Diagnostics of Inflammatory Bowel Disease using Fecal Microbiota

Diagnostic Markers and Commercial Potential

Caroline Jevanord Frøyland

Master Thesis in Applied & Commercial Biotechnology

Faculty of Education and Natural Sciences

HEDMARK UNIVERSITY COLLEGE

2010

Preface

This master thesis was performed at the Hedmark University College in the period between October 2009 and November 2010. It has been a pleasure working with the thesis and it is pleasant to learn that the work and recommendations presented, have resulted in consideration of a patent application and filing of complain against a US patent application.

The thesis could not been accomplished without the help and support from a number of people which I would like to thank. First I would like to express my sincere gratitude to my advisor, Professor Knut Rudi, for his always positive and encourage guidance and support of my graduate study. I am also grateful to my thesis advisor, Professor Lars Monrad-Krohn, for advice and dedicated interest on the commercial part of the thesis.

Employees at Ahus represented by Morten Vaten are thanked for preparing and delivering the patient material. Patients participating in the study should also be thanked. I hope and believe the scientific community together with commercial companies soon will develop and provide better diagnostic tools and treatment for inflammatory bowel disease.

Additionally I want to thank Genetic Analysis AS for allowing me to write this thesis. Ragnhild Nestestog from whom I have learned the experimental skills and Morten Isaksen are thanked for valuable advices.

My fellow class mates should also be thanked for an unforgettable study time.

Special thanks are given to my dear son, Henrik. Finally Sturla, my dear husband, should be thanked. The thesis would not have be accomplished or even started without his great support.

Abstract

Establishing the diagnosis of Inflammatory Bowel Disease (IBD) with its two main sub forms Crohn's Disease (CD) and Ulcerative colitis (UC) are based on medical history, clinical evaluation, laboratory tests, endoscopy, radiology and histology. However no gold standard exists. The lack of appropriate diagnostic tools leads to delayed and incorrect treatment of IBD patients. A substantial amount of patients diagnosed as CD are later reclassified as UC and opposite. Also the type of colitis remains unclassified in many patients. In addition, non-IBD patients presenting with similar symptoms as IBD are unnecessarily investigated with invasive tests leading to increased hospitals costs.

The cause of IBD is not yet completely described, but most evidence points to a combination of genetic predisposition, immunological factors, environmental triggers, and gastro intestinal (GI) microbes. However, neither the types of microbes responsible for the diseases nor changes in the microbiota as a result of the diseases have been sufficiently identified. The aim of this thesis was to evaluate the potential of using the fecal microbiota for IBD diagnostics. This was achieved through a combination of a literature study, lab study and investigations of the commercial potential including a patent search.

The literature study revealed conflicting evidence related to the amounts of bacteria in IBD patients relative to controls. Nevertheless, a majority of the articles agreed in decreased amounts of Clostridia species and increased amounts of Gammaproteobacteria species in the GI microbiota of IBD patients.

The lab study comprised an evaluation of a genetic test, GA-map™, commercialized by Genetic Analysis AS (GA). By using variable regions in the 16S rRNA gene**,** simultaneous detection and identification of multiple bacteria in a complex mixture of DNA is possible. Probes and analytic methods are suitable for several types of diagnostic tests among other IBD. A sequence analysis of fecal samples from 152 IBD patients and 105 non-IBD controls was performed. Significantly a probe detecting increased relative amounts of Proteobacteria and Bacteroidetes species was identified as a new possible diagnostic test for CD patients.

A search in European and American patent databases revealed several patents related to IBD diagnostics. Especially important, a patent application from George Mason University

comprised claims referring to IBD diagnostics by using the microbial community of the digestive tract and lumen. If issued, this application could influence the freedom to operate to companies focusing on bacterial markers in IBD diagnostics. Few other patents or patent applications from the search query include claims for identification of bacteria in fecal samples.

A concluding remark from examining the commercial potential of IBD diagnostics in this thesis is to tailor make a diagnostic test separating IBD from irritating bowel syndrome (IBS), a common functional disease frequently confused with IBD. Based on estimations of price and profit per test, a €7Mill research budget was recommended for the normal case scenario. Finally it is concluded that development of a diagnostic test based on fecal microbiota has a commercial potential within the proposed framework.

Table of Contents

Appendices

1. Glossary and Abbreviations

1.1 Glossary

16S rRNA gene	The 16S ribosomal RNA gene is a subunit of prokaryotic ribosomes frequently used in phylogenetic studies due to its highly difference between conserved regions in bacteria and archaea	(Pei, et al., 2009).
Appendicectomy	The surgical removal of appendix	(History of medicine, 2010)
Colon	The last part of the digestive system	(SEER Training Modules, 2010)
Commensal	Symbiotic relationship between two organisms where one organism benefits while the other neither benefit nor is harmed	(Baker, Love, & Ferguson, 2009)
Cytokine	Any of a group of small, short-lived proteins that are released by one cell to regulate the function of another cell, thereby serving as intercellular chemical messengers	(Cytokine, 2010)
Diagnostic sensitivity	The percentage of persons who have the disorder of interest correctly detected as such by the assay	(Saah & Hoover, 1997)
Diagnostic specificity	The percentage of persons who do not have the condition of interest correctly detected as such by the assay	(Saah & Hoover, 1997)
Dysbiosis	The condition of having microbial imbalances on or within the body	(Baker, et al., 2009)
Etiology	The cause, set of causes, or manner of causation of a disease or condition	(Oxford Dictionary of English, 2010)
Fissure	Crack-shaped wounds	(Engelsk medisinsk ordbok, 2010)
Fistula	An abnormal or surgically made passage between a hollow or tubular organ and the body surface, or between two hollow or tubular organs	(Oxford Dictionary of English, 2010)
Freedom to operate	Used to describe that testing or commercializing of a product, can be done without infringing valid intellectual property rights of others	(Nagori & Mathur, 2009)
Functional disease	A disease in which there is an abnormal change in the function of an organ, but no structural alteration in the tissues involved	(Functional disease, 2010)
Homeostasis	Any self-regulating process by which biological systems tend to maintain stability while adjusting to conditions that are optimal for survival	(Homeostasis, 2010)
Ileum	The final and longest segment of the small intestine	(Ileum, 2010)
Incidence	The number of new individuals who contract a disease during a particular period of time	(Engelsk medisinsk ordbok, 2010)
Infection	The detrimental colonization of a host organism by a foreign species. The process of infecting or the state of being infected	(Engelsk medisinsk ordbok, 2010) (Oxford Dictionary of English, 2010)
Inflammation	Inflammation is part of the non-specific immune response that occurs in reaction to any type of bodily injury	(Ferrero-Miliani, Nielsen, Andersen, & Girardin, 2007)
Microbiota	The micro-organisms of a particular site	(Oxford Dictionary of English, 2010)
Mucosa	A thin membrane that cover the inside of e.g. the digestive tract.	(Engelsk medisinsk ordbok, 2010)
Pathogenesis	The manner of development of a disease	(Oxford Dictionary of English, 2010)
Prevalence	A measurement of all individuals affected by the disease within a particular period of time	(Engelsk medisinsk ordbok, 2010)
Proctitis	Inflammatory infection of the anus and rectum	(proctitis, 2010)
Relapse	Renewed flare, recurrence	(Medisinsk ordbok, 2010)
Remission	A temporary diminution of the severity of disease or pain	(Oxford Dictionary of English, 2010)
Toxic megacolon	An acute non-obstructive enlargement of the colon, seen in advanced UC and CD.	(Farthing, 2003)

1.2 Abbreviations

16S	16 Svedberg (sedimentation coefficient)
23S	23 Svedberg (sedimentation coefficient)
Ahus	Akershus University Hospital
AIEC	Adherent invasive E. coli
ANCA	Nuclear anti-neutrophil cytoplasmic antibodies
ANOVA	Analysis of variance
ASCA	Anti-Saccharomyces cerevisiae antibodies
bp	Base pair
cANCA	Classical anti-neutrophil cytoplasmic antibodies
CARD15	Caspase recruitment domain family, member 15
CCD	Carge-coupled device
CD	Crohns disease
cDNA	complementary DNA
CRP	C-reactive protein
CT	Computed tomography
CV	Coefficient of variation
ddNTP	dideoxynucleotide
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
ECCO	European Crohn's and Colitis Organisation
EPO	European Patent Office
ESR	Erythrocyte sedimentation rate
EXO I	Exonuclease I
GA	Genetic Analysis AS
GI	Gastro intestinal
HS	Healthy subjects
IBD	Inflammatory bowel disease
IBDU	Inflammatory bowel disease unclassified
IBS	Inflammatory bowel syndrome
IPR	Intellectual property rights
MAP	Mycobacterium avium spp. paratuberculosis
MRI	Magnetic resonance imaging
MWW	Mann-Whitney-Wilcoxon-test
NADPH	Nicotinamide adenine dinucleotide phosphate-oxidase
NASBA	Nucleic Acid Sequence Based Amplification
NCF	Neutrophil cytosol factor 4
NOD2	Nucleotide-binding-oligomerisation-domain2
OD	Opposition division
pANCA	Peri-nuclear anti-neutrophil cytoplasmic antibodies
PCR	Polymerase chain reaction
PNA	Peptide nucleic acid
RFU	Relative fluorescence unit
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
ROI	Return on investment
SAP	Shrimp alkaline phosphatase
SBFT	Small bowel follow-through
SCFA	Short-chain fatty acid
SD	Standard deviation
SNE	Single nucleotide extension
Spp.	Species (plural)
SSU	Small subunit
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor-alpha
T-RFLP	Terminal restriction fragment length polymorphism
TTGE	Temporal temperature gradient electrophoresis
UC	Ulcerative colitis
USPTO	United States Patent and Trademark Office

2. Problem description

The adult human gastro intestinal (GI) tract is 9 meters long and refers to the structures from the mouth to the anus. It plays an important role in human health where the main functions are digestion of food ensuring nutrition to the body, absorption of water and excretion of waste material (Dahl & Rinvik, 2010). Several diseases and conditions can affect the GI tract, one of these are Inflammatory Bowel Disease (IBD).

Consisting of Crohn's Disease (CD) and Ulcerative Colitis (UC), IBD are relapsing and chronic inflammation of the gastro intestinal tract. The symptoms can be diarrhea, rectal bleeding, abdominal pain, weight loss, fever, fatigue, and/or anemia. No cure exists (D. C. Baumgart & Sandborn, 2007). The cause of IBD has not been found, but most evidence points to a combination of genetic predisposition, immunological factors, environmental triggers and conditions, and GI microbes (Sartor, 2006).

Diagnostics of IBD is comprehensive. The current IBD diagnostics are mainly based on medical history, clinical evaluation, laboratory tests, endoscopy, radiology and histology (E F Stange, et al., 2006; E. F. Stange, et al., 2008)

Several conditions can be confused with IBD. Patients having diffuse symptoms as abdominal pain and/or diarrhea represent a common diagnostic challenge for primary doctors (Dubinsky, Johanson, Seidman, & Ofman, 2002). As many as 20% of the population in the western world have these symptoms (Suleiman & Sonnenberg, 2001). Most patients suspected to have IBD and undergo standard invasive tests (e.g. endoscopy) have a functional disease, and the majority of these have Irritable Bowel Syndrome (IBS) (Dubinsky, et al., 2002). Up to 40% of patients undergoing endoscopy have IBS, and this accounts for 0,5% of the yearly total health care cost in the US (Camilleri & Williams, 2000). In addition, it is difficult to distinguish CD from UC. Approximately 10% of the IBD patients are misclassified (Reese, et al., 2006). Furthermore, in 10-15% of the IBD patients the type of colitis remains unclassified (Nikolaus & Schreiber, 2007). The development of a noninvasive test that can screen for IBD and separate CD from UC is of high value. By recognizing the patients in whom invasive diagnostic testing can be avoided, it would both gain the patients health and reduce the unnecessary resource use and costs (Dubinsky, et al., 2002). Despite of this there is no such test existing today.

Increasing amounts of evidence show disturbed GI microbiota in IBD patients. A comprehensive culture-independent study revealed significant differences between microbiota of small intestine from CD, UC and non-IBD samples (Frank, et al., 2007). Studies using fecal samples indicate differences in the microbial communities between IBD patients and controls, but the relevance of fecal bacteria in IBD pathogenesis and diagnostics remains unclear (Swidsinski, et al., 2002; Swidsinski, Weber, Loening-Baucke, Hale, & Lochs, 2005). If a dysbiotic pattern in feces could be identified in IBD patients, it can be utilized as a non-invasive diagnostic test. But controversy exists nevertheless, whether mucosal or fecal microbiota are suitable for IBD diagnostics (Bibiloni, et al., 2008; Swidsinski, et al., 2002; Swidsinski, et al., 2005; Tannock, 2008).

A major challenge in microbial diagnostics is the simultaneous detection and identification of multiple bacteria in a complex mixture. Utilizing DNA based methods for this purpose has been a solution, but still many challenges exist. Genetic Analysis AS (GA) is a Norwegian company which is trying to solve this problem. A new method for parallel detection of huge amounts of different bacteria is being commercialized. The method, called GA-map™ is principally based on the detection of nucleic acid sequences in the 16S rRNA gene. The gene consists of conserved universal regions and variable regions. Microbiota DNA is isolated from stool samples. Using primers to the universal regions of 16S rRNA the sequence is amplified by PCR followed by binding of oligo nucleotide probes to the variable regions of the sequence. Next bound probe is selectively labeled. Subsequently the probes are hybridized to a known sequence on a microarray and finally detected. So far neither bacteria nor probes for an IBD test have been identified.

GA is now evaluating the possibilities of commercializing an IBD test using the GA-map™ technology. Prior starting research and development of a diagnostic test, investigating the commercial potential is important, especially to companies. Challenges with the current diagnostics and more general evaluations toward whether it is a product which is needed has to be identified. An indication whether a good return on investment (ROI) can be expected has to be calculated. This can be achieved through identification of the number of patients, expected market share, estimated profits and a proposal of research budget.
In biotechnology patenting is important. Ahead of commercializing a diagnostic test, search for other inventive tests is important. Freedom to operate and possibilities for strong intellectual property rights (IPR) have to be assessed.

3. Purpose and aim

The purpose of this master thesis was to find the diagnostic and commercial potential of using fecal microbiota in IBD diagnostics.

The specific aims and ways to achieve these were:

-Reveal challenges in the current diagnostics of IBD.
This was done through identification of ways to diagnose IBD and related issues, assisted by the literature currently available on the subject.

-Identify gastro intestinal bacteria suitable for IBD diagnostics.
This was accomplished by a literature study classifying amounts of fecal and mucosa associated bacteria as increased, equal or decreased relative to controls.

-Evaluate whether selected bacterial probes can be used for IBD diagnostics on fecal samples.
The probes were selected based on the literature study. A lab study was then performed, analyzing sequences from 152 IBD patients and 105 non-IBD controls.

-Reveal the commercial potential of improved IBD diagnostics.
A calculation method for a research budget in order to assure a reasonable ROI was established. This included identifying the number of possible tests sold yearly, and estimations of market share and profit.

-Identify European and American patent documents relevant to IBD diagnostics.
This was accomplished by initially finding an optimal search profile by performing an iterative search processes. Next the search query was submitted in the Esp@cenet and the United States Patent and Trademark Office (USPTO) patent databases respectively.

4. Inflammatory bowel diseases

IBD is a chronic disease that affects the GI tract. Two major types are CD and UC. CD and UC have several clinical and pathological differences, but many similarities exist (Bouma & Strober, 2003). As previously described sufferers might experience several GI symptoms, including diarrhea, rectal bleeding, abdominal pain, weight loss, fever, fatigue, and/or anemia. Extra intestinal manifestation such as joint, skin and eye disorders might also be present (E F Stange, et al., 2006).

IBD can cause lethal conditions such as perforation, rectal bleeding not responding to treatment and toxic megacolon. Increased mortality has been shown in CD, but not in UC. Patients also have an increased risk of colon cancer after 8-10 years of diagnosis. But the overall survival has improved. In the 1950s only 80% of the patients survived a 10 year period, today the patients have a normal life expectancy (D. C. Baumgart & Sandborn, 2007; Shanahan & Bernstein, 2009).

The conditions consist of periods of remission and relapse. The time between flare-ups can be weeks or years, but some patients have persistent symptoms (E F Stange, et al., 2006; E. F. Stange, et al., 2008).

CD affects the whole thickness of the bowel wall. It can involve any part of the GI tract from the mouth to the anus, particularly the most distal part of the small intestine (ileum) as well as the proximal part of the large intestine (colon) (*Figure 1*). Unlike UC, there may be unaffected bowel between areas of active disease called skip lesions. Severe complications may include narrowing of parts of the intestine (strictures), abnormal tunnels that connect organs (fistulas) and cracks in the anal skin (fissures) (Figure 2) (D. C. Baumgart & Sandborn, 2007).

UC is characterized by diffuse inflammation of the colonic mucosa, the innermost layer that is in direct contact with the fecal flow. The disease always starts in the rectum (Figure 1 and *Figure 3*) and can extend as a continuous inflammation to the whole length of the colon (Tannock, 2008). However, some patients develop inflammation in the ileum and in 10-15% of the cases CD or UC cannot be distinguished (Nikolaus & Schreiber, 2007).

Although microbial pathogens have been postulated to cause CD and UC since their original descriptions (Sartor, 2008), it is now generally accepted that human IBD pathogenesis arises from, and is perpetuated by, interactions between host genetic and immune factors, environmental triggers, and GI microbes as previously described (Frank, et al., 2007; Sartor, 2008; Underhill & Braun, 2008).

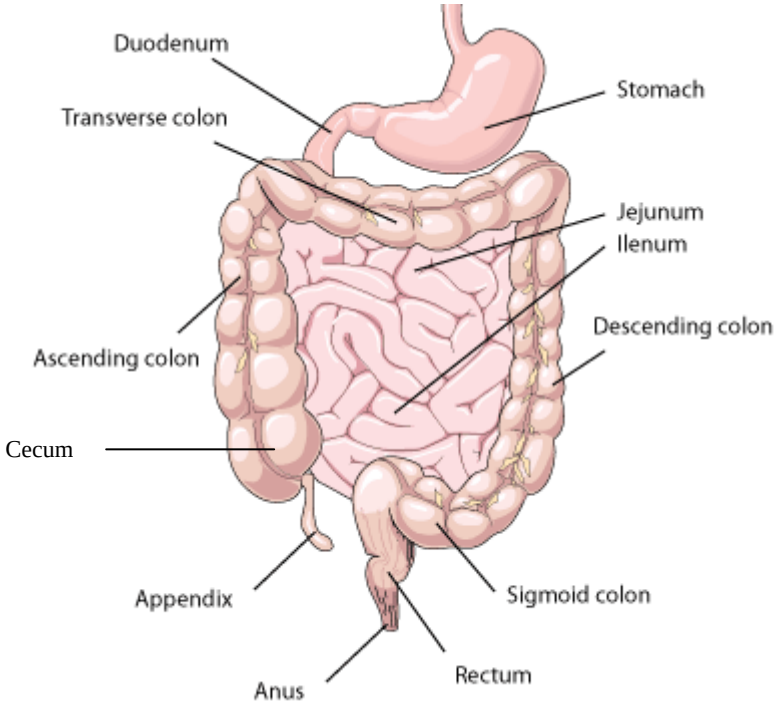

Figure 1 Anatomy of the gastro intestinal tract.

4.1 Epidemiology

IBD typically occurs between the ages of 15 and 35, but diagnosis can be made at any age. Men and women are equally affected (E F Stange, et al., 2006; E. F. Stange, et al., 2008). The disease is basically found in developed countries with increasing incidence rates in developing countries. As many as 1.4 million persons in the United States and 2.2 million persons in Europe suffer from these diseases (D. C. Baumgart & Sandborn, 2007; Loftus, 2004). Interestingly race seems to play an important role in risk for IBD. For example prevalence for CD among Hispanics living in North America is ten times less than those for white individuals. Nonetheless, lifestyle and environmental effects are suggested to contribute

to disease development as prevalence of IBD rises over time after migration e.g. Chinese people living in Hong Kong compared with Chinese living in mainland China (D. C. Baumgart & Carding, 2007).

4.2 Host genetic factors

A positive family history is currently the single largest risk factor for the diseases. Studies with monozygotic twins show a concordance rate for CD as high as 58% and 6-17% for UC, indicating an important role of genetic factors on the pathogenesis of IBD (Bouma & Strober, 2003). Several genes seem to have an impact on the pathogenesis. So far twelve chromosomal regions have been identified and some of them named IBD1-9. CD and UC share several of these loci (Gaya, Russell, Nimmo, & Satsangi, 2006). The nucleotide-binding-oligomerisation-domain 2 (NOD2) is thought to be involved in the recognition of bacteria (Bouma & Strober, 2003). Mutations in the Caspase recruitment domain family, member 15 (*CARD15)* gene, which encodes NOD2, is present in 10-15% of CD patients. These mutations are thought to result in disturbance in immunologic responses to commensal intestinal microbiota (Baumgart & Carding, 2007).

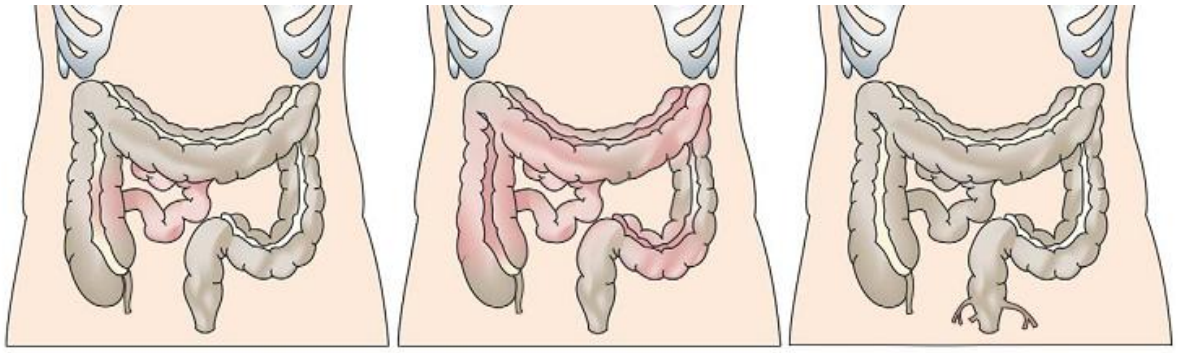

Figure 2 Anatomy of Crohn's disease affecting ileum and cecum (left), colon and ileum (in the midle) and fistulising Crohn's disease (right) (D. C. Baumgart & Sandborn, 2007).

Mutations or polymorphisms in IBD-associated genes can molecularly lead to, down-regulating of tight junction components, up-regulating of pore-forming components or increased epithelial apoptosis which finally contribute to increased mucosal permeability and possibly inflammation. Yet it is unclear whether inflammation or increased mucosal permeability comes first in the IBD pathogenesis (Packey & Sartor, 2008).

4.3 Immunobiology

The normal human colon contains 10^{11} to 10^{12} bacteria per gram of material (Sartor, 2008). The reported number of individual species in the GI tracts varies from 500 to more than 40 000. (Neish, 2009; Sartor, 2008; Sears, 2005) Despite these circumstances, the normal intestine remains healthy. This is due to several immunological factors primarily those of the mucosa. Epithelial barrier is the first line of defense. It consists of a complex network of lymphoid and non-lymphoid cell populations and humoral factors. Dentritic cells express NODs and toll-like-reseptors (TLR) which plays an important role in distinguishing commensals and pathogens and in activating or silencing T-cell response (Baumgart & Carding, 2007).

In IBD the well balanced immune system is disturbed at all levels. Antigens from the intestinal lumen are allowed to pass the mucosa to underlying tissue where they trigger and maintain inflammation. Commensal antigens are falsely detected as pathogens. Numerous leucocytes enter from the mucosal blood vessels and released chemokines attract more inflammatory cells which amplify and perpetuate the inflammation. Tissue damage results from the release of numerous injurious mediators (Baumgart & Carding, 2007). Which of the antigen being responsible for this are so far unknown, but likely this arises from bacteria in the GI tract (Frank, et al., 2007).

4.4 Environmental and lifestyle risk factors

4.4.1 Improved hygiene

Improved hygiene has been hypothesized to contribute to IBD. Low exposure to bacteria during childhood particularly, could lead to inappropriate immunologic responses later in life. The hypothesis comes from the observation of the striking increased number of IBD patients appearing simultaneously as improvement of the hygiene in the western world over the last century. Also, the number of IBD cases in poor countries rises as they develop. Exposure to microorganisms seems to be necessary for programming the immune system to respond properly to allergens, microbial and other antigens in the GI tract. The exposure appears to be

especially important in the early childhood to create the accurate balance between pro-inflammatory and tolerance-inducing immune cells (Koloski, Bret, & Radford-Smith, 2008).

Hot tap water, pet ownership, use of refrigerator, smaller family size, day care attendance and high socioeconomic status have all been suspected as risk factors for developing IBD. However the most promising protective factors so far have been Helminthic infection, *Helicobacter pylori* exposure, breastfeeding, lack of antibiotic use, and sibship. All representing exposure to bacteria or factors altering which bacteria will thrive in the gut. As an example lactoferrin found in human milk is an iron-binding protein with direct antibiotic effect on *Escherichia coli* and *Staphylococcuss spp*. (D. C. Baumgart & Carding, 2007; Koloski, et al., 2008). But conflicting evidence exists and these needs further investigation. It is hypothesized that having older siblings represent an increased risk for UC while having younger siblings is associated with decreased risk of CD. This is due to a higher risk of getting an infection from an older sibling at an early age and re- exposure of protective micro-organisms from younger siblings respectively (Montgomery, Lambe, Wakefield, Pounder, & Ekbom, 2002). The hypothesis that childhood vaccinations increase the risk of IBD by altering the maturation of the intestinal and systemic immune system has later been discredited (D. C. Baumgart & Carding, 2007; Koloski, et al., 2008).

4.4.2 Commensal colonization

Other states that it is not the absence of pathogens, but the lack of commensal colonization in the gut which needs consideration (Bernstein & Shanahan, 2008). From the moment we are born the colonizing of the sterile baby gut begins. Hence vaginal or cesarean delivery influences the timing and composition of the microbial progression. During the first 2-3 years of life the symbiotic relation is established (D. C. Baumgart & Carding, 2007). How this affect the pathogenesis of IBD is not fully understood.

Diet has a huge impact on the human gut microbiota. The diet over the last century has changed dramatically compared to earlier times in the western world. Higher amounts of vegetables, fruits, fish, dietary fiber and long-chain omega-3 fatty acids have been reported to significantly protect from CD, but further investigation is needed (Bernstein & Shanahan, 2008).

4.4.3 Smoking and appendectomy

While smoking complicates the course of the disease in CD, nicotine surprisingly seems to have a protective effect on UC. It is associated with less aggressive course of the UC disease. Some of the reasons are suggested to be increased mucus production and improved intestinal barrier function. CD smokers on the other hand, probably get an increased amount of neutrophils into the intestinal mucosa. Additionally appendectomy might be protective against UC while the opposite is true for CD where it is associated with raised risk of strictures (D. C. Baumgart & Carding, 2007).

4.5 Management

IBD patients are urged to have close contact with a medical doctor and specialist. The medical treatment is not curative thus the goal is to maintain remission and stop flare ups. In addition IBD patients must be treated for anemia and nutritional deficiencies (Baumgart & Sandborn, 2007).

Medical management for UC are quite comprehensive and includes medical treatments which provide anti inflammatory effect (5-aminosalicylic acid (5-ASA)), stop acute flare ups (glukocorticoides), reduce the lymphocyte count and maintain remission (immune modifying cytostatica, Azathioprin/6-mercaptopurine), and monoclonal antibodies which causes programmed cell death of certain lymphocytes and is used in maintenance and acute treatment (anti-tumor necrosis factor α (anti-TNFα) agent, Infliximab) (Norsk legemiddelhåndbok, 2009).

UC is typically classified as mild, moderate or severe and treated based on this. The extent of the disease determines delivery way of therapy oral or topical (suppositories). Severe colitis requires close interaction between gastroenterologists and surgeons to indicate surgery if the complications gets life threatening. UC can be cured by surgical removal of the colon (colectomy). Depending on the age, overall health state and disease stage of the patient a surgical procedure are being choose. The whole colon and rectum can be removed and an opening on the abdomen through which feces is emptied into a pouch (ileostomy). Other common surgery for UC patients are a comprehensive procedure, ileo-pouch anal anastomosis, in which the small intestine is attached to the anal sphincter muscle. This

surgery allows the passage of the feces through the anus to be reestablished (Baumgart & Sandborn, 2007).

Contrarily, surgery is not curative in patients with CD. Nevertheless surgery is required in nearly half of the patients after 5-10 years. Indications for surgery include part or complete bowel obstruction, fistulas, abscess, bleeding and/or non-function medical treatment. In the simplest surgery, short segment of the intestine with active disease or narrowing is removed and the remaining bowel is joined together.

