Comparative Effectiveness Research Review Disposition of Comments Report

Research Review Title: *Comparative Effectiveness of Fecal DNA Testing in Screening for Colorectal Cancer in Average Risk Adults*

Draft review available for public comment from August 1, 2011 to August 29, 2011.

Research Review Citation Lin JS, Webber EM, Beil TL, Goddard KA, Whitlock EP. Fecal DNA Testing in Screening for Colorectal Cancer in Average-Risk Adults. Comparative Effectiveness Review No. 52. (Prepared by the Oregon Evidence-based Practice Center under Contract No. HHS-290-2007-10057-I.) AHRQ Publication No. 12-EHC022-EF. Rockville, MD: Agency for Healthcare Research and Quality. February 2012. Available at: www.effectivehealthcare.ahrq.gov/reports/final.cfm.

Comments to Research Review

The Effective Health Care (EHC) Program encourages the public to participate in the development of its research projects. Each comparative effectiveness research review is posted to the EHC Program Web site in draft form for public comment for a 4-week period. Comments can be submitted via the EHC Program Web site, mail or E-mail. At the conclusion of the public comment period, authors use the commentators' submissions and comments to revise the draft comparative effectiveness research review.

Comments on draft reviews and the authors' responses to the comments are posted for public viewing on the EHC Program Web site approximately 3 months after the final research review is published. Comments are not edited for spelling, grammar, or other content errors. Each comment is listed with the name and affiliation of the commentator, if this information is provided. Commentators are not required to provide their names or affiliations in order to submit suggestions or comments.

The tables below include the responses by the authors of the review to each comment that was submitted for this draft review. The responses to comments in this disposition report are those of the authors, who are responsible for its contents, and do not necessarily represent the views of the Agency for Healthcare Research and Quality.

Source: http://www.effectivehealthcare.ahrq.gov/index.cfm/search-for-guides-reviews-and-reports/?pageaction=displayproduct&productID=971
Published Online: February 2012

Commentator & Affiliation	Section	Comment	Response
Public Comment - David Ahlquist	Executive Summary	ES-1 Background, paragraph 3 Thus far a single company, Exact Sciences, has been the major manufacturer and commercial developer of fecal DNA testing in the US (Table 1). Please correct: Exact Sciences has NOT to date been a manufacturer of fecal DNA test kits. Please change this and all other instances describing Exact Sciences as a "manufacturer" in this document: Please change to: Thus far a single company, Exact Sciences, has been the major developer of fecal DNA testing in the US (Table 1).	We have changed all instances of referring to Exact Sciences incorrectly as a "manufacturer" and replaced with "developer" instead. We have noted that LabCorp is the manufacturer for ColoSure.
Public Comment - David Ahlquist	Executive Summary	ES-1 Background paragraph 3 - Currently, only onefecal DNA test, ColoSure, is commercially available. This test is a single marker fecal DNA assay for methylated vimentin developed by Exact Sciences and distributed by LabCorp. Marketing for commercially available ….. Comment ES-1-1: Please correct as below: Colosure was developed and is distributed by LabCorp. Please change to: Currently, only one fecal DNA test, ColoSure, is commercially available. This test is a single marker fecal DNA assay for methylated vimentin developed and distributed by LabCorp. Marketing for commercially available….	We have changed all instances of referring to Exact Sciences incorrectly as a "manufacturer" and replaced with "developer" instead. We have noted that LabCorp is the manufacturer for ColoSure.

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Public Comment - David Ahlquist	Executive Summary	ES-1 , paragraph 1 - The ACS-MSTFACR's recommendation was based on lower-quality evidence that was excluded from the review conducted on behalf of the USPSTF.4 Comment ES-4-1 Please correct ALL instances in this document related a possible SIP request made to Exact Sciences that was neither received by Exact. Exact has no record of having received a SIP request as part of this review that was generated by a vendor under contract from the AHRQ/EPC process. Please indicate that Exact did provide all available information through the TEP process and collaborated fully with AHRQ in providing information for this review. In the future, please address all correspondence related to AHRQ/EPC requests for information to: Dr. Barry M. Berger, CMO Exact Sciences Corporation 441 Charmany Drive Madison, WI 53719 Please change phrase to: Additional unpublished literature was requested and received from Exact through the Technical Expert Panel process.	We have corrected all instances of SIP request to Exact Sciences. We have been explicit in the report that identification of data from Exact Sciences was through our TEP process, and that a SIP request was sent to LabCorp.
Public Comment - David Ahlquist	Executive Summary	ES-1, Table 1 (T1) Please update table 1 Comment ES1-T1-1- Table 1 - Exact announced the new marker panel that will be tested in Cologuard (referred to as "Next generation" or as "version 3" in this table) on August 2 2011 Methylation markers(2):BMP3 and NDRG4, KRAS (7 point mutations), quantitative fecal hemoglobin ELISA (FIT) and a logistical analytic formula Information on the 2011-2012 pivotal study can be found at http://www.clinicaltrials.gov/ct2/show/NCT01397747?term=Deep-C&rank=1	We have updated Table 1 and the test of the report with this new detail about Cologuard.
Public Comment - David Ahlquist	Executive Summary	ES-2 Table 2 (T2) Edits and Comments on Table 2 Comment ES2 T2 -1 – There is a typo in the value reported for the prevalence of CRC in the Ahlquist study should be: 0.5% (19/3764 = .0050)	Typo corrected.

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Public Comment - David Ahlquist	Executive Summary	ES-2 Table 2 (T2) Edits and Comments on Table 2 Comment ES2 T2-2 – Because fecal DNA does not distinguish between a finding of CRC and adenoma, the specificity value is applicable to both. This may seem awkward and imprecise but the alternative would be to include precursor lesions as false positives when considering specificity for CRC and similarly, including precursor lesions as false positives when considering specificity for CRC and similarly, including precursor lesions as false positives when considering specificity for precursor lesions. This is why specificity in the studies, historically, considers a colon free of neoplasia or with only diminutive polyps as the specificity comparator.	We have considered this comment. In our results table we calculated specificity for CRC and CRC plus advanced adenomas. We understand that fecal DNA test do not distinguish between these two entities, however clinicians may want to know how good the tests is at picking up CRC versus CRC plus pre-cancerous lesions. A similar argument could be made for FOBT (that is does not distinguish between CRC or adenoma but is still informative to know the specificity of CRC versus CRC plus advanced adenomas).
Public Comment - David Ahlquist	Executive Summary	ES-2 Table 2 (T2) Edits and Comments on Table 2 Comment ES2-T2-3 – Imperiale did report 95% CI for specificity though he expressed specificity as the detection of normals rather than as 1-normal detection: 5.6% (95% CI 4.5 – 6.9%), which translates to 94.4% specificity (95% CI 93.1 – 95.5%).	This specificity reported in our tables were calculated for CRC and CRC plus advanced adenomas, the specificity reported in the Imperiale study was for any lesion.

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Public Comment - David Ahlquist	Executive Summary	ES-3 – Table 3 (T3) …very low sensitivities were reported for FOBT which are not consistent with previous best known estimates see Comment ES 3-T3-1 ….Persons 65 years of age and over were disproportionately represented in the study population see Comment ES 3-T3-2 Comment ES3-T3-1: Given that Hemoccult II performance has been shown to be deficient, it was removed as a screening option in the 2008 USPSTF guidelines. The performance of Hemoccult II (HOII) in both the Imperiale and Ahlquist studies likely are better representations of the performance of HOII in clinical practice than older studies. In Imperiale, the cards were prepared and read by 81 sites in the same manner as they were done in everyday practice. In Ahlquist, trained readers reviewed the cards centrally. In both cases the test performed poorly. If these studies were deficient, one would expect that effect to also be seen with elevated specificity, which was not seen. With the advent of FIT and an editorial accompanying the Morikawa paper (Gastroenterol 2004) Dr. Jim Alison references the HOII results of the Imperiale study and uses it to compare with the Morikawa FIT performance. Perhaps, in light of the above the comment it would be most fair to simply state that: …very low sensitivities were reported for FOBT which are not consistent with previous estimates	We have changed our wording to simply state that the very low sensitivities were reported for FOBT which are not consistent with previous estimates. We have also included a sentence in the discussion to explain the discrepancies in estimates.
Public Comment - David Ahlquist	Executive Summary	Comment ES 3-T3-2 ….Persons 65 years of age and over were disproportionately represented in the study population The powering of the study was driven by CRC's and the prevalence of occult CRC in the screening population increases with age. The study recruitment goal was matched to this prevalence curve. If a population proportionate study was done, it would take approximately 20-30% more subjects to find the same number of CRC's and the vast majority would still be in the 65 and older group. The effect of NOT using an age-enriched population was the major reason for the low number of CRC's in Ahlquist (19 CRC's). Ahlquist initially did not follow this approach and the study enrolled predominantly low prevalence younger patients with very rare CRC's, the enrollment process was shifted to older patients later in the study.	We understand the research design rationale for oversampling older adults. However we have mentioned this in the context of applicability (external validity), we did not state that this was problematic for quality (internal validity).

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Public Comment - David Ahlquist	Executive Summary	Page ES- 8 Evidence gaps and future research, paragraph 1 The most critical evidence gap for fecal DNA testing to screen for CRC is the lack of Appropriately designed diagnostic accuracy studies applicable to currently available fecal DNA testing. At a minimum, clinical decision making should be based upon evidence from test validation studies conducted in the intended population (i.e., asymptomatic screening population) for which the test is proposed. Comment ES8-2 - Please consider additional information to include here: To close this gap in the future, such a study for the new stool DNA test has already begun enrollment (DeeP-C study, [http://www.clinicaltrials.gov/ct2/show/NCT01397747?term=Deep-C&rank=1], which will generate both appropriate clinical and technical validity data to support a submission to FDA for pre-market clearance or approval.	We have kept the discussion of evidence gaps separate from future research in the report. We have added detail of the DeeP-C study and clinicaltrials.gov identifier to the future research section.
Public Comment – James Allison	Executive Summary	This is a very thorough and fair evaluation of the current state of knowledge of the usefulness of a fecal DNA test in screening average risk populations for colorectal cancer. It is a very useful document for all companies with a hopeful screening test for colorectal cancer. The sections on evidence gaps, acceptability of testing, analytic validity and future research are particularly good. If it is read carefully, companies wishing to market their screening tests would save a lot of time and money. I particularly like the fairness you have shown to those working on new and perhaps better versions of the Fecal DNA test by saying this review will likely be out of date as new tests and evidence supporting them becomes available within the next 2 years.	No response needed.
Public Comment – James Allison	Executive Summary	I have one minor comment on P. ES-11 where you describe the fecal immunochemical test. In only one FIT is the sample collected with a brush. Different FITs have different collection techniques including probe, stick and brush. They also have wet sampling (specimen deposited into liquid buffer) and dry sampling (specimen placed on a card).	We have added these points on different collection techniques to the suggested place (glossary).

