The Public Health Evidence for FDA Oversight of Laboratory Developed Tests: 20 Case Studies

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Office of the Commissioner
Food and Drug Administration

November 16, 2015

Executive Summary

Laboratory developed tests (LDTs) serve an increasingly important role in health care today. They also have become significantly more complex and higher risk, with several notable examples of inaccurate tests placing patients at otherwise avoidable risk.

While laboratories that offer LDTs are subject to the Federal Food, Drug, and Cosmetic Act (FD&C Act), in addition to the Clinical Laboratory Improvement Amendments (CLIA), FDA has generally exercised enforcement discretion towards these tests (i.e., generally not enforced applicable provisions under the FD&C Act and FDA regulations).

Therefore, most laboratories that offer LDTs follow only the regulatory requirements of CLIA, which are intended to regulate the operations of laboratories, but are not specifically intended to regulate in vitro diagnostic devices. Despite the contention from some that "CLIA is enough," all of the tests described as problematic in this report were offered from laboratories following the minimum requirements of CLIA.

We examined events involving 20 LDTs that illustrate, in the absence of compliance with FDA requirements, that these products may have caused or have caused actual harm to patients. In some cases, due to false-positive tests, patients were told they have conditions they do not really have, causing unnecessary distress and resulting in unneeded treatment. In other cases, the LDTs were prone to false-negative results, in which patients' life-threatening diseases went undetected. As a result, patients failed to receive effective treatments.

Other LDTs provided information with no proven relevance to the disease or condition for which they are intended for use, while still others are linked to treatments based on disproven scientific concepts. In addition to patient harm, inaccurate or unreliable tests can be costly to society. We estimated these costs, if sufficient data were available.

I. Introduction

A. Background

In 1976, as part of the Medical Device Amendments to the Federal Food, Drug, and Cosmetic Act (FD&C Act), Congress gave FDA the authority to regulate in vitro diagnostics (IVDs) as medical devices.[1] Laboratory developed tests (LDTs) are the subset of IVDs intended for clinical use that are designed, manufactured, and used in a single laboratory, as opposed to other IVDs made by a conventional manufacturer and used by many laboratories.

An IVD meets the device definition irrespective of where and by whom it is manufactured; LDTs are therefore subject to the requirements of the FD&C Act and fall under FDA jurisdiction. Historically, however, FDA has generally used its enforcement discretion to not enforce the device provisions of the FD&C Act for LDTs.

In the 1970s, LDTs were limited in number and used fairly simple technologies, typically to diagnose rare diseases and conditions in small numbers of patients.

As technology and science have advanced, LDTs have increased in complexity and availability and are now used to diagnose common, serious medical conditions, including cancer and heart disease. Others guide therapy for these and other conditions, while still others predict one's personal risk of developing a particular disease. Some individual laboratories that initially developed LDTs have now morphed into separate businesses that market complex tests nationwide. These activities take place in much larger populations than the local or limited patient populations who may have used these products four decades ago.

As the field of medicine evolves, the need for accurate, reliable, and clinically meaningful tests is essential. For instance, the advancement of Precision Medicine,[*] depends upon accurate diagnosis in order to better target therapies. But inaccurate or unreliable LDTs and unsupported or disproven claims can undermine progress in Precision Medicine and other fields.

While certain LDTs have undoubtedly brought benefits to many patients, the increase in complexity and patient volume brings a concomitant risk that patients will be harmed – and, in fact, have been harmed – and highlights the need for appropriate oversight. It is not the intention of this report to undermine the value of LDTs, but rather to highlight that the current oversight framework is inadequate and, hence, why FDA has proposed to increase its oversight for these tests.[2]

FDA oversight for LDTs is needed to address several serious concerns:

- Lack of evidence supporting the clinical validity of tests.

[*] Precision Medicine encompasses prevention and treatment strategies that take individual variability into account.

Most fundamentally, patients expect that any test administered or ordered by their health care provider will generate a result upon which they can base decisions that can affect their life and health. Inaccurate tests can result in the failure to detect life-threatening diseases, might cause patients to elect unproven therapies over proven ones, or can cause patients unnecessarily to undergo uncomfortable and even dangerous procedures. The examples presented in this report illustrate each of these circumstances. FDA oversight would help ensure that regulated tests are supported by rigorous evidence, thus assuring patients and health care providers that they can have confidence in the test result.

- Deficient adverse event reporting.

Device adverse event reporting requirements provide a mechanism by which adverse events (serious injuries, deaths, malfunctions likely to cause/contribute to serious injuries/deaths) associated with use of a medical device can be reported by a manufacturer to the FDA and tracked. This is an important tool both for manufacturers (to identify problems with their test systems that may develop over time) as well as FDA (to identify potential public health issues and to take regulatory action, as appropriate). Currently, information on adverse events associated with LDTs is not systematically collected or reported.

- No premarket review of performance data.

The time to evaluate the safety and effectiveness of a product is before it is marketed, not after it has been used by thousands of patients. Premarket review, particularly for high-risk LDTs, is all the more important in the absence of satisfactory adverse event reporting.

- Unsupported manufacturer claims.

A critical part of premarket review is the assurance that manufacturer claims are supported by the available data. In the absence of appropriate oversight, unsupported claims have the potential to seriously mislead patients and health care providers. The cases reviewed provide several examples of such claims.

- Inadequate product labeling.

Without FDA review and oversight of LDT labeling, the labeling may not provide adequate information for patients and providers, including adequate information on interpreting a test result and determining whether and when follow-up testing is necessary.

- Lack of transparency.

In the absence of appropriate oversight, patients and health care providers may be unaware of any scientific basis for manufacturer claims or any support that the LDT performs as claimed. Indeed, patients and providers may not even be aware that an uncleared/unapproved LDT has been used or that an FDA-cleared/approved test could have been used instead.

- Uneven playing field.

Laboratories and other IVD manufacturers that go through the process of conducting the research necessary to validate their devices and seek premarket review are placed at an unfair disadvantage when their LDT competitors do not follow the same standards to support their claims and the safety and efficacy of their device. Under the status quo, manufacturers have every incentive not to seek FDA clearance/approval, and the public is thus denied the advantages and improvements in scientific rigor the research and review process ensures.

- Threats to the scientific integrity of clinical trials.

Clinical investigators studying other products often rely on LDTs to select patients for participation in a clinical trial and, if the patient is enrolled, whether to provide the patient with a particular treatment. If the tests are inaccurate, the scientific conclusions derived from these trials may also be inaccurate.

- No comprehensive listing of all LDTs currently being used.

This prevents an overall assessment of the LDTs on the market, including the extent of inaccurate or unreliable LDTs.

It is often claimed that FDA regulation of LDTs is unnecessary because the tests are sufficiently regulated under the Clinical Laboratory Improvement Amendments (CLIA). While CLIA created requirements that are essential for ensuring that laboratories and their personnel maintain standards of high quality (i.e., it is primarily concerned with the process of testing), compliance with CLIA regulation alone does not ensure that the diagnostic devices themselves are safe and effective.[2] As noted by the Centers for Medicare and Medicaid Services (CMS), which oversees the CLIA program:

> The CMS' analytical validity review is intended to determine if a specific test finds what it is supposed to find (i.e. the analyte it is intended to detect) when laboratories perform testing on patient specimens. Therefore, the analytical validation must be performed by the laboratory intending to use the test on patient specimens. Furthermore, the laboratory's analytical validation of an LDT is reviewed during its routine biennial survey – after the laboratory has already started testing. Moreover, the routine CLIA survey does not include a review of the clinical validation of a LDT – that is, the accuracy with which the test identifies, measures, or predicts the presence or absence of a clinical condition or predisposition in a patient.
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> In contrast, the FDA's review of analytical validity is done prior to the marketing of the test system, and, therefore, prior to the use of the test system on patient specimens in the clinical diagnosis/treatment context. Further, the FDA's analytical validity review is more in-depth and more comprehensive than that of the CLIA program, and it is focused on the test system's safety and effectiveness. As a result, FDA review may uncover errors in test design or other problems with a test system. Also, while CMS' CLIA program does not address the clinical validity of any test, FDA's premarket review of a test system includes an assessment of clinical validity.[3]

FDA's experience with non-LDT IVDs gives a sense of the issues that may arise with LDTs. In 2014 alone, FDA issued 31 warning letters to IVD manufacturers for various reasons, including adulteration due to violations of quality system regulations. That year, inspections of IVD manufacturers identified problems such as inadequate design validation and inadequate investigation of devices failures. In addition, FDA classified and reviewed 313 product recalls that were performed by the IVD industry. The same sorts of problems would be expected with LDTs, but could go undetected because there is generally no premarket review and limited adverse event reporting for LDTs. In fact, these problems may be more common because laboratories that produce LDTs may not follow key aspects of the quality system regulations, such as design controls and supplier controls.

B. Public Health Statistics: understanding public health problems

To better understand the issues surrounding the evaluation of LDTs and the cases presented in this report, a basic public health vocabulary related to diagnostic test performance is provided below.

I. True positive: A person who has a positive test result and actually has the disease/condition.
II. False positive: A person who has a positive test result but does not actually have the disease/condition.
III. True negative: A person who has a negative test result and actually does not have the disease/condition.
IV. False negative: A person who has a negative test result but actually has the disease/condition.
V. Sensitivity is the ability to of a test to detect the disease when it is present; it is defined as # true positives/# with the disease/condition.
VI. Specificity is the ability of a test to exclude the disease when it is absent; it is defined as # true negatives/# without the disease/condition.
VII. Positive Predictive Value (PPV) is the likelihood that a positive test truly represents the presence of the disease/condition; it is defined as # true positives/# all positives. A test has a high PPV if a large percentage of people who get a positive test result actually have the disease/condition.
VIII. Negative Predictive Value (NPV) is the likelihood that a negative test truly represents the absence of the disease/condition; it is defined as # true negatives/# all negatives. A test has a high NPV if a large percentage of people who get a negative test result really don't have the disease/condition.
IX. "Analytic Validity" refers to acceptably demonstrated performance in the measurement or detection characteristics of a test: how well the test measures or identifies something in a person, such as a protein or a gene mutation.
X. "Clinical Validity" is the acceptably demonstrated association of a test result with the presence or absence of the target disease/condition.

C. Glossary

ASD	Autism Spectrum Disorder
CDC	U.S. Centers for Disease Control and Prevention
CDRH	Center for Devices and Radiological Health, FDA
cfDNA	Cell-Free DNA
CHD	Coronary Heart Disease
CML	Chronic Myelogenous Leukemia
DNA	Deoxyribonucleic Acid
EUA	Emergency Use Authorization
FDA	U.S. Food and Drug Administration
HER2	Human Epidermal Growth Factor Receptor 2
HPV	Human Papillomavirus
IDE	Investigational Device Exemption
IOM	Institute of Medicine
LDT	Laboratory Developed Test
PCR	Polymerase Chain Reaction
PSA	Prostate Specific Antigen
RNA	Ribonucleic Acid

RT-PCR Reverse Transcription-Polymerase Chain Reaction
SNP Single Nucleotide Polymorphism

II. Case Studies of Problematic LDTs

To assess the public health impact of problematic LDTs, we identified 20 well-documented cases from publicly available information in medical journals, media reports and FDA Warning Letters. FDA is limited in its ability to identify such cases as adverse events on LDTs have generally not been reported to the Agency. FDA is aware of additional cases, but these are not included in this report because either the data demonstrating that the test is faulty are more limited or because these include confidential commercial information, which the Agency is prohibited by law from releasing to the public.