The medical treatment of CD has similarities with UC treatment, but in addition antibiotics (metronidazole) might be used for a short period of time. Patients might also be asked to follow a dietary treatment low on fat and high on fiber. Probe nutrition can be necessary for some CD patients (Norsklegemiddelhåndbok, 2009).

5. Relationship between IBD and microorganisms

Few studies have so far enlightened the important role of microbiota in human health. A study showed that obese people had a decreased portion of Bacteroidetes in the gut, compared to lean people (Ley, Turnbaugh, Klein, & Gordon, 2006). Some research results also presents disturbed microbiota in asthmatic airways (Hilty, et al.).

The highest cell densities in any ecosystem are represented by the intestinal microbiota (Sokol, et al., 2009). It is dominated by microorganisms as bacteria, but fungi, and archaea are also present (Sokol, Lay, Seksik, & Tannock, 2008). The relationship between the microbiota and humans are believed to have coevolved over millennia. Complex immune mechanisms have been optimized so the host intestine remains healthy. The microbe benefits from stable nutrient supply, while the host benefit from metabolic ability and exclusion of less benign microbes (Neish, 2009). In the human intestine, bacteria constitute ten times greater numbers than human cells. Totally the intestinal microbiota contains at least 100 times as many genes as the human genome (Sartor, 2008). Therefore the integrated microorganisms have been called the forgotten organ due to their role in the GI tract where they synthesize essential amino acids and vitamins, and process components of otherwise indigestible contributions to human diet such as plant polysaccharides (Neish, 2009).

Further the intestinal commensal bacteria are essential in protection and homeostasis. Protection of the mucosa involves bacteria-bacteria interactions comprising physical exclusion, nutrient competition with less benign bacteria and a crosstalk between the microbiota and specialized epithelial cells (Sokol, et al., 2009). Disturbance of the normally stable GI microbiota are therefore predicted to adversely affect the health of the host (Frank, et al., 2007). Studies of experimental animal models of IBD reveal that germ-free animals show few signs of inflammation; experimental colitis is exhibited only when the animal is exposed to natural microbial communities. Likewise human studies have shown a response of IBD patients to antibiotic and probiotic treatment (Hecht, 2008). In CD patients inflammation most commonly appears in the gut locations where bacterial concentrations are high. Furthermore diversion of the fecal stream from the lumen is associated with improvement of the inflammation, indicating a role for bacteria in the IBD pathogenesis (Baker, Love, & Ferguson, 2009).

Albeit the types of GI microbes which influence IBD pathogenesis not yet have been adequately described, some bacterial species have been proposed to have a role. The discovery of *H. pylori* and its role in gastritis and peptic ulcer disease, awarded with The Nobel Prize in 2005, has stimulated the search for microbes as possible causes of other chronic inflammatory conditions like IBD (Peterson, Frank, Pace, & Gordon, 2008, (Inflammatory Bowel Disease, 2009).

5.1 Spesific miroorganisms in IBD

Relatively few studies reports single organisms to be responsible for IBD, especially few for UC. Additionally, conflicting results are reported, complicating the picture. Whether it is reasonable to expect a single organism to be able on its own to produce the myriad of signs and symptoms associated with IBD, should be considered. It has been speculated that the difficulty of finding one organism responsible for IBD is that more than one organism is involved (Lowe, Yansouni, & Behr, 2008). However, some organisms have been proposed to have a role in IBD pathogenesis.

5.1.1 Mycobacterium avium spp. paratuberculosis

Johne's disease, a wasting disease in cattle, has histopathological similarities with CD in humans. When *Mycobacterium avium* spp. *paratuberculosis* (MAP) was found to be the etiological agent of Johne's disease, huge effort was done to culture MAP from CD tissue. The investigations were considerably intensified when MAP was found in tap water and milk. But associations found between MAP and the etiology of CD was later contradicted in several studies (Sartor, 2008). Yet it is possible that MAP selectively colonize ulcers in the intestines of CD patients, especially patients with gene defects e.g. NOD2 (Packey & Sartor, 2008).

5.1.2 Escherichia coli

Functional alterations of commensal bacteria include enhanced epithelial adherence, invasion, and resistance to killing by the immune system or obtaining virulence factors. This can result in increased stimulation of innate and adaptive immune responses and subsequently create an inflammation. *E. coli* is a predominant bacterial species in the normal human intestinal flora

where it plays an important role in the intestinal homeostasis. In CD higher concentrations and antibody titres of *E. coli* has been found in the intestinal lumen and serum respectively. In 80% of microdissected granulomas of CD patients *E. coli* DNA was found (Packey & Sartor, 2008). Some *E. coli* strains can acquire virulence factors via horizontal transfer of DNA and cause a broad spectrum of diseases. Adherent invasive *E. coli* (AIEC) are abnormally predominant in CD. It can adhere and invade to the intestinal epithelium, Furthermore AIEC can replicate inside vacuoles in macrophages without killing the host cell and induce the release of TNF-α, a cytokine causing among other apoptosis and induction of inflammation (Darfeuille-Michaud & Colombel, 2008). The functional changes of AIEC might be one of the reasons why there are increased amounts of Enterobacteriae (including *E. coli*) associated with the mucosa in CD (Sartor, 2008). Whether AIEC represents a primary insult or rather a secondary contributor, remains to be established (Lowe, Yansouni, & Behr, 2008).

5.1.3 Faecalibacterium prausnitzii

Faecalibacterium prausnitzii is producing butyrate, an anti-inflammatory agent (Manichanh, et al., 2006). A reduction of *F.prausnitzii* is associated with a higher risk of postoperative recurrence of ileal CD, a study revealed. Interestingly *F.prausnitzii* was found to exhibit anti-inflammatory effects in both cellular and mice models. It even tended to reduce the intestinal dysbiosis in the mice model. Nonetheless the study showed that the strong anti-inflammatory effect of *F.prausnitzii* not was due to butyrate (Sokol, Pigneur, et al., 2008).

5.1.4 Fungi

Although most available results indicate a connection between bacteria and IBD pathogenesis, fungi microflora might also add valuable information. Anti-*Saccharomyces cerevisiae* antibodies (ASCA) are directed against common fungal cell wall epitope (Underhill & Braun, 2008). ASCA are mainly present in CD but might also be found in UC (E F Stange, et al., 2006; E. F. Stange, et al., 2008). Among other *S. cerevisiae* and *Candida albicans* are said to stimuli the development of ASCA, but the exact way this is done is unknown (Underhill & Braun, 2008).

5.2 Gastro intestinal microbiota in IBD

In contrast to the interpretation of IBD pathogenesis by a single microorganism, the other approach is to find compositional shifts in bowel bacterial communities. A change in the composition of the intestinal microbiota has been proposed to have a role in IBD pathogenesis. But changes in the composition of microbiota could also be a result of the disease. Nonetheless discrepancies between IBD and control GI microbiota could be used in a diagnostic test. Before one can state a GI microbiota to be disturbed one need to know what is normal. Large studies are now trying to enlighten the human core microbiota, and more information will be revealed in the years to come (Tap, et al., 2009).

Bacterial diversity within the human colon and feces is greater than previously described, and most of it is novel. Differences between individuals are significantly greater than intrasubject differences, with the exception of variation between stool and adherent mucosal communities. Variation associated with time, diet and health status have not been adequately described. (Eckburg, et al., 2005).

All bacteria are divided in taxonomic ranks from phylum to species via class, order, family, and genus. Metagenomic studies reports that most bacteria in human GI tract are represented by only four phyla regardless of disease state: *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* (Eckburg, et al., 2005; Frank, et al., 2007; Ley, Turnbaugh, Klein, & Gordon, 2006). Nearly half of the sequences have been shown to belong to just two subgroups: The order Bacteroidales and the family Lachnospiraceae (Frank, et al., 2007).

Neither UC nor CD is characterized by a uniform, stereotypical microbiota (Frank, et al., 2007). Hence it is unclear whether microbiota of the human intestine alone can be used to diagnose IBD, or whether human genetic, serologic and immunologic factors have to be included.

5.2.1 Temporal variation of the gastro intestinal microbiota

Healthy humans have relatively stable temporal compositions of GI microbiota; but the microbial content between persons differs considerably (Sokol, et al., 2008). In the case of CD patients in remission, the stability of the fecal microbiota varies greatly over time

(Scanlan, et al., 2006). Even though microflora have a high degree of diversity in both situations, dominant microflora varies markedly between remission and flare (Scanlan, Shanahan, O'Mahony, & Marchesi, 2006; Seksik, et al., 2003). Additionally IBD patients have shown higher concentrations of bacteria in mucosal and fecal samples (Sokol, et al., 2008).

In contrast, a notably decrease in operational taxonomic units (OTU) in IBD fecal and mucosal microbiota, mainly reflected by fewer types of bacteria in the Firmicutes phylum have been reported. While Fusobacteria and Verrucomicrobia phyla are associated with healthy subjects, increased number of Bacteroidetes is associated with IBD (Eckburg, et al., 2005).

5.2.2 Composition of gastro intestinal microbiota and bacterial metabolism

Despite differences in GI microbes between persons, the same metabolic functions can be accomplished by several bacterial species (Sokol, et al., 2008). Butyrate is a short-chain fatty acid (SCFA) which is metabolized by mainly *Firmicutes* and *Bacteroides* species. SCFA accounts for up to 10% of calories in a Western diet each day. Butyrate also functions as anti-inflammatory agent by inhibition of NF-κB activation. Loss of butyrate producers might therefore interfere with the balance between host epithelial cells and resident microorganisms, leading to the development of CD associated ulcerations. Other known butyrate producers are microorganisms in *Clostridium leptum* and *Clostridium coccoides* groups (Manichanh, et al., 2006). Loss of butyrate producers in IBD patients are observed in several studies (Gophna, Sommerfeld, Gophna, Doolittle, & Veldhuyzen van Zanten, 2006; Manichanh, et al., 2006; Sokol, et al., 2009)

Bacteria which produce hydrogen sulfide can inhibit butyrate oxidation and hence cause colonic lesions. Higher concentrations of hydrogen sulfide producing bacteria have been reported in the microbiota of active UC patients compared to those in remission (Sokol, et al., 2008).

6. IBD Diagnostics

As there is no single pathogenic marker of IBD found, no gold standard for making the diagnosis exists. Rather the recommended diagnosis of CD and UC is based on clinical evaluation, laboratory findings and medical history and is confirmed by negative microbiological test, endoscopy and histological findings. Several diseases presenting similar symptoms, needs to be ruled out. Most patients suspected to have IBD and undergo standard invasive tests have a functional disease and the majority of these have irritable bowel syndrome (IBS) (Dubinsky, et al., 2002). IBS differ from IBD in that it does not cause inflammation, ulcers or organic damage to the GI tract. IBS diagnostics are based on symptoms and evaluation of organic abnormality (Grundmann & Yoon). Treatment of IBD includes diet, psychotherapy, and medications like antidiarrheal agents, anti spasmodics and antidepressants (Kennedy, et al., 2006; Villanueva, Dominguez-Munoz, & Mearin, 2001).

6.1 Medical history and clinical evaluation

Because the symptoms of IBD are mixed, depending on the localization and severity of the disease, diagnostic problems often occur. Diarrhea, abdominal pain and/or weight loss are the main symptoms of CD. In UC bloody diarrhea is the primary symptom, but it might also be present in CD. Patients with UC might also have symptoms as rectal bleeding and rectal urgency. Passage of mucus and pus are more common in UC than CD. Additionally symptoms of malaise, loss of appetite and/or fever are common in CD but could be a sign of severe attack in UC. Fistulae are common in CD and may occur in UC. Furthermore, several symptoms associated with constipation are described by UC patients. Common extraintestinal manifestations in IBD are abnormalities of the musculoskeletal system (E F Stange, et al., 2006; E. F. Stange, et al., 2008).

Conditions that produce similar symptoms need to be ruled out. Recent antibiotic use may indicate pseudomembranous colitis, travel abroad might cause infectious colitis and abdominal pain relived with bowel movements could represent IBS (Langan, Gotsch, Krafczyk, & Skillinge, 2007). Loose stools for more than six weeks normally differentiate IBD from infectious diseases. Patients are questioned carefully about medication, recent travel, the onset of symptoms, nocturnal diarrhea, incontinence, food intolerances, contact

with enteric illnesses, sexual practice, smoking, family history of IBD, and history of appendicectomy. Also a general examination is preformed (E F Stange, et al., 2006; E. F. Stange, et al., 2008).

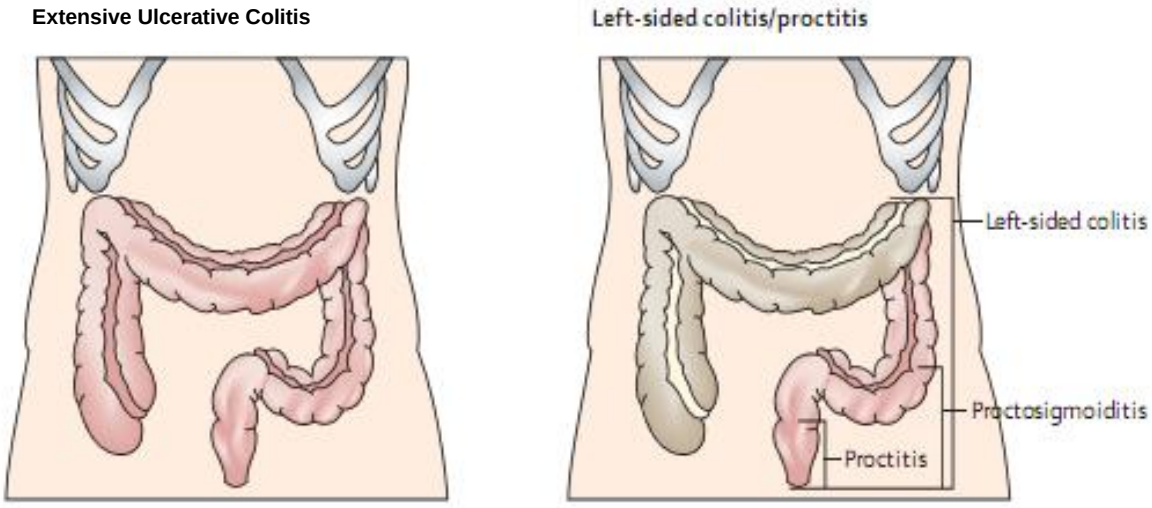

Figure 3 Anatomy of Ulcerative Colitis affecting the whole colon (left) and partially affected colon (right) (D. C. Baumgart & Sandborn, 2007).

6.2 Laboratory Tests

6.2.1 Blood Tests

Initially, several blood samples are investigated comprising a full blood count, serum urea, creatinine, electrolytes, liver enzymes, iron studies, and C-reactive protein (CRP). (E F Stange, et al., 2006; E. F. Stange, et al., 2008). In IBD the blood count might show anemia from long term rectal bleeding, thromocytosis as a result of chronic inflammatory response. CRP might be elevated as a result of chronic inflammation. A basic metabolic profile may demonstrate electrolyte abnormalities from persistent diarrhea (Langan, et al., 2007; E F Stange, et al., 2006; E. F. Stange, et al., 2008). Nevertheless no sign of chronic inflammation might be found in UC (Nikolaus & Schreiber, 2007)

The single most robust serological biomarker of CD is anti-*Saccharomyces cerevisiae* antibodies (ASCA). Another marker is peri-nuclear anti-neutrophil cytoplasmic antibodies (pANCAs). The markers have insufficient sensitivity to serve as a screening tool for populations with low prevalence of disease. However, used in combination it is utilized as a

confirmation test when IBD is assigned, to differentiate CD from UC. pANCA is found in half of the UC patients (E. F. Stange, et al., 2008). Likewise ASCA can be detected in 35-50% in the CD patients and less than 1% of the UC patients (Nikolaus & Schreiber, 2007). The specificity of ASCA for CD and pANCA for UC is rather high with 89-97% and 89% reported respectively (Nikolaus & Schreiber, 2007).

Studies report that CRP has a diagnostic sensitivity of 70-100% for CD and 50-60% for UC (Vermeire, Van Assche, & Rutgeerts, 2006).

6.2.2 Stool Examination

Microbiological testing is recommended to exclude infectious diarrhea caused by common pathogens including specific assays for *Clostridium difficile* toxin, *Campylobacter* spp and *E. coli* 0157:H7. In addition other stool test may be tailored for patients who recently have traveled abroad (E F Stange, et al., 2006; E. F. Stange, et al., 2008).

Calprotectin is a fecal marker and represents migration of leukocytes to the GI tract. It is a sensitive marker for inflammation in the GI tract. Further it can differentiate IBS from IBD and it has been suggested to skip endoscopies in patients with normal calprotectin levels and IBS symptoms. It can be used to measure disease activity and predict relapse in CD. It has been proponed that the marker could replace colonoscopy in follow-up of CD (Jahnsen, Røseth, & Aadland, 2009). Nevertheless several other conditions as cancer, infections and polyps can give a positive calprotectin test (Vermeire, et al., 2006).

6.2.3 Genotyping

Genotyping is not recommended in UC since it is a complex disease and mutations in identified disease associated genes not guarantee disease development. Nor absence of these mutations is sufficient for remaining healthy (E. F. Stange, et al., 2008). *CARD15* mutations have been associated with CD, but low predictive value and missing preventive treatment have led to the absence of routine genetic testing (E F Stange, et al., 2006).

6.3 Endoscopy

Endoscopy is used to establish the diagnosis and extent of UC and CD. Some types of endoscopy are proctosigmoidoscopy for rectum, colonoscopy for the large bowel and the lower colon and ileoscopy for the colon up to the ileum. Patients with upper GI symptoms might need to perform an upper endoscopy, oesopagogastroduodenoscopy, but this is not recommended as a screening procedure (Nikolaus & Schreiber, 2007). The endoscope is passed through the rectum or mouth. It has a small camera attached to a flexible tube and tissue samples can be taken with a biopsy forceps inserted through the tube (Endoscopy. (2010). In Encyclopædia Britannica. Retrieved February 19). Biopsies from several sites in the colon, rectum and ileum are obtained (E. F. Stange, et al., 2008). The procedure time is about 20 minutes (Terheggen, et al., 2008).

A colonoscopy or ileoscopy with biopsy are recommended for the examination of patient suspected to have IBD. Endoscopic appearance as distribution and shape of lesions differentiate CD from UC in most cases (Nikolaus & Schreiber, 2007). If the condition of an IBD patient is severe, there is a danger for perforation and fast medical management might be crucial. In this situation it is advised to postpone the examination, alternatively perform sigmoidoscopy (E F Stange, et al., 2006). In IBD patients severe complications as bleeding and perforation occurs in 0,7% while minor complications as intense flatulence , tachycardia, allercic raction etc. occurs in 3,9% of the endoscopic procedures (Terheggen, et al., 2008). The need for surgery as a result of endoscopy is 0,42% (Froehlich, Gonvers, Vader, Dubois, & Burnand, 1999). Other shortcomings of endoscopy are the invasive nature of the method, the discomfort of the patient and the expenses. Additionally it is not an objective test.

Wireless capsule endoscopy might be preformed for the diagnosis of CD after ileoscopy. It is swallowed and images are being recorded as the capsule pass the upper GI tract and small bowel (Bourreille, et al., 2009). Further studies are needed to confirm the diagnostic relevance.

6.3.1 Endoscopic Features

UC causes continuous inflammation from rectum and a variable length of the colon. The inflammation has a clear macroscopically boundary to healthy tissue. Typical features are

ulcerations, loss of the vascular pattern, exudates, and granularity (Figure 4). Severity is reflected by the presence of spontaneous bleeding, deep ulcerations and mucosal bleeding to light touch. But no endoscopic feature is specific for UC (E. F. Stange, et al., 2008). UC are restricted to the colon except of rare occasions when the whole colon is affected "back-wash ileitis" can be seen. The distribution of UC is classified according to macroscopic findings using endoscopy. In proctitis the inflammation is limited to the rectum. Inflammation from rectum to the splenic flexure is characterized as left-sided, while inflammation which passes this location is called extensive UC (Figure 3). When the diagnosis is set, the disease activity is classified as remission, mild, moderate or severe according to parameters as puls, temperature, hemoglobin, erythrocyte sedimentation rate (ESR), CRP and number of bloody stools pr day.

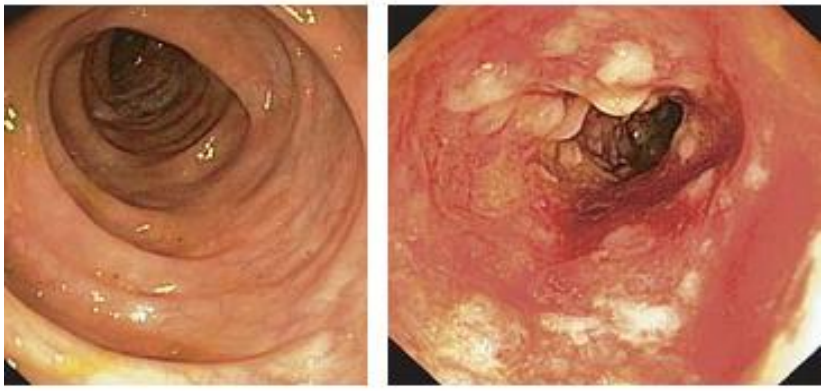

Figure 4 Endoscopic features of UC. Left: Transverse colon in a UC patient in remission. Typical post-inflammatory changes- i.e., loss of fine vascular pattern. Right: Descending colon: Extensive UC with irregular surface due to extensive ulcerations, spontaneous haemorrhage, and pseudopolys (D. C. Baumgart & Sandborn, 2007).

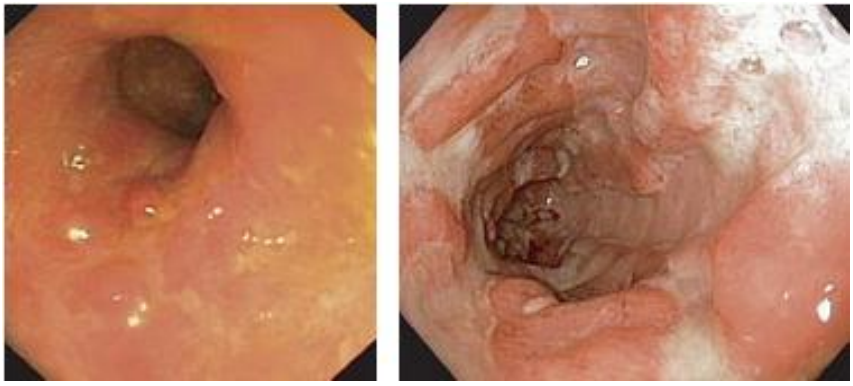

Figure 5 Endoscopic features of CD. Left: Terminal ileum of a CD patient: Friability of mucosa with erythema, loss of ramifying vascular pattern, and lymphoid hyperplasia. Right: Sigmoid colon of a CD patient: Linear and serpinguous ulcerations and patchy inflammation ultimately resulting in cobblestone pattern (D. C. Baumgart & Sandborn, 2007).

The features of CD are normal mucosa within inflamed areas, anal lesions and deep ulcerations with swelling of the surrounding tissue (cobble stoning) (Figure 5). The ulcers can vary in depths from the submucosa through the muscle layer (E F Stange, et al., 2006). Frequently CD patients have small bowel involvement. But 15-23% of the patients have inflammation restricted to the colon and this could confuse the diagnosis with UC (Nikolaus & Schreiber, 2007).

6.4 Histology

The presence of granuloma, focal inflammation or architectural abnormalities found in biopsies is a main feature of CD. In contrast UC features are diffuse crypt irregularity, reduced crypt numbers and general crypt epithelial polymorphs (E F Stange, et al., 2006). The microscopic features are dependent upon how early the biopsies were obtained. In early biopsies no pathological signs might be found (E. F. Stange, et al., 2008).

6.5 Radiology

6.5.1 Small bowel follow-through, computed tomography, and magnetic resonance imaging

Although it is not a diagnostic test for IBD, abdominal radiography is useful in patients where endoscopy is not recommended. Small bowel follow-through (SBFT) and small bowel enema has been the standard for detection of small bowel involvement in CD or to confirm a normal upper GI tract in UC patients. SBFT requires an experienced radiologist and main findings are often only observed during fluoroscopy. In addition the radiation dose is relative high and should preferably not be used in children (Nikolaus & Schreiber, 2007; E F Stange, et al., 2006).

Several other techniques exists for small bowel investigations including among other ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) (E F Stange, et al., 2006). MRI can differentiate active inflammation from fibrosis. As SBFT, MRI can be used to detect stenosis, anorectal fistulae and abscesses in IBD. MRI have higher costs than SBFT, but because of non-ionizing radiation and increasing higher diagnostic precision MRI

can become the method of choice for detection of small bowel involvement in CD (Nikolaus & Schreiber, 2007). CT gives additional information especially in patients where the disease is severe such as thickening of the bowel wall. CT includes radiation and other method should be considered in young patients (E F Stange, et al., 2006).

6.5.2 Ultrasound

Sonography can detect inflamed areas in both small bowel and colon with high diagnostic precision based on bowel wall diameter and blood flow. Based on this, quiescent disease can be separated from moderate to severe disease. Pathological findings like enlarged lymph nodes, abscesses, stenoses, and fistulae can be detected. It is a quick and inexpensive screening method for IBD and can also be used to evaluate complications. The sensitivity for primary detection of CD with sonographic techniques is 75-95% with a specificity of 67-100%, depending on the operator (Nikolaus & Schreiber, 2007).

6.6 Innovative diagnostic procedures identifying intestinal microbial populations using the 16S rRNA gene

Several methods exist for the identification of bacteria in a certain microbiota. Previously, phenotypic determination of bacterial species by cultivation was the only way. Through the evolution intestinal bacteria has adapted to the metabolism of the host and other bacteria in the ecosystem, hence they are almost impossible to cultivate in pure culture. Only 10-50% of bacteria in the gut can be identified by this method (Zoetendal, Rajilic-Stojanovic, & de Vos, 2008). Thanks to new culture-independent tools, huge amounts of new data can be revealed. Especially technologies based on 16S rRNA sequence information have been a solution.

6.6.1 Identification of bacteria by 16S rRNA

Ribosomes comprise two rRNA subunits, called small and large subunit, which work together during protein synthesis (Schluenzen, et al., 2000). In prokaryotes these consist of, among other, 16S and 23S rRNA respectively (Pei, et al., 2009). While 23S rRNA catalyzes peptide bonds, 16S rRNA initiates contact with mRNA, decode mRNA via tRNA and control the interaction between codon and anticodon (Schluenzen, et al., 2000). Throughout the evolution, the gene functions and sequence of rRNA genes have remained relatively

conserved with the exception of some variable regions. The variable regions of 16S and 23S rRNA are used to identify and differentiate species. PCR primers are designed for the universal regions surrounding the variable regions of the gene. In this way sequence and species differences can be detected (Zoetendal, et al., 2008). The nature of the gene allows simultaneously detection of many species. Primers and probes can be designed to detect species, genra, family or even order.

Small subunit (SSU) rRNA genes are found in all organisms. Because of these properties, the gene was used in the construction of the "tree of life" where the Archaea was discovered as the third "domain of life" in addition to Bacteria and Eucarya. So far more than 400 000 SSU rRNA sequences are available in databases (Zoetendal, et al., 2008). The two prokaryotic rRNA genes have a length of 1542 (16S) and 2904bp (23S) (Srikanta & Simpson, 2005) which is sufficient length for comparative sequence analysis (Zoetendal, et al., 2008). Due to the short sequence, the 16S rRNA gene is used more frequently in the identification of prokaryotes than 23S rRNA. The properties of the gene make it suitable for detection of microbiota in different types of samples. Samples from the intestines as fecal and mucosal samples can be utilized to detect bacteria in i.e. IBD diagnosis.

6.6.2 Challenges with the simultaneously detection of a complex mixture of DNA from different organisms

Methods such as flow cytometry, Real-Time PCR and mass spectrometry, can utilize the rRNA gene, but is limited to a low number of tests carried out simultaneously. The two first because of limited number of fluorochromes (Rudi & Jakobsen, 2003). Denaturing gradient gel electrophoresis (DGGE) is another option, but because of low throughput, hetero-duplex formation and the difficult comparison of gels, this method has also been causing problems. Terminal restriction fragment length polymorphism (T-RFLP) is widely used, but is imprecise and has low solubility. Standard microarray for 16S rRNA has been shown to provide a high number of cross hybridization. Non-specific labeling of samples with DNA array hybridization has been tested, however it do not identify many bacteria at a time (Rudi & Jakobsen, 2003; Rudi, Skulberg, Skulberg, & Jakobsen, 2000). For this purpose next generation sequencing can become an option, but so far many challenges exist. Short reads and base calling errors makes it difficult to assemble the sequences. Furthermore, an

unnecessary, large volume of data is generated and needs to be handled. Additionally the costs are high (Chistoserdova, 2010).

