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-CONFIDENTIAL-

ColonView CLINICAL TRIAL

A new-generation immunochemical fecal occult blood test [Biohit ColonView quick test] compared to guaiac-based test (Hemoccult®SENSA®) in detection of proximal and distal colorectal neoplasia among colonoscopy referral patients.

Jointly Executed by:

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Research Team:

Names Open.

Summary

Background: Colorectal cancer (CRC) is the third common malignancy, with over 1.2 million new cases and over 600.000 annual deaths being recorded worldwide. However, CRC meets the criteria of a screening condition; it is highly prevalent and develops (in most cases) through well defined precursors (adenomatous polyps). CRC screening can impact both i) the primary prevention (finding precursor lesions with malignant potential) and ii) the secondary prevention (detecting early stage cancers amenable to treatment). Commonly used CRC screening tests include **guaiac-based fecal occult blood test (FOBT),** FS (flexible sigmoidoscopy), and colonoscopy, all shown to reduce CRC mortality. Currently available FOB tests are not specific for human blood, however, leading to false positive results and unnecessary referrals to colonoscopy, if subjects are not compliant with diet restrictions before sampling. To circumvent this problem, different fecal **immunochemical tests (FITs)** have been introduced since the early 1990's. Still the studies comparing the performance of FOBTs and FITs are relatively few, and report contradictory results; some suggest that the sensitivity of FIT assays is substantially better than that of FOBT, with comparable or even superior specificity, just to be refuted by other studies. Clearly, more studies are mandatory to evaluate the performance of the new-generation FITs in comparison to the most sensitive guaiac-based FOBTs on the market. One of these new-generation FITs was recently launched by a Finnish company (Biohit Oyj, Helsinki), with the proprietary name **Biohit ColonView quick test**, intended for rapid detection of occult blood in stool samples.

Objective: To compare Biohit Oyj's Biohit ColonView quick test with guaiac-based test (Hemoccult®SENSA®) in detection of fecal occult blood as surrogate of clinically significant colorectal neoplasia.

Study Design: This study is a clinical trial comparing Biohit ColonView quick test with Hemoccult®SENSA® (Beckman Coulter®) in colonoscopy-referral patients to establish their performance indicators in detection of significant neoplasia in the proximal and distal colon (adenomas, carcinomas, AN).

Methods: Subjects (with no age and gender restriction) are enrolled among the patients referred for colonoscopy at the outpatient Department of Gastroenterology, Hospital X (City Y, Country Z). After consenting to participate, the patients are given the necessary sampling material with detailed instructions how to collect, handle, store and deliver the collected stool samples to the test laboratory (Hospital X). Both ColonView quick test and Hemoccult®SENSA® tests are processed in the laboratory of Hospital X. All stool samples (for both tests) must be collected before admission to colonoscopy. Colonoscopy (used as gold standard) is performed according to conventional procedures, and findings classified by the Paris classification. All detected lesions (polyps, tumors) are biopsied or removed if technically feasible. Biopsies are interpreted using the WHO classification for colorectal tumors. Statistical analyses include calculation of the performance indicators separately i) for the three tests (ColonView Hb, ColonView Hb/Hp, Hemoccult), and ii) for individual study endpoints, including ROC analysis, and controlling for potential confounders (other conditions as a cause of fecal occult blood). As the final step, data from this clinical setting will be translated to a hypothetical screening setting, using simulations with varying population prevalence of the different study endpoints.

Specific Aims: The single most important objective of this study is to establish which of the two tests has the best sensitivity/specificity balance in detecting the study endpoints. These study endpoints include the following: i) adenoma in the proximal colon, ii) adenoma in the distal colon, iii) adenocarcinoma in the proximal colon, iv), adenocarcinoma in the distal colon, and v) advanced neoplasia (AN) at both anatomic sites. For all study endpoints, sensitivity (SE), specificity (SP), negative predictive value (NPV), positive predictive value (PPV) and AUC (area under ROC curve) will be calculated for all three tests. In this context, we will also assess: 1) the rate of unnecessary referrals for colonoscopy (false positive rate; 1-PPV) following a positive FOBT and FIT; 2) the rate of colonoscopies to be avoided after a negative FIT and FOBT (true negative rate; NPV), and 3) the rate of clinically significant conditions that are missed by these two tests (i.e., false negative rate; 1-SE). Simulations will be used to estimate the performance characteristics of these tests in real-life screening setting for CRC and its precursors.

Study execution and time table: The study plan necessitates a review by the institutional review board (IRB). Given that among these consecutive colonoscopy referral patients, a 10-15% test-positive rate is anticipated, we estimate that a minimum of 500 subjects must be screened to make the study adequately powered for the study endpoints. With the current colonoscopy frequency at Hospital X, this will take about ? months. Because only a minor delay in the laboratory arm of the study, we expect that the full database will be available at conclusion of the clinical study arm.

Impact of the study: We anticipate to obtain unbiased (i.e., 100% confirmed by the gold standards) estimates for the performance of each three tests (ColonView Hb, ColonView Hb/Hp, Hemoccult®SENSA®) in detecting the target lesions. These unbiased estimates can be used in data simulation, translating the results to a real-life screening setting with different population prevalence of the study endpoints. This approach is expected to disclose the similarities and disparities that are to be anticipated between the FOBT- and FIT-based screening strategies for the lesions (adenoma, carcinoma) located at different anatomic sites (proximal and distal colon).

1. BACKGROUND

1.1.Descriptive epidemiology

Colorectal cancer or colorectal adenocarcinoma (CRC) is a malignant tumor arising within the walls of the large intestine, including the segments in the 1) caecum, 2) ascending colon, 3) transverse colon, 4) descending colon, 5) sigmoid, and 6) rectum. Tumors in locations 1-3 are called proximal CRC, while those of 4-6 are distal CRC. CRC does not include tumors in the anus or the small intestine. Adenomas are benign epithelial tumors that are considered precancerous lesions. Adenomas can present with a different degrees of dysplasia and different histological characteristics (tubular, tubule-villous, and villous) associated with increasing malignant potential. Carcinoma *in situ* (CIS) refers to adenomas with severe dysplasia, while lesions that invade the muscularis mucosa are considered adenocarcinomas. The concept advanced neoplasia (AN) refers to a composite outcome, including adenocarcinoma, adenomas 10mm or greater in diameter, adenomas with high-grade dysplasia, and those with ≥25% of villous histology.

CRC continues to be a major global disease burden. In 2008, CRC ranked in the third place among the most common malignancies, with over 1.2 million new cases diagnosed and over 600.000 annual deaths being recorded worldwide (1). In Europe, the corresponding figures are 432.000 and 212.000, making CRC the single most frequent malignancy and second only to lung cancer in annual cancer deaths (1). In the US, CRC ranks the 3rd most common cancer, with 153.000 incident cases and 52.000 cancer deaths (1,2). Similarly, in Asia, CRC holds the 3rd position among most frequent cancers, with over 500.000 new cases and 267.000 annual deaths (1). In Finland, CRC is the second most common cancer among women (1240 new cases), and third most common among males (1246 cases)(3). The global age-adjusted incidence and mortality rates of CRC are 17.2/100.000 and 8.2/100.000, respectively (1).

There is good evidence e.g. from the US, that increasing age, male sex, and Black race are associated with an increased incidence of CRC (2). Accordingly, age-adjusted incidence rates for CRC are higher in men than women, and Blacks have the highest incidence of CRC among the racial/ethnic subgroups in the US. Blacks also have a disproportionately high disease-specific mortality (4). Interestingly, age, sex, and race/ethnicity also appear to influence on the anatomic distribution of CRC (5). Data from the NCI's Surveillance, Epidemiology, and End Results Program (SEER)(2) demonstrate a proximal migration of CRC over the past two decades, which is

attributed to a decrease in incidence of distal CRCs, and an aging population in which proximal lesions are more common (5). This proximal migration appears in both men and women, and in Whites and Blacks. This difference between the two races was not evident during the 1970's.

