

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

GENEOSCOPY, INC.,
Petitioner,

v.

EXACT SCIENCES CORPORATION,
Patent Owner.

Case No.: IPR2024-00459
U.S. Patent No. 11,634,781

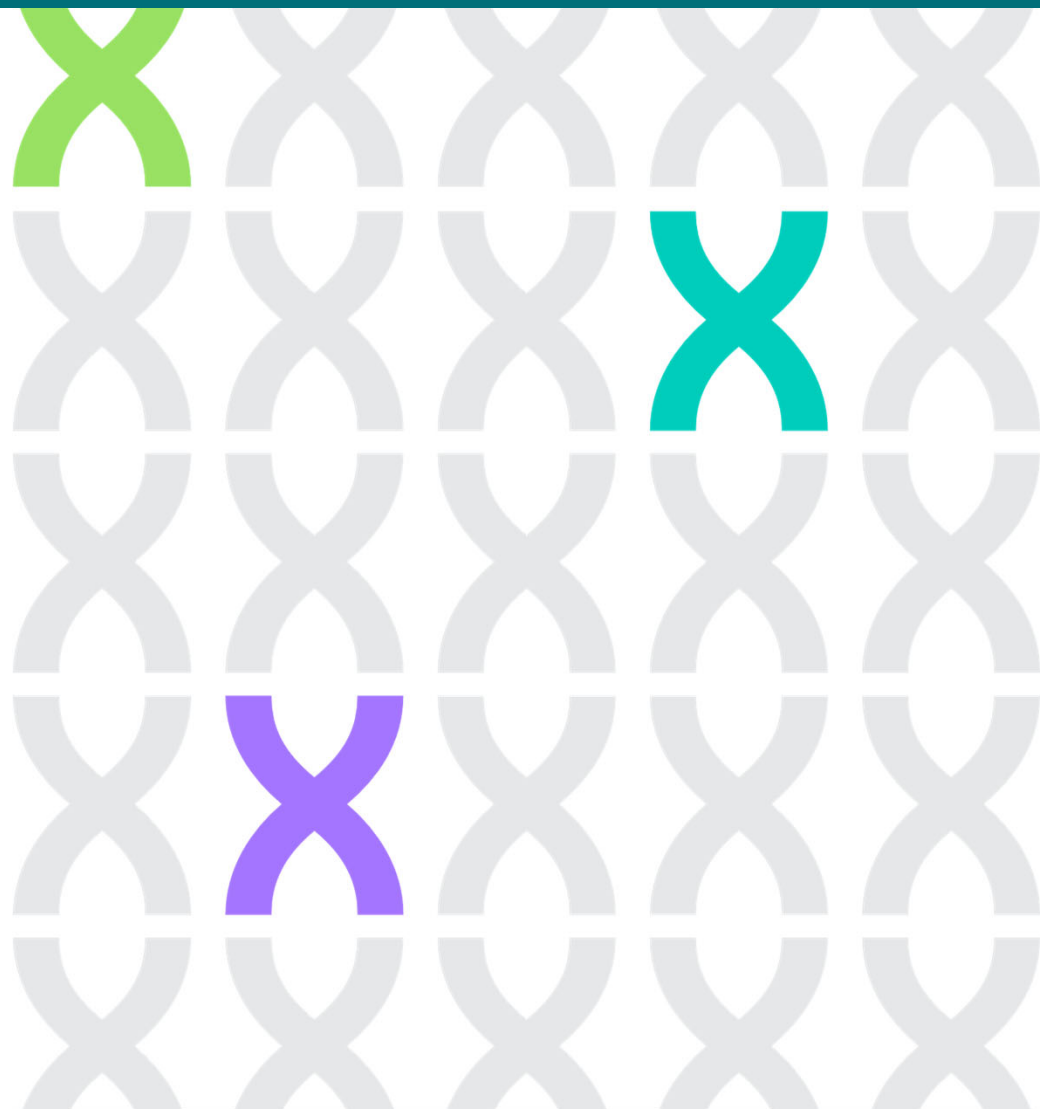
PATENT OWNER'S DEMONSTRATIVES



Geneoscopy, Inc. v. Exact Sciences Corp.
IPR2024-00459
U.S. Patent No. 11,634,781

Patent Owner Exact Sciences' Demonstratives

Hearing: May 1, 2025



Grounds

Ground	Challenged Claims
I. Lenhard, Vilkin, and Itzkowitz	1-9, 11, 14-20
II. Lenhard, Vilkin, Itzkowitz, and Kanaoka	12 and 13
III. Lenhard, Vilkin, Itzkowitz, and Derks	10
IV. Shuber and Vilkin	1-9, 11, 14-20
V. Shuber, Vilkin, and Kanaoka	12 and 13
VI. Shuber, Vilkin, and Derks	10

Summary Of Disputed Issues

Geneoscopy Failed To Meet Its Burden To Show Motivation To Combine

- **Lenhard concluded DNA-only was preferred approach**
 - Single DNA marker was more sensitive than DNA + FOBT combination
 - Authors did not conclude DNA + FOBT combination was worth pursuing
- **Vilkin iFOBT alone was already 100% sensitive for CRC**
 - No motivation to go backward
- **Itzkowitz teaches reducing complexity/cost with two DNA markers already more sensitive than Lenhard DNA+FOBT**
 - No motivation to increase complexity for worse sensitivity
- **No “art-recognized” strategy to modify Shuber with Vilkin**
- **No showing of motivation to use two containers with two buffers**

Law Requires Consideration Of Entirety Of The Art

- **No possible CRC sensitivity improvement over Vilkin's 100%**
- **No expectation of improvement in detection of advanced adenomas**
- **Proposed combination of DNA + FOBT test would:**
 - Be more complex and costly than test for single class of markers
 - Require conflicting collection methodologies
 - At-home patient interaction with stool and undesirable refrigeration of multiple samples vs. lab collection for immediate processing
 - Reduce specificity leading to unnecessary colonoscopies and decrease patient trust and acceptance

Strong Objective Indicia Show Geneoscopy's Hindsight Bias

- **No dispute that:**
 - Cologuard practices the claims
 - Cologuard is a commercial success
 - Others failed in attempting to develop a non-invasive CRC screening test with sufficient diagnostic accuracy that is acceptable to patients
 - Cologuard met the long-felt but unmet need for such a CRC screening test
 - Industry has praised Cologuard
- **Geneoscopy's only argument—lack of nexus—fails**
 - Cologuard success results directly from claimed invention
 - No evidence other patents are relevant to Cologuard success
 - No evidence marketing is responsible for Cologuard success

Without Claims As Roadmap, No Reason To Selectively Focus On Portions Of Art



1. A method of processing a freshly-collected fecal sample **without freezing**, the method comprising:
 - a) collecting a fecal sample from a human subject, wherein the fecal sample is **collected at home** by the human subject by **defecation directly into a sealable collection vessel**;
 - b) **removing a portion** of the fecal sample to a separate sealable container to produce a **removed portion** and a **remaining portion** of the fecal sample;
 - c) combining the **removed portion** of the fecal sample in the separate sealable container with a **buffer that prevents denaturation or degradation of blood proteins found in a fecal sample**, and sealing the sealable container; and
 - d) combining the **remaining portion** of the fecal sample in the sealable collection vessel **with a stabilizing buffer**, and sealing the sealable collection vessel.

EX1001at 45:21-37

3. A method of processing a fecal sample, the method comprising:
 - a) obtaining a pair of portions of a fecal sample collected from a human subject, the pair of portions comprising:
 - i) a **sealed sealable container containing a removed portion** of a fecal sample and a buffer; and
 - ii) a **sealed sealable collection vessel containing a remaining portion** of a fecal sample and a stabilizing buffer,
 the pair of portions **obtained by the method of claim 1**;
 - b) testing the **removed portion** of the fecal sample for an amount of a blood protein present in the **removed portion**;
 - c) extracting nucleic acid from the **remaining portion** of the fecal sample; and
 - d) testing the nucleic acid for an amount of a human nucleic acid.

EX1001at 45:45-61

Geneoscopy Ignores Key Characteristics Of A CRC Screening Test

Comparative evaluation of a new bedside faecal occult blood test in a prospective multicentre study

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These authors contributed equally to this study.

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SUMMARY

Background Faecal occult blood testing is an established method of colorectal neoplasia screening. Guaiac-based tests are limited by poor patient compliance, low sensitivity, specificity and positive predictive value. Newer immunochemical-based tests, accurate but tedious, require a well-established laboratory set up. There is need for simpler immunochemical tests that can be performed at the out-patient clinic.

Aim

To compare the performance characteristics of a new bedside immunochemical test strip device with a sensitive Guaiac-based and established immunochemical test for detection of faecal occult blood in patients undergoing colonoscopy.

Methods

A total of 389 consecutive patients from four centres who were referred for colonoscopy also provided the stool samples for detection of occult blood without dietary restrictions. Stool tests performed were (i) Guaiac-based, (ii) immunochemical enzyme-linked immunosorbent assay and (iii) bedside immunochemical strip test.

Results

At the optimal threshold level, the sensitivity and specificity of the bedside immunochemical strip test for detection of significant colorectal neoplasia (adenomas >1.0 cm and carcinomas) were 60% and 95%, respectively.

Conclusions

This bedside immunochemical strip test proved to be a simple, convenient, non-combustible and accurate tool with similar performance characteristics for detection of any bleeding lesion including colorectal neoplasia when compared with an established immunochemical faecal occult blood test.

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Geneoscopy Exhibit 1029, Page 1

CRC a lucrative health goal. An ideal screening test must be non-invasive, acceptable to patients, simple, have high sensitivity and specificity, inexpensive, cost-effective, easy to implement across a large population and should be effective in reducing morbidity and mortality from CRC in the screened population.⁶

EX1029 at 2: Sur-Reply at 3

Geneoscopy Failed To Meet Its Burden To Show Motivation To Combine Or Modify References

Lenhard: Geneoscopy's Main Obviousness Reference Grounds I-III

Analysis of Promoter Methylation in Stool: A Novel Method for the Detection of Colorectal Cancer

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needed. We explored the potential of a single epigenetic marker in comparison with fecal occult blood testing (FOBT) for the discrimination of patients with CRCs and adenomas from those without. Methods: Methylation-

EX1004 at 1; POR at 8

- Goal: To **compare** results of a single DNA marker to FOBT, **not combine** them

Lenhard: Conclusion

In summary, this study shows HIC1 as a sensitive and specific marker well suited for stool-based CRC screening. Combination of HIC1 with a few additional sensitive and specific methylation markers may allow for the highly sensitive and specific stool-based detection of CRCs and adenomas. Evaluation of other genes known to be methylated in CRC such as hMLH1⁵⁴ and HPP1,⁵⁵ identification of other useful methylation markers, prospective screening studies, as well as further simplifications and automatization of the assays used, clearly are indicated.

EX1004 at 7; POR at 27; EX2022 at 67, 103

► Lenhard's conclusion was to combine multiple DNA markers, **NOT** to combine DNA and protein

POR at 12; EX2022 (Backman Dec.), ¶ 67

Analysis of Promoter Methylation in Stool: A Novel Method for the Detection of Colorectal Cancer

KONSTANZE LENHARD,* GUIDO T. BOMMER,* SILKE ASHUTAY,* ROLF SCHAUER,*

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Background & Aims: Detection of tumor-derived DNA alterations in stool is an intriguing new approach with high potential for the noninvasive detection of colorectal cancer (CRC). Because of heterogeneity of tumors, many multiple markers distributed throughout the human genome need to be analyzed. This is labor intensive and does not allow for high throughput screening. Therefore, markers with high sensitivity and great specificity are needed. We explored the potential of a single epigenetic marker in comparison with fecal occult blood testing (FOBT) for the discrimination of patients with CRCs and adenomas from those without. Methods: Methylation-specific polymerase chain reaction (PCR) was performed to analyze hypermethylated in cancer 1 (HIC1) promoter methylation status in a blinded fashion in stool samples from 26 patients with CRC, 13 with adenoma >1 cm, 9 with hyperplastic polyps, 9 with chronic inflammatory bowel disease, and 32 with endoscopically normal colons. Results: Ninety-seven percent of the stool samples contained amplifiable DNA. Forty-two percent of the samples from patients with CRC and 32% of the samples from patients with colorectal adenoma >1 cm were positive for HIC1 promoter methylation. No methylated HIC1 promoter DNA was detected in the stool DNA from patients with endoscopically normal colon or hyperplastic polyps. Conclusions: The epigenetic marker HIC1 promoter methylation carries high potential for the remote detection of CRCs. We predict that a panel of merely a few genetic and epigenetic markers will be required for the highly sensitive and specific detection of CRCs and adenomas in fecal samples from affected patients.

Colorectal cancer (CRC) is one of the leading causes of cancer-related morbidity and mortality.¹ About 50% of patients die within 5 years of being diagnosed. This mainly is attributable to late presentation with locally advanced or metastatic disease in one third of cases, precluding curative surgery.² The majority of CRCs follow the adenoma-carcinoma sequence, requiring a time period of usually more than 10 years.³ Detection

of early disease and preinvasive adenomatous lesions leads to a decrease of CRC-related mortality.⁴⁻⁷ Therefore, there is a strong rationale for screening programs. Sigmoidoscopy, colonoscopy, and fecal occult blood test (FOBT) alone or in combination with endoscopy, are recommended for screening of the average-risk population.⁸ However, FOBT, when applied regularly, can decrease CRC mortality by only 15%-19%.⁹ Compliance with endoscopic screening is not satisfactory. Overall, in 1998, only 37% of eligible adults in the United States had been screened for CRC in the previous 3 years.¹⁰

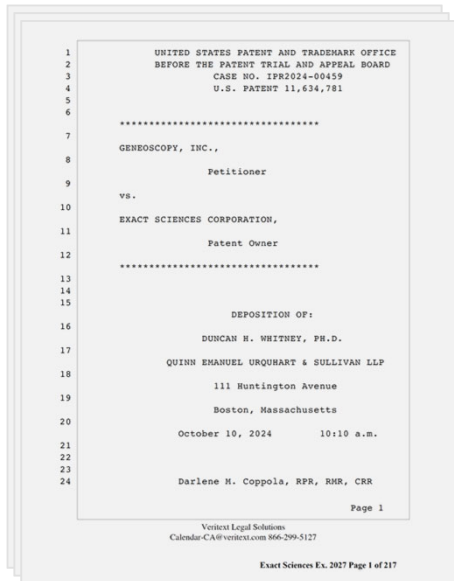
Detection of tumor-derived genetic changes in stool is a promising new approach for CRC screening. Studies published so far focus on the detection of mutations in oncogenes,¹¹⁻¹³ and tumor-suppressor genes,¹⁴⁻¹⁷ as well as changes in microsatellite markers.¹⁸⁻²⁰ Two recent studies required the analysis of up to 15 different genetic markers²¹ or 144 oligomers from each stool²² for the detection of 91% and 63% of cancers and 82% and 50% of adenomas >1 cm, respectively. This precludes their application for routine high throughput screening owing to high labor intensity and costs. The identification of new markers with high sensitivity and specificity is needed. So far, little attention has been paid to DNA hypermethylation as a potential stool marker for CRC.