6.6.3 Fecal and mucosal samples in the detection of gastro intestinal microbiota

Materials used for testing the microflora of the human intestine are heavily discussed. Conflicting evidence exists whether fecal microbiota differs substantially from that of the GI mucosa. High similarity profiles of biopsy-, aspirate- and faeces associated bacteria have been presented (Bibiloni, et al., 2008; Tannock, 2008) while other report that some microbes are associated with the gut wall and these are likely a more critical factor than fecal microbes in promoting IBD pathogenesis (Frank, et al., 2007) The existence of a so called biofilm more adherent to the intestinal mucosa in patients with IBD than healthy humans further suggests the relevance of biopsy (Swidsinski, Weber, Loening-Baucke, Hale, & Lochs, 2005). Biofilms, with their inherent resistance to antibiotics and host immune attack, have increasingly been identified as sources of many recalcitrant bacterial infections (e.a periodontal disease, endocarditis)(Swidsinski, et al., 2005). Patients usually undergo bowel cleansing before endoscopy and residuals pools in the large bowel. This aspirate contaminates, in all likelihood, the endoscope and the collected tissue (Tannock, 2008). Further, a study found no clear bacterial infiltration of the mucosa in IBD patients. Even in patients with severe inflammation, bacteria were not found below the epithelial layer. Biofilms were also found in regions without signs of inflammation. Crossing of the mucus and bacterial adherence is therefore considered taking place before mucosal destruction. The diversity of mucosal bacteria was additionally found to be low compared to the fecal bacteria (Swidsinski, et al., 2005).

Absolute numbers of microbial species vary greatly a long the gut, ranging from 10^{2-3} cell/g content in the proximal ileum and jejunum to 10^{7-8} in the distal ileum and 10^{11}-10^{12} in the ascending colon (Neish, 2009, Sartor, 2008). Results from several studies on GI mucosa reveal that the same bacterial phyla dominate in the stomach, small intestine, and colon, although their relative abundance and details of the component species vary as a function of position along the gut (Peterson, et al., 2008). Mucosal biopsies, unlike stool, provide samples collected from regions of the intestinal tract where inflammation occurs (Tannock, 2008). In contrast, the fecal microbiota is thought to represent the microbiology of the distal large

bowel and it has been stated that it do not reflect neither the colon nor the terminal ileum (Sokol, et al., 2008). Knowing that CD can occur anywhere in the digestive tract from mouth to anus (Tannock, 2008) revealing where in the GI tract the microbiota of stool samples reflect is important. Anyway, as previously described, differences in the fecal microbiota between IBD patients and controls have been reported. Fecal samples with their easily availability are preferred instead of biopsies. But so far no such test is commercially available.

7. GA-map™ - technology description

The Norwegian company Genetic Analysis AS (GA) is commercializing a patented technology, named GA-map™. To diagnose IBD, GA is seeking to identify an IBD specific bacteria composition in fecal samples. Generally, GA-map™ involves detection of sequences in the 16S rRNA gene by binding of oligonucleotide probes and selective labeling of the bound probe. Subsequently, the probes are hybridized to a known sequence followed by detection, see Figure 6 (Rudi & Jakobsen, 2003).

The method can be used in a complex mixture of DNA from different organisms. Microbiotas in fields such as water, soil, buildings, health, veterinary and food products can be explored using this method. In health, GA wants to survey the human intestinal flora in terms of stomach ulcers, allergies, obesity, bowel cancer, energy capture and IBD.

The method described in the patent, US6617138 "Nucleic acid detection method", has a general profile and multiple customizations to the invention are possible. Different approaches are being optimized.

7.1.1 Target sequences, primers and probes

The target sequences for detection may be of numerous types: DNA, PCR amplified DNA, native DNA, reverse transcribed DNA (cDNA), single-or double stranded DNA, linear or circular. Moreover, the target sequence might be RNA such as mRNA, or rRNA. (Rudi & Jakobsen, 2003)

Primary, a method using PCR amplified rRNA gene sequences, have been developed. Other possible amplification methods are; in vivo cloning, Nucleic Acid Sequence Based Amplification (NASBA) for amplification of RNA and ligase chain reaction. The main focus is the 16S rRNA gene, but the method might also be compatible with other genes.

The Cover-all™ universal 16S rRNA primers pair developed by GA, can be used to amplify most bacteria in the human gut. Further the company has developed a computer program, the GA-map ProbeTool™, for the identification of specific areas within the 16S rRNA gene suitable for probes. The probes used are complementary to the variable regions of 16S rRNA.

The length of the oligonucleotide probes are approximately 20 nucleotides. The lengths of the probes are optimized to be sufficiently long for specific binding, and reasonably short to avoid unnecessary chemical synthesis. Amounts of probe are optimized in terms of qualitative and quantitative detection (Rudi & Jakobsen, 2003).

7.1.2 Single nucleotide extension (SNE)

DNA is extracted from the fecal samples and the 16S rRNA gene is amplified using primers targeting universal regions in the gene. Excess primers and unincorporated nucleotides are removed by Exonuclease I (EXO I) and Shrimp Alkaline Phosphatase (SAP) treatment respectively. Subsequently, the oligonucleotide probe binds to the target sequence in the sample with normal base pairing and forms a complex (probe-sequence) (Kostić T & L., 2007).

Labeling of the probe is template dependent. Extension with fluorescently labeled nucleotide will only occur in the presence of target sequence. A dideoxynucleotide (ddNTP) is incorporated to the bound probe by the polymerase. Further polymerization is prohibited because of the nature of ddNTPs having a 3'hydroxyl group which is modified or missing (Kostić T & L., 2007). The method can be performed using one dideoxynukleotid, or a mix of ddGTP, ddCTP, ddTTP and ddATP. If only one ddNTP is present it may be a higher risk of misincorporation despite the usual base pairing rules. Several different ddNTP'er in the reaction reduces the likelihood of misincorporation and thus the background signal and noise on the array.

Several types of labeling or reporter molecules of the probe are possible. Examples of labeling are chemiluminescent, radioactivity, chromogenic, enzymatic, antibody, and fluorescence. The use of fluorescently labeled ddNTP are under development (Rudi & Jakobsen, 2003).

7.1.3 Specificity and sensitivity

Compared to other DNA based methods, GA-map ™ can perform with higher specificity, lower noise and lower background signal. The main reason is the specific binding of the probe

followed by the incorporation of fluorescent labeled ddNTP with specific polymerase. Methods where the probe is marked in advance will result in lower specificity. In the case of

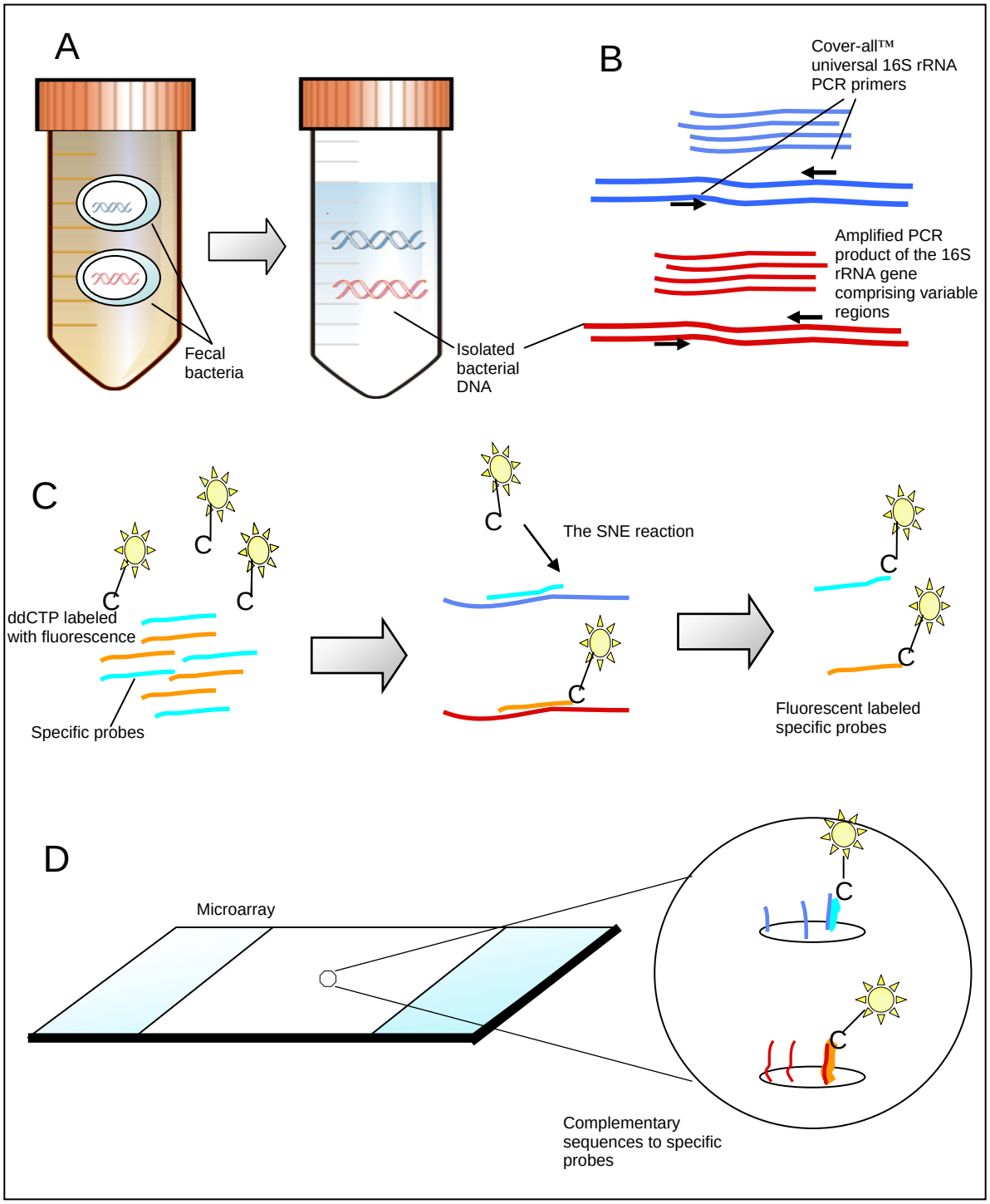

Figure 6 The GA-map™ technology. DNA is isolated from the fecal sample (A). Variable regions of the 16S rRNA gene are amplified by Cover-all™ 16S rRNA universal PCR primers (B). Specific probes are bound to a complementary sequence and extended with a single fluorescence labeled nucleotide. Subsequently the probe is separated from the sequence by denaturation (C). The specific probes are hybridized to complementary probes spotted on a microarray (D). Step C and D are patent protected and licensed by GA (Rudi & Jakobsen, 2003).

normal base pairing the corresponding nucleotide is preferred and specificity increased. The sensitivity can be improved by increasing the number of cycles in the labeling step and the use of a thermal stable polymerase is presupposed. The optimal number of cycles is approximately 10. Additional cycles may be used even in quantitative detection, using a competitive PCR, where a competing sequence is added in specific amounts to the reaction containing the same primer-template sequence (Rudi & Jakobsen, 2003).

7.1.4 Hybridization

The SNE reaction is followed by the hybridization reaction. First the complex (probe-PCR product) is separated by denaturation. This can be e.g. high pH or high temperature (Rudi & Jakobsen, 2003). High temperature denaturation is used in the method now being developed. Subsequently, the oligonucleotide is hybridized to a complementary sequence on a microarray. The microarray allows simultaneous detection of a high number (thousands) of different oligonucleotide attached to a glass or silicon chip (Passarge, 2001). Other options of solid surfaces are particles, gels, filters, membranes, fibers, capillaries, microtitre strips, tubes, plates or wells. The probe sequences on the microarray can be designed with areas not completely complementary to the probe, it can e.g. be truncated or contain a longer sequence. But the sequence must be sufficiently complementary to allow binding of the probe.
The complementary sequence can be DNA or PNA (Peptide Nucleic Acid) (GeneticAnalysis, 2010; Rudi & Jakobsen, 2003).

7.1.5 Detection

The detection can be quantitative, qualitative or semi-quantitative. The signals are detected according to what form of labeling used. Fluorescent labeling is detected by a laser scanner. Monochrome light emits, and fluorescent signal are detected by a charge-coupled device (CCD) camera. The image created is analyzed and each spot is quantified. The signals can be treated with a variety of statistical applications for qualitative and quantitative measurements. Finally, the signals will reflect the relative content of target sequences, the bacteria it contains, and the number of bacteria in a sample (GeneticAnalysis, 2010; Kostić T & L., 2007; Rudi & Jakobsen, 2003).

8. Potential bacterial target for the GA-map™

A literature study was performed to reveal what is known about the relationship between IBD and a change in the GI microbiota. Articles presenting results from bacterial markers of IBD in feces and mucosal samples were studied. A complete overview of the findings is presented in Appendix A and Appendix B and a summary is presented in Table 1.

8.1.1 Variation in the gastro intestinal microbiota of IBD patients

Conflicting evidence is presented in the literature. The most uniform results reports reduced amounts of *C. coccoides* in stools from CD patients (Mangin, et al., 2004; Seksik, et al., 2003; Sokol, et al., 2009) Additionally a study reported reduced amounts in UC versus controls (Sokol, et al., 2006). Further, consistent results have also reported reduced amounts of another *Clostridium* species, namely *C. leptum* (Mangin, et al. 2004; Manichanh, et al., 2006; Prindiville, Cantrell, & Wilson, 2004; Scanlan, et al., 2006; Sokol et al., 2006). While reduced amounts in active-IBD were found, this was not true for patients in remission, a study showed (Sokol et al., 2009). Furthermore, Clostridium cluster IV and XI and subcluster XIVa are decreased in CD versus healthy subjects (Andoh, et al., 2009).

Using fecal samples *Bacteroidetes vulgatus* was the only species shared by all 4 patients in a study. Especially it was high in the two patients with *CARD15* mutation and disease located in the ileum (Mangin, et al. 2004). Also in mice *B. vulgatus* was found to be positive associated (Ye, et al., 2008).

Enterobacteria were detected in feces from all patients but none of the controls (Seksik, et al., 2003). Similarly the order Enterobacteriales has also been reported in increased amounts in fecal samples from IBD patients (Andoh, et al., 2009).

Faecalibacterium prausnitzii was found to be reduced in active IBD in a study using quantitative real-time PCR on fecal samples (Sokol, et al,. 2009). While the opposite was reported in a study using mucosal samples (Swidsinski, et al., 2005).

Firmicutes phylum was reduced in both biopsy and fecal samples of CD and active IBD patients (Gophna, Sommerfeld, Gophna, Doolittle, & Veldhuyzen van Zanten, 2006; Manichanh, et al., 2006; Sokol, et al., 2009). Interestingly the Firmicutes/Bacteroidetes ratio has been presented as reduced in active IBD (Sokol, et al., 2009).

Confunding results have been reported from numerous studies of species in the Bacteroidetes phylum and Bacteroides genus. Increased, equal and similar amounts have been presented (Appendix A and Appendix B).

Table 1 Bacteria status reported in IBD patients relative to controls

Class	Feces			Mucosa		
	Increased	Decreased	Equal	Increased	Decreased	Equal
Actinobacteria		3	5	5		
Alphaproteobacteria				3		
Bacilli	5	2	5	3		
Bacteroida	4	2	4	8	8	1
Clostridia		17	3	5	10	
Gammaproteobacteria	3		5	6		4

9. Evaluation of selected GA-map™ probes for IBD diagnostics

In order to search for bacterial probe candidates for the GA-map™ IBD array, a laboratory study were performed. Samples from 257 individuals were hybridized with five probes in separate reactions. The probes were extended by a fluorescence labeled ddNTP and detection was performed by capillary gel electrophoresis as described below. Hence, the following are a simplified version of the GA- map™ previously described.

This is the first study to use the GA developed protocols and probes on adult patient samples. The study shows the potential of diagnosing IBD by using 16S rRNA probes in fecal samples. Most previous IBD studies utilizing 16S rRNA in fecal samples, include few number of patients (<50). These previous studies include patients which have initiated their medical treatment, although most exclude patients who have taken relevant medication for a period of time (month) before collection of sample. The present study includes 152 patients and 105 controls.

9.1 Materials and methods

9.1.1 Patients and samples

The patient samples used in this study were a cohort from the Norwegian IBSEN study and were kindly distributed by Akershus University Hospital (Ahus). The samples comprise newly diagnosed untreated IBD patients and non-IBD patients. In total 257 patients were included in the study (Table 2). The inclusion criteria for the IBD cohort were abdominal symptoms including diarrhea and/or blood in the stools for more than 10 days, endoscopic or radiological signs of inflammation as well as histological signs of chronic inflammation. Exclusion criteria were infection of pathogenic gut bacteria (excluding mycobacterium avium), findings of parasites, cysts and eggs. In addition patients with cancer, hematological or hepatological disorders, significant cardiovascular, neurological, or repiratory comorbidity, and other chronic inflammation than IBD were excluded from the study (cases and controls).

9.1.2 Classification of patients

The IBD patients were further classified as CD, UC or IBD unclassified (IBDU).

Patients were diagnosed as CD based on the presence of two or more of the following criteria:

- Clinical features including abdominal pain, diarrhoea and weight loss.
- Macroscopic appearance at operation or endoscopy: Segmental, discontinuous, and/or patchy lesions with or without rectal involvement, discrete or apthous ulcerations, fissuring or penetrating lesions, cobblestone or strictures.
- Radiological evidence of stenosis in the small bowel, segmental colitis or findings of fistulae.
- Histologic evidence of transmural inflammation or epithelial granulomas with giant cells.

The diagnosis of UC was based on the presence of three or more of the following criteria:

- A history of diarrhoea and or blood/pus in stool
- Macroscopic appearance at endoscopy, with continuous mucosal inflammation affecting the rectum in continuity with some or the entire colon.
- Microscopic features on biopsy compatible with UC.
- No suspicion of CD on small bowel roentgenography, ileocolonoscopy, or biopsy.

Patients with inconclusive or divergent endoscopy and histopathology according to CD or UC criteria were classified as IBDU.

The non-IBD cohort was derived from patients referred to colonoscopy, not suffering from IBD.

Table 2 Characteristics of patients

	Children	Adults	Total number of patients
UC	13	50	63
CD	29	36	65
IBDU	3	21	24
Non-IBD	25	80	105

9.1.3 Universal polymerase chain reaction

Collection of stool samples, DNA extraction and PCR products from the 257 fecal samples had previously been derived at Ahus. The samples were stored at -80ºC. DNA had been extracted using QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). Using the GA universal 16S rRNA primers, PCR conditions were as follows: HotFirePol 1.25U, B2 buffer 1x, MgCl2 2.5mM, dNTP 200µM (Solis BioDyne, Tartu, Estonia), GA Cover -all™ 16S rRNA forward primer 0,2µM, GA Cover -all™ 16S rRNA reverse primer 0,2µM (Genetic Analysis, Aas, Norway), DNA template 10-100ng. Each reaction was done in a final volume of 25µL. The reactions were performed using a standard PCR machine and the conditions were as follows: 95°C for 15 min, followed by 30 cycles at 95°C for 30s, 55°C for 30s, 72°C for 1min 20s, followed by a final elongation at 72°C for 7 min.

The PCR products were treated with Exonuclease (EXOI) 3U and Shrimp Alkaline Phosphatase (SAP) 8U in order to remove excess primers and unincorporated nucleotides respectively. Subsequently the samples were incubated at 37°C for 2h followed by 80°C for 15 min.

9.1.4 Probes

Four probes previously generated by the GA-map ProbeTool™ were chosen on the basis of the literature study (AppendixA, Appendix B). The hybridization of the probes had previously been tested against target species among 262 bacterial strains. Bacterial hits are described in Table 3. Additionally a universal probe was used.

9.1.5 Endlabeling

A bacterial strain specific probe was hybridized to complimentary sequences in the sample and extended with one base pair using fluorescent ddCTP (Tamra) in an end-labeling reaction (Single Nucleotide Extention, SNE). All samples were hybridized with five probes (*Table 3*) in separate reactions. The reaction was performed with a final volume of 10µL. The conditions were as follows: HOT Termpiol DNA polymerase 0,25U, HOT Termipol Reaction Buffer C 1x, $MgCl_2$ 4mM (Solis BioDyne, Tartu, Estonia), ddCTP Tamra 0,4µM (Jena Bioscience, Jena, Germany), GA probe 0,1 µM (Genetic Analysis, Aas, Norway),

EXOI-SAP treated template 2 µL. The samples were loaded on a thermal cycler: 95°C for 12 min, followed by 10 cycles at 95°C for 20s and 60°C 35s.

Residual nucleotides and phosphate groups from the 5'end were removed by SAP, 1U per 10 µL end-label product. Further the samples were incubated in a thermal cycler at 37°C for 1h followed by 80°C for 15min.

9.1.6 Capillary gel electrophoresis

Next, 1µL of each SAP treated end-labeling product was transferred to a MicroAmp™ Optical 96-Well Reaction Plate and mixed with 9 µL Hi-Di™ formamide and 0,5 µL GeneScan™ 120 LIZ™ Size Standard (Applied Biosystems, Foster City, CA, USA) Subsequently, the samples were denaturated at 95°C for 5min. Capillary gel electrophoresis were performed using an AB 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Fluorescent signals were measured and represent semi-quantitative signal of previously bound probe. The samples were analyzed using GeneMapper ® Software v4.1 (Applied Biosystems, Foster City, CA, USA). Default settings were used including a cut off value of 100 relative fluorescent units (RFU).

9.1.7 Quantification of PCR products

Quantification of the PCR products was performed using Quant-iT PicoGreen ds DNA Reagent and Kits as previously described (Invitrogen, 2008) (Invitrogen, Carlsbad, CA, USA). In order to make a standard curve, Lambda DNA standard was diluted in 1x TE and Quant-iT PicoGreen Reagent to the following final concentrations: (1,0 ng/µl, 0,1 ng/µl, 0,01 ng/µl, 0,001 ng/µl and 0 ng/µl).

Furthermore, 2 µl of each sample was diluted in 100 µl Quant-iT PicoGreen Reagent and 98 1xTE. A control sample (Lambda DNA standard) with known concentration was measured on each run. Each standard, control and sample was analyzed in triplicate. Fluorescent signal were detected using FluoStar Optima (BMG Labtecth, Offenburg, Germany). The quantification of PCR product was done by the Mars Data Analysis software version 1.10 (BMG Labtecth, Offenburg, Germany). Standard deviation (SD) and Coefficient of variation (CV) was calculated of the RFU. Standards and controls was accepted at <10% CV and samples at<33% CV. Additionally the control was accepted at <3SD from the standard curve.

The RFU of the blank was subtracted from the average RFU signal of each sample. Further the concentration of the sample PCR product was determined by the standard curve (y=53897x+27,082).

9.1.8 Statistical Methods

The patient samples was randomly distributed in three 96 well plates according to patient status. To further normalize potentially plate to plate variation, sample RFU values were transformed to percent value of mean RFU values per plate. Further, normalization of variable concentration of PCR product was done. The sample value was divided by the concentration of PCR product as measured by Quant-iT PicoGreen. Mean, median and SD of normalized signals from UC, CD, IBDU and control individuals are presented in
Table 5.

For probe signal between patient groups comparisons, 1-way analysis of variance (ANOVA) was performed. Non-parametric distributed data significances were confirmed using the Mann-Whitney-Wilcoxon-test (MWW). Further, Tukey's Honestly Significant Differences (HSD) Test was done for pairwise comparisons of the patient groups. The calculations were done using PASW Statistics 18 (SPSS Inc . Chicago, Illinois, USA). Statistical significance was accepted at $P< 0,05$.

Percent sensitivity (True positive / (true positive + false negative) and specificity (true negative/(true negative + false positives) was calculated for significant probe in patients versus controls. True positives being patients correctly identified as such, and false negative being patients diagnosed as non-IBD. Similarly true negative is non-IBD patients diagnosed as such, and false positives are non-IBD patients diagnosed as IBD.

9.2 Results

The probes hybridized to sequences in all cohorts. The signal intensity between probes varied greatly. While the IG0028 probe had a mean fluorescent intensity of 289 the Universal probe had a mean intensity of 4116. Due to among other weak fluorescent signal, samples were removed from the study (Table 4).

Table 3 Phylogenetic distribution of the probes. Probes are assumed to cover all species within a given family

GAprobe	phylum	class	order	family
IG0005	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae
			Aeromondales	Aeromonadaceae
			Pseudomonadales	Pseudomonadaceae
		Epsilonproteobacteria	Camylocacterales	Helicobacteraceae
		Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae
	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae
IG0028	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae
				Corynebacteriaceae
				Micrococcaceae
				Streptomycetaceae
			Bifidobacteriales	Bifidobacteriaceae
AG0006	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae
				Corynebacteriaceae
				Micrococcaceae
				Streptomycetaceae
			Bifidobacteriales	Bifidobacteriaceae
		Coriobacteridae	Coriobacteriales	Coriobacteriaceae
	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae
				Porphyromonadaceae
				Prevotellaceae
		Flavobacteria	Flavobacteriales	Flavobacteriaceae
		Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae
	Firmicutes	Bacilli	Bacillales	Staphylococcus
			Lactobacillales	Lactobacillaceae
		Clostridia	Clostridiales	Blautia
				Clostridiaceae
				Clostridiales Family XI. Incertae Sedis
				Eubacteriaceae
				Lachnospiraceae
				Peptostreptococcaceae
				Ruminococcaceae
		Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae
	Verrucomicrobia	Lentisphaerae	Victivallales	Victivallaceae
AG0012	Actinobacteria	Actinobacteridae	Actinomycetales	Corynebacteriaceae
				Streptomycetaceae
		Coriobacteridae	Coriobacteriales	Coriobacteriaceae
	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae
	Firmicutes	Clostridia	Clostridiales	Veillonellaceae
		Bacilli	Lactobacillales	Lactobacillaceae
	Fusobacteria	Fusobacteria (class)	Fusobacterales	Fusobacteriaceae
	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae
		Gammaproteobacteria	Pseudomonadales	Pseudomonadales

9.2.1 Probe quantification

Mean signal between group variance of the probes is presented in Table 6. In particular a significant *P*-value in probe IG0005 was observed. No significant differences were predicted using the Universal, IG0028, AG0006, or AG0012 probe. The MWW test showed similar

significance for the IG0005 probe (Asymp Sig. (2-tailed) <0,0005). The Tukey HSD test (Table 7) further showed a significant increase in the IG0005 probe in CD patients compared to the control group (*P*=3,56E-4). Although not significant, low *P*-values was observed between CD-UC (*P*=0,089) and between CD-IBDU (*P*=0,065). No significant values among other patient cohorts were found in IG0028, AG0006 and AG0012 probes (data not shown).

The distribution of IG0005 positive bacteria indicates two distinct peaks (Figure 7). Overall, no significant difference in RFU was found between adult and child (ANOVA). However, when analyzing the IG0005 probe signals separately (HSD), adult samples showed significant difference between CD and controls (p=0,003) while no significant value was found in the child cohort relative to controls (p=0,274). Accuracy data for the IG0005 probe was calculated (Table 8). According to this sample set the IG0005 used as a diagnostic marker in feces, could detect 58,3% of the CD patients.

Table 4 Valid and removed number of samples

	Universalprobe	IG0028	IG0005	AG0006	AG0012
Valid	218	65	250	253	252
Removed	39	192	7	4	5

Table 5 Profiling of fecal samples from UC, CD, IBDU and control individuals. Normalized signals.

		Universal	IG0028	IG0005	AG0006	AG0012
UC	Mean	10.572	11.452	10.085	9.916	10.953
	Median	6.393	8.438	7.685	9.168	8.743
	Std. Deviation	10.5529	8.3777	8.3404	4.0916	6.0368
CD	Mean	11.235	11.540	13.645	11.162	9.961
	Median	9.960	10.269	15.231	10.212	9.193
	Std. Deviation	7.1232	6.5789	8.8931	5.3880	4.0141
IBDU	Mean	9.643	10.621	8.651	10.373	10.191
	Median	7.472	9.555	4.857	8.570	9.310
	Std. Deviation	7.9973	5.7026	8.1694	6.7009	4.4649
Control	Mean	10.421	13.613	8.153	10.102	10.772
	Median	9.398	11.176	4.371	9.163	9.909
	Std. Deviation	7.6171	8.6919	8.0041	3.9719	5.2750

Table 6 The 1-way ANOVA between patient group comparisons. Normalized signals.

GA probe	*P* =
Universal	0.884
IG0028	0,773
IG0005	**0,001**
AG0006	0,444
AG0012	0,680

Table 7 Par wise comparisons of cohorts. Tukey HSD Test for the GA developed probe IG0005 sig. values.

	UC	**CD**	**IBDU**	**Control**
UC	-			
CD	0,089	-		
IBDU	0,891	0,065	-	
Control	0,474	**3.56E-4**	0,994	-

Table 8 Accuracy data for differentiating Crohn's disease from controls using the IG0005 probe with a cut off value of 12 of normalized values.