1.2.Risk factors

Most cases of CRC are sporadic, with 75% of cases developing in average-risk persons, and only some 20% of cases are found in persons with some type of family history (6,7). The remainder of cases develop in persons who have predisposing inflammatory bowel disease (e.g. ulcerative colitis) or a known genetic mutation, including familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC). Case-control and cohort studies indicate an approximately two-fold increase in CRC risk for persons with a first-degree relative (e.g., parent, sibling, or child) with CRC. This increased risk is also applicable to first-degree relatives of individuals with colorectal adenomas (8). CRC may be associated with non-genetic risk factors, such as smoking or obesity, although evidence is limited to case-control and cross-sectional data (6-8). There has been substantial progress in understanding the molecular genetics of CRC, and these scientific advances underpin the efforts to develop DNA testing (fecal or plasma) for CRC detection.

1.3.Natural history

It is estimated that at least 95% of all CRC cases arise from pre-existing polypoid or flat adenomas (9). The notion of an adenoma-carcinoma sequence stems from observations of a markedly elevated CRC risk among patients with hereditary polyposis syndromes, and from observational studies showing an estimated 60 to 90% reduction in CRC incidence after polypectomy during colonoscopy or FS (flexible sigmoidoscopy)(10). The most convincing evidence from FS studies comes from well designed case-control studies that have demonstrated a decrease in CRC mortality, and in some cases, in CRC incidence. The landmark case-control study by Selby and colleagues (11) found a 60% reduction in mortality from distal CRC over 10 years in persons who underwent sigmoidoscopy with polypectomy, compared to matched contemporaneous controls. Despite some uncertainty around the magnitude of this benefit, these studies provide the best available estimates for the impact of polypectomy on CRC incidence (10,11).

1.3.1.Significance of the polyp size

While there is general agreement that the risk of local cancer, or progression to cancer, for

polyps 10 mm or larger is sufficient to require immediate removal, the necessity and benefit of removing small polyps is not clear (12,13). Sensitivity estimates for optical methods (e.g., CTC, FS, and colonoscopy) depend on the threshold for the size of polyp considered clinically meaningful. The threshold for polyp size also determines the number of colonoscopy referrals that will result from primary CT Colonography (CTC) and other visualization-only screening methods. No large observational studies are available to determine the consequences of untreated adenomas. Similarly, the natural history of smaller adenomas, particularly those of different sizes (e.g., 5 mm or under, 6 to 9 mm), is practically unknown. The tendency towards net growth or regression may vary by polyp size and histology, as well as by other characteristics such as patient age, tumor location, and number of lesions (14). Some colonoscopy database studies suggest that the prevalence of CRC in lesions <6 mm in diameter ranges from zero to 0.8%, and in those with 6–9 mm, from 0.4% to 1.1% (15). While advanced neoplasia (AN) seems to be somewhat more common than CRC in small polyps, the clinical significance of AN remains poorly understood.

1.3.2.Significance of advanced neoplasia (AN)

Several studies have estimated the accuracy of optical screening methods not only for CRC, but also for AN. By definition, AN is a composite endpoint for three conditions: i) an adenoma 10mm or larger in size, ii) a smaller adenoma with at least 25% of villous histology, or iii) adenomas containing high-grade dysplasia or invasive carcinoma. It is important to understand both the impact of polypectomy of ANs on the risk of future CRC, and conversely, the impact of leaving an AN lesion intact on the risk of future CRC. In a long-term follow-up study after polypectomy (n=1618 patients), the risk of subsequent CRC was increased at least 3-fold in patients with tubule-villous, villous, or large (≥10 mm) adenomas (i.e., AN), as compared with other types of recto-sigmoid adenomas (16). Similarly, the frequency of invasive carcinomas in polyps with surface villous histology ranged from 10 to 40%, as compared with 6 to 23% among polyps with surface tubule-villous histology, 2 to 5% in polyps with surface tubular histology, as well as 34% among polyps with high-grade dysplasia (17). Importantly, however, there are no prospective studies on the natural history of AN, and no longitudinal studies have validated the clinical benefit of targeting AN in screening populations. The prevalence of flat and depressed (non-polypoid) adenomas in screening populations is largely unknown, as is the prevalence of dysplasia in these lesions, and the risk of CRC associated with these lesions is currently under dispute.

1.4.Screening of colorectal cancer

Among few human cancers, CRC meets the criteria of a screening condition; it is a highly prevalent disease, with well established preclinical period during which the majority of CRC develops from precursor lesions, i.e., adenomatous polyps of AN. Thus, screening for CRC can impact both i) the primary prevention (finding precancerous polyps that could later become malignant) and ii) the secondary prevention (detecting early cancers that can be more effectively treated). Based on evidence from randomized controlled trials (RCT), a screening program using simple, reasonably acceptable, guaiac fecal occult blood (FOB) screening tests reduces CRC mortality when used with repeated application over time and endoscopic follow-up of positive results (18). Also other screening approaches are recommended, based on extrapolation from the evidence of RCTs (19). However, no current CRC screening tests are without flaws, including potential harms, limited accessibility, or imperfect acceptability to patients. Ongoing research aims to make more accurate screening tests available to further improve CRC screening programs (20). While there is general consensus that CRC screening reduces disease-specific mortality, newer screening tests have created uncertainty about the optimal methods for CRC screening in the general population (19,20).

1.4.1.Methods available for CRC screening

CRC screening tests in common use include home FOBT (fecal occult blood test), FS (flexible sigmoidoscopy), and colonoscopy (19,20,21). In many countries, the US included, the use of colonoscopy for CRC screening has increased recently while the use of FS has decreased, in part due to the changed policies of coverage by health insurances (22). Public perceptions of accuracy also play an important role in this issue. Significant variation in community CRC screening practices, which may impact effectiveness of screening, has also been reported. Similarly, there appears to be variation in practice for follow-up of positive FOBT (e.g., using FS instead of colonoscopy)(23). Lastly, there remains significant variation in operator characteristics for endoscopies, both FS and colonoscopy, which may affect test characteristics for screening and confirmatory endoscopy.

1.4.2. Performance of the different CRC screening tests

1.4.2.1.Flexible sigmoidoscopy (FS) and colonoscopy

The most comprehensive treatise on the performance of different methods used for CRC screening is to be found in the systematic review of Whitlock et al. 2008, published in two

separate reports (19,20). According to this comprehensive review, in community settings, FS (with or without biopsy to determine colonoscopy referral) has an estimated sensitivity of 58% to 75% for CRC in the entire colon and an estimated sensitivity of 72% to 86% for AN. Variations in these estimates are likely due to differences in examiner skills and the patient's risks for proximal lesions in the unexamined colon (19,20). While colonoscopy remains the most accurate screening test for CRC at a single application, recent studies have confirmed that colonoscopy misses polyps and may also miss CRC. Colonoscopy also presents a higher risk for harms than other tests. Serious harms from community endoscopies are about 10 times more common with colonoscopy (3.1 per 1,000 procedures) than with FS (3.4 per 10,000 procedures) (19,20).

1.4.2.2.Guaiac-based fecal occult blood tests (FOBT)

During the past, different guaiac-based FOBTs have been very popular methods in CRC screening in many countries (19,20). These tests detect fecal occult blood based on peroxidase activity of hemoglobin (Hb)-derived heme groups, but, unfortunately, are not specific for human blood. In addition to human blood, these guaiac-based tests can also trace animal blood derived from food, and in addition, peroxidases derived from some raw vegetables. This can lead to false positive results and unnecessary referrals to colonoscopy. In addition, these tests are usually not highly sensitive, which can also lead to false negative results.

Not unexpectedly, the data on the efficacy of FOBT screening are controversial (19,20). While two studies with long-term follow-up of biennial FOBT screening suggest CRC mortality reduction by 13 to 21%, after 8 to 13 years of screening, another two trials did not show mortality benefit until after 15–18 years of screening. In a recent meta-analysis from the Cochrane Collaboration, the overall estimate of CRC mortality reduction by biennial FOBT screening was 15%, using either random- or fixed-effect models (RR=0.85, 95%CI: 0.78-0.92)(18). However, this analysis did not incorporate the recently reported data from one of these trials, suggesting that CRC mortality benefit is no longer statistically significant at 17 years when deaths due to CRC treatment are included (RR=0.89, 95%CI: 0.78-1.01)(24). When critically assessed in the recent systematic review (19,20), a meta-analysis of all four FOBT screening trials indicated no benefit for all-cause mortality (RR=1.00, 95%CI: 0.99-1.03)(18,24). This made the authors to suggest that CRC screening is not be expected to reduce all-cause mortality in these ongoing FOBT trials (19,20).