These cases are grouped according to their primary problem; many cases have more than one problematic aspect. For five of these cases, economic assessments of impact were conducted by FDA's Economics Staff.

A. Tests that Yield Many Positive Results when the Disease or Condition is not Actually Present (False-Positives)

i. Lyme Disease Diagnostic Tests

Category	LDT Characteristics
LDT Name	Lyme disease antigen and culture tests
Description	Test to detect portions of the bacterium that causes Lyme disease or antibodies to the bacterium
Purpose	Diagnose Lyme disease
Target Population	Patients with symptoms suggestive of Lyme disease
Alternatives	Over 80 FDA-cleared diagnostic tests
LDT Problem 1	In clinical use, large numbers of patients with positive tests do not have Lyme disease
Clinical Consequence	Patients with false-positive tests may be treated with unnecessary medications; delayed diagnosis of true underlying condition
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards
Cost Impact of Inaccuracy	$1,226 per case

Lyme disease is caused by infection with the bacterium *Borrelia burgdorferi*, transmitted to humans by the bite of an infected tick. The diagnosis is based on a history of exposure to ticks along with typical symptoms, including fever, fatigue, muscle, and joint aches, and a characteristic rash.[4] CDC recommends a two--test process to detect antibodies against *B. burgdorferi*.[5] If an initial enzyme-linked immunosorbent assay test is positive or indeterminate, it is followed by a confirmatory Western Blot test.

A patient is only diagnosed with Lyme disease if the confirmatory Western Blot is positive. As of May 2015, over 80 initial and confirmatory diagnostic blood tests for Lyme disease had been cleared by FDA.[6] Patients diagnosed with Lyme disease are treated with oral or intravenous antibiotics for 2-4 weeks. This relieves symptoms in 80%-90% of patients, but can lead to harmful side effects, including nausea, allergic reactions,[7] and intravenous site infection.[8] A falsely positive diagnosis of Lyme disease can lead to patients experiencing harmful side effects without clinical benefit, an increase in the risk of creating infectious organisms resistant to the antibiotics used to treat Lyme disease, and delay in the diagnosis of a patient's true underlying condition.

Between 2000 and 2005, a "Dot Blot" test for urine antigens against Lyme disease was offered, claiming a 97% "true positive rate," although this term does not have a clear meaning in public health terms.[9] An independent evaluation conducted in 2001 ran the test five times for the same 10 healthy subjects (i.e., 50 tests) and found that the test was consistently falsely positive in all tests run for two subjects (10 false-positive tests) and gave contradictory results on at least two pairs of tests for 8 subjects (i.e., at least 16 false-positive tests), leading to the conclusion that at least half of all test results were incorrect or uninterpretable, and that this test should not be used for Lyme disease detection.[9]

Further research also indicated that, because of lack of a clear correlation with clinical disease, urine tests in general are not appropriate for the diagnosis of Lyme disease,[10] but sales continued with between 50,000 and 70,000 tests sold in 2005.[11] Early that year, however, the LDT was implicated in 8 reports of false-positive diagnoses.[11]

Diagnostic tests for Lyme antigens in the blood also have been marketed. One marketed between 2003 and 2005 was prone to false-positives. On the basis of false-positive results, two couples underwent months of unnecessary treatment with antibiotics and other alternative medications.[12] After litigation, a judge awarded them a total of $30 million in damages.[12]

In April 2014, CDC issued a warning related to a Lyme disease culture test.[13] The Agency had conducted a review[14] that "raised serious concerns about false-positive results caused by laboratory contamination and the potential for misdiagnosis." Consequently, CDC recommended that only FDA-cleared/approved diagnostics for Lyme disease be used.

FDA estimated the cost of a false-positive diagnosis as the direct medical treatment costs for a patient with early-stage Lyme disease. The most relevant and comprehensive estimate (in Year 2000 dollars) comes from a study of Lyme disease patients with varying severities of disease and includes the costs of health care provider visits, consultation, serologic testing, therapy, hospitalization, and out-of-pocket costs of prescription and non-prescription drugs.[15] Updating the mean per-patient costs to current dollars using the medical care component of the Consumer Price Index yields an estimate of $1,226[16] for the cost to society for each case.[17]

ii. OvaCheck Ovarian Cancer Screening and Detection Test

Category	LDT Characteristics
LDT Name	OvaCheck
Description	Blood test and companion algorithm to create a genetic profile of markers displayed by cancer cells
Purpose	Screen and detect ovarian cancer
Target Population	Women at risk for ovarian cancer
Alternatives	Other biomarkers or physical symptoms
LDT Problem 1	No validation that test predicts or detects ovarian cancer
LDT Problem 2	Inflated accuracy claims by the manufacturer
Clinical Consequence	Women with false-positive tests may undergo unnecessary surgery to remove healthy ovaries
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims; assurance of consistent manufacturing practices and standardized instrument calibration
Cost Impact of Inaccuracy	Not estimated; not brought to market

Ovarian cancer is one of the more common and deadly cancers, with 14,000 deaths per year in the United States.[18] There is currently no reliable screening test for ovarian cancer, so most women are diagnosed only after the disease has spread widely, resulting in the poor prognosis.[19] Depending on the

extent of disease spread, treatment can include surgery to remove the ovaries, the uterus, and any visible cancer, followed by chemotherapy and sometimes radiation.[20] Effective early detection would reduce the mortality from ovarian cancer, but a screening test that over-diagnoses the disease will lead to extensive medical workups and potential unnecessary treatment. Importantly, a test that was prone to false-positives would be readily discerned in this clinical situation – the surgical specimen would have no cancer cells. But false-positives related to other tests might not be as easily detected.

In the late 1990s, a group of federal scientists believed, based on their research, that they had developed a new protein signature that could detect early ovarian cancer. OvaCheck, a commercial test, was developed using a technique called mass spectrometry that could distinguish between blood samples from ovarian cancer patients and those from healthy women based on that protein signature.[21] Subsequently, researchers derived a positive predictive value (PPV) of 94%,[22,23] suggesting that only 1 out of every 17 positive OvaCheck tests would be a false-positive.

However, the reported PPV was based on an incorrect ovarian cancer prevalence of almost 50% (derived using the ratio of 50 cancer cases to 66 controls in their study, a ratio the researchers determined by who they decided to enroll), rather than the true prevalence of 1 case for every 2,500 women in the screening population, greatly inflating the PPV from the true 0.8% to the reported 94%. Thus, in clinical practice, fewer than 1 per 100 women who test positive would actually have ovarian cancer.[24,25] Some of these false-positives would likely be detected by subsequent workup, but a subset would likely proceed to surgery.

In February 2004, FDA's Center for Devices and Radiological Health issued a letter to the company indicating the need for premarket review of the device.[26] Responding to public pressure, the investigators made their data public, but independent analyses found that the results were not reproducible and that calibration of the mass spectrometry instrument was faulty.[27] Other researchers claimed that the algorithm to interpret test results was not valid, and that the analytic methodology was flawed.[28]

The device was not brought to market in the U.S. and so an accounting of costs associated with a false-positive diagnosis is not possible. However, the cost could have been similar to that for OvaSure and PreOvar (see below). But for the intervention of FDA and others, women could have been exposed to this test and many would have been incorrectly diagnosed and possibly treated for ovarian cancer that they did not have.

iii. OvaSure™ Ovarian Cancer Screening Test

Category	LDT Characteristics
LDT Name	OvaSure Screening Test
Description	Blood test on fourbiomarkers based on initial research in the published literature reporting an association with ovarian cancer
Purpose	Screen for and detect ovarian cancer
Target Population	Women at risk for ovarian cancer
Alternatives	Other biomarkers or physical symptoms
LDT Problem 1	No validation that test predicts or detects ovarian cancer

LDT Problem 2	Inflated PPV claims by the manufacturer, so many patients with a positive test won't have the disease
Clinical Consequence	Women with false-positive tests may undergo unnecessary surgery to remove healthy ovaries
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims
Cost Impact of Inaccuracy	$12,578 per ovary removal after false-positive

In an effort to develop better cancer detection methods, researchers at Yale published a study on a test that they claimed had a PPV of 99.3%[29] (i.e., almost all positive test results appeared to represent actual ovarian cancer patients). The test, OvaSure, and a companion interpretation algorithm were marketed to screen for early stage ovarian cancer in high-risk women, beginning in June 2008.[29]

However, the PPV was derived using the ratio of cancer cases to controls in a single study (46%, a ratio the researchers determined by who they decided to enroll), rather than from the prevalence of the disease in the screening population. This meant that the PPV of 99.3% was inflated beyond what would be experienced in clinical practice. For example, the true PPV dropped to 6.5% if the actual population prevalence of 0.04% was used,[30] meaning that only 1 in 15 patients who tested positive actually had the disease and the remaining 14 women with a positive result could undergo unnecessary surgery to remove healthy ovaries, if subsequent workup did not rule out the disease.

In July 2008, the Society of Gynecologic Oncologists issued a formal statement that additional clinical validation of the test's effectiveness was needed before it should be offered outside a research study.[31] In light of these concerns, FDA notified the manufacturer in August 2008 that it considered OvaSure to be a "high-risk test that has not received adequate clinical validation, and may harm the public health."[32] The manufacturer did not provide further validation, prompting FDA to issue another letter, this time a warning letter.[33] In October 2008, OvaSure was pulled from the market.[30]

Using data from the Healthcare Cost and Utilization Project (HCUP), a nationally representative sample of hospital discharges, the average cost of surgery to remove the ovaries would be $9,200 per patient. We assumed an average hospital stay of 3 days, a post-surgical recovery of 10 work days, and estimated the value of an hour's work at $31.52.[34] Assuming a woman would miss 13 days of work, the total value of lost productivity per patient would be $3,378 ($31.52 x 8 x 13). Adding the direct medical cost yields a total cost to society of $12,578 per patient receiving a false-positive result. This estimate does not account for the costs, economic and psychosocial, of infertility and hormone replacement therapy for women who needlessly had their ovaries removed.

iv. PreOvar KRAS-Variant Ovarian Cancer Screening Test

Category	LDT Characteristics
LDT Name	PreOvar KRAS-Variant Test
Description	Blood or saliva test for KRAS-variant genetic mutation
Purpose	Identify women with elevated risk of ovarian cancer; guide treatment for ovarian cancer patients
Target Population	Women at risk for and with a diagnosis of ovarian cancer

Alternatives	Other biomarkers or medical history to assess personal risk and likelihood of response to therapy
LDT Problem 1	Lack of validation that KRAS-variant correlates with cancer risk and therapeutic response
LDT Problem 2	Faulty data analysis
Clinical Consequence	Women with false-positive tests may undergo unnecessary surgery to remove healthy ovaries; women with ovarian cancer may receive other inappropriate treatments
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims; evaluation of company data analyses
Cost Impact of Inaccuracy	$12,578 per ovary removal after false-positive

A certain variant in the KRAS gene has been reported in one publication to impart an elevated risk of ovarian cancer, particularly in women with a positive family history.[35] PreOvar, an LDT to detect the KRAS-variant mutation to aid in the prediction of ovarian cancer risk (for at-risk women undergoing screening) and to predict response to treatment (for those with a cancer diagnosis), was offered beginning in 2010.[36]

In September 2010, the Society of Gynecologic Oncology (SGO) released a statement that the test was developed and marketed to the public with insufficient clinical validation.[37] Concerned that the initial study was too small to generate a definitive assessment of ovarian cancer risk, researchers from the Ovarian Cancer Association Consortium performed an independent evaluation of over 21,000 subjects, finding no evidence of an association between the KRAS-variant and ovarian cancer.[38]

Amid growing controversy, the Consortium conducted a new analysis using its database of controls, cancer cases and patients with gene variants, and concluded that a group of variants, including KRAS, were not predictive of ovarian cancer. The authors suggested that earlier associations may have been due to small sample size or associations between the KRAS variant and other factors.[39,40] Despite these actions from the scientific community, this test remains on the market,[41] and the company's website states that the test "results are >99.9% accurate,"[41] placing women at risk of being incorrectly told that they have a high risk of ovarian cancer or a better chance of responding to therapies.