	CD patients	Control patients
Diagnosed as CD	35	28
Diagnosed as non-CD	25	77
Total number of patients	60	105
	Sensitivity	Specificity
	58,3%	73,3%

9.3 Discussion

The IG0005 probe was found to be significantly increased in CD samples compared to controls. The IG0005 probe hybridizes to species in the Enterobacteriaceae, Aeromonadaceae, Pseudomonadaceae, Helicobacteraceae, Desulfovibrionaceae, and Flavobacteriaceae family (Table 3). The result is supported by previous studies demonstrating increased numbers of Gammaproteobacteria in fecal samples from CD patients compared to controls. Significantly, increased levels ($P<0,05$) of Enterobacteriales was observed in a study using digested 16S rRNA genes determined by T-RFLP (Andoh, et al., 2009). In a semi –quantitative

microbiological study, a significant increase in total score of *E. coli* in active CD compared to UC, inactive CD, and controls was reported (Giaffer, Holdsworth, & Duerden, 1991). Further signals from Enterobacteria were detected in all CD fecal samples (17) but none in the healthy controls (16) using dot blot hybridization. Further no significant difference in Enterobacteria between CD patients in remission and flare was found (Seksik, et al., 2003).

An increased level of Enterobacteriales is also reported by several studies on the IBD mucosa (M. Baumgart, et al., 2007; Frank, et al., 2007; Gophna, et al., 2006; Swidsinski, et al., 2002) Interestingly the number of *E.coli* in situ correlated with severity of CD according to a study by Baumgart et al. (M. Baumgart, et al., 2007).

Two distinct peaks in the IG0005 histogram (Figure 7) were observed. It might indicate the existence of two sub groups of CD patients. One group containing increased amounts of IG0005 positive bacteria, the other group with similar amounts as controls. Higher quantities of mucosa-associated *E. coli* have previously been shown in subgroups of Crohn's iletis (Darfeuille-Michaud, et al., 2004; Martinez-Medina, et al., 2009). It could be hypothesized that the CD patients with increased amounts of the IG0005 in the fecal sample, reflect AIEC associated with ileal CD.

Diagnostic accuracy for the IG0005 probe was calculated (Table 8). Serologic markers (pANCA, ASCA) currently used in diagnosing IBD, have similar sensitivity values. A combination of ASCA(+) with pANCA(-) test in CD have been observed with 54,6%, 92,8% for sensitivity and specificity, respectively (Reese, et al., 2006).

To be used as a diagnostic marker the specificity should be enhanced. This could be done by modifying the IG0005 probe sequence in order to exclude non disease specific bacteria hits. A combination of probes on the GA-map^{TM} array could also enhance the diagnostic accuracy for CD. The predictive values of the test depend critically on the prevalence of CD in the patients being tested (Altman & Bland, 1994). Hence, in a diagnostic setting, the use of certain criteria for offering the test to a patient is crucial.

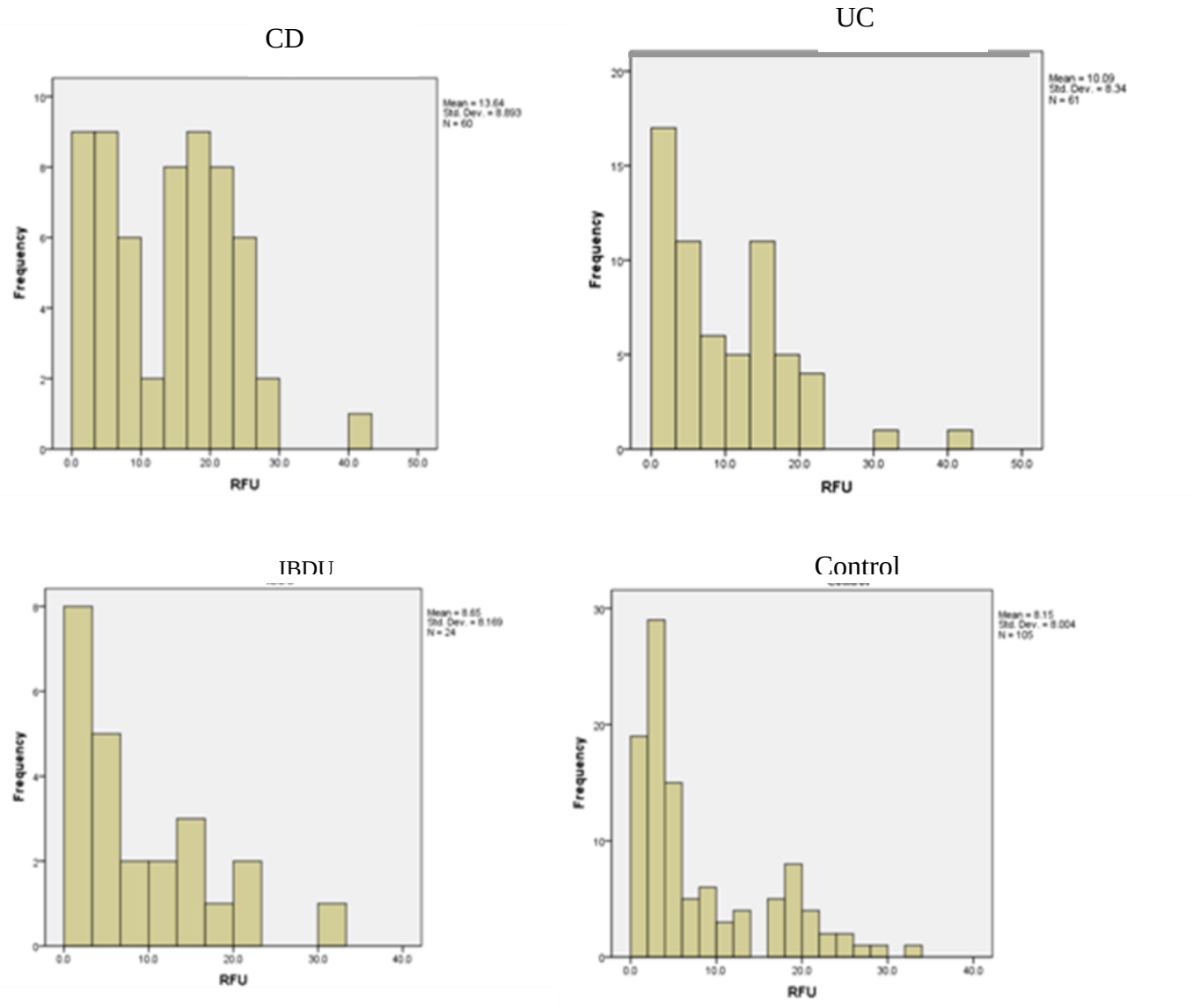

Figure 7 Distribution of RFU in the IG0005 probe in CD, UC, IBDU, and control patients

Actinobacteria have previously been found in decreased levels in fecal samples from CD patients (Table 1). This was not confirmed in this study. The IG0028, AG0006 and AG0012 probe which comprise complementarities to Actinobacteria was not observed with significantly decreased levels. Only 25% of the samples hybridized with IG0028 probe had fluorescent signal intensity above the cut off value. A reevaluation of this probe should be performed with optimized probe conditions. In IBD, species within the Firmicutes and Bacteroidetes phyla has been observed in decreased and increased levels respectively. Especially Clostridia and Bacteroida species have been observed with respectively decreased and increased levels in CD. The AG0006 and AG0012 probe both comprise sequences

complementary to species including Actinobacteria, Bacteroidetes, and Firmicutes phyla (Table 3). It could be hypnotized that these probes covers too many bacterial species to reveal diagnostic relevant information.

Finally, IG0005 was identified as a candidate probe for the GAmap™ array separating CD patients from controls. Nevertheless the results should be confirmed in three parallels as the samples in this study were performed once. If confirmed, the probe could add valuable information to current diagnostic markers in IBD.

10. Examination of commercial potential and patent situation of IBD diagnostics

10.1 Commercial potential of improving IBD diagnostics

10.1.1 Direct and indirect cost in IBD

Cost of disease studies often includes direct and indirect costs. The indirect health costs comprise decreased work productivity and disability while the direct health costs comprise the expenditures incurred by the health care system in managing a disease (Camilleri & Williams, 2000). IBD afflicts young adults during higher education, in their economical productive years and careers. Lifelong medication is required and often surgery is necessary leading to costs for the society. Direct health care costs are increasing and affects the national health care resources (Odes, 2008).

Varying costs are being reported for IBD in different countries and studies, primarily because of the high prices in private medical practice. Studies report a total economical burden of CD to be $10-15 billion in the US and € 2-16 billion in Europe (Yu, Cabanilla, Wu, Mulani, & Chao, 2008). In Germany cost diaries reports a mean 4-week cost per patient of €1425 for CD and €1015 for UC. The indirect cost accounted for 64% and 54% for CD and UC respectively (Stark, Konig, & Leidl, 2006). Annually CD patients cost ~$18 500 in the United States and €2800-6900 in other Western countries. Indirect costs accounted for 28 and 64-69% in the US and Europe respectively (Yu, et al., 2008).

10.1.2 Costs of IBD diagnostics

Each year a high number of patients with functional diseases are suspected to have IBD and unnecessarily undergo invasive testing (Suleiman & Sonnenberg, 2001).
Few studies have investigated the clinical and economical effect on different IBD diagnostic strategies. A European cost study with 10 years follow-up, found mean (median) costs of IBD diagnostics of €257 (€95) per patient-year. The expenditures were highest in the first patient year at €734 per patient. CD diagnostics were significantly more expensive than UC diagnostics (Odes, et al., 2006). Similarly a study from the US showed that laboratory

procedures accounted for 3.6% ($470) of total health care costs in 12 months after the index diagnosis in UC patients (Bickston, Waters, Dabbous, Tang, & Rahman, 2008).

A way to reduce the number of patients who unnecessarily underwent invasive testing including colonoscopy and biopsies with histological examination, UGI and SBFT, has been indicated in a study by Dubinsky et al. The criteria were a sequential serodiagnostic screening of ANCA, ASCA followed by a confirmatory assay comprising pANCA and classical anti-neutrophil cytoplasmic antibodies (cANCA). In fact the study showed a reduction of 39% of invasive testing and similarly a reduction of $550 per patient and an increase of diagnostic accuracy of 2%. Additionally the saved costs might be higher as indirect costs and other direct costs was not considered (Dubinsky, et al., 2002).

This may also suggest a potential cost-effective role for other IBD diagnosing methods depending on the overall cost and accuracy of the test. Initially the price per GA-map ™ test is assumed to be in the range between 1 000 and 2 000 NOK ($160-$330) by GA. The threshold, in the mentioned study, for a cost-effective role of an initial test before invasive testing was $650 and $1205 with base case costs of $53.67 and $146.49 for the existing primary and confirmatory assays respectively. Hence the estimated price for the GA-map™ test is within the threshold for cost effectiveness of testing before initiating invasive testing. Yet, this depends on the prevalence of IBD patients with suggestive symptoms and final sensitivity and specificity of the test. The same study found that a 80% reduction of the costs of invasive testing or a prevalence of IBD of at least 83%, were the threshold where a serodiagnostic strategy no longer was cost-effective (Dubinsky, et al., 2002). Nevertheless one should keep in mind that this study is using a price list from year 2000, hence the costs probably differ ten years later. Interestingly the primary assay had a sensitivity of 80% and a specificity of 50%. Contrary the confirmatory assay had a sensitivity of 65% and a specificity of 95% (Dubinsky, et al., 2002).

New non-invasive procedures and inventive ways to distinguish IBS from IBD could also lower the overall costs of diagnostics. In fact IBS can be ruled out by a diagnostic probability of more than 80% without invasive tests, a study showed. Also, simply by questioning patient about symptoms and history in addition to the physical examination a sensitivity and specificity of 65% and 100% respectively of IBS was achieved (Suleiman & Sonnenberg, 2001).

10.2 Specifications in IBD diagnostics

There has been a search for laboratory markers of IBD for the last decades without great success (Vermeire, et al., 2006). An ideal marker should have many qualities. In terms of performance it should be fast and easy to perform, not invasive, cheap, and reproducible between labs and individuals. Beside diagnose IBD it should also assess disease activity and severity, and determine the prognosis and therapy response.

Since the symptoms often are subjective the test should objectively assess disease activity and severity. It needs to separate non-IBD from IBD (specificity) and correctly identify IBD patients as having the disease (sensitivity). If possible it should identify persons with risks of IBD.

10.3 Calculated number of patients tested for IBD without having the disease

Abdominal pain and diarrhea is a common cause for contacting a physician. Several diseases can cause these symptoms, both functional and organic. IBS is a functional GI disorder commonly confused with CD and UC. In the western world it is assumed that 10-20% of the adults have irritable bowel syndrome (IBS) in which 30% seek medical assistance (Maxion-Bergemann, Thielecke, Abel, & Bergemann, 2006; Suleiman & Sonnenberg, 2001). Nonetheless up to 50% who have IBS undergo examination of the colon to exclude cancer and IBD (Gunnarsson & Simren, 2008).

Number of patient tested for IBD without having the disease is not easily available. Rather it can be assumed that the number of patients is several times higher than new patients diagnosed with IBD annually. The European mean incidence rates per 100 000 was 5.6 for CD and 10.4 for UC in patients aged 15-64 (Binder, 2004). Roughly calculated this means more than 115 000 persons are diagnosed with IBD in Europe annually, (assuming a European population of 731 000 000) (Europe, 2010). Moreover, 22-43 million Europeans with IBS seek medical assistance each year. Likewise US with its 308 million inhabitants have similar incidence rates meaning 50,000 new IBD patients each year. Similarly it can be

calculated that 9-18 million persons in the US suffering from IBS seeks medical assistance each year.

10.3.1 Symptoms and final diagnosis after endoscopy in children

A study comprising children showed correlations between main symptom before examination and final diagnosis after endoscopy. Of the children with blood in the stool as the main symptom, 49% did not have a GI disease, 28% had IBD, while the resuming had other diseases. Of the children presenting with chronically diarrhea as the main symptom 30% did not have a GI disease, 21% had IBD, 16% had celiac disease and the remaining had other diseases. Of the children with abdominal pain as main symptom 19% was diagnosed with unspecific abdominal pain as their final diagnosis, 9,5 % had IBD, 5,4% had IBS, 19% had celiac disease (Perminow, Rydning, Jacobsen, & Frigessi, 2000).

10.4 The usability of new diagnostic tools in different settings of IBD

In IBD, mainly four goals for initiation of a diagnostic procedure exist. These comprise establishing of the disease (IBD), assigning of CD or UC, guiding therapeutic decisions, and detect complications that require treatment (Nikolaus & Schreiber, 2007). Challenges on all these stages exist. The diagnostic accuracy of a test decides on which level of the diagnostic course it can be used. If the diagnostic sensitivity is high it might be used as a primary test. If the diagnostic specificity is high it could be used confirmatory.

10.4.1 Establishing of the disease

As previously described, research results indicate a difference in stool microbiota of IBD patients relatively to those of healthy persons. The biggest market for an IBD test is as a screening test. Though, even if the test is as good as or even better than endoscopy in diagnosing IBD, an invasive procedure might be necessary in some cases, in order of excluding other diseases like GI cancer and celiac disease. A positive IBD test might have to be confirmed by an invasive test to reveal the extent of the disease. But if the test could separate a substantial number of IBS patient from IBD, the test could be useful. Further investigation is needed to find indications for performing such a screening test.

10.4.2 Assigning of CD or UC

Proper treatment of IBD is important. Knowing that 10% of patients first diagnosed as UC five years later are rediagnosed as CD or discounted (Packey & Sartor, 2008) there is a need for a new method. Contrary 10-15% of the CD patients are being reclassified as UC during the first year (E F Stange, et al., 2006). Further, 10-15% of the IBD patients remain unclassified (Nikolaus & Schreiber, 2007). Additionally, about 5-10% of patients undergoing ileo-pouch anal anastomosis surgery with a diagnosis of UC at the time of surgery are subsequently diagnosed with CD (Fleshner, Vasiliauskas, Melmed, & Targan, 2008). Some results indicate different microbiota in stools of IBD patients especially CD patients (Chapter 8).

10.4.3 Guiding therapeutic decisions

A test using microbiota might also be useful in guiding of therapeutic decisions. Mesalamine (5-ASA) lowers sulfide concentrations (Packey & Sartor, 2008). Higher concentrations of hydrogen sulfide producing bacteria have been reported in the microbiota of active UC patients compared to those in remission (Sokol, et al., 2008). UC patients are warranted to take follow-up endoscopies to find the time to stop taking mesalamine and start maintenance therapy or as a outcome parameter for biological therapy (Nikolaus & Schreiber, 2007). If the GI microbiota is proved to respond to different therapy, a GA-map^{TM} array could be costume made to guide therapeutic decisions.

A US study showed that medical expenditures (sum of inpatient, outpatient, emergency room, and prescription drug spending) accounted for $18,963 and $15,020 for CD and UC respectively. Infliximab entered the arena for treating severely affected CD patients in 1998. UC patients now also receive Infliximab. There are an increasingly trend to introduce Infliximab earlier in the therapeutic regimen as it reduces hospitalization and surgery. Infliximab is very expensive. It can cost $19,000 to $22,000 a year per patient (Gibson, et al., 2008). Thus a test guiding the physician in which patients who should receive biologic therapy, when to start, predicting response, when to end therapy and when to change to other types of therapy, is desirable.

10.4.4 Detect complications that require treatment

Charges for hospitalization, surgery and biological therapy comprises a large percentage of the health care costs in IBD. Keeping a patient in remission therefore means more than increased quality of life to the patients, it also reduces the costs related to medical and surgical hospitalization (Odes, 2008). Indeed a study showed that patients with severe CD had 3-9 fold higher costs than patients in remission (Yu, et al., 2008). A test improving therapeutic guiding and detection of complications would enhance health related quality of life for IBD patients and reduce the health care costs.

10.5 Profits and research budget

When developing a commercial diagnostic test, it is important to have an economic framework for research and development. Estimated profits for a patent period can be used as an indication of commercial potential and what should be invested in research and development. The following presentation of possible scenarios includes estimates, risks and uncertainties. In order to estimate the profit of the GA-map™ test, a business plan must be developed. However, this was out of the scope of this thesis, so a simplified method for budget estimates is presented.

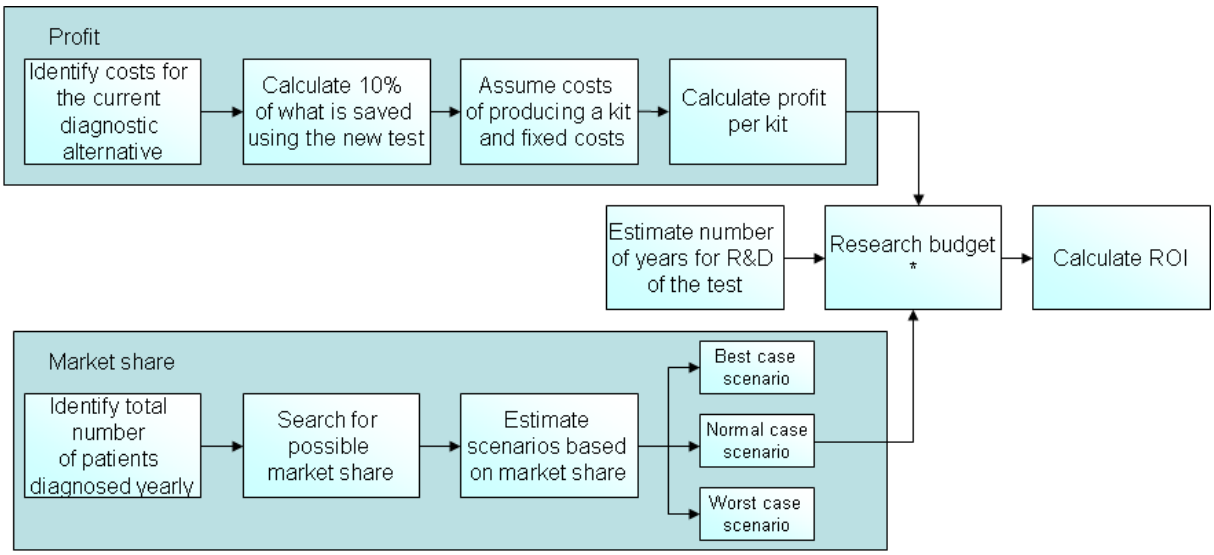

*Figure 8 Method for estimating research budget and ROI. *50% of profit in the normal case scenario for the R&D period is calculated as a research budget*

To estimate the profit, research budget and ROI for a diagnostic test, a calculation method was developed (Figure 8).

10.5.1 Market

Forecasting the market share of a future product is a challenge and depends on several factors such as how much funding will be spent on marketing. What market share can GA expect? As an example, a Norwegian company, NorDiag ASA, is selling a genetic test for early diagnosis of GI cancer based on human DNA in fecal samples (Genefec™). The test was launched in 2002 and four years later the market share was 11% in Norway, 2 % in the Nordic countries and 0,09% in Europe (Nordiag, 2010). Another comparison is an American company Myriad Genetics Inc. with their breast cancer diagnostic test, BRACAnalysis. The test is used by 10% of the American obstetrician/gynecologist (Seekingalpha, 2010). The market share depends on the performance of the diagnostic test. In this calculation it is assumed that the test perform as good as or better than endoscopy. Further an assumption was that it can be sold as a kit for screening IBD by differentiate between IBD and IBS and be used to avoid endoscopies especially in IBS patients. The potential market for GA-map™ is presented in Table 9 and three different scenarios based on different market share are presented in Table 11.

Table 9 Market for an IBD screening test

	Number of patients yearly	Number of patients in a 20 year period*
Patients diagnosed with IBD in USA and Europe¤	165 000	3 300 000
Endoscopies performed on IBS patients in USA and Europe§	15 500 000	310 000 000
Sum market	15 665 000	313 300 000

¤ Based on chapter 10.3
§ Assuming 50% of the calculated IBS patients (10.3) undergo an endoscopy examination (Gunnarsson & Simren, 2008)
*Period of a patent

10.5.2 Price

A recommended way to determine the price for a new inventive product is 10% of the expenditures of the existing alternatives (Monrad-Krohn, Personal communication). But factors like the degree of reimbursement from European national health authorities or American insurance companies, can affect the price. The recommended price in Table 10 does

not include labor costs. Even though additional costs as hospitalization may be saved as a result of new diagnostics, such cost was not included in the calculation of the price. Price and calculated profits are presented in Table 10.

Table 10 Calculated profits pr GA-map™ test sold

Recommended price for GA-map™ kit*	€70
Estimated marginal costs of a GA-map™ kit **	€20
Gross margin§	€50
Estimated share of fixed cost (30%)	€15
Profits per GA-map™ kit	€35

*Assuming the existing diagnostic alternative corresponds to expenditures the first patient year, €734 (Odes, et al., 2006) Labor is not included
**Cost of producing one GA-map test is assumed to cost €10 by GA, but in this calculation €20 is used
§Gross margin: Price – marginal costs

10.5.3 Profit estimates

The margin depends on a number of factors e.g. competition, volume, turnover rate and business talent of the entrepreneurs (Utgård & Refsum, 2007). The competition further heavily depends on the patent situation. Additionally, the marginal costs depend on the complexity of the test. Few probes in e.g. GA-map™, result in lower marginal costs. Price, margin and there by profit will be highest in the first years, but might decrease as competing diagnostic tools become available.

Table 11 Scenarios for a patent period

	Worst case scenario 0,01% market share in Europe and USA	Normal case scenario 0,5% market share in Europe and USA	Best case scenario 10% market share in Europe and USA
Tests sold to IBD patients 20 years§	330	16 500	330 000
Tests sold to IBS patients 20 years§	31 000	1 550 000	31 000 000
Sum tests sold next 20 years§	31 330	1 566 500	31 330 000
Yearly profit *	€55 000	€2,7 Mill	€55 Mill
Sum profit the next 20 years*	1,1 Mill	€55 Mill	€1 100 Mill

§Number of tests is based on Table 9
*Profits are based on Table 10

10.5.4 Investing in research

In order to assure reasonable ROI a calculation of the threshold for research budget should be performed. The period of development is assumed to be three years for the diagnostic test. Additionally 2-3 years are necessary for an approval from Food and Drug Administration (FDA). In most situations a long period must be calculated to penetrate the market, but in this

calculation a simplified model was used. If one further assumes that 50% of the profits for five years are budgeted on research and development, this totally accounts for €7 Mill. in the normal case scenario (Table 11). In return this leads to a 38% ROI of research (grossly simplified calculations for guidance only). This is considered as acceptable numbers to recommend commercialization.

10.6 Examination patent situation in IBD diagnostics

In biotechnology patents plays an important role. Particularly it is important for the advancement of innovation. Investors can get a security for their funds and may be compensated for high development costs.

The US is traditionally one of the main markets for biotechnology and therefore all development within the US patent law, are of interest to the biotechnology industry worldwide. Europe has also become an important biotech market. Since 1977 it has been possible to apply for a joint patent in Europe (EPO, 2010b). Biotechnology is a field of constant development, and patenting of some parts of it is controversial. Especially the patenting of genes and genetic diagnostics has been publicly discussed. Some of these patents have resulted in litigations. Modification of the patent law or how it is interpreted may be a result. Biotechnology companies may benefit from following this development. A description of the European and American patent system is presented in Appendix C.

The nature of the patent system allows others to learn what has already been invented. Resources can be saved and competitors or potential business partners might be found by searching for patents and patent application in patent databases. An indication whether a diagnostic test for IBD based on fecal microbiota has a commercial potential may be found by search in patent databases.

To find new inventive IBD diagnostic methods, search in European and American patent databases were performed.

10.5.5 Issued US patents

To find an optimal search profile an iterative search process was performed (Appendix D). The query comprised words in the title and abstract of the documents in the database. In addition application date was set to the last ten years. The following search was performed in the United States Patent and Trademark Office (USPTO) Patent full-text and image database (http://patft.uspto.gov/netahtml/PTO/search-adv.htm): ((((ABST/"inflammatory bowel disease" OR ABST/Crohn) OR ABST/"Ulcerative colitis") AND ABST/diagn$ OR ABST/analy$ OR ABST/detect$ AND APD/20000101->20100803). 54 patents were found (

Appendix H), in which 36 were referring to IBD diagnostics. The remaining patents were primarily related to treatment of IBD or treatment and diagnostics of non-IBD diseases.

Even though most patents do not specify a certain biologic material for detecting IBD, a majority of the found patents might utilize blood samples. In a few patents the use of fecal samples in the diagnosis of IBD patients is claimed. The claimed fecal samples are primarily used for lactoferrin detection. Lactoferrin is a detection marker for leukocytes in fecal samples, and elevated levels are associated with IBD in contrast to IBS (James Hunter Boone, David Maxwell Lyerly, Tracy Dale Wilkins, & Richard Littleton Guerrant, 2009; Boone, 2007). Interestingly exhaled gas after digestion of a controlled amount of radio labeled sugars is also used in some of the patents (Lin & Pimentel, 2004; Lin & Pimentel, 2006). Another patent use sera for the detection of antibodies for *Fusobacteria varium* to diagnose UC patients, (Sato, Okusa, & Okayasu, 2010). Other patents comprise detection of sequences from an unknown microbial organism from the human gut (Braun & Sutton, 2001, 2004). None of these patents used 16S rRNA in diagnostics; in fact few of the patents were related to detection of bacteria (Table 12).

Nevertheless, human gene and protein sequences and antibodies like ASCA, ANCA, anti-outer membrane protein C (anti-OmpC) and anti-I2 antibody, accounted for most of the relevant US patents (Table 12). The gene patents comprising presence or absence of haplotypes, mutations and gene expression.

10.5.6 US patent application

The same search profile as previously described was used for the USPTO patent application full text and image database (http://appft.uspto.gov/netahtml/PTO/search-adv.html) with the exception of application date, APD/20050101->20100803. The query resulted in 101 hits (Appendix F). Of the 101 patent applications 74 were considered relevant.

The vast majority of the applications use serum and blood for IBD diagnostics, but colon biopsy, urine, and stool samples are also used. Among the most represented patent applications found are human gene or polypeptide and antibodies to ASCA, pANCA, outer membrane protein C (OmpC), anti I-2 polypeptide, anti-CBir1. Among the gene expression applications are methods predicting responsiveness of infliximab treatment in UC patients (US20090054253, US20080293582). Another inventive application was prostaglandin in urine which can be measured to separate pre-remission from the remission state of UC (Fujiwara, Isao, & Hayashi, 2009).

A US patent application claiming the use of rRNA sequences in order of detecting bacteria was found, "Composition and methods for diagnosing colon disorders, US 20090197249". The invention relates to diagnosing, prognosticating, or monitoring disease progression of IBD by detecting bacterial rRNA genes in mucosal tissue or lumen samples. Among the species are *Acinetobacter* sp. of *Pseudomonas* group, *Bacteroides* sp. of the *Bacteroides* Group, *Ruminoccocus* sp. of the *C. Coccoides* Group, Clostridium sp. of the *Clostridium botulinum* Group and *Enterococus* sp. of the *Enterococcus* Group. Among other *E. coli* is used in the diagnosis of pouchitis (Gillevet, 2009). If issued, this patent application, filed by the US George Mason University November 1, 2005, could reduce the freedom to operate in IBD diagnostics using fecal microbiota.