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Public Comment - Barry Berger	Executive Summary	ES-1 Background, paragraph 3 – Thus far a single company, Exact Sciences, has been the major manufacturer and commercial developer of fecal DNA testing in the US (Table 1). Please correct: Exact Sciences has NOT to date been a manufacturer of fecal DNA test kits. Please change this and all other instances describing Exact Sciences as a "manufacturer" in this document: Please change to: Thus far a single company, Exact Sciences, has been the major developer of fecal DNA testing in the US (Table 1).	We have changed all instances of referring to Exact Sciences incorrectly as a "manufacturer" and replaced with "developer" instead. We have noted that LabCorp is the manufacturer for ColoSure.
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Public Comment - Barry Berger	Executive Summary	ES –4 paragraph 2 Additional unpublished literature was sought via a Scientific Information Packet (SIP) request to Exact Sciences. Comment ES-4-1 - Please correct ALL instances in this document related a possible SIP request made to Exact Sciences that was neither received by Exact. Exact has no record of having received a SIP request as part of this review that was generated by a vendor under contract from the AHRQ/EPC process. Please indicate that Exact did provide all available information through the TEP process and collaborated fully with AHRQ in providing information for this review. In the future, please address all correspondence related to AHRQ/EPC requests for information to: Dr. Barry M. Berger, CMO, Exact Sciences Corporation, 441 Charmany Drive, Madison, WI 53719. Please change phrase to: Additional unpublished literature was requested and received from Exact Sciences through the Technical Expert Panel process.	We have corrected all instances of SIP request to Exact Sciences. We have been explicit in the report that identification of data from Exact Sciences was through our TEP process, and that a SIP request was sent to LabCorp.

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Public Comment – Margaret Piper	Executive Summary	Any suggested edits/additional emphasis from the points below that are acted upon should be changed in parallel, if applicable, in the Executive Summary.	None of the below suggested edits pertained to the Executive Summary.
Public Comment - David Ahlquist	Introduction	Page 1 Adenoma to Colorectal Cancer Progression …..Although there is some variation in the exact definition, advanced Adenomas generally refer to adenomas 1 cm or greater, or withvillous components (tubulovillous or villous), or with high-grade or severe dysplasia. Comment Page 1-1 – Serrated polyps (especially, sessile serrated adenomas/polyps) have been increasingly appreciated as critical precursor lesions, particularly in the right colon. (Noffsinger AE Serrated polyps and colorectal cancer: new pathway to malignancy. Annual Review of Pathology Mechanisms of Disease 2009, 4:343-64, Leggett B, Whitehall V, Role of the serrated pathway in colorectal cancer pathogenesis, Gastroenterol 2010, 138:2088-2100) and may lead to up to 35% of CRC's (Snover DC, Update on the serrated pathway to colorectal carcinoma, Human Pathology 2011, 42:1-10). Importantly, these lesions are harder to detect on colonoscopy as they may be "flat" and harder to identify. Importantly, they are associated with molecular biomarkers that can be detected on fecal DNA analysis in the screening setting. (Hussain FTN, Yab TC, Harrington JJ, Taylor WR, Smyrk TC, Mahoney DW, Zou H, Ahlquist DA, Non invasive detection of serrated colorectal polyps by stool assay of methylated Vimentin and mutant BRAF genes, abstract, DDW 2010)	We did not change our definition of advanced adenomas, as none of the definitions we have seen (or used by the studies) explicitly include "serrated polyps". We recognize the important of the growing appreciation of "flat lesions" and this is acknowledged in the introduction and the discussion of the report.

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Public Comment - David Ahlquist	Introduction	Page 3 …. Assay development has had to focus on improving the analytic sensitivity (or lower limit of detection) of test methodology and technology. Techniques have been developed to better preserve stool DNA (e.g., buffer to stabilize DNA) and extract DNA from stool. In addition, techniques to enrich target DNA by selective capture from stool followed by digital or emulsion PCR have been developed (e.g., BEAMing and digital melt curve analysis) and seem promising in improving assay sensitivity.16,18 See comment Comment Page 3 -2 – Based on the findings of the BEAMing assay, which indicated the required discrimination of signal to noise at the 1:5000 to 1:1000 level for detecting DNA mutations in a background of normal human DNA (Diehl 2008, Gastroenterology) an analytic platform was developed to meet that need: quantitative allele specific real-time target and signal amplification (QuARTS®). This platform has been incorporated into Cologuard, which is the subject of the ongoing pivotal study (DeeP-C) (Zou et al., 2010, AACC abstract). Information on the 2011-2012 pivotal study (Deep-C) can be found at http://www.clinicaltrials.gov/ct2/show/NCT01397747?term=Deep-C&rank=1	We have added QuARTS to the discussion section under future research (where we also identify studies from conference abstracts). We have not included it into the results, as no studies meeting our inclusion criteria for QuARTS were identified.
Public Comment - David Ahlquist	Introduction	Page 3, paragraph 3.… Thus far a single company, Exact Sciences, has been the major manufacturer and commercial developer of fecal DNA testing in the US. See comment Comment Page 3-3: Exact Sciences has NOT to date been a manufacturer of stool DNA based test kits. Thus far a single company, Exact Sciences, has been the major developer and licensor of fecal DNA based testing for colorectal cancer screening in the U.S.	We have changed all instances of referring to Exact Sciences incorrectly as a “manufacturer” and replaced with “developer” instead. We have noted that LabCorp is the manufacturer for ColoSure.

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Public Comment - David Ahlquist	Introduction	Page 3, paragraph 3 Thus far a single company, Exact Sciences, has been the major manufacturer and commercial developer of fecal DNA testing in the US. Since developing their prototype, the manufacturer has marketed two tests, PreGen Plus (2003-2008) and ColoSure (the only commercially available test currently in the US) (Table 1). Please revise: Exact Sciences has NOT to date been a manufacturer of fecal DNA test kits. Thus far a single company, Exact Sciences, has been the major developer of fecal DNA testing in the US. Since developing their prototype, (further developed and offered as a LDT [PreGen Plus] by LabCorp 2003-2008), Exact has licensed technology to Lab Corp, who manufactures and markets ColoSure , the only commercially available test currently in the US (Table 1).	We have changed all instances of referring to Exact Sciences incorrectly as a "manufacturer" and replaced with "developer" instead. We have noted that LabCorp is the manufacturer for ColoSure.
Public Comment - David Ahlquist	Introduction	Page 3 - The major analytic advances included the use of technologies to isolate human DNA targets, that improve the analytic sensitivity or lower limit of detection of these assays (BEAMing, QuARTS) Comment Page 3-5……. Additional language to consider -- The major analytic advances included the optimization use of technologies to isolate human DNA targets, development of new methods that improve the analytic sensitivity or lower limit of detection of these assay (BEAMing, QuARTS), and the identification of highly discriminant marker panels to increase sensitivity for CRC and precursor lesions.	We have added QuARTS to the discussion section under future research (where we also identify studies from conference abstracts). We have not included it into the results, as no studies meeting our inclusion criteria for QuARTS were identified.
Public Comment - David Ahlquist	Introduction	Page 6 Table I - see previous comments on Table 1 see previous comments - Comment ES1-T1-1	Duplicate comment.

Commentator & Affiliation	Section	Comment	Response
Public Comment - David Ahlquist	Introduction	Page 7, paragraph 1 Only one fecal DNA test for the detection of adenomas and colorectal tumors is currently commercially available. This test, ColoSure, is regulated by the Centers for Medicare and Medicaid Services under the Clinical Laboratory Improvement Amendments (CLIA) of 1988 developed by Exact Sciences and distributed by LabCorp Comment P7-1 Clarifications needed, Exact Sciences did NOT develop and does NOT provide ColoSure, change to: Only one fecal DNA test for the detection of adenomas and colorectal tumors is currently commercially available. This test, ColoSure®, was developed and is provided by LabCorp as a laboratory developed test and is regulated by the Centers for Medicare and Medicaid Services under the Clinical Laboratory Improvement Amendments (CLIA) of 1988.	We have changed all instances of referring to Exact Sciences incorrectly as a "manufacturer" and replaced with "developer" instead. We have noted that LabCorp is the manufacturer for ColoSure.
Public Comment - David Ahlquist	Introduction	Page 7, paragraph 1 … is approved as a direct-to consumer test .. Comment P7-2… We do not understand this term nor the intention. Was the intention to indicate that direct to consumer advertising is allowed vs. "over the counter" or consumer generated request for test? Test of this nature are generally not approved for direct purchase by consumers. Consumers must go through a licensed clinician, which may be a physician, NP, or PA, depending on the regulations of the state with authority, who must order the test and follow up with the patient. Certain internet test access portals employ physicians who would order tests not directly available to consumers, at a consumer's request. This may appear to be being sold directly to the consumer, but it is not. While there are fecal occult blood tests that can be purchased over the counter in drug stores and performed and results sent to patients directly – however this is not so for high complexity tests (under CLIA) like fecal DNA.	We have removed the term direct-to-consumer.
Public Comment - David Ahlquist	Introduction	Page 7- As a laboratory developed (homebrew) test, ….. Comment P7-3 - the term "Home brew" has been phased out of use and has been replaced with "laboratory developed test (LDT)". We suggest removing the term "home-brew" to reflect current terminology.	We have removed the term "home-brew".

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Public Comment - David Ahlquist	Introduction	Page 7 - ColoSure is not subject to regulation by the U.S. Food and Drug Administration (FDA) and has not obtained FDA clearance or approval. Currently, there are no fecal DNA tests approved by the FDA for screening or diagnosing of CRC. Historically, the FDA's oversight of genetic testing has been focused on commercial test kits. Comment P7-4 –All LDT's are subject to FDA oversight, according to FDA, but FDA has chosen to "exercise enforcement discretion" for these LDT testing services. We suggest changing this section to read: ColoSure has not obtained FDA clearance or approval. Currently, there are no fecal DNA tests approved by the FDA for screening or diagnosing of CRC. Historically, the FDA's oversight of genetic testing has been focused on commercial test kits.	We have changed the wording of this sentence and noted that the FDA does have oversight but has chosen to "exercise enforcement discretion".
Public Comment - David Ahlquist	Introduction	Page 7- Correction to timeline and components of new fecal DNA test panel A new fecal DNA test from Exact Sciences is projected to be available in 2012 (Table 1) Although the actual markers are not known, it is clear that the molecular are different from the current test version and will include an immunohistochemical assay for fecal hemoglobin).37 It is yet unclear if this fecal immunohistochemical test (FIT), is similar or different from other currently available FITs. Comment P7-5, Please revise this section to read: Data from the average risk screening population pivotal study utilizing a new fecal DNA test from Exact Sciences is projected to be available in late 2012 (Table 1) and should appear in a publication in 2013. The molecular markers include NDRG4, BMP3, point mutations (7) in k-ras Exon 2, a fecal immunohistochemical test (FIT) for fecal hemoglobin and a logistic analytic model.37 The fecal immunohistochemical test, while optimized for this assay, is similar to other currently available ELISA based FITs.	We have added this updated information to Table 1.
Public Comment – James Allison	Introduction	Adenoma to CRC Progression – In the first sentence you make it seem like a polyp and an adenoma are different entities. Many polyps are adenomas but some like the hyperplastic polyp or juvenile polyp are not. The term "neoplastic" polyp would cover all adenomas.	We have changed the term to "neoplastic polyp".

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Public Comment – James Allison	Introduction	Screening of Colorectal Cancer – Clinicians generally refer to FIT as fecal immunochemical test and not fecal immunohistochemical test. I think one term should be used consistently and all of my papers and most of the ones I review refer to the test as a fecal immunochemical test. I do have an email in to two highly regarded lab chemists in the UK who will be able to tell me if there is a meaningful difference between these two terms.	We have changed the wording throughout the report (and tables) from immunohistochemical to immunochemical.
Public Comment – James Allison	Introduction	Though certainly not necessary for this review, you might consider adding that almost of the increased screening rate has been due to colonoscopy screening in the insured population since colonoscopy was approved as a CMS covered test for Medicare reimbursement. No such increase has occurred in the uninsured/underserved population. (See slides below)	We have considered these slides, but did not include the data as it is not central to the report.
Public Comment – James Allison	Introduction	Rationale and current practice - The paragraph on the differences between guidelines and the reasons is very important and, the consequences should be an important lesson for those who make guideline recommendations without good evidence behind them. A lot of people lost money investing in the company lobbying for guideline coverage of the fecal DNA test and a lot of hopeful patients were dismayed to learn these recommended tests were no longer available	No response needed.
Public Comment – James Allison	Introduction	Evolution of fecal DNA testing for CRC screening -The information about what fecal DNA tests are available now (only Colosure) and the limited evidence it has behind it is very important for consumers and investors. So is the information about what is known about the next-generation sDNA version 3.0 and what it will take in terms of findings and time to earn it a recommended status in evidence based guidelines and CMS approval for reimbursement in Medicare patients.	No response needed.

Commentator & Affiliation	Section	Comment	Response
Public Comment - Barry Berger	Introduction	Page 1 Adenoma to Colorectal Cancer Progression …..Although there is some variation in the exact definition, advanced adenomas generally refer to adenomas 1 cm or greater, or with villous components (tubulovillous or villous), or with high-grade or severe dysplasia. Comment Page 1-1 – Serrated polyps (especially, sessile serrated adenomas/polyps) have been increasingly appreciated as critical precursor lesions, particularly in the right colon. (Noffsinger AE Serrated polyps and colorectal cancer: new pathway to malignancy. Annual Review of Pathology Mechanisms of Disease 2009, 4:343-64, Leggett B, Whitehall V, Role of the serrated pathway in colorectal cancer pathogenesis, Gastroenterol 2010, 138:2088-2100) and may lead to up to 35% of CRC's (Snover DC, Update on the serrated pathway to colorectal carcinoma, Human Pathology 2011, 42:1-10). Importantly, these lesions are harder to detect on colonoscopy as they may be "flat" and harder to identify. Importantly, they are associated with molecular biomarkers that can be detected on fecal DNA analysis in the screening setting. (Hussain FTN, Yab TC, Harrington JJ, Taylor WR, Smyrk TC, Mahoney DW, Zou H, Ahlquist DA, Non invasive detection of serrated colorectal polyps by stool assay of methylated Vimentin and mutant BRAF genes, abstract, DDW 2010)	We did not change our definition of advanced adenomas, as none of the definitions we have seen (or used by the studies) explicitly include "serrated polyps". We recognize the important of the growing appreciation of "flat lesions" and this is acknowledged in the introduction and the discussion of the report.

Commentator & Affiliation	Section	Comment	Response
Public Comment - Barry Berger	Introduction	Page 3 …. Assay development has had to focus on improving the analytic sensitivity (or lower limit of detection) of test methodology and technology. Techniques have been developed to better preserve stool DNA (e.g., buffer to stabilize DNA) and extract DNA from stool. In addition, techniques to enrich target DNA by selective capture from stool followed by digital or emulsion PCR have been developed (e.g., BEAMing and digital melt curve analysis) and seem promising in improving assay sensitivity.16,18 See comment Comment Page 3 -2 – Based on the findings of the BEAMing assay, which indicated the required discrimination of signal to noise at the 1:5000 to 1:1000 level for detecting DNA mutations in a background of normal human DNA (Diehl 2008, Gastroenterology) an analytic platform was developed to meet that need: quantitative allele specific real-time target and signal amplification (QuARTS®). This platform has been incorporated into Cologuard, which is the subject of the ongoing pivotal study (DeeP-C) (Zou et al., 2010, AACC abstract). Information on the 2011-2012 pivotal study (Deep-C) can be found at http://www.clinicaltrials.gov/ct2/show/NCT01397747?term=Deep-C&rank=1	We have added QuARTS to the discussion section under future research (where we also identify studies from conference abstracts). We have not included it into the results, as no studies meeting our inclusion criteria for QuARTS were identified.
Public Comment - Barry Berger	Introduction	Page 3, paragraph 3 - Thus far a single company, Exact Sciences, has been the major manufacturer and commercial developer of fecal DNA testing in the US. See comment Comment Page 3-3 : Exact Sciences has NOT to date been a manufacturer of stool DNA based test kits. Thus far a single company, Exact Sciences, has been the major developer and licensor of fecal DNA based testing for colorectal cancer screening in the U.S.	We have changed all instances of referring to Exact Sciences incorrectly as a “manufacturer” and replaced with “developer” instead. We have noted that LabCorp is the manufacturer for ColoSure.

Commentator & Affiliation	Section	Comment	Response
Public Comment - Barry Berger	Introduction	Page 3, paragraph 3 Thus far a single company, Exact Sciences, has been the major manufacturer and commercial developer of fecal DNA testing in the US. Since developing their prototype, the manufacturer has marketed two tests, PreGen Plus (2003-2008) and ColoSure (the only commercially available test currently in the US) (Table 1). Please revise: Exact Sciences has NOT to date been a manufacturer of fecal DNA test kits. Thus far a single company, Exact Sciences, has been the major developer of fecal DNA testing in the US. Since developing their prototype, (further developed and offered as a LDT [PreGen Plus] by LabCorp 2003-2008), Exact has licensed technology to Lab Corp, who manufactures and markets ColoSure , the only commercially available test currently in the US (Table 1).	We have changed all instances of referring to Exact Sciences incorrectly as a "manufacturer" and replaced with "developer" instead. We have noted that LabCorp is the manufacturer for ColoSure.
Public Comment - Barry Berger	Introduction	Page 3 - The major analytic advances included the use of technologies to isolate human DNA targets, that improve the analytic sensitivity or lower limit of detection of these assays (BEAMing, QuARTS) Comment Page 3-5……. Additional language to consider -- The major analytic advances included the optimization use of technologies to isolate human DNA targets, development of new methods that improve the analytic sensitivity or lower limit of detection of these assay (BEAMing, QuARTS), and the identification of highly discriminant marker panels to increase sensitivity for CRC and precursor lesions.	We have added QuARTS to the discussion section under future research (where we also identify studies from conference abstracts). We have not included it into the results, as no studies meeting our inclusion criteria for QuARTS were identified.
Public Comment - Barry Berger	Introduction	Page 6 Table I see previous comments on Table 1 see previous comments Comment ES1-T1-1	Duplicate comment.

Commentator & Affiliation	Section	Comment	Response
Public Comment - Barry Berger	Introduction	Page 7, paragraph 1 - Only one fecal DNA test for the detection of adenomas and colorectal tumors is currently commercially available. This test, ColoSure, is regulated by the Centers for Medicare and Medicaid Services under the Clinical Laboratory Improvement Amendments (CLIA) of 1988 developed by Exact Sciences and distributed by LabCorp Comment P7-1 Clarifications needed, Exact Sciences did NOT develop and does NOT provide ColoSure, change to: Only one fecal DNA test for the detection of adenomas and colorectal tumors is currently commercially available. This test, ColoSure®, was developed and is provided by LabCorp as a laboratory developed test and is regulated by the Centers for Medicare and Medicaid Services under the Clinical Laboratory Improvement Amendments (CLIA) of 1988.	We have changed all instances of referring to Exact Sciences incorrectly as a "manufacturer" and replaced with "developer" instead. We have noted that LabCorp is the manufacturer for ColoSure.
Public Comment - Barry Berger	Introduction	Page 7, paragraph 1 - is approved as a direct-to consumer test.. Comment P7-2 - We do not understand this term nor the intention. Was the intention to indicate that direct to consumer advertising is allowed vs. "over the counter" or consumer generated request for test? Test of this nature are generally not approved for direct purchase by consumers. Consumers must go through a licensed clinician, which may be a physician, NP, or PA, depending on the regulations of the state with authority, who must order the test and follow up with the patient. Certain internet test access portals employ physicians who would order tests not directly available to consumers, at a consumer's request. This may appear to be being sold directly to the consumer, but it is not. While there are fecal occult blood tests that can be purchased over the counter in drug stores and performed and results sent to patients directly – however this is not so for high complexity tests (under CLIA) like fecal DNA.	We have removed the term direct-to-consumer.

Commentator & Affiliation	Section	Comment	Response
Public Comment - Barry Berger	Introduction	Page 7 - ColoSure is not subject to regulation by the U.S. Food and Drug Administration (FDA) and has not obtained FDA clearance or approval. Currently, there are no fecal DNA tests approved by the FDA for screening or diagnosing of CRC. Historically, the FDA's oversight of genetic testing has been focused on commercial test kits. Comment P7-4 – All LDT's are subject to FDA oversight, according to FDA, but FDA has chosen to "exercise enforcement discretion" for these LDT testing services. We suggest changing this section to read: ColoSure has not obtained FDA clearance or approval. Currently, there are no fecal DNA tests approved by the FDA for screening or diagnosing of CRC. Historically, the FDA's oversight of genetic testing has been focused on commercial test kits.	We have changed the wording of this sentence and noted that the FDA does have oversight but has chosen to "exercise enforcement discretion".
Public Comment - Barry Berger	Introduction	Page 7 Correction to timeline and components of new fecal DNA test panel A new fecal DNA test from Exact Sciences is projected to be available in 2012 (Table 1) Although the actual markers are not known, it is clear that the molecular are different from the current test version and will include an immunohistochemical assay for fecal hemoglobin).37 It is yet unclear if this fecal immunohistochemical test (FIT), is similar or different from other currently available FITs. Comment P7-5 - Please revise this section to read: Data from the average risk screening population pivotal study utilizing a new fecal DNA test from Exact Sciences is projected to be available in late 2012 (Table 1) and should appear in a publication in 2013. The molecular markers include NDRG4, BMP3, point mutations (7) in k-ras Exon 2, a fecal immunohistochemical test (FIT) for fecal hemoglobin and a logistic analytic model.37 The fecal immunohistochemical test, while optimized for this assay, is similar to other currently available ELISA based FITs.	We have added this updated information to Table 1.
Public Comment – Margaret Piper	Introduction	P2. I don't quite understand the information provided in the parens of this sentence: "The ACS-MSTF-ACR recommendation was based on lower-quality evidence that was excluded from the review conducted on behalf of the USPSTF, which has more stringent inclusion and quality criteria (e.g., case-control studies of screening accuracy or lack of a reference standard)."	We have deleted the parenthetical.

Commentator & Affiliation	Section	Comment	Response
Public Comment – Margaret Piper	Introduction	P2-3. Suggested edit for clarity: "Although the presence of these alterations does not guarantee a progression to cancer, it is thought that these molecular markers can identify the adenomas most likely to develop into cancer, in addition to detecting early stages of CRC."	We have revised the text as suggested.
Public Comment – Margaret Piper	Introduction	P3. EITHER, "Some of the most common (and well studied) DNA markers in stool include mutations in APC, KRAS, and TP53. . ." OR, "Some of the most common (and well studied) tests for DNA markers in stool include mutational analysis of APC, KRAS, and TP53. . ." (adjust rest of sentence according to choice)	We have revised the text as suggested.
Peer Reviewer 1	Introduction	Fine.	No response needed.
Peer Reviewer 2	Introduction	The introduction does not discuss important limitations of currently available fecal tests, which would seem important. This is an important omission, because some of the limitations of fecal DNA also apply to other forms of fecal testing. 1. gFOBT has been studied in RCT, but has poor sensitivity, and virtually no data on programmatic adherence in clinical practice. Since program adherence is crucial to effectiveness, this is a major gap in knowledge. 2. high-sensitivity guaiac and FIT have not been studied in RCT. As the document points out, there is little standardization of FIT	We agree this is an important topic, we have added a few sentences in the **discussion** to address the limitations (in evidence) of other fecal tests.
Peer Reviewer 3	Introduction	This section of the Review provides a concise summary of CRC development, screening in general and fecal DNA testing, in particular.	No response needed.
Peer Reviewer 4	Introduction	yes to all	No response needed.
Peer Reviewer 5	Introduction	Fine as written.	No response needed.

Commentator & Affiliation	Section	Comment	Response
Public Comment - David Ahlquist	Methods	Page 10 Literature Search Strategy - Additional unpublished literature was sought via a Scientific Information Packet (SIP) request to Exact Sciences (the developer of the only currently available fecal DNA test). Comment P10-1 - As indicated above - Exact Sciences provided data through the TEP process and did not receive a SIP request. We suggest revising to the following if appropriate: Additional unpublished literature was solicited from LabCorp, (the developer and provider of Colosure, the only currently available fecal DNA test).	We have corrected all instances of SIP request to Exact Sciences. We have been explicit in the report that identification of data from Exact Sciences was through our TEP process, and that a SIP request was sent to LabCorp.
Public Comment - Barry Berger	Methods	Page 10 Literature Search Strategy - Additional unpublished literature was sought via a Scientific Information Packet (SIP) request to Exact Sciences (the developer of the only currently available fecal DNA test). Comment P10-1 - As indicated above - Exact Sciences provided data through the TEP process and did not receive a SIP request. We suggest revising to the following if appropriate: Additional unpublished literature was solicited from LabCorp, (the developer and provider of Colosure, the only currently available fecal DNA test).	We have corrected all instances of SIP request to Exact Sciences. We have been explicit in the report that identification of data from Exact Sciences was through our TEP process, and that a SIP request was sent to LabCorp.
Public Comment – Margaret Piper	Methods	P11. Suggested edits for clarity: "We initially excluded case-control studies and cohorts in high-risk patients as this study design and distorted selection of patients has been shown to overestimate sensitivity.39,40 However, because of a paucity of included studies to address Key Questions 1 and 2, we decided to identify and examine the excluded case-control or cohort studies in high-risk patients, keeping in mind the inherent biases of these study designs."	We have revised the text as suggested.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer 1	Methods	Search strategy generally adequate, as are inclusion and exclusion criteria. Note the authors exclude case control studies. There are numerous, but the CRC cases have already been diagnosed; therefore, they do not truly represent a screening context, which is the subject of the report. However, there is the following consideration, which it is not clear the authors have taken into account. In general with screening markers, one wants to evaluate the marker in a screening (pre-diagnosed and asymptomatic) population, as mentioned above. However, it is difficult to accrue large numbers of diseased in such a population, as is acknowledged. In 2 relatively large prospective, studies, only 19 and 31 CRC cases were accrued. With CRC, though, where there is already an effective screening modality, it may be possible to validly utilize diagnosed cases (and a case-control design) when estimating sensitivity. Specifically, if cases are diagnosed from a screening colonoscopy and the fecal DNA is performed shortly thereafter (before surgery), then these probably represent a valid surrogate for a screening population with prevalent cancer, i.e., a valid surrogate from which to calculate sensitivity. For example, in the study by Itzkowitz (2008), cases subjects were subjects with CRC diagnosed at colonoscopy and their fecal samples were taken 6-14 days after colonoscopy and before pre-surgical bowel prep. However, it is not clear in the article whether all (or the majority) of the above colonoscopies were purely for screening purposes. Other case-control studies, though, may (or may not) be more clear on this point. A case-control study would not be valid to estimate specificity per se, since subjects with small or hyperplastic polyps would have had those removed, which could affect later DNA test performance. However, such a design could provide estimates of the false positive rate in those with normal colonoscopy (no polyps). This could be informative about the overall specificity of the fecal DNA test.	Although we agree with the reviewer that adequate numbers of cancers is difficult to accrue, we have not changed the review exclusion of case-control studies. Empiric data (from others and from this report) has shown consistently that this study design has led to overestimation of test performance. We have added a sentence to the discussion that acknowledges that nested case-control designs, case-control studies nested in “screening” cohorts, in which the stool sample is taken before colonoscopy is reasonable. However, stool samples taken after colonoscopy is problematic as colonoscopies often biopsy or remove suspicious lesions (no just small or hyperplastic lesions).

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer 1	Methods	Finally, it is not clear, but it also seems that case-control studies were not considered when evaluating patient preferences/acceptability. Here again, it seems that evaluation of these outcomes could be done in a valid way with case-control studies. Itzkowitz et al (2008) does also report on patient acceptability, though it is flawed for a reason un-related to the case control design (no report of survey response rate).	Case-control studies were considered for Key Question 5 (pt acceptability). The article Itzkowitz 2007 was re-evaluated and we have included it in our results, although it does not change the conclusion for this key question.
Peer Reviewer 2	Methods	Methodology is strong	No response needed.
Peer Reviewer 3	Methods	The Methods are outlined in considerable detail.	No response needed.
Peer Reviewer 3	Methods	Search strategies are thoroughly explained; inclusion and exclusion criteria are appropriate.	No response needed.
Peer Reviewer 3	Methods	The definitions and diagnostic criteria for the outcome measures are appropriate as are the statistical methods.	No response needed.
Peer Reviewer 4	Methods	yes to all	No response needed.
Peer Reviewer 5	Methods	Yes	No response needed.
Public Comment - David Ahlquist	Results	Page 20 The most recent study by Ahlquist and colleagues published in 2008 was a manufacturer funded diagnostic accuracy study conducted in a large cohort (n enrolled=4482) of 50 to 80 year olds at average risk for CRC (Table 5).34 Comment P20-1: Correction suggested as the Ahlquist study was an NCI funded study: The most recent study by Ahlquist and colleagues published in 2008 was an NCI-funded diagnostic accuracy study conducted in a large cohort (n enrolled=4482) of 50 to 80 year olds at average risk for CRC (Table 5).34	The Ahlquist study appears to have had both NCI and industry funding, we have amended the sentence to reflect both funding mechanisms.

Commentator & Affiliation	Section	Comment	Response
Public Comment - David Ahlquist	Results	Page 20 The study evaluated a precommercial stood DNA test (SDT-1, pre-commercial version of PreGen Plus), which was subsequently changed during the study to a different multimarker panel (SDT- 2). Comment P20-2: SDT-2 was added as a substudy in order to evaluate newer marker approaches to fecal DNA analysis that evolved after the study had begun. The study itself was not changed. Essentially, the biorepository of specimens generated by the base study was used to study the next generation marker combinations (SDT-2). Please note typo in report “stood” should be “stool”	We have changed the sentence and fixed the typo.
Public Comment - David Ahlquist	Results	Page 20 After reviewing interim results on the first 2497 participants, it was decided to implement a newer test, SDT-2. The next 1267 participants were part of a case-control study design in which the SDT-2 test was run on a subset of patients (n analyzed=217) Comment P20-3: The interim data look was precipitated by a decision to add Hemoccult SENSA as a comparator. The specimens used for SDT-2 were taken as a subset of the entire study population. This study took many years to accrue sufficient patients to power the study, resulting in the evolution of technology over the course of the recruitment period.	We have reworded the sentence to accurately reflect the details provided by the reviewer (who is the lead author of this included study).

Commentator & Affiliation	Section	Comment	Response
Public Comment - David Ahlquist	Results	P 20-21 - Although the lack of adherence to dietary and medication restrictions should, in theory, not decrease sensitivity of FOBT, given the very low sensitivities reported in this study (as compared to other, more generally accepted, estimates for Hemoccult II and Hemoccult Sensa),102 the quality and applicability of all FOBT test results are questionable. Comment P20-21-1 Consider changing this section: the quality and applicability of all FOBT test results are questionable. Consider revising to: HOII performance in Ahlquist was similar to that seen in Imperiale and lower than that seen in older studies Note: The poor performance of HOII in Ahlquist and also in Imperiale is likely much more reflective of HOII performance in actual clinical practice, given the size of both studies, the fact that all subjects had a reference colonoscopy and Ahlquist had HOII interpreted centrally by expert readers and Imperiale had HOII performed as it was in daily practice at each of the 81 sites. In the Ahlquist study, the Hemoccult manufacturer actually turored the laboratory technicians on the optimal performance and interpretation of the test prior to the study. Dr. James Allison quotes the Imperiale HOII performance as indicative of gFOBT performance in his editorial accompanying the 2004 Morikawa paper published in Gastroenterology. The fact that USPSTF subsequently eliminated low sensitivity gFOBT's like HOII from the guidelines for CRC screening supports is supportive of this view. HOII performance as reported in the very large studies of Ahlquist and Imperiale was likely truly representative of performance in daily practice and the criticism may not be warranted. Please also see similar criticism in Table 7 page 25	We have changed our wording to simply state that the very low sensitivities were reported for FOBT which are not consistent with previous estimates. We have also included a sentence in the discussion to explain the discrepancies in estimates.
Public Comment - David Ahlquist	Results	Page 21 - See my changes - About 50 percent more patients (641 vs. 426) did not provide an adequate sample for fecal DNA testing as compared to Hemoccult II, which may signal differences infeasibility or acceptability to patients. Comment P21-1: The Schroy preference study used the cohort of subjects who completed HOII, fecal DNA and colonoscopy. Fecal DNA testing was preferred over FOBT, a finding supported by a subsequent study using CRC screen naïve subjects. The 641 subjects primarily included patients who simply did not perform the test rather than those who submitted an inadequate sample.	We have considered this sentence, but no changes were made. The Schroy study is discussed as part of Key Question 4.

Commentator & Affiliation	Section	Comment	Response
Public Comment - David Ahlquist	Results	Page 22 - inclusion of a mostly older population (in which three quarters of the study population was over 65 years of age), Comment P22-1: Note concerning the study limitation based on the age distribution of study participants. Consider reconsider. Note: The experienced CRC prevalence in average risk screening studies increases dramatically with increasing age, approximately 1CRC /1000 subjects at age 50 and 10CRC's /1000 subjects at age 65. Designing studies of reasonable size that are powered by the number of subjects with CRC requires incorporating age related prevalence factors into study design. To date, there is no evidence of age related effects on analytic sensitivity and specificity for DNA biomarkers. By designing the study enrollment to mirror the age related biology of the disease, the number of subjects screened can be decreased and the required study power can be achieved more efficiently. This design has been approved by both FDA and CMS and was recently copied by the Pre-Sept average risk CRC screening study of plasma Sept in 9. NPV and PPV will vary with prevalence and thus performance related to those metrics must be calculated taking age related prevalence into account.	We understand the research design rationale for oversampling older adults. However we have mentioned this in the context of applicability (external validity), we did not state that this was problematic for quality (internal validity).
Public Comment - David Ahlquist	Results	Page 26 – Key Question 4. Lack of analytic validity data on Colosure. We did not receive any information in the form of the Scientific Information Packet that was requested from Exact Sciences. For analytic validity, Comment P26-1 Please correct the statement - Please correct this statement to read: Additional unpublished literature was requested and received from Exact Sciences through the TEP process. Note: For data related to the currently available fecal DNA test AHRQ should contact LabCorp for information on technical validity of Colosure as LabCorp is the developer and sole provider of Colosure and as such is the only entity that would have such data Note: Exact Sciences did not receive any request for a SIP. As the latest version of fecal DNA testing is engaged in a pivotal average risk screening study (DeeP-C study) for FDA pre-market clearance or approval, there will be full analytic validity data generated and included in the FDA submission. Such data will be available in the future.	We have corrected all instances of SIP request to Exact Sciences. We have been explicit in the report that identification of data from Exact Sciences was through our TEP process, and that a SIP request was sent to LabCorp.

Commentator & Affiliation	Section	Comment	Response
Public Comment - David Ahlquist	Results	Page 27 - The applicability of this experiment is also poor given that the accuracy study was conducted in plasma samples, rather than stool samples, and methyl-BEAMing does not appear to be used in the assay evaluated by Ahlquist and colleagues (included in Key Question 2), or in the currently available methylated vimentin test. Comment P27 -1: Note: BEAMing is an exquisitely sensitive analytic assay that quantifies the DNA biomarker strand count in stool or plasma on a per gm or ml basis. It has not yet been reduced to clinical practice. However, the technique provided critical insight that led to improved DNA isolation techniques by demonstrating the strand length of mutated and altered sequences (very small) and the signal to noise ratio discrimination required of a test for mutated or alter fecal DNA in a background of normal (Wild type) DNA (1:10,000). The QuARTS platform discussed above was designed to discriminate MT and WT at the level of 1:10,000. Both Diehl in 2008 (Gastroenterol) and Li in 2009 studied BEAMing in both stool and plasma. Diehl studied 16 matched pairs of stool/plasma and reported the superiority of stool as a test matrix over plasma. Earlier studies had shown, as well, that plasma DNA biomarker levels in normal controls and patients with precursor CRC lesions that are not invasive had similar levels of plasma DNA biomarkers. This demonstrates that plasma will not be a CRC screening strategy with direct potential to prevent CRC through precursor lesion detection. Precursors identified subsequent to a “positive” plasma CRC screening assay, based on insights from the BEAMing data, would most likely be the result of random failure of plasma assay specificity rather than a reflection of the sensitivity of the assay.	We have changed our sentence to reflect that this technology has not yet been applied to fecal DNA tests that have been in clinical practice.

Commentator & Affiliation	Section	Comment	Response
Public Comment – David Ahlquist	Results	Page 41 – Through conversations with our TEP, conference presentations, and information from investment conference telephone calls, we understand that Exact Science is currently developing a new assay, a multimarker fecal DNA test plus FIT, Cologuard. This test is expected to be available within the next couple of years. To our knowledge it includes a combination of different markers (methylation markers, mutations in KRAS exon 2) and a FIT, and uses new proprietary technology (Table 1). However, the details of the assay are still unknown. Comment P36-2: Please correct the phrase with the following additional information. Through conversations with our TEP, conference presentations, and information from investment conference telephone calls, we understand that Exact Science is currently developing a new assay, a multi-marker fecal DNA test plus FIT. This test includes two methylation markers (NDRG4 and BMP-3), 7 point mutations in Kras Exon 2, a FIT, and a logistical analytic model. The pivotal study is currently ongoing.	We have updated our text to reflect this new information available about Cologuard.
Public Comment – James Allison	Results	This section raises the very important issue that there must be evidence of safe screening intervals for any screening test being considered for evidence based guideline recommendation. To market a test recommending testing every (x) years is not ethical unless and until there is evidence showing this in the peer reviewed literature.	No response needed.
Public Comment – James Allison	Results	p. 21 - I am fairly certain that the Ahlquist study had NCI and industry funding and that should be mentioned if confirmed.	We have changed the sentence to reflect funding from NCI as well as industry.

Commentator & Affiliation	Section	Comment	Response
Public Comment – James Allison	Results	P. 21 – The real problem with the results of Hemoccult II use in the Imperiale study is likely the large number of different sites (81) with no oversight/standardization of lab development of Hemoccult II tests. Accurate interpretation of results for Hemoccult II requires training and supervision especially when interpreting borderline results Niv Y. Fecal Occult blood test: the importance of proper evaluation. J Clin Gastroenterol, 1990; 12:393-395. Fleisher M, Winawer SJ, Zauber AG, Smith C, Schwartz MK. Accuracy of fecal occult blood test interpretation: National Polyp Study Work Group. Ann Intern Med, 1991; 114: 875-876. Selinger RRE, Norman S, Dominitz JA. Failure of Health Care Professionals to Interpret Fecal Occult Blood Tests Accurately. Am J Med, 2003; 114:64-67.	We have included this explanation in the discussion of report when talking about the discrepancies between "best estimates" for HOII and HOII performance in the Ahlquist and Imperiale studies. We have also cited these references.
Public Comment - Barry Berger	Results	Page 20 The study evaluated a precommercial stood DNA test (SDT-1, pre-commercial version of PreGen Plus), which was subsequently changed during the study to a different multimarker panel (SDT- 2). Comment P20-2: SDT-2 was added as a substudy in order to evaluate newer marker approaches to fecal DNA analysis that evolved after the study had begun. The study itself was not changed. Essentially, the biorepository of specimens generated by the base study was used to study the next generation marker combinations (SDT-2).	We have changed the sentence and fixed the typo.
Public Comment - Barry Berger	Results	Please note typo in report "stood" should be "stool"	Typo has been corrected.

Commentator & Affiliation	Section	Comment	Response
Public Comment - Barry Berger	Results	Page 20 - After reviewing interim results on the first 2497 participants, it was decided to implement a newer test, SDT-2. The next 1267 participants were part of a case-control study design in which the SDT-2 test was run on a subset of patients (n analyzed=217) Comment P20-3: The interim data look was precipitated by a decision to add Hemoccult SENSA as a comparator. The specimens used for SDT-2 were taken as a subset of the entire study population. This study took many years to accrue sufficient patients to power the study, resulting in the evolution of technology over the course of the recruitment period.	We have reworded the sentence to accurately reflect the details provided by the reviewer (who is the lead author of this included study).

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Public Comment - Barry Berger	Results	P 20-21 Although the lack of adherence to dietary and medication restrictions should, in theory, not decrease sensitivity of FOBT, given the very low sensitivities reported in this study (as compared to other, more generally accepted, estimates for Hemoccult II and Hemoccult Sensa),102 the quality and applicability of all FOBT test results are questionable. Comment P20-21-1: Consider changing this section: the quality and applicability of all FOBT test results are questionable. Consider revising to: HOII performance in Ahlquist was similar to that seen inImperiale and lower than that seen in older studies Note: The poor performance of HOII in Ahlquist and also inImperiale is likely much more reflective of HOIIperformance in actual clinical practice, given the size ofboth studies, the fact that all subjects had a referencecolonoscopy and Ahlquist had HOII interpreted centrally byexpert readers and Imperiale had HOII performed as it was in daily practice at each of the 81 sites. In the Ahlquist study, the Hemoccult manufacturer actually turored the laboratory technicians on the optimal performance and interpretation of the test prior to the study. Dr. James Allison quotes the Imperiale HOII performance as indicative of gFOBT performance in his editorial accompanying the 2004 Morikawa paper published in Gastroenterology. The fact that USPSTF subsequently eliminated low sensitivity gFOBT's like HOII from the guidelines for CRC screening supports is supportive of this view. HOII performance as reported in the very large studies of Ahlquist and Imperiale was likely truly representative of performance in daily practice and the criticism may not be warranted. Please also see similar criticism in Table 7 page 25	We have changed our wording to simply state that the very low sensitivities were reported for FOBT which are not consistent with previous estimates. We have also included a sentence in the discussion to explain the discrepancies in estimates.

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Public Comment - Barry Berger	Results	Page 21 - See my changes: About 50 percent more patients (641 vs. 426) did not provide an adequate sample for fecal DNA testing as compared to Hemoccult II, which may signal differences in feasibility or acceptability to patients. Comment P21-1: The Schroy preference study used the cohort of subjects who completed HOII, fecal DNA and colonoscopy. Fecal DNA testing was preferred over FOBT, a finding supported by a subsequent study using CRC screen naïve subjects. The 641 subjects primarily included patients who simply did not perform the test rather than those who submitted an inadequate sample.	We have considered this sentence, but no changes were made. The Schroy study is discussed as part of Key Question 4.
Public Comment - Barry Berger	Results	Page 22 -…..inclusion of a mostly older population (in which threequarters of the study population was over 65 years of age), Comment P22-1: Note concerning the study limitation based on the age distribution of study participants. Consider reconsider. Note: The experienced CRC prevalence in average risk screening studies increases dramatically with increasing age, approximately 1CRC /1000 subjects at age 50 and 10CRC's /1000 subjects at age 65. Designing studies of reasonable size that are powered by the number of subjects with CRC requires incorporating age related prevalence factors into study design. To date, there is no evidence of age related effects on analytic sensitivity and specificity for DNA biomarkers. By designing the study enrollment to mirror the age related biology of the disease, the number of subjects screened can be decreased and the required study power can be achieved more efficiently. This design has been approved by both FDA and CMS and was recently copied by the PreSept average risk CRC screening study of plasma Septin 9. NPV and PPV will vary with prevalence and thus performance related to those metrics must be calculated taking age related prevalence into account.	We understand the research design rationale for oversampling older adults. However we have mentioned this in the context of applicability (external validity), we did not state that this was problematic for quality (internal validity).

Commentator & Affiliation	Section	Comment	Response
Public Comment - Barry Berger	Results	Page 22 - …The fecal test had zero percent sensitivity, testing positive in none of the 31 participants with advanced colorectal neoplasia (seven patients with invasive CRC) (Table 6). The highest rate of mutant KRAS was reported in participants with a negative colonoscopy (7.5 percent). Important study limitations include bias in the spectrum of patients self selecting for colonoscopy, and the lag-time between stool collection and clinical diagnosis that could have affected test performance (Table 7). Comment P22-1: DNA isolation and analytic technique are critical to test performance. Mutated K-ras is present in approximately 35% of CRC's and, while a finding of 0/7 positives in stool is possible, it is highly unlikely using proper technique. Similarly, the 7.5% false positive rate I also high for k-ras alone and this too speaks to analytic performance issues. While self-selection bias may have changed the prevalence of CRC in the patient population, there is no evidence to suggest that such a bias would contribute to the number of CRC's that either have or do not have K-ras mutations. There are no behavioral features known to date that are directly predictive of the underlying molecular biomarkers associated with colonic neoplasia.	All clinical validity (clinical test performance) is based on the analytic validity of the test. We have not changed the text in response to this comment.

Commentator & Affiliation	Section	Comment	Response
Public Comment - Barry Berger	Results	Page 26 – Key Question 4.- Lack of analytic validity data on Colosure…We did not receive any information in the form of the Scientific Information Packet that was requested from Exact Sciences. For analytic validity, Comment P26-1 Please correct the statement – Please correct this statement to read: Additional unpublished literature was requested and received from Exact Sciences through the TEP process. Note: For data related to the currently available fecal DNA test AHRQ should contact LabCorp for information on technical validity of Colosure as LabCorp is the developer and sole provider of Colosure and as such is the only entity that would have such data Note: Exact Sciences did not receive any request for a SIP. As the latest version of fecal DNA testing is engaged in a pivotal average risk screening study (DeeP-C study) for FDA pre-market clearance or approval, there will be full analytic validity data generated and included in the FDA submission. Such data will be available in the future.	We have corrected all instances of SIP request to Exact Sciences. We have been explicit in the report that identification of data from Exact Sciences was through our TEP process, and that a SIP request was sent to LabCorp.

Commentator & Affiliation	Section	Comment	Response
Public Comment - Barry Berger	Results	Page 27 - ……The applicability of this experiment is also poor given that the accuracy study was conducted in plasma samples, rather than stool samples, and methyl-BEAMing does not appear to be used in the assay evaluated by Ahlquist and colleagues (included in Key Question 2), or in the currently available methylated vimentin test. Comment P27 -1: Note: BEAMing is an exquisitely sensitive analytic assay that quantifies the DNA biomarker strand count in stool or plasma on a per gm or ml basis. It has not yet been reduced to clinical practice. However, the technique provided critical insight that led to improved DNA isolation techniques by demonstrating the strand length of mutated and altered sequences (very small) and the signal to noise ratio discrimination required of a test for mutated or alter fecal DNA in a background of normal (Wild type) DNA (1:10,000). The QuARTS platform discussed above was designed to discriminate MT and WT at the level of1:10,000. Both Diehl in 2008 (Gastroenterol) and Li in 2009 studied BEAMing in both stool and plasma. Diehl studied 16 matched pairs of stool/plasma and reported the superiority of stool as a test matrix over plasma. Earlier studies had shown, as well, that plasma DNA biomarker levels in normal controls and patients with precursor CRC lesions that are not invasive had similar levels of plasma DNA biomarkers. This demonstrates that plasma will not be a CRC screening strategy with direct potential to prevent CRC through precursor lesion detection. Precursors identified subsequent to a "positive" plasma CRC screening assay, based on insights from the BEAMing data, would most likely be the result of random failure of plasma assay specificity rather than a reflection of the sensitivity of the assay.	We have changed our sentence to reflect that this technology has not yet been applied to fecal DNA tests that have been in clinical practice.
Public Comment – Margaret Piper	Results	P53. Need to edit: "Although the specificity of SDT-2 was not reported, SDT-2 had a positivity rate of 16 percent (95% CI, 8 to 24 percent) in persons with normal colonoscopies, and that the positivity rate increase with age."	We have edited the grammatical error.
Public Comment – Margaret Piper	Results	P53, Table 8. "Ability present in the sample of assay to measure the target substance when potentially interfering or cross-reacting substances are present in the sample"	We have edited the grammatical error.

Commentator & Affiliation	Section	Comment	Response
Public Comment – Margaret Piper	Results	P53, KQ4 Analytic validity: I agree with including an analysis of analytic validity in systematic reviews of diagnostics in general, and in this one in particular. However, not all agree and some view it as extraneous, assuming the impact will be seen in the analyses of clinical validity and utility. I suggest that in addition to the definition provided, a clear explanation of why an analysis of analytic validity is included and important would be very helpful. [Note, the explanation in the Discussion section comes late, and though it is helpful, it is incomplete]	
Peer Reviewer 1	Results	In general, the results are adequately presented, with sufficient detail. With respect to overlooked studies, see comment in above section on Methods. There are a few specific comments below.	
Peer Reviewer 1	Results	It is stated - "However, test performance outcomes for SDT-2 (Ahlqiust) were rated poor quality" (page 47). It is not clearly specified why these outcomes were rated poor quality.	We have added a parenthetical (see below) to direct the readers to rationale for "poor quality" which was detailed in a following paragraph (and is also included in a quality table).
Peer Reviewer 1	Results	It is stated - "The two studies found different sensitivities, although the confidence intervals overlapped." (page 43, line 32-33). Confidence intervals (1-p) overlapping is not the same as the null hypothesis being rejected at the alpha=p level. Confidence intervals can overlap and the null hypothesis can still be rejected. Here a simple analysis of the 2 by 2 tables of sensitivities shows the null hypothesis is not rejected at p=0.05 (p=0.11 Chi-squared; p=0.17 2-sided Fisher exact test).	We agree with the reviewer's comment. However our point was simply the imprecision around the estimates, we have reworded this sentence to more clearly state our point.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer 1	Results	In table 4, with respect to Ahlquist et al, the report states that weighted sensitivity for cancer alone and for advanced adenomas alone for STD-2 were not reported. While they were not reported per se, they are easily calculated. For cancer alone, there in fact is no need to weight since all cancers were included in the analysis. For cancers, the paper shows (Table 4) a sensitivity of 58% (95% CI 36-80). For advanced adenomas, where some (adenomas 1-2cm) were only 50% sampled, the weights are based on the overall distribution of screen-relevant neoplasia given in table 1; they are given in table 3 footnote. These are 0.18 for adenomas > 2 cm and .68 for adenomas 1-2. The footnote gives the weight for cancer plus HGD as 0.13; from Table 1, since there are 19 cancers and 20 HGD, the weight for HGD would be (20/39)*0.13=0.07. Then the sensitivity would be the weighted average of the sensitivities for HGD, adenomas 1-2 cm and adenomas > 2cm, with the above weights. From table 4, sensitivity of adenomas > 2 cm is 57% and sensitivity for adenomas 1-2 cm is 34%. Sensitivity is reported combined for cancer plus HGD. However, since sensitivity for cancer is given as 58% for n=19 and sensitivity for cancer+HGD is given as 49% for n=39, it follows that 11/19 of cancers were positive and 19/39 cancer +HGD were positive; thus 8 of 20 (40%) HGD were positive. Then sensitivity for advanced adenomas =(0.07*40+.18*57+.68*34)/(.07+.18+.68)=39%	We have confirmed this calculation with our statistician and agree that this is a valid way to estimate a weighted sensitivity. We have changed our Table to include the “calculated weighted sensitivity”.
Peer Reviewer 1	Results	With respect to the Imperiale et al., 2004 study, the specificity seems to be incorrectly computed by the authors of the report, since they did not utilize the sampling weights from the study. Subjects with normal colonoscopy and minor polyps were sampled for inclusion into the fecal DNA component at around 60% and 40%, respectively. Thus, specificity for CRC and CRC+adenomas should take into account those weights. Correct specificities would be 92.8% (instead of 92.4) and 93.6% (instead of 93.8). Confidence intervals can be calculated since the weights and denominators are known.	We have corrected our estimates using the “weighted” specificity, and calculated CI using standard formula.
Peer Reviewer 2	Results	Detail is appropriate	No response needed.
Peer Reviewer 3	Results	The Results Section is impressive. The included studies are evaluated critically and objectively. The Tables are comprehensive and informative. I am not aware of any significant studies that have been overlooked.	No response needed.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer 4	Results	No significant studies were overlooked. Yes to all other questions.	No response needed.
Peer Reviewer 5	Results	Yes	No response needed.
Public Comment - David Ahlquist	Discussion	Page 36, Evidence Gaps. - Presently, there is no such evidence on test performance (diagnostic accuracy and inaccuracy) in a screening population for either currently available testing or soon to be available testing. Evidence about optimal screening intervals, analytic validity, and acceptability of adherence (helpful in understanding the implementation of screening and the real world effectiveness of screening) are also generally….. Comment P36-1 – Note: This is to be expected for the latest test in development as it is 1) not available commercially and 2) currently the subject of a pivotal study to supply that evidence for review. As indicated earlier, this evidence gap will be filled as part of the submission for FDA pre-market clearance or approval. The fact that there is no evidence currently for a test that is not yet in the market is not unusual. What is important is that such information will be available when the test is available. I strongly suggest considering a change in this sentence to reflect that timing issue. Suggested Change to: Presently, there is no such evidence on test performance (diagnostic accuracy) and analytic validity in a screening population for the currently available test Colosure. Such evidence will be included as part of the planned FDA submission for the next generation test, which will be prior to its availability clinically. Evidence for optimal screening intervals is generated over years of clinical experience with newly implemented tests, supplemented, initially intervals are suggested by the results of modeling. Similarly, while initial preference and adherence with a new test can be studied, programmatic adherence occurs over time and can only be studied after the test is in routine clinical use.	We have considered the reviewer's comments. We believe that all of these conceptual points have been addressed in the discussion. We therefore have not reworded the text.

Commentator & Affiliation	Section	Comment	Response
Public Comment - David Ahlquist	Discussion	P. 38 - We located only three relevant studies despite searching nonpublished and grey literature and requesting additional information from Exact Sciences. We found no evidence on the overall analytic validity of methylated vimentin fecal DNA testing. Please revise: Exact Sciences does NOT have nor had access to any information that relates to Colosure. Exact Sciences provided all available materials to AHRQ/EPC through the TEP process. SUGGESTED CHANGE: We located only three relevant studies despite searching non-published and grey literature. We found no evidence on the overall analytic validity of methylated vimentin fecal DNA testing.	We have corrected all instances of SIP request to Exact Sciences. We have been explicit in the report that identification of data from Exact Sciences was through our TEP process, and that a SIP request was sent to LabCorp.

Commentator & Affiliation	Section	Comment	Response
Public Comment - Barry Berger	Discussion	Page 36, Evidence Gaps - …*Presently, there is no such evidence on test performance (diagnostic accuracy and inaccuracy) in a screening population for either currently available testing or soon to be available testing. Evidence about optimal screening intervals, analytic validity, and acceptability of adherence (helpful in understanding the implementation of screening and the real world effectiveness of screening) are also generally…..* Comment P36-1 – Note: This is to be expected for the latest test in development as it is 1) not available commercially and 2) currently the subject of a pivotal study to supply that evidence for review. As indicated earlier, this evidence gap will be filled as part of the submission for FDA pre-market clearance or approval. The fact that there is no evidence currently for a test that is not yet in the market is not unusual. What is important is that such information will be available when the test is available. I strongly suggest considering a change in this sentence to reflect that timing issue. Suggested Change to: Presently, there is no such evidence on test performance (diagnostic accuracy) and analytic validity in a screening population for the currently available test Colosure. Such evidence will be included as part of the planned FDA submission for the next generation test, which will be prior to its availability clinically. Evidence for optimal screening intervals is generated over years of clinical experience with newly implemented tests, supplemented, initially intervals are suggested by the results of modeling. Similarly, while initial preference and adherence with a new test can be studied, programmatic adherence occurs over time and can only be studied after the test is in routine clinical use.	We have considered the reviewer's comments. We believe that all of these conceptual points have been addressed in the discussion. We therefore have not reworded the text.

Commentator & Affiliation	Section	Comment	Response
Public Comment - Barry Berger	Discussion	P. 38 - We located only three relevant studies despite searching nonpublished and grey literature and requesting additional information from Exact Sciences. We found no evidence on the overall analytic validity of methylated vimentin fecal DNA testing. Please revise: Exact Sciences does NOT have nor had access to any information that relates to Colosure. Exact Sciences provided all available materials to AHRQ/EPC through the TEP process. SUGGESTED CHANGE: We located only three relevant studies despite searching non-published and grey literature. We found no evidence on the overall analytic validity of methylated vimentin fecal DNA testing.	We have corrected all instances of SIP request to Exact Sciences. We have been explicit in the report that identification of data from Exact Sciences was through our TEP process, and that a SIP request was sent to LabCorp.
Public Comment - Barry Berger	Discussion	Page 41 – Through conversations with our TEP, conference presentations, and information from investment conference telephone calls, we understand that Exact Science is currently developing a new assay, a multimarker fecal DNA test plus FIT, Cologuard. This test is expected to be available within the next couple of years. To our knowledge it includes a combination of different markers (methylation markers, mutations in KRAS exon 2) and a FIT, and uses new proprietary technology (Table1). However, the details of the assay are still unknown. Comment P36-2: Please correct the phrase with the following additional information. Through conversations with our TEP, conference presentations, and information from investment conference telephone calls, we understand that Exact Science is currently developing a new assay, a multi-marker fecal DNA test plus FIT. This test includes two methylation markers (NDRG4 and BMP-3), 7 point mutations in Kras Exon 2, a FIT, and a logistical analytic model. The pivotal study is currently ongoing.	We have updated our text to reflect this new information available about Cologuard.
Public Comment – Margaret Piper	Discussion/ Conclusion	P37: “Therefore, the most critical evidence gap for fecal DNA testing to screen for CRC is the lack of appropriately designed diagnostic accuracy studies applicable to currently available fecal DNA testing.”	We have revised the text as suggested.
Public Comment – Margaret Piper	Discussion/ Conclusion	P37: “we do not expect any clinically significant harms other than the (unnecessary) downstream effects of testing and complications from testing resulting from false positives, or clinically significant sequelae from missed diagnosis resulting from false negatives.” I suggest that the downstream harms described are clinically significant; but relative to other tests that have the same harms, no more problematic.	We have added a sentence to clarify that the downstream harms from false positives should be considered in comparison to other stool based testing.

Commentator & Affiliation	Section	Comment	Response
Public Comment – Margaret Piper	Discussion/ Conclusion	p 39: Edit? "However, there are a few important limitations in scope and timing of this review."	We have revised the text as suggested.
Public Comment – Margaret Piper	Discussion/ Conclusion	P39: "Finally, given the rapidly evolving nature of fecal DNA testing, this review will likely be out of date in the near future (although the framing of issues will not), as new tests and evidence supporting them become available in the next 1 to 2 years." Suggest removing the phrase in parens and developing the thought more fully in a following sentence or two, as this is an important contribution of the review. What are the most important issues discussed for new tests/test versions going forward? Why should the concepts in this review be the starting point for future reviews?	We have split the last sentence to state 1) the review will be out of date in the next 1-2 years, 2) that the issues laid out in the report will be helpful in framing future reviews. We have not reiterated the details about important evidence gaps in this section (focused on limitations). The following section on future research does start with an opening paragraph restating the most important evidence gaps going forward.
Peer Reviewer 2	Discussion/ Conclusion	The summary includes mention of performance of Hemoccult II and FIT. The description of FIT performance is problematic and not easily summarized in one sentence. As the authors note, there are many FIT tests, and little/no standardization of technique or threshold of positivity. If the authors applied the standards they used to critique fecal DNA to FIT, I think the conclusion would be that data are insufficient to recommend FIT. I realize the charge was to evaluate fecal DNA, but if comparisons with other fecal tests are to be presented, then it should be fair and balanced. There are serious issues of performance and adherence with both FIT and gFOBT	We have added a sentence to the discussion stating the limitations of the other tests (guaiac and immunochemical based) but are not able to address in depth as it is out of scope for this review.
Peer Reviewer 3	Discussion/ Conclusion	The Discussion is balanced and clear with respect to deficiencies in studies performed to date and limitations in conclusions drawn.	No response needed.
Peer Reviewer 3	Discussion/ Conclusion	The future research section is well constructed and relatively easily translatable into new research.	No response needed.
Peer Reviewer 3	Discussion/ Conclusion	The investigators did not omit any important literature.	No response needed.
Peer Reviewer 4	Discussion/ Conclusion	yes to all	No response needed.
Peer Reviewer 5	Discussion/ Conclusion	Yes	No response needed.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer 1	Conclusion	The implications of the major findings are clearly stated, as are the limitations. In terms of future research, the authors cite an ongoing study. However, since prospective studies are very expensive and logistically challenging to recruit enough CRC cases, the authors should discuss the extent to which appropriate case-control studies may be used to estimate sensitivity (see above text on methods). These would be studies where cancers were identified on screening colonoscopy and the fecal DNA test done shortly thereafter before surgery	We have added a sentence to the discussion that acknowledges that nested case-control designs, case-control studies nested in "screening" cohorts, in which the stool sample is taken before colonoscopy is reasonable. However, stool samples taken after colonoscopy is problematic as colonoscopies often biopsy or remove suspicious lesions (no just small or hyperplastic lesions).
Public Comment - Barry Berger	General	In contrast to previous fecal DNA tests, the combination of the clinical data from DeeP-C and other data generated to fulfill the requirements of the FDA approval process will prospectively address the majority of data gaps raised in the review. Exact Sciences will submit the Cologuard data from DeeP-C in conjunction with extensive analytic validity documentation to the FDA for consideration of premarket approval or clearance for Cologuard. We believe this approach should set the standard for any future CRC screening tests. By way of comparison, the current and only clinically available stool DNA test, developed and offered by Laboratory Corporation of America, is a laboratory developed test service (ColoSure™) 6 using a single marker, aberrant methylation of the Vimentin gene, as the biomarker. It has not been cleared or approved by FDA but is regulated by CLIA, which also requires the development analytic validity data. We request that this Review clarifies that LabCorp is the manufacturer, developer and provider of ColoSure in the marketplace. Exact Sciences does not provide materials or technical support and as such is not the manufacturer nor developer of the ColoSure test. We request that this also be rectified throughout the report. In summary, Exact Sciences appreciates the efforts of the authors of this review as well as the opportunity to provide additional requested data on the new multi-target assay and the timeline of data availability for use in subsequent analyses by USPSTF and other groups involved with CRC screening guidelines. While a later review could have included the data from the DeeP-C study, this review remains a valuable road map for anticipating and prospectively filling data gaps. Further, it provides a historical platform from which to view the enhanced performance of multi-marker based CRC screening tests for CRC precursor and CRC	We have changed all instances of referring to Exact Sciences incorrectly as a "manufacturer" and replaced with "developer" instead. We have noted that LabCorp is the manufacturer for ColoSure.

Commentator & Affiliation	Section	Comment	Response
		detection. We look forward to continuing our collaborative working relationship with AHRQ and EPC as additional data is generated and future reviews occur. Yours Truly, Barry M. Berger, MD FCAP Barry M. Berger, MD FCAP Chief Medical Officer Exact Sciences Corporation References: 1. HEDIS measures 2010 www.NCQA.org 2. Cologuard marker panel: Methylation markers (2) – NDRG4 and BMP-3, K ras point mutations in Exon 2 (7) -, quantitative fecal hemoglobin (ELISA), and a logistic model 3. Sensitive Quantification of Vimentin Methylation with a Novel Methylation Specific qInvader Technology, Zou, H; Allawi H, Cao X;, Domanico M; Harrington J; Taylor W, Yab T, Ahlquist D, Lidgard G. AACC annual meeting 20 July, 2010, [poster # D-144]. Note: This developmental data describing the highly sensitive and specific analytic platform used for DNA biomarker detection using quantitative allele-specific real-time target and signal amplification (QuARTS™) was presented in abstract form. 4. JNCI 2009, 101(18):1225-1227. 5. Next Generation Stool DNA Testing for Detection of Colorectal Neoplasia: Early Marker Evaluation - Poster Presentation. Exact Sciences Corporation. 2011; Colorectal Cancer Biology to Therapy, Ahlquist D, Domanico M, Mahoney DW, et al Abstract PR9, AACR, Phila Pa Oct29, 2010. 6. www.labcorp.com LabCorp Test Number 480430 [ColoSure™]	
Public Comment – Margaret Piper	General	Very well organized and presented review. Relatively minor edits and suggestions below. Also, suggest a quick update to this review when the Exact Sciences test and trial results are fully available	We have conducted a bridge search to update this review. Our review will be finalized before the availability of the Exact Sciences new test and results of DeeP-C.
Peer Reviewer 1	General	General Comments: The key questions are appropriate and clearly stated. The target audience is identified. The report has some limitations on clinical utility, based on the fact the fecal DNA testing is a rapidly evolving field (see below for further comments).	No response needed.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer 2	General	The key questions are appropriate. In my opinion, there is a missing question relevant to this particular technology. "What is the meaning of a positive fecal DNA test and a negative colonoscopy?" Is this an indication of a false positive fecal DNA test or is it possible that colonoscopy was not sensitive enough to detect some pathology? It strikes me that if a mutation is detected in stool, it is real. Then the question is one of significance.	We agree that this is an important clinical question. We have added this point to the "limitations" section of the report.
Peer Reviewer 3	General	The report has limited clinical importance; its impact is lessened because of the absence of information from new studies that have just begun using recently developed methodology.	No response needed.
Peer Reviewer 3	General	The target and audience are clearly defined.	No response needed.
Peer Reviewer 3	General	The key questions are appropriate and explicitly stated.	No response needed.
Peer Reviewer 3	General	It is apparent that great care was exercised in evaluation of published and unpublished information.	No response needed.
Peer Reviewer 4	General	yes to all	No response needed.
Peer Reviewer 5	General	a. General Comments: Yes. I noticed a few minor typos. Suggest having a technical editor or science writer do a careful read of the report to pick up and correct these. Otherwise, the report is clearly written and well organized.	Our report has been sent to a technical editor before being finalized.
Public Comment – Margaret Piper	Abstract	Pv: Suggested edit: "Results. Despite the availability of numerous excluded initial validation studies of fecal DNA testing, we found only three studies that examined the test accuracy of fecal DNA testing in screening populations; numerous initial validation studies were excluded due to use of highly selected patient populations." It might be discussed later in the document that such initial validation studies are a useful first step in test development (if a test is poorly discriminative in highly selected disease-positive and disease-negative patients, it is unlikely to discriminate well in a screening population) but are ONLY a first "discovery" step that must be followed by studies in the intended population to give an accurate representation of performance.	Revised text to include suggested parenthetical. We believe our discussion already addresses the problems of initial validation studies, and that they are not sufficient to estimate test performance in the intended population.

Commentator & Affiliation	Section	Comment	Response
Public Comment – Margaret Piper	Future	P41: Section might best be organized into bullet points, with greater explanation of reasons for each. With regard to CISNET's microsimulation models, what are the strengths and limitations in the suggested role? Is this evidence? If not, is evidence needed?	We decided not to change the formatting of this section. We did not include a deeper discussion of CISNET's models, many of the limitations are the same for any "models" versus direct evidence. The question of "are decision models evidence" is a philosophical one and depends on the stakeholder (if they would consider it evidence or not), and therefore we have not changed the text, which simply states that "…the issue of considering the net benefit of fecal DNA testing compared to the best CRC screening alternative(s) may require some degree of modeling" if comparative effectiveness trials reporting health outcomes (direct evidence) is not available given the rapid evolution of fecal DNA testing.
Public Comment – Margaret Piper	Future	P41: Upcoming Studies – please provide clinicaltrials.gov Identifier for Exact Sciences Trial; note that primary comparison is to colonoscopy, secondary comparison is to FIT	We have included the clinicaltrials.gov identifier for this trial.
Peer Reviewer 1	Clarity / Usability	Clarity and Usability: In general the report is well structured and the main points are clearly presented. As the authors note, the field of fecal DNA testing is rapidly evolving. Additionally, as also admitted, the study results analyzed do not exactly match current clinical practice, i.e., the version of the test in the studies analyzed is different from the (only) test that is commercially available. Therefore, it may be difficult, now, and into the 1-2 year future, for the report to inform policy/practice decisions.	No response needed.
Peer Reviewer 1	Clarity / Usability	Numerous typos and very awkward presentation. 1. In Table 7, under Ahlquist, Quality Concerns – "but did not presented weighted … ") 2. Page 37 of main report, under Table 12, 1st sentence - "Therefore, the most critical evidence gap for fecal DNA testing to screen for CRC the lack of appropriately designed diagnostic accuracy studies .. ". 3. First full sentence, page 38 of main report, beginning "Based on two .. ".	Typos corrected. The report has also been sent to our editor for a final proofread.
Peer Reviewer 2	Clarity / Usability	This is a well-structured report	No response needed.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer 3	Clarity / Usability	The report is well structured and organized.	No response needed.
Peer Reviewer 3	Clarity / Usability	I agree with these comments (ES-8,9): "The most critical evidence gap for fecal DNA testing is the lack of appropriately designed diagnostic accuracy studies applicable to currently available fecal DNA testing". "The limitations in this review are primarily from the limitations in the primary research (small body of variable, often poor quality studies) and the evolving nature of fecal DNA testing.... The conclusions can be used to inform future policy and/or practice decisions.	No response needed.
Peer Reviewer 4	Clarity and Usability	Clear and well organized. Main points and conclusions are clearly stated, as are policy/practice implications of the findings.	No response needed.
Peer Reviewer 5	Clarity and Usability	The conclusions section is oriented more to a future research agenda rather than clinical practice, which is appropriate given the poor existing evidence base for this technology.	No response needed.