1.4.2.3.Fecal immunochemical tests (FIT)

In Japan, the pioneering country of CRC screening, different fecal immunochemical tests (FITs) have been the principal screening method since the early 1990's (25). In the recent systematic review (19,20), altogether 12 types of FITs were identified in the literature, representing 20 different proprietary names. Due to the major differences in test methodology, the authors were unable to assess all FITs as a class, however, but had to satisfy with a sub-group analysis (19,20).

In their systematic assessment, four individual FITs (Magstream/HemeSelect; FlexSure OBT/Hemoccult ICT; OC-Hemodia; Monohaem) showed higher sensitivity for CRC (61% to 91% percent) than estimates for (guaiac-based) non-rehydrated Hemoccult II (25% to 38%), with somewhat reduced specificity (91% to 97%, respectively). Sensitivity for ANs or large adenomas is less commonly reported, but ranges between 20% and 67% for FITs, which is comparable or superior to the sensitivity for non-rehydrated Hemoccult II (19,20). Fewer acceptable-quality studies are available on another FIT (HemoccultSENSA), and although it appears to improve sensitivity for CRC (64% to 80%), it seems to have a lower specificity (87% to 90%). The authors concluded that in the lack of test accuracy results indicating clearly superior test sensitivity with comparable specificity, determining the trade-offs between sensitivity and specificity of these newer fecal tests in organized CRC screening programs requires further studies (19,20).

1.5.Studies comparing FOBT and FIT tests

During the recent past, a number of studies have been published where the performance of guaiac-based FOBT has been compared with the fecal immunochemical tests (FIT) in detecting different study endpoints of CRC screening (26-32). In their study of 8,104 patients, Allison et al. (26) compared two guaiac tests (Hemoccult II, Hemoccult II SENSA) with HemeSelect (a FIT). They found that the sensitivity of Hemoccult II (37.1%; 95%CI, 19.7-54.6), was clearly inferior to that of HemeSelect (68.8%; 95%CI, 51.1-86.4), and the same was true with test specificity as well: 86.7% and 94.4%, respectively (26). In an earlier study, Frommer et al. (27) concluded that immunological detection of occult blood in fecal samples seems to show more adenomas and carcinomas (particularly early lesions) than the Hemoccult II kit and has a rate of false positive results that is acceptably low (27). In another recent study, comparing a new bedside immunological test strip device (Prevent ID CC) with a sensitive guaiac-based test (Hemoccult), and an established immunochemical test (Human Hb ELISA)(28), the sensitivity and specificity of the beside immunochemical strip test for detection of adenomas and CRC were 60% and 95%,

which favorably competes with an established immunochemical fecal occult blood test and exceeds the performance of the guaiac-based test (28). In contrast, Rozen et al. found a guaiac-based test (HemoccultSENSA) to be more sensitive but less specific than a FIT (FlexSure) in detecting any colorectal neoplasms (29).

Sieg et al. (1999) compared the Hb test to a newly developed immuno-chemiluminometric (ILMA) assay for quantifying the hemoglobin-haptoglobin (Hb/Hp) complex in feces which shows high stability against degradation (30). In a cohort of 621 patients, the sensitivity for detecting CRC proved to be 87% with Hb. With the Hb/Hp complex, it was 87% at a cutoff level of 1.5 microg/g feces, 83% at 2.0 microg/g feces, and 78% at 2.5 and 3.0 microg/g feces. The sensitivity for detecting large adenomatous polyps was 54% with Hb, 76% with the Hb/Hp complex at a cutoff point of 1.5 microg/g feces, 73% at 2.0, and 2.5 microg/g feces, and 65% at 3.0 microg/g cutoff. The optimal cutoff point for the Hb/Hp complex was estimated to be 2.0 microg/g stool. The specificity for Hb (99%) was significantly higher than that for the Hb/Hp complex at 2.0 microg/g feces (96%). These authors concluded that immunological determination of the Hb/Hp complex in feces has a comparable sensitivity as the fecal Hb assay for CRC, and a significantly higher sensitivity for adenomatous polyps, but at the expense of significantly lower specificity (30).

Results in alignment of these were recently reported by van Rossum et al. (2008), who compared a guaiac-based FOBT and a FIT in the first population-based study on a random sample of 20,623 individuals, randomized to either Hemoccult-II or OC-Sensor (31). Of the 10,993 tests returned, 117 FOBTs (2.4%) were positive vs. 339 (5.5%) of FITs. Cancer and advanced adenomas were found, respectively, in 11 and 48 of FOBTs and in 24 and 121 of FITs. Differences in PPV for cancer and advanced adenomas were, respectively, 2.1% (P=0.4) and -3.6% (P=0.5). Differences in specificities favor FOBT and were, respectively, 2.3% (P<0.01) and -1.3% (P<0.01). Differences in intention-to-screen detection rates favor FIT and were, respectively, 0.1% (P<0.05) and 0.9% (P<0.01). Taken together, the number-to-scope to find 1 cancer was comparable between the two tests. However, participation and detection rates for advanced adenomas and cancer were significantly higher for FIT than FOBT, the latter significantly underestimates the prevalence of advanced adenomas and cancer in the screening population compared with FIT (31).

Finally, Wong et al. (2003) compared the performance characteristics of a guaiac-based FOBT (Hemoccult SENSA) and a FIT (FlexSure OBT) in a Chinese population referred for colonoscopy (32). The sensitivity, specificity and positive predictive value (PPV) for the detection of significant colorectal neoplasia were 91%, 70% and 18% for Hemoccult SENSA and 82%, 94% and 47% for FlexSure OBT. The specificity and PPV were significantly higher for FlexSure OBT than for Hemoccult SENSA ($P<0.001$ and $P=0.016$, respectively). The authors concluded that PPV of the FIT for detection of colorectal neoplasia was 29% better than that of the sensitive FOBT, and the poor specificity of the guaiac-based test (without dietary restriction), makes it less useful for CRC screening in a Chinese population (32).

Taken together, the studies comparing the performance of FOBT and FIT tests show in part contradictory results (19,20,26-32). The data suggest, however, that the sensitivity of FIT assays is substantially better than that of FOBT. In some studies, the specificity of the two assays is comparable, whereas in some others, also the specificity of FITs seems to be clearly superior to that of FOBTs. In the only RCT conducted so far, the evidence suggests that the performance of FITs is clearly superior to FOBTs in detecting any type of colorectal neoplasia (31). Clearly, more studies are mandatory to evaluate the performance of the new-generation FITs in comparison to the most sensitive guaiac-based FOBTs on the market, in both a clinical setting and particularly as screening tools for CRC. One of these new-generation FITs was recently launched by a Finnish company (Biohit HealtCare Oyj), with the proprietary name **Biohit ColonView quick test**, intended for rapid detection of occult blood in stool samples.

1.6.Biohit ColonView quick test

Biohit ColonView quick test is a visual test, using immunochromatography for quick and qualitative detection of human hemoglobin (Hb) and hemoglobin/haptoglobin complex (Hb/Hp) in stool samples. Hb molecule consists of 2 pairs of peptide (α- and β-globins) chains and 4 heme groups, each with one atom of iron. Free Hb may separate into $\alpha-\beta$ molecules, which are bound to a protein called haptoglobin (Hp). Hb/Hp complex plays an important role in the retrieval of Hb from lysed erythrocytes and is relatively stable against acid and proteolytic degradation. This means that the Hb/Hp complex can be detected even after longer passage through the bowel, increasing the chance that also the blood mixed with larger intestinal polyps and proximal CRCs can be detected. There is evidence that detection of the Hb/Hp complex displays a significantly

increased sensitivity in recognition of colorectal adenomas and carcinomas, when combined with Hb detection (30,33).