Another application comprised an assay for nucleic acids of two bacteria genes namely serine protease autotransporter (SPATE) family and antigen 43 (Ag43). Ag43 is a surface adhesin promoting bacterial biofilm formation due to cell-to-cell aggregation. SPATEs are potentially important in IBD because they exhibit functions like degradation of the barrier function of the gut, and cleavage of proteins in the enterocyte, which are phenotypes associated with IBD. Conserved and specific regions to virulent enteric strains of *E.coli* can be detected in fecal samples as a biomarker of IBD (Krause & Bernstein, 2009).

10.5.7 European patent documents

Relevant patents and patent applications in Europe were found by search in the EPO's patent database Esp@cenet (http://ep.espacenet.com/advancedSearch?locale=en_EP). The database has some limitations compared to the USPTO database. Only one member of each patent family is shown and a smaller number of words are allowed in the search query. As a result, a limited number of European patent documents have identified in this study. The worldwide database was chosen for the following query (Appendix E): "inflammatory bowel disease?" or crohn* or "ulcerative colitis" (diagnos* or analys* or detect* or determin*) in the title or abstract AND EP as the publication number AND 2005:2010 as the publication date. Twenty applications were found in which two were written in a foreign language and was not considered (Appendix G).

Table 12 Number of patents using various markers

	Esp@cenet	US application	US patent
Antibody	7	29	9
Cytokine	1	4	1
Human gene** and/or polypeptide	7	31	12
Human protein	2	6	8
Bacterial protein		1	
Metabolite	1	1	
Gas	1	2	2
Cell*		3	1
Glycans§	1	2	
Bacterial gene** and/or polypeptide	1	3	4
Other	1	2	2

The number of markers do not sum up to number of patents due to multiple markers used in several of the patent documents.
** "Gene" also comprising nucleic acid sequences, alleles, gene expression etc.
* Cells e.g: antigen presenting cell that expresses polypeptides or T cells that are specific for cells expressing such polypeptides.
§ Glycans e.g.: oligosaccharide in immunoglobulin G (IgG), O-linked glycans etc.

Several ways of diagnosing IBD are presented in the considered applications (Table 12). Antibodies, gene expression, gene variants and genotypes are represented in most documents.

The expression of anti-CBir, an antibody against flagellin, is associated with Crohn disease but not UC and is found in about 40% of patients with Crohn disease who are ASCA-negative. In addition the detection of inflammation markers in biopsy, prostaglandin metabolite in urine, glycans and oligo saccharide in blood are claimed ways to diagnose IBD. Interestingly lactoferrin measured in fecal samples obtained at two different times, are claimed to improve the detection of IBD compared to one measurement (J. H.. Boone, D. M. Lyerly, T. D. Wilkins, & R.L. Guerrant, 2009).

10.5.8 Current focus in patenting of IBD diagnostics

Few patents related to bacterial genes or polypeptides (Table 12) were found. Rather the current focus in IBD diagnostics seems to be human genes or polypeptides/polynucleotides, and antibodies. A trend seems to be an increase of applications claiming methods with the use of gene expression and human DNA variations which are more represented than among the found issued patents. Also an increased interest of antibodies is indicated by number of found patent applications.

10.5.9 Patent assignees in IBD diagnostics

Two American assignees, Cedars-Sinai Medical Center and Prometheus Laboratories Inc, have in particular numerous patents and applications covering both treatment and diagnostics. Cedars-Sinai Medical Center have a broad field of diagnostics and have patents comprising the detection of the *CARD15* gene, interleukins, small intestinal bacterial overgrowth by measuring exhaled gas, antibodies like ASCA, pANCA, IgA anti-OmpC. The focus on antibodies and other interest concerning IBD diagnostics and therapy is shared by Prometheus. Their patent documents were found in all three databases (Appendix F, Appendix G,

Appendix H). Especially interesting are their invention using an algorithm based on the levels of IBD markers like ANCA, ASCA-IgA, ASCA-IgG, anti-OmpC antibody, anti-I2 antibody, and pANCA in serum in IBD diagnostics. With low sensitivity and specificity one

by one these antibodies gives a complex picture of IBD. But calculated simultaneously they may add valuable information in the diagnostics of IBD. It is stated that the algorithm also can be trained to detect complications and the efficacy of therapeutic treatment.
The autumn 2010 Prometheus and Tarrot Laboratories, a business unit of Cedars-Sinai Medical Center, announced exclusive agreement for CD treatment, to focus on identifying genetic or serologic markers associated with clinical responses to anti-TNF (Prometheuslabs).

Another company with several patents is Techlab Inc. which uses lactoferrin, ASCA and ANCA in feces. Lately the company Exagen diagnostics Inc. have filed several applications on distinguishing IBD and IBS by gene expression. Of all the relevant US patents nine have universities as assignees and six of the relevant US patent applications had a university as assignee. Patents from such an institution might be easier to license compared to that of corporations.

11. Recommendations for future development in IBD diagnostics based on the GA-map™ technology

11.1 Future development of IBD tests

11.1.1 Recommended sample sets

To find suitable fecal markers, comprehensive and well designed studies needs to be accomplished. Today most culture independent studies on fecal microbiota as markers of IBD include few patients and controls. The reproducibility of relative amounts of bacteria in IBD is also poor between articles. This might be due to different sample set used as well as methods. Patient samples are often from long term patients on medications. These are circumstances which can lead to modification of the fecal microbiota. Hence samples from these patients are probably not ideal for studies with the goal of early detection of IBD or assigning CD or UC. Compared to these studies, the sample set used in the present study are unique in quantity and quality. Access to these samples and sample data should be secured by an agreement with Ahus. But other sample sets should also be considered. Since microbiota stability is low in CD, revealing the temporal differences in fecal samples from such patients would be interesting.

11.1.2 Target selection

In this thesis, the CD patients could be detected with sensitivity and specificity of 58,3% and 73,3 % respectively, using one single probe. Adding more probes could enhance these values to match or even be better than those of existing non-invasive tests. Research should be done to reveal a potential etiologic effect of organisms detected by IG0005. Further the effect of serologic status, disease activity, disease location, and *CARD15* genotype should be analyzed in the relation to increased IG0005 levels.

The IG0005 probe was designed to many species. Probes designed to lower phylogenetic levels within this probe should be tested. Similarly the other probes tested in this thesis should also be redesigned to species at lower phylogenetic levels.

Furthermore, other possible targets uniformly reported (presented in chapter 8, Appendix A and Appendix B) should be tested on a patient material.

11.1.3 Type of test to be developed

Based on the high numbers of IBS patients compared to IBD patients, a diagnostic test should focus on differentiating IBD from IBS, in order to exclude IBS patients from having endoscopy unnecessarily. The need for performing other laboratory tests in advance of an IBD test based on fecal microbiota should be investigated. Further the level of the tests sensitivity and specificity is crucial for its use. If GA-map™ should be used as a screening test the specificity have to be high. The prevalence of IBD in the patient population (IBD and e.g. IBS patients) tested has to be limited to avoid false positive.

The fecal microbiota of children differs substantially from that of the adults (Palmer, Bik, DiGiulio, Relman, & Brown, 2007). Hence different tests to children and adult have to be developed.

11.1.4 Challenges of GA-map™

Number of probes
Using the GA-map™ technology, multiple probes can be detected at once. If only a few probes are necessary to diagnose IBD, cheaper and more efficient technologies than GA-map™ should be utilized. The threshold of the number of probes in which GA-map™ no longer is reasonable should be investigated.

Bacterial virulence
The 16s rRNA PCR do not differentiate between strains or identify functional alterations. Therefore, virulence factors, toxin production and the reciprocal regulation of bacterial and host epithelial genes must be investigated by other methods (Packey & Sartor, 2008). An opportunity is to measure 16S rRNA genes followed by virulence genes.

Price and performance

The preliminary price for a test is set to €110-€230 ($160-$330) by GA. This is within the range for what is acceptable before an endoscopy procedure, but considerably more expensive than the recommended price per test (€70) presented in this thesis. The price per test should therefore be reconsidered.

Estimated time of GA-map™ is in the range 24 to 48 hours and experienced personnel are required. Reproducibility of the test remains to be established. To achieve high reproducibility GA may use only a few centers which specialize in doing the analysis.

It is questionable whether a test solely based on microbiota totally can replace the use of endoscopy in IBD. To give a proper therapeutic management it is required to find to which extend the inflammation of the intestine is.

Challenges using the 16S rRNA gene

One of the advantages of using the 16S rRNA gene for identification of bacteria is that cultivation is not necessary. Nonetheless, it has been stated that only concentrations above 10^3 cfu/uL can be reliably detected techniques by using universal 16S rRNA PCR (Swidsinski, et al., 2002). Another challenge with the 16S rRNA gene is that multiple copies of this gene are often present in a bacterium. These copies can differ in sequence, leading to identification of multiple ribotypes for a single organism (Case, et al., 2007).

Furthermore it has been stated that it might be more useful to identify bacterial antigens against which the immune cells of CD and UC patients react, rather than to determine shifts in community composition (Tannock, 2008).

11.2 Commercial remarks

According to EPO, significant commercial potential depends on a number of factors (Table 13) (EPO, 2008). Beside, products are categorized as exceptional, good or unexceptional. The author of this thesis has the opinion that a diagnostic test for IBD based on fecal microbiota

represents an exceptional product which can set new diagnostic standards and possibly dominate the market.

Table 13 Significant commercial potential

Most companies look for (EPO, 2008):	The thesis author's comment related to GA-map™ IBD test
Strong IP or monopoly situation	GA should secure strong IP comprising patents, trademarks, copyright, secrecy and non-disclosure agreements. Additionally monitoring patent infringements is important. Several patents related to improved IBD diagnostics have been identified in this thesis. However, few of the patents were related to IBD diagnostics using fecal microbiota.
Good return on investment	In the normal case scenario a yearly profit of €2,7 Mill. was calculated. The return of investment is estimated to be €1,35Mill. yearly the first five years followed by €2,7Mill. the subsequent years. This result in 38% ROI which is reasonable.
A clear, low-risk route to market	Intensive research is needed in most diagnostic industries, representing a higher risk route to market.
Something consumers will want in preference to competing products	Depending on analytic sensitivity and specificity, it is assumed that patients, doctors and health authorities prefer a diagnostic test based on fecal microbiota as an alternative to invasive tests such as endoscopy. If a diagnostic alternative utilizing markers in blood is launched, it will probably outperform a fecal test. People prefer not to work with feces (Ingrid Alfheim, BMI, Personal communication).

All methods diagnosing IBD are potential competitors of a GA-map™ IBD test. Search in patent databases should be done frequently. One reason is the possibility to file a complaining against applications (see Appendix C). The US patent application filed by US George Mason University is an example. A complain against this application should be considered. Otherwise, licenses can usually be bought to affordable prices, especially from universities.

11.2.1 Patent strategy

Different patent strategies exist. A company may be taken more seriously financially and get a higher credibility by patenting even small inventions. This type of patenting might function as a warning to small companies speculating in starting IBD diagnostics. Larger companies may buy a license from the patentee. Patent applications on single probe sequences for the identification of intestinal bacteria in IBD diagnostics, might be a challenge to be issued because of previously publicized literature. As an alternative GA might patent a combination of probes to the IBD GA-map™ test, even though infringement of this type of patent are easily avoided. Based on the results of this thesis it is recommended to consider applying for a patent comprising the IG0005 probe.

Patents have many assets other than just being a protective measure. Based on renewal data the general mean value of a patent have been estimated to $78 000 in the US. Further patents issued to small patentees are much less valuable than those issued to large corporations. The cause is probably because economical force is needed to litigate. Litigated patents are more valuable, as are highly cited patents (Bessen, 2008).

11.2.2 Additional search in patent databases to be performed

Search for international applications filed under the PCT of the World Intellectual Property Organization (WIPO) was not performed in the master thesis. Search in WIPO's patent database, Patentscope, could add valuable information.

The European patent database, esp@cenet, is not meant for detailed search. As only one member of each patent family is displayed, more thorough search for European issued patents should be done. Access to more complex databases is commercially available.

This thesis did not include patent search for specific technologies which could replace the GA-map™ technology. Hence such search should be performed to further identify the freedom to operate.

12. Conclusion

This thesis presents an overview of GI bacteria as potential biologic markers in diagnostics of IBD. Results from the lab study revealed a significantly increased level of the IG0005 probe in CD patients relative to controls. The probe was increased in 58,3% of the CD patients showing similar sensitivity as the existing serum markers. As a result of this thesis, the IG0005 probe, detecting species in the Proteobacteria and Bacteroidetes phyla, is suggested as a new diagnostic marker in IBD diagnostics. Applying for a patent protection of the probe is recommended.

Supported by the evaluations done in this thesis, it is concluded that a screening test based on fecal microbiota separating IBD from IBS, has a commercial potential. However, if the test shall dominate the market it needs to show similar or better diagnostic accuracy as endoscopy.

Several patent documents related to IBD diagnostics was found in European and American patent databases. Hardly any patents or patent applications claimed the use of fecal microbiota in order of diagnosing IBD. Nevertheless, a US patent application from the George Mason University comprised claims which could affect the freedom to operate to methods using fecal microbiota in IBD diagnostics.

13. References

Altman, D. G., & Bland, J. M. (1994). Statistics Notes: Diagnostic tests 2: predictive values. *BMJ, 309*(6947), 102-.

Andoh, A., Tsujikawa, T., Sasaki, M., Mitsuyama, K., Suzuki, Y., Matsui, T., et al. (2009). Faecal microbiota profile of Crohn's disease determined by terminal restriction fragment length polymorphism analysis. *Alimentary Pharmacology & Therapeutics, 29*(1), 75-82.

European Patent Convention, 83 C.F.R. (2007). Retrieved 04.19.2010, on http://www.epo.org/patents/law/legal-texts/html/epc/2000/e/ma1.html

Baker, P. I., Love, D. R., & Ferguson, L. R. (2009). Role of gut microbiota in Crohns disease. *Expert Review of Gastroenterology and Hepatology, 3*, 535-546.

Barton, J. H. (2006). Emerging patent issues in genomic diagnostics. *Nat Biotech, 24*(8), 939-941.

Baumgart, D. C., & Carding, S. R. (2007). Inflammatory bowel disease: cause and immunobiology. *The Lancet, 369*(9573), 1627-1640.

Baumgart, D. C., & Sandborn, W. J. (2007). Inflammatory bowel disease: clinical aspects and established and evolving therapies. *The Lancet, 369*(9573), 1641-1657.

Baumgart, M., Dogan, B., Rishniw, M., Weitzman, G., Bosworth, B., Yantiss, R., et al. (2007). Culture independent analysis of ileal mucosa reveals a selective increase in invasive Escherichia coli of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum. *Isme Journal, 1*(5), 403-418.

Bernstein, C. N., & Shanahan, F. (2008). Disorders of a modern lifestyle: reconciling the epidemiology of inflammatory bowel diseases. *Gut, 57*(9), 1185-1191.

Bessen, J. (2008). The value of U.S. patents by owner and patent characteristics. *Research Policy, 37*(5), 932-945.

Bibiloni, R., Tandon, P., Vargas-Voracka, F., Barreto-Zuniga, R., Lupian-Sanchez, A., Rico-Hinojosa, M. A., et al. (2008). Differential clustering of bowel biopsy-associated bacterial profiles of specimens collected in Mexico and Canada: What do these profiles represent? [Article]. *Journal of Medical Microbiology, 57*(1), 111-117.

Bickston, S. J., Waters, H. C., Dabbous, O., Tang, B. X., & Rahman, M. I. (2008). Administrative claims analysis of all-cause annual costs of care and resource

utilization by age category for ulcerative colitis patients. [Article]. *Journal of Managed Care Pharmacy, 14*(4), 352-362.

Binder, V. (2004). Epidemiology of IBD during the twentieth century: an integrated view. *Best Practice & Research in Clinical Gastroenterology, 18*(3), 463-479.

Boone, J. H., Lyerly, D. M., Wilkins, T. D., & Guerrant, R. L. (2009). Method for monitoring gastrointestinal inflammation in persons with inflammatory bowel disease (IBD) US7,560,240. USPTO.

Boone, J. H., Lyerly, D. M., Wilkins, T. D., & Guerrant, R. L. (2009). Method for monitoring persons with IBD using total endogeneous lactoferrin as a marker (EP2071332 (A1)).

Boone, J. H., Lyerly, D. M., Wilkins, T. D. Guerrant, R. L., (2007). Method for differentiating irritable bowel syndrome from inflammatory bowel disease (IBD) and for monitoring persons with IBD using total endogenous lactoferrin as a marker US 7,192,724. USPTO.

Bouma, G., & Strober, W. (2003). The immunological and genetic basis of inflammatory bowel disease. *Nature Reviews Immunology, 3*(7), 521-533.

Bourreille, A., Ignjatovic, A., Aabakken, L., Loftus Jr, E. V., Eliakim, R., Pennazio, M., et al. (2009). Role of small-bowel endoscopy in the management of patients with inflammatory bowel disease: an international OMED-ECCO consensus. *Endoscopy, 41*(07), 618-637.

Braun, J., & Sutton, C. (2001). IBD-associated microbial nucleic acid molecules US6,320,037. USPTO.

Braun, J., & Sutton, C. (2004). IBD-associated microbial nucleic acid molecules US6,759,530. USPTO.

Camilleri, M., & Williams, D. E. (2000). Economic burden of irritable bowel syndrome - Proposed strategies to control expenditures. *Pharmacoeconomics*, 17(4), 331-338.

Chistoserdova, L. (2010). Recent progress and new challenges in metagenomics for biotechnology. [Review]. *Biotechnology Letters, 32*(10), 1351-1359.

Dahl, H. A., & Rinvik, E. (2010). Menneskets funksjonelle anatomi. *Cappelen akademiske forlag*.

Darfeuille-Michaud, A., Boudeau, J., Bulois, P., Neut, C., Glasser, A. L., Barnich, N., et al. (2004). High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease. [Article]. *Gastroenterology, 127*(2), 412-421.

Darfeuille-Michaud, A., & Colombel, J. F. (2008). Pathogenic Escherichia coli in inflammatory bowel diseases. *Journal of Crohns & Colitis, 2*(3), 255-262.

Dubinsky, M. C., Johanson, J. F., Seidman, E. G., & Ofman, J. J. (2002). Suspected inflammatory bowel disease - The clinical and economic impact of competing diagnostic strategies. *American Journal of Gastroenterology, 97*(9), 2333-2342.

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., et al. (2005). Diversity of the human intestinal microbial flora. *Science, 308*(5728), 1635-1638.

Elliott, G. (2007). Basics of US patents and the patent system. . *Aaps Journal, 9*(3), E317-E324.

Endoscopy. (2010). In Encyclopædia Britannica. Retrieved 02.19.2010, from Encyclopædia Britannica Online: https://bib.hihm.no/eb/eb/article-9032622.

EPO (2008). Significant commercial potential Retrieved 10.29.2010, from http://www.epo.org/topics/innovation-and-conomy/handbook/assessing/potential.html

EPO (2010a). Guidelines for Examination in the European Patent Office, Retrieved 05.15.2010, from http://documents.epo.org/projects/babylon/eponet.ns-f/0/7ffc755ad943703dc12576f00054cacc/$FILE/guidelines_2010_complete_en.pdf

EPO (2010b). Member States of the European Patent Organisation, from http://www.epo.org/about-us/epo/member-states.html

Europe (2010). *Encyclopædia Britannica* Retrieved 05.10.2010, from http://search.eb.com

Farthing, M. J. G. (2003). Severe inflammatory bowel disease: Medical management. *Digestive Diseases, 21*(1), 46-53.

Fleshner, P. R., Vasiliauskas, E. A., Melmed, G., & Targan, S. R. (2008). 20100105044. USPTO.

Frank, D. N., St. Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz, N., & Pace, N. R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the National Academy of Sciences, 104*(34), 13780-13785.

Froehlich, F., Gonvers, J. J., Vader, J. P., Dubois, R. W., & Burnand, B. (1999). Appropriateness of gastrointestinal endoscopy: Risk of complications. *Endoscopy, 31*(8), 684-686.

Fujiwara, M., Isao, O., & Hayashi, Y. (2009). Method for clinical staging of ulcerative colitis or interstitial pneumonia and reagent kit for the same US 20090130775. USPTO.

GeneticAnalysis (2010). Technical Description, Retrived 06012010 from http://www.genet-analysis.com/Default.aspx

Giaffer, M. H., Holdsworth, C. D., & Duerden, B. I. (1991). The assessment of fecal flora in patients with inflammatory bowel-disease by a simplified bacteriological technique. *Journal of Medical Microbiology, 35*(4), 238-243.

Gibson, T. B., Ng, E., Ozminkowski, R. J., Wang, S. H., Burton, W. N., Goetzel, R. Z., et al. (2008). The Direct and Indirect Cost Burden of Crohn's Disease and Ulcerative Colitis. *Journal of Occupational and Environmental Medicine, 50*(11), 1261-1272.

Gillevet, P. (2009). Compositions and methods for diagnosing colon disorders. US 20090197249. USPTO.

Gophna, U., Sommerfeld, K., Gophna, S., Doolittle, W. F., & Veldhuyzen van Zanten, S. J. O. (2006). Differences between Tissue-Associated Intestinal Microfloras of Patients with Crohn's Disease and Ulcerative Colitis. *Journal of Clinical Microbiology, 44*(11), 4136-4141.

Grundmann, O., & Yoon, S. L. Irritable bowel syndrome: Epidemiology, diagnosis and treatment: An update for health-care practitioners. *Journal of Gastroenterology and Hepatology, 25*(4), 691-699.

Gunnarsson, J., & Simren, M. (2008). Efficient diagnosis of suspected functional bowel disorders. *Nature Clinical Practice Gastroenterology & Hepatology, 5*(9), 498-507.

Hilty, M., Burke, C., Pedro, H., Cardenas, P., Bush, A., Bossley, C., et al. Disordered Microbial Communities in Asthmatic Airways. *PLoS ONE, 5*(1), e8578.

Inflammatory Bowel Disease (2009), *In Encyclopædia Britannica*, Retrived 10.29.09 from https://search.eb.com

Invitrogen (2008). Quant-iT™ PicoGreen ® dsDNA Reagent and Kits, Retrived 05.01.2010 from http://probes.invitrogen.com/media/pis/mp07581.pdf

Jahnsen, J., Røseth, A. G., & Aadland, E. (2009). Measurement of calprotectin in faeces. *Tidsskr Nor Legeforen, nr 8*(129), 743-745.

Kennedy, T. M., Chalder, T., McCrone, P., Darnley, S., Knapp, M., Jones, R. H., et al. (2006). Cognitive behavioural therapy in addition to antispasmodic therapy for irritable bowel syndrome in primary care: randomised controlled trial. *Health Technology Assessment, 10*(19), 1-.

Koloski, N. A., Bret, L., & Radford-Smith, G. (2008). Hygiene hypothesis in inflammatory bowel disease: A critical review of the literature. *World Journal of Gastroenterology, 14*(2), 165-173.

Kostić T, W. A., Rubino S, Delogu G, Uzzau S, Rudi K, Sessitsch A,, & L., B. (2007). A microbial diagnostic microarray technique for the sensitive detection and identification of pathogenic bacteria in a background of nonpathogens. *Analytical Biochemistry 360*, 244-254.

Krause, D. O., & Bernstein, C. N. (2009). Microbial markers of inflammatory bowel disease US20090305267. USPTO.

Langan, R. C., Gotsch, P. B., Krafczyk, M. A., & Skillinge, D. D. (2007). Ulcerative colitis: Diagnosis and treatment. *American Family Physician, 76*(9), 1323-1330.

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Microbial ecology - Human gut microbes associated with obesity. *Nature, 444*(7122), 1022-1023.

Lin, H. C., & Pimentel, M. (2004). Methods of diagnosing irritable bowel syndrome and other disorders caused by small intestinal bacterial overgrowth. US 6,805,852. USPTO.

Lin, H. C., & Pimentel, M. (2006). Methods of diagnosing and treating small intestinal bacterial overgrowth (SIBO) and SIBO-related conditions. US0147496 A1. USPTO.

Loftus, E. V. (2004). Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology, 126*(6), 1504-1517.

Lowe, A. M., Yansouni, C. P., & Behr, M. A. (2008). Causality and gastrointestinal infections: Koch, Hill, and Crohn's. *Lancet Infectious Diseases, 8*(11), 720-726.

Mangin, I., Bonnet, R., Seksik, P., Rigottier-Gois, L., Sutren, M., Bouhnik, Y., et al. (2004). Molecular inventory of faecal microflora in patients with Crohn's disease. *Fems Microbiology Ecology, 50*(1), 25-36.

Manichanh, C., Rigottier-Gois, L., Bonnaud, E., Gloux, K., Pelletier, E., Frangeul, L., et al. (2006). Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut, 55*(2), 205-211.

Martinez-Medina, M., Aldeguer, X., Lopez-Siles, M., Gonzalez-Huix, F., Lopez-Oliu, C., Dahbi, G., et al. (2009). Molecular Diversity of Escherichia coli in the Human Gut: New Ecological Evidence Supporting the Role of Adherent-Invasive E. coli (AIEC) in Crohn's Disease. [Article]. *Inflammatory Bowel Diseases, 15*(6), 872-882.

Maxion-Bergemann, S., Thielecke, F., Abel, F., & Bergemann, R. (2006). Costs of irritable bowel syndrome in the UK and US. *Pharmacoeconomics, 24*(1), 21-37.

Montgomery, S. M., Lambe, M., Wakefield, A. J., Pounder, R. E., & Ekbom, A. (2002). Siblings and the risk of inflammatory bowel disease. *Scandinavian Journal of Gastroenterology, 37*(11), 1301-1308.

Nagori, B. P., & Mathur, V. (2009). Basics of Writing Patent Non-Infringement and Freedom-to-Operate Opinions. [Article]. *Journal of Intellectual Property Rights, 14*(1), 7-13.

Neish, A. S. (2009). Microbes in Gastrointestinal Health and Disease. [Review]. *Gastroenterology, 136*(1), 65-80.

Nelson, A. (2004). Obviousness or Inventive Step as Applied to Nucleic Acid Molecules: A Global Perspective *North Carolina Journal of law & technology 6*(1).

Nikolaus, S., & Schreiber, S. (2007). Diagnostics of inflammatory bowel disease. *Gastroenterology, 133*(5), 1670-1689.

Nordiag (2010). Nordiag ASA Hordaland på Børs-investorseminar. Retrived 11.11.2010 from http://www.bergen-chamber.no/uploads/NORDIAG.pdf

Norgesbank (2010). Styringsrenten, from http://www.norges-bank.no/

Norsklegemiddelhåndbok (2009). *Norsk legemiddelhåndbok.* Retrived 04032010 from http://www.legemiddelhandboka.no/xml/.

Odes, S. (2008). How expensive is inflammatory bowel disease? A critical analysis. *World Journal of Gastroenterology, 14*(43), 6641-6647.

Odes, S., Vardi, H., Friger, M., Wolters, F., Russel, M. G., Riis, L., et al. (2006). Cost analysis and cost determinants in a European inflammatory bowel disease inception cohort with 10 years of follow-up evaluation. *Gastroenterology, 131*(3), 719-728.

Oxford Dictionary of English (2010). Retrieved 06.08.2010, from http://www.ordnett.no/21.html

Packey, C. D., & Sartor, R. B. (2008). Interplay of commensal and pathogenic bacteria, genetic mutations, and immunoregulatory defects in the pathogenesis of inflammatory bowel diseases. *Journal of Internal Medicine, 263*(6), 597-606.

Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A., & Brown, P. O. (2007). Development of the Human Infant Intestinal Microbiota. *PLoS Biol, 5*(7), e177.

Palombi, L. (2009). *Gene Cartels:Biotech Patents in the Age of Free Trade*: Edward Elgar Pub.

Passarge, E. (Ed.) (2001). *Color Atlas of Genetics* (2 ed.). Thieme.

Pei, A., Nossa, C. W., Chokshi, P., Blaser, M. J., Yang, L., Rosmarin, D. M., et al. (2009). Diversity of 23S rRNA Genes within Individual Prokaryotic Genomes. *PLoS ONE, 4*(5), e5437.

Perminow, G., Rydning, A., Jacobsen, C. D., & Frigessi, A. (2000). Gastrointestinale endoskopier av barn. *Tidsskr Nor Lægeforen*(120), 3503-3506.

Prindiville, T., Cantrell, M., & Wilson, K. H. (2004). Ribosomal DNA sequence analysis of mucosa-associated bacteria in Crohn's disease. *Inflammatory Bowel Diseases, 10*(6), 824-833.

Prometheuslabs. 2010, Retrived 08.21.2010 from http://www.prometheuslabs.com/

Reese, G. E., Constantinides, V. A., Simillis, C., Darzi, A. W., Orchard, T. R., Fazio, V. W., et al. (2006). Diagnostic precision of anti-Saccharomyces cerevisiae antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *American Journal of Gastroenterology, 101*(10), 2410-2422.