Methylation of CpG islands of promoters leads to silencing of transcription of the affected gene.²³⁻²⁵ Methyltransferase polymerase chain reaction (PCR) (MSP)²⁶ has been used successfully to detect DNA methylation in primary tumors and in various body fluids.²⁷ The feasibility of amplification of methylated

Abbreviations used in this paper: FOBT, fecal occult blood test; HIC1, hypermethylated in cancer 1; MSP, methylation-specific PCR; PCR, polymerase chain reaction.

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DOI: 10.1053/j.gastro.2003.05.024

Geneoscopy's Expert: Lenhard's Conclusion Is To Use Only DNA Markers



Q. So Lenhard's conclusion is to suggest a panel of a few genetic and epigenetic markers, correct?

A. That's what he has written under the "Conclusion" section.

EX2027 (Whitney 10/10/24 Dep. Tr.) at 41:13-18

Q. Lenhard does not include a suggestion to combine HIC1 with FOBT in this conclusory paragraph . . . of his article?

A. In that paragraph, he does not mention FOBT.

EX2027 (Whitney 10/10/24 Dep. Tr.) at 57:10-17

Q. There is no reference to a blood protein test in the conclusions of the Lenhard abstract, correct?

A. Again in the two sentences that are described under the "Conclusions" part of the abstract to describe this whole study, it does not have that.

EX2054 (Whitney 1/9/25 Dep. Tr.) at 131:14-20

Lenhard: Single DNA Marker Was More Sensitive Than Combined Markers



been reported.^{26,27} In one study evaluating several potentially methylated genes, SFRP2, SFRP5, and PGR were found to be methylated differentially in the stool of patients with CRC.²⁷ Of these markers, SFRP2 was found to be the most sensitive fecal methylation marker, detecting 77%–90% of CRCs. However, specificity of SFRP2 methylation was quite poor, at 77%. Therefore, identification of both sensitive and highly specific methylation markers is required before MSP can be evaluated as a stool-based screening procedure for CRC in prospective studies.

EX1004 at 2; POR at 12, 26; EX2022 at 98

Vilkin: iFOBT Alone Already Achieves 100% Sensitivity For CRC

Performance Characteristics and Evaluation of an Automated-Developed and Quantitative, Immunochemical, Fecal Occult Blood Screening Test

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¹Gastroenterology Department, Rabin Medical Center, Beilinson Hospital, Petach Tikva; ²Tel Aviv University Medical School, Tel Aviv, Israel; and ³Gastroenterology Unit, Clalit Health Services, Tel Aviv, Israel

OBJECTIVES: Quantitative fecal occult blood colorectal cancer (CRC) screening tests (iFOBT) are limited for low sensitivity and nonspecificity for human hemoglobin (Hb). Automated-developed, immunochemical, human Hb iFOBT (iFOBT) is specific, eliminates diet restrictions, and the quantitative allows selection of a threshold for colonoscopy. Aims were to determine 1) test reproducibility; 2) test stability; 3) interoperator daily iFOBT variation; 4) test sensitivity and specificity for neoplasia in 100 symptomatic high-risk patients undergoing colonoscopy; and 5) to correlate fecal Hb measurements with findings.

METHODS: The desktop instrument OC-Sensor (Eiken, Japan) automatically develops and quantitates 50 tests, 10 for Hb. Patients prepared three tests, which were quantified and then 1) repeatedly re-examined; 2) stored at 4°C or 20°C or 28°C and repeatedly examined; and 3) fecal Hb levels were correlated with colonoscopy findings.

RESULTS: Five iFOBTs re-examined five times in 1 day had no significant measurement changes. Thirty tests stored for 21 or more days had a decay rate of 0.3% ± 0.4 at 4°C (NS), 2.2% ± 1.7 at 20°C (NS), and 3.7% ± 1.4 at 28°C (p < 0.05). There were no significant variations between the three daily iFOBTs (NS). At the recommended 100 ng Hb/mL threshold, all six cases of CRCs and 20 out of 28 cases of advanced adenomas were detected. Evaluated together their sensitivity and specificity were 76.5% and 95.3%.

CONCLUSIONS: Desktop, automated-developed, quantitative iFOBT is now available. Replagated OC-Sensor samples are stable for 21 days, easy to prepare and develop and, at the 100 ng Hb/mL threshold, have high sensitivity, specificity, and negative predictive values for significant neoplasia. Suitability for population CRC screening awaits further evaluation.

(Am J Gastroenterol 2005;100:2519–2525)

The standard guaiac fecal occult blood test (g-FOBT) is limited for its low sensitivity for significant colorectal neoplasia (colorectal cancer (CRC) and advanced adenomatous polyps (AAP)), and low specificity due to nonspecificity for human hemoglobin (Hb). It is also limited by the possibility of inaccurate development and evaluation by inadequately trained personnel (1–4).

The introduction of central laboratory and office-developed, immunochemical, fecal occult blood tests (i-FOBT) specific for human Hb improved specificity (5–11). However, for the office-developed tests, the i-FOBT manufacturers have predetermined the degree of sensitivity for Hb, and the occasional experience with more complex office-developed tests is not conducive for large-scale screening and maintaining quality control (1–4, 8, 11).

The automated-developed and quantitative i-FOBT is human Hb specific, eliminates the need for diet restrictions, and the Hb quantification allows selection of a variable threshold level for follow-up colonoscopy (5–7, 9–12).

The introduction of such methodology, for large-scale screening, requires evaluation of the instrument and its performance characteristics. In addition, we need to evaluate its true sensitivity and specificity for colorectal neoplasia versus colonoscopy in different clinical settings and determine which level of fecal Hb should trigger follow-up colonoscopy.

Specific aims were to determine 1) test-developed reproducibility; 2) effect of temperature and duration of storage on fecal sample stability; 3) interoperator daily i-FOBT test variation; 4) sensitivity and specificity for neoplasia found

Table 3. Sensitivity for Significant Colorectal Neoplasia (N = 34)* for Each I-FOBT† Prepared

I-FOBT Positive	Cancer (%)	Cancer + AAP (%)
Sample 1	83.3	52.9
Sample 2	66.7	52.9
Sample 3	100	61.8
Samples 1 and 2	83.3	64.7
Samples 1, 2, and 3	100	76.5

*Six colorectal cancers (CRC) and 28 advanced adenomatous polyps (AAP).

†At the 100 ng Hb/mL threshold.

EX1005 at 7; POR at 14; Sur-Reply at 2

Petitioner's Combinations Defy Logic

Vilkin

iFOBT only – **100% for CRC**

Table 3. Sensitivity for Significant Colorectal Neoplasia (N = 34)* for Each I-FOBT† Prepared

I-FOBT Positive	Cancer (%)	Cancer + AAP (%)
Sample 1	83.3	52.9
Sample 2	66.7	52.9
Sample 3	100	61.8
Samples 1 and 2	83.3	64.7
Samples 1, 2, and 3	100	76.5

* Six colorectal cancers (CRC) and 28 advanced adenomatous polyps (AAP).

† At the 100 ng Hb/mL threshold.

EX1005 at 7; POR at 14, 29

Itzkowitz

Nucleic acid only – **87.5% for CRC**

72.5% and 86.9%, respectively. The optimal combination of vimentin methylation plus DIA resulted in **87.5% sensitivity** and 82% specificity; cancers were detected regardless of stage or location. False-positive vimentin methylation was associ-

EX1006 at 1; POR at 15, 29

Lenhard

HIC1/gFOBT – **65% for CRC**

89.9%, $P = .102$). The combination of both assays resulted in increased detection rates for **CRCs (65%; 95% CI, 44%–83%)**. Although the combined test detected all localized cancers, no increase in the sensitivity for adenoma was seen.

EX1004 at 6; POR at 12, 29

- A POSA would not go back to a worse combination test (Lenhard); they would stick to the already more accurate single-marker-category tests (Itzkowitz and Vilkin)

No Reason To Choose Vilkin iFOBT Over A High-Sensitivity gFOBT



Based on data from these 6 studies, it appears that there are no clear patterns of superior performance in overall test performance between a high-sensitivity guaiac-based test (Hemoccult SENSA) and a variety of FIT.

EX1013 at 7; POR at 36

Itzkowitz: Using Only Two DNA Markers Reduces Assay Complexity

Improved Fecal DNA Test for Colorectal Cancer Screening

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Background & Aims: Fecal DNA testing has shown greater sensitivity than guaiac-based assays blood tests for noninvasive colorectal cancer (CRC) screening. The prototype assay (version 1), which analyzed 22 gene mutations and DNA integrity assay (DIA), showed a sensitivity of 52% for CRC detection and a specificity of 94% in average-risk individuals. The present study was conducted to determine the sensitivity and specificity of a second-generation assay (version 2) that uses improved DNA extraction/analysis techniques and a new promoter methylation marker. **Methods:** Forty patients with CRC and 122 subjects with normal colonoscopy provided stool samples to which DNA preservation buffer was added immediately. DNA was purified using gel-based capture, and analyzed for the original panel of 22 mutations, DIA, and 2 new promoter methylation markers. **Results:** By using DNA that was optimally preserved and purified from stool, the sensitivity of the prototype version 1 assay increased to 73.5% because of enhanced performance of DIA. Vitaminin gene methylation alone provided sensitivity and specificity of 72.5% and 86.5%, respectively. The optimal combination of vitaminin methylation plus DIA resulted in 87.5% sensitivity and 87% specificity; centers were detected regardless of stage or location. False-positive vitaminin methylation was associated with older age. **Conclusions:** An improved fecal DNA test that incorporates only 2 markers shows much higher sensitivity for CRC. The new assay is easier to perform and should be less costly, thereby facilitating its use for noninvasive CRC screening.

Screening for colorectal cancer (CRC) is arguably the most effective intervention for preventing any cancer. Unfortunately, despite the recommendations of all major medical societies, fewer than half of eligible individuals older than age 50 have undergone CRC screening.¹⁻³ In the United States, colonoscopy is being used increasingly as a primary screening tool because of its excellent diagnostic accuracy and ability to remove precancerous and early cancerous lesions. However, the availability of an accurate, noninvasive screening test might increase compliance with CRC screening guidelines by individuals who are reluctant to undergo more invasive tests, or situations in which colonoscopy screening is not feasible or readily available.

Several studies have shown the feasibility of detecting colon tumor-specific products in the stool.⁴⁻⁶ The markers in these studies represent alterations of genes involved in the predominant chromosomal instability pathway (such as APC, p53, and

Economic models based on the prototype version 1 assay have provided various scenarios whereby fecal DNA testing could become comparable with colonoscopy with respect to cost effectiveness.^{2,4} Our results suggest that with only 2 markers (vimentin+DY) providing a sensitivity of 88%, the assay complexity can be reduced significantly, thereby decreasing cost and facilitating the development of an assay kit that could be managed by local clinical laboratories. At the present time, fecal

EX1006 at 7; POR at 28-29

- Itzkowitz teaches benefit of only two DNA markers, not combining different classes of markers with different collection processes and different buffers

Itzkowitz: Small Number Of Markers From Same Class Reduces Cost/Management



ated with older age. **Conclusions:** An improved fecal DNA test that incorporates only 2 markers shows much higher sensitivity for CRC. The new assay is easier to perform and should be less costly, thereby facilitating its use for noninvasive CRC screening.

EX1006 at 1; Sur-Reply at 8-9

cost effectiveness.²⁴ Our results suggest that with only 2 markers (vimentin+DY) providing a sensitivity of 88%, the assay complexity can be reduced significantly, thereby decreasing cost and facilitating the development of an assay kit that could be managed by local clinical laboratories. At the present time, fecal

EX1006 at 7; Sur-Reply at 9

- Combining different classes of markers with different collection processes and buffers would be more costly and difficult to manage

Shuber – Geneoscopy's Main Obviousness Reference Grounds IV-VI



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WHITNEY, Duncan, H. [US/US]; 20 Read Road, Sudbury, MA 01776 (US).

EX1009

Shuber: Already Disclosed Up To 98% Sensitivity For Adenomas



adenomas. Therefore, detecting adenomas is particularly useful for identifying early signs or risks of colorectal cancer (e.g., cancerous and precancerous lesions or growths in the colon).

EX1009 at 13:7-8; POR at 18

In one embodiment, a combination of all of the above loci may be used to detect adenomas with a greater than 95% sensitivity (e.g., greater than 98% sensitivity).

EX1009 at 14:15-16

Geneoscopy's Additional References Fail To Show Art-Recognized Strategy

- Geneoscopy cites **three additional** references to piece together an even more complicated purported motivation to combine:
 - Nishikawa includes **one sentence** speculating (without data) that testing for *K-ras* mutation could be combined with FOBT or other DNA tests
 - Kutzner speculates whether DNA and gFOBT (non-quantitative) should be used together; states its data do not allow conclusions on that approach
 - Levin questions “whether **or not**” combining DNA and FOBT would “improve sensitivity **without adversely affecting specificity**”

Ex. 2022 (Backman Dec.), ¶¶ 128-132; POR at 43-46; Sur-Reply at 15-16

Kanaoka



existing fecal occult blood test. More specifically speaking, a method of detecting a tumor marker for diagnosing colon cancer which comprises collecting biological sample which is immediately frozen using liquid nitrogen in some cases, homogenizing the sample in the presence of an inhibitor of an RNA digesting enzyme to give a suspension, extracting RNA from the obtained suspension, subjecting the extracted RNA to reverse transcription to give cDNA, amplifying the obtained cDNA and then detecting the thus amplified cDNA.

EX1007 at Abstract; POR at 16

[0065] COX-2 was detected in 27 cases among the 30 colon cancer cases (caeca: 2/2, ascending colon: 3/5, descending colon: 1/1, sigmoid colon: 7/7, rectum: 12/13; early cancer: 2/3, advanced cancer: 25/27), but was not detected in any of 22 cases in the control group (sensitivity: 90%, specificity: 100%).

[0066] In the immunological fecal occult blood test, 23 among 28 colon cancer cases and 3 among 22 control cases were positive (sensitivity: 82.1%, specificity: 86.3%).

EX1007 at ¶¶ 65-66; POR at 16-17, 49

Geneoscopy Failed To Meet Its Burden To Show Motivation For Two Separate Buffers Or Containers

Itzkowitz: Discourages Manipulating Stool Into Portions

Improved Fecal DNA Test for Colorectal Cancer Screening

STEVEN H. ITZKOWITZ,¹ LINA JANDORF,² RANDALL BRAND,³ LINDA RABENECK,³ PAUL C. SCHROY III,¹ STEPHEN SONTAG,⁴ DAVID JOHNSON,⁴ JOEL SKOLETSKY,^{1,2} KRIS DURKEE,^{1,2} SANFORD MARKOWITZ,^{1,2} AND ANTHONY SHUBERT^{1,2}

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Background & Aims: Fecal DNA testing has shown greater sensitivity than guaiac-based stool tests for noninvasive colorectal cancer (CRC) screening. The prototype assay (version 1), which analyzed 22 gene mutations, achieved a sensitivity of 92% and specificity of 52% for CRC detection and a specificity of 96% in average-risk individuals. The present study was conducted to improve the assay (version 2) that used improved DNA stabilization/isolation techniques and a new promoter methylation assay. **Methods:** Forty patients with colorectal tumors with normal colonoscopy provided stool samples to which DNA preservation buffer was added immediately. DNA was purified using gel-based capture, and analyzed for 22 gene mutations and 10 CpG sites for methylation. **Results:** By using DNA that was optimally preserved and purified from stool, the sensitivity of the assay was improved to 96% and specificity to 92% because of enhanced performance of DNA. Vincentin gel purification also provided sensitivity and specificity of 92.8% and 92%, respectively. The use of a 100% methanol in methylation plus DNA resulted in 87.5% sensitivity and 82% specificity; cancers were detected regardless of stage and tumor size. **Conclusions:** An improved fecal DNA test that incorporates only 2 markers shows much higher sensitivity than guaiac-based stool tests. The assay should be less costly than the current assay and should be less complex.