This might, in turn, place women being screened at risk for undergoing unnecessary diagnostic and surgical procedures, and expose women with ovarian cancer to potentially inappropriate treatment. The cost of ovarian removal following a false-positive diagnosis would be identical to that for OvaSure (see above) and thus is not duplicated here.

v. Whooping Cough (Pertussis) Diagnostic PCR Test

Category	LDT Characteristics
LDT Name	Whooping Cough (Pertussis) PCR Test
Description	Single or multiple target PCR tests
Purpose	More rapid and improved diagnosis of whooping cough
Target Population	People who have been exposed to whooping cough; those suspected to have whooping cough

Alternatives	Bacterial culture; FDA-cleared PCR tests; blood antibody test
LDT Problem 1	In clinical use, patients with positive test may not have whooping cough
Clinical Consequence	Patients with false-positive tests were incorrectly diagnosed and incorrectly treated
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims
Cost Impact of Inaccuracy	Not estimated

Pertussis, or whooping cough, is a bacterial illness spread through coughing and sneezing by an infected person. The CDC reports that between 300,000 and 626,000 adults get whooping cough each year in the U.S.[42] Historically, the disease was diagnosed by its hallmark symptoms, which are cold-like complaints along with prolonged bouts of coughing that can lead to vomiting. Because of its ability to compromise the airway, whooping cough can be deadly to infants, but is also dangerous for elderly or frail patients.

It is generally treated with a course of antibiotics; these drugs can also prevent the disease in people who have been exposed but are not ill, a standard practice known as "post-exposure prophylaxis." The antibiotics used are common, but can have side effects ranging from nausea, vomiting and headache to rare, but potentially fatal, irregularities in the heart's rhythm. A vaccine (Tdap) is estimated by CDC to be 85-90% effective initially in preventing new whooping cough cases, but vaccine efficacy wanes over several years, putting people who are exposed to pertussis at risk of infection.

Doctors can diagnose whooping cough with a variety of tests, some of which are FDA-cleared, including bacterial culture, a blood antibody test, and tests on samples from the nose and throat to detect bacterial DNA. Culture is considered the most accurate diagnostic test. Rapid detection tests identify pertussis by matching a clinical sample to one or two "target" gene sequences using a technique called polymerase chain reaction (PCR). If one target is used, additional testing often is necessary. They also allow public health officials to act quickly to prevent an outbreak. However, standardization and central oversight of the quality of those PCR tests not cleared by FDA is lacking.

In March 2006, a health worker at the Dartmouth University hospital in New Hampshire presented to a clinic with a 3-week history of classic symptoms and was diagnosed with whooping cough on a single-target LDT PCR, started on antibiotics, and furloughed for 5 days.[43] The subsequent investigation identified 15 workplace contacts of the initial case who had respiratory illness and a positive or equivocal result on the LDT PCR. The hospital suspected a whooping cough outbreak. The expanding investigation led to the identification of additional symptomatic patients, and all laboratory workers at the hospital were provided with post-exposure antibiotic prophylaxis and vaccination.

The hospital then began testing all symptomatic people with the LDT PCR. By June, 134 cases of suspected whooping cough had been identified. The PCR test was positive or equivocal in 98 of these cases (73%), and an additional 36 cases were identified using symptoms alone. Twenty-seven of these 134 cases were submitted for culture. None were positive. Substantial resources were invested to investigate and control this suspected outbreak.

Overall, 978 workers with symptoms were treated, and, out of an abundance of caution, furloughed from their posts as they waited to see if their PCR test would be negative; 1,311 close contacts of

suspected cases were given post-exposure prophylaxis antibiotics, and 4,524 workers were vaccinated. As a result, many people who did not have whooping cough lost productive days of work, and were exposed to treatments that can have harmful side effects. The risk of developing antibiotic-resistant organisms was increased.

B. Tests that Yield Many Negative Results when the Disease or Condition is Actually Present (False-Negatives)

i. Oncotype DX HER2 Breast Cancer RT-PCR Test

Category	LDT Characteristics
LDT Name	Oncotype DX HER2 RT-PCR
Description	Rapid PCR test for tumor HER2 receptors
Purpose	Use HER2 receptor level to guide treatment
Target Population	Newly diagnosed Stage I and II breast cancer patients
Alternatives	FDA-approved HER2 receptor tests
LDT Problem 1	Test has poor sensitivity – many tests reported as normal HER2 levels will actually have high HER2 levels
Clinical Consequence	Patients with false-negative tests won't receive appropriate treatment, and cancer may progress
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards
Cost Impact of Inaccuracy	$775,278 estimated cost per false-negative case

The majority of breast cancers test positive for particular hormone receptors, proteins that bind their corresponding hormone and stimulate the cancer to grow. About 1/4th[44] to 1/5th[45] of breast cancer patients have on their tumors more than the normal amount of a certain type of receptor, known as human epidermal growth factor receptor 2 (HER2). These women have higher rates of breast cancer recurrence and mortality (i.e., a worse prognosis) than those who are HER2-negative. Cancer doctors, therefore, recommend that every patient with invasive breast cancer undergo HER2 testing.

Women who over-express HER2 receptors are treated with one or more drugs such as trastuzumab (Herceptin®) in addition to chemotherapy. Trastuzumab targets HER2 receptors and improves tumor response rates, time to progression, and survival.[46] However, it also can cause serious though rare heart disease,[47] and is costly, estimated by the Journal of Oncology at approximately $100,000 for a year of therapy in 2006 dollars.[45] The majority of tests used to detect HER2 protein or gene amplification are LDTs, but, at least in the past, approximately 20% of tests may have been inaccurate,[45] creating concern that some invasive breast cancer patients may be exposed to treatments that are less than optimal when the test fails to detect high HER2 levels.

Starting in 2008, Genomic Health began adding results from the HER2 test, which is part of the 21-gene signature that makes up the Oncotype DX Breast Cancer test, to their test report. The Oncotype Dx test provides individualized breast cancer treatment options and recurrence risk estimates based on the genes expressed in a tumor.[48,49] The RNA-based form of HER2 test is not included in the guidelines issued by the American Society of Clinical Oncology (ASCO) as a test to be used to decide whether

trastuzumab is indicated. Instead, ASCO focuses on FDA-approved tests known as fluorescence in situ hybridization (FISH) tests and immunohistochemistry (IHC) tests for detecting HER2 gene amplification and protein overexpression, respectively.[50]

The underlying issue is that there is no demonstrated direct correlation between number of RNA copies of the gene, the basis for Oncotype Dx HER2 RT-PCR, and the number of protein copies on the cell surface. As a consequence, it is not possible to infer that high or low amounts of RNA correspond to high or low amounts of HER2 protein.

In 2011, a group of prominent pathologists from three independent laboratories found discrepancies between this HER2 RT-PCR and the FDA-approved tests. The LDT reported large numbers of tumors that tested positive on FISH-HER2 as equivocal (33% of FISH-positive cases) or negative (39% of FISH-positive cases).[51] In 2014, the LDT missed all three HER2-positive patients included in a study, diagnosing two as negative and one as equivocal. As a result, the two patients who tested HER2-negative failed to receive trastuzumab, placing them at higher risk for cancer progression.[52]

We estimated the social cost when patients fail to receive appropriate trastuzumab therapy by multiplying the number of years a patient could gain from appropriate cancer treatment by the value of a statistical life-year (VSLY). Standard estimates for the VSLY are $129,213, $258,426, and $387,639.[53,54,55] Research has shown that the projected life expectancy is 3 years longer for HER2-positive patients who receive trastuzumab in addition to chemotherapy, compared to those receiving chemotherapy alone.[56] Multiplying the 3 life-years gained from therapy by the middle VSLY value of $258,426 allows us to estimate the cost to society for each patient who fails to receive trastuzumab as $775,278.

ii. Human Papillomavirus Test using SurePath Collection Medium

Category	LDT Characteristics
LDT Name	SurePath Collection Medium for cervical samples collected for Human Papillomavirus (HPV) Test
Description	Collection medium to permit DNA or RNA test to detect HPV strains conferring high risk for cervical cancer
Purpose	To determine or assist in the management of pre-cancerous lesions of the cervix
Target Population	Women with an equivocal Pap Test; women over 25 tested for HPV alone; women over 30 if tested with Pap Test and HPV test simultaneously for cervical cancer screening
Alternatives	FDA-approved combinations of cervical sample collection media and HPV assay
LDT Problem 1	Use of test with unknown sensitivity
Clinical Consequence	Patients with false-negative test results may receive improper patient management; pre-cancerous cells may progress to cancer and patients may require more extreme medical interventions.
Potential Impact of FDA Oversight	Assurance there is premarket review of test performance

Cost Impact of Inaccuracy	Not estimated

The majority of cervical cancer is caused by an infection with one or more "high risk" strains of the human papillomavirus (HPV). Current guidelines[57] state that women should be screened regularly[58] for cervical cancer, as periodic screening has been shown to reduce mortality from cervical cancer.[57] To date, FDA has approved 6 HPV tests; these tests are approved with specific collection media that permit transport to the testing location. For women with equivocal Pap test results, all these approved assays have negative predictive values (NPVs) above 99%, so a negative test can avert colposcopy, an examination of the cervix that is the next step in the workup of suspected cervical cancer.