Rudi, K., & Jakobsen, K. S. (2003). Nucleic acid detection method US Patent No. 6617138. USPTO

Rudi, K., Skulberg, O. M., Skulberg, R., & Jakobsen, K. S. (2000). Application of Sequence-Specific Labeled 16S rRNA Gene Oligonucleotide Probes for Genetic Profiling of Cyanobacterial Abundance and Diversity by Array Hybridization. *Appl. Environ. Microbiol., 66*(9), 4004-4011.

Sartor, R. B. (2006). Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nature Clinical Practice Gastroenterology & Hepatology, 3*(7), 390-407.

Sartor, R. B. (2008). Microbial influences in inflammatory bowel diseases. *Gastroenterology, 134*(2), 577-594.

Sato, N., Okusa, T., & Okayasu, I. (2010).. Therapeutic agent for ulcerative colitis: US Patent No 7,700,106. USPTO.

Scanlan, P. D., Shanahan, F., O'Mahony, C., & Marchesi, J. R. (2006). Culture-independent analyses of temporal variation of the dominant fecal microbiota and targeted bacterial subgroups in Crohn's disease. *Journal of Clinical Microbiology, 44*(11), 3980-3988.

Schluenzen, F., Tocilj, A., Zarivach, R., Harms, J., Gluehmann, M., Janell, D., et al. (2000). Structure of Functionally Activated Small Ribosomal Subunit at 3.3 Å Resolution. *Cell, 102*(5), 615-623.

Sears, C. L. (2005). A dynamic partnership: Celebrating our gut flora. *Anaerobe, 11*(5), 247-251.

Seekingalpha (2010). Myriad Genetics - A Diagnostics Heavy Hitter, from http://seekingalpha.com/article/115611-myriad-genetics-a-diagnostics-heavy-hitter

Seksik, P., Rigottier-Gois, L., Gramet, G., Sutren, M., Pochart, P., Marteau, P., et al. (2003). Alterations of the dominant faecal bacterial groups in patients with Crohn's disease of the colon. *Gut, 52*(2), 237-242.

Shanahan, F., & Bernstein, C. N. (2009). The evolving epidemiology of inflammatory bowel disease. *Current Opinion in Gastroenterology, 25*(4), 301-305.

Sokol, H., Lay, C., Seksik, P., & Tannock, G. W. (2008). Analysis of bacterial bowel communities of IBD patients: What has it revealed? *Inflammatory Bowel Diseases, 14*(6), 858-867.

Sokol, H., Seksik, P., Furet, J. P., Firmesse, O., Nion-Larmurier, L., Beaugerie, L., et al. (2009). Low Counts of Faecalibacterium prausnitzii in Colitis Microbiota. *Inflammatory Bowel Diseases, 15*(8), 1183-1189.

Sokol, H., Seksik, P., Rigottier-Gois, L., Lay, C., Lepage, P., Podglajen, I., et al. (2006). Specificities of the fecal microbiota in inflammatory bowel disease. *Inflammatory Bowel Diseases, 12*(2), 106-111.

Srikanta, G., & Simpson, J. (2005). Nonbridging phosphate oxygens in 16S rRNA important for 30S subunit assembly and association with the 50S ribosomal subunit. *RNA Journal, 11*, 657-667.

Stackebrandt, E., & Goebel, B. M. (1994). Taxonomic Note: A Place for DNA-DNA Reassociation and 16S rRNA Sequence Analysis in the Present Species Definition in Bacteriology. *Int J Syst Bacteriol, 44*(4), 846-849.

Stange, E. F., Travis, S. P. L., Vermeire, S., Beglinger, C., Kupcinkas, L., Geboes, K., et al. (2006). European evidence based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. *Gut, 55*(suppl 1), i1-i15.

Stange, E. F., Travis, S. P. L., Vermeire, S., Reinisch, W., Geboes, K., Barakauskiene, A., et al. (2008). European evidence-based Consensus on the diagnosis and management of ulcerative colitis: Definitions and diagnosis. *Journal of Crohn's and Colitis, 2*(1), 1-23.

Stark, R., Konig, H. H., & Leidl, R. (2006). Costs of inflammatory bowel disease in Germany. *Pharmacoeconomics, 24*(8), 797-814.

Suleiman, S., & Sonnenberg, A. (2001). Cost-effectiveness of endoscopy in irritable bowel syndrome. *Archives of Internal Medicine, 161*(3), 369-375.

Swidsinski, A., Ladhoff, A., Pernthaler, A., Swidsinski, S., Loening-Baucke, V., Ortner, M., et al. (2002). Mucosal flora in inflammatory bowel disease. *Gastroenterology, 122*(1), 44-54.

Swidsinski, A., Weber, J., Loening-Baucke, V., Hale, L. P., & Lochs, H. (2005). Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. *Journal of Clinical Microbiology, 43*(7), 3380-3389.

Tannock, G. W. (2008). The search for disease-associated compositional shifts in bowel bacterial communities of humans. *Trends in Microbiology, 16*(10), 488-495.

Tap, J., Mondot, S., Levenez, F., Pelletier, E., Caron, C., Furet, J. P., et al. (2009). Towards the human intestinal microbiota phylogenetic core. *Environmental Microbiology, 11*(10), 2574-2584.

Terheggen, G., Lanyi, B., Schanz, S., Hoffmann, R. M., Bohm, S. K., Leifeld, L., et al. (2008). Safety, feasibility, and tolerability of ileocolonoscopy in inflammatory bowel disease. *Endoscopy, 40*(8), 656-663.

Underhill, D., & Braun, J. (2008). Current understanding of fungal microflora in inflammatory bowel disease pathogenesis. *Inflammatory Bowel Diseases, 14*(8), 1147-1153.

USPTO (2001). Utility Examination Guidelines, from http://www.uspto.gov/web/offices/com/sol/notices/utilexmguide.pdf

USPTO (2009). Glossary. Retrieved 05.27.2009 from http://www.uspto.gov/main/glossary/index.html

Utgård, O., & Refsum, H. (Eds.). (2007). *Fra idé til ny virksomhet*: Universitetsforlaget.

Vermeire, S., Van Assche, G., & Rutgeerts, P. (2006). Laboratory markers in IBD: useful, magic, or unnecessary toys? *Gut, 55*(3), 426-431.

Villanueva, A., Dominguez-Munoz, J. E., & Mearin, F. (2001). Update in the therapeutic management of irritable bowel syndrome. *Digestive Diseases, 19*(3), 244-250.

WIPO (2010). Patents: Frequently Asked Questions, from http://www.wipo.int/patentscope/en/patents_faq.html#protection

Yu, A. P., Cabanilla, L. A., Wu, E. Q., Mulani, P. M., & Chao, J. D. (2008). The costs of Crohn's disease in the United States and other Western countries: a systematic review. *Current Medical Research and Opinion, 24*(2), 319-328.

Zoetendal, E. G., Rajilic-Stojanovic, M., & de Vos, W. M. (2008). High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. *Gut, 57*(11), 1605-1615.

Appendix A Variation of bacterial organisms in fecal samples from IBD patients vs. controls

Organism	Class	IBD, CD or UC compared to controls	Techniques used in the article	Refferences
bifidobacteria	Actinobacteria	decreased	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
bifidobacteria	Actinobacteria	decreased	TTGE, dot blot hyb	(Seksik, et al., 2003)
bifidobacteria	Actinobacteria	decreased	quantitative real-time PCR	(Sokol, et al., 2009)
Lactobacillus	Bacilli	decreased	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
Bacteroides fragilis	Bacteroida	decreased	DGGE	(Scanlan, et al., 2006)
Clostiridium cluster IV	Clostridia	decreased	T-RFLP (16S rRNA)	(Andoh, et al., 2009)
Clostiridium cluster XI	Clostridia	decreased	T-RFLP (16S rRNA)	(Andoh, et al., 2009)
Clostridium family	Clostridia	decreased	T-RFLP (16S rRNA)	(Andoh, et al., 2009)
Clostiridium subcluster XIVa	Clostridia	decreased	T-RFLP (16S rRNA)	(Andoh, et al., 2009)
Clostridium coccoides	Clostridia	decreased	TTGE, dot blot hyb	(Seksik, et al., 2003)
Clostridium leptum group	Clostridia	decreased	quantitative real-time PCR	(Sokol, et al., 2009)
Clostridium coccoides group	Clostridia	decreased	quantitative real-time PCR	(Sokol, et al., 2009)
Faecalibacterium prausnitzii	Clostridia	decreased	quantitative real-time PCR	(Sokol, et al., 2009)
Clostridium coccoides group (ClusterXIVa)	Clostridia	decreased	cloning, sequencing	(Mangin, et al., 2004)
Clostridium leptum group	Clostridia	decreased	cloning, sequencing	(Mangin, et al., 2004)
Clostridium coccoides	Clostridia	decreased	DGGE	(Scanlan, et al., 2006)
Clostridium leptum	Clostridia	decreased	DGGE	(Scanlan, et al., 2006)
Clostridium leptum group	Clostridia	decreased	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)
Clostridium coccoides	Clostridia	decreased	FISH + flow cytometry	(Sokol, et al., 2006)
Clostridium leptum	Clostridia	decreased	FISH + flow cytometry	(Sokol, et al., 2006)
Bacteroides	phylum	decreased	TTGE, dot blot hyb	(Seksik, et al., 2003)
Firmicutes phylum	phylum	decreased	quantitative real-time PCR	(Sokol, et al., 2009)
Firmicutes/Bacteroidetes ratio	phylum	decreased	quantitative real-time PCR	(Sokol, et al., 2009)
Firmicutes	phylum	decreased	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)
Bacteroides	phylum	decreased	standard culture	(Krook, et al., 1981)
Total bacteria		decreased	FISH + flow cytometry	(Sokol, et al., 2006)
Additivity		decreased	FISH + flow cytometry	(Sokol, et al., 2006)
Gram-positive		decreased	FISH + flow cytometry	(Sokol, et al., 2006)
Bifidobacterium subgroup	Actinobacteria	equal	T-RFLP (16S rRNA)	(Andoh, et al., 2009)
bifidobacteria	Actinobacteria	equal	DGGE	(Scanlan, et al., 2006)
Actinobacteria	Actinobacteria	equal	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)
Atopobium group	Actinobacteria	equal	FISH + flow cytometry	(Sokol, et al., 2006)
Bifidobacterium subgroup	Actinobacteria	equal	FISH + flow cytometry	(Sokol, et al., 2006)
Lactobacillales	Bacilli	equal	T-RFLP (16S rRNA)	(Andoh, et al., 2009)
viridans streptococci	Bacilli	equal	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
Lactobacillus	Bacilli	equal	TTGE, dot blot hyb	(Seksik, et al., 2003)
Lactobacillus johnsonii	Bacilli	equal	OFRG (Oligonucleotide Fingerprinting of rRNA Genes)	(Ye, et al., 2008)
Prevotella	Bacteroida	equal	T-RFLP (16S rRNA)	(Andoh, et al., 2009)

Organism	Class	IBD, CD or UC compared to controls	Techniques used in the article	Refferences
Bacteroides fragilis	Bacteroida	equal	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
Bacteroides vulgatus	Bacteroida	equal	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
Bacteroidetes phylum	Bacteroida	equal	quantitative real-time PCR	(Sokol, et al., 2009)
Clostridia	Clostridia	equal	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
Clostridium leptum	Clostridia	equal	TTGE, dot blot hyb	(Seksik, et al., 2003)
Clostridium coccoides group	Clostridia	equal	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)
Klebsiella	Gammaproteobacteria	equal	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
Proteus spp	Gammaproteobacteria	equal	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
E.coli	Gammaproteobacteria	equal	quantitative real-time PCR	(Sokol, et al., 2009)
E. Coli	Gammaproteobacteria	equal	standard culture	(Krook, et al., 1981)
Enterobacteria	Gammaproteobacteria	equal	FISH + flow cytometry	(Sokol, et al., 2006)
Bacteroides	phylum	equal	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
Bacteroides	phylum	equal	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)
Bacteroides	phylum	equal	FISH + flow cytometry	(Sokol, et al., 2006)
anaerobic flora		equal	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
yeast		equal	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
Total number of bacterial		equal	quantitative real-time PCR	(Sokol, et al., 2009)
aerobic bacteria		equal	standard culture	(Krook, et al., 1981)
anaerobic bacteria		equal	standard culture	(Krook, et al., 1981)
Gram-negative		equal	FISH + flow cytometry	(Sokol, et al., 2006)
Enterococcus faecalis	Bacilli	increased*	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
Bacteroides vulgatus (and relatives)	Bacteroida	increased	cloning, sequencing	(Mangin, et al., 2004)
Unclassified Porphyromonadaceae	Bacteroida	increased	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)
Enterobacteriales	Gammaproteobacteria	increased	T-RFLP (16S rRNA)	(Andoh, et al., 2009)
E. coli	Gammaproteobacteria	increased	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
Enterobacteria	Gammaproteobacteria	increased	TTGE, dot blot hyb	(Seksik, et al., 2003)
Bacteroides	phylum	increased	T-RFLP (16S rRNA)	(Andoh, et al., 2009)
total aerobe		increased	standard culture, semi-quantitative method	(Giaffer, et al., 1991)
E. faecalis	Bacilli	increased / decreased*	quantitative real-time PCR	(Sokol, et al., 2009)
Prevotella subgroup	Bacteroida	Increased number of ribotypes**	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)
Atopobium group	Actinobacteria	Reduced number of ribotypes**	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)
Bacteroides fragilis subgroup	Bacteroida	Reduced number of ribotypes**	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)
"Other Firmicutes groups		Reduced number of ribotypes**	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)
Bifidobacterium subgroup	Actinobacteria	Similar number of ribotypes**	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)
Bacteroides distasonis subgroup	Bacteroida	Similar number of ribotypes**	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)

Organism	Class	IBD, CD or UC compared to controls	Techniques used in the article	Refferences
Delta proteobacteria	Delta proteobacteria	Similar number of ribotypes**	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)
Gamma proteobacteria	Gammaproteobacteria	Similar number of ribotypes**	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)
Proteobacteria	phylum	Similar number of ribotypes**	Clone library, macroarray, sequencing , FISH	(Manichanh, et al., 2006)

*Isolated from significantly fewer patients with active CD than quiescent CD or UC.

**Ribotypes: Organisms whose 16S rRNA sequences are at least 97% identical are commonly considered to be the same ribotype (Stackebrandt & Goebel, 1994)

Appendix B Variation of bacterial organisms in mucosal samples from IBD patients vs. controls

Organism	Class	IBD, CD or UC compared to controls	Techniques used in the article	Refferences
Butyrate-producing bacterium A2–A165	-	decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Bacteroidetes	Bacteroida	decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Alistipes sp. WAL 8169 Bacteroidetes; Bacteroidales	Bacteroida	decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Bacterium mpn-isolate group 5 Bacteroidetes; Bacteroidales	Bacteroida	decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Bacteroides thetaiotaomicron Bacteroidetes; Bacteroidales	Bacteroida	decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Bacteroides acidofaciens	Bacteroida	decreased	cloning, sequencing	(Prindiville, et al., 2004)
Prevotella nigrescens	Bacteroida	decreased	cloning, sequencing	(Prindiville, et al., 2004)
Bacteroides_5 (Bacteroides fragilis)	Bacteroida	decreased	cloning, sequencing	(Prindiville, et al., 2004)
Bacterium mpn-isolate group 18 Firmicutes; Lachnospiraceae	Clostridia	decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Bacterium mpn-isolate group 19 Firmicutes; Lachnospiraceae	Clostridia	decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Butyrate-producing bacterium L2—L7 Firmicutes; Lachnospiraceae	Clostridia	decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Butyrate-producing bacterium SR1/1 Firmicutes; Lachnospiraceae	Clostridia	decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Butyrate-producing bacterium SS2/1 Firmicutes; Lachnospiraceae	Clostridia	decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Clostridium nexile Firmicutes; Lachnospiraceae	Clostridia	decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Firmicutes; Lachnospiraceae	Clostridia	decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Lachnospiraceae	Clostridia	decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Firmicutes	phylum	decreased	cloning, sequencing	(Gophna, et al., 2006)
total bacteria		decreased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Lachnospiraceae bacterium A4 (Gp66)	Clostridia	decreased	OFRG (Oligonucleotide Fingerprinting of rRNA Genes)	(Ye, et al., 2008)
Lachnospiraceae bacterium A4 (GpC2)	Clostridia	decreased	OFRG (Oligonucleotide Fingerprinting of rRNA Genes)	(Ye, et al., 2008)
Barnesiella viscericola	Bacteroida	decreased	OFRG (Oligonucleotide Fingerprinting of rRNA Genes)	(Ye, et al., 2008)
Clostridium leptum	Clostridia	decreased	cloning, sequencing	(Prindiville, et al., 2004)
Akkermansia muciniphilia	Verrucomicrobiae	decreased	OFRG (Oligonucleotide Fingerprinting of rRNA Genes)	(Ye, et al., 2008)
Bacteroides	phylum	dominant	FISH + in situ quantification of mucosal bacteria	(Swidsinski, et al., 2005)

Organism	Class	IBD, CD or UC compared to controls	Techniques used in the article	Refferences
Bacteroides vulgatus	Bacteroida	equal	cloning, sequencing	(Gophna, et al., 2006)
E.coli	Gammaproteobacteria	equal	cloning, sequencing	(Gophna, et al., 2006)
Gamma proteobacteria	Gammaproteobacteria	equal	cloning, sequencing	(Gophna, et al., 2006)
Enterobacteriaceae	Gammaproteobacteria	equal	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Gamma proteobacteria	Gammaproteobacteria	equal	FISH	(Swidsinski, et al., 2008
Bacteroides	phylum	equal	FISH	(Swidsinski, et al., 2002
Total bacteria (universal probe)		equal	FISH	(Swidsinski, et al., 2013
Acidimicrobidae Ellin7143 Actinobacteria	Actinobacteria	increased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Actinobacteria	Actinobacteria	increased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Actinobacterium GWS-BW-H99 Actinobacteria 62.5	Actinobacteria	increased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Actinomyces oxydans Actinobacteria 48.9	Actinobacteria	increased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Nocardioides sp. NS/27 Actinobacteria 61.8	Actinobacteria	increased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Drinking-water bacterium Y7 Alphaproteobacteria 76.0	Alphaproteobacteria	increased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Novosphingobium sp. K39 Alphaproteobacteria 57.8	Alphaproteobacteria	increased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Sphingomonas sp. AO1 Alphaproteobacteria 49.6	Alphaproteobacteria	increased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Bacillus licheniformis Firmicutes; Bacilli 49.7	Bacilli	increased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Enterococcus faecium	Bacilli	increased	cloning, sequencing	(Prindiville, et al., 2004)
Bacteroides fragilis	Bacteroida	increased	cloning, sequencing	(Gophna, et al., 2006)
Bacteroidetes	Bacteroida	increased	cloning, sequencing	(Gophna, et al., 2006)
Bacteroides distasonis	Bacteroida	increased	FISH + in situ quantification of mucosal bacteria	(Swidsinski, et al., 2005)
Bacteroides fragilis	Bacteroida	increased	FISH + in situ quantification of mucosal bacteria	(Swidsinski, et al., 2005)
Bacteroides-Prevotella	Bacteroida	increased	FISH + in situ quantification of mucosal bacteria	(Swidsinski, et al., 2005)
Bacteroides-Prevotella, Bacteroides fragilis	Bacteroida	increased	FISH + in situ quantification of mucosal bacteria	(Swidsinski, et al., 2005)
Ruminococcus gnavus	Clostridia	increased	cloning, sequencing	(Prindiville, et al., 2004)
Eubacterium rectale	Clostridia	increased	FISH + in situ quantification of mucosal bacteria	(Swidsinski, et al., 2005)
Fusobacterium prausnitzii	Clostridia	increased	FISH + in situ quantification of mucosal bacteria	(Swidsinski, et al., 2005)
Clostridium	Clostridia	increased	quantitative PCR + sequence analysis	(Swidsinski, et al., 2005
Peptostreptococci	Clostridia	increased	quantitative PCR + sequence analysis	(Swidsinski, et al., 2009
Klebsiella pneumonia	Gammaproteobacteria	increased	cloning, sequencing	(Gophna, et al., 2006)
Acientobacter junii	Gammaproteobacteria	increased	cloning, sequencing	(Gophna, et al., 2006)
Gamma proteobacterium	Gammaproteobacteria	increased	Library construction,	(Frank, et al., 2007)

Organism	Class	IBD, CD or UC compared to controls	Techniques used in the article	Refferences
DD103 Gammaproteobacteria 50.9			sequence analysis, Q-PCR	
Pseudomonas straminea Betaproteobacteria 53.7	Gammaproteobacteria	increased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Enterobacteriaceae	Gammaproteobacteria	increased	Culture	(Swidsinski, et al., 2006
Enterobacteriaceae	Gammaproteobacteria	increased	quantitative PCR + sequence analysis	(Swidsinski, et al., 2007
Proteobacteria	phylum	increased	cloning, sequencing	(Gophna, et al., 2006)
Proteobacteria	phylum	increased	Library construction, sequence analysis, Q-PCR	(Frank, et al., 2007)
Bacteroides acidofaciens	Bacteroida	increased	OFRG (Oligonucleotide Fingerprinting of rRNA Genes)	(Ye, et al., 2008)
Streptococci	Bacilli	increased	quantitative PCR + sequence analysis	(Swidsinski, et al., 2010
Bacteroides	phylum	increased	Culture	(Swidsinski, et al., 2003
Bacteroides	phylum	increased	quantitative PCR + sequence analysis	(Swidsinski, et al., 2004
Total aerobes		increased	Culture	(Swidsinski, et al., 2011
Total anaerobes		increased	Culture	(Swidsinski, et al., 2012
Bacteroides vulgatus	Bacteroida	increased	OFRG (Oligonucleotide Fingerprinting of rRNA Genes)	(Ye, et al., 2008)
Ruminococcus schinkii (GpC1)	Clostridia	increased	OFRG (Oligonucleotide Fingerprinting of rRNA Genes)	(Ye, et al., 2008)
Clostridium ramosum	Clostridia	increased	OFRG (Oligonucleotide Fingerprinting of rRNA Genes)	(Ye, et al., 2008)

Appendix C American and European patent legislation

Patenting is a way to give inventors exclusive rights for their invention for a limited period of time, usually 20 years. The intention of the patent system is to promote advances in science and technology and give new and useful products and processes which the citizens can benefit from (Elliott, 2007). To achieve this, the patentee needs to disclose a description of the invention in the application. In this way inventions are available to the public allowing further development. In return the inventor can prevent others from making, using, selling, or distributing the patented invention without permission (WIPO, 2010). In addition to the disclosed description a patent contains claims, drawings, and abstract. The claims are the most essential part of the patent. Only what is set forth in the claims is protected by the patent (Elliott, 2007).

C.1 European and US patents

The United States Patent and Trademark Office (USPTO) are issuing patents in the US. In Europe it is possible to obtain patent in the thirty seven member countries of the European Patent Convention by filing one single application to the European Patent Office (EPO) (EPO, 2010b). EPO is not an EU institution.

C.1.1 Interpretation of the US and European Patent law

The key laws for the approval of U.S. patents are 35 USC Section 101, 102, 103, and 112. They describe requirement as inventions patentable, novelty, non-obviousness and specification respectively. Generally the invention or discovery must be novel, useful and non-obvious compared to earlier inventions. But an improvement of an earlier invention is also patentable (35 USC section 101).

In the United States so-called "everything under the sun made by man" is patentable. This is often cited although it is misleading. The full quote reads “A person may have “invented” a machine or a manufacture, which may include anything under the sun made by man but it is

not necessarily patentable under section 101 unless the conditions of the title are fulfilled (H.R. Rep. No. 1923, 82d Cong., 2d Sess. 6 (1952).

In 2001 Utility examination guidelines were published by the USPTO. The guidelines were made to assist patent examiners and do not have the force and effect of the law. Several tests from the Supreme Court were adopted. E.g it requires that the application contains at least one specific, substantial and credible use of the invention. Additionally the guidelines stress the utility to be credible to a person skilled in the art. The claimed inventions must have a practical purpose, a real world use in currently available form. This excludes a utility as non-specific utilities as e.g. "landfill" or "throw-away". Further, an object which is only useful of further research is not considered to have a substantial utility. Developments occurring after the filing date of an application are of no significance regarding what one skilled in the art believed as of the filing date (USPTO, 2001). Patent laws in the US have a broad view of what is applicable and the judiciary does not require that an invention will be better than previous inventions (Baillie, Richards, & Cord, 2009). It is not a requirement in law that the inventor may know how or why the invention works (USPTO, 2001).

European Patent Convention (EPC) is a legal system in which European patents are granted. The patentable subject must have a technical character, be new, involve an inventive step, be susceptible of industrial application, and be sufficiently disclosed to the skilled person. Mathematical methods or formulae, computer programs and business methods are some innovations which are not patentable under the EPC. Especially discoveries, methods for performing mental acts, new plant or animal varieties or inventions contrary to order public or morality as cloning of human life are excluded from patentability in Article 52 (2).

C.1.2 The legal basis and differences of the European and US patent system

The European and US patent laws are slightly different. The legal basis of the patent system in US is set forth in The Constitution Article 1, Section 8, Clause 8 "The Congress shall have power (...) to promote the Progress of Science and useful Arts, by securing for limited Times to Authors and Inventors the exclusive Right to their respective Writings and discoveries". In Europe discoveries are not considered patentable and it is expected an inventive step. According to the European Patent Convention (EPC) Article 52 paragraph 1: "European

patents shall be granted for any inventions, in all fields of technology, provided that they are new, involve an inventive step and are susceptible of industrial application".

In the US a patent might be granted to the first person who invents while the first to apply for an invention can get a patent in Europe (USPTO, 2009). Furthermore, in the US inventors can freely publish and sell the invention for one year prior to the application (35 USC 102). However, by doing so the invention is not suitable for a patent in Europe (Article-54-EPC, 2007). The US patent law requires that the best way to practice the invention is described in the patent application (35 USC 112). In contrast, the Europe patent law requires a description of at least one way of practicing the invention. But it is not required that it is the best way to perform the invention (Article-83-EPC, 2007). In this way the patentee can keep some secrets of the invention in the European system.

C.1.3 Validity and Opposition opportunities of granted patents

Patents granted by the EPO automatically become valid in each of the member counties and get the same rights as a national patent in those EPC countries. During the first nine months after a patent is granted under the EPC it is possible to anyone to start a procedure against the patent to get it annulled in all the countries at once. Patent disputes are handled within the EPO and not in the courts. The patentee and the opponents can debate by exchanging written arguments followed by an oral proceeding. The Opposition Division (OD) of the EPO will take a decision based on these arguments. Both parties can appeal the decision. Once again written and oral arguments are made before a final decision is made by the Board of Appeal. Later, nine months after granting, separate proceedings in each country are necessary to get a patent annulled (EPO, 2008).

Because the US patent law is a federal statue a patent granted in the US are valid in all states of the USA. Anyone can complain about the validity of a granted patent. Then it is up to the patentee to give reasons to the USPTO examiner why the patent should remain valid. The opponent is not a part of this discussion. Appeals for patent decisions are made by the Federal Circuit Court of Appeals, which is a special court for patent cases. United States Supreme Court is the highest federal court in the United States, it has ultimate authority to interpret and decide questions of federal laws including the federal constitution (Berg, s.a.). Judge adopted

here will also be important for the interpretation of the patent law. As an example genes have been patentable since the case of Diamond v. Chakrabarty in 1980, (Falciola, 2000).

C.1.4 Description

According to both EPC and US patent law, the application needs to have a description disclosed in such a way that an educated and trained person can perform the invention (35USC 112, EPC article 83). Nowadays patent specifications are filed with as little as possible detailed information about the invention. Contrary, background information about possible usage is vast, especially in biotechnology and pharmaceutical industry. This is done in order to avoid narrowing of the patent protection by the examiner or the courts. As a result claims and applications are complex and huge resulting in a nearly impossible job for examiners to find what the inventor has done to advance the technology. Now applicants are asked to describe more clearly what is invented.

C.2 Patentability of Genes

The patentability of genes is a controversial topic. It is widely discussed how the genetic information should be treated to serve the greater good raising questions touching upon innovation policy, social policy, medical ethics, economic policy, and the ownership of what some view as our common heritage. When the US Company Celera Genomics managed to map the whole human genome and later attempted to patent it, it led to public outrage. The US President Clinton and UK Prime Minister Blair jointly condemned it, stating that the human genome belongs to no man and that it is a resource that should be freely available to all researchers (Palombi, 2009). This is however not yet been adopted by the US and European patent law.

C.2.1 Patentability of genes in the US

Currently USPTO have a practice in which patents on genes are granted as long as the DNA is in an isolated form, making it no different from any other chemical compound. However many feels that this way to interpret the law is a “lawyer’s trick”. On the other hand other claims that isolated human DNA constitutes patentable subject matter and fears that banning

this would negatively affect innovation and scientific research of human therapeutics and personalized medicine (Barton, 2006).

