container.
2. Stool sample is sent or delivered to the laboratory within 4 days.
3. Temperatures during transport should never exceed 30°C.
4. Store faeces samples at -20°C.
5. Thaw frozen stool samples at room temperature.
6. Weigh (tare) the empty screw cap tube together with the inoculation loop.
7. Take out approx. 100 mg (between 40 - 120 mg) faeces by means of the inoculation loop, and place into a screw-cap tube.
8. Weigh tube and loop with faeces and calculate net faeces weight (between 40 - 120 mg).
9. Break off the loop handle, leaving the loop with faeces and a 4-6 cm handle inside the screw cap tube.
10. Add pre-diluted extraction buffer (weight/volume ratio 1:50), e.g. 100 mg faeces + 4.9 ml diluted extraction solution. (See table in the Technical advice and customer service). Close the tube.

11. Shake or mix vigorously for 30 seconds by means of a mixer.
12. Homogenise 25 ± 5 minutes on a shaker or roller. The loop inside the tube will act as an agitator.

13. Transfer the homogenate (1 ml) to an Eppendorf tube and centrifuge for 20 min. at 10,000g at RT using a bench-top centrifuge (e.g. Heraeus Biofuge 13).
14. Transfer 0.5 ml of the clear extract supernatant to new Eppendorf tube.
15. The extracts may be tested immediately. Extracts may be stored (max. 3 months at -20°C) for later measurement.

Procedure

1. Ensure that all reagents reach room temperature (20-25°C).
2. Thaw frozen samples at room temperature.
3. Dilute samples 1:50 (20 μl sample + 980 μl dilution buffer). For further dilution of high concentration samples, dilute the sample to a final 1: 250 dilution (e.g. 200 μl of the 1:50 dilution + 800 μl dilution buffer).
4. Suggested plate layout in duplicates is shown below. Fit the strip holder with the required number of micro ELISA strips. Use uncoated strips to complete the strip holder if the washer requires a full plate. Blank, standards and controls must be included in each run.
5. Add 100 μl of Dilution buffer to wells A1-B1 (blank).
6. Add 100 μl of each standard in duplicate wells (C1-D1, E1-F1, G1-H1, A2-B2, C2-D2).
7. Add 100 μl of each control in duplicate wells (E2-F2, G2-H2).
8. Mix diluted sample well before application to the plate and add 100 μl of each sample in duplicate wells (A3-B3, C3-D3, ...).
9. Cover plate with plate cover and incubate the plate at room temperature for 45 ± 5 min.
10. At the end of the incubation time, wash the plate by adding 0.4 ml of diluted washing solution to each well. Remove as much liquid as possible. Repeat this step two more times up to a total of 3 washing steps. Avoid blocking of aspiration or filling probes. After the final aspiration, invert plate and tap gently on absorbent tissue to ensure complete removal of washing solution.

11. Add 100 µl conjugate to each well.

12. Cover plate with plate cover and incubate the plate at room temperature for 45 ± 5 min.

13. Repeat washing step as above (see 10).

14. Add 100 µl substrate solution to each well.

Note: a multi-channel pipette is recommended in order to avoid variation in substrate development time. Avoid also the formation of bubbles during substrate pipetting.

15. Incubate the plate at room temperature for approx. 30 minutes in a dark place or wrap the plate with an aluminium foil.

16. Read the O.D. values by means of an ELISA reader at 405 nm. When standard 5 reaches an OD value between 1.2-1.5, the reaction should be read with an automatic EIA reader or stopped by adding 100 µl NaOH 1M stop solution. Plates stopped with NaOH 1M may be stored at 4°C for 24 hours.

Assay calibration and quality control
1. A new standard curve is used with each run.
2. The plate background should be < 0.15 OD.
3. Control 1 and 2 are to be included in each run.

Calculation of test results
Calculate the mean optical densities (OD) of all duplicates. Subtract the mean Blank OD from all values. Plot the standard curve with actual calprotectin concentration of standards as ng/ml and corresponding mean OD values on an xy system. The readings of the Samples from the Standard curve is corrected for the dilution and converted to mg/kg by multiplying 2.5 (e.g. a reading of 50 ng/ml becomes 125 mg/kg). If samples are further diluted this must be compensated for during calculations. Concentrations can also be determined by use of a computer linked to the ELISA reader.

Reference values
Clinical studies\(^{(2,5,15,17)}\) gave the following values:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Median (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal healthy reference</td>
<td>25</td>
</tr>
<tr>
<td>Colorectal cancers</td>
<td>350</td>
</tr>
<tr>
<td>Inflammatory bowel disease (CD &amp; UC)</td>
<td>1722</td>
</tr>
</tbody>
</table>

Interpretation of results
Samples giving values above 50 mg/kg are regarded as having a positive PhiCal test. In healthy adults the median value is about 25 mg/kg. The median value in patients with colorectal cancers is about 350 mg/kg. Patients with active, symptomatic inflammatory bowel disease have levels between 200 and 20 000 mg/kg.

References

**Developed by:**
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Under US patents 4,833,074; 5,455,160; and 6,225,072 B1.
PhiCal is a US registered trade mark, Reg. No. 1,932,460.

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**PhiCal Test**
96 tests, code 9032
Date of preparation:
Doc PhiCal 001 / Rev 1 /03.02.05
**Technical Advice**

**Preparation of stool samples**

**Weighing and sample dilution**
For a rapid sample dilution regime the table below gives the volume of extraction solution added to a given amount of stools (weight + volume = 1+49).

<table>
<thead>
<tr>
<th>Stools (mg)</th>
<th>Extraction solution (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>5.9</td>
</tr>
<tr>
<td>115</td>
<td>5.6</td>
</tr>
<tr>
<td>110</td>
<td>5.4</td>
</tr>
<tr>
<td>105</td>
<td>5.2</td>
</tr>
<tr>
<td>100</td>
<td>4.9</td>
</tr>
<tr>
<td>95</td>
<td>4.7</td>
</tr>
<tr>
<td>90</td>
<td>4.4</td>
</tr>
<tr>
<td>85</td>
<td>4.2</td>
</tr>
<tr>
<td>80</td>
<td>3.9</td>
</tr>
<tr>
<td>75</td>
<td>3.7</td>
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<tr>
<td>70</td>
<td>3.4</td>
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<tr>
<td>65</td>
<td>3.2</td>
</tr>
<tr>
<td>60</td>
<td>2.9</td>
</tr>
<tr>
<td>55</td>
<td>2.7</td>
</tr>
<tr>
<td>50</td>
<td>2.5</td>
</tr>
<tr>
<td>45</td>
<td>2.2</td>
</tr>
<tr>
<td>40</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**ELISA assay procedure**

**Washing**
1. Avoid blocking of aspiration probes.

**Pipetting**
1. Use disposable tips.
2. Avoid contamination of the pipette.
3. Avoid air bubbles in the tips.

**Substrate**
1. Store substrate working solution in the dark.
2. Colour of substrate working solution should be colourless or pale, not yellow.
3. Use separate reservoir for conjugate and substrate if different than originals vials.
4. Change vessel between each plate. Use multichannel pipettes to eliminate variations in substrate development time. Avoid carry-over from conjugate to substrate if only one pipette is used.

**Conjugate**
1. Mix content of vial prior to use (do not shake).

**Extraction and homogenisation**
1. Vortex mixing time should be as accurate as possible (30 seconds).
2. Shaking time not shorter than 20 minutes. Reduce delay before pipetting.
3. Recommended batch sizes: 10, 20, 30 or 40 samples
4. Centrifugation: for ease of harvesting and handling, make sure the pellet is compact at the bottom of the centrifuge tube.
5. Do not change centrifugation time and g force. Check g force and rpm.

**Harvesting of faeces extract supernatant**
1. Harvest the top half of the clear supernatant, approx. 500 µl.
2. Avoid contact with the pellet as aggregates or particles can cause erroneous calprotectin values.