The Biohit ColonView quick test is based on an immunochromatographic method, in which both Hb and Hb/Hp complexes are specifically recognized through specific antibody reactions (**ANNEX 1**). The Test Cassette strip is pre-coated with anti-human Hb and anti-human Hp antibodies on the Test region (T) and goat anti-mouse antibodies on the Control region (C). An anti-human Hb/Hp complex antibody-colloidal gold conjugate pad is placed at the end of the membrane. When human Hb/Hp complexes are present in the stool sample dissolved in buffered saline, the mixture of colloidal gold conjugate and extracted sample moves along the membrane, chromatographically by capillary action.

In the case of a positive result, the molecules from the stool sample loaded with gold-marked antibodies attach to the test band (T) and are visible by means of a pink/red coloration. In the case of a negative result, there are no hemoglobin molecules that can attach to the test band (T) as complexes and therefore, there can also be no coloration of the test band (T). If the control strip (C) turns red/pink in color, this shows that the sample has been correctly taken and has migrated properly, indicating that the test is technically valid.

1.7.Guaiac-based fecal occult blood test (Hemoccult®SENSA®)

According to its manufacturer (Beckman Coulter®), the Hemoccult®SENSA® test is a rapid, convenient and qualitative method for detecting fecal occult blood which may be indicative of gastrointestinal disease. The Hemoccult®SENSA® test is recommended for use as a diagnostic aid during routine physical examinations, for hospital patients to monitor for gastrointestinal bleeding in patients with iron deficiency anemia or recuperating from surgery, peptic ulcer, ulcerative colitis and other conditions, and in screening programs for CRC when the Patient Instructions are closely followed. Serial fecal specimen analysis is recommended when screening asymptomatic patients (34,35).

Like all guaiac-based assays, this test is based on an old concept that the gum guaiac, which is a natural resin extracted from the wood of *Guaiacum officinale*, is useful in detecting occult blood. The Hemoccult®SENSA® test is based on the oxidation of guaiac by hydrogen peroxide to a blue-colored compound. The heme portion of Hb, if present in the fecal specimen, has peroxidase

activity which catalyzes the oxidation of alpha-guaiaconic acid (active component of the guaiac paper) by hydrogen peroxide (active component of the developer) to form a highly conjugated blue quinone compound (36).

The Hemoccult®SENSA® test, like its predecessor, the Hemoccult® test, is a simplified and standardized variation of the laboratory guaiac procedure for detection of occult blood. The Hemoccult®SENSA® test formulation includes an enhancer which makes the test more sensitive and more readable than other guaiac-based tests. The Hemoccult®SENSA® test more reliably detects abnormal bleeding associated with gastrointestinal disorders than standard guaiac tests. As a result, it will have a higher sensitivity for disease but also a higher false-positive rate among non-diet compliant patients. Hemoccult®SENSA® positive test results appear as more stable, intense blue color reactions than the results of other guaiac tests, improving overall readability and precision. As with other guaiac tests, accuracy depends upon the status of the patient at the time the specimen is taken and may be affected by interfering substances.

2. STUDY DESIGN

The present study is a clinical trial, comparing the Biohit ColonView quick test (Biohit Oyj, Helsinki, Finland) and the sensitive guaiac-based FOBT (Hemoccult®SENSA®) in detection of occult fecal blood as indicator of clinically significant colorectal neoplasia. The conditions representing the endpoints in this study include the following: i) adenoma in the proximal colon, ii) adenoma in the distal colon, iii) adenocarcinoma in the proximal colon, iv), adenocarcinoma in the distal colon, and v) advanced neoplasia (AN; for definition, see Section 1.1.) at both anatomic sites. As additional endpoints, all cases of diverticulosis, angiectasia, and inflammatory bowel disease will be recorded, because potential confounding factors for FOBT (false positives).

2.1.Aims of the study

The single most important objective of this study is to compare the performance characteristics of a new-generation FIT (Biohit ColonView quick test) with those of a sensitive FOBT (Hemoccult®SENSA®) in detecting fecal occult blood as surrogate marker of significant colorectal neoplasm, i.e., the various conditions listed as study endpoints in Section 2. This main goal consists of several specific aims in this study.

1. Sensitivity (SE), specificity (SP), negative predictive value (NPV), positive predictive value (PPV) and AUC (area under ROC curve) for Biohit ColonView quick test (separately fob Hb and Hb/Hp complex)* and Hemoccult®SENSA® test in detecting **adenomas (with different size) in the proximal colon**.

2. SE, SP, NPV, PPV and AUC for Biohit ColonView quick test and Hemoccult®SENSA® test in detecting **adenomas (with different size) in the distal colon**.

3. SE, SP, NPV, PPV and AUC for Biohit ColonView quick test and Hemoccult®SENSA® test in detecting **adenocarcinomas in the proximal colon**.

4. SE, SP, NPV, PPV and AUC for Biohit ColonView quick test and Hemoccult®SENSA® test in detecting **adenocarcinomas in the distal colon**.

5. SE, SP, NPV, PPV and AUC for Biohit ColonView quick test and Hemoccult®SENSA® test in detecting **advanced neoplasia (AN) in both proximal and distal colon.**

6. In this context, we will also assess: 1) the rate of unnecessary referrals for colonoscopy (false positive rate; 1-PPV) following a positive Biohit ColonView quick test and Hemoccult®SENSA® test; 2) the rate of colonoscopies to be avoided after a negative FIT and FOBT examination (true negative rate; NPV), and 3) the rate of clinically significant diseases (conditions) that are missed by these two tests (i.e., false negative rate; 1-SE).

7. The results obtained (points 1-5) from this unbiased 100% colonoscopy (and gastroscopy)-confirmed clinical setting will be translated to a screening setting (always subject to verification bias), using statistical modeling simulating the settings with different population prevalence of the above listed study endpoints (adenoma, carcinoma, AN). The aim is to estimate the performance characteristics of these tests in real-life screening setting for CRC and its precursors.

2.2.Patients

This clinical trial is conducted in collaboration between Biohit Oyj (Helsinki, Finland) and Department of Gastroenterology, Hospital X (City Y, Country Z)(hereafter called "the Partners"). The study is performed exclusively in Hospital X, supervised by a steering committee consisting of members from both research Partners.

Enrolment of the patients in the study will take place at Hospital X, including consecutive patients (with no age limit), attending the Outpatient Department of Endoscopy for an appointment to colonoscopy. The cohort to be screened is 500 subjects, including adequate numbers of all conditions (see: Section 2, above) used as study endpoints.

Patient enrollment is taking place in two steps. In brief, the potentially eligible patients are identified among the outpatient clinic attendants referred for colonoscopy with different indications. At this stage, every patient will be asked to read the patient information sheet, and consent the study by signing a written consent to participate. Those consenting to participate will be given or delivered by mail a DELIVERY BOX, containing all necessary material for sampling, as well as instructions for sample collection, handling and mailing, as described below (**ANNEX 2**).

Eligible patients are all adult females and males, irrespective whether symptomatic or asymptomatic, who 1) have been scheduled for diagnostic colonoscopy in the clinic, and ii) who have read the Patient Information Form and given a written consent to participate (**ANNEX 3**).

The following patients should be considered **non-eligible**: 1) Those patients who refuse to participate; 2) patients in whom the colonoscopic examination remains unsatisfactory (to be judged by the endoscopist), as well as 3) all patients who have visible blood in the stools regularly or frequently (see: the data collection form)(**ANNEX 4**).

2.2.1.Patient instructions

Proper conduction of and reliable results from the Biohit ColonView quick test and Hemoccult®SENSA® tests necessitate some preparatory measures of the patients. Before consenting to participate, all patients are offered a Patient Information Form, describing the principles of the study (ANNEX 3). Having consented to participate, each subject will receive (personally or by mail) detailed instructions on the sampling, their proper handling, storage and

delivery to the test laboratory, all included in the Delivery Box.

Being a FIT test, Biohit ColonView quick test does not necessitate any major preparatory steps of the patient, or compliance with any restrictions in the daily dietary habits or daily medication. Hemoccult®SENSA®, in contrast, being a guaiac-based FOBT, necessitates compliance with certain restrictions in the daily diet and daily medication. To make the comparison of these two basically different tests as unbiased as possible, the Delivery Box also contains detailed instructions for the patient preparation as well as precautions of the restrictions in diet and daily medication, as described in **ANNEX 2**. **Importantly, we preserve the option for any patient who feels unsecure or clearly non-compliant with the instructions of Hemoccult®SENSA® sampling, to refrain from the stool sampling for this test, and only submit the ColonView sample**. This will inevitably lead to lower return of Hemoccult®SENSA® samples, but at the same time, it offers an advantage for comparing these two tests without bias caused by the non-compliant dietary and medication restrictions inherent to guaiac-based FOBTs.