RNA and DNA sequences can be patented if they meet the statutory requirements for patentability. Every patent holder on a diagnostic sequence or marker may block others from using the sequence. Licensing of sequences is possible, but it is not required to provide a license (Barton, 2006). A DNA sequence itself is not patentable because the sequence only describes information about a molecule. An isolated and purified DNA molecule, however, may be patentable because it is considered a composition of matter. A patent application on genes is granted by the general rules in which the use of the isolated gene must be presented in the claims. If other develops new and not obvious ways to use the patented sequence, they have opportunity to patent the new method (USPTO, 2001).

Recently the Court of Appeals for the Federal Circuit (CAFC) supported USPTO in the decision that expressed sequence tags (EST) are DNA fragments without specific and substantial utility (in re Fisher) (Elliott, 2007).

C.2.2 Patentability of genes in EPC countries

In 1998 the European Parliament and Council stated that: the human body, at the various stages of its formation and development, and the simple discovery of one of its elements, including the sequence or partial sequence of a gene,...'' are not patentable but that ''an element isolated from the human body or otherwise produced by means of a technical process, including the sequence or partial sequence of a gene may constitute a patentable invention, even if the structure of that element is identical to that of a natural element''. This directive was taken up in the EPC under Rule 29 of the implementing regulations. Discoveries which are non patentable subject matter under article 52 (2) should be interpreted in the light of rule 29 (2).

C.2.3 Obviousness of gene sequences

If a claimed DNA molecule is obvious or not, depends on whether a molecule that has the given structure of DNA is obvious to an educated and trained person at the time the invention

was made. The amount of work load done to describe the DNA molecule is not significant in order for a sequence to be patentable (USPTO, 2001). “Patentability shall not be negatived by the manner in which the invention was made” (Section 103 US). In other words patent on gene sequences can not be denied because of the method used was of previous art according to US law. In contrast EPO requires what is referred to as “an inventive step”. A gene discovered by using known methodologies does not provide an inventive step and hence is obvious. If the isolated gene possess unexpected or improved features the Board of Appeal can accept an inventive step (Nelson, 2004).

The EPO Guidelines state that to ”find a substance freely occurring in nature is also a mere discovery and therefore unpatentable. However, if a substance found in nature first has to be isolated from its surroundings and a process for obtaining it is developed, that process is patentable. Moreover, if the substance can be properly characterized either by its structure, by the process by which it is obtained or by other parameters and is ”new” in the sense of having no previously recognized existence, then the substance may be patentable (EPO, 2010a).

C.2.3 Patentability of Gene Probes

In the Utility Guide lines of 2001 the USPTO attempts that genes are patentable, but raise the need for specific, substantial, and credible utility for gene fragments. Further probes are also patentable, but in order to satisfy the utility requirement it needs to be specific, substantial, and credible. A nucleic acid fragment claimed a utility as a probe does not pass the specific utility test. In order to pass, it is necessary to identify the particular gene. Further it is not sufficiently specific to describe a probe as diagnostic- the condition diagnosed also needs to be identified. A substantial utility is one that defines a “real world” use. For example a probe is not patentable if it is only useful for further research. But a probe which is used for a diagnostic purpose like IBD is defined as substantial useful (USPTO, 2001).

Whether a probe is credible, depends if a person skilled in the art would accept the invention as currently available for such use. One of the core questions of the patent examiner is if additional knowledge is necessary before invention can be used. A nucleic acid used as a probe for the 16S rRNA would likely satisfy the credibility requirement (Duke L. 2001).

Appendix D Iterative patent search process in the USPTO Patent Full-Text and Image Database and Patent Application Full Text and Image Database

Search query	number of hits	Comment
APD/1/1/2000->8/3/2010 AND ABST/"inflammatory bowel disease" AND ABST/diagn$	15	Few hits, use additional disease expressions
((((ABST/"inflammatory bowel disease" OR ABST/Crohn) OR ABST/"Ulcerative colitis") AND ABST/diagn$) AND APD/20000101->20100803):	49	Try additional expressions related to diagnose
(ABST/"inflammatory bowel disease" OR ABST/Crohn OR ABST/"Ulcerative colitis") AND (ABST/diagn$ OR ABST/analy$) AND (APD/20000101->20100803)	50	Try additional expressions related to diagnose
(ABST/"inflammatory bowel disease" OR ABST/Crohn OR ABST/"Ulcerative colitis") AND (ABST/diagn$ OR ABST/analy$ OR ABST/detect$) AND (APD/1/1/2000->8/3/2010)	56	OK

ABST: Abstract, APD:Application date, $:Truncation mark

Appendix E Iterative patent search process for the EPO's Esp@cnet database

Search query	number of hits	Comment
"inflammatory bowel disease?" or crohn* or "ulcerative colitis" in the title or abstract AND EP as the publication number AND 2005:2010 as the publication date	192	Too many
("inflammatory bowel disease?" or crohn* or "ulcerative colitis") AND diagnos* in the title or abstract AND EP as the publication number AND 2005:2010 as the publication date	14	Too few?
("inflammatory bowel disease?" or crohn* or "ulcerative colitis") AND (diagnos* or analy*) in the title or abstract AND EP as the publication number AND 2005:2010 as the publication date	15	Too few?
("inflammatory bowel disease?" or crohn* or "ulcerative colitis") (diagnos* or analy* or detect*) in the title or abstract AND EP as the publication number AND 2005:2010 as the publication date	20	Ok

* Truncation; multiple digits,?;Truncation; one digit

Appendix F Patents found in the USPTO patent application database

PUB. APP. NO.	Title	Assignee		Description	Specimen	Type of marker
20090197249	COMPOSITIONS AND METHODS FOR DIAGNOSING COLON DISORDERS	GEORGE MASON UNIVERSITY Fairfax VA	***	The invention relates to microbial community present in the digestive tract and lumen in normal subjects and IBD. It also relates to diagnosing, to determine a therapeutic regimen, to determine the onset of active disease and to determine the predisposition to the disease.	Colonic mucosal tissue, lumen sample	Bacterial gene and/or polypeptide
20100129838	METHODS FOR PREDICTION OF INFLAMMATORY BOWEL DISEASE (IBD) USING SEROLOGIC MARKERS	Prometheus Laboratories Inc. San Diego CA	**	Classifying a sample from a pediatric individual as an IBD sample using a statistical algorithm and/or empirical data	Sample	Antibodies
20090221007	DIAGNOSIS, PREVENTION AND TREATMENT OF CROHN'S DISEASE USING THE OMPC ANTIGEN	Cedars-Sinai Medical Center Los Angeles CA	**	Diagnosing CD in a subject by determining the presence or absence or IgA anti-OmpC antibodies	Serum	Antibodies
20090221006	DIAGNOSIS, PREVENTION AND TREATMENT OF CROHN'S DISEASE USING THE OMPC ANTIGEN	Cedars-Sinai Medical Los Angeles CA	**	Diagnosing CD in a subject by determining the presence or absence or IgA anti-OmpC antibodies	Serum	Antibodies
20080182280	METHODS OF DIAGNOSING INFLAMMATORY BOWEL DISEASE	Prometheus Laboratories Inc. San Diego CA	**	Differentiating between a clinical subtype of IBD such as CD and UC using a statistical algorithm and/or empirical data.	Serum, plasma, whole blood, and stool.	Antibodies
20080131439	METHODS OF DIAGNOSING INFLAMMATORY BOWEL DISEASE	Prometheus Laboratories Inc. San Diego CA	**	Differentiating between a clinical subtype of IBD such as CD and UC using a statistical algorithm and/or empirical data.	Serum, plasma, whole blood, and stool.	Antibodies
20070196931	THERAPEUTIC AGENT FOR ULCERATIVE COLITIS	Nobuhiro SATO Suginami-Ku JP	**	A method for making a diagnosis of UC caused by *Fusobacterium varium* in a patient.	Serum	Antibodies
20070161065	DIAGNOSIS, PREVENTION AND TREATMENT OF CROHN'S DISEASE USING THE OmpC ANTIGEN	Cedars-Sinai Medical Los Angeles CA	**	Diagnosing CD in a subject by determining the presence or absence or IgA anti-OmpC antibodies	Serum	Antibodies
20060205014	Method for diagnosing and prognosing inflammatory bowel disease and Crohn's disease	-	**	Diagnosing and prognosing IBD or CD by measuring levels of antibodies to glycans in a biological sample.	Sample	Antibodies
20060154276	Methods of diagnosing inflammatory bowel disease	Prometheus Laboratories Inc. San Diego CA	**	Differentiating between a clinical subtype of IBD such as CD and UC using a statistical algorithm and/or empirical data.	Serum	Antibodies
20060083686	Therapeutic agent for ulcerative colitis	Sato; Nobuhiro Suginami-Ku JP	**	A method for making a diagnosis of UC caused by *Fusobacterium varium* in a patient.	Serum	Antibodies
20060003392	Methods of diagnosing inflammatory bowel disease	Prometheus Laboratories Inc. San Diego CA	**	Differentiating between a clinical subtype of IBD such as CD and UC using a statistical algorithm and/or empirical data.	Serum	Antibodies
20100099124	Method for Diagnosing Diseases Based on Levels of Anti-Glycan Antibodies	Glycominds, LTD Global Park IL	**	Diagnosing CD or anti-phospholipid syndrome by measuring levels of antibodies to glycans in a biological sample	Biological sample	Antibody

PUB. APP. NO.	Title	Assignee		Description	Specimen	Type of marker
20100021455	METHODS FOR DIAGNOSIS AND TREATMENT OF CROHN'S DISEASE	CEDARS-SINAI MEDICAL CENTER Los Angeles CA	**	Diagnosis of CD is accomplished by determining the presence of the anti-CBir1 expression or determining the presence of anti-CBir1 expression and detection of the presence of pANCA. Treatment methods include antigen-directed therapy targeting CBir1 flagellin and manipulating the bacteria in the colon and/or small intestine.	Sample	Antibody
20100015600	METHOD FOR DIAGNOSING AND TREATING CROHN'S DISEASE	-	**	Diagnosing or determining predisposition to CD by detecting overexpression of the CD66c receptor.	Biological sample	Antibody
20090305267	MICROBIAL MARKERS OF INFLAMMATORY BOWEL DISEASE	UNIVERSITY OF MANITOBA Winnipeg MB	**	Method for diagnosing IBD or determining susceptibility to developing IBD, comprising the step of assaying for serine protease autotransporter (SPATE) or antigen 43 (Ag43) or both, in an enteric bacteria-containing sample from the subject, wherein the presence of SPATE in the sample indicates that the subject has IBD or is susceptible to developing IBD.	Enteric bacteria-containing sample; tissue, stool sample, or intestinal wash.	Bacterial gene and/or polypeptide
20090142778	COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF INFLAMMATORY BOWEL DISEASE	Corixa Corporation Seattle WA	**	Diagnosis, prevention and/or treatment of IBD by bacterial polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides	Biological sample	Bacterial gene and/or polypeptide, cells, antibody
20090311694	Splice variants of human IL-23 receptor (IL-23R) mRNA and use of a delta 9 isoform in predicting inflammatory bowel diseases	Medical Diagnostic Laboratoiries, LLC Hamilton NJ	**	Method of predicting CD by measuring .DELTA.9 isoform of IL-23R.	Biological sample, blood, colon tissue	Cytokine
20060205012	Diagnostic test	Meso Scale Technologies, LLC Gaithersburg MD	**	Diagnostic tests for the identification of the IBDs by the detection of cytokines	Sample	Cytokines
20090311707	O-GLYCANS AS DIAGNOSTIC MARKERS FOR INFLAMMATORY BOWEL DISEASE	-	**	Diagnostic methods for inflammatory bowel disorders comprising assessing expression, structure and/or function of O-glycans in a sample from a subject, as well as antibodies to such molecules.	Colon lavage, a fecal sample, a colorectal swab, or a colon tissue sample.	Glycans
20090186371	METHOD FOR DETERMINATION OF INFLAMMATORY BOWEL DISEASE	OSAKA UNIVERSITY Suita-shi, Osaka JP	**	Differential diagnosis of IBD which comprises, determining the relative ratio of G0 oligosaccharide	Serum	Glycans
20100190162	METHODS OF USING SINGLE NUCLEOTIDE POLYMORPHISMS IN THE TL1A GENE TO PREDICT OR DIAGNOSE INFLAMMATORY BOWEL DISEASE	CEDARS-SINAI MEDICAL CENTER Los Angeles CA	**	Diagnosing or predicting susceptibility to IBD by determining the presence or absence of genetic variants in the TNFSF15 locus.	Biological sample	Human gene and/or polypeptide
20100184967	Biomarkers for inflammatory bowel disease and Irritable Bowel Syndrome	EXAGEN DIAGNOSTICS, INC. Albuquerque NM	**	Diagnosing or distinguishing IBD and irritable bowel syndrome by expression levels of certain genes.	Blood	Human gene and/or polypeptide
20100184625	Biomarkers for inflammatory bowel disease and Irritable Bowel Syndrome	EXAGEN DIAGNOSTICS, INC. Albuquerque NM	**	Diagnosing and/or distinguishing IBD and irritable bowel syndrome by expression levels of certain genes	Blood	Human gene and/or polypeptide
20100184050	DIAGNOSIS AND TREATMENT OF INFLAMMATORY BOWEL DISEASE IN THE PUERTO RICAN POPULATION	CEDARS-SINAI MEDICAL CENTER Los Angeles CA	**	Diagnosis and treatment of IBD by determining the presence or absence of a risk variant of different gene loci	Biological sample	Human gene and/or polypeptide

PUB. APP. NO.	Title	Assignee		Description	Specimen	Type of marker
20100152062	Biomarkers for inflammatory bowel disease and Irritable Bowel Syndrome	EXAGEN DIAGNOSTICS, INC. Albuquerque NM	**	Diagnosing and/or distinguishing IBD and irritable bowel syndrome by expression levels of certain genes	Blood	Human gene and/or polypeptide
20100099083	CROHN DISEASE SUSCEPTIBILITY GENE	GENIZON BIOSCIENCES INC VILLE ST-LAURENT CA	**	The ATG1611 gene and pharmacogenomics, diagnostics, patient therapy and the use of genetic haplotype information to predict an individual's susceptibility to CD and/or their response to a particular drug or drugs.	Biological sample	Human gene and/or polypeptide
20100081129	Genemap of the human genes associated with crohn's disease	-	**	Pharmacogenomics, diagnostics, patient therapy and the use of genetic haplotype information to predict an individual's susceptibility to CD and their response to drugs, so that drugs tailored to genetic differences of population groups may be developed and administered to the appropriate population.	Sample	Human gene and/or polypeptide
20100055700	ROLE OF IL-12, IL-23 AND IL-17 RECEPTORS IN INFLAMMATORY BOWEL DISEASE	CEDARS-SINAI MEDICAL CENTER Los Angeles CA	**	Diagnosing or predicting susceptibility or protection against IBD in an individual by determining the presence or absence of genetic variants in the genes for IL-12, IL-23, and/or IL-17 receptors.	Sample	Human gene and/or polypeptide
20100021917	METHODS OF USING GENES AND GENETIC VARIANTS TO PREDICT OR DIAGNOSE INFLAMMATORY BOWEL DISEASE	CEDARS-SINAI MEDICAL CENTER Los Angeles CA	**	Diagnosing or predicting susceptibility to IBD by determining the presence or absence of genetic variants.	Sample	Human gene and/or polypeptide
20090233306	Biomarkers for inflammatory bowel disease and Irritable Bowel Syndrome	EXAGEN DIAGNOSTICS, INC. Albuquerque NM	**	Diagnosing or distinguishing IBD and irritable bowel syndrome by expression levels of certain genes.	Blood	Human gene and/or polypeptide
20090233305	Biomarkers for inflammatory bowel disease and Irritable Bowel Syndrome	EXAGEN DIAGNOSTICS, INC. Albuquerque NM	**	Diagnosing or distinguishing IBD and irritable bowel syndrome by expression levels of certain genes	Blood	Human gene and/or polypeptide
20090233304	Biomarkers for inflammatory bowel disease and Irritable Bowel Syndrome	EXAGEN DIAGNOSTICS, INC. Albuquerque NM	**	Diagnosing or distinguishing IBD and irritable bowel syndrome by expression levels of certain genes	Blood	Human gene and/or polypeptide
20090155788	GENE EXPRESSION MARKERS FOR INFLAMMATORY BOWEL DISEASE	-	**	Detecting the presence of IBD in gastrointestinal tissues or cells by gene expression	Tissue biopsy	Human gene and/or polypeptide
20090111102	METHODS FOR DETECTING INFLAMMATORY BOWEL DISEASE	Genentech, Inc. South San Francisco CA	**	Detecting the presence of IBD in gastrointestinal tissues or cells of a mammal by detecting increased expression of LY6 genes, relative to a control.	Tissue or cells	Human gene and/or polypeptide
20090081658	GENEMAP OF THE HUMAN GENES ASSOCIATED WITH CROHN'S DISEASE	-	**	Pharmacogenomics, diagnostics, patient therapy and the use of genetic haplotype information to predict an individual's susceptibility to CD or their response to a particular drugs, so that drugs tailored to genetic differences of population groups may be developed and administered to the appropriate population.	Sample	Human gene and/or polypeptide
20090054253	Markers and Methods for Assessing and Treating Ulcerative Colitis and Related Disorders Using 66 Gene Panel		**	A method for prognostic or diagnostic assessment of a gastrointestinal-related disorder, such as UC, in a subject correlates the presence, absence, and/or magnitude of a gene in a sample with a reference standard to determine the presence or severity of the disorder, and the response to treatment for the disorder.	Colon biopsy, blood	Human gene and/or polypeptide

PUB. APP. NO.	Title	Assignee		Description	Specimen	Type of marker
20080293582	Markers and Methods for Assessing and Treating Ulcerative Colitis and Related Disorders Using a 43 Gene Panel	-	**	A method for prognostic or diagnostic assessment of a gastrointestinal-related disorder, such as UC, in a subject correlates the presence, absence, and/or magnitude of a gene in a sample with a reference standard to determine the presence and/or severity of the disorder, and/or the response to treatment for the disorder.	Colon biopsy, blood	Human gene and/or polypeptide
20070042364	Target genes for inflammatory bowel disease	-	**	The invention pertains to a method to identify patients susceptible to IBD by testing for single nucleotide polymorphisms (SNPs) in two genes, FLJ21425 and CSF1R, which were shown to be susceptibility genes for IBD, especially CD.	Biological sample	Human gene and/or polypeptide
20060205022	Method for diagnosing and prognosing inflammatory bowel disease and crohn's disease	-	**	Diagnosing and prognosing IBD or CD by measuring levels of antibodies to glycans in a biological sample.	Sample	Human gene and/or polypeptide and antibodies
20100015156	DIAGNOSIS OF INFLAMMATORY BOWEL DISEASE IN CHILDREN	CEDARS-SINAI MEDICAL CENTER Los Angeles CA	**	Diagnosing and predicting disease progression of CD by determining the presence or absence of CARD15 variants and serological markers.	Biological sample	Human gene and/or polypeptide, antibodies. Bacterial protein
20100129386	Composotions And Methods For The Identification And Treatment Of Immune-Mediated Inflammatory Diseases	-	**	Therapy and diagnosis of IBD by bacterial polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides	Biological sample	Human gene and/or polypeptide, cells
20100144903	METHODS OF DIAGNOSIS AND TREATMENT OF CROHN'S DISEASE	CEDARS-SINAI MEDICAL CENTER Los Angeles CA	**	Diagnosing and/or predicting susceptibility to CD by determining the presence or absence of risk haplotypes in IL23R, IL17A, IL17RA and/or IL12RB1 locus. In another embodiment, the invention provides methods of diagnosing and/or predicting susceptibility to CD in an individual by determining the presence or absence of risk haplotype at the IL12RB2 locus.	Biological sample	Human gene and/or polypeptides
20070020660	Expression profiles of peripheral blood mononuclear cells for inflammatory bowel diseases	-	**	The identification of PBMC- and IBD-associated biomarkers that may be used to diagnose IBD, and optionally, distinguish between PBMCs isolated from a patient with CD and PBMCs isolated from a patient with UC.	Blood	Human gene and/or polypeptide, antibodies and proteins
20090253155	Method For Diagnosing Irritable Bowel Syndrome and Monitoring inflammatory bowel disease	TECHLAB, INC. BLACKSBURG VA	**	Differentiating irritable bowel syndrome from IBD by determining the level of total endogenous human lactoferrin in clinical specimens	Fecal sample	Human protein
20100183521	METHODS OF DETECTING AND TREATING COLON DISORDERS	UNIVERSITY OF SOUTH FLORIDA Tampa FL	**	Determining whether Lipopolysaccharide Responsive Beige-like Anchor (LRBA) is underexpressed in the peripheral blood mononuclear cells (PBMCs), wherein LRBA underexpression is indicative of the colon disorder.	Blood	Human protein
20090176244	METHODS AND COMPOSITIONS FOR THE DIAGNOSIS OF CROHN'S DISEASE	-	**	Diagnose, monitor, or determine the efficacy of treatment for CD. The methods involve determining the presence, absence, or level of zonulin in a subject sample.	Blood	Human protein

PUB. APP. NO.	Title	Assignee		Description	Specimen	Type of marker
20090130775	METHOD FOR CLINICAL STAGING OF ULCERATIVE COLITIS OR INTERSTITIAL PNEUMONIA AND REAGENT KIT FOR THE SAME	OKAYASU; Isao Sagamihara-shi JP HAYASHI; Yuzo Sagamihara-shi JP Mutsunori FUJIWARA Minato-ku JP FUJIREBIO INC. Chuo-ku JP	**	Measurement of the value of main metabolites of prostaglandin E (PGE-MUM) concentration in urine to separate the pre-remission phase from the remission phase of UC.	Urine	Metabolite
20050260155	Compositions and methods for treatment of ulcerative colitis	-	**	The identification of TCP-1 gamma as a protein whose expression is decreased in patients with UC and CD. The protein interacts with hTM5, which is involved in the pathogenesis of UC.	Blood	Human protein
20100203042	RECOMBINANT ANTI-VLA4 ANTIBODY MOLECULES	BIOGEN IDEC MA INC. Cambridge MA	*	Humanized recombinant anti-VLA-4 antibodies disclosed can be useful in methods of diagnosing and localizing sites of inflammation.	Sample	Antibody
20070298447	Method for diagnosing immunologic food sensitivity	-	*	A method for diagnosing an immunologic food sensitivity comprising the steps of: collecting a fecal sample; screening the fecal sample to detect the presence of an antibody to a particular food substance; and diagnosing an immunologic food sensitivity based on the presence of the antibody.	Fecal sample	Antibody
20090123478	Human antibody molecules for IL-13	-	*	Diagnosis or treatment of IL-13 related disorders	Sample	Antibody
20080267959	Anti-Il13 Human Antibodies	Novartis AG	*	Methods for using anti-IL-13 antibody molecules in diagnosis or treatment of IL-13 related disorders, such as asthma, atopic dermatitis, allergic rhinitis, fibrosis, IBD and Hodgkin's lymphoma.	Sample	Antibody
20070231266	Diagnosis of macrophage mediated disease	-	*	Treating or monitoring/diagnosing a disease state mediated by activated macrophages in a myriad of different diseases	Biological sample	Cell
20100061987	High Affinity Antibodies Against HMGB1 and Methods Of Use Thereof	MedImmune, LLC Gaithersburg MD	*	Inhibiting the release of a proinflammatory cytokine from a vertebrate cell, and for inhibiting an inflammatory cytokine cascade in a patient. Also suitable as diagnostic antibodies.	Biological sample	Antibodies
20090169546	HIGH AFFINITY ANTIBODIES AGAINST HMGB1 AND METHODS OF USE THEREOF	MedImmune, LLC Gaithersburg MD	*	Inhibiting the release of a proinflammatory cytokine from a vertebrate cell, and for inhibiting an inflammatory cytokine cascade in a patient. Also suitable as diagnostic antibodies.	Biological sample	Antibodies
20060099207	High affinity antibodies against HMGB1 and methods of use thereof	MedImmune, LLC Gaithersburg MD	*	Inhibiting the release of a proinflammatory cytokine from a vertebrate cell, and for inhibiting an inflammatory cytokine cascade in a patient. Also suitable as diagnostic antibodies.	Biological sample	Antibodies
20090142806	INTERLEUKIN-17F ANTIBODIES AND OTHER IL-17F SIGNALING ANTAGONISTS AND USES THEREFOR	WYETH MADISON NJ	*	Diagnosing, prognosing, monitoring the progress of, and treating and/or preventing disorders related to IL-17F signaling.	Sample	Cytokines
20080241130	METHODS AND COMPOSITIONS FOR MODULATING IL-17F/IL-17A BIOLOGICAL ACTIVITY	-	*	The invention is also directed to novel methods for diagnosing, prognosing, monitoring, preventing, and/or treating IL-17F/IL-17A-associated disorders	Sample	Cytokines

PUB. APP. NO.	Title	Assignee		Description	Specimen	Type of marker
20080233134	Antibodies That Specifically Bind to Chemokine Beta-4	HUMAN GENOME SCIENCES, INC. Rockville MD	*	Antibodies and related molecules that specifically bind to chemokine beta-4. Such antibodies have uses, for example, in wound healing and in the diagnosis, prevention, and treatment of a number of diseases including IBD. The invention also relates to nucleic acid molecules encoding anti-chemokine beta-4 antibodies, vectors and host cells containing these nucleic acids, and methods for producing the same.	Serum	Antibodies
20100209507	METHODS OF DIAGNOSING AND TREATING SMALL INTESTINAL BACTERIAL OVERGROWTH (SIBO) AND SIBO-RELATED CONDITIONS	CEDARS-SINAI MEDICAL CENTER Los Angeles CA	*	A method of detecting small intestinal bacterial overgrowth (SIBO) in a human subject, comprising:detecting the relative amounts of methane, hydrogen, and at least one sulfur-containing gas in a gas mixture exhaled by said human subject, after said human subject has ingested a controlled quantity of a substrate, said gas mixture being at least partially produced by the intestinal microflora of said human subject.	Gas mixture exhaled by a human, serum	Gas
20060147496	Methods of diagnosing small intestinal bacterial overgrowth (SIBO) and SIBO-related conditions	Cedars-Sinai Medical Center Los Angeles CA	*	A method of detecting small intestinal bacterial overgrowth (SIBO) in a human subject, comprising:detecting the relative amounts of methane, hydrogen, and at least one sulfur-containing gas in a gas mixture exhaled by said human subject, after said human subject has ingested a controlled quantity of a substrate, said gas mixture being at least partially produced by the intestinal microflora of said human subject.	Gas mixture exhaled by a human, serum	Gas
20060251659	Method for screening molecules that restore NOD1 activity in cells containing an NOD2 mutation that reduces or eliminates NOD1 activity	-	*	A method for identifying a molecules which modulates the activity of Nod pattern recognition molecules	Cell	Human gene and/or polypeptide
20060229272	Human G-protein coupled receptor, HGPRBMY11, and variants thereof	-	*	The invention relates to diagnostic and therapeutic methods for applying novel HGPRBMY11, HGPRBMY11v1, and/or HGPRBMY11v2 polypeptides in various diseases including CD	Biological sample	Human gene and/or polypeptide
20060105381	Polymorphisms of the OCTN1 cation transporters associated with inflammatory bowel disorders	-	*	Method for diagnosing IBD, using genetic markers that are implicated in severe, early-onset CD.	Biological sample, tissue	Human gene and/or polypeptide
20090137783	CNGH0010 Specific Polynucleotides, Polypeptides, Antibodies, Compositions, Methods and Uses	-	*	Novel polypeptides (CNGH0010), antibodies and polynucleotides for therapeutic and diagnostic purposes in among other IBD	Biological sample	Human gene and/or polypeptide and antibodies
20100167285	METHODS AND AGENTS FOR EVALUATING INFLAMMATORY BOWEL DISEASE, AND TARGETS FOR TREATMENT	-	*	Diagnostic and therapeutic agents for IBD including methods, polynucleotides, polypeptides, and antibodies relating to variants of, and polymorphisms in, the nel-like 1 precursor (NELL1), as well as the 5p13.1 locus, and other genes associated with IBD.	Biological sample	Human gene and/or polypeptides
20100130526	Methods for Disease Therapy	-	*	A method of identifying a phenotype-linked variant genomic sequence in an individual to be used for various diagnostic, prognostic, and/or therapeutic applications.	Biological sample	Human gene and/or polypeptides