In addition to the currently available FDA-approved HPV tests, some laboratories are using cervical samples collected in the SurePath collection medium, even though this medium has only been approved for Pap testing, but not for HPV testing. The manufacturer of the SurePath collection medium has not publicly reported the NPV or the PPV for any HPV tests used with cervical samples in the SurePath medium.[59]

In June 2012, it issued a technical bulletin to laboratories stating that the use of cervical samples in the SurePath collection medium for HPV testing "may, under certain conditions, provide false-negative results."[60] In 2013, an investigative journalist reported that labs were using HPV test results derived from cervical samples that had been collected in the SurePath medium, despite the warning.[59]

Under existing guidelines, a false-negative test result could lead to the absence of patient follow-up and, ultimately, to preventable cancer progression. For this reason, the professional societies that set U.S. cervical cancer screening guidelines specifically recommend against the use of LDTs for cervical cancer screening.[57]

C. Tests with the Potential to Yield both Many False-Positive and False-Negative Results

i. Noninvasive Prenatal Testing (A.K.A. cell-free DNA testing)

Category	LDT Characteristics
LDT Name	Noninvasive prenatal cell-free DNA testing (NIPT, or cfDNA)
Description	Blood test to identify traces of fetal chromosomes in maternal blood
Purpose	To detect a range of fetal chromosomal abnormalities
Target Population	Pregnant women concerned about a fetal chromosomal abnormality
Alternatives	Invasive testing, including amniocentesis and chorionic villi sampling; "quad testing" of multiple substances combined with ultrasound imaging
LDT Problem 1	Lack of clinical validation that tests detect and predict fetal abnormalities at an appropriate rate
LDT Problem 2	Many false-positive results when used in the general population

Clinical Consequence	Women with false-positive results may abort a normal pregnancy; women with false-negative results may deliver a child with an unanticipated genetic syndrome
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims
Cost Impact of Inaccuracy	Not estimated

Human cells normally have 23 pairs of chromosomes. A fetus with an extra chromosome ("trisomy"), a condition with unknown cause, is usually incompatible with life, leading to miscarriage. However, infants can survive if they are born with a trisomy of one of three chromosomes: 21 (Down syndrome), 18 (the rare Edwards syndrome) and 13 (the very rare Patau syndrome). While children with Down syndrome may lead relatively independent lives, the vast majorities of infants with Edwards and Patau syndromes have significant birth defects and die within a year of birth.[61]

Mothers who are over 35 or have a previous genetically abnormal pregnancy are at elevated risk for a trisomy pregnancy. Two standard non-invasive screening tests are offered to every pregnant woman: a first trimester ultrasound measuring the thickness of the fetal neck fold and a second trimester quadruple marker screening blood test.[62] A woman who is at high risk or has a positive screening test typically undergoes one of two invasive diagnostic tests to examine fetal chromosomes: chorionic villus sampling, which examines tissue from the early placenta, and amniocentesis, which samples the amniotic fluid through a needle inserted into the uterus.[63] Both involve a risk of miscarriage and fetal malformation, so it is important not to undertake them without adequate justification.

Noninvasive prenatal testing (NIPT) offers women who test positive on an initial non-invasive test or are otherwise at high risk the promise of avoiding the dangers of an invasive diagnostic procedure. There is typically some exchange of blood between mother and fetus due to microscopic bleeding within the placenta, and NIPT can detect traces of fetal chromosomes in maternal blood and determine whether a trisomy is present.[64]

At least four companies in the U.S. have recently begun offering these tests, using a technique called cell-free DNA testing (cfDNA). Marketing materials cite very high accuracy rates. One company claims that its test has a "very low false-positive rate,"[65] while another company claims a specificity of 99.9% for trisomy 18 (1 out of every 1000 results expected to be a false-positive) and 99.95% for trisomy 13 (5 out of every 10,000 results expected to be a false-positive).[66]

However, trisomy 18 and 13 are so rare (1 in 5,000 for trisomy 18 and 1 in 10,000 for trisomy 13) that even these high specificities should yield more false-positive than true-positive results, requiring follow-up testing for confirmation.[64] A clinical case series describes 8 women who received false-positive NIPT results for trisomy 18 and 13, including one patient who terminated her pregnancy after screening positive for trisomy 13, but was found to have a normal pregnancy on post-abortion testing.[67] Further testing showed the fetus had normal chromosomes.

A 2014 investigative report described three families who considered abortions based on what further testing showed to be false-positive results.[68,69] A study of one test calculated a PPV of 83% for 4 tested genetic conditions, and found that 22 (6%) of women who received positive results obtained abortions without a follow-up invasive diagnostic test.[70] Citing concern that these tests could be used in the

general, low-risk population with resulting low PPVs, the American College of Obstetricians and Gynecologists issued a statement in December 2012 that NIPT should not be offered to such women.[63]

Although the main concern is over the test's PPV for the rarer trisomies, in 2012, a patient reported a false-negative result to FDA after she received normal NIPT results and unexpectedly delivered an infant with trisomy 21.[71] Additional cases were documented in an investigative report in the Boston Globe in 2014.[72]

D. The Factor Detected has no Clear Relevance to the Disease

i. Fibromyalgia FM/a Diagnostic Test

Category	LDT Characteristics
LDT Name	FM/a Test
Description	Blood test to identify immune cytokine markers
Purpose	Claimed to diagnose and quantify fibromyalgia
Target Population	Patients with suspected fibromyalgia, based on a physician's history and physical exam
Alternatives	Clinician history and physical examination
LDT Problem 1	Biomarker not adequately shown to be associated with fibromyalgia
LDT Problem 2	Improper clinical trial design to validate test
Clinical Consequence	Patients with false-positives may take inappropriate medications; may delay diagnosis and treatment for underlying conditions
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims
Cost Impact of Inaccuracy	Not estimated

Fibromyalgia is a syndrome consisting of fatigue and a body-wide reduced pain threshold, and commonly occurs in association with psychiatric symptoms including anxiety and depression.[73] Most patients with the diagnosis are women, and, although there is no cure, symptoms can be treated with FDA-approved medications (e.g., pregabalin (Lyrica), duloxetine (Cymbalta), and milnacipran (Savella)) along with exercise and behavioral therapy.[74] The condition does not have a known cause and is typically diagnosed based on a physician's history and physical examination.

A small 2012 study (110 fibromyalgia patients, 91 controls) reported a lower-than-normal cytokine immune response among patients with fibromyalgia compared to controls.[75] The FM/a Test appeared on the market within months of this publication, offered as an LDT claiming to diagnose the disease by documenting altered immunity as an explanation of symptoms experienced by fibromyalgia patients.[76] The manufacturer suggests that the test diagnoses the disease, and markets the test for people undergoing a diagnostic workup of fibromyalgia.[76]

A number of critiques of the study methodology appeared subsequently.[77] These included concerns that the control group was inadequate[78] and that the authors had not adjusted for other conditions known

to change cytokine levels that may have been present at different rates in the fibromyalgia and control groups.[79] The company failed to conduct clinical trials to verify that the immune system deficiency reported in the study was clinically relevant to fibromyalgia. Approximately 1,000 tests were sold for $744 apiece during the first month the test was offered.[78]

Making an inaccurate diagnosis of fibromyalgia can be especially harmful when the patient may be suffering from a different, treatable condition with similar symptoms. Such conditions include Lyme disease and rheumatoid arthritis, for which effective therapies exist. Moreover, patients wrongly diagnosed with fibromyalgia may take unnecessary medications for that condition and be exposed to associated adverse effects.

ii. KIF6 Genotyping Test to Predict Heart Disease Risk and Statin Therapy Response

Category	LDT Characteristics
LDT Name	KIF6 "Statincheck" Genotyping Assay
Description	Genotype test for KIF6 variant
Purpose	Predict risk of heart disease and response to statin therapy
Target Population	Patients at risk for coronary heart disease (CHD)
Alternatives	Standard history, standard laboratory work-up and behavioral risk factor assessment
LDT Problem 1	Biomarker not adequately shown to be predictive of CHD or of statin response
LDT Problem 2	Test incorrectly validated
LDT Problem 3	Unproven product claims
Clinical Consequence	Over- or under-treatment with statins
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims
Cost Impact of Inaccuracy	Not estimated

Therapy with a class of drugs known as statins reduces the risk of heart attack and death from heart disease by about 1/3 in patients with coronary heart disease (CHD).[80] However, side effects of these drugs range from muscle pain and cramping to more serious reactions such as nerve damage, mood, sleep and cognitive impairment, and, rarely, muscle breakdown leading to kidney failure.

In 2007 and 2008, two population-based observational cohort studies suggested that a certain genetic variant, the KIF6 genotype, imparted a moderately elevated risk of cardiovascular events.[81,82] The findings from three medium-sized, randomized controlled trials by the same group of authors supported this relationship,[83,84] although the size of effect was modest, and one trial found that only KIF6 carriers (those with only one copy of the gene) had an increased risk in a sub-group, but those who were homozygous for the KIF6 variant (i.e., had two copies of the gene) were not, a paradoxical finding.[85] However, a large meta-analysis of 19 case-control studies found no association between KIF6 status and risk of CHD events.[86]

In a related line of research, results from two medium-sized, randomized, controlled trials suggested that KIF6 carriers experienced a greater reduction in CHD when placed on statin therapy (compared with placebo) than experienced by non-carriers.[87] A third randomized, controlled trial found a greater relative reduction in risk only in one sub-group of patients,[85] and a fourth trial's results supported the reduction in risk for a similar sub-group,[88] but only measured KIF6 status in approximately half of the patients on statins.[80] A fifth trial reported that KIF6 status had no impact upon response to statin therapy.[89] Finally, the large definitive WPS trial of 18,348 patients found no relationship between KIF6 status and statin response.[80] By 2010, more than 150,000 tests had been performed.[90]

In April 2011, FDA informed the manufacturer that its submission for premarket device approval was not approvable, stating that the evidence submitted was insufficient to support the test's safety and effectiveness in determining risk of heart disease or in predicting statin response.[91]

Inaccurate assessment of patient risk or likelihood of responding to statin therapy could lead to overtreatment, with an associated risk of adverse events, as well as undertreatment, with the risk of failing to prevent cardiovascular events and deaths.[80] The company withdrew its marketing application. However, the KIF6 test remains on the market as an LDT, and the manufacturer's website continues to make a claim, unsupported by the evidence, that KIF6 carriers may have elevated CHD risk and demonstrate favorable response to statins.[92]

iii. Target Now Cancer Biomarker Test

Category	LDT Characteristics
LDT Name	Target Now
Description	Molecular test to detect 20 cancer biomarkers for a range of tumor types
Purpose	Profile a cancer and suggest chemotherapy
Target Population	Patients with refractory or recurrent cancer
Alternatives	Follow standard chemotherapy regimens
LDT Problem 1	List of suggested treatments generated by the test have not necessarily been shown to have an impact for a patient's particular cancer
LDT Problem 2	List of suggested treatments generated by test have not been studied in combination
LDT Problem 3	Improper clinical trial design to validate test
Clinical Consequence	Patients may forego standard cancer therapy for unproven alternative therapy, with related risk of serious adverse events
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims
Cost Impact of Inaccuracy	Not estimated

Scientists increasingly recognize that cancers have unique molecular profiles that can be used to personalize therapies. The Target Now test, first offered in 2008, uses multiple technologies to provide molecular profiles for a variety of cancers. It then generates a list of suggested drugs, shown in the scientific literature to target those biomarkers, but not necessarily demonstrated to have clinical effect

in the particular cancers affecting each patient.[93] If two targets are identified that are associated with what the manufacturers consider a well-tolerated drug combination, that combination is suggested.[94]