Katz, the microsatellite instability pathway [30a,26], and markers of abnormal apoptosis. Studies using stool samples from patients already known to have colon cancer, adenomas, or a normal colon report sensitivities of 62%-91% for CRC, 27%-82% for advanced adenomas, and specificities of 93%-96% in individuals with a normal colonoscopy.³⁷ These encouraging data suggest that a large, prospective study of stool samples from 4000 average-risk, asymptomatic individuals older than age 50. The results showed a higher sensitivity for detecting cancer with the fecal DNA test compared with Hemoccult II (fecal occult blood test) (94% vs. 82%, respectively) and with comparable specificity (94.4% vs. 95.2%, respectively). Despite superior sensitivity over Hemoccult II, the prototype fecal DNA test (version 1) revealed lower than expected sensitivity, most owing to a significantly low rate of sensitivity for the DNA mismatch repair (DMM) component of the test. We learned that the suboptimal performance of DNA was a result of DNA degradation during transport of specimens to the laboratory, despite precautions such as immediate chilling of samples and

Since that time, pilot studies have shown that several technical and conceptual advances could improve fecal DNA testing. First, adding a DNA-stabilizing buffer to the stool immediately on defecation was shown to prevent DNA degradation for several days and enhance the performance of DNA. Second, a gel-based DNA capture approach, rather than the original bead-based technology, allowed for enhanced extraction of DNA from stool.³ Finally, promoter methylation has become recognized as a key pathway by which colon cancers develop.

Screening for colorectal cancer (CRC) is arguably the most effective intervention for preventing any cancer. Unfortunately, despite the recommendations of all major medical societies, fewer than half of eligible individuals older than age 50 have undergone CRC screening.¹⁻³ In the United States, colonoscopy is being used increasingly as a primary screening tool because of its excellent diagnostic accuracy and ability to remove precancerous and early cancerous lesions. However, the availability of an accurate, noninvasive screening test might increase compliance with CRC screening guidelines by individuals who are reluctant to undergo more invasive tests, or situations in which colonoscopy screening is not feasible or readily available.

Abbreviations used in this paper: CI, confidence interval; CRC, colorectal cancer; DNA, DNA integrity assay; DY, locus D (5p22) and locus Y (10p11.23); HLTf, Helicase-like Transcription Factor; MSP, methylation-specific PCR.

Several studies have shown the feasibility of detecting colon tumor-specific products in the stool.⁸ The markers in these studies represent alterations of genes involved in the predominant chromosomal instability pathway (such as APC, p53, and

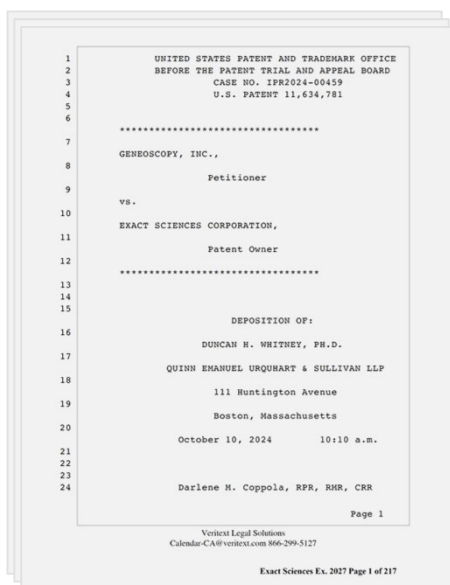
doi:10.1016/j.cgh.2006.10.006

Inherent in the success of any screening test is patient acceptance. Our preliminary experience with the new fecal DNA test in which patients add stabilization buffer to stool confirms a very high degree of patient satisfaction. These findings support results from the previous multicenter study in which subjects preferred fecal DNA testing to both the fecal occult blood test and colonoscopy.¹⁶

Abbreviations used in this paper: CI, confidence interval; CRC, colorectal cancer; DIA, DNA integrity assay; DY, locus D (5p21) and locus Y (LOC911599); HLTf, Helicase-like Transcription Factor; MSP, methylation-specific polymerase chain reaction; NC, normal colonoscopy; PCR, polymerase chain reaction.

EX1006 at 6-7; POR at 35; EX2022 (Backman Dec.), ¶ 114

No Evidence That Two Separate Buffers (Rather Than One) Were Needed



Q. Let me ask again.

Is it -- in your opinion, would it be impossible to form a buffer that would stabilize both nucleic acids and proteins?

A. Again, my opinions are formed by reviewing data which illustrates to me what is possible. I have not viewed data, in my recollection, that would allow me to answer that question.

EX2027 (Whitney 10/10/24 Dep. Tr.) at 148:14-22; POR at 39
(objection omitted)

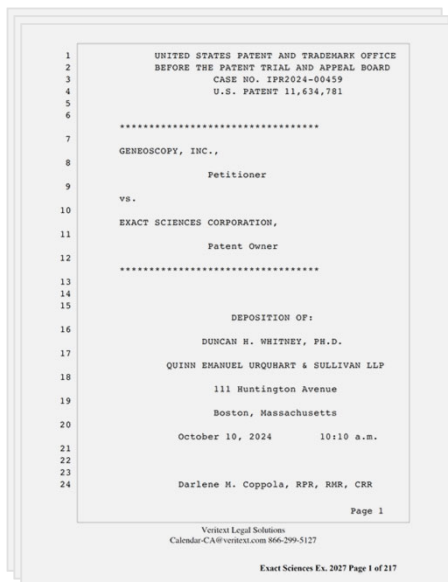
No Evidence That Two Separate Buffers (Rather Than One) Were Needed

Q. You said that part of your opinion would be based on -- is it correct that you do not, as you sit here today, have an opinion on whether it is possible to create a buffer that stabilizes both nucleic acid and blood protein?

A. **As I sit here today, I don't recall a set of experiments that I would refer to to be able to allow me to conclude that there are buffers that could simultaneously stabilize DNA and protein.**

Q. Is it likewise the case that you cannot, as you sit here today, identify anything that would say that you could not create such a buffer?

A. **I'm not forming an opinion on whether or not it's possible.**



EX2027 (Whitney 10/10/24 Dep. Tr.) at 149:18-150:13; POR at 39
(objections omitted)

No Evidence That Vilkin “Hb stabilizing buffer” Would Not Stabilize Nucleic Acid

Performance Characteristics and Evaluation of an Automated-Developed and Quantitative, Immunochemical, Fecal Occult Blood Screening Test

Alon Vilkin, M.D.,¹ Paul Rosen, MB, BS,^{2,3} Zohar Levi, M.D.,³ Amal Wakeel, B.Sc.,¹ Eran Masor, M.D.,² Shimon Birkenfeld, M.D.,² and Yaron Yu, M.D.^{1,3}
¹Gastroenterology Department, Rabin Medical Center, Beilinson Hospital, Petach Tikva; ²Tel Aviv University Medical School, Tel Aviv, Israel; and ³Gastroenterology Unit, Clalit Health Services, Tel Aviv, Israel

OBJECTIVES: Gastric fecal occult blood colorectal cancer (CRC) screening tests (FOBT) are limited for low sensitivity and nonspecificity for human hemoglobin (Hb). Automated-developed, immunochemical, human Hb FOBT (iFOBT) is specific, eliminates diet restrictions, and Hb quantification allows selection of a threshold for colonoscopy. Aims were to determine 1) test reproducibility; 2) test stability; 3) interoperator daily iFOBT variation; 4) test sensitivity and specificity for neoplasia in 100 symptomatic high-risk patients undergoing colonoscopy; and 5) to correlate fecal Hb measurements with findings.

METHODS: The desktop instrument OC-Sensor (Eiken, Japan) automatically develops and quantitates 50 tests. In 10 Hb. Patients prepared three tests, which were quantified and then 1) repeatedly re-examined; 2) stored at 4°C or 20°C or 28°C and repeatedly examined; and 3) fecal Hb levels were correlated with colonoscopy findings.

RESULTS: Five iFOBTs re-examined five times in 1 day had no significant measurement changes. Thirty tests stored for 21 or more days had a decay rate of $0.29\% \pm 0.4$ at 4°C (NS), $2.2\% \pm 1.7$ at 20°C (NS), and $3.7\% \pm 1.4$ at 28°C ($p < 0.05$). There were interoperator variations between the three daily iFOBTs (NS). At the recommended 100 ng Hb/mL threshold, all six cases of CRCs and 20 out of 28 cases of advanced adenomas were detected. Evaluated together their sensitivity and specificity were 76.5% and 95.3%.

CONCLUSIONS: Desktop, automated-developed, quantitative iFOBT is now available. Refrigerated OC-Sensor samples are stable for 21 days, easy to prepare and develop and, at the 100 ng Hb/mL threshold, have high sensitivity, specificity, and negative predictive values for significant neoplasia. Suitability for population CRC screening awaits further evaluation.

(Am J Gastroenterol 2005;100:2519–2525)

The standard guaiac fecal occult blood test (g-FOBT) is limited for its low sensitivity for significant colorectal neoplasia (colorectal cancer [CRC] and advanced adenomatous polyps [AAP]), and low specificity due to nonspecificity for human hemoglobin (Hb). It is also limited by the possibility of inaccurate development and evaluation by inadequately trained personnel (1–4). The introduction of central laboratory and office-developed, immunochemical, fecal occult blood tests (i-FOBT) specific for human Hb improved specificity (5–11). However, for the office-developed tests, the i-FOBT manufacturers have predetermined the degree of sensitivity for Hb, and the occasional experience with more complex office-developed tests is not conducive for large-scale screening and maintaining quality control (1–4, 8, 11).

The automated-developed and quantitative i-FOBT is human Hb specific, eliminates the need for diet restrictions, and the Hb quantification allows selection of a variable threshold level for follow-up colonoscopy (5–7, 9–12). The introduction of such methodology, for large-scale screening, requires evaluation of the instrument and its performance characteristics. In addition, we need to evaluate its true sensitivity and specificity for colorectal neoplasia versus colonoscopy in different clinical settings and determine which level of fecal Hb should trigger follow-up colonoscopy. Specific aims were to determine 1) test-developer reproducibility; 2) effect of temperature and duration of storage on fecal sample stability; 3) interoperator daily i-FOBT test variation; 4) sensitivity and specificity for neoplasia found

Fecal Sampling

The fecal test sampling device is shaped like a small test tube with the fecal probe inserted into it and sealing it. The probe has a serrated tip, which is poked into the stool and then pushed back into the tube, past a scrapper, and through a membrane into the sample cup. These remove most of the excess feces and leave the stool collected (about 10 mg) into the serrations (Fig. 1). The tip is then put in a closed amount of Hb stabilizing buffer. Each sample tube has a unique bar

EX1005 at 4; POR at 38

No Evidence Itzkowitz “DNA stabilizing buffer” Would Not Stabilize Protein



Sample Collection

ning the presurgical bowel preparation. Subjects were given detailed instructions and a special stool collection kit that is mounted on the toilet bowl. Immediately after defecation, subjects added 250 mL of a DNA-stabilizing buffer⁷ to a stool specimen of at least 50 g. Only 10 patients provided less than

EX1006 at 6-7; POR at 35; EX2022 (Backman Dec.), ¶ 114

Separating Sample Would Increase Complexity And Undesirability

Vilkin

temperatures, even when homes are air-conditioned. The double zip-lock bags we provided answered some of the screeners' hesitancy about keeping the prepared test transiently in their home refrigerator. This packaging could be further improved.

EX1005 at 8; Sur-Reply at 11-12

Hoepffner

CRC a lucrative health goal. An ideal screening test must be non-invasive, acceptable to patients, simple, have high sensitivity and specificity, inexpensive, cost-effective, easy to implement across a large population and should be effective in reducing morbidity and mortality from CRC in the screened population.⁶

EX1029 at 2; Sur-Reply at 3

Itkowitz

ated with older age. **Conclusions:** An improved fecal DNA test that incorporates only 2 markers shows much higher sensitivity for CRC. The new assay is easier to perform and should be less costly, thereby facilitating its use for noninvasive CRC screening.

Inherent in the success of any screening test is patient acceptance. Our preliminary experience with the new fecal DNA

EX1006 at 1, 6, Sur-Reply at 8-9; POR at 15

Law Requires Consideration Of Both Advantages And Disadvantages In The Collective Art

Benefits Lost And Gained Must Be Considered

“But immediately after the sentence quoted by HPC, Winner continues: ‘[T]he benefits, both lost and gained, should be weighed against one another’ 202 F.3d at 1349 n.8 (emphasis added). That is consistent with the longstanding principle that the prior art must be considered for all its teachings, not selectively.”

Henny Penny Corp. v. Frymaster LLC, 938 F.3d 1324, 1332 (Fed. Cir. 2019); Sur-Reply 1, 3

“In fact, the prior art could contain one reference suggesting a combination and others critiquing or otherwise discouraging the same. Even a single reference can include both types of statements, and we have held that it is error to fail to consider the entirety of the art.”

Arctic Cat Inc. v. Bombardier Recreational Prods., Inc., 876 F.3d 1350, 1360 (Fed. Cir. 2017); Sur-Reply at 1, 3-4

No Improvements Gained From Combination

Benefit Purportedly Gained	Benefit Not Actually Gained
Potential for increased sensitivity for CRC	No possible improvement over Vilkin's 100% CRC sensitivity. EX1005 at 7; Sur-Reply at 2 No expectation of improvement in detection of advanced adenomas – i.e., preventing cancer. EX1004 at 1, 6; POR at 10-11

Drawbacks Far Outweighed Any Arguable Benefits Gained From Combination

Benefit Purportedly Gained	Drawbacks from Combination
Potential for increased sensitivity for CRC	More costly and more complex than test for single marker class. EX2022 ¶113; POR at 35-36 More patient interaction with stool sample/less patient acceptability than Itzkowitz alone. EX1006 at 6-7; EX2022 at ¶112; POR at 35 Vilkin samples required objectionable at-home refrigeration. EX1005 at 4, 8; POR at 34 Reduced specificity – more false positives leads to more unnecessary colonoscopies. EX1004 at 4; EX2022 at ¶¶ 61-107; POR at 31; see <i>also</i> EX1007 at [0065][0066]; POR at 49

Geneoscopy Ignores Undesirability Of At-Home Separation Of Fecal Sample

Lenhard Discloses Collection At Laboratory, Not At Home

Analysis of Promoter Methylation in Stool: A Novel Method for the Detection of Colorectal Cancer

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THOMAS BRÄULETZ,[†] BURKHARD GÖKE,* ROLF LAMERZ,* and FRANK T. HOLLIG*

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Background & Aims: Detection of tumor-derived DNA alterations in stool is an intriguing new approach with high potential for the noninvasive detection of colorectal cancer (CRC). Because of heterogeneity of tumors, usually multiple markers distributed throughout the human genome need to be analyzed. This is labor intensive and does not allow for high throughput screening. Therefore, markers with high sensitivity and good specificity are needed. We explored the potential of a single epigenetic marker in comparison with fecal occult blood testing (FOBT) for the discrimination of patients with CRCs and adenomas from those without. **Methods:** Methylation-specific polymerase chain reaction (PCR) was performed to analyze hypermethylated in cancer 1 (HIC1) promoter methylation status in a blinded fashion in stool samples from 26 patients with CRC, 13 with adenoma >1 cm, 9 with hyperplastic polyps, 9 with chronic inflammatory bowel disease, and 32 with endoscopically normal colon. Results: Nearly 90% percent of the stool samples contained amplifiable DNA. Forty-two percent of the samples from patients with CRC and 21% of the samples from patients with colorectal adenoma >1 cm were positive for HIC1 promoter methylation. No methylated HIC1 promoter DNA was detected in the stool DNA from patients with endoscopically normal colon or hyperplastic polyps. **Conclusions:** The epigenetic marker HIC1 promoter methylation carries high potential for the remote detection of CRC. We propose that a panel of merely a few genetic and epigenetic markers will be required for the highly sensitive and specific detection of CRC and adenomas in stool samples from affected patients.

Colorectal cancer (CRC) is one of the leading causes of cancer-related morbidity and mortality.¹ About 40% of patients die within 5 years of being diagnosed. This mainly is attributable to late presentation with locally advanced or metastatic disease in one third of cases, precluding curative surgery.² The majority of CRC follow the adenoma-carcinoma sequence, requiring a time period of usually more than 10 years.³ Detection

of early disease and precancerous adenomatous lesions leads to a decrease of CRC-related mortality.^{4,5} Therefore, there is a strong rationale for screening programs. Sigmoidoscopy, colonoscopy, and fecal occult blood test (FOBT) alone or in combination with endoscopy, are recommended for screening of the average-risk population.⁶ However, FOBT, when applied regularly, can decrease CRC mortality by only 15%–35%.^{7–9} Compliance with endoscopic screening is low worldwide. Overall, in 1998, only 37% of eligible adults in the United States had been screened for CRC in the previous 3 years.¹⁰

Detection of tumor-derived genetic changes in stool is a promising new approach for CRC screening. Studies published so far focus on the detection of mutations in oncogenes,^{11–13} and tumor-suppressor genes,^{14–16} as well as changes in microsatellite markers.^{17–19}

Two recent studies required the analysis of up to 13 different genetic markers²⁰ or 144 fragments from each stool²¹ for the detection of 91% and 61% of cancers and 82% and 30% of adenomas >1 cm, respectively. This precludes their application for routine high-throughput screening owing to high labor intensity and costs. The identification of new markers with high sensitivity and specificity is needed. So far, little attention has been paid to DNA hypermethylation as a potential stool marker for CRC.

Methylation of CpG islands of promoters leads to silencing of transcription of the affected gene.^{22–25} Methylation-specific polymerase chain reaction (PCR) (MS-PCR)²⁶ has been used successfully to detect DNA methylation in primary tumors and in various body fluids.²⁷ The feasibility of amplification of methylated

rectal tumors before stool sampling. Samples were received within 10 hours after defecation at the laboratory, aliquoted, and stored at -80°C .

EX1004 at 2; POR at 8; Sur-Reply at 13-14; EX2022 ¶¶55-56

Abbreviations used in this paper: FOBT, fecal occult blood test; HIC1, hypermethylated in cancer 1; MS-PCR, methylation-specific PCR; PCR, polymerase chain reaction.

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1542-3556/05/1503-0000
DOI: 10.1053/j.gastro.2004.08.024X

Vilkin: Petitioner Relies On Results, But Ignores How Authors Got There

Vilkin concludes that they “found this desktop, automated-developed and quantified Hb version of the immunochemical occult blood test to provide a highly sensitive test for detecting significant colorectal neoplasia with an acceptable specificity and consequent high negative predictive value.” EX1005 p.2524.

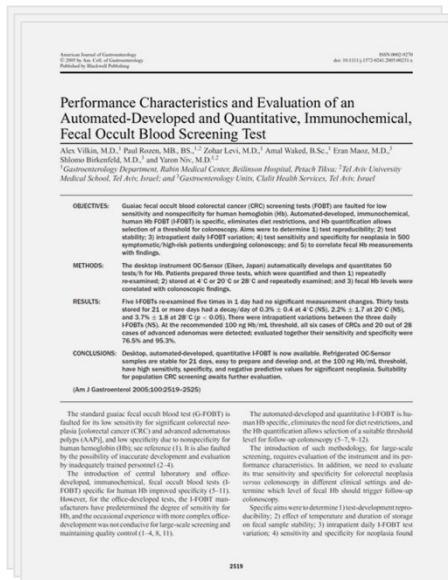
Pet. at 16

Lenhard’s assay. EX1080 ¶57; EX1006 114. Likewise, Vilkin’s disclosure faulting gFOBT like the one used in Lenhard for low sensitivity would motivate a POSA to use Vilkin’s more sensitive iFOBT in its place. EX1080 ¶57; EX1005 2519.

Reply at 7-8

Lenhard’s gFOBT has a demonstrably lower sensitivity (35%) than the iFOBT of Vilkin (65%-79%, depending on the Hb threshold applied). EX1004 146; EX1005

Reply at 13



Patient Acceptance Is Critical

Improved Fecal DNA Test for Colorectal Cancer Screening

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¹Department of Medicine and Oncological Sciences, Mount Sinai School of Medicine, New York, New York; ²Gastroenterology Division, Exton/Jonheworth Healthcare, Exton, PA; ³Florida Sunnybrook Regional Cancer Centre, University of Toronto, Toronto, Ontario, Canada; ⁴Gastroenterology Division, Boston University School of Medicine, Boston, Massachusetts; ⁵Gastroenterology Section, New Veterans Affairs Hospital, New, Bronx; ⁶Gastroenterology Division, Eastern VA Medical School, Norfolk, Virginia; ⁷Exact Sciences Corporation, Marlborough, Massachusetts; ⁸Howard Hughes Medical Institute and Department of Medicine, Case Western Reserve University, Cleveland, Ohio

[illegible][illegible]

Screening for colorectal cancer (CRC) is arguably the most effective intervention for preventing any cancer. Unfortunately, despite the recommendations of all major medical societies, fewer than half of eligible individuals older than age 50 have undergone CRC screening.¹⁻³ In the United States, colonoscopy is being used increasingly as a primary screening tool because of its excellent diagnostic accuracy and ability to remove precancerous and early carcinous lesions. However, the availability of an accurate, noninvasive screening test might increase compliance with CRC screening guidelines by individuals who are reluctant to undergo more invasive tests, or situations in which colonoscopy screening is not feasible or readily available.

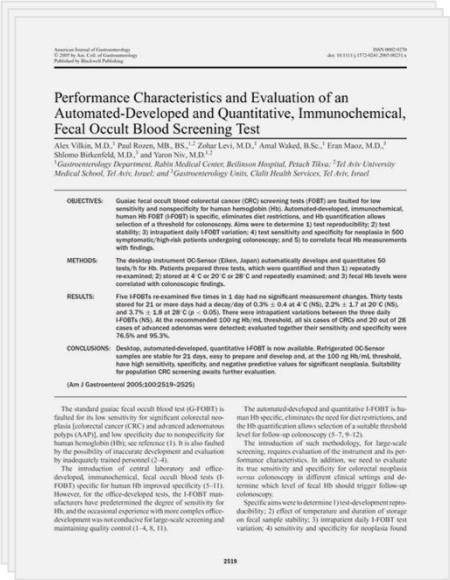
Abbreviations used in this paper: CI, confidence interval; CRC, colorectal cancer; DA, DNA integrity assay; DV, locus D (5q21) and locus Y (10q91.139); HLT, Helicase-like Transcription Factor; MSP, methylation-specific polymerase chain reaction; NC, normal colonoscopy; PCR, polymerase chain reaction.

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1542-3565/07/\$32.00
doi:10.1016/j.jgh.2006.10.006
Gastroscopy Exhibit 1006, Page 1

Inherent in the success of any screening test is patient acceptance. Our preliminary experience with the new fecal DNA

EX1006 at 6; POR at 35

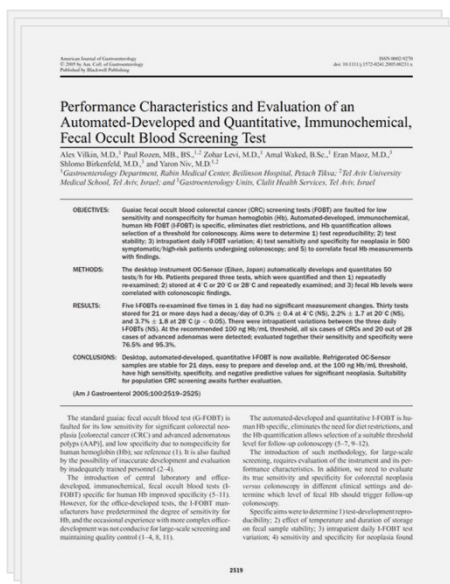
Vilkin: Collect Three Consecutive Stool Samples, Refrigerate At Home



name and date on the tube. The examinee is requested to prepare three consecutive daily samples during the week before colonoscopy examination. Samples are double-closed in zip-lock bags and kept in the refrigerator until returned to the developing laboratory where they are also kept at 4°C until development.

EX1005 at 3, 4; POR at 23

Vilkin: At-Home Refrigeration Of Three Samples Is A Requirement



in Table 3. The best results were obtained when all three tests were performed and utilized for analysis.

EX1005 at 6; POR at 13

temperatures, even when homes are air-conditioned. The double zip-lock bags we provided answered some of the screeners' hesitancy about keeping the prepared test transiently in their home refrigerator. This packaging could be further improved.

EX1005 at 8; Sur-Reply at 12

Vilkin: Home Refrigeration Caused Hesitancy Among Users

Performance Characteristics and Evaluation of an Automated-Developed and Quantitative, Immunochemical, Fecal Occult Blood Screening Test

Alon Vilkin, M.D.,¹ Paul Rosen, MB, BS,^{2,3} Zohar Levi, M.D.,³ Amal Wakeel, B.Sc.,¹ Eran Masor, M.D.,³ Shimon Birkenfeld, M.D.,⁴ and Yaron Yu, M.D.^{1,3}
¹Gastroenterology Department, Rabin Medical Center, Beilinson Hospital, Petach Tikva; ²Tel Aviv University Medical School, Tel Aviv, Israel; and ³Gastroenterology Unit, Clalit Health Services, Tel Aviv, Israel

OBJECTIVES: Quantitative fecal occult blood (FOBT) screening tests (FOBTs) are limited for low sensitivity and nonspecificity for human hemoglobin (Hb). Automated-developed, immunochemical, human Hb FOBT (i-FOBT) is specific, eliminates diet restrictions, and the quantitative allows selection of a threshold for colonoscopy. Aims were to determine 1) test reproducibility; 2) test stability; 3) integrated daily i-FOBT variation; 4) test sensitivity and specificity for neoplasia in 100 symptomatic high-risk patients undergoing colonoscopy; and 5) to correlate fecal Hb measurements with findings.

METHODS: The desktop instrument OC-Sensor (Eiken, Japan) automatically develops and quantitates 50 tests. In 10 Hb. Patients prepared three tests, which were quantified and then 1) repeatedly re-examined; 2) stored at 4°C or 20°C or 28°C and repeatedly examined; and 3) fecal Hb levels were correlated with colonoscopy findings.

RESULTS: Five i-FOBTs re-examined five times in 1 day had no significant measurement changes. Thirty tests stored for 21 or more days had a decay rate of $0.3\% \pm 0.4$ at 4°C (NS), $2.2\% \pm 1.7$ at 20°C (NS), and $3.7\% \pm 1.4$ at 28°C ($p < 0.05$). There were no significant variations between the three daily i-FOBTs (NS). At the recommended 100 ng Hb/mL threshold, all six cases of CRCs and 20 out of 28 cases of advanced adenomas were detected. Evaluated together their sensitivity and specificity were 76.5% and 95.3%.

CONCLUSIONS: Desktop, automated-developed, quantitative i-FOBT is now available. Refrigerated OC-Sensor samples are stable for 21 days, easy to prepare and develop and, at the 100 ng Hb/mL threshold, have high sensitivity, specificity, and negative predictive values for significant neoplasia. Suitability for population CRC screening awaits further evaluation.
(Am J Gastroenterol 2005;100:2519–2525)

The standard guaiac fecal occult blood test (g-FOBT) is limited for its low sensitivity for significant colorectal neoplasia (colorectal cancer [CRC] and advanced adenomatous polyps [AAPs]) and low specificity due to nonspecificity for human hemoglobin (Hb) and false positivity. It is also limited by the possibility of inaccurate development and evaluation by inadequately trained personnel (1–4). The introduction of central laboratory and office-developed, immunochemical, fecal occult blood tests (i-FOBTs) specific for human Hb improved specificity (5–11). However, for the office-developed tests, the i-FOBT manufacturers have predetermined the degree of sensitivity for Hb, and the occasional experience with more complex office-developed tests is not conducive for large-scale screening and maintaining quality control (1–4, 8, 11).

The automated-developed and quantitative i-FOBT is human Hb specific, eliminates the need for diet restrictions, and the Hb quantification allows selection of a variable threshold level for follow-up colonoscopy (5–7, 9–12). The introduction of such methodology, for large-scale screening, requires evaluation of the instrument and its performance characteristics. In addition, we need to evaluate its true sensitivity and specificity for colorectal neoplasia versus colonoscopy in different clinical settings and determine which level of fecal Hb should trigger follow-up colonoscopy. Specific aims were to determine 1) test-development reproducibility; 2) effect of temperature and duration of storage on fecal sample stability; 3) integrated daily i-FOBT test variation; 4) sensitivity and specificity for neoplasia found

(3). The storage of the prepared I-FOBT in a refrigerator, until development, is important for maintaining the test stability. This is critical in countries with high ambient temperatures, even when homes are air-conditioned. The double zip-lock bags we provided answered some of the screeners' hesitancy about keeping the prepared test transiently in their home refrigerator. This packaging could be further improved.

EX1005 at 8; POR at 34

Shuber: Limited Discussion Of Sample Collection



In any of the aspects of the invention described herein, including the examples, a sample (e.g., a stool sample) may be directly deposited into a container (e.g., a sealable container) that already contains stabilization solution. The amount of solution may be sufficient to cover the sample or a portion thereof. Alternatively, stabilization solution may be added to a container when (e.g., at the same time, immediately after, or within minutes, hours, or a day) a biological sample (e.g., a stool sample) is deposited in the container. The amount of solution added may be sufficient to cover the entire sample or at least a portion thereof (e.g., 10%, 25%, 50%, or more). In one embodiment, an amount of solution sufficient to fill the container may be added. In some embodiment, a container may be between about 25 mls and about 1,000 mls (e.g., between about 50 mls and about 500 mls, between about 100 mls and about 400 mls, or about 250 mls). However, smaller or larger containers may be used. A container with sample and stabilization solution may be sealed for storage/shipping.

EX1009 at 29:7-19; POR at 19

No Motivation To Modify Any Of These Methodologies With An Aspect Of Another

Lenhard

rectal tumors before stool sampling. Samples were received within 10 hours after defecation at the laboratory, aliquoted, and stored at -80°C .

EX1004 at 2; POR at 8

Vilkin

name and date on the tube. The examinee is requested to prepare three consecutive daily samples during the week before colonoscopy examination. Samples are double-closed in zip-lock bags and kept in the refrigerator until returned to the developing laboratory where they are also kept at 4°C until development.

EX1005 at 4; POR at 13

Itzkowitz

mounted on the toilet bowl. Immediately after defecation, subjects added 250 mL of a DNA-stabilizing buffer⁷ to a stool specimen of at least 50 g. Only 10 patients provided less than 50 g of stool, and, of these, 3 subsequently provided an adequate second specimen. The specimen was shipped at room temperature overnight using a coded identifier provided by an external clinical research organization (Carestat Inc., Newton, MA) to keep the laboratory blinded to the clinical source. The

EX1006 at 2; PET at 19-20

Shuber

In any of the aspects of the invention described herein, including the examples, a sample (e.g., a stool sample) may be deposited into a container (e.g., a sealable container) that already contains stabilization solution. The amount of solution may be sufficient to cover the sample or a portion thereof. Alternatively, stabilization solution may be added to a container when (e.g., at the same time, immediately after, or within minutes, hours, or a day) a biological sample (e.g., a stool sample) is deposited in the container. The amount of solution added may be sufficient to cover the entire sample or at least a portion thereof (e.g., 10%, 25%, 50%, or more). In one embodiment, an amount of solution sufficient to fill

EX1009 at 29:7-19; POR at 19

- No motivation to start with any of these different methods and add or exclude others

Geneoscopy Ignores Failure To Improve Advanced Adenoma Detection

Lenhard

EX1004 at 1; POR at 1

Levin

EX1013 at 4; Sur-Reply at 7

Goal Of Screening = Early Detection

Shuber

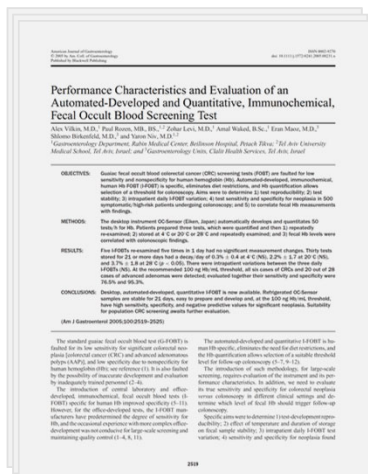
However, many cancers (e.g., carcinomas such as colorectal carcinomas) are thought to develop from adenomas. Indeed, a majority of colon cancers are thought to develop from adenomas. Therefore, detecting adenomas is particularly useful for identifying early signs or risks of colorectal cancer (e.g., cancerous and precancerous lesions or growths in the colon).

EX1009 at 13:7-8: POR at 18

Vilkin

specificity of 95.3% (1). This means that in this group of high-risk and symptomatic patients, all cases of cancer and most advanced adenomas would be detected and a negative test would provide a very high degree of certainty that there was no clinically significant colorectal neoplasia at this round of screening.

EX1005 at 8; POR at 28



Lenhard: Adding gFOBT Did Not Increase Adenoma Sensitivity

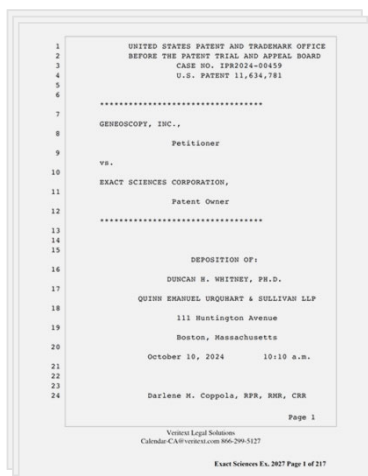


Table 2. Clinicopathologic Data and HIC1 Methylation Status of Patients With Adenomatous Polyps

No.	Sex	Age (y)	Diagnosis	HIC1 methylation	FOBT
27	M	72	Tubular adenoma, ascending colon, 3 cm	+	—
28	F	63	Tubulovillous adenoma, ascending colon, hepatic flexure, sigmoid colon, 1–1.5 cm	—	—
29	M	63	Tubulovillous adenoma, ascending colon, 2 cm	—	—
30	M	76	Tubulovillous adenoma, ascending and transverse colon, 1, 3, 4 cm	+	+
31	M	83	Tubulovillous adenoma, transverse colon, 3 cm	—	—
32	F	65	Tubulovillous adenoma, sigmoid colon, 1.5 cm	—	—
33	M	72	Tubulovillous adenoma, ascending colon, 1 cm	—	—
34	F	60	Tubular adenoma, sigmoid colon, 1.5 cm	—	—
35	M	69	Tubular adenoma, 1.5 cm, ascending colon	—	—
36	M	70	Tubulovillous adenoma, sigmoid colon, 1 cm	—	—
37	M	64	Tubulovillous adenoma, rectum, 1 cm	—	—
38	M	72	Adenoma, cecum, 2 cm	+	—
39	F	63	Tubulovillous adenoma, sigmoid colon, 2 cm	+	—

M, male; F, female.

EX1004 at 3; POR at 27



Q. So the **FOBT** added no sensitivity to -- with respect to the **adenomas**, correct?

A. In this small study, in this table, **it did not add any sensitivity.**

EX2027 (Whitney 10/10/24 Dep. Tr.) at 52:23-53:3

Shuber: Already Disclosed Up To 98% Sensitivity For Adenomas



adenomas. Therefore, detecting adenomas is particularly useful for identifying early signs or risks of colorectal cancer (e.g., cancerous and precancerous lesions or growths in the colon).

EX1009 at 13:7-8; POR at 18

In one embodiment, a combination of all of the above loci may be used to detect adenomas with a greater than 95% sensitivity (e.g., greater than 98% sensitivity).

EX1009 at 14:15-16; POR at 18-19

Geneoscopy Ignores Sacrifice Of Specificity

Petitioner's References Emphasize Importance Of Specificity

Lenhard

at 77%. Therefore, identification of both sensitive and highly specific methylation markers is required before MSP can be evaluated as a stool-based screening procedure for CRC in prospective studies.

uation will compromise specificity severely. In addition, low specificity of a test results in a low positive predictive value and consecutively in high rates of unnecessarily performed colonoscopies.

EX1004 at 2, 7; POR at 11-12

Vilkin

The clinical evaluation demonstrated a high sensitivity of 76.5% for all significant colorectal neoplasia and acceptable specificity of 95.3%.

EX1005 at 7; POR at 13, 27

Petitioner's References Emphasize Importance Of Specificity

Nishikawa

tions is low among cancers as a whole. However, the technique is highly specific, noninvasive, and cost-effective, and should provide a more sensitive and specific tool for mass screening of colorectal cancer than is currently available, especially if used in combination with fecal occult blood testing and other methods for detecting genetic abnormalities.

EX1011 at 6; Sur-Reply at 15

Levin

required. Another question worthy of consideration is whether or not including a sensitive gFOBT or FIT at the time of testing would improve sensitivity without adversely affecting specificity. In a recent retrospective anal-

EX1013 at 9; POR at 45

Young

rate. FITs provide for an improved sensitivity/specificity ratio; in other words, they can achieve better sensitivity without an unacceptable deterioration in specificity.

EX1024 at 4; POR at 32; PET at 22

Lenhard: Decreasing Specificity Is Undesirable

Analysis of Promoter Methylation in Stool: A Novel Method for the Detection of Colorectal Cancer

HONSTANZE LENHARD,* GUIDO T. BONNIER,* SILKE ASUTAY,* ROLF SCHAUER,* THOMAS BRÄULETZ,² BURKHARD GÖKE,* ROLF LAMERZ,* and FRANK T. KOLLIGS*

*Department of Medicine I, and ²Department of Surgery, Heinrich-Heine-University of Düsseldorf, Germany, and the Institute of Pathology, University of Göttingen, Göttingen, Germany

Background & Aims: Detection of tumor-derived DNA alterations in stool is an intriguing new approach with high potential for the noninvasive detection of colorectal cancer (CRC). Because of heterogeneity of human, usually multiple markers distributed throughout the human genome need to be analyzed. This is labor intensive and does not allow for high throughput screening. Therefore, markers with high sensitivity and good specificity are needed. We explored the potential of a single epigenetic marker in comparison with fecal occult blood testing (FOBT) for the discrimination of patients with CRCs and adenomas from those without. **Methods:** Methylation-specific polymerase chain reaction (PCR) was performed to analyze hypermethylated in cancer 1 (HIC1) promoter methylation status in a blinded fashion in stool samples from 26 patients with CRC, 13 with adenoma >1 cm, 9 with hyperplastic polyps, 9 with chronic inflammatory bowel disease, and 32 with endoscopically normal colon. **Results:** Ninety-seven percent of the stool samples contained amplifiable DNA. Forty-two percent of the samples from patients with CRC and 31% of the samples from patients with colorectal adenoma >1 cm were positive for HIC1 promoter methylation. No methylated HIC1 promoter DNA was detected in the fecal DNA from patients with endoscopically normal colon or hyperplastic polyps. **Conclusions:** The epigenetic marker HIC1 promoter methylation carries high potential for the sensitive detection of CRCs. We propose that a panel of merely a few genetic and epigenetic markers will be required for the highly sensitive and specific detection of CRCs and adenomas in fecal samples from affected patients.

Colorectal cancer (CRC) is one of the leading causes of cancer-related morbidity and mortality.¹ About 60% of patients die within 5 years of being diagnosed. This mainly is attributable to late presentation with locally advanced or metastatic disease in one third of cases, precluding curative surgery.² The majority of CRCs follow the adenoma-carcinoma sequence, requiring a time period of usually more than 10 years.³ Detection

of early disease and precancerous adenomatous lesions leads to a decrease of CRC-related mortality.^{4,5} Therefore, there is a strong rationale for screening programs. Sigmoidoscopy, colonoscopy, and fecal occult blood test (FOBT) alone or in combination with endoscopy, are recommended for screening of the average-risk population.⁶ However, FOBT, when applied regularly, can decrease CRC mortality by only 15%–35%.^{7–9} Compliance with endoscopic screening is not uniform. Overall, in 1998, only 37% of eligible adults in the United States had been screened for CRC in the previous 3 years.

Detection of tumor-derived genetic changes in stool is a promising new approach for CRC screening. Studies published so far focus on the detection of mutations in oncogenes,^{10–12} and tumor-suppressor genes,^{13–16} as well as changes in microsatellite markers.¹⁷ Two recent studies required the analysis of up to 15 different genetic markers¹⁸ or 148 fragments from each stool¹⁹ for the detection of 91% and 61% of cancers and 82% and 50% of adenomas >1 cm, respectively. This precludes their application for routine high throughput screening owing to high labor intensity and costs. The identification of new markers with high sensitivity and specificity is needed. So far, little attention has been paid to DNA hypermethylation as a potential stool marker for CRC.

Methylation of CpG islands of promoters leads to silencing of transcription of the affected gene.^{20–22} Methylation-specific polymerase chain reaction (PCR) (MS-PCR)²³ has been used successfully to detect DNA methylation in primary tumors and in various body fluids.²⁴ The feasibility of amplification of methylated

Abbreviations used in this paper: FOBT, fecal occult blood test; HIC1, hypermethylated in cancer 1; MS-PCR, methylation-specific PCR; FOBT, fecal occult blood test.

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1542-3566/05/030301
DOI: 10.1053/j.gastro.2005.06.004

tations. This shows that methylation markers as well as genetic markers need to be selected carefully to achieve both high sensitivity and specificity. Choosing sensitive markers that already are positive in a premalignant situation will compromise specificity severely. In addition, low specificity of a test results in a low positive predictive value and consecutively in high rates of unnecessarily performed colonoscopies.

EX1004 at 7; POR at 31

Lenhard: High Specificity Is Required For CRC Screening

does not allow for high through-put screening. Therefore, markers with high sensitivity and **good specificity** are needed. We explored the potential of a single epigenetic marker in comparison with fecal occult blood testing (FOBT) for the discrimination of patients with CRCs and adenomas from those without. *Methods:* Methylation-

EX1004 at 1; POR at 11

at 77%. Therefore, identification of both sensitive and **highly specific** methylation markers is required before MSP can be evaluated as a stool-based screening procedure for CRC in prospective studies.

EX1004 at 2; POR at 11

Discussion

Highly sensitive, specific, and easily analyzable markers are required for noninvasive stool-based CRC screening. We show here that DNA methylation can be

EX1004 at 6; POR at 11

Analysis of Promoter Methylation in Stool: A Novel Method for the Detection of Colorectal Cancer

KONSTANZE LENHARD,* GUIDO T. BOMMER,* SILKE ASLUTAY,* ROLF SCHAUER,*

THOMAS BRÄULETZ,¹ BURKHARD GÖKE,* ROLF LAMERZ,* and FRANK T. HOLLIG*

¹Department of Medicine I, and *Department of Surgery, Heinrich-Heine-University of Düsseldorf, Germany, and the *Institute of Pathology, University of Erlangen, Erlangen, Germany

Background & Aims: Detection of tumor-derived DNA alterations in stool is an intriguing new approach with high potential for the noninvasive detection of colorectal cancer (CRC). Because of heterogeneity of tumors, usually multiple markers distributed throughout the human genome need to be analyzed. This is labor intensive and does not allow for high through-put screening. Therefore, markers with high sensitivity and good specificity are needed. We explored the potential of a single epigenetic marker in comparison with fecal occult blood testing (FOBT) for the discrimination of patients with CRCs and adenomas from those without. **Methods:** Methylation-specific polymerase chain reaction (MSP) was performed to analyze hypermethylated in cancer 1 (HIC1) promoter methylation status in a blinded fashion in stool samples from 26 patients with CRC, 13 with adenoma >1 cm, 9 with hyperplastic polyps, 9 with chronic inflammatory bowel disease, and 32 with endoscopically normal colon. Results: Nearly 90% percent of the stool samples contained amplifiable DNA. Forty-two percent of the samples from patients with CRC and 21% of the samples from patients with colorectal adenoma >1 cm were positive for HIC1 promoter methylation. No methylated HIC1 promoter DNA was detected in the stool DNA from patients with endoscopically normal colon or hyperplastic polyps. Conclusion: The epigenetic marker HIC1 promoter methylation confers high potential for the remote detection of CRC. We propose that a panel of merely a few genetic and epigenetic markers will be required for the highly sensitive and specific detection of CRC and adenomas in fecal samples from affected patients.

Colorectal cancer (CRC) is one of the leading causes of cancer-related morbidity and mortality.¹ About 80% of patients die within 5 years of being diagnosed. This mainly is attributable to late presentation with locally advanced or metastatic disease in one third of cases, precluding curative surgery.² The majority of CRCs follow the adenoma-carcinoma sequence, requiring a time period of usually more than 10 years.³ Detection

of early disease and precancerous adenomatous lesions leads to a decrease of CRC-related mortality.^{4,5} Therefore, there is a strong rationale for screening programs. Sigmoidoscopy, colonoscopy, and fecal occult blood test (FOBT) alone or in combination with endoscopy, are recommended for screening of the average-risk population.⁶ However, FOBT, when applied regularly, can decrease CRC mortality by only 15%–35%.^{7,8} Compliance with endoscopic screening is low worldwide. Overall, in 1998, only 37% of eligible adults in the United States had been screened for CRC in the previous 5 years.⁹

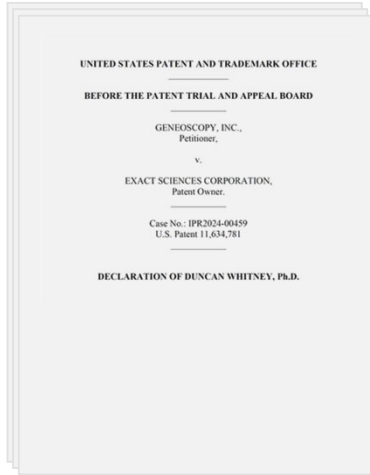
Detection of tumor-derived genetic changes in stool is a promising new approach for CRC screening. Studies published so far focus on the detection of mutations in oncogenes,^{10–12} and tumor-suppressor genes,^{13–15} as well as changes in microsatellite markers.^{16–18} Two recent studies required the analysis of up to 13 different genetic markers¹⁹ or 144 fragments from each stool²⁰ for the detection of 91% and 61% of cancers and 82% and 50% of adenomas, 91 cm, respectively. This precludes their application for routine high-throughput screening owing to high labor intensity and costs. The identification of new markers with high sensitivity and specificity is needed. So far, little attention has been paid to DNA hypermethylation as a potential stool marker for CRC.

Methylation of CpG islands of promoters leads to silencing of transcription of the affected gene.^{21–23} Methylation-specific polymerase chain reaction (MSP)^{24,25} has been used successfully to detect DNA methylation in primary tumors and in various body fluids.²⁶ The feasibility of amplification of methylated

Abbreviations used in this paper: FOBT, fecal occult blood test; HIC1, hypermethylated in cancer 1; MSP, methylation-specific polymerase chain reaction.

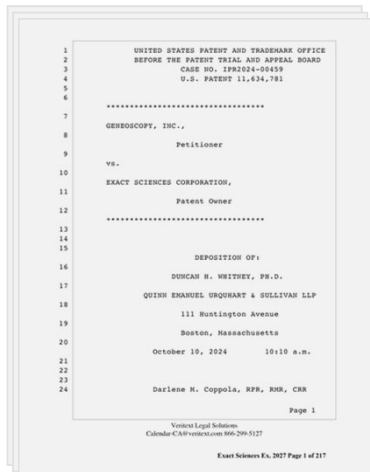
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1542-3566/05/\$30.00
DOI: 10.1053/j.gastro.2004.06.024

Geneoscopy's Expert: High Specificity Is Desirable



not have the disease being tested for). Having a high specificity is desirable in fecal CRC tests, because patients who wrongly test positive are often subjected to an unnecessary medical procedures and potentially unnecessary treatment.

EX1002 (Whitney Dec.) ¶ 29; POR at 4



Q. You agree that having a high specificity is desirable in fecal CRC tests because patients who wrongly test positive often are subjected to an unnecessary medical procedure and potentially unnecessary treatment?

A. I agree – excuse me. I agree with that, yes.

EX2027 (Whitney 10/10/24 Dep. Tr.) at 35:24-36:7

Experts Agree: High Specificity Is Required

Vadim Backman, Ph.D. Exact Sciences' Expert

“low specificity results in an unacceptable number of false positives which then lead to unnecessary colonoscopies.”

Ex. 2022 (Backman Decl.) ¶ 30; POR at 4

“False positives also have significant patient impact. See, e.g., Ex. 1033 at 12-13. If a screening test has low specificity, then both health care providers and patients would understand that a positive test result may not be indicative of disease. This, in turn, may reduce incentive to follow up with the recommended colonoscopy after a positive screening test result.”

Ex. 2022 (Backman Decl.) ¶ 107; POR at 4

Duncan H. Whitney Geneoscopy's Expert

- A. The -- what's required of a good test, as we were discussing before, is to have high specificity in order to avoid false positives and to have high sensitivity to avoid false negatives. These are relative terms, and so the combination of specific biomarkers and the benefit of what happens when you combine different biomarkers is something that would need to be explored.

EX2027 (Whitney 10/10/24 Dep. Tr.) at 133:12-15; POR at 4

Lenhard: Combining DNA With Protein Decreases Specificity

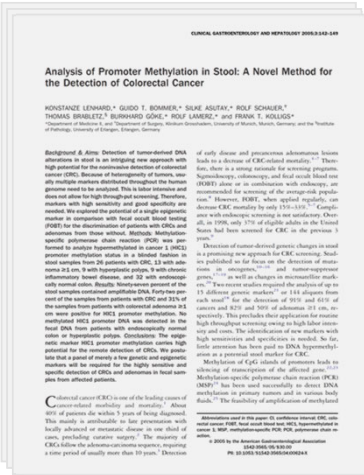
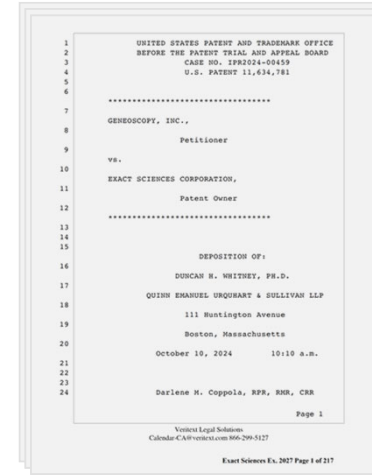


Table 3. HIC1 Methylation Status of Healthy Controls and Patients With Nonneoplastic Disease

No.	Sex	Age	Diagnosis	HIC1 methylation	FOBT
40	F	61	Normal	—	+
41	F	61	Normal	—	—
42	F	73	Normal	—	—
43	M	63	Normal	—	—
44	M	47	Normal	—	—
45	F	41	Normal	—	—
46	M	53	Normal	—	—
47	F	59	Normal	—	+
48	F	73	Normal	—	—
49	M	36	Normal	—	—
50	M	71	Normal	—	—
51	F	65	Normal	—	—
52	F	60	Normal	—	—
53	F	66	Normal	—	—
54	M	80	Normal	—	—
55	M	70	Normal	—	—
56	F	27	Normal	—	—
57	F	57	Normal	—	—
58	M	55	Normal	—	ND
59	M	23	Normal	—	—
60	F	73	Normal	—	—
61	F	79	Normal	—	—
62	M	68	Normal	—	—
63	F	78	Normal	—	—
64	F	38	Normal	—	—
65	F	62	Normal	—	—
66	M	76	Normal	—	—
67	F	61	Normal	—	—
68	M	23	Normal	NA	—
69	M	74	Normal	—	—
70	M	60	Normal	—	—
71	F	49	Normal	—	—
72	M	59	Hyperplastic polyp	—	—
73	F	66	Hyperplastic polyp	—	—
74	F	67	Hyperplastic polyp	—	—
75	F	65	Hyperplastic polyp	—	—
76	F	58	Hyperplastic polyp	NA	—
77	F	76	Hyperplastic polyp	—	—
78	F	54	Hyperplastic polyp	—	—
79	F	76	Hyperplastic polyp	—	—
80	F	60	Hyperplastic polyp	—	—
81	M	47	Ulcerative colitis	+	+
82	M	64	Ulcerative colitis	—	+
83	M	27	Ulcerative colitis	—	—
84	M	67	Ulcerative colitis	—	+
85	M	53	Ulcerative colitis	—	—
86	M	51	Ulcerative colitis	—	—
87	F	44	Crohn's disease	—	—
88	M	30	Crohn's disease	—	—
89	M	37	Crohn's disease	—	—

F, female; M, male; ND, not determined; NA, no amplification.



Q. And then there are four more false positives in the FOBT column; is that right?

A. That's correct.

Q. So the combination, therefore, reduced the specificity with more false positives; is that right?

A. That would seem to be the case, yes.

EX2027 (Whitney 10/10/24 Dep. Tr.) at 54:19-55:1

EX1004, Table 3; POR at 12

Petitioner Incorrectly Assumes POSA Would Have Sacrificed Specificity

AGA INSTITUTE

The following article contains new recommendations for colorectal cancer screening, the first set we have published since 2003 (Winawer S, Fletcher R, Rex D, et al. Colorectal cancer screening and surveillance: clinical guidelines and rationale—update based on new evidence. *Gastroenterology* 2003;124:544–560.) The current recommendations have emerged through the participation of multiple national societies, taking into consideration newly emerging technologies. Please note the US Multi-Society Task Force (USMTF) represents the American Gastroenterological Association (AGA) Institute, the American Society for Gastrointestinal Endoscopy, and the American College of Gastroenterology. Commissioned originally by the American Cancer Society, this compendium will be published concurrently in CA: A Cancer Journal for Clinicians and reprinted in the June issue of Radiology.

Screening and Surveillance for the Early Detection of Colorectal Cancer and Adenomatous Polyps, 2008: A Joint Guideline From the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology

EX1013 at 1

required. Another question worthy of consideration is whether or not including a sensitive gFOBT or FIT at the time of testing would improve sensitivity without adversely affecting specificity. In a recent retrospective anal-

EX1013 at 9; Sur-Reply at 13



Levin Cites Kutzner



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The following article contains new recommendations for colorectal cancer screening, the first set we have published since 2003 (Winawer S, Fletcher R, Rex D, et al. Colorectal cancer screening and surveillance: clinical guidelines and rationale—update based on new evidence. Gastroenterology 2003;124:544–560.) The current recommendations have emerged through the participation of multiple national societies, taking into consideration newly emerging technologies. Please note the US Multi-Society Task Force (USMTF) represents the American Gastroenterological Association (AGA) Institute, the American Society for Gastrointestinal Endoscopy, and the American College of Gastroenterology. Commissioned originally by the American Cancer Society, this compendium will be published concurrently in CA: A Cancer Journal for Clinicians and reprinted in the June issue of Radiology.

Screening and Surveillance for the Early Detection of Colorectal Cancer and Adenomatous Polyps, 2008: A Joint Guideline From the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology

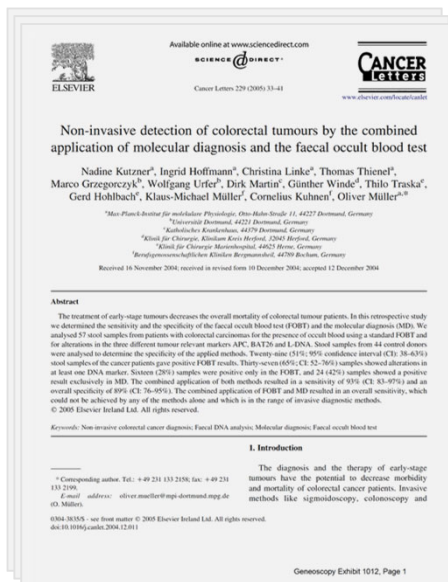
EX1013 at 1

required. Another question worthy of consideration is whether or not including a sensitive gFOBT or FIT at the time of testing would improve sensitivity without adversely affecting specificity. In a recent retrospective analysis of stool samples from patients with CRC and donor controls, combined results from a standard gFOBT and a panel of DNA markers (APC, BAT26, and L-DNA) resulted in a combined sensitivity for cancer of 93% and specificity of 89%.⁷⁶

76. Kutzner N, Hoffmann I, Linke C, et al. Noninvasive detection of colorectal tumours by the combined application of molecular diagnosis and the faecal occult blood test. Cancer Lett 2005; 229:33–41.

EX1013 at 9, 22; POR at 45; Sur-Reply at 16

Kutzner: No Conclusion On Combination Of Nucleic Acid And FOBT



alone. Regarding the low cost and the little technical expense of the FOBT, we suggest that MD and FOBT should complement rather than replace each other. The sensitivity of the combined application of MD and FOBT is in the range reported for invasive methods. This leads to the question whether both methods should be used together in broad diagnostic screening programs. We are aware that our retrospective data do not allow any conclusion on the potential of this approach in diagnostic screening.

EX1012 at 8; POR at 44-45; Sur-Reply at 16

**References Collectively Would Have
Discouraged POSA From The Claimed Method**

Geneoscopy Ignores The Implications Of The Art As A Whole

- Based on combinations of references, POSA would have expected:
 - Combining different marker classes would:
 - **increase** complexity/cost including for different containers and buffers
 - **not increase** adenoma sensitivity
 - **decrease** CRC sensitivity over iFOBT
 - **decrease** specificity
 - Requiring patients to manipulate and refrigerate stool multiple times would **decrease** patient acceptance
- Geneoscopy ignores how a POSA would view the collective teachings of the references together in combination

Objective Indicia Of Non-Obviousness Show Geneoscopy Relies On Hindsight

Common Sense: If The Claims Were So Obvious, Why Did It Take So Long?

May 14, 2004
Shuber



EX1009

Feb. 2005
Lenhard



EX1004

Nov. 2005
Vilkin



EX1005

Jan. 2007
Itzkowitz



EX1007

Still not a
recognized strategy
to increase sensitivity

2004

2005

2006

2007

2008

2009

May 2008
Levin – Joint Guideline

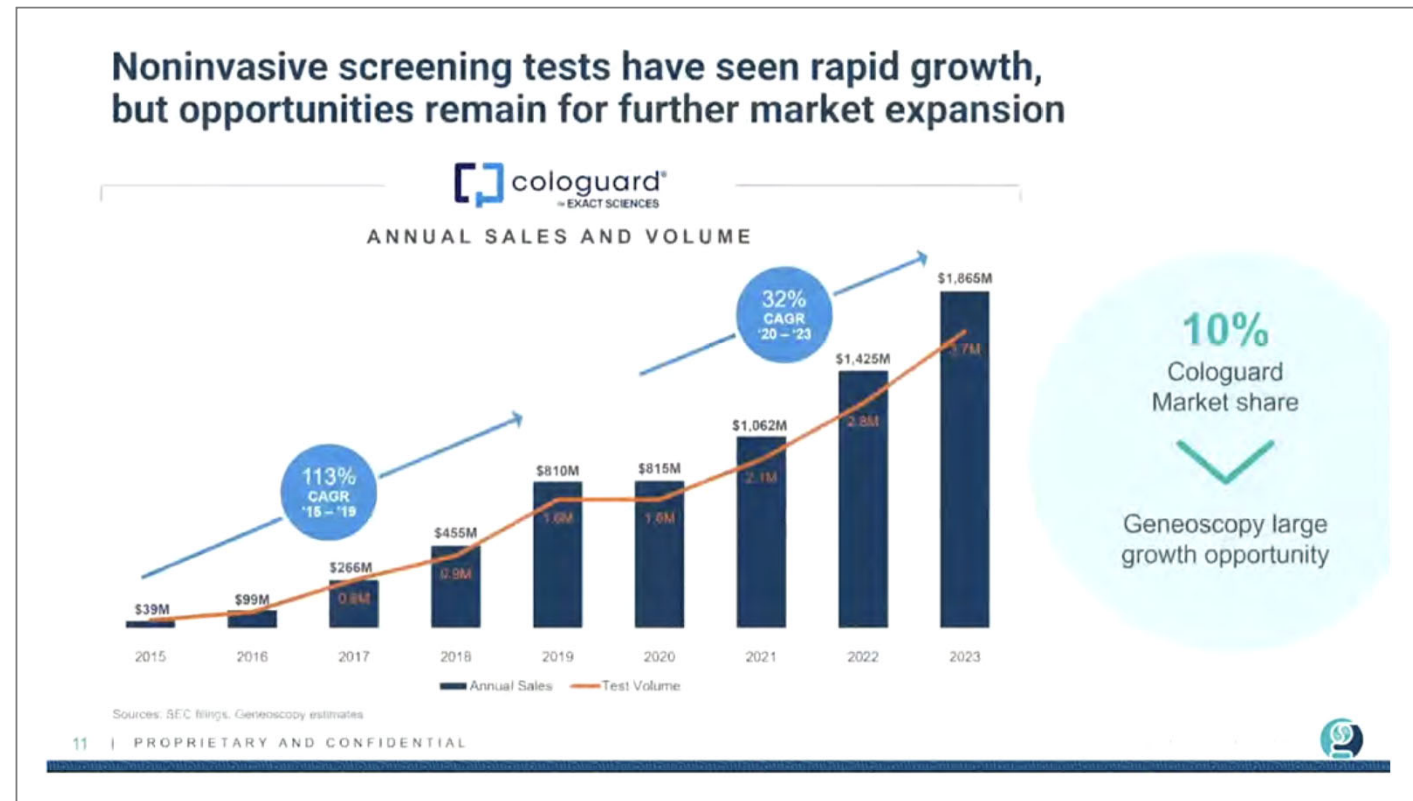
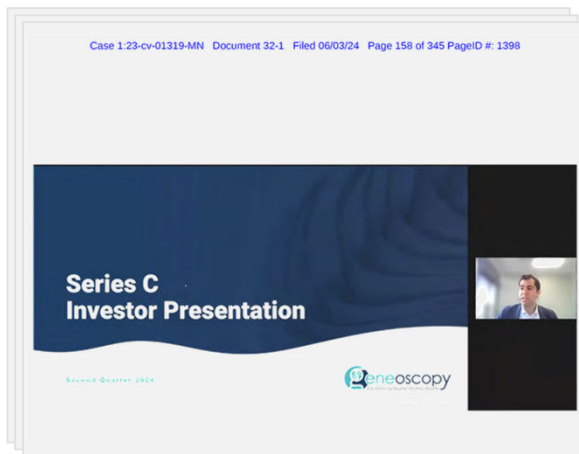
Another question worthy of consideration is whether or not including a sensitive gFOBT or FIT at the time of testing would improve sensitivity without adversely affecting specificity.

EX1013

December 31, 2008
Exact Sciences Down to
Only 4 Employees
EX2035 at 18

February 3, 2009
Filing Date for
Provisional Application
EX1001

No Dispute – Cologuard Is A Commercial Success



EX2029 at 11; POR at 7-8

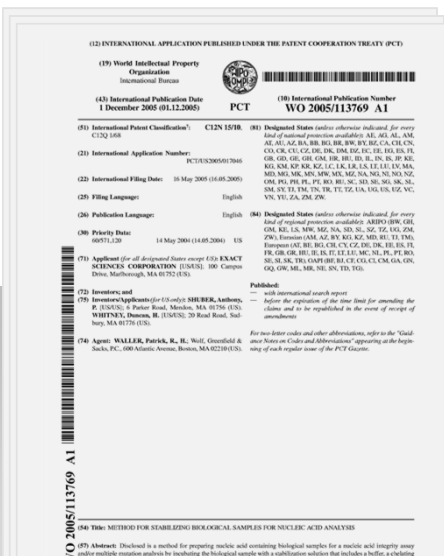
No Dispute – Others Tried And Failed



EX1004



EX1006



EX1009



EX1043

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

64

No Dispute – Cologuard Met A Long-Felt Need

More than 55% of Americans between the ages of 45 and 85 who are at average risk for colorectal cancer are not up-to-date with screening according to the American Cancer Society's ("ACS") colorectal cancer screening guidelines. We believe our Cologuard test helps more people get screened for colorectal cancer. Internal studies have shown that approximately 40% of surveyed Cologuard users were previously unscreened for colorectal cancer.

EX2040 at 5; POR at 61

disparities. The number of people screened with the mt-sDNA test further increased in 2019, with 1.7 million patients successfully screened using mt-sDNA testing [8].

EX2049 at 2; POR at 61

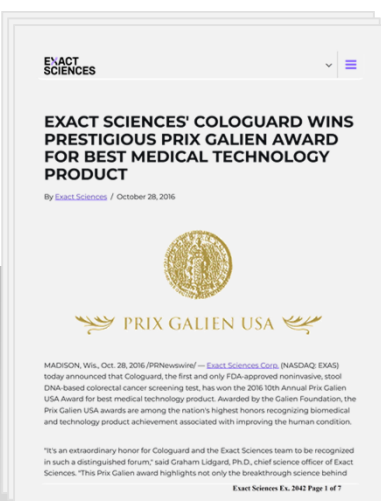
FIT adherence. A recent study of cross-sectional adherence in a large, national sample (n = 368,494) of Medicare beneficiaries reported an mt-sDNA test completion rate of 71%.⁵⁵ This rate exceeds by 58% the modeled FIT screening adherence rate of 45%,⁵⁴ and is well above the proposed cost-effectiveness threshold from Naber and colleagues.⁵³

EX2050 at 10; POR at 61

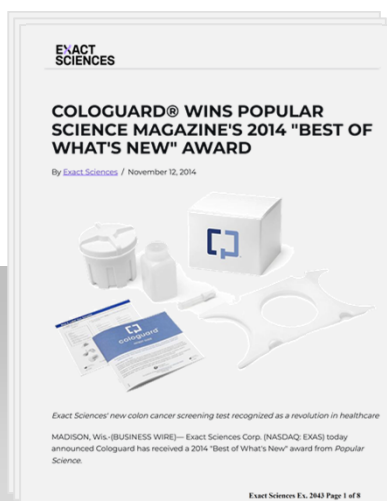
neoplastic findings at diagnostic colonoscopy [43•]. Furthermore, we recently presented data from a cohort of over 2000 MT-sDNA-positive patients showing that approximately 25% had never been screened for CRC and were > 10 years overdue [44]. While there may be many reasons for patients to go without screening, these findings suggest that MT-sDNA is attracting previously non-adherent patients, a critical outcome in the ongoing long-term fight to reduce CRC-attributable mortality.

EX2052 at 4; POR at 61

No Dispute – Cologuard Is Subject Of Praise And Awards



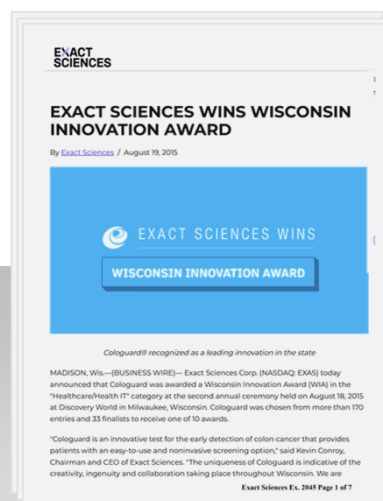
EX2042



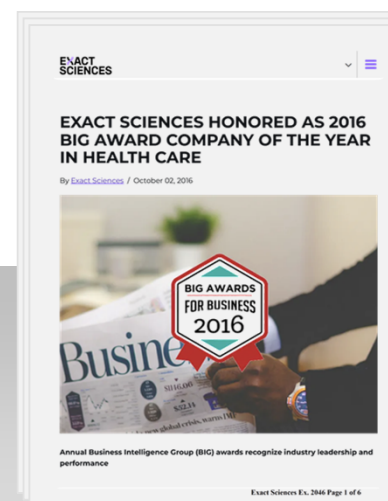
EX2043



EX2044



EX2045



EX2046

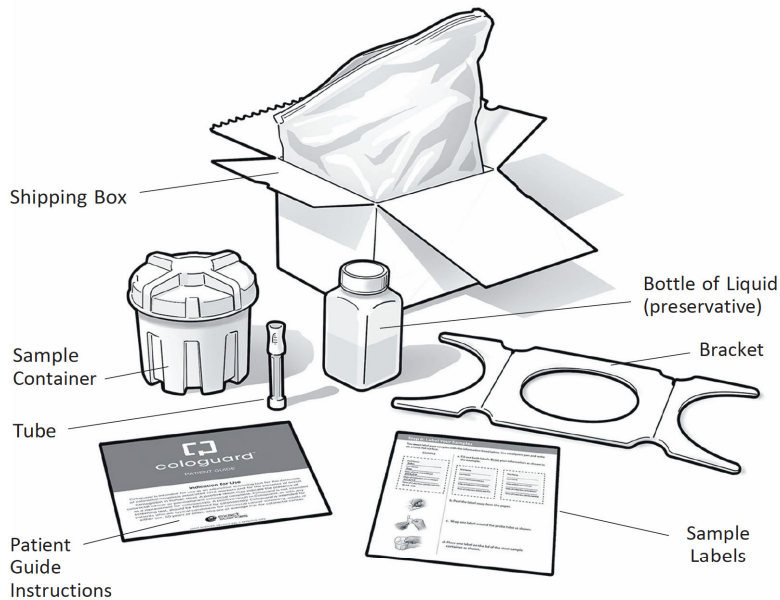
POR at 62-63

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

66

Common Sense Belies Petitioner's Denial Of Copying

What Is Inside the Cologuard Collection Kit



- The bottle of liquid contains a preservative (less than 10% EDTA in Tris buffered solution).
- The tube contains a 10% albumin in Tris buffered detergent solution with an antimicrobial agent.

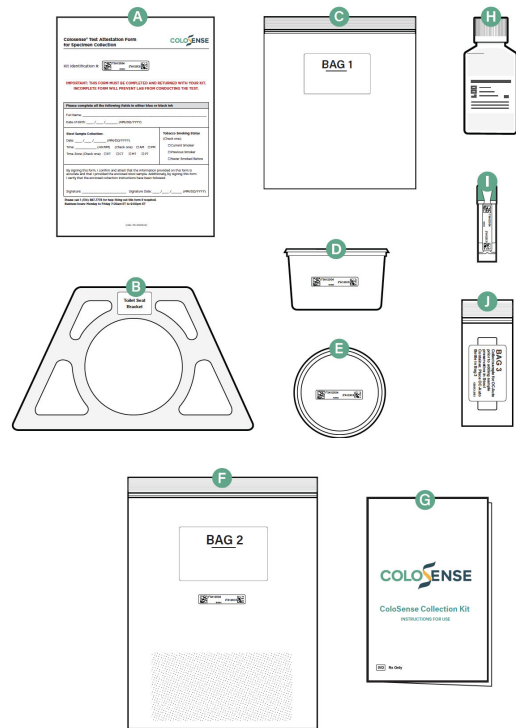
Page 8

For questions or help call [Exact Sciences at 1-844-870-8878](tel:1-844-870-8878)
www.cologuardtest.com
 Rx Only

Exact Sciences Ex. 2032 Page 8 of 139

EX2032 at 8; POR at 51

ColoSense Collection Kit Contents



EX2053 at 34; POR at 64

Geneoscopy's Arguments On Objective Indicia Fail

- Geneoscopy does not dispute objective indicia of non-obviousness:
 - Cologuard's commercial success
 - Failure of others
 - Long-felt but unmet need
 - Industry Praise for Cologuard
- Geneoscopy ***only argues lack of nexus***

Geneoscopy Ignores Proper Framework For Nexus

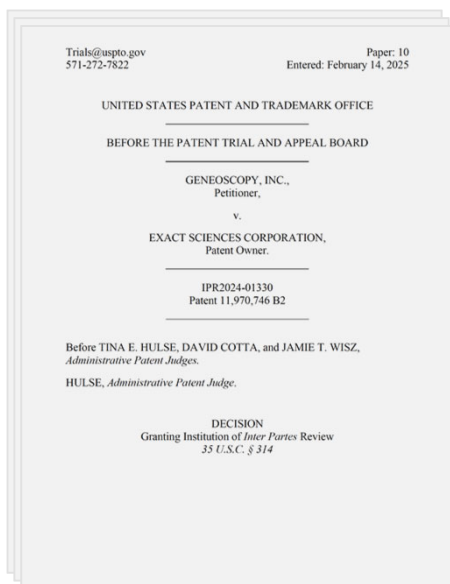
We presume a nexus “when the patentee shows that the asserted objective evidence is tied to a specific product and that product ‘embodies the claimed features, and is coextensive with them.’ ”

* * *

“The coextensive requirement does not require a patentee to prove perfect correspondence between the product and a patent claim.”

Quanergy Sys. Inc. v. Velodyne Lidar USA, Inc., 24 F.4th 1406, 1417 (Fed. Cir. 2022) (citations omitted);
Sur-Reply at 19

Presumption Of Nexus – Institution Decision In IPR2024-01330



At this stage of the proceeding, we find Patent Owner has the better position that a presumption of a nexus exists between Patent Owner’s evidence related to Cologuard and the ’746 Patent claims. We find the Federal Circuit’s discussion in *Teva Pharmaceuticals Int’l GmbH v. Eli Lilly & Co.*, 8 F.4th 1349 (Fed. Cir. 2021) to be instructive. The Federal Circuit

the two portions of the fecal sample. *See, e.g.,* Ex. 1001, claim 3. Thus, at this stage of the proceeding, we are persuaded that Cologuard “embodies the claimed features, and is coextensive with them” such that a presumption of nexus applies. *See Brown & Williamson Tobacco Corp. v. Philip Morris Inc.*, 229 F.3d 1120, 1130 (Fed. Cir. 2000).

IPR2024-01330, Paper 10 at 30-31; Sur-Reply at 19

Cologuard Patient Guide: '781 Patent Covers Cologuard Workflow



cologuard™

PATIENT GUIDE

Indication for Use

Cologuard is intended for the qualitative detection of colorectal neoplasia associated DNA markers and for the presence of occult hemoglobin in human stool. A positive result may indicate the presence of colorectal cancer (CRC) or advanced adenoma (AA) and should be followed by diagnostic colonoscopy. Cologuard is indicated to screen adults of either sex, 50 years or older, who are at typical average-risk for CRC. Cologuard is not a replacement for diagnostic colonoscopy or surveillance colonoscopy in high risk individuals.



For questions or help call **Exact Sciences at 1-844-870-8878**
www.cologuardtest.com
 Rx Only

Exact Sciences
Madison WI USA

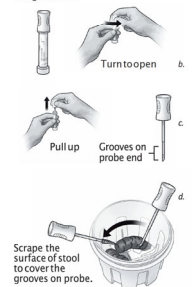
LBL-0207rev3

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EX2032 (Cologuard Patient Guide);
EX2022 at 143-154; POR at 51-55

Step 4: Scrape the Stool Sample

You must scrape the stool sample with the probe to get another small sample for the Cologuard test.



- Lift the tube out of the box.
- Turn the white tube cap and unscrew it.
- Pull the probe from the tube.
 - You may have to pull hard.
- Scrape the surface of your stool sample until the end of the probe has stool on it.
 - Your stool sample may look different from the stool sample pictured.

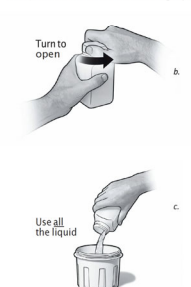
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Step 5: Prepare Stool Sample Container for Shipping

The stool sample must have a preservative poured onto it to make sure the lab can test it. Then, the container must be closed tightly for shipping.

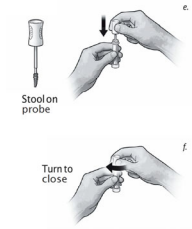


- Lift the bottle of liquid preservative out of the box.
- Hold the bottle and turn the cap to unscrew it.
 - Caution:** Do **not** drink the liquid.
 - If the liquid from the bottle touches your skin or eyes, wash with water.
- Pour **all** the liquid in the bottle into the container with the stool.
 - The empty bottle and cap can be recycled or thrown in the trash.

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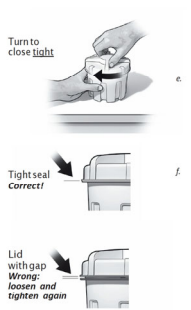


- Place the probe back into the open end of the tube.
- Turn the cap to close.
- Set the tube down.

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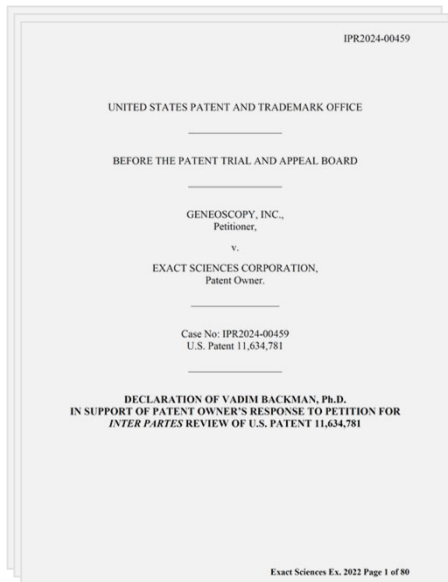
- Hold the stool sample container on a hard, flat surface.
- Place the lid on top of the container.
 - Make sure the lid is straight so it will close tightly.
- Turn the lid to tighten until it does not tighten anymore.

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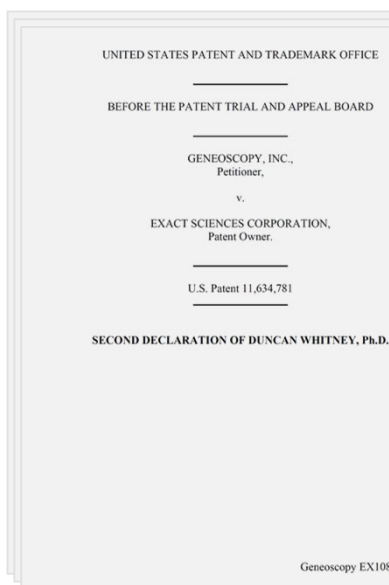
Cologuard's Success Directly Results From The Claimed Inventions



155. In my opinion, the Cologuard test would not have been adopted to the extent that it has been if it did not satisfy two critical aspects of screening tests: diagnostic accuracy and uptake. As illustrated in the discussion of the claims above, the claimed invention of the '781 patent is essential to both of these criteria that are necessary for both clinical and commercial success of a diagnostic product such as a CRC screening test. The addition of buffers to each of the remaining and

EX2022 ¶ 155; POR at 56-57

Petitioner's Cursory Arguments Regarding Other Patents



105. Cologuard identifies on its website 54 Exact patents unrelated to the '781 patent that cover components of Cologuard and its use.¹⁶⁹ An additional eight patents were identified on Exact's webpage as covering Cologuard in 2014, which I understand are now expired.¹⁷⁰ The claims of these many patents are directed to numerous distinct components of Cologuard, including those set forth below:

- **Patents related to general use of stool in CRC screening:** *e.g.*, U.S. Pat. Nos. 5,741,650, 5,952,178, 6,303,304, 6,586,177.
- **Patents related to containers for collecting and storing stool samples:** *e.g.*, U.S. Pat. Nos. 9,211,112, 9,750,482, 10,265,054, 10,486,158, 10,702,250, 10,894,256.
- Patents related to DNA stabilizing buffer: *e.g.*, U.S. Pat. Nos. 6,551,777, 8,722,330.
- **Patents related to DNA isolation methods:** *e.g.*, U.S. Pat. Nos. 6,268,136, 6,406,857, 8,993,341, 8,999,176, 9,000,146, 9,057,098, 9,163,278, 9,169,511, 9,657,330, 9,631,228, 9,845,491, 9,856,515, 10,047,390, 10,822,639.

- **Patents related to DNA markers:** *e.g.*, U.S. Pat. Nos. 8,673,555, 8,969,046, 9,121,070, 9,127,318, 9,163,278, 9,169,511, 9,399,800, 9,783,856, 9,803,249, 9,845,491, 10,435,753.
- Patents related to methods of preparing DNA for methylation analysis: *e.g.*, U.S. Pat. Nos. 9,315,853, 10,144,953, 10,704,083.
- **Patents related to methods of testing for methylated DNA:** *e.g.*, U.S. Pat. Nos. 8,916,344, 9,637,792, 10,519,510, 10,590,489, 10,808,286.
- **Patents related to methods of detecting DNA mutations:** *e.g.*, U.S. Pat. Nos. 8,361,720, 8,715,937, 9,024,006, 9,121,071, 9,212,392, 9,290,797, 9,376,721, 10,000,817, 10,106,844, 10,590,489.
- **Patents related to control molecules used with DNA assays:** *e.g.*, U.S. Pat. Nos. 9,212,392, 10,106,844, 10,138,524, 10,253,358, 10,889,867.

EX1080 at ¶ 105; Sur-Reply at 22

No Evidence Other Cited Patents Are Relevant

Q. Did you do an analysis of whether Cologuard practices the claims of all of those 60 patents?

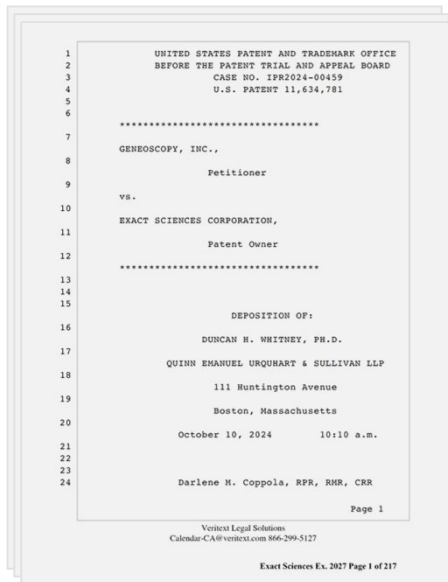
A. No, I did not.

D.I. 197-1, Ex. B (Whitney 1/9/25 Dep. Tr.) at 57:13-16

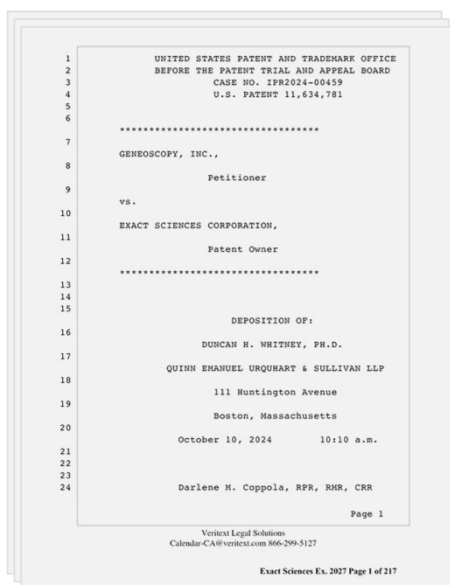
Q. But you don't actually know one way or another whether the 60 patents were written for the sake of protecting some aspects of Cologuard?

A. I think one -- in order to answer that question, one would have to look at each of those individual patents, which you have already asked me about, and you would have to do a careful analysis of what exactly is claimed and described in each of those patents and compare that to all of the aspects of the Cologuard product itself. I have not done that analysis.

EX2054 (Whitney 1/9/25 Dep. Tr.) at 58:16-59:5



No Evidence Other Cited Patents Are Relevant



Q. So you don't have an opinion as to whether or not **those 60 patents** are the reason for patient uptake or accuracy of the test?

A. I have an opinion on patient uptake of Cologuard, but I don't have -- **I'm not asserting that there is a direct connection between the patient uptake and those 60 patents, either in my declaration or here.**

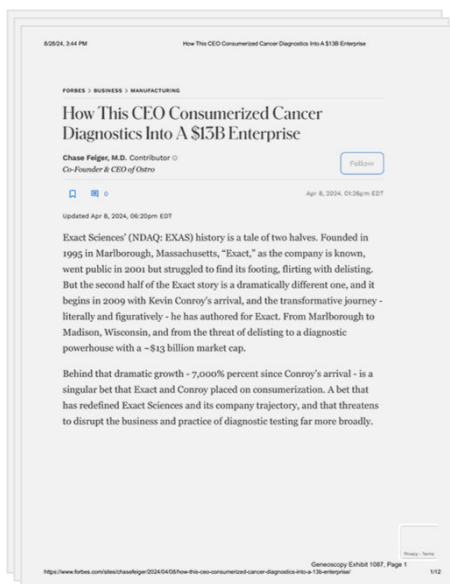
EX2054 (Whitney 1/9/25 Dep. Tr.) at 61:18-62:3; Sur-reply at 20

Q. Are you aware of whether any of **those 60 patents** you reference in Paragraph 9 of your second declaration are expired?

A. I know that **some of the patents that we reference were expired. I don't recall which ones or how many.**

EX2054 (Whitney 1/9/25 Dep. Tr.) at 59:6-12

Evidence Shows That Marketing Is Not The Reason For Success



To be clear, Conroy and Exact have not relied solely - or even primarily - on DTC tactics to turn Cologuard into a powerhouse. Their playbook has included more traditional elements, including innovation, discipline, and grit, that have led to Cologuard achieving adoption by more than 10 million patients over the past decade. “Getting into the clinical guidelines required

EX1087 at 7; Sur-Reply at 23

Petitioner's Marketing Arguments Are Unsupported

Q. So sitting here today, you can't identify any Exact Sciences marketing materials that you considered relating to your opinion?

A. I don't recall specifically which marketing materials I did refer to, if I referred to them.

Q. Do you want to check your Materials Considered and see?

A. Sure. . . . Yeah, I don't believe I see any marketing materials on here or advertising. Is that what you said?

Q. Advertising or marketing materials, either one?

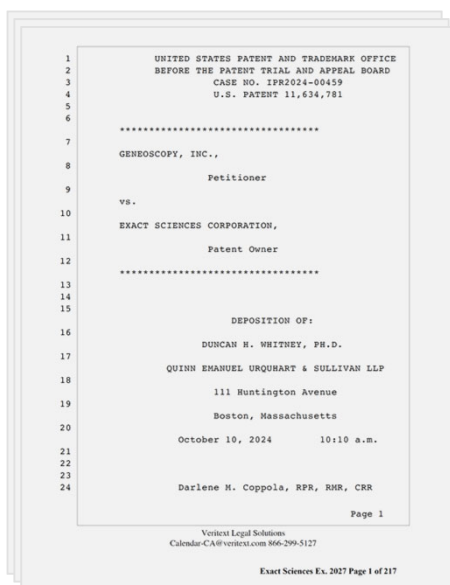
A. No, I don't believe I see anything on here.

EX2054 (Whitney 1/9/25 Dep. Tr.) at 138:20-139:18

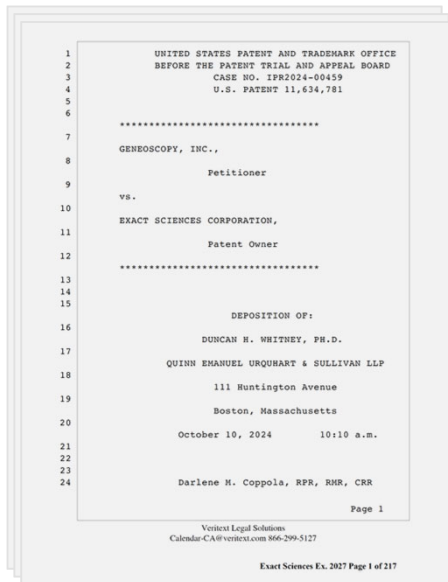
Q. So sitting here today, you can't identify any particular Exact Sciences marketing or advertising materials that you considered in connection with your opinions?

A. I couldn't point to any in particular.

EX2054 (Whitney 1/9/25 Dep. Tr.) at 140:11-15



Efficacy Data Was Critical To Commercial Success



Q. And what is your opinion about that, not about the FDA, but about whether the medical community would prescribe Cologuard if there was not data to support its use?

A. I see. I see what you're getting at. I believe that the -- let me think about the way I want to answer this. **I believe there must be evidence that provides some convincing aspect of utility of a test in order for people to prescribe it.**

EX2054 (Whitney 1/9/25 Dep. Tr.) at 143:3-14; Sur-Reply at 23

Cologuard's Success Is Due To The Merits Of The Invention

With respect to the objective indicia of nonobviousness, while there is evidence that marketing and financing played a role in the success of Hybritech's kits, as they do with any product, it is clear to us on the entire record that the commercial success here was due to the merits of the claimed invention. It cannot be argued on this record that Hybritech's success would have been as great and as prolonged as admittedly it has been if that success were not due to the merits of the invention. The evidence is that these kits compete successfully with numerous others for the trust of persons who have to make fast, accurate, and safe diagnoses. This is not the kind of merchandise that can be sold by advertising hyperbole.

Hybritech Inc. V. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1382 (Fed. Cir. 1986); Sur-Reply at 23-24

THANK YOU

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. § 42.6(e), the undersigned hereby certify that Patent Owner's Demonstratives were served on April 28, 2025 by e-mailing copies to:

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Date: April 28, 2025

Respectfully submitted,

By: /s/ James Glass
James Glass (Reg. No. 46,729)

Counsel for Patent Owner