PUB. APP. NO.	Title	Assignee		Description	Specimen	Type of marker
20100093552	USE AND IDENTIFICATION OF BIOMARKERS FOR GASTROINTESTINAL DISEASES		*	Identification of biomarkers for gastrointestinal diseases and provides methods utilizing the biomarkers for in drug discovery, monitoring of treatment efficacy, and diagnostics.	Mucosal tissue	Human gene and/or polypeptides
20080044422	CNGH0010 Specific Polynucleotides, Polypeptides, Antibodies, Compositions, Methods and Uses	-	*	Novel polypeptides (CNGH0010), antibodies and polynucleotides for therapeutic and diagnostic purposes in among other IBD.	Biological sample	Human gene and/or polypeptides
20100136574	METHOD FOR DETECTING A CHRONIC INFLAMMATORY-ASSOCIATED DISEASE	-	*	Early detection marker for chronic or acute inflammatory-associated diseases	Fluid sample; serum, plasma, or cerebrospinal fluid.	Human protein
20070166768	Method for predicting sepsis or an acute infectious inflammatory response	-	*	Methods for detecting chronic and acute inflammatory-associated diseases by detecting CAP37 proteins in a body fluid.	Fluid sample comprising serum, plasma, or cerebrospinal fluid	Human Protein
20080260640	Use of Precursors Of Tachykinins and/or Their Fragments in Medical Diagnostic	SPHINGOTEC GMBH Borgsdorf DE	*	The use of protachykinin and/or fragments thereof that can be isolated from body fluids, tissues or other biological samples and used as a marker peptide for medical diagnosis of diseases/disorders.	Body fluids, tissues and/or other biomaterials	Polypeptides antibody
20090186022	Organic Compounds	Novartis AG	*/-	Methods for using anti-hTSLP antibody molecules in diagnosis or treatment of hTSLP related disorders		Antibody
20090280055	Use of Fluorine-Containing Compounds for Diagnostic Purposes Using Imaging Methods	Heinrich-Heine Universitat Dusseldorf Dusseldorf DE	*/-	Administering a fluorine-containing compound to a subject and imaging the compound for the detection of an inflammatory process		Imaging
20100184728	MATERIALS AND METHODS FOR TREATMENT AND DIAGNOSIS OF DISORDERS ASSOCIATED WITH OXIDATIVE STRESS	THERAPEUTIC RESEARCH LLC Memphis TN	-			
20100105044	ILEAL POUCH-ANAL ANASTOMOSIS (IPAA) FACTORS IN THE TREATMENT OF INFLAMMATORY BOWEL DISEASE	CEDARS-SINAI MEDICAL CENTER Los Angeles CA	-			
20090054380	Methods for diagnosing and treating a mycobacterium avium subspecies paratuberculosis infection	-	-			
20090012048	Methods for diagnosing and treating a mycobacterium avium subspecies paratuberculosis infection	-	-			
20080311122	Antagonists of Hmgb1 and/or Rage and Methods of Use Thereof	Medimmune, LLC Gaithersburg MD	-			
20080288227	Algorithms to predict clinical response, adherence, and shunting with thiopuriness	UNIVERSITY OF MICHIGAN, Ann Arbor MI	-			
20070032426	Therapeutic and diagnostic methods for ulcerative colitis and associated disorders	-	-			

PUB. APP. NO.	Title	Assignee	Description	Specimen	Type of marker
20100222303	REVERSE-TURN MIMETICS AND METHOD RELATING THERETO	Choongwae Pharma Corporation Seoul KR	-		
20100120758	REVERSE-TURN MIMETICS AND METHOD RELATING THERETO	Choongwae Pharma Corporation Seoul KR	-		
20100081655	REVERSE-TURN MIMETICS AND METHOD RELATING THERETO	Choongwae Pharma Corporation Seoul KR	-		
20100029630	REVERSE-TURN MIMETICS AND METHOD RELATING THERETO	Chongwae Pharma Corporation Seoul KR	-		
20090325994	METHODS OF TREATING DIARRHEA CAUSED BY SMALL INTESTINAL BACTERIAL OVERGROWTH	CEDARS-SINAI MEDICAL CENTER Los Angeles CA	-		
20090291884	Proteins for use in diagnosing and treating infection and disease	-	-		
20080138396	Treatment and diagnosis of macrophage mediated disease	-	-		
20080014184	METHODS OF TREATING FIBROMYALGIA CAUSED BY SMALL INTESTINAL BACTERIAL OVERGROWTH	CEDARS-SINAI MEDICAL CENTER Los Angeles CA	-		
20070248598	Recombinant anti-VLA4 antibody molecules	Biogen Idec MA Inc. Cambridge MA	-		
20070043052	Reverse-turn mimetics and method relating thereto	Choongwae Pharma Corporation Seoul KR	-		
20060084655	Reverse-turn mimetics and method relating thereto	Choongwae Pharma Corporation Seoul KR	-		
20060029550	Methods of treating irritable bowel syndrome and other disorders caused by small intestinal bacterial overgrowth	CEDARS-SINAI MEDICAL CENTER Los Angeles CA	-		
20050272123	Novel human G-protein coupled receptor	Solvay Pharmaceuticals B.V. CP Weesp NL	-		
20050272122	Novel human G-protein coupled receptor	Solvay Pharmaceuticals B.V. CP Weesp NL	-		
20050266529	Novel human G-protein coupled receptor	Solvay Pharmaceuticals B.V. Weesp NL	-		
20100166789	PROTEINS FOR USE IN DIAGNOSING AND TREATING INFECTION AND DISEASE	The Regents of the University of Colorado Denver CO	-		
20090253645	METHODS AND MATERIALS FOR TREATING AND PREVENTING INFLAMMATION OF	-	-		

PUB. APP. NO.	Title	Assignee	Description	Specimen	Type of marker
	MUCOSAL TISSUE				
20080206251	Methods and Compositions to Treat and Detect Misfolded-SOD1 Mediated Diseases	-	-		
20080014185	METHODS OF TREATING DIARRHEA AND BLOATING CAUSED BY SMALL INTESTINAL BACTERIAL OVERGROWTH	CEDARS-SINAI MEDICAL CENTER 8700 Beverly Boulevard Los Angeles CA 90048	-		

Relevance: -less interesting, *relevant, **interesting, ***very interesting

Appendix G Patent documents found in the European patent database Espacenet

Publication number	Title	Applicant(s)		Description	Type of specimen used	Type of marker
EP1955070 (A2)	METHODS OF DIAGNOSING INFLAMMATORY BOWEL DISEASE	PROMETHEUS LAB INC [US]	**	Diagnosing the presence or severity of IBD and for stratifying IBD in an individual by determining the level of one or more IBD markers in a sample from the individual and calculating an index value using an algorithm based upon the level of the IBD markers.	Serum	Antibody
EP1554580 (A2)	INFLAMMATORY BOWEL DISEASE AND IRRITABLE BOWEL SYNDROME IBD-FIRST CHEK DIAGNOSTIC PANEL	TECHLAB INC [US]	**	A method for differentiation of IBS from IBD followed by distinguishing UC from CD by testing lactoferrin anti-Saccharomyces cerevisiae antibodies (ASCA) and anti-neutrophil cytoplasmic antibodies (ANCA) in fecal samples	Feces	Antibody, human protein
EP1819827 (A2)	METHODS FOR DIAGNOSIS OF CROHN'S DISEASE	CEDARS SINAI MEDICAL CENTER [US]	**	Diagnosis of CD by determining the presence of the anti-CBir1 expression or presence and detection of the pANCA.	Sample	Antibody
EP1539791 (A2)	METHOD FOR DISTINGUISHING ULCERATIVE COLITIS FROM CROHN S DI SEASE BY DETECTING THE PRESENCE OF FECAL ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA)	TECHLAB INC [US]	**	Differentiation of ulcerative colitis from Crohn's disease and other gastrointestinal illnesses using the presence of anti-neutrophil cytoplasmic antibodies (ANCA) as a marker of ulcerative colitis	Feces, whole blood, serum, plasma, human bodily fluid and human tissue.	Antibody
EP1780215 (A1)	CROHN'S DISEASE ANTIBODY EPITOPE PEPTIDE AND REAGENT FOR TESTING CROHN'S DISEASE	TOAGOSEI CO LTD [JP]; UNIV KURUME [JP]	**	A peptide characteristic of an epitope to a Crohn's disease antibody.	Blood	Antibody human peptide
EP2034305 (A1)	METHOD FOR DETERMINATION OF INFLAMMATORY BOWEL DISEASE	UNIV OSAKA [JP]	**	A method for the differential diagnosis of inflammatory bowel disease by determining the relative ratio of G0 oligosaccharide to G2 oligosaccharide in a serum IgG oligosaccharide fraction.	Periferal blood	Glycan
EP2224012 (A1)	Compositions and methods for the therapy and diagnosis of inflammatory bowel disease	CORIXA CORP [US]	**	Diagnosis, prevention and/or treatment of IBD. Comprising one or more bacterial polypeptides, immunogenic portions, polynucleotides that encode such polypeptides, antigen presenting cell expressing such polypeptides.	Biological sample	Bacterial gene and/or polypeptide
EP1642135 (A1)	METHOD FOR DIAGNOSING INFLAMMATORY BOWEL DISEASE	ASTRAZENECA AB [SE]	**	A method for the diagnosis of IBD or determining susceptibility to develop IBD, by detecting variant DLG5.	Sample	Human gene and/or polypeptide
EP1534855 (A1)	METHOD AND KIT FOR THE DIAGNOSIS OF ULCERATIVE COLITIS	INDEX PHARMACEUTICALS AB [SE]	**	Differentiation between ulcerative colitis and Crohn's disease by a multi-gene approach where gene expression profiles in biopsy samples obtained from inflamed, and optionally also non-inflamed, areas in the intestines of a patient are studied.	Biopsy	Human gene and/or polypeptide
EP2132342 (A1)	A METHOD FOR DETERMINING THE GENOTYPE AT THE CROHN'S DISEASE LOCUS	UNIV LIEGE [BE]; CT HOSPITALIER UNIVERSITAIRE D [BE]; COMMISSARIAT ENERGIE ATOMIQUE [FR]	**	A method for determining the genotype of a human individual at the 5p13.1 CD risk locus,	Sample	Human gene and/or polypeptide

Publication number	Title	Applicant(s)		Description	Type of specimen used	Type of marker
EP2071332 (A1)	Method for monitoring persons with IBD using total endogeneous lactoferrin as a marker	TECHLAB INC [US]	**	Monitioring IBD by meassuring lactoferrin in two fecal samples obtained at two different times	Feces	Human protein
EP2050761 (A1)	Galactosylated peptides, their preparation and use in autoimmune diseases diagnosis	TOSCANA BIOMARKERS S R L [IT]	*	The invention refers to galactosylated peptides formed of 11-21 amino acids capable of identifying autoantibodies in several autoimmune diseases	Sample, serum	Antibody
EP1811303 (A2)	Methods of diagnosing and treating irritable bowel syndrome and other disorders	CEDARS SINAI MEDICAL CENTER [US]	*	Detecting the presence of small intestinal bacterial overgrowth (SIBO) by measuring exhaled gas mixture after ingesting a controlled quantity of a labeled substrate	Exhaled gas, cellular, fluid, fecal, or gaseous matter	Gas
EP2140021 (A2)	METHODS FOR DETECTING INFLAMMATORY BOWEL DISEASE	GENENTECH INC [US]	*	Method of detecting the presence of inflammatory bowel disease in gastrointestinal tissues or cells of a mammal by detecting increased expression of LY6 genes in the tissues or cells relative to a control.	Tissue or cells	Human gene and/or polypeptide
EP1557428 (A1)	Antibodies against a human stromal derived factor (SDF) and pharmaceutical composition containing them	ONO PHARMACEUTICAL CO [JP]	*	Novel polypeptides produced by hematopoietic cells and DNAs encoding them for the treatment and diagnosis of among other UC	-	Human gene and/or polypeptide
EP2185936 (A2)	DIAGNOSIS, STAGING AND MONITORING OF INFLAMMATORY BOWEL DISEASE	ISS IMMUNE SYSTEM STIMULATION [SE]	*	A method of differentiating between active and inactive IBD in a patient and methods of determining the presence of CD and UC in a patient using inflammation markers in biopsies	Biopsy	Human gene and/or polypeptide, protein, cytokine and antibody
EP1870708 (A1)	METHOD FOR CLINICAL STAGING OF ULCERATIVE COLITIS OR INTERSTITIAL PNEUMONIA AND REAGENT KIT FOR THE SAME	FUJIREBIO KK [JP]; FUJIWARA MUTSUNORI [JP]; OKAYASU ISAO [JP]; HAYASHI YUZO [JP]	*	A method that measures the value of main metabolites of prostaglandin E (PGE-MUM) concentration in urine and judges stages between the pre-remission phase of and the remission phase of ulcerative colitis	Sample	Metabolite
EP1653231 (A1)	MEASURING APPARATUS FOR ULCERATIVE COLITIS DIAGNOSIS/PROGNOSTIC TEST AND METHOD OF MEASURING	MATSUSHITA ELECTRIC IND CO LTD (JP) TOKYO UNIVERSITY OF PHARMACY A (JP)	*	A measurement apparatus for ulcerative colitis diagnosis and prognostic test	Sample	Other
EP1950222 (A1)	Method for detecting antibodies in body fluids via an immune reaction with glycoprotein 2 (GP2) from zymogen granula of the pancreas for a differential diagnosis of inflammatory bowel diseases and chronic pancreatitis	GA GENERIC ASSAYS GMBH [DE]	§			
EP1800134 (A2)	METHOD FOR DIAGNOSING AND TREATING CROHN'S DISEASE	UNIV D AUVERGNE CLERMONT 1 [FR]	§	Diagnosing or determining predisposition Crohn's disease, by detecting an overexpression of the CD66c receptor		

Relevance: -less interesting, *relevant, **interesting, ***very interesting, § not read, patent document written in foreign language

Appendix H Patents found in the USPTO issued patent database

PAT. NO.	Title	Assignee		Description	Type of specimen used	Type of marker
6,320,037	IBD-associated microbial nucleic acid molecules	The Regents of the University of California (Oakland, CA)	***	The present invention provides nucleic acid and amino acid sequence of the novel I-1 and I-2 polypeptides, which are associated with human IBD.	Sample	Bacterial gene and/or polypeptide
6,759,530	IBD-associated microbial nucleic acid molecules	The Regents of the University of California (Oakland, CA)	***	*Diagnosing* and treating *IBD* using the IBD-associated I-1 and I-2 antigens	Whole blood, plasma, saliva, or other bodily fluid or tissue, preferably serum	Bacterial gene and/or polypeptide
7,759,079	Methods of diagnosing inflammatory bowel disease	Prometheus Laboratories Inc. (San Diego, CA)	**	Diagnosing the presence or severity of inflammatory bowel disease. Monitoring the efficacy of IBD therapy, monitoring the progression or regression of IBD, and optimizing therapy in an individual having IBD.	Biological sample	Antibody
7,662,569	Methods of assessing Crohn's disease patient phenotype by I2 serologic response	Cedars-Sinai Medical Center (Los Angeles, CA)	**	Determining the likelihood of being susceptible to a fibrostenotic subtype of Crohn's disease by determining the presence or absence in the subject of IgA anti-I2 antibodies, a NOD2 variant, anti-Saccharomyces cerevisiae antibodies (ASCA), IgA anti-OmpC antibodies, or perinuclear anti-neutrophil cytoplasmic antibodies (pANCA)	Sample	Antibody
7,608,414	Method for diagnosing and prognosing inflammatory bowel disease and crohn's disease	Glycominds, Ltd (IL)	**	Diagnosing and prognosing IBD or CD by measuring levels of antibodies to glycans in a biological sample	Serum	Antibody
7,592,150	Method for diagnosing diseases based on levels of anti-glycan antibodies	Glycominds, Ltd (IL)	**	Methods for diagnosing CD or anti-phospholipid syndrome by measuring levels of antibodies to glycans in a biological sample.	Biological fluid	Antibody
7,358,058	Method and agents for the diagnosis and therapy of chronic inflammatory intestinal disease	B.R.A.H.M.S Aktiengesellschaft (Henningsdorf, DE)	**	Diagnosis, early diagnosis, differential diagnosis, assessment of the severity and therapy-accompanying monitoring and prognosis of chronically inflammatory intestinal diseases in which the presence and/or the amount of one or more antibodies which bind to ribosomal proteins, in particular P0 and L5, are determined in the serum, plasma, tissue samples and/or stool.	Biological sample	Antibody
7,138,237	Diagnosis, prevention and treatment of Crohn's disease using the OmpC antigen	Cedars-Sinai Medical Center (Los Angeles, CA) The Regents of the University of California (Oakland, CA)	**	Determining the presence or absence of IgA anti-OmpC antibodies in the subject, where the presence of the IgA anti-OmpC antibodies indicates that the subject has *Crohn's* disease.	Sample	Antibody
6,777,197	Method and test kit for measuring immunoglobulins reactive with amylase as indication of crohn's disease	Wakamoto Pharmaceutical Co., Ltd. (Tokyo, JP)	**	Detection of the presence of immunoglobulins reactive with amylase.	Blood	Antibody
7,700,106	Therapeutic agent for ulcerative colitis	Sato; Nobuhiro (Tokyo, JP) Wakamoto Pharmaceutical Co., Ltd. (Tokyo, JP)	**	Preliminarily determining that the probability for developing or having ulcerative colitis by detecting Fusobacterium varium anitbodies in patient sera.	Sera	Antibody

PAT. NO.	Title	Assignee		Description	Type of specimen used	Type of marker
7,183,065	Diagnosis, prevention and treatment of ulcerative colitis, and clinical subtypes thereof, using microbial UC pANCA antigens	The Regents of the University of California (Oakland, CA)	**	Microbial UC pANCA antigens and methods of diagnosing UC and methods of inducing tolerance in a pANCA-positive patient with UC using a histone H1-like antigen. Methods of diagnosing UC and methods of inducing tolerance in a pANCA-positive patient with UC using a Bacteroides antigen also are provided.	Biological sample	Antigen
7,361,733	Compositions and methods for the therapy and diagnosis of inflammatory bowel disease	Corixa Corporation (Seattle, WA)	**	Compositions and methods for the therapy and diagnosis of Inflammatory Bowel Disease comprising one or more bacterial polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides.	Biological sample	Bacterial gene and/or polypeptide
6,805,852	Methods of diagnosing irritable bowel syndrome and other disorders caused by small intestinal bacterial overgrowth	Cedars-Sinai Medical Center (Los Angeles, CA)	**	A method of diagnosing irritable bowel syndrome by detecting the presence of small intestinal bacterial overgrowth (SIBO) by measuring exhaled gas mixture after ingesting a controlled quantity of a labeled substrate.	Intestinal sampling, cellular, fluid, fecal, or gaseous matter	Gas
7,419,782	Methods of using a major histocompatibility complex class III haplotype to diagnose Crohn's disease	Cedars-Sinai Medical Center (Los Angeles, CA	**	A method of diagnosing or predicting susceptibility to an autoimmune disease in an individual by determining the presence or absence in the individual of a 2-2-4 haplotype at the Notch4, HSP70-HOM and D6S273 loci.	Blood	Human gene and/or polypeptide
6,858,391	Nod2 nucleic acids and proteins	Regents of the University of Michigan (Ann Arbor, MI)	**	Methods of identifying individuals at increased risk of developing CD by Nod2 protein and nucleic acid detection.	Sample	Human gene and/or polypeptide
6,762,021	Method for diagnosis of crohn's disease	Mitsubishi Pharma Corporation (Osaka, JP)	**	Analyzing the level of gene expression.	Colon tissue or ileum tissue	Human gene and/or polypeptide
6,632,617	Tumor-associated antigen	Incyte Corporation (Palo Alto, CA)	**	The invention is based on the discovery of a tumor-associated antigen (TUAN) which is useful in the diagnosis and treatment of inflammatory and cell proliferative disorders, in particular, Crohn's disease and colon cancer.	Biological sample	Human protein
6,590,089	RVP-1 variant differentially expressed in Crohn's disease	Incyte Genomics, Inc. (Palo Alto, CA)	**	Mammalian cDNA which encodes a mammalian RVP-1 variant (MAPOP-1) useful in the diagnosis and treatment of cell proliferative disorders, particularly cancers, and autoimmune disorders, particularly Crohn's disease.	Sample	Human gene and/or polypeptide
7,560,240	Method for monitoring gastrointestinal inflammation in persons with inflammatory bowel disease(IBD)	TechLab, Inc. (Blacksburg, VA)	**	A method for aiding in differentiating irritable bowel syndrome from inflammatory bowel disease by determining the level of total endogenous human lactoferrin in clinical specimens	Feces, mucus and bile	Human protein
7,192,724	Method for differentiating irritable bowel syndrome from inflammatory bowel disease (IBD) and for monitoring persons with IBD using total endogenous lactoferrin as a marker	Techlab, Inc. (Blacksburg, VA)	**	Method for quantitating the level of total endogenous human lactoferrin in clinical specimens, such as feces, mucus and bile, to monitor gastrointestinal inflammation in persons having inflammatory bowel disease.	Feces	Human protein
6,627,458	Nuclear envelope protein recognized by atypical p-ANCA in patients with inflammatory bowel disease and autoimmune liver diseases	The Trustees of Columbia University in the City of New York (New York, NY)	**	A method for determining nuclear envelope protein of neutrophils and myeloid cells capable of binding of a typical p-antineutrophil cytoplasmic antibodies (p-ANCA)	Body fluid, serum	Human protein and cells

PAT. NO.	Title	Assignee		Description	Type of specimen used	Type of marker
7,375,192	Antibodies that specifically bind to chemokine beta-4	Human Genome Sciences, Inc. (Rockville, MD)	*	The present invention relates to antibodies and related molecules that specifically bind to CK-B4. Such antibodies have uses, for example, in the prevention and treatment of cancer as well as immune system diseases	Biological sample	Antibody
7,557,194	Antibody materials for an IBD-associated polypeptide	The Regents of the University of California (Oakland, CA	*	Nucleic acid and amino acid sequence of I-1 and I-2 polypeptides, which are associated with humanIBD. Methods of diagnosing and treating IBD using the IBD-associated I-1 and I-2 antigens	Sample	Bacterial gene and/or polypeptide
7,531,310	Methods of diagnosing Crohn's disease by measuring expression level of RNA encoding human G-protein coupled receptor, HGPRBMY11	Bristol-Myers Squibb Company (Princeton, NJ)	*	Methods of diagnosing Crohn's disease by measuring expression level of RNA encoding human G-protein coupled receptor, HGPRBMY11	Colon test sample	Human gene and/or polypeptide
7,479,479	CNGH0010 specific polynucleotides, polypeptides, antibodies, compositions, methods and uses	Centocor, Inc. (Malvern, PA)	*	Polypeptides (CNGH0010) useful for therapeutic and diagnostic formulations, administration and devices.	Sample	Human gene and/or polypeptide
7,326,687	CNGH0010 specific polynucleotides, polypeptides, antibodies, compositions, methods and uses	Centocor, Inc. (Malvern, PA)	*	CNGH0010 polypeptides, variants, and fragments useful for therapeutic and diagnostic formulations.	Sample	Human gene and/or polypeptide
7,151,165	Antibodies immunospecific for a novel human G-protein coupled receptor family	Solvay Pharmaceutical B.V. (Weesp, NL)	*	Polynucleotides, polypeptides more particularly G-protein coupled receptor (GPCR) and the use and production of them. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides and a method for screening compounds capable to act as an agonist or an antagonist of G-protein coupled receptor IGS4, and to the cognate ligand of IGS4.	Sample	Human gene and/or polypeptide
7,049,422	Tumor necrosis related receptor, TR7	SmithKline Beecham Corporation (Philadelphia, PA)	*	TR7 polypeptides and polynucleotides and methods for producing such polypeptides and diagnostic assays	Sample	Human gene and/or polypeptide
7,771,932	Method for identification of Tr1 lymphocytes regulators by the presence and over-expression of specific molecules and application thereof	TxCell (Nice, FR)	*	A method for identifying Tr1-regulatory lymphocytes present in a biological sample comprising lymphocytes. A method for in vitro prognosis or diagnosis of an autoimmune or inflammatory disease.	Biological sample comprising lymphocytes	Human gene and/or polypeptide, and protein
7,655,480	Method for predicting sepsis or an acute infectious inflammatory response	The Board of Regents of the University of Oklahoma (Norman, OK)	*	Early detection marker for chronic or acute inflammatory-associated diseases.	Serum, plasma, or cerebrospinal fluid	Human protein and cytokines
6,998,255	Human G-protein coupled receptor	Solvay Pharmaceuticals B.V. (CP Weesp, NL)	*	The invention relates to polynucleotides, polypeptides more particularly G-protein coupled receptor (GPCR) and the use and production of them. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides and a method for screening compounds capable to act as an agonist or an antagonist of G-protein coupled receptor IGS4, and to the cognate ligand of IGS4.	Sample	Human gene and/or polypeptide, and protein
6,800,446	Therapeutic and diagnostic methods for ulcerative colitis and associated disorders	University of Medicine & Dentistry of New Jersey (New Brunswick, NJ)	*	Diagnostic method for detecting inflammatory bowel disease and ulcerative colitis comprising detecting CEP-hTM5 complexes with antibodies against CEP and hTM5 in a colonic	Colonic tissue	Human protein

PAT. NO.	Title	Assignee		Description	Type of specimen used	Type of marker
				tissue		
7,736,622	Methods of diagnosing small intestinal bacterial overgrowth (SIBO) and SIBO-related conditions	Cedars-Sinai Medical Center (Los Angeles, CA)	*	A human subject ingests a controlled quantity of an isotope-labeled sugar, and gas mixture exhaled are detected and used for diagnosis purpose.	Gas mixture exhaled by an human	Gas
6,787,303	Identification of a novel retrovirus associated with primary sclerosing cholangitis and autoimmune hepatitis	Alton Ochsner Medical Foundation (New Orleans, LA)	*	Detecting the presence or absence of a Primary Sclerosing Cholangitis, PSC, associated retroviral nucleic acid molecule to indicate PSC, Autoimmune Hepatitis, Crohn's disease, and ulcerative colitis.	Biological sample	Retroviral gene
7,138,250	Method of determining thiopurine methyltransferase activity	Prometheus Laboratories, Inc. (San Diego, CA)	*	Methods for determining thiopurine methyltransferase activity in order to individualize dosages of 6-mercaptopurine therapy.	Sample	Human gene and/or polypeptide
6,576,438	Method of determining thiopurine methyltransferase activity	Prometheus Laboratories, Inc. (San Diego, CA)	*	Determining thiopurine methyltransferase (TPMT) activity in a subject.	Sample, a preferred sample is whole blood	Human protein
7,048,906	Methods of diagnosing and treating small intestinal bacterial overgrowth (SIBO) and SIBO-related conditions	Cedars-Sinai Medical Center (Los Angeles, CA)	-			
7,585,504	High affinity antibodies against HMGB1 and methods of use thereof	MedImmune, LLC (Gaithersburg, MD)	-			
7,781,169	Diagnosis of gluten sensitive enteropathy and other autoimmunopathies	-	-			
7,740,854	Treatment of macrophage mediated disease	Purdue Research Foundation (West Lafayette, IN)	-			
7,671,054	Reverse-turn mimetics and method relating thereto	Choongwae Pharma Corporation (Seoul, KR)	-			
7,605,240	Methods of treating diarrhea and bloating caused by small intestinal bacterial overgrowth	CedaraSinai Medical Center (Los Angeles, CA)	-			
7,585,862	Reverse-turn mimetics and method relating thereto	Choongwae Pharma Corporation (Seoul, KR)	-			
7,585,838	Methods of treating fibromyalgia caused by small intestinal bacterial overgrowth	Cedars-Sinai Medical Center (Los Angeles, CA)	-			
7,576,084	Reverse-turn mimetics and method relating thereto	Choongwae Pharma Corporation (Seoul, KR)	-			
7,566,711	Reverse-turn mimetics and method relating thereto	Choongwae Pharma Corporation (Seoul, KR)	-			
7,482,003	Treatment for asthma with .alpha.4-specific antibodies	Biogen Idec MA Inc. (Cambridge, MA)	-			
7,452,857	Methods of treating irritable bowel syndrome and other disorders caused by small intestinal bacterial overgrowth	Cedars-Sinai Medical Center (Los Angeles, CA)	-			

PAT. NO.	Title	Assignee	Description	Type of specimen used	Type of marker
7,232,822	Reverse-turn mimetics and method relating thereto	Choongwae Pharma Corporation (Seoul, KR)	-		
7,157,086	Treatment for inflammatory bowel disease with .alpha.4-specific antibodies	Biogen Idec MA Inc. (Cambridge, MA)	-		
7,122,336	Therapeutic and diagnostic methods for ulcerative colitis and associated disorders	University of Medicine & Dentistry of New Jersey (New Brunswick, NJ)	-		
7,056,686	Method of diagnosing fibromyalgia caused by small intestinal bacterial overgrowth	Cedars-Sinai Medical Center (Los Angeles, CA)	-		
6,555,566	Methods and materials for treating and preventing inflammation of mucosal tissue	Mayo Foundation for Medical Education and Research (Rochester, MN)	-		
6,399,573	Interleukin-1 receptor antagonist beta (IL-1rabeta)	SmithKline Beecham Corporation (Philadelphia, PA)	-		

Relevance: -less interesting, *relevant, **interesting, ***very interesting