By the end of December 2010, more than 12,550 tests had been sold.[95] That year, in a single uncontrolled study of 86 patients with recurrence of various metastatic cancers, 66 patients had tumors that generated biomarker targets detected by the test and received treatment according to the list of suggested drugs generated by the test.[94] At four months, 14 patients had not experienced progression, and 18 experienced a longer time to progression than they had on the regimens in use when they enrolled in the study.[94] This study was small and had no control arm, and so provides little evidence of clinical validity.[95,96]

Patients undergoing this test may forego standard treatment and opt for the list of alternatives put forth on the test report, even though the test has not been clinically validated and these treatments have not necessarily been clinically proven, in combination or in the context of the patient's particular cancer. The study reported no treatment-related deaths, but did report nine treatment-related serious adverse events, such as anemia, dehydration, pancreatitis, and nausea and vomiting.[94]

iv. Prolaris Prostate Cancer Biomarker Test

Category	LDT Characteristics
LDT Name	Prolaris
Description	Prognostic genetic panel of 46 genes from tumor biopsy
Purpose	Predict risk of recurrence and death, and to guide treatment
Target Population	Men with localized prostate cancer
Alternatives	Prostate specific antigen (PSA) test, Gleason score
LDT Problem 1	Test not evaluated for its ability to meaningfully improve clinical outcomes
LDT Problem 2	Insufficient evidence for manufacturer marketing claims
Clinical Consequence	Patients potentially receive inappropriate cancer treatment
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims
Cost Impact of Inaccuracy	Not estimated

Prostate cancer is diagnosed in 233,000 men in the U.S. a year and is associated with 29,480 deaths annually.[97] For a man diagnosed with prostate cancer, the so-called Gleason score, based on biopsy cancer cell characteristics, along with the prostate specific antigen (PSA) level provide a standard measure of cancer severity and guide treatment decisions. Treatment options depend on expected severity and range from "watchful waiting" (withholding treatment but monitoring the patient carefully) for men with low-risk tumors, to hormone therapy, surgery, radiation or chemotherapy for men with symptomatic or metastatic cancer. Side effects of these interventions include urinary incontinence, sexual impotence, and infertility. About 10% of all prostate cancer patients elect watchful waiting, but experts hypothesize that up to 40% of patients may actually qualify for that approach.[98]

In an effort to further differentiate low- from high-risk patients, the Prolaris test was introduced as an LDT in March 2010. The genetic panel measures expression of 46 genes correlated with prostate cancer cell proliferation,[99] generating a score that, when combined with the Gleason score and the PSA level, is

claimed to predict the 10-year risk of prostate cancer progression and the risk of death.[100] We would expect those with poorer predicted outcome scores are more likely to elect active treatment.

The test is being used to make patient management decisions. In a 2014 study, 65% of more than 300 physicians (mostly urologists) caring for men diagnosed with prostate cancer reported that the test score influenced their initial treatment plan.[101] After receiving the test score, 37% of physicians who initially planned to recommend active interventions recommended watchful waiting instead, while 30% recommended active intervention instead of watchful waiting.[101] However, no study has prospectively examined whether these treatment decisions represent clinically appropriate management of prostate cancer.[102] As a result, patients could be either over-or undertreated for prostate cancer.

v. Chronic Fatigue Syndrome XMRV Test

Category	LDT Characteristics
LDT Name	XMRV-Chronic Fatigue Syndrome (CFS) Test
Description	PCR tests to detect mouse virus with reported link to CFS
Purpose	Detect "cause" of CFS
Target Population	Patients with CFS or suggestive symptoms
Alternatives	Clinical diagnosis based on symptoms
LDT Problem 1	Evidence that mouse virus is linked to CFS was based on contaminated study samples, and has since been disproved
LDT Problem 2	No verification that patients with positive test have CFS
Clinical Consequence	Patients with positive tests may take non-indicated antiviral drugs
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims
Cost Impact of Inaccuracy	Not estimated

Chronic Fatigue Syndrome (CFS) is a disorder with unknown cause. In October 2009, a study published in the journal *Science* suggested a possible association between the newly-discovered *xenotropic Moloney murine leukemia virus-related virus* (XMRV) and CFS.[103] Three laboratories began offering a rapid PCR blood test as an LDT to detect XMRV.[104,105,106] When a September 2010 study[107] reported similar findings linking XMRV to CFS, the public became concerned that CFS could be transmitted through blood.[108] By December 2010, the American Red Cross,[109] Canada, Australia, New Zealand, and several European countries had banned blood donations from CFS patients.[110]

However, one manufacturer publicly discontinued its test in April 2010[111] when internal validation studies failed to replicate an association between XMRV and CFS. Several studies in Europe and China and two case-control studies from CDC in 2010[112] and from the University of Utah in 2011[113] found no association between XMRV and CFS. A study that attempted to repeat the testing on 15 XMRV-positive samples in 9 labs across the country failed to confirm XMRV in patients with CFS.[114] Two of the initial authors re-examined the samples and ascribed some of the findings to contamination.[115] The initial study was retracted by the editor of *Science* in December 2011.[116]

A number of CFS patients who tested positive for XMRV on these LDTs started off-label use of antiretroviral drugs,[117] which can be associated with potentially serious side effects, from nausea and vomiting to disorders of the blood and nervous systems.[118] Patients were subjected to stigma as many of the world's blood banks banned blood from CFS patients. The ban has since been reversed.

E. Tests Linked to Treatments Based on Disproven Scientific Concepts

i. CARE Clinics Autism Biomarkers Test

Category	LDT Characteristics
LDT Name	CARE Clinics BioMarkers
Description	Genetic biomarker and heavy metal intoxication test
Purpose	To determine the cause for Autism Spectrum Disorder (ASD) and to recommend treatment
Target Population	Children with ASD
Alternatives	American Psychiatric Association-approved behavioral and developmental diagnostic criteria for ASD
LDT Problem 1	No evidence that "causes" identified by the test correlate with ASD
LDT Problem 2	No evidence that recommended treatments improve ASD outcomes
Clinical Consequence	Children undergo inappropriate and harmful treatment based on test results
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims; protection of children from unproven therapies
Cost Impact of Inaccuracy	$66.1 million

Autism spectrum disorder (ASD) is a developmental disability that manifests along a broad clinical range, from a child with low measured intelligence, frequent repetitive behaviors, and limited communication to one with gifted intellectual and communicative faculties, average social interactions, and mild repetitive behaviors.[119] It is assumed to have multiple causes, although most have not yet been identified, and it does not have a recognized diagnostic test. Instead, doctors evaluate a child's behavior and development to make a diagnosis using criteria developed by the American Psychiatric Association.

In 2001, the Center for Autistic Spectrum Disorders was founded, opening affiliated CARE Clinic laboratories in Texas and in Florida. The CARE Clinics began marketing a number of tests for children with ASD, including a panel of biomarkers along with a heavy metal toxicity test that purported to identify the causes of a child's autism. These causes were in turn linked to a CARE Clinics Health Blueprint™ treatment plan, which included chelation, hyperbaric oxygen, and intravenous vitamin therapy. (Chelators bind to heavy metals such as lead and mercury and remove them from the body through the urine.)

These interventions for ASD have been discredited by the medical community, indicating no evidence for a role for heavy metal chelation in either preventing or treating ASD, and including a warning of the dangers and lack of scientific basis of chelation therapy for children with ASD from the American Academy of Pediatrics.[120] Of these interventions, chelation products are particularly popular and may have notable toxicities,[121] including allergic reactions, dehydration, and kidney failure.[122]

According to tax returns described on the Autism Watch website, the Center for Autistic Spectrum Disorders and the CARE Clinics received $9.8 million for these tests for 2,027 children reportedly tested between 2004 and 2007.[121] Compared with a child with a comparable behavioral condition, a family with a child with ASD will pay an additional annual average of $1,759 for health care,[123] $5,659 for lost family income,[124] and $7,562 for special education costs,[123,125] for a total of $14,980. We assumed that the children were only misdiagnosed until the end of 2007, the last year we have numbers for the Center.

Given this assumption, 427 children were misdiagnosed for four years, 150 were misdiagnosed for three years, 150 were misdiagnosed for two years, and 1,300 were misdiagnosed for one year, for a total of 3,758 misdiagnosed years. Assuming that all these children tested had other psychiatric conditions but were instead being treated as if they had autism, these incorrect diagnoses would have resulted in a cost of $56.3 million ($14,980 x 3,758). Together, the costs of the unnecessary tests and the improper diagnosis and treatment of these children accounted for an estimated total cost of $66.1 million ($56.3 million + $9.8 million).

ii. Heavy Metal Chelation Challenge Test

Category	LDT Characteristics
LDT Name	Various heavy metal challenge tests
Description	Urine chelation challenge test to measure levels of heavy metals
Purpose	To detect chronic heavy metal poisoning
Target Population	The general public
Alternatives	Routine blood screening without challenge test, for high-risk children
LDT Problem 1	In clinical use, patients with positive urine chelation challenge tests may not have heavy metal toxicity
LDT Problem 2	Manufacturer claims unsupported by evidence
Clinical Consequence	False-positive results may lead to the administration of inappropriate, unproven or dangerous therapies
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims
Cost Impact of Inaccuracy	Not estimated

Heavy metals such as lead and mercury are ubiquitous in the environment, and thus trace amounts are common in urine, hair, or blood samples, although blood levels are the most reliable and must be assessed prior to treatment.[126] While heavy metal exposures to high doses over a short time period can cause acute poisoning and death, long-term exposure to low levels also may cause chronic poisoning and health problems.[127]

Lead and mercury are two of the most common heavy metals involved in poisoning, and they can cause vague abdominal complaints, organ damage, and developmental and neurological problems.[126,128,129,130] Elevated blood levels of heavy metals can be treated with chelation therapy.[131]

A "provoked chelation challenge test" uses the same chelating agents and measures heavy metals in the urine;[132] these are always higher than those measured without provocation. There are no accepted standards for interpreting provoked results,[133] although they have been used to justify chelation therapy to treat conditions claimed by some, often without satisfactory evidence, to be associated with heavy metal toxicity, including heart disease, Alzheimer's disease, and Autism Spectrum Disorders.[122] The American Academy of Pediatrics discourages the use of chelation as a treatment for children with Autism Spectrum Disorders, stating that the practice is particularly dangerous and lacks any scientific evidence of benefit.[120]

For example, one laboratory manufactures a provoked chelation challenge test but gives results using a normal, non-provoked reference scale, and the outcome may then result in recommendations for intravenous chelation therapy. This inappropriate use of chelation therapy can be costly, unnecessary, and harmful, as chelators also bind important minerals such as calcium and iron and remove them from the body. Chelation products also can cause allergic reactions, dehydration, and kidney failure.[122] Oregon's public health department has reported three deaths associated with chelation therapy,[132] and CDC reported the deaths of three individuals with heart attacks due to low blood calcium following chelation therapy between 2003 and 2005.[134] One of these was a 5-year-old who was being treated for autism. It is unknown whether these patients were exposed to any of the LDTs in question.

F. Tests that Undermined Drug Approval or Drug Treatment Selection

i. Omapro Companion Diagnostic to New Leukemia Medication

Category	LDT Characteristics
LDT Name	Omapro Companion Diagnostic
Description	Genetic test to detect T315I gene mutation in cancerous cells of chronic myelogenous leukemia (CML)
Purpose	To enroll research participants in a clinical trial and determine sub-population most likely to benefit from a new drug
Target Population	Adults with treatment-resistant CML and the T315I mutation
Alternatives	None
LDT Problem 1	Lack of standardized LDTs leads to unreliable selection of patients for clinical trial enrollment
LDT Problem 2	Drug sponsor used two different, non-comparable LDTs to enroll patients in a clinical trial
LDT Problem 3	Researchers did not obtain the proper investigational device exemption needed to carry out a research study
Clinical Consequence	Delay in approval of a drug with some benefit for treatment-resistant CML patients
Potential Impact of FDA	Assurance the test is appropriately validated for use in the

Oversight	clinical trial
Cost Impact of Inaccuracy	Not estimated

Chronic myelogenous leukemia (CML) is a cancer of the blood and immune system for which first-line treatment is a tyrosine kinase inhibitor (e.g., imatinib). Although treatment prolongs life, 30%-40% of patients become treatment-resistant within seven years.[135] Up to 20% of treatment-resistant patients, or 250 to 300 patients a year, have a T351I mutation in their cancerous cells.[135]

One manufacturer initiated a clinical trial that planned to test a new drug (Omapro; omacetaxine mepesuccinate) that targets cancer cells in a different way than the typical treatment and was designed specifically to target the CML sub-population with the T351I mutation. The study used two different LDTs to screen CML patients and enrolled only those with the mutation in the trial.[136]

The drug showed some benefit,[137] but the LDTs used different techniques, with different measurement ranges, and their ability to detect the mutation had not been validated, so it was not possible to compare enrollment strategies based on the two different tests. This prevented valid assessment of the drug's efficacy in the group with the mutation. In 2010, FDA's Oncologic Drug Advisory Committee voted against Omapro approval for this subgroup,[136] and the drug was not brought to market at that time.

In October 2012, after further consideration of the drug's potential as a treatment option for CML patients,[138] FDA granted approval of the same drug, under the name Synribo, for CML patients who had failed all other treatment, regardless of their T351I mutation status.[139] It remains unclear whether the drug's efficacy is confined to the subgroup with the mutation. Regardless, approval of the drug was delayed for two years as a result of issues with the LDTs.

ii. Duke University Chemotherapy Assessment Test

Category	LDT Characteristics
LDT Name	Duke University Chemotherapy Assessment
Description	Genetic assay of tumor cells
Purpose	Assign clinical trial patients to chemotherapy treatment according to test results
Target Population	Patients with ovarian, lung and breast cancer
Alternatives	Existing clinical guidelines for treatment of ovarian, lung and breast cancers
LDT Problem 1	Errors in data management and analysis
LDT Problem 2	Lack of clinical validation that test predicts response
Clinical Consequence	Patients enrolled in trials took unproven, potentially inadequate treatments
Potential Impact of FDA Oversight	Assurance the test is appropriately validated for use in the clinical trial
Cost Impact of Inaccuracy	Not estimated

In October 2006, cancer researchers at Duke University Medical Center published data from multi-gene expression studies on cells from patients with ovarian, lung and breast cancer.[140] The data suggested

that results from gene expression panels, implemented as LDTs, could predict individuals' responses to specific chemotherapy regimens.[140] Without further validation of predictive validity for the laboratory's LDTs, three clinical trials were conducted, using LDT results to allocate patients to chemotherapy treatments.[141]

As patients were being enrolled, members of the scientific community interested in using the tests in their own practices attempted to validate the initial study findings and discovered data management errors. Scientific rigor dictates that a test should be developed with one set of data, and validated on an entirely separate set of data in order to avoid over-estimation of performance. The Duke investigators, however, allowed overlap of the data sets,[142] which produced overestimates of test accuracy. In addition, outside researchers uncovered basic spreadsheet errors in the data used to select the genes for inclusion in the test,[143] as a consequence of which the test results were non-reproducible.[143,144] These errors also led to the inappropriate inclusion of at least 14 of the 50 genes in the LDT.[142]

The trials continued until a separate issue regarding false academic claims made by one of the principal investigators in the trials drew media attention to the earlier criticisms of the test. In response, the Institute of Medicine (IOM) conducted a special investigation of this and several similar situations, issued a statement[145] that internal oversight mechanisms within the university had failed, and that the most basic conclusions of the test were invalid.

In the IOM's assessment, greater FDA oversight and involvement may have uncovered errors and validation issues before the test was used in clinical trials. At a minimum, said the IOM, researchers should discuss LDTs with FDA prior to initiating validation studies, particularly when the test is intended for future clinical use.[146] In the ensuing months, the three trials were discontinued and 27 papers describing the test's performance were partially or completely retracted. This illustrates that publication in a peer-reviewed medical journal, even a prestigious one as in this case, is not equivalent to validation, because reviewers typically do not have access to the underlying data and often are not sufficiently expert in complex algorithms to identify errors. On November 9, 2015, the Office of Research Integrity in the Department of Health and Human Services concluded that one of the principal investigators had "engaged in research misconduct."[147]

As a consequence of the use of this insufficiently validated LDT, cancer patients were exposed to potentially inappropriate chemotherapy.

G. Other Unvalidated Tests

i. Vitamin D Deficiency Test

Category	LDT Characteristics
LDT Name	Vitamin D Test
Description	Liquid chromatography tandem mass spectrometry
Purpose	To determine a patient's vitamin D blood level
Target Population	The general population in early marketing claims; people at risk for Vitamin D toxicity or deficiency in current materials
Alternatives	FDA-cleared vitamin D tests from other manufacturers
LDT Problem 1	Faulty calibration of device

LDT Problem 2	Inadequate validation that test results correlate with clinical vitamin D excess or deficiency
LDT Problem 3	Lack of standardization of LDT between testing sites
Clinical Consequence	Over- or under-treatment of Vitamin D excess or deficiency
Potential Impact of FDA Oversight	Assurance of consistent manufacturing practices and standardized instrument calibration; assurance the test meets minimum performance standards
Cost Impact of Inaccuracy	Not estimated

Vitamin D is the product of sun exposure, but it can also be obtained through the diet and in dietary supplements. It maintains bone strength and supports the immune and nervous systems, as well as maintains proper levels of certain minerals (calcium, phosphorous) that the body needs to carry out its normal functions. Low levels have been associated with cancer, heart disease, and disorders of the immune system, although whether these relationships are causal is less clear.[148,149]

Between 2006 and 2007, a testing company changed its vitamin D test from an FDA-cleared test to an LDT that relied on a specialized mass spectrometry instrument, with the expectation that the LDT would be more accurate. However, company officials later reported faulty calibration of the instruments and failure to follow standard test procedures at four of the seven laboratories offering vitamin D testing.[150] Most of the inaccurate tests reported an inflated vitamin D level, leading some patients not to take potentially beneficial supplements. However, some tests reported a falsely low vitamin D level, which could lead patients to take unnecessary supplements – and risk vitamin D toxicity, which includes elevated calcium blood levels, nausea, vomiting, and kidney damage.[151] The company marketed the test heavily, including in a 2009 video produced with UCLA doctors that promoted the importance of testing for vitamin D.[152] In January 2009, the company notified thousands of physicians that the vitamin D test results of at least one of each of their patients from the prior two years was inaccurate and recommended retesting.[150,153] The editor of a pathology newsletter quoted in *The New York Times* coverage described these events as "the largest patient test recall I'm aware of in my 20 years in the business."[150]

ii. OncoVue Genetic Breast Cancer Risk Test

Category	LDT Characteristics
LDT Name	OncoVue
Description	Genetic test combining individual mutation profile with personal history
Purpose	Predict inherited breast cancer risk
Target Population	Women without breast cancer
Alternatives	Gail Model for predicting risk of breast cancer
LDT Problem 1	Specificity not assessed
LDT Problem 2	Lack of validation of test performance in clinical use
Clinical Consequence	Patients with elevated scores may undergo unnecessary mastectomy or tamoxifen prophylaxis; patients with low scores may have a false sense of security and forego recommended screenings

Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims
Cost Impact of Inaccuracy	Not estimated

Factors such as age, environment, and personal medical history make up the Gail model, which is relatively well-validated for use to predict a woman's future risk of breast cancer. Ninety to ninety-five percent of breast cancer is caused by behavioral and environmental factors such as cigarette smoke, high levels of hormone exposure, obesity, and lack of exercise.[154] The remaining 5% is inherited.[154] Researchers have discovered single genes that carry an increased risk of breast cancer, such as the relatively rare BRCA genes; much of the remaining genetic risk may be explained by interaction between inherited genetic DNA variations called single nucleotide polymorphisms (SNPs).[155,156]

In 2006 a new LDT, OncoVue, was introduced to detect a group of 22 SNPs. The test was intended to combine multiple SNPs with the Gail model to predict a woman's future risk of breast cancer.[157]

Data from two abstracts (not otherwise reported in a peer-reviewed medical journal) showed that the LDT identified 56 of 169 breast cancer cases (33% sensitivity), compared to the 37 (22% sensitivity) that were predicted to be high risk in a retrospective application of the Gail model.[158,159] Because all patients in this study had cancer, its specificity in detecting patients without cancer has not been studied and is unknown. Without further testing, the test was marketed to the public.

Some observers believe that the use of such SNP panels to screen the population for breast cancer risk is premature.[160,161] For example, a woman with a low-risk score may be given a false sense of security and forego recommended screenings and a woman given a high-risk score might, in error, choose to undergo more intensive screening with ultrasound or MRI, take tamoxifen or raloxifene to prevent breast cancer, or even consider having her breasts removed to prevent cancer.[162]

iii. BrafV600E Genetic Mutation Test to Guide Melanoma Treatment

Category	LDT Characteristics
LDT Name	BrafV600E Mutation Tests
Description	PCR test to detect V600E BRAF gene mutation in melanomas
Purpose	Select patients for treatment with vemurafenib
Target Population	Patients with metastatic melanoma
Alternatives	FDA-approved diagnostic to detect V600E BRAF mutation
LDT Problem 1	Lack of evidence to support manufacturer claims that LDT performs better than alternatives
Clinical Consequence	Patients may inappropriately be administered vemurafenib
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims
Cost Impact of Inaccuracy	Not estimated

Melanoma is a cancer of the skin that has a median survival of eight months if it spreads.[163] Tumors can have a genetic mutation called BRAF V600E, which is present in approximately 50% of metastatic melanoma cases.[164] A class of drugs including vemurafenib, shown to increase overall and progression-free survival in these patients compared to traditional chemotherapy, is approved by FDA for the

treatment of patients with unresectable or metastatic melanoma with the BRAF V600E mutation, as long as the mutation was detected by an FDA-approved test.[165]

In August 2011, FDA approved the in vitro diagnostic Roche Cobas® 4800 test as a commercially distributed companion diagnostic to detect the BRAF V600 mutation,[166] whereupon at least nine laboratories[167,168] announced that they were offering their own LDT version of the BRAF test for the same intended use. Some of these detected several additional mutations that had not been shown to predict response to vemurafenib.

Some of the other manufacturers claimed improved performance over the FDA-approved test. For example, one manufacturer claimed that its LDT "surpasses other commercially-available tests."[167] In addition, some claimed that their test was more sensitive (i.e., it could detect the V600 mutation when a lower fraction of tumor cells had the mutation), but we have no knowledge whether mutation frequencies as low as those detected by the LDTs predict clinical response to vemurafenib. Thus patients could be recommended for vemurafenib therapy with perhaps no benefit. None of these LDTs have been approved by FDA, and FDA is not aware of evidence to support claims of superior performance or even to support the use of these LDTs to identify patients who may benefit from vemurafenib. Insurance companies may not cover vemurafenib if it is started after using an LDT, so patients may have to pay out-of-pocket.[169]

III. Conclusion

In this report, we have reviewed events related to 20 LDTs in which patients have been demonstrably harmed or may have been harmed by tests that did not meet FDA requirements. Tests that are inaccurate, unreliable, or have unproven or disproven claims expose patients to a range of harms. These include patients told incorrectly that they have life-threatening diseases and others whose life-threatening diseases have gone undetected.

Despite arguments from some that "CLIA is enough," all of the tests described as problematic in this report were offered from laboratories following the minimum requirements of CLIA. Specifically, CLIA does not:

- Ensure the safety and effectiveness of LDTs prior to marketing.
- Assess the quality of the design and manufacture of devices.
- Ensure test labeling provides adequate directions for use.
- Require truth in marketing materials and other labeling.
- Require adverse event reporting.
- Permit removal of unsafe devices from the market.
- Require informed consent for patients participating in clinical studies of LDTs.
- Establish procedures for the conduct of such studies.

These cases, therefore, highlight the need for greater FDA oversight of LDTs that is appropriately tailored so that it is complementary and does not duplicate the oversight currently provided under CLIA. Greater FDA oversight is needed to promote access to LDTs that provide benefits to patients and the health care system, while helping to ensure patients are not unduly exposed to harm.

[1] 21 U.S.C. 321(h). http://www.gpo.gov/fdsys/pkg/USCODE-2013-title21/html/USCODE-2013-title21-chap9-subchapII-sec321.htm. Accessed April 16, 2015.
[2] Food and Drug Administration. Draft Guidance for Industry, Food and Drug Administration Staff, and Clinical Laboratories: Framework for Regulatory Oversight of Laboratory Developed Tests (LDTs). October 3, 2014. http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM416685.pdf. Accessed December 1, 2014.
[3] Centers for Medicare and Medicaid Services. Laboratory developed tests (LDTs): Frequently asked questions. October 22, 2013. https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/LDT-and-CLIA_FAQs.pdf. Accessed June 8, 2015.
[4] Centers for Disease Control and Prevention. Signs and symptoms of Lyme disease. April 12, 2011. http://www.cdc.gov/lyme/signs_symptoms/index.html. Accessed November 16, 2014.
[5] Centers for Disease Control and Prevention. Two-step laboratory testing process. April 12, 2011. http://www.cdc.gov/lyme/diagnosistesting/LabTest/TwoStep/. Accessed November 16, 2014.
[6] U.S. Food and Drug Administration. Devices at FDA. December 8, 2014. http://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm. Accessed December 1, 2014.
[7] Wormser GP, Dattwyler RJ, Shapiro ED. The clinical assessment, treatment, and prevention of Lyme disease, Human granulocytic anaplasmosis, and babesiosis: Clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis*. 2006;43(9):1089-134.
[8] Centers for Disease Control and Prevention. Lyme disease Treatment. April 12, 2011. http://www.cdc.gov/lyme/treatment/index.html. Accessed November 14, 2014.
[9] Klempner MS, Schmid CH, Hu L, et al. Intralaboratory reliability of serologic and urine testing for Lyme disease. *Am J Medicine*. 2001;110(3):217-9.
[10] Rauter C, Mueller M, Diterich I, et al. Critical evaluation of urine-based PCR assay for diagnosis of Lyme borreliosis. *Clin Diagn Lab Immunol*. 2005;12(8):910–7.
[11] Hurley D, Santora M. Unproved Lyme disease tests prompt warnings. *New York Times*. August 23, 2005. http://www.nytimes.com/2005/08/23/health/policy/23lyme.html?pagewanted=all&_r=0. Accessed November 14, 2014.
[12] Margolies D. Who's left to pay this big verdict? *Kansas City Star*, May 25, 2009. http://www.aldf.com/Bowen_Laboratory_Settlement.pdf. Accessed November 20, 2014.
[13] Nelson C, Hojvat S, Johnson B, et al. Concerns regarding a new culture method for *Borrelia burgdorferi* not approved for the diagnosis of Lyme disease. *Morbidity and Mortality Weekly Report* 2014; 63:333.
[14] Johnson BJ, Pilgard MA, Russell TM. Assessment of new culture method for detection of *Borrelia* species from serum of Lyme disease patients. *Journal of Clinical Microbiology* 2014;52:721–4.
[15] Zhang X, Martin IM, Peña AC, Hopkins AB, Wroth L, Fix AD. Economic impact of Lyme disease. *Emerging Infect Dis*. 2006;12(4):653-660. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3294685/. Accessed April 16, 2015.
[16] $1,226 = ($464 x 1.59 + $307 x 1.59).
[17] U.S. Department of Labor, Bureau of Labor Statistics (BLS). CPI Databases. 2013. http://www.bls.gov/cpi/tables.htm. Accessed March 26, 2013.
[18] Surveillance, Epidemiology, and End Results Program (SEER). SEER Stat Fact Sheets: Ovary Cancer. 2014. http://seer.cancer.gov/statfacts/html/ovary.html. Accessed November 5, 2014.
[19] Sfakianos GP, Havrilesky LJ. A review of cost-effectiveness studies in ovarian cancer. *Cancer Control*. 2011;18(1):59-64.
[20] Society of Gynecologic Oncologists. Ovarian Cancer: Treatment Options. 2014. https://www.sgo.org/patients-caregivers-survivors/patients/ovarian-cancer-what-are-the-treatment-options/. Accessed December 1, 2014.
[21] Institute of Medicine. Evolution of Translational Omics: Lessons Learned and the Path Forward. Appendix A, Case Studies. The National Academies Press (US). 2012. http://www.nap.edu/catalog/13297/evolution-of-translational-omics-lessons-learned-and-the-path-forward. Accessed May 28, 2015.

[22] Petricoin E, Ardekani A, Hitt B, et al. Use of proteomic patterns in serum to identify ovarian cancer. *Lancet*. 2002;359:572-7.
[23] Liotta L, Petricoin E, Ardekani A et al. General keynote: proteomic patterns in sera serve as biomarkers of ovarian cancer. *Gynecol Oncol.* 2003;88(1 Pt 2):S25-8; discussion S37-42.
[24] Pearl DC. Proteomic patterns in serum and identification of ovarian cancer. *Lancet*. 2002;360(9327):169-70.
[25] Elwood M. Proteomic patterns in serum and identification of ovarian cancer. *Lancet.* 2002;360(9327):170.
[26] Gutman SI. Letter to Levine PJ, President and CEO, Correlogic Systems, Inc. Food and Drug Administration, Center for Devices and Radiological Health. February 18, 2004. https://web.archive.org/web/20040711090448/http:/www.fda.gov/cdrh/oivd/letters/021804-correlogic.html. Accessed July 14, 2015.
[27] Baggerly K, Morris J, Coombs K. Reproducibility of SELDI-TOF protein patterns in serum: comparing data sets from different experiments. *Bioinformatics.* 2004;20(5):777-85.
[28] Diamandis E. Analysis of serum proteomic patterns for early cancer diagnosis: drawing attention to potential problems. *J Natl Cancer Inst. 2004;*96(5):353-5.
[29] Visintin I, Feng Z, Longton G, et al. Diagnostic markers for early detection of ovarian cancer. *Clinical Cancer Research*. 2008;14:1065-72.
[30] Buchen L. Missing the mark: Why is it so hard to find a test to predict cancer? *Nature*. 2011;471:428-32.
[31] Society of Gynecologic Oncologists Statement Regarding OvaSure(TM). July 2, 2008. https://www.sgo.org/wp-content/uploads/2012/09/Statement-on-OvaSure.pdf. Accessed December 1, 2014.
[32] Food and Drug Administration Center for Devices and Radiological Health. OvaSure (TM) Manufacturer Letter. August 2008. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/IVDRegulatoryAssistance/ucm125130.htm. Accessed December 1, 2014.
[33] Food and Drug Administration Center for Devices and Radiological Health. Warning Letter. Inspections, Compliance, Enforcement, and Criminal Investigations. September 8, 2008. http://www.fda.gov/ICECI/EnforcementActions/WarningLetters/2008/ucm1048114.htm. Accessed December 1, 2014.
[34] U.S. Department of Labor, Bureau of Labor Statistics (BLS). National Industry-Specific Occupational Employment and Wage Estimates for Healthcare Practitioners and Technical Occupations (290000). May 2011. http://www.bls.gov/oes/. Accessed March 26, 2013.
[35] Ratner E, Lu L, Boeke M, et al. A KRAS-variant in ovarian cancer acts as a genetic marker of cancer risk. *Cancer Res*. 2010;70(16):6509-15.
[36]MiraDX. (No date). The KRAS-Variant & Cancer Risk. http://miradx.com/preovar-kras-variant-test-populations/. Accessed December 1, 2014.
[37] Society of Gynecologic Oncology. "PreOvar" Ovarian Cancer Risk Assessment Test. September 2010. https://www.sgo.org/newsroom/position-statements-2/preovar-ovarian-cancer-risk-assessment-test/. Accessed December 1, 2014.
[38] Pharaoh PD, Palmeiri RT, Ramus SJ, et al. Ovarian Cancer Association Consortium. The role of KRAS rs61764370 in invasive epithelial ovarian cancer: implications for clinical testing. *Clin Cancer Res*. 2011;17(11):3742-50.
[39] Risch HA, Berchuck A, Pharaoh PDP, et al. Ovarian Cancer Association Consortium. KRAS rs61764370 in Epithelial Ovarian Cancer - Response. *Clin Cancer Res*. 2011;17:6601.
[40] Permuth-Wey J, Chen Z, Tsai YY, et al. MicroRNA processing and binding site polymorphisms are not replicated in the Ovarian Cancer Association Consortium. *Cancer Epidemiol Biomarkers*. 2011;20:1793.
[41] MiraDX. (No date). FAQ. https://miradx.com/faq/. Accessed November 20, 2014.
[42] Centers for Disease Control and Prevention (CDC). Preventing Tetanus, Diptheria, and Pertussis among adults: Use of Tetanus toxoid, reduced Diptheria toxoid and acellular Pertussis vaccine. Morbidity and Mortality Weekly Report, Recommendations and Reports. 2006;55(RR17):1-33. http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5517a1.htm.

[43] Centers for Disease Control and Prevention. Outbreaks of respiratory illness mistakenly attributed to Pertussis-*New Hampshire, Massachusetts, and Tennessee*, 2004-2006. *MMWR Morb Mortal Wkly Rep*. August 24, 2007;56(33):837-42. http://www.cdc.gov/MMWR/preview/mmwrhtml/mm5633a1.htm. Accessed May 28, 2015.
[44] Cancer Connect. Oncotype DX accurately determines HER2 status in breast cancer. http://news.cancerconnect.com/oncotype-dx-accurately-determines-her2-status-in-breast-cancer/. Accessed November 6, 2014.
[45] Wolff AC, Hammond MEH, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Journal of Clinical Oncol*. 2006;25(1):118-45. http://jco.ascopubs.org/content/25/1/118.full.pdf+html. Accessed May 28, 2015.
[46] National Cancer Institute. FDA Approval for Trastuzumab. July 2013. http://www.cancer.gov/cancertopics/druginfo/fda-trastuzumab. Accessed December 8, 2014.
[47] The American Society of Clinical Oncology (ASCO). HER2 testing for breast cancer. CancerNet. http://www.cancer.net/research-and-advocacy/asco-care-and-treatment-recommendations-patients/her2-testing-breast-cancer. Accessed November 6, 2014.
[48] Butkus, B. Oncotype DX study raises questions about RT-PCR testing for HER2 expression. GenomeWeb. December 2011. https://www.genomeweb.com/pcrsample-prep/oncotype-dx-study-raises-questions-about-rt-pcr-testing-her2-expression. Accessed December 5, 2014
[49] Genomic Health. Oncotype DX. 2014. http://www.genomichealth.com/en-US/OncotypeDX.aspx#.VIXKRskwA3Q. Accessed December 8, 2014.
[50] Guideline Summary: American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for human epidermal growth factor receptor HER2 Testing in breast cancer. *Journal of Oncology Practice*. 2007;3(1):48-50. http://jop.ascopubs.org/content/3/1/48.full. Accessed May 28, 2015.
[51] Dabbs DJ, Klein ME, Mohsin SK, et al. High false-negative rate of HER2 quantitative reverse transcription polymerase chain reaction of the Oncotype DX test: An independent quality assurance study. *J Clin Oncol.* 2011;29:4279-85.
[52] Park MM, Ebel JJ, Zhao W, Zynger DL. ER and PR immunohistochemistry and HER2 FISH versus oncotype DX: implications for breast cancer treatment. *Breast J*. 2014;20(1):37-45.
[53] Cutler D. Are we finally winning the war on cancer? *J Economic Perspectives*. 2008;22:3-26.
[54] Murphy KM, Topel RH. The value of health and longevity. *J of Political Economy*. 2006;114(5):871-904.
[55] 74 Federal Register 33030. July 9, 2009.
[56] Garrison LP, Lubeck D, Lalla D, et al. Cost-effectiveness analysis of trastuzumab in the adjuvant setting for treatment of HER2-positive breast cancer. *Cancer*. 2007;110(3):489-98.
[57] Saslow D, Solomon D, Lawson HW, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *Am J Clin Path.* 2012;137(4):516-42.
[58] CDC, USPSTF, and ACOG guidelines state that, starting at age 21, a woman at average risk of cervical cancer should be screened with a Pap test alone every three years; starting at the 30th birthday, cytology and HPV testing should be performed concurrently every five years. A woman 30 years of age or older can continue to be screened with the Pap Test alone every three years, but the professional societies prefer co-testing.
[59] Ortega, B. False-negative results found in HPV testing. The Arizona Republic. January 18, 2013. http://www.azcentral.com/news/articles/20130112hpv-testing-false-negative.html. Accessed December 1, 2014.
[60] BD Diagnostics. Letter to Laboratory Directors, June 8, 2012. http://archive.azcentral.com/ic/pdf/0113-bd-bulletin.pdf. Accessed February 27, 2015.
[61] National Institutes of Health. Genetics Home Reference. November 2013. http://ghr.nlm.nih.gov/condition/trisomy-13. Accessed December 1, 2014.
[62] Society of Maternal Fetal Medicine. Incorporating first-trimester Down syndrome studies into prenatal screening: executive summary of the National Institute of Child Health and Human Development workshop. 2004. https://www.smfm.org/publications/128-incorporating-first-trimester-down-syndrome-studies-into-prenatal-

screening-executive-summary-of-the-national-institute-of-child-health-and-human-development-workshop. Accessed December 1, 2014.

[63] The American College of Obstetricians and Gynecologists. Committee opinion: Noninvasive prenatal testing for fetal aneuploidy. December 2012. http://www.acog.org/Resources-And-Publications/Committee-Opinions/Committee-on-Genetics/Noninvasive-Prenatal-Testing-for-Fetal-Aneuploidy. Accessed December 1, 2014.

[64] Nicolaides KH, Syngelaki A, Ashoor G, et al. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. *Am J Obstet Gynecol*. 2012:374.e1-374.e6.

[65] Sequenom. MaterniT21 Plus. 2014. http://laboratories.sequenom.com/maternit21plus/prenatal-test-information-for-providers. Accessed December 1, 2014.

[66] Illumina. Verifi™ prenatal test. 2014. http://www.verifitest.com/healthcare-professionals/. Accessed December 1, 2014.

[67] Mennuti MT, Cherry AM, Morrissette JJD, Dugoff L. Is it time to sound the alarm about false-positive cell-free DNA testing for fetal aneuploidy? *Am J Obstet Gynecol*. 2013;209(5):415-9.

[68] Daley B. Oversold and misunderstood: Prenatal screening tests prompt abortions. New England Center for Investigative Reporting. http://features.necir.org/prenatal-testing. Accessed December 22, 2014.

[69] Donaldson-James S. Prenatal tests have high failure rate, triggering abortions. NBC News. December 14, 2014. http://www.nbcnews.com/health/womens-health/prenatal-tests-have-high-failure-rate-triggering-abortions-n267301. Accessed December 22, 2014.

[70] Dar P, Curnow KJ, Gross SJ, et al. Clinical experience and follow-up with large scale single-nucleotide polymorphism-based noninvasive prenatal aneuploidy testing. *American J Obstet Gynecol*. 2014;211(5):527.e-527.e17.

[71] U.S. Food and Drug Administration. MAUDE Adverse Event Report: SEQUENOM MATERNIT21. March 2012. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfMAUDE/detail.cfm?mdrfoi__id=2903746. Accessed December 1, 2014.

[72] Daley B. Oversold prenatal tests spur some to choose abortions. *Boston Globe*, December 14, 2014. http://www.bostonglobe.com/metro/2014/12/14/oversold-and-unregulated-flawed-prenatal-tests-leading-abortions-healthy-fetuses/aKFAOCP5N0Kr8S1HirL7EN/story.html. Accessed April 11, 2015.

[73] Medscape. Fibromyalgia: New criteria improve diagnosis. February 2014. http://www.medscape.com/viewarticle/820613. Accessed December 1, 2014.

[74] WebMD. What is fibromyalgia? (No date) http://www.webmd.com/fibromyalgia/guide/what-is-fibromyalgia. Accessed December 1, 2014

[75] Behm FG, Gavin IM, Karpenko O, et al. Unique immunologic patterns in fibromyalgia. *BMC Clinical Pathology*. 2012;12:25.

[76] EpicGenetics. FM/A(R): A simple and definitive test for FM. 2014. http://thefibromyalgiatest.com/?p=7006. Accessed May 28, 2015.

[77] Wolfe F. Junk science – Junk ethics. (Blog post). February 2013. http://www.fmperplex.com/2013/02/25/junk-science-junk-ethics/. Accessed November 25, 2014.

[78] Bonislawski A. Despite researcher criticism, EpicGenetics' fibromyalgia test does $750K in sales in first month. April 2013. GenomeWeb. https://www.genomeweb.com/proteomics/despite-researcher-criticism-epicgenetics-fibromyalgia-test-does-750k-sales-firs. Accessed November 25, 2014.

[79] Tarkan L. Questions arise over new diagnostic test for fibromyalgia. Fox News. February 2013. http://www.foxnews.com/health/2013/02/26/questions-arise-over-new-diagnostic-test-for-fibromyalgia/. Accessed November 25, 2014.

[80] Hopewell JC, Parish S, Clarke R, et al. for the MRC/BHF Heart Protection Study Collaborative Group. No impact of KIF6 genotype on vascular risk and statin response among 18,348 randomized patients in the Heart Protection Study. *J Am Coll Cardiol*. 2011;57:2000-7. http://www.sciencedirect.com/science/article/pii/S0735109711007261. Accessed May 28, 2015.

[81] Shiffman D, Chasman DI, Zee RY, et al. A kinesin family member 6 variant is associated with coronary heart disease in the Women's Health Study. *J Am Coll Cardiol*. 2008;51:444-8.

[82] Morrison AC, Bare LA, Chambless LE, et al. Prediction of coronary heart disease risk using a genetic risk score: the Atherosclerosis Risk in Communities Study. *Am J Epidemiol*. 2007;166:28-35.
[83] Shifman D, Sabatine MS, Louie JZ, et al. Effect of pravastatin therapy on coronary events in carriers of the KIF6 719Arg allele from the cholesterol and recurrent events trial. *Am J Cardiol*. 2010;105:1300-5.
[84] Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N Engl J Med*. 1995;333(20):1301-7.
[85] Iakoubova OA, Robertson M, Tong CH, et al. KIF6 Trp719Arg polymorphism and the effect of statin therapy in elderly patients: Results from the PROSPER study. *Eur J Cardiovasc Prev Rehabil*. 2010;17:455-61.
[86] Assimes TL, Holm H, Kathiresan S, et al. Lack of association between the Trp719Arg polymorphism in kinesin-like protein-6 and coronary artery disease in 19 case-control studies. *J Am Coll Cardiol*. 2010;56:1552-63. http://content.onlinejacc.org/cgi/content/abstract/j.jacc.2010.06.022v1. Accessed May 28, 2015.
[87] Iakoubova OA, Tong CH, Rowland CM, et al. Association of the TRP719Arg polymorphism in kinesin-like protein 6 with myocardial infarction and coronary heart disease in 2 prospective trials: the CARE and WOSCOPS trials. *J Am Coll Cardiol.* 2008;51:435-43.
[88] Iakoubova OA, Sabatine MS. Rowland CM, et al. Polymorphism in KIF6 gene and benefit from statins after acute coronary syndromes: results from the PROVE-IT-TIMI 22 study. *J Am Coll Cardiol*. 2008;51:449-55.
[89] Ridker PM, MacFadyen JG, Fonseca FA, et al. Number needed to treat with rosuvastatin to prevent first cardiovascular events and death among men and women with low low-density lipoprotein cholesterol and elevated high-sensitivity C-reactive protein: justification for the use of statins in prevention: an intervention trial evaluating rosuvastatin (JUPITER). *Circ Cardiovasc Outcomes*. 2009;2(6):616-23.
[90] Topol EJ, Damani SB. The KIF6 collapse. Journal of the American College of Cardiology 2010;56:1564-6.
[91] GenomeWeb Daily News. FDA tells Celera KIF6 unapprovable without changes. April 21, 2011. https://www.genomeweb.com/dxpgx/fda-tells-celera-kif6-test-unapprovable-without-changes. Accessed December 1, