Title: Miscellaneous Genetic and Molecular Diagnostic Tests

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<table>
<thead>
<tr>
<th>Populations</th>
<th>Interventions</th>
<th>Comparators</th>
<th>Outcomes</th>
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<tr>
<td>Individuals:</td>
<td>Interventions of interest are:</td>
<td>Comparators of interest are:</td>
<td>Relevant outcomes include:</td>
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<tr>
<td>• With various conditions thought to be hereditary or with a known genetic component</td>
<td>• Testing with a miscellaneous genetic or molecular diagnostic test*</td>
<td>• Usual care without genetic or molecular diagnostic testing</td>
<td>• Test accuracy</td>
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<td>• Change in disease status</td>
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| Celiac PLUS, ColonSentry, Crohn's Prognostic, DecisionDx-Melanoma, DecisionDx-Thymoma, DNA Methylation Pathway Profile, GI Effects (Stool), IBD sgi Diagnostic, ImmunoGenomic Profile, ResponseDx: Colon, SEPT9 methylated DNA (eg, ColoVantage, Epi proColon), TransPredict Fc gamma 3A; Know Error.

**DESCRIPTION**

There are numerous commercially available genetic and molecular diagnostic tests. This evidence review evaluates miscellaneous genetic and molecular diagnostic tests not addressed in a separate review. If a separate evidence review exists, then conclusions reached there supersede conclusions in this review. The main criterion for inclusion in
this review is that there is limited evidence on the clinical validity for the test. As a result, these tests do not have clinical utility and the evidence is insufficient to determine the effect on health outcomes.

**Background**

**Tests Addressed in This Evidence Review**

Tests that are assessed in this evidence review are listed in Table 1.

**Table 1:** Genetic and Molecular Diagnostic Tests in This Evidence Review

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Manufacturer</th>
<th>Date Added</th>
<th>Diagnosis</th>
<th>Risk Assessment</th>
<th>Prognosis</th>
<th>Treatment Response</th>
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*Castle: Castle Biosciences; Dxcs: Diagnostics; Gxcs: Genetics.
  a In a joint venture with Innovative Diagnostic Laboratory.
  b For example, ColoVantage®, Epi proColon®.
  c ARUP, Quest, Clinical Genomics, Epigenomics.

**Diagnostic Tests**

**Multiple Conditions**

Single-nucleotide polymorphisms (SNPs) are the most common type of genetic variation, and each SNP represents a difference in a single nucleotide in the DNA sequence. Most commonly, SNPs are found in the DNA between genes and can act as biological markers of genes and disease association. When SNPs occur within a gene or a gene regulatory region, they can play a more direct role in disease by affecting the gene’s function. SNPs may predict an individual’s response to certain drugs, susceptibility to environmental factors, and the risk of developing certain diseases.

DNA specimen provenance assays can be used to confirm that tissue specimens are correctly matched to the patient of origin. Specimen provenance errors may occur in up to 1% to 2% of pathology tissue specimens, and have serious negative implications for patient care if the error is not corrected. Analysis of DNA microsatellites from tissue specimens can be performed by analyzing long tandem repeats (LTR), and comparing the LTRs of the tissue specimen to LTRs from a patient sample.
**Test Description: DNA Methylation Pathway Profile**
The DNA Methylation Pathway Profile (Great Plains Laboratory, Lenexa, KS) analyzes SNPs associated with certain biochemical processes, including methionine metabolism, detoxification, hormone imbalances, and vitamin D function. Intended uses for the test include clarification of a diagnosis suggested by other testing and as an indication for supplements and diet modifications.

**Test Description: Know Error DNA Specimen Provenance Assay**
The Know Error test (Strand Diagnostics, Indianapolis, IN) compares the LTRs of tissue samples with LTRs from a buccal swab of the patient. The intended use of the test is to confirm tissue of origin and avoid specimen provenance errors due to switching of patient samples, mislabeling, or sample contamination.

**Celiac Disease**
Celiac disease (previously called sprue, celiac sprue, gluten-sensitive enteropathy, gluten intolerance, nontropical sprue, idiopathic steatorrhea) is an immune-based reaction to gluten (water insoluble proteins in wheat, barley, rye) that primarily affects the small intestine. Celiac disease occurs almost exclusively in patients who carry at least 1 human leukocyte antigen (HLA) DQ2 or DQ8 allele; negative predictive value (NPV) of having neither allele exceeds 98%. Serum antibodies to tissue transglutaminase (TTG), endomysium, and deamidated gliadin peptide (DGP) support a diagnosis of celiac disease, but diagnostic confirmation requires duodenal biopsy taken when patients are on a gluten-containing diet.

**Test Description: Celiac PLUS**
Celiac PLUS (Prometheus Therapeutics & Diagnostics, San Diego) is a panel of 2 genetic and 5 serologic markers associated with celiac disease. Per the manufacturer, Celiac PLUS is a diagnostic test that also stratifies future risk of celiac disease. Genetic markers, HLA DQ2 and DQ8, are considered predictive of the risk of developing celiac disease; serologic markers—immunoglobulin A (IgA) anti-TTG antibody, IgA anti-endomysial antibodies, IgA anti-DGP antibodies, IgG anti-DGP, and total IgA—are considered diagnostic for celiac disease. Celiac PLUS is intended for patients at risk for disease (eg, with an affected first-degree relative) or with symptoms suggestive of disease.

**Irritable Bowel Syndrome**
Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder that affects 10% to 20% of the general population in the United States and worldwide. Symptoms include abdominal pain and/or bloating associated with disordered bowel habit (constipation, diarrhea, or both). Pathophysiology is poorly understood but may be related to chronic low-grade mucosal inflammation and disturbances in GI flora. Recommended treatments include dietary restriction and pharmacologic symptom control. Probiotics—living microorganisms that promote health when administered to a host in therapeutic doses—are being investigated as a treatment for IBS. Several
systematic reviews of randomized controlled trials (RCTs) have found evidence to support efficacy,\textsuperscript{8,9,11,14-16} but results from recent RCTs have been mixed.\textsuperscript{17-22} This discrepancy may be due in part to differential effects of different probiotic strains and doses.\textsuperscript{8}

**Test Description: GI Effects Comprehensive Stool Profile**
The GI Effects Comprehensive Stool Profile (Genova Diagnostics, Asheville, NC) is a multianalyte stool assay.\textsuperscript{23} The test uses polymerase chain reaction (PCR) to quantify 26 commensal gut bacteria, and standard biochemical and culture methods to measure levels of other stool components (eg, lipids, fecal occult blood) and potential pathogens (ova and parasites, opportunistic bacteria, yeast). The test is purported to optimize management of gut health and to differentiate IBS from inflammatory bowel disease (IBD).

**Inflammatory Bowel Disease**
IBD is an autoimmune condition characterized by inflammation of the bowel wall, and clinical symptoms of abdominal pain, diarrhea and associated symptoms. Crohn disease (CD) and ulcerative colitis (UC) are the 2 main entities under the category of IBD. The diagnosis is typically made by endoscopy or colonoscopy with biopsy and histologic analysis. This requires a semi-invasive procedure; as a result, a blood test to diagnose IBD could avoid the need for the procedures.

**Test Description: IBD sgi Diagnostic**
IBD sgi Diagnostic (Prometheus Therapeutics & Diagnostics, San Diego, CA) is a panel of 17 serologic (n=8), genetic (n=4), and inflammatory biomarkers (n=5). A proprietary algorithm produces an IBD score; results are reported as consistent with IBD (consistent with UC, consistent with CD, or inconclusive for UC vs CD) or not consistent with IBD. The test is intended for use in patients with clinical suspicion of IBD.

**Colon Cancer**
Early detection of colorectal cancer (CRC) reduces disease-related mortality, yet many individuals do not undergo recommended screening with fecal occult blood test or colonoscopy. It is thought that a simpler screening blood test may encourage screening and decrease mortality, although this has not been proved. Serum biomarkers that are shed from colorectal tumors have been identified and include Septin 9 hypermethylated DNA (\textit{SEPT9}). Septin 9 protein is involved in cell division, migration, and apoptosis, and acts as a tumor suppressor; when hypermethylated, expression of \textit{SEPT9} is reduced.

**Test Descriptions: SEPT9 Methylated DNA**
ColoVantage (various manufacturers) blood tests for serum \textit{SEPT9} methylated DNA are offered by several laboratories (ARUP Laboratories, Quest Diagnostics, Clinical Genomics). Epi proColon (Epigenomics, Berlin) received FDA approval in the United States in April 2016. Epigenomics has licensed its Septin 9 DNA biomarker technology to ARUP and Quest. ColoVantage and Epi proColon are both PCR assays; however,
performance characteristics vary across tests, presumably due to differences in methodology (eg, DNA preparation, PCR primers, probes). Sensitivity as high as 90%, with 88% specificity and 99.9% NPV (4% positive predictive value [PPV]) have been reported for ColoVantage.24,25 By comparison, reported sensitivity and specificity for Epi proColon were 68% and 80%, respectively.26 Serum SEPT9 methylated DNA testing is intended for individuals 50 years of age or older who have an average risk of colorectal cancer.25

### Risk Assessment

#### Celiac Disease

**Test Description: ImmunoGenomic Profile**

The ImmunoGenomic Profile (Genova Diagnostics, Asheville, NC) is a buccal swab test that evaluates SNPs in 6 genes associated with immune function and inflammation: interleukin (IL)-10, IL-13, IL-1β, IL-4, IL-6, and tumor necrosis factor α.27 According to the company website, variations in these genes “can affect balance between cell (TH-1) and humoral (TH-2) immunity, trigger potential defects in immune system defense, and stimulate mechanisms underlying chronic, overactive inflammatory responses… The test uncovers potential genetic susceptibility to: Asthma, Autoimmune Disorders, Certain Cancers, Allergy, Infectious Diseases, Bone Inflammation, Arthritis, Inflammatory Bowel Disease, Heart Disease, Osteopenia, and Helicobacter pylori infection (cause of ulcers)…”

#### Colorectal Cancer

A cofounder of the biotechnology firm GeneNews developed a patented platform technology based on the sentinel principle.28 The sentinel principle posits that because blood interacts with all bodily tissues, “subtle changes occurring in association with injury or disease, within the cells and tissues of the body, may trigger specific changes in gene expression in blood cells reflective of the initiating stimulus.”28 In this way, blood cells (specifically, leukocytes) may act as sentinels of disease. In studies that led to the formulation of this principle, investigators compared gene expression (total RNA levels) in blood samples with catalogued genes from 9 different organs (brain, colon, heart, kidney, liver, lung, prostate, spleen, stomach) and estimated that 66% to 82% of genes encoded in the human genome are expressed in human leukocytes.28

**Test Description: ColonSentry**

ColonSentry (GeneNews, Ontario; Innovative Diagnostic Laboratory, Richmond, VA) is a PCR assay that uses a blood sample to detect expression of 7 genes found to be differentially expressed in CRC patients compared with controls29: ANXA3, CLEC4D, TNFAI/P6, LMNB1, PRRG4, VNN1, and IL2RB. Per the company website, these genes are early-warning signs of colon cancer, and test results can indicate the odds of having CRC compared with an average-risk person.30 An average-risk person is defined as one who is “at least 50 years old, is asymptomatic for CRC, has no personal history of benign colorectal polyps, colorectal adenomas, CRC, or inflammatory bowel disease,
and does not have a first-degree relative with CRC.”30 The test is intended for use in adults who are averse to colonoscopy and/or fecal occult blood testing. “Because of its narrow focus, the test is not expected to alter clinical practice for patients who comply with recommended screening schedules.”31

**Prognostic Tests**

**Crohn Disease**
Recent studies have identified serologic32 and genetic33,34 correlates of aggressive CD that is characterized by fistula formation, fibrostenosis, and the need for surgical intervention. Prometheus has developed a blood test that aims to identify patients with CD who are likely to experience an aggressive disease course.

*Test Description: Crohn’s Prognostic*
Crohn’s Prognostic (Prometheus Therapeutics & Diagnostics, San Diego, CA) is a panel of 6 serologic (n=3) and genetic (n=3) biomarkers. Limited information about the test is available on the manufacturer’s website.

**Cutaneous Melanoma**
Cutaneous melanoma represents less than 5% of skin malignancies but results in the most skin cancer deaths. The incidence of cutaneous melanoma continues to increase, and it is currently the sixth most common cancer in the United States. Standard treatment options for stage 1 and 2 melanoma are excision with or without sentinel lymph node examination. Current risk factors to predict localized tumor aggression include Breslow tumor thickness, tumor ulceration, and mitotic rate of the tumor cells. The likelihood of regional lymph node involvement increases with increasing tumor thickness, and significantly negatively impacts the rate of survival.

*Test Description: DecisionDx-Melanoma*
DecisionDx-Melanoma (Castle Biosciences, Friendswood, TX) is a gene expression profile test with a signature of 31 genes, 28 discriminating genes and 3 control genes. The test is used to measure risk of metastasis in patients with stage I and II cutaneous melanoma and classifies tumors into 2 groups of risk of metastasis—low or high (classes 1 and 2, respectively). The test purports to give an independent prediction of tumor metastatic risk, independent of currently used metrics of risk assessment (eg, Breslow thickness, ulceration status, and mitotic rate; American Joint Committee on Cancer [AJCC] stage, sentinel lymph node biopsy [SLNB] status), so that patients with high-risk stage 1 or 2 disease can undergo more aggressive surveillance treatment than they would have otherwise received. The test is intended to provide additional prognostic information to current staging methods (AJCC stage, SLNB).

**Thymomas and Thymic Carcinomas**
Thymomas and thymic carcinomas are rare epithelial tumors of the thymus. Most are diagnosed in individuals between 40 and 60 years of age. Thymic epithelial tumors range from histologically benign tumors to microscopically or macroscopically invasive...
low- or high-grade malignant tumors. However, even tumors that are histologically benign can behave aggressively.

**Test Description: DecisionDx-Thymoma**

DecisionDx-Thymoma (Castle Biosciences, Friendswood, TX) is a gene expression profile test that measures the activity of 23 genes within the thymic tumor. Its intended use is to distinguish between thymic carcinoma and thymoma, and to predict tumor aggressiveness by likelihood that the tumor will metastasize.

**Tests for Genetic Variants That Alter Response to Treatment or to an Environmental Factor**

**Colon Cancer**

**Test Description: ResponseDX: Colon**

Response Genetics (Los Angeles, CA) currently markets 2 colon cancer genetic panels to guide treatment selection, as well as separate tests for 11 genes associated with colon cancer prognosis and/or treatment response. The Driver Profile panel comprises PCR mutation testing in *KRAS, BRAF,* and mismatch repair genes (microsatellite instability), plus *NRAS* exon 2 and 3 sequencing. These gene tests are reviewed elsewhere (see evidence reviews 2.04.08 and 2.04.53), and this panel is not considered here. The ResponseDX: Colon test comprises the 4 tests in the Driver Profile plus: *EGFR* expression; *PI3K* exon 1, 9, and 20 sequencing; *TS* expression; *ERCC1* expression; *UGT1A1* SNP testing (rs8175347, rs4148323); *VEGFR2* expression; and *MET* amplification by fluorescence in situ hybridization. Evidence for clinical validity and clinical utility of the ResponseDX: Colon test was sought.

**Non-Hodgkin Lymphoma**

Rituximab is a humanized IgG monoclonal antibody against the CD20 antigen, which is commonly expressed on B lymphocytes. It is FDA-approved for treatment of non-Hodgkin lymphoma, chronic lymphocytic leukemia, and nononcologic uses (eg, rheumatoid arthritis). Although rituximab has demonstrated improved response and survival rates in combination chemotherapy regimens in patients with follicular lymphoma, chronic lymphocytic leukemia, and diffuse large B-cell lymphoma than chemotherapy alone (not all patients responded). Altered binding to lymphocyte-bound rituximab by cytotoxic effector cells (eg, natural killer cells, macrophages) has been identified as a mechanism of reduced rituximab efficacy. Effector cells with a Val158Phe substitution mutation in their surface receptors for IgG molecules (eg, rituximab) have impaired binding affinity, and cellular cytotoxicity is reduced. A genetic test for the Val158Phe mutation of the gene that encodes the IgG receptor on effector cells (*FCGR3A*) has been developed and investigated as a means of predicting response to rituximab.

**Test Description: TransPredict Fc gamma 3A**

TransPredict Fc gamma 3A (formerly PGxPredict:Rituximab; Transgenomic, Omaha, NE) is a PCR assay that uses a blood sample to detect the Val158Phe mutation of the
*FCGR3A* gene. For patients who are homozygous for valine, the test reports a high likelihood of response to rituximab; for all other patients (homozygous for phenylalanine or heterozygous), the test reports an average probability of response. The test is intended for patients with follicular, CD20-positive, B-cell non-Hodgkin lymphoma who are being considered for treatment with rituximab.

**Regulatory Status**
Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general standards of the Clinical Improvement Act (CLIA). Genetic tests reviewed in this policy are available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of these tests.
POLICY
A. All of the tests listed in this policy are considered experimental / investigational and grouped according to the categories of genetic testing:
   1. Diagnostic testing
   2. Risk assessment
   3. Prognostic testing
   4. Genetic variants that alter response to treatment or to an environmental factor

RATIONALE
The literature review was performed through July 5, 2016 (see Appendix Table 1 for genetic testing categories). Evidence reviewed for the tests listed in Table 1 is presented after the following sections that outline general principles and categories of genetic tests.

General Principles of Genetic Tests
The test should be cleared or approved by the U.S. Food and Drug Administration (FDA) or performed in a Clinical Laboratory Improvement Amendment–certified laboratory.

Peer-reviewed literature on test performance and indications for the test should be available. Evaluation of genetic tests focuses on 3 main principles:
   (1) Analytic validity (technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent)
   (2) Clinical validity (diagnostic performance of the test [sensitivity, specificity, positive and negative predictive values] in detecting clinical disease)
   (3) Clinical utility (how results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes)

Categories of Genetic Tests
Medical criteria listed after each category define the circumstances in which testing for a genetic or heritable disorder may be considered clinically useful.

Diagnostic Tests
Diagnostic testing for genetic or heritable mutations in a symptomatic individual refers to molecular diagnosis defined by the presence of a known pathologic mutation. For purposes of genetic testing, a symptomatic individual is defined as an individual with a clinical phenotype that correlates with a known pathologic mutation.

Criteria
- An association of the marker with the disorder has been established; AND
- Symptoms of the disease are present; AND
- A definitive diagnosis cannot be made based on history, physical examination, pedigree analysis, and/or standard diagnostic studies/tests; AND
- Clinical utility of a diagnosis has been established, e.g., by demonstrating that a definitive diagnosis will lead to changes in clinical management of the condition, changes in surveillance, or changes in reproductive decision making, and the changes will lead to
improved health outcomes; AND
• Establishing the diagnosis by genetic testing will end the clinical workup for other disorders.

Risk Assessment
Risk assessment for genetic and heritable mutations is done for:
• Predictive and presymptomatic types of testing are used to detect gene mutations associated with disorders that appear after birth, usually later in life. These tests can be used in individuals with a family history of a genetic disorder, but who themselves have no features of the disorder at the time of testing. Predictive testing can identify mutations that increase an individual’s risk of developing disorders with a genetic basis, such as certain types of cancer or cardiovascular disease. Presymptomatic testing can determine whether a person will develop a genetic disorder, before any signs or symptoms appear, by determining whether an individual has a genetic mutation that may lead to development of the disease.

Criteria
• Predictive and presymptomatic testing:
  o An association of the marker with future disorder has been established; AND
  o Clinical utility has been established, e.g., by demonstrating that testing will lead to improved health outcomes based on prevention or early detection strategies.

Prognostic Tests
Prognostic testing of diagnosed disease is done to predict natural disease course (e.g., aggressiveness, risk of recurrence, death). This type of testing uses gene expression of affected tissue to predict the course of disease.

Criteria
• An association of the marker with the natural history of the disease has been established; AND
• Clinical utility of identifying the mutation has been established, e.g., by demonstrating that testing will lead to changes in clinical management of the condition or changes in surveillance.

Tests for Genetic Variants That Alter Response to Treatment or to an Environmental Factor
There are 3 main types of tests to identify genetic variants that alter response to treatment or to an environmental factor:
• Constitutional (germline) testing to detect genetic variants that alter risk of treatment response, adverse events, drug metabolism, drug effectiveness, etc., e.g., cytochrome p450 testing (also referred to as pharmacogenomics).
• Tissue-specific or tumor testing to detect mutations that predict response to a certain type of treatment, e.g., ALK mutation testing in non-small-cell lung cancer to predict response to crizotinib.
• Testing for genetic mutations that adversely affect response to exposures in the environment that are ordinarily tolerated (e.g., G6PD deficiency, genetic disorders of immune function, aminoacidopathies).
Criteria
- Constitutional (germline) testing:
  - Association of the marker with a phenotype/metabolic state that relates to drug efficacy or adverse drug reactions has been established; AND
  - Clinical utility has been established, e.g., by demonstrating that results of the genetic test will impact clinical decision making and will be expected to yield improved clinical outcomes for the patient based on drug selection or dosage.
- Tissue-specific or tumor testing:
  - Association of a mutation with response to a particular drug has been established; AND
  - Clinical utility has been established, e.g., by demonstrating that the patient is a candidate for targeted drug therapy that is associated with a specific mutation.

Diagnostic Tests

Multiple Conditions
DNA Methylation Pathway Profile
No full-length, peer-reviewed studies of the DNA Methylation Pathway Profile were identified.

Clinical Validity
Evidence for clinical validity is lacking.

Clinical Utility
Direct and indirect evidence for clinical utility are lacking.

Section Summary: DNA Methylation Pathway Profile
No studies were identified that evaluated this test.

Know Error Specimen Provenance Assay
Clinical Validity
Evidence for clinical validity of the Know Error Specimen Provenance Assay is lacking. There is some evidence on the application of short tandem repeat testing for specimen provenance assays in general, but these data are not specific to the Know Error test.

Clinical Utility
Direct evidence for clinical utility is lacking. It is not possible to construct an indirect chain of evidence for clinical utility due to the lack of clinical validity.

Section Summary: Know Error Specimen Provenance Assays
There is a lack of published evidence on the use of the Know Error test to confirm tissue of origin. Studies are needed that compare use of Know Error to standard laboratory quality measures, and that demonstrate a reduction in specimen provenance errors associated with use of Know Error.

Celiac Disease
Celiac PLUS
In 2013, the American College of Gastroenterology (ACG) published an evidence-based diagnostic algorithm for patients with high (>5%) or low (<5%) probability of celiac disease. In both groups of patients, immunoglobulin A (IgA) anti-tissue transglutaminase (anti-TTG)
antibody is “the preferred single test for detection of celiac disease in individuals over the age of 2 years” (strong recommendation based on a high level of evidence); sensitivity and specificity of the anti-TTG IgA are both approximately 95%. For patients with high probability of disease, initial diagnostic workup comprises duodenal biopsy and anti-TTG IgA. If both tests are negative, celiac disease is unlikely; if both are positive, celiac disease is diagnosed. If results are discrepant, further workup including human leukocyte antigen (HLA) DQ2 and DQ8 genotyping and total IgA level to rule out IgA deficiency is recommended. For patients with low probability of disease, initial diagnostic workup comprises anti-TTG IgA level and total IgA level. With 1 exception, combining several serologic tests rather than obtaining IgA anti-TTG alone is not recommended due to substantially reduced specificity for only marginally increased sensitivity (weak recommendation based on moderate evidence). In children younger than 2 years of age, however, combination testing with IgA anti-TTG and anti-DGP (both IgA and IgG) is recommended due to reduced test performance in this age group (strong recommendation based on moderate evidence). A strong recommendation (based on moderate evidence) against routine HLA DQ2 and DQ8 testing in the initial diagnostic workup of celiac disease is made; targeted HLA DQ2 and DQ8 testing is recommended for select clinical situations (eg, discrepant serology and biopsy results [strong recommendation based on moderate evidence]).

No studies of the combined serologic and genetic Celiac PLUS test were identified.

Clinical Validity
Celiac PLUS tests for genetic and serologic factors known to be associated with celiac disease. All 7 test components are included in an evidence-based diagnostic algorithm developed by a professional medical society. However, algorithmic testing is individualized according to baseline risk of disease and is done sequentially, rather than simultaneously as in Celiac PLUS. Information about clinical validity of obtaining several serologic and genetic tests at once (i.e., Celiac PLUS) is lacking; improved sensitivity and reduced specificity may be expected.

Clinical Utility
No studies examining the clinical utility of Celiac PLUS were identified. Factors that support an indirect chain of evidence for prognostic or diagnostic utility are lacking. A comparison of clinical and/or histopathologic outcomes using either Celiac PLUS or ACG’s published diagnostic algorithm would be required to demonstrate improved health outcomes with Celiac PLUS.

Section Summary: Celiac Disease
No studies examining the clinical utility of Celiac PLUS were identified. Factors that support an indirect chain of evidence for prognostic or diagnostic utility are lacking. A comparison of clinical and/or histopathologic outcomes using either Celiac PLUS or ACG’s published diagnostic algorithm would be required to demonstrate improved health outcomes with Celiac PLUS.

Irritable Bowel Syndrome
GI Effects Comprehensive Stool Profile
Two manufacturer-sponsored studies were published in 2014. Goepp et al conducted a retrospective cohort study to determine the frequency of abnormal fecal biomarkers among patients with IBS symptoms. Records from Genova Diagnostics were reviewed to identify patients with ICD-9 codes for at least 1 of 13 IBS symptoms who had available results of a fecal biomarker panel (N=2256). Quantitative stool culture for Lactobacillus and Bifidobacterium (“beneficial bacteria”) indicated low growth in 73% of patients, parasites in 8%, and elevated
eosinophil protein X, elevated calprotectin, and low pancreatic elastase in 14%, 12%, and 7%, respectively. The authors interpreted these biochemical findings to support diagnoses of food allergies, inflammation, and exocrine pancreatic insufficiency, respectively.

Parsons et al (2014) conducted a retrospective matched cohort study to compare direct medical costs of care for patients with IBS who underwent fecal biomarker testing with those of matched controls. Investigators searched medical and pharmacy claims from a national pharmacy benefits manager for IBS-related diagnosis codes; patients who also had fecal panel test codes and data for up to 1 year after testing (n=132) were compared with propensity-matched (for age, sex, diagnosis code[s], and baseline medical and pharmacy utilization) controls from the same database who had IBS-related diagnosis codes but no fecal test codes. Outcomes of interest were diagnostic and medical service costs determined from claims data. At baseline, laboratory costs were higher in tested groups than controls. At 30, 90, and 365 days after testing, total medical costs, GI procedural costs including imaging, and laboratory costs were higher in controls. For example, at 90 days after testing, GI procedural costs were $26 less than baseline utilization in the tested cohort and $165 more than baseline in the control cohort.

Clinical Validity
No studies were identified that assessed the accuracy of the GI Effects fecal panel for diagnosing IBS or for documenting “gut health,” a concept that may be difficult to define given large interindividual variability in gut flora.

Clinical Utility
Clinical trials demonstrating net health benefit with the GI Effects fecal panel were not identified. Because probiotics are not currently a standard treatment of IBS, impacts of test results on disease management are uncertain; that is, an indirect chain of evidence for clinical utility of the test cannot be established.

Section Summary: Irritable Bowel Syndrome
Evidence for clinical validity and clinical utility of the GI Effects Comprehensive Stool Profile is lacking. Two claims-based, retrospective studies evaluated abnormal fecal marker prevalence and costs associated with use of the test. This evidence is insufficient to demonstrate net health benefit with use of the test.

Inflammatory Bowel Disease
IBD sgi Diagnostic™
The IBD sgi Diagnostic™ product monograph includes an extensive bibliography that documents associations of the 17 component markers, individually and in combination, with UC and/or CD. Development and performance characteristics of the 17-marker panel are described without citation, and it is unclear what standard criterion was used for diagnosis. Overall sensitivity for IBD, UC, and CD is reported as 74%, 98%, and 89%, respectively; specificity is reported as 90%, 84%, and 81%, respectively; receiver operating characteristic (ROC) analysis showed greater discrimination with the 17-marker panel (area under the curve [AUC], 0.871) compared with any individual marker (greatest AUC=0.690 for IgA anti-Saccharomyces cerevisiae antibodies [ASCA]). Test performance characteristics for distinguishing UC from CD were not provided.
In a 2012 review of the monograph, Shirts et al\textsuperscript{43} observed that serologic tests for ASCA-IgA, ASCA-IgG, and atypical perinuclear anti-neutrophil cytoplasmic antibody are standard of care in the diagnostic workup of IBD,\textsuperscript{44,45} although not all investigators include these tests in recommended diagnostic strategies.\textsuperscript{46-49} These 3 markers are included in the 17-marker panel. Based on a meta-analysis of 60 studies (total N=11,608), pooled sensitivity and specificity of the 3-test panel were 63% and 93%, respectively, for diagnosing IBD.\textsuperscript{50} Because the product monograph does not include a comparison of the 17-marker panel with the 3-marker panel, incremental improvement in diagnosis with the 17-marker panel is unknown. Shirts et al calculated an AUC for the 3-marker panel of 0.899.

**Clinical Validity**

Published evidence supports associations of each marker in the 17-marker panel, alone and in combination, with IBD diagnosis. Based on manufacturer data, accuracy for IBD diagnosis of the 17-marker panel exceeds that of each component marker, but the relevant comparison—with a panel of 3 markers that has good discrimination for IBD—was not included; subsequent analysis suggests that the panels may perform similarly. Performance characteristics for the 17-marker panel to distinguish UC from CD were not provided.

**Clinical Utility**

No studies examining the clinical utility of IBD sgi Diagnostic™ were identified.

**Section Summary: Inflammatory Bowel Disease**

No studies examining the clinical utility of IBD sgi Diagnostic™ were identified. Although manufacturer data supports clinical validity of the test for diagnosing IBD, this evidence is insufficient to support an indirect chain of evidence for clinical utility due to lack of details about study methodology and lack of replication of the findings. For distinguishing UC from CD, clinical validity has not been established; therefore, an indirect chain of evidence for clinical utility for this purpose cannot be established.

**Colorectal Cancer**

**SEPT9 Methylated DNA**

There is a fairly large literature on the association of \textit{SEPT9} methylation with colon cancer in general. In case-control studies involving more than 3000 patients, overall sensitivity of \textit{SEPT9} DNA methylation screening was 60% to 70% and specificity was 89%.\textsuperscript{24,25,51-53} Modifications to ColoVantage methodology increased sensitivity to 90% with little decrement to specificity.\textsuperscript{24,25,54} A systematic review published in 2016 reviewed 39 studies on the diagnostic performance of \textit{SEPT9} methylation for detecting colon cancer.\textsuperscript{55} The combined sensitivity was 62% (95% confidence interval [CI], 56% to 67%) and the combined specificity was 91% (95% CI, 89% to 93%). There was no significant impact on the accuracy of testing according to target gene number, tumor stage, geographical region, or method of analysis.

Only 1 test has received FDA approval, the Epi proColon test, which was approved in 2016 for use in average-risk patients who decline other screening methods. There is a smaller number of studies that have specifically evaluated the performance of the commercially available Epi proColon test. A case-control study that compared Epi proColon with fecal immunochemical testing (FIT) for CRC screening enrolled 102 patients with CRC and 199 patients who presented for screening.\textsuperscript{56} Colonoscopy was the reference standard. Sensitivity and specificity were 73% and 82% for Epi proColon, respectively, and 68% and 97%, respectively, for FIT. In 290 paired
samples, sensitivity of the 2 tests was similar (≈70%), but specificity of Epi proColon was lower (81% vs 97%). Similar results were observed in a subsequent retrospective case-control study that used a second-generation version of the test.57

In 2014, Church et al reported an international prospective screening study of Epi proColon®, called PRESEPT.58 Patients 50 years of age or older with average risk of CRC who were scheduled for colonoscopy were enrolled (N=7941). Of these, 1516 (19%) were selected for laboratory analysis in stratified random sampling; colonoscopy identified 53 patients (3%) with invasive adenocarcinoma, 315 (21%) with advanced adenoma, 210 (14%) with nonadvanced adenoma, and 938 (62%) with no evidence of disease. Overall sensitivity, specificity, PPV, and NPV for Epi proColon® detection of invasive adenocarcinoma were 48%, 92%, 5%, and 100%, respectively. Sensitivity for advanced adenoma was low (11%). As observed by the study investigators, detection of only half of preclinical cancers and a small proportion of advanced adenomas limits clinical utility of the test.

Tham et al (2014) reported on a smaller prospective cohort study in Singapore (N=150).59 Investigators measured methylation levels of 7 genes, including SEPT9, in patients with stage I-III CRC who underwent curative resection. Blood samples were collected 1 week before and 6 months and 1 year after surgery. At median follow-up of 59 months (range, 5-79 months), 43 patients (29%) developed recurrence. Although a statistically significant association between methylated SEPT9 level at 1 year and recurrence was found, interpretation of this result is limited by lack of correction for multiple comparisons. Additionally, cutoff values for a positive test were determined by median levels rather than prespecified. ROC analysis using optimized cutoffs for SEPT9 at 1 year yielded an AUC of 0.70 (95% confidence interval [CI], 0.58 to 0.82). AUC for carcinoembryonic antigen at 1 year was similar (AUC=0.69 [95% CI, 0.57 to 0.80]).

Orntoft et al published a case-control study examining whether the prognostic information is impacted by other clinical and demographic variables.60 One hundred fifty cases of CRC were matched with 150 controls from a database of 4698 individuals undergoing colonoscopy for evaluations of CRC. The variables examined, together with the results of the Epi proColon test, were age, sex, comorbidities, tumor site, and tumor stage. The overall sensitivity of Epi proColon was 73% (95% CI, 64% to 80%). Sensitivity varied by tumor stage. The sensitivity was 37% for stage I tumors; 91% for stage II; 77% for stage 3; and 89% for stage IV. In addition to tumor stage, age and comorbidities impacted the accuracy of testing. Elderly patients (>65 years old) had both lower sensitivity and specificity of testing. Patients with arthritis had decreased sensitivity, while patients with coronary artery disease and diabetes had decreased specificity. Diabetes was particularly associated with a positive test, with an odds ratio of 5.2 (95% CI, 1.4 to 19.1) for a positive test compared to patients without diabetes.

Clinical Validity
Evidence for clinical validity of CRC screening includes case-control studies and 2 prospective screening studies. These studies reported that sensitivity of testing ranges from 60% to 80% and specificity from 85% to 95%. Test performance characteristics were better in case-control studies, suggesting that tests intended for screening be prospectively tested in the screening setting.58 One study indicated that age and comorbidities were potential confounders of testing. Based on results from these studies, the clinical validity of SEPT9 methylated DNA screening is limited by low sensitivity and low PPV of the test. The sensitivity of the test is lower than
imaging screening strategies. Compared to stool-based strategies, the sensitivity is in the same range and the specificity is lower.

**Clinical Utility**

Studies comparing survival outcomes in patients who undergo CRC screening with SEPT9 methylated DNA testing versus standard screening were not identified. Such comparative studies with clinically meaningful outcomes (survival) are necessary to demonstrate incremental improvement in net health outcome compared with current standard screening approaches (FIT, colonoscopy) and to address lead-time bias for cancers identified through screening. Because evidence for clinical validity is currently lacking, an indirect chain of evidence establishing clinical utility of SEPT9 methylated DNA cannot be established.

**Section Summary: Colorectal Cancer**

There is a need for further studies comparing survival outcomes in patients screened with SEPT9 methylated DNA testing (ColoVantage, Epi proColon) compared to other screening methods. Such comparative studies with clinically meaningful outcomes (eg, survival) are necessary to demonstrate improvement in net health outcome compared with current standard screening approaches (FIT, colonoscopy) and to address lead-time bias for cancers identified through screening. Evidence on clinical validity has reported that the test has a lower sensitivity than other screening methods. Clinical utility is uncertain. If the test is restricted only to patients who would otherwise not be screened, outcomes will be improved. However, if the test is used as a substitute for other screening tests that have higher sensitivity, outcomes may be worse.

**Risk Assessment**

**Immunologic Disorders**

**ImmunoGenomic Profile**

No full-length, peer-reviewed studies of the ImmunoGenomic Profile were identified.

**Clinical Validity**

Evidence for clinical validity is lacking.

**Clinical Utility**

Direct and indirect evidence for clinical utility are lacking.

**Section Summary: Immunologic Disorders**

Evidence for clinical validity and utility of the ImmunoGenomic Profile to predict risk of developing arthritis, asthma, allergies, or other chronic inflammatory disorders is currently lacking.

**Colorectal Cancer**

**ColonSentry**

Marshall et al (2010) conducted a genome-wide association study in 189 whole blood samples (98 controls, 91 patients with CRC) and identified 45 differentially expressed gene biomarker candidates using microarray hybridization. Through logistic regression and bootstrapping (subsampling with replacement) in a training set of 232 samples (120 controls, 112 patients with CRC), 7 genes were selected for further development. Sensitivity, specificity, PPV, and NPV for detecting CRC were 82%, 64%, 68%, and 79%, respectively. AUC was 0.80 (95% CI, 0.74
to 0.85). In a test set of 410 samples (208 controls, 202 patients with CRC), sensitivity, specificity, PPV, and NPV were 72%, 70%, 70%, and 72%, respectively. AUC was 0.80 (95% CI, 0.76 to 0.84). The authors applied subsequent Bayesian modeling to incorporate the prevalence of CRC in the average-risk population (0.7%) and proposed relative risk categories to further stratify average-risk patients for CRC screening. Because of its cross-sectional design, follow-up of controls to determine which strata developed CRC was not reported, limiting conclusions that can be drawn about the accuracy of the test for risk prediction. In a subsequent publication, the investigators reported test performance stratified by left- versus right-sided cancers and tumor stage.\(^{31}\)

Yip et al (2010) conducted a similar cross-sectional study in 210 blood samples (111 controls, 99 CRC) from patients in Malaysia.\(^{29}\) The Malaysian population has different ethnic groups with different CRC incidences (e.g., 0.02% in Chinese Malaysians, 0.01% in ethnic Malays), and CRC in Asian populations is more likely to be nonpolypoid (i.e., flat or depressed) compared with western populations in whom the test was developed. Sensitivity and specificity for detecting CRC were 61% and 77%, respectively. AUC was 0.76 (95% CI, 0.70 to 0.82). With optimized cut points, sensitivity and specificity were 72% and 71%, respectively. As previously, the cross-sectional design of the study limits conclusions that can be drawn.

**Clinical Validity**
Two cross-sectional studies do not permit full characterization of the ability of ColonSentry® to predict CRC risk.

**Clinical Utility**
No studies examining the clinical utility of ColonSentry® were identified. Factors that support an indirect chain of evidence for predicting CRC risk are lacking, primarily because evidence for clinical validity of the test is lacking.

**Section Summary: Colorectal Cancer**
ColonSentry® is intended to stratify patients with average CRC risk who are averse to screening to identify those who may be at increased risk and therefore choose screening. However, 2 cross-sectional studies are insufficient to demonstrate the risk predictive ability of the test; that is, clinical validity has not been established. Direct and indirect evidence of clinical utility is currently lacking.

**Prognostic Tests**

**Crohn Disease**
**Crohn's Prognostic**
No studies of the 6-marker panel were identified.

**Clinical Validity**
Evidence for clinical validity is lacking.

**Clinical Utility**
Direct and indirect evidence for clinical utility is lacking.
Section Summary: Crohn's Disease
Direct and indirect evidence for clinical utility of the Crohn's Prognostic test to identify individuals likely to have an aggressive disease course is currently lacking.

Cutaneous Melanoma
DecisionDx-Melanoma

Clinical Validity
To develop the DecisionDx-Melanoma gene panel, Gerami et al (2015) conducted a meta-analysis of published studies that identified differential gene expression in metastatic versus nonmetastatic primary cutaneous melanoma. Of 54 identified genes, investigators selected 20 for further PCR analysis based on chromosomal location. Five genes from the DecisionDx-UM gene panel were added based on analysis of metastatic and nonmetastatic primary cutaneous melanoma, and 2 probes (for both the 3' and 5' ends) of the BRCA1-associated protein 1 gene, BAP1, which has been associated with the metastatic potential of uveal melanoma, also were added. Finally, 4 genes with minimal variation in expression level between metastatic and nonmetastatic primary cutaneous melanoma were added as controls. The 31-gene panel was applied to 3 cohorts using archived formalin-fixed, paraffin-embedded primary cutaneous melanoma tissue. Patients had minimum follow-up of 5 years unless there was a well-documented metastatic event, including positive sentinel lymph node biopsy. Information about treatments received was not provided.

The first cohort (development set) included 107 patients with stage 1 or 2 primary melanoma from 3 U.S. centers. The second and third cohorts included 161 additional patients with stage 0-4 disease from 7 U.S. centers (total N=268). Thirty-four patients (20%) without evidence of metastasis had less than 5 years of follow-up. Test performance characteristics in the 3 cohorts are summarized in Table 2. For 78 patients in the third cohort (test set) with AJCC stage 1 or 2 cutaneous melanoma who had either a metastatic event or had more than 5 years of follow-up without metastasis, 5-year disease-free survival (DFS) was 98% for class 1 patients and 37% for class 2 patients; PPV and NPV were 67% and 94%, respectively. For 220 patients with AJCC stage 1 or 2 cutaneous melanoma in the combined training and test cohorts, DecisionDx-Melanoma classified 84% of patients who did not develop metastasis as class 1 and 89% of patients who developed metastasis as class 2 (sensitivity, 90%; specificity, 84%; PPV=72%; NPV=95%). Median duration of follow-up for these 220 patients was not reported.

Table 2. DecisionDx-Melanoma Test Performance Characteristics in Gerami et al (2015)62

<table>
<thead>
<tr>
<th></th>
<th>Development Set</th>
<th>Training Set</th>
<th>Test Set</th>
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<tbody>
<tr>
<td>Total N</td>
<td>107</td>
<td>164</td>
<td>104</td>
</tr>
<tr>
<td>Class 1, n (%)</td>
<td>64 (60%)</td>
<td>88 (54%)</td>
<td>61 (59%)</td>
</tr>
<tr>
<td>Class 2, n (%)</td>
<td>43 (40%)</td>
<td>76 (46%)</td>
<td>43 (41%)</td>
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</table>

5-year disease-free survival

<table>
<thead>
<tr>
<th></th>
<th>Development Set</th>
<th>Training Set</th>
<th>Test Set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>100%</td>
<td>91%</td>
<td>97%</td>
</tr>
<tr>
<td>Class 2</td>
<td>38%</td>
<td>25%</td>
<td>31%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>85%</td>
<td>89%</td>
</tr>
<tr>
<td>Specificity</td>
<td>78%</td>
<td>80%</td>
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<td>Positive predictive value</td>
<td>56%</td>
<td>75%</td>
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<tr>
<td>Negative predictive value</td>
<td>100%</td>
<td>89%</td>
<td>93%</td>
</tr>
<tr>
<td>AUC</td>
<td>0.93</td>
<td>0.91</td>
<td>0.91</td>
</tr>
</tbody>
</table>

AUC: area under the receiver operating characteristic curve.

In a subsequent study of patients with melanoma who had undergone SLNB, Gerami et al (2015) compared prognostic classification by DecisionDx-Melanoma with biopsy results.63
Patients comprised a convenience sample of 217 patients from a database of 406 patients who had previously been tested with DecisionDx-Melanoma. Patients who had undergone SLNB were eligible for the current study and may have overlapped with patients in the Gerami et al study discussed above. Most patients (73%) had negative SLNB, and 27% had positive SLNB. Five-year overall survival for SLNB-negative patients was 70% versus 62% for SLNB-positive patients. DecisionDx-Melanoma classified 76 tumors (35%) as low-risk (class 1) and 141 tumors (65%) as high-risk (class 2). Five-year overall survival for class 1 patients was 89% versus 55% for class 2 patients. Within the group of SLNB-negative patients, 5-year overall survival was 91% in class 1 patients versus 55% in class 2 patients. Within the group of SLNB-positive patients, 5-year overall survival was 77% in class 1 patients versus 57% in class 2 patients.

Section Summary: Clinical Validity
Two studies using archived tumor specimens suggested that DecisionDx-Melanoma may provide incremental prognostic information for patients with melanoma compared with current staging methods (AJCC staging, SLNB). However, these studies were small and may have enrolled similar patient sets; follow-up may have been inadequate to determine DFS in some patients; and because details about treatments received were not provided, potential confounding by treatment cannot be assessed. The findings require replication in larger, independent cohorts, ideally with homogenous treatment histories.

Clinical Utility
Direct evidence for clinical utility is limited. Berger et al published a retrospective study of 156 consecutive patients from 6 institutions who had cutaneous melanoma and were evaluated with the DecisionDx-Melanoma test. This study used chart review to describe changes in management, and examined whether management changes were associated with DecisionDx-Melanoma results. The frequency of clinic visits, imaging tests, referrals, and blood work was measured before and after results of DecisionDx were available. For patients with class 1 results, there was reduced utilization in 40 of 42 patients; for patients with class 2 results, there was increased utilization for 74 of 79. The difference in management changes by test class was statistically significant (p<0.001).

An indirect chain of evidence to evaluate clinical utility cannot be constructed primarily because robust evidence for clinical validity is lacking. Additionally, it is unclear whether changes in clinical management that may result from DecisionDx-Melanoma test results would impact net health outcome, given current management guidelines for patients with melanoma.

Section Summary: Cutaneous Melanoma
Evidence for clinical validity and utility of the DecisionDx-Melanoma test to identify individuals likely to have an aggressive disease course is currently lacking. Some evidence on clinical validity has been published and reports that the gene expression profile can identify groups of patients with different metastatic risk. This evidence is limited by small, selected patient samples and the lack of independent validation. There is minimal evidence on clinical utility, with 1 retrospective study reporting that test results are associated with utilization measures. An indirect chain of evidence is not possible given the lack of sufficient clinical validity.
Thymomas and Thymic Carcinomas

DecisionDx-Thymoma
No full-length, peer-reviewed studies of DecisionDx-Thymoma were identified.

Clinical Validity
Evidence for clinical validity is lacking.

Clinical Utility
Direct and indirect evidence for clinical utility is lacking.

Section Summary: Thymomas and Thymic Carcinomas
Evidence for clinical validity and utility of the DecisionDx-Thymoma test to identify individuals likely to have an aggressive disease course is currently lacking.

Tests for Genetic Variants That Alter Response to Treatment or to an Environmental Factor

Colon Cancer

ResponseDX: Colon
No full-length, peer-reviewed studies of the ResponseDX: Colon test were identified.

Clinical Validity
Evidence for clinical validity is lacking.

Clinical Utility
Direct and indirect evidence for clinical utility is lacking.

Section Summary: Colon Cancer
Evidence for clinical validity and utility of the Comprehensive Colon Profile to guide treatment selection in patients with colon cancer is currently lacking.

Non-Hodgkin's Lymphoma

TransPredict Fc Gamma 3A
In a multicenter study from France, Cartron et al (2002) compared objective response rates (including unconfirmed complete remission) among 49 previously untreated patients with follicular lymphoma who received rituximab.65 Ten patients (20%) had the homozygous valine genotype of FCGR3A, 17 patients (35%) were homozygous for phenylalanine, and 22 patients (45%) were heterozygotes. At 2 months, objective response rate was 100% in valine homozygotes, 70% in phenylalanine homozygotes, and 64% in heterozygotes (Fisher exact test, p=0.09). At 12 months, objective response rate was 90%, 59%, and 45%, respectively (Fisher exact test, p=0.06). At both time points, the difference in response rate between valine homozygotes and phenylalanine carriers (homo- and heterozygotes) was statistically significant. With median follow-up of 35 months, there was no statistical difference in 3-year PFS between valine homozygotes and phenylalanine carriers (56% vs 35%, respectively).

In a multicenter study from Korea, Kim et al (2006) compared objective response rates in 198 patients with diffuse large B-cell lymphoma who received first-line CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone; n=85) or rituximab plus CHOP (R-CHOP; n=113).66 Fifty-three patients (47%) were valine homozygotes, 6 patients (5%) were phenylalanine carriers (homo- and heterozygotes) was statistically significant.
homozygotes, and 54 patients (48%) were heterozygotes. In the R-CHOP group, complete response rate was statistically greater in valine carriers than in phenylalanine carriers (88% in valine homozygotes, 50% in phenylalanine homozygotes, 79% in heterozygotes, respectively; Fisher exact test, p=0.002). In the CHOP group, response rates were similar across genotypes. With median follow-up of 420 days, no difference in event-free or overall survival across genotypes was found in either treatment group.

Subsequent, larger studies have not shown an association between FCGR3A genotype and outcomes in patients with follicular lymphoma67-70 or chronic lymphocytic leukemia71 who received rituximab plus chemotherapy. Smaller studies in rituximab-treated patients with diffuse large B-cell lymphoma,72-74 mantle cell lymphoma,75 and posttransplant lymphoproliferative disorder76 also have reported no association.

Two meta-analyses were identified, which came to different conclusions about the association of FCGR2A and FCGR3A SNPs and response to rituximab. In 2016, Ghesquieres et al published a patient-level meta-analysis from 2 large patient cohorts of B-cell lymphoma (total N=1034 patients).77 FCGR3A status did not correlate with OS, and there was a marginally significant trend toward worse event-free survival for patients with FCGR3A (hazard ratio, 0.87; 95% CI, 0.76 to 0.99; p=0.04). Febrile neutropenia was more common in patients with FCGR3A mutations (39%) compared to patients with FCGR2A SNPs (29%) or no SNPs (32%; p=0.04). A 2014 meta-analysis from Korea assessed the association between FCGR3A and IL-6 genotype and response to biologic therapies in patients with rheumatoid arthritis.78 Literature was searched through January 2014, and 3 studies of FCGR3A and rituximab were included (total N=500 patients). Study quality was not assessed. A statistically significant association between FCGR3A genotype and response to rituximab was not observed (odds ratio for nonresponse, valine homozygotes vs all other patients, 0.59; 95% CI, 0.10 to 3.4; p=0.55); statistical heterogeneity was considerable (I²=82%).

Clinical Validity
Small studies in patients with non-Hodgkin lymphoma suggested that the Val158Phe polymorphism of the FCGR3A gene may predict response to rituximab therapy, although survival outcomes did not differ by genotype. However, in subsequent, larger studies in rituximab-treated patients with follicular lymphoma and chronic lymphocytic leukemia, this finding was not replicated. Studies in other types of non-Hodgkin lymphoma also have reported no association between FCGR3A genotype and outcomes. Meta-analysis of studies in rheumatoid arthritis did not find an association between FCGR3 genotype and response to rituximab.

Clinical Utility
No studies examining the clinical utility of TransPredict Fc gamma 3A were identified. Factors that support an indirect chain of evidence for predicting response to rituximab are lacking, primarily because evidence for clinical validity of the test is lacking.

Section Summary: Non-Hodgkin’s Lymphoma
No studies examining the clinical utility of TransPredict Fc gamma 3A were identified. Factors that support an indirect chain of evidence for predicting response to rituximab are lacking, primarily because evidence for clinical validity of the test is lacking.
Summary of Evidence
For individuals with various conditions thought to be hereditary or with a known genetic component who receive testing with a miscellaneous genetic or molecular diagnostic test (eg, Celiac PLUS, ColonSentry, Crohn's Prognostic, DecisionDx-Melanoma, DecisionDx-Thymoma, DNA Methylation Pathway Profile, GI Effects (Stool), IBD sgi Diagnostic, ImmunoGenomic Profile, ResponseDX: Colon, SEPT9 methylated DNA [eg, ColoVantage, Epi proColon], TransPredict Fc gamma 3A; Know Error), the evidence consists of case series, cross-sectional studies, diagnostic accuracy studies, and cohort studies. Relevant outcomes are test accuracy and validity, symptoms, change in disease status, and morbid events. The lack of clinical utility of these tests is based on criteria outlined in evidence review 2.04.91 (general approach to genetic testing). Also, 1 or more of the following factors are present: (1) there is no or extremely limited published data addressing the test; and/or (2) there is insufficient evidence demonstrating clinical validity of the test. For each test addressed herein, a literature review is conducted. The literature review was not comprehensive, but sufficient to establish lack of clinical utility. A test will be removed from this evidence review and addressed separately if it is determined that enough evidence has accumulated to reevaluate its potential clinical utility. The evidence is insufficient to determine the effects of the technologies on health outcomes.

Practice Guidelines and Position Statements
Diagnostic Tests: Multiple Conditions
No guidelines or statements were identified.

Diagnostic Tests: Celiac Disease
In 2013, American College of Gastroenterology (ACG) published an evidence-based consensus algorithm for the diagnosis and management of celiac disease. A recommendation for genetic testing using a multigene panel test (eg, Celiac PLUS) was not included.

Diagnostic Tests: Irritable Bowel Syndrome
American Gastroenterological Association
A 2014 evidence-based American Gastroenterological Association (AGA) guideline for pharmacologic management of IBS did not review probiotic treatment.

American College of Gastroenterology
AGG practice guidelines for ulcerative colitis (2010) and for Crohn disease (2009) did not contain recommendations for multimarker panels that include genetic tests to facilitate diagnosis or prognosis.

British Dietetic Association
A 2012 evidence-based British Dietetic Association guideline for dietary management of IBS in adults recommended considering probiotics “secondary to other second-line advanced dietary interventions,” such as reduced intake of fermentable carbohydrates (grade of recommendation: B [based on 4 randomized controlled trial and 1 observational study with high risk of bias]).

National Institute for Health and Care Excellence
A 2008 evidence-based National Institute for Health and Care Excellence guideline for diagnosis and management of IBS in primary care stated that testing for fecal ova and parasites is unnecessary to confirm the diagnosis in patients who meet IBS diagnostic criteria.
guideline also stated: “People with IBS who choose to try probiotics should be advised to take
the product for at least 4 weeks while monitoring the effect. Probiotics should be taken at the
dose recommended by the manufacturer.” This guideline is currently being updated.80

Diagnostic Tests: Colorectal Cancer
Current National Comprehensive Cancer Network (NCCN) guidelines for colon cancer (v.2.2015)
do not include a recommendation for genetic testing (eg, SEPT9 methylated DNA) for early
detection of colon cancer.81,82

Risk Assessment: Multiple Conditions
No guidelines or statements were identified.

Risk Assessment: Colorectal Cancer
National Comprehensive Cancer Network
NCCN guidelines for CRC screening (v.1.2015) include category 2A recommendations for CRC
screening in average-risk individuals using high-sensitivity or immunohistochemical-based stool
testing, flexible sigmoidoscopy with or without interval stool testing, or colonoscopy.83
Colonoscopy is recommended for screening individuals with increased risk. No recommendation
for genetic or molecular testing of average-risk individuals was included.

American College of Physicians
Based on its review of U.S. guidelines, the American College of Physicians (ACP) issued a
guidance statement in 2012 on screening for CRC.84 For average-risk adults ages 50 to 75
years, ACP recommended using a stool-based test, flexible sigmoidoscopy, or optical
colonoscopy for screening. For high-risk patients, ACP recommended using optical colonoscopy.
No recommendation for genetic or molecular testing of average-risk individuals was included.

Prognostic Tests: Crohn Disease
No guidelines or statements were identified.

Prognostic Tests: Cutaneous Melanoma
NCCN guidelines for melanoma (v.3.2015) state that “while there is interest in newer prognostic
molecular techniques such as gene expression profiling to differentiate melanomas at low-
versus high-risk for metastasis, routine genetic testing of primary melanoma (before or
following sentinel lymph node biopsy) is not recommended outside of a clinical trial.”85,86

Prognostic Tests: Thymomas and Thymic Carcinomas
NCCN guidelines for thymomas and thymic carcinomas (v.1.2015) do not address the use of
gene expression profiling of tumors of the thymus.

Tests for Genetic Variants: Colon Cancer
Although current NCCN guidelines for colon cancer (v.2.2015) consider the clinical utility of
genetic testing for specific genes to guide treatment selection (eg, “EGFR testing has no
demonstrated predictive value; therefore routine EGFRT testing is not recommended”), gene
panels for colon cancer are not addressed.81,82
Tests for Genetic Variants: Non-Hodgkin Lymphoma

National Comprehensive Cancer Network
Current NCCN guidelines for non-Hodgkin lymphomas (v.2.2015) do not include a recommendation for genetic testing (eg, TransPredict Fc gamma 3A) to predict response to rituximab therapy.87,88

American College of Rheumatology
Current (2012) American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis do not address FCGR3 testing.89

U.S. Preventive Services Task Force Recommendations
Unless otherwise indicated for the diagnostic, risk assessment, prognostic, and genetic variant tests that alter response to treatment or an environmental factor, no U.S. Preventive Services Task Force (USPSTF) recommendations for genetic or molecular tests have been identified.

Risk Assessment: Colorectal Cancer
USPSTF is currently updating its recommendation for CRC screening in adults.90 Existing recommendations include a grade A recommendation (high certainty that the net benefit is substantial) for CRC screening using fecal occult blood testing, sigmoidoscopy, or colonoscopy beginning at age 50 years and continuing until age 75 years.91

No USPSTF recommendations for genetic or molecular tests for colon cancer have been identified.

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

CPT/HCPCS
81327 SEPT9 (Septin9) (eg, colorectal cancer) methylation analysis
81382 HLA Class II typing, high resolution (ie, alleles or allele groups); one locus (eg, HLA-DRB1, -DRB3/4/5, -DQB1, -DQA1, -DPB1, or -DPA1), each
81401 Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81479 Unlisted molecular pathology procedure
82397 Chemiluminescent assay
82784 Gammaglobulin (immunoglobulin); IgA, IgD, IgG, IgM, each
83520 Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
84999 Unlisted chemistry procedure
86021 Antibody identification; leukocyte antibodies
86140 C-reactive protein;
86255  Fluorescent noninfectious agent antibody; screen, each antibody
87045  Culture, bacterial; stool, aerobic, with isolation and preliminary examination (eg, KIA, LIA), Salmonella and Shigella species
87046  Culture, bacterial; stool, aerobic, additional pathogens, isolation and presumptive identification of isolates, each plate
87075  Culture, bacterial; any source, except blood, anaerobic with isolation and presumptive identification of isolates
87102  Culture, fungi (mold or yeast) isolation, with presumptive identification of isolates; other source (except blood)
87177  Ova and parasites, direct smears, concentration and identification
87209  Smear, primary source with interpretation; complex special stain (eg, trichrome, iron hemotoxylin) for ova and parasites

87328  Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative, multiple-step method; cryptosporidium
87329  Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative, multiple-step method; giardia
87336  Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative, multiple-step method; Entamoeba histolytica dispar group
87798  Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism

**Diagnoses**
Experimental / Investigational for all diagnoses related to this medical policy.

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**REVISIONS**

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<td>01-07-2016</td>
<td>Policy added to the bcbsks.com web site on 12-08-2015 with an effective date of 01-07-2016.</td>
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<tr>
<td>08-17-2016</td>
<td>Updated Description section.</td>
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<td>Updated Rationale section.</td>
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<td>▪ Removed CPT code: 88347.</td>
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<td>Updated References section.</td>
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<tr>
<td>01-01-2017</td>
<td>In Coding section:</td>
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<tr>
<td></td>
<td>▪ Added CPT code: 81327 (New code, effective January 1, 2017).</td>
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REFERENCES


43. Shirts B, von Roon AC, Tebo AE. The entire predictive value of the prometheus IBD sgi diagnostic product may be due to the three least expensive and most available components. Am J Gastroenterol. Nov 2012;107(11):1760-1761. PMID 23160303


69. Weng WK, Levy R. Immunoglobulin G Fc receptor polymorphisms do not correlate with response to chemotherapy or clinical course in patients with follicular lymphoma. Leuk Lymphoma. Sep 2009;50(9):1494-1500. PMID 19672774


82. Vijzelaar R, van der Zwan E, van Gammeren A, et al. Rapid Detection of the Three Celiac Disease Risk Genotypes HLA-DQ2.2, HLA-DQ2.5, and HLA-DQ8 by Multiplex Ligation-
PMID 26798991


Other References
1. Blue Cross and Blue Shield Pathology Liaison Committee, July 2016.
2. Blue Cross and Blue Shield Surgery Liaison Committee, February 2016.

APPENDIX

Appendix 1. Categories of Genetic Testing Addressed

<table>
<thead>
<tr>
<th>Category</th>
<th>Addressed</th>
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</thead>
<tbody>
<tr>
<td>1. Testing of an affected individual’s germline to benefit the individual</td>
<td>Yes</td>
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<tr>
<td>1a. Diagnostic</td>
<td>X</td>
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<tr>
<td>1b. Prognostic</td>
<td>X</td>
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<tr>
<td>1c. Therapeutic</td>
<td>X</td>
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<tr>
<td>Category</td>
<td>Addressed</td>
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<tr>
<td>-------------------------------------------------------------------------</td>
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<tr>
<td>2. Testing cancer cells from an affected individual to benefit the individual</td>
<td>X</td>
</tr>
<tr>
<td>2a. Diagnostic</td>
<td>X</td>
</tr>
<tr>
<td>2b. Prognostic</td>
<td>X</td>
</tr>
<tr>
<td>2c. Therapeutic</td>
<td>X</td>
</tr>
<tr>
<td>3. Testing an asymptomatic individual to determine future risk of disease</td>
<td>X</td>
</tr>
<tr>
<td>4. Testing of an affected individual’s germline to benefit family members</td>
<td></td>
</tr>
<tr>
<td>5. Reproductive testing</td>
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<tr>
<td>5a. Carrier testing: preconception</td>
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</tr>
<tr>
<td>5b. Carrier testing: prenatal</td>
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<tr>
<td>5c. In utero testing: aneuploidy</td>
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<tr>
<td>5d. In utero testing: mutations</td>
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</tr>
<tr>
<td>5e. In utero testing: other</td>
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<tr>
<td>5f. Preimplantation testing with in vitro fertilization</td>
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