2.3.Methods

2.3.1.Sample collection for Biohit ColonView quick test and Hemoccult®SENSA® test

All sample collection for these two tests will be done by the consented patients at home, following the detailed (but simple) instructions given or delivered to each patient as a part of the Delivery Box (ANNEX 2). It is recommended that as the first step, the patient who is intended to start his/her sampling, should fill the Patient Information Form (**ANNEX 5**) as complete as possible. After completion of the sample collection in three consecutive days, the patient should deliver the sample box without delay to the test laboratory, following the instructions for handling, packing and delivery given in ANNEX 2.

2.3.2.Sample processing in the test laboratory

Due to the basically different principles of the Biohit ColonView quick test and Hemoccult®SENSA® test, also the sample processing in the test laboratory is different. For completeness of this study protocol, the sample processing of both tests is illustrated in **ANNEX 6** and **ANNEX 7**.

2.3.3.Evaluation of ColonView quick test and Hemoccult®SENSA® test results

Prerequisite for reliable results of both tests is an adequately collected, handled, stored, delivered and processed stool sample (ANNEX 2). When all these steps are properly conducted, interpretation of the results of both tests is straightforward, as illustrated in ANNEX 6 and ANNEX 7, for ColonView and Hemoccult®SENSA®, respectively. Because ColonView quick test consists of two different components, it can be treated as a 2-test assay (for human Hb and Hb/Hp complex), and as such, the test performance indicators can be calculated separately for both components.

2.4.Colonoscopy and biopsy procedures

All patients examined with the ColonView quick test and Hemoccult®SENSA® tests, will be subjected to **colonoscopy**, providing the histological confirmation to be used as the gold standard in calculating the performance indicators for the two tests. In the case of completely normal colonoscopy, however, biopsies will not be taken, and in such a case, normal colonoscopy is used as the gold standard indicating a negative result regarding the study endpoints (Section 2).

The colonoscopy will be done according to the usual practice, and in each patient, a record will be made of all findings as a regular colonoscopy report, as explained in **ANNEX 4**. Of key importance is an accurate record of all study endpoints (adenomas, carcinomas and ANs), including their number, size and exact locations. In classifying the colonoscopy findings, the **Paris classification** should be used (37). According to this classification, all superficial colorectal lesions (=lesions with no invasion) are called type 0 neoplastic lesions, with different variants. These include polypoid (Ip and Is), non-polypoid (IIa, IIb, and IIc), non-polypoid and excavated (III)(p=pedunculated; s=sessile; IIa=slightly elevated; IIb=flat; IIc=slightly depressed; III=excavated or ulcer).

2.4.1.Biopsy protocols

In the cases with no objective findings in colonoscopy, no biopsies will be taken. In positive colonoscopy, however, all polyps and tumors will be biopsies (or removed if small enough). In addition, biopsy confirmation will be made for all other lesions listed in ANNEX 4 as potential confounders (interfering conditions) of the tests under study. The biopsy sites as well as accurate description of the biopsied lesions (polyp, carcinoma, other), should be given in the

colonoscopy report. The biopsy site should be given at the level of the exact anatomic location (caecum, ascendens, transversum, descendens, sigma, recto-sigmoid, rectum), although the final evaluation will be done separately only for the dichotomized variable (proximal and distal colon).

2.4.2.Preparation of the microscopy slides

The biopsies from formalin bottles/tubes are embedded in paraffin using the routine procedures at the Pathology Laboratory of Hospital X. The blocks are cut into 4-µ-sections, and stained with hematoxylin eosin (HE) and PAS and Ab-PAS for routine diagnosis.

2.4.3.Interpretation of the biopsies

All colonoscopy biopsies are examined by the expert pathologists at Hospital X, among the daily routine samples. The diagnoses are reported using the standard WHO classification of colorectal neoplasia. In addition to their size, all polyps must be classified as hyperplastic polyps or adenomas. All adenomas are further classified according to their histological growth pattern as tubular, tubule-villous, villous and serrate adenomas. In the new WHO classification, several sub-categories of the latter are used, including microvacuolar hyperplastic polyp, goblet cell rich hyperplastic polyp, sessile serrate adenoma, traditional serrate adenoma. A composite entity know as advanced neoplasia (AN) contains adenocarcinoma, adenomas 10mm or greater in diameter, adenomas with high-grade dysplasia, and those with ≥25% of villous histology. For this reason, an estimate on the grade of dysplasia should be given of all diagnosed adenomas.

Following the routine diagnosis of all colonoscopy biopsies at the Department of Pathology, all pathological findings will be reviewed by the second pathologist in the research group (Prof. Kari Syrjänen, Biohit). In case of major discrepancy, he will consult Prof. Pentti Sipponen, MD, PhD, (Helsinki, Finland) who is a recognized international authority in the field of gastrointestinal pathology, and member of the Scientific Advisory Board of Biohit Oyj.

2.5.Statistical Analyses

All statistical analyses will be performed using the SPSS 25.0.0.1 for Windows (IBM, NY, USA) and STATA/SE 15.1 software (STATA Corp., Texas, USA). The descriptive statistics will be conducted according to routine procedures. Performance indicators (sensitivity, specificity, positive predictive value, PPV, negative predictive value, NPV and their 95%CI) of the two tests will be

calculated separately for each study endpoint, using the STATA/SE software and the *diagti* algorithm introduced by Seed et al. (2001)(38). This algorithm also calculates the area under ROC (Receiver Operating Characteristics) called AUC [(SE+SP)/2], for each study endpoint. Because Biohit ColonView quick test consists of two components, these performance indicators can be calculated separately for Hb and Hb/Hp complex, increasing the flexibility of this assay. Significance of the difference between AUC values is estimated using the roccomb test (STATA) with 95%CI.

Before closing the cohort enrolment, careful power analyses will be conducted to estimate the statistical power of this study. It has been estimated that with the 10-15% prevalence of positive FIT or FOBT test among colonoscopy referral patients, we can expect 50 to 75 patients testing positive in a cohort of 500 subjects. With the test sensitivity level of 80%, that would yield some 40 to 60 cases of clinically significant pathologies (study endpoints). This number is more than enough to give 100% power for each test in calculating the difference in effect size in the disease/no disease setting (e.g. in two-sample proportion test). This sample size of 60 (but not 40) would also probably be powered enough (at 80% level) to compare the three tests in detection of clinically significant disease, within the effect size difference of e.g. 0.8 vs. 0.55, i.e. 80% detection (by test 1) vs. 55% detection (by test 2). Any effect size difference smaller than this necessitates more cases to maintain the power of the study at the acceptable 80% level.

As the final step, data from this clinical setting (n=500) will be translated to a hypothetical screening setting of 10.000 subjects, using simulations varying the population prevalence of the different study endpoints (derived from the published literature). To do that, we need to make two assumptions: 1) test sensitivity remains the same as in our colonoscopy series, and 2) the false positive rate (FPR) also remains unaffected. These assumptions are based on the reasoning that the i) test sensitivity remains the same, irrespective whether you examine colonoscopy referral patients (high disease prevalence) and a random (screening) population, and ii) the same happens with the FPR, i.e., equal proportion of tests will give false positive result in both settings.

3.ETHICAL ISSUES

The study design and its execution do not involve any significant ethical issues except those in other clinical studies of similar type. The study protocol will be submitted for approval to the

Institutional Ethical Committee of Hospital X, and the study is conducted in accordance with the Declaration of Helsinki.

Patients are enrolled among consecutive outpatients referred for colonoscopy at the outpatient Department of Hospital X. Thus, they represent regular outpatients who have different indications for colonoscopic examination. All patients must sign the informed consent for their participation. While consenting to participate in the study, the patients agree to collect, handle, store and deliver stool samples in three consecutive days, collected by the sampling kits of the FIT and FOBT compared in this study. When the results of the ColonView quick test and HemoccultSENSA tests, colonoscopy and biopsies are available, the patients will be informed about the results following the usual hospital practices, including an explanation of the practical consequences of these test results.

4.TIME TABLE

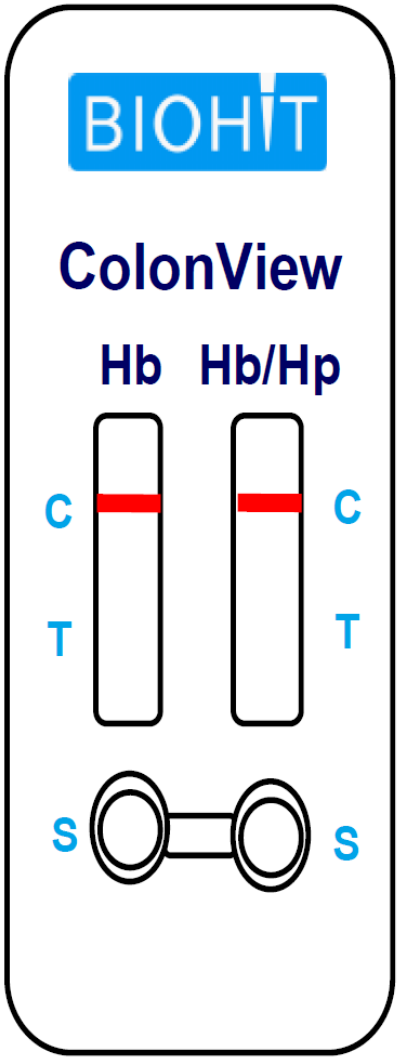
The necessary preparations for the study execution at Hospital X will start immediately when the hospital has reached the agreement with Biohit Oyj. The study plan necessitates a review by the institutional review board (IRB, Ethical Committee) before permission to start. Given that the subjects in the study will be enrolled among consecutive patients referred for colonoscopy at the Outpatient Department of Hospital X, a 10-15% test-positive rate is anticipated. To accumulate a minimum number of conditions listed as study endpoints, we estimate that a minimum of 500 subjects must be screened by the two tests and colonoscopy, which at the current colonoscopy rate will take a minimum of ? months. The laboratory arm of this study is expected to proceed online with the progress of patient enrollment, delivery of collected stool samples and performed colonoscopies. There will be the usual minor delay (of days) due to the biopsy examinations at the Department of Pathology, until the results of all examinations (FIT & FOBT, colonoscopy, biopsy) of each subject are available and entered into the study database.

5. PROJECTED COSTS TO BE COVERED BY Biohit Oyj

To be agreed by Biohit and Hospital X.

ANNEX 1:

Biohit ColonView quick test



The Biohit ColonView quick test (30) is based on a immunochromatographic method, in which both Hb and Hb/Hp complexes are specifically recognized through specific antibody reactions. The Test Cassette strip is pre-coated with anti-human Hb and anti-human Hp antibodies on the Test region (T) and goat anti-mouse antibodies on the Control region (C). An anti-human Hb/Hp complex antibody-colloidal gold conjugate pad is placed at the end of the membrane. When human Hb/Hp complexes are present in the sample, dissolved in buffered saline, the mixture of colloidal gold conjugate and extracted sample moves along the membrane, chromatographically by capillary force.

In a positive test, the molecules from the stool sample loaded with gold-marked antibodies attach to the test band (T) and become visible by means of a pink/red coloration. In a negative test, there are no Hb molecules that can attach to the test band (T) as complexes and therefore, there is no coloration of the test band (T). When the control strip (C) turns red/pink in color, this indicates that the sample has been correctly taken and has migrated properly, indicating that the test is technically valid.

ANNEX 2.

PATIENT INSTRUCTIONS

1.Clinical Study

You have been referred for a diagnostic endoscopy of your large bowel (colonoscopy), and you have kindly consented to participate in the ongoing clinical study, where two different methods for detecting fecal occult blood will be directly compared. These tests for fecal occult blood are used in diagnosis of gastrointestinal tract disorders, and the ongoing study aims to assess, which of these two tests is more accurate in the population of this country. Those two tests to be compared are Biohit ColonView quick test (30) and Hemoccult® SENSA®.

The entire research team is very grateful for your important contribution to this study!

2. The content of the package given or delivered to you

The sample package that you received is intended for delivery of your samples to the test laboratory. This box contains the following items:

- Personal data form
- ColonView sample collection kit (ColonView Sample Collection Kit)
- Hemoccult® SENSA® sample collection kit (green paper pack)
- Two empty minigrip bags, one of which has a soft paper sheet.

3. Personal Data Form

Your sampling package also contains a personal data form. It is important that all data in this form are accurately filled. Mail the form together with the samples, as instructed later.

4. Collecting the stool samples

All stool samples must be collected BEFORE appointment to colonoscopy. We need samples from three different stools collected from three different (consecutive) days as follows:

- Day 1: The first stool sample: Hemoccult ja ColonView samples from the same stool.
- Day 2: The second stool sample: Hemoccult ja ColonView samples from the same stool.
- Day 3: The third stool sample: Hemoccult ja ColonView samples from the same stool.

These three days should, if possible, be three consecutive days. In any case, the samples should be collected within one week time (max).

4.How to collect the samples in all three days

IMPORTANT: Use the same stools collected onto the stool collection paper (ColonView Sample Collection Kit) for collecting **both** the ColonView- and Hemoccult SENSA samples.

4.1.Biohit ColonView quick test 30 samples

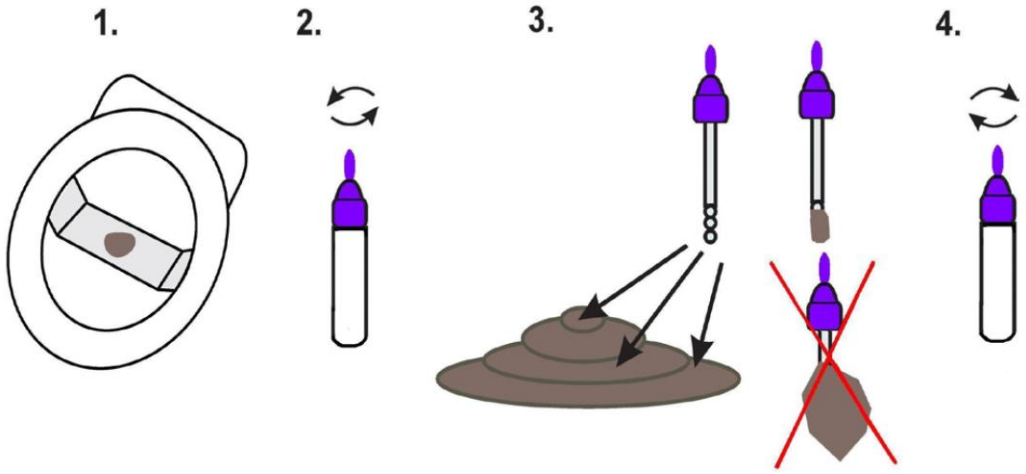


Figure 1. Instructions for collecting the stool samples for both tests

1. Collect a random sample of stool in a clean dry container or receptacle, for example the Stool Collection Paper provided with the Sample Collection Bag. Use the Stool Collection Paper as follows:

- Peel off the liner covering the adhesive tape on each end of the collection paper.
- Lift toilet seat. Unfold collection paper and place on the rim of toilet bowl. Secure the adhesive tabs on the collection paper to the sides of the toilet rim.
- Make bowel movement onto collection paper.

2. Unscrew the colored cap and remove the Sample Collection Tube applicator stick. Be careful not to spill or spatter solution from container.

3. Collect random samples by inserting the applicator stick into the stool specimen. Take samples from various surfaces of the stool specimen.

4. Re-insert the applicator stick into the tube and screw the cap tightly. Be careful not to break the tip of the Sample Collection Tube (colored end).

- If you are using the Stool Collection Paper, release adhesive tabs and flush the collection paper with stool.

5. Write your name and the date of sample collection in the space provided on the Sample Collection Tube.

6. Return the specimen for testing promptly by mail or in person. The specimen(s) can be stored in the refrigerator (2-8 °C) for no more than 11 days, or at room temperature (max. 25 °C) for no more than 5 days.

4.2. Hemoccult®SENSA® samples

The stool sample for Hemoccult® SENSA® can be collected using the same instructions as given in **Figure 1.** However, the manufacturer of this test has given more detailed instructions for people who collect their samples for Hemoccult® SENSA® test. These instructions are enclosed here MERELY, because if you are not compliant with these, you should simply NOT collect the sample for Hemoccult® SENSA® test, but ONLY for the ColonView test.

If you feel sure that you are compliant with the instructions listed in the next sections, you can take the sample for Hemoccult® SENSA® test, using the specimen collection kit following the manufacturer´s instructions.

- take one sample card and write your name and sampling day in front of the card (lines preserved for that).
- Open the cover slip of the card from the same side (under the name).
- Then, move a small piece of stools using the stick and spread it over the sample window A of the card (left side).

- Do the same and move a tiny piece of stools by stick and spread it over the sample window B (right side).
- Close the cover slip and put the sampling card into one of the minigrip bags (the one with no paper), to wait for the other samples.
- Store the sample at room temperature.

The unused (excess) stool can be flushed by the toilet. Also the stool collection paper can be discarded in the same way.

4.2.1.Patient preparation and instructions

IMPORTANT: Patients should follow these PATIENT INSTRUCTIONS at least 7 days prior to and continuing through the test period.

- For accurate test results, apply samples from bowel movements collected on **three different days** to slide.
- Do not collect sample, if blood is visible in your stool or urine (e.g., menstruation, active hemorrhoids, urinary tract infection). **Contact your doctor.**
- For the most accurate test results, collect each stool sample before contact with the toilet bowl water. You may use any clean, dry container.
- Return completed slides to your doctor or laboratory no later than 14 days after your first sample collection.
- Protect slides from heat, light, and volatile chemicals (e.g., ammonia, bleach, bromine, iodine, household cleaners).
- Remove toilet bowl cleaners from toilet tank and flush twice before proceeding.

Drug Guidelines

- For ***seven*** days before and during the stool collection period, **avoid** non-steroidal anti-inflammatory drugs such as ibuprofen, naproxen or aspirin (more than one adult aspirin a day).
- Acetaminophen (Tylenol*****) can be taken as needed.
- For ***three*** days before and during the stool collection period, **avoid** vitamin C in excess of 250 mg a day from supplements, and citrus fruits and juices.

Diet Guidelines

- For ***three*** days before and during stool collection period, **avoid** red meats (beef, lamb and liver).
- Eat a well balanced diet including fiber such as bran cereals, fruits and vegetables.

Notes:

1. Please talk to your doctor or pharmacist if you have any questions about medications you take regularly: 100% of RDA of vitamin C for an adult is 60 mg a day; Some iron supplements contain vitamin C in excess of 250 mg.

4.2.2.Specimen collection

The Hemoccult®SENSA® test requires only a small fecal specimen. The specimen is applied to the guaiac paper of the Hemoccult®SENSA® slide as a **THIN SMEAR** using the applicator stick provided. Hemoccult®SENSA® Slides are best developed no sooner than 3 days after sample application. This allows any fruit and vegetable peroxidases present in the sample to degrade. Slides containing samples may be stored up to 14 days at room temperature (15 to 30°C) before developing. However, patients using the Hemoccult®SENSA® test should be instructed to return the slides to the physician or laboratory immediately after preparing the last test.

4.2.3.Interfering substances

In general, patients should be carefully instructed to not ingest foods and vitamins which can cause false-positive or false-negative test results for at least 72 hours before and through the test period.

Substances which can cause false-positive test results:

- Red meat (beef, lamb and liver)
- Aspirin (greater than 325 mg/day) and other non-steroidal anti-inflammatory drugs such as ibuprofen, indomethacin and naproxen
- Corticosteroids, phenylbutazone, reserpine, anticoagulants, antimetabolites, and cancer chemotherapeutic drugs
- Alcohol in excess
- The application of antiseptic preparations containing iodine (povidone/iodine mixture)

Dietary iron supplements **will not** produce false-positive test results with Hemoccult® SENSA® tests. Acetaminophen is not expected to affect test results.

Substances which can cause false-negative test results:

- Ascorbic acid (vitamin C) in excess of 250 mg per day
- Excessive amounts of vitamin C enriched foods, citrus fruits and juices
- Iron supplements which contain quantities of vitamin C in excess of 250 mg per day

5. When you have collected all the samples during the three days:

1. When all samples have been collected, enclose the two minigrip bags (ColonView sampling vials in ONE, and HemoccultSENSA samples in ANOTHER), into the SAMPLE DELIVERY BOX. Enclose also the filled **Personal Data Form** in the box.
2. Close the DELIVERY BOX and bring it to the local post office during the same day when the last samples were collected, or latest by the next day. **NOTICE, the delivery box (postage prepaid) MUST be mailed from a Post Office and not left into a common Mail Box.**

6. NOTICE:

The names of all participants in this study, the test results as well as the information in the patient data collection form are strictly confidential, and will be used for research purpose only, collectively without patient identification.

ANNEX 3.

PATIENT INFORMATION SHEET

Respected patient,

You have been scheduled an appointment for colonoscopy in your hospital. In this context, we kindly ask you to participate in a research project where we compare two different commercially available diagnostic tests for detection of fecal occult blood, used in diagnosis of different gastrointestinal disorders. A company from Finland, Biohit Oyj has developed a new-generation immunological test for fecal occult blood (FOBT), which is likely to be more accurate than the tests in current use and based on different testing strategy. This new test is called Biohit ColonView quick test 30, consisting of two components, i.e., detection of Hb and Hb/Hp complex. In this new study, we intend to compare this new test with the most sensitive existing test (Hemoccult®SENSA®), currently used for fecal occult blood testing in this country.

The performance of the two tests is evaluated by comparing their results with the findings in the colonoscopy that you will soon undergo. We assure you that while evaluating the test results, your colonoscopy results are treated fully anonymously and confidentially, including all other information of your health that is possibly related to detection of fecal occult blood.

If you agree to participate, we kindly ask you to take samples of stools in three different days, for both the ColonView että HemoccultSENSA tests, following the simple instructions that you will receive. It is important to take the samples **before** you drink the liquid that makes the bowel empty for colonoscopy, and deliver the samples by mail to the test laboratory. Your colonoscopy will be performed as scheduled, and taking the stool samples does not interfere with this examination in any way.

You have the full right to refuse participating in this study, and it does not affect your colonoscopy examination or your eventual other tests or future treatments. This stool examination does not cost you anything, and you will receive the test results to your home address within a few months.

To make your participation possible, we need your written consent (below). Thank you very much for your valuable contribution to this study!

Consent to participate:

I have been explained the purpose and goals of this study, including my own contribution in it. I hereby agree to provide the required stool samples for this study, and permit their use for research purposes. I also give my consent to use all such information of my health that is potentially useful for the study purposes and its goals, however, in the way that my personal information will not be disclosed at any stage to anybody outside the research group conducting this study.

_____ _____

Date and Place: Patient's Signature Name

_____

Patient's Social Security Number

Consent received by:

_____

Signature Name

ANNEX 4.

PATIENT DATA COLLECTION FORM

Data item	Response	Options
General:		
Age		Years
Gender		1=Female; 2=Male
Height		in centimeters
Weight		in kilograms
BMI		Body Mass Index (to be calculated)
Origin		1=Native; 2=Descendant of native; 3=Descendent of immigrant; 4=Immigrant
If 3 or 4, from where?		Name the country
History:		
Family history of CRC		1=Yes; 2=No
Family history of FAP		1=Yes; 2=No
Family history of HNPCC		1=Yes; 2=No
Family history of other cancer		1=Yes; 2=No
If yes, please define		Which cancer, in whom, when, outcome….?
Past history of polyps/adenomas		1=Yes; 2=No
If yes, define		when, where, how many, size, treatment?
Inflammatory Bowel Disease		0=None; 1=Crohn's disease; 2=Ulcerative colitis
If 1 or 2, define		How long, treatment, controls?
Any (other) systemic disease		Please define
Life-style factors:		
Obese		1=Yes; 2=No
Sedentary lifestyle		1=Yes; 2=No
Smoking status		Smoker: 0=never; 1=past; 2=current
Alcohol consumption		0=No alcohol; 1=social drinker; 2=regular; 3=abuse
Dietary habits:		
Balanced diet		1=Yes; 2=No
Unbalanced diet		1=Yes; 2=No
Rich in		1=fats, 2=meat, 3=fish
Poor in		1=fruits; 2=vegetables; 3=fibers
Symptoms:		
Abdominal pain		1=Yes; 2=No
Flatulence		1=Yes; 2=No
Obstipation		1=Yes; 2=No
Diarrhea		1=Yes; 2=No
Recent changes in bowel habits		1=Yes; 2=No
Rectal bleeding or blood in stools		0=never; 1=a few times per month; 2= on weekly basis; 3=daily (this is **exclusion criteria**)
Regular Medication:		
Use of mini-ASA or NSAID >3 times per week?		1=Yes; 2=No
Regular use of other medicines		List

Clinical findings:		
Haemorrhoids		1=external hemorrhoids; 2=internal hemorrhoids; 3=anal bleeding at examination
Diverticles		1=single lesions in left colon, 2=single lesions throughout colon, 3=numerous lesions in left colon, 4=numerous lesions throughout colon
Angiectasies		1=single lesions in left colon, 2=single lesions throughout colon, 3=numerous lesions in left colon, 4=numerous lesions throughout colon; 5=bleeding angiectasia
Inflammatory bowel disease		Localization and grading following normal colonoscopy practice
Polyps:		
Number		Total number
Size		Minimum to maximum
Location		1=Right colon; 2=Transverse colon; 3=Left colon; 4=Sigma; 5=Rectum; 6=Multiple site, list
Tumors:		
Number		Total number
Size		Minimum to maximum
Location		1=Right colon; 2=Transverse colon; 3=Left colon; 4=Sigma; 5=Rectum; 6=Multiple site, list
Visible bleeding on colonoscopy		1=Yes; 2=No
Colonoscopy findings explain positive FOBT/FIT result (endoscopist's judgment)		1=Yes; 2=No
Liquid used for emptying the bowel		Name of liquid used
Other observations		

ANNEX 5.

PERSONAL DATA FORM

This form will be filled on the occasion of colonoscopy. This form will be annexed to the colonoscopy report and stored in the patient file (blinded to the test laboratory).

First name: _____

Family name: _____

Social Security Number: _____

Telephone: _____

Address: _____

_____

_____

ANNEX 6.

SAMPLE PROCESSING OF Biohit ColonView quick test IN THE LABORATORY

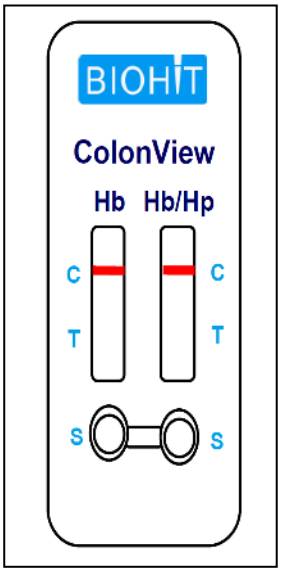
1. The Test Cassette and the Sample Collection Tube containing the stool sample should be brought to room temperature (20 ... 30°C) at least 10 minutes before testing.

2. Take the required number of Test Cassettes from the foil packaging only immediately before performing the test. Mark the Test Cassette with the name of the patient or with another form of identification.

3. Carefully shake the Sample Collection Tube to ensure that the stool sample mixes properly with the saline solution.

4. Take a paper towel and break the seal of the Sample Collection Tube with rotary motion or use a pair of scissors to cut the seal. Hold the collection tube upright and add 3 drops of the solution into both round sample windows (S) of the Test Cassette.

5. Read the results after 5 minutes. Strongly positive results may be evaluated even sooner. Evaluate the result within a maximum of 15 minutes.



Quality Control or Internal Procedural Control

The test also contains a procedural control. A colored line that appears in the control region (C) shows that each test is performed correctly. It is not unusual that the background turns slightly yellowish in color during the testing, depending on the color of the stool sample. This is acceptable, as long as evaluation of the test results is not adversely affected.

A clear background in the observation window is considered an internal negative control. However, when the stool samples are tested, the background may appear slightly yellowish due to the original color of the stool samples. This is acceptable as long as it does not interfere with the interpretation of test result. The test is invalid if the background fails to clear and obscures the reading of the result.

Evaluation of the test results

Test result is evaluated as "positive", if two lines appear.

Positive: 2 pink-colored lines appear; one in the control region (C) and one in the test region (T) in either Hb and/or Hb/Hp tests. If you are testing strongly positive samples, the intensity of the control line may be reduced. It is not recommended to compare the intensity of the lines.

Negative: 1 pink-colored line appears in the control region (C) only, in either Hb and/or Hb/Hp tests.

Invalid: If no red line appears in the control (C) region in either Hb and/or Hb/Hp tests, this is a sign that the test is not functioning properly, or that the test materials are not correct. In this case, repeat the test with a new Test Cassette or contact the manufacturer for technical support.

ANNEX 7

SAMPLE PROCESSING OF Hemoccult®SENSA® TEST IN THE LABORATORY

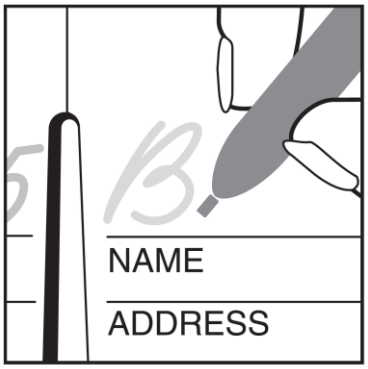
Developing the Test

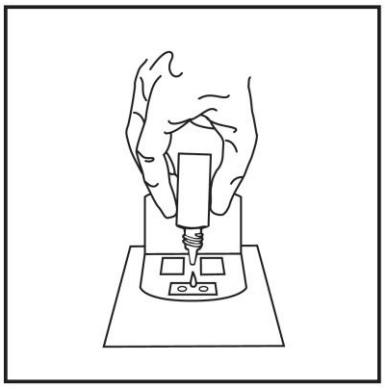
• Slides are best developed no sooner than three days after sample application to allow for degradation of any fruit and vegetable peroxidases that may be present in the fecal sample. However, if immediate testing is required, wait 3 to 5 minutes before developing.

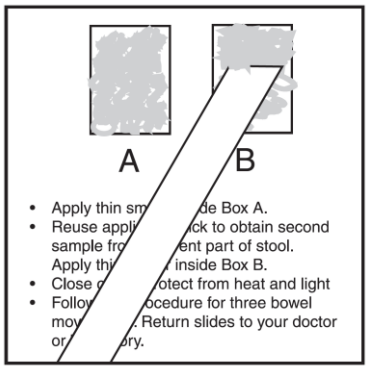
• Open back of slide and apply two drops of Hemoccult® SENSA® Developer to guaiac paper directly over each smear.

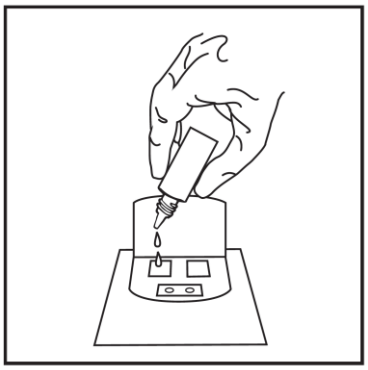
• **Read results within 60 seconds.**

Any trace of blue on or at the edge of the smear is positive for occult blood.









Developing the Performance Monitor® Feature (Quality Control)

• The Performance Monitor® areas must be developed on every slide.

• Apply **one drop** of Hemoccult® SENSA® Developer between the positive and negative Performance Monitor® areas.

• **Read results within 10 seconds.**

If the slide and developer are functional, a blue color will appear in the positive Performance Monitor® area and no blue will appear in the negative Performance Monitor® area.

• Neither the intensity nor the shade of the blue from the Positive Performance Monitor® area should be used as a reference for the appearance of positive test results.

• Any blue originating from the positive Performance Monitor® area should be ignored when reading the sample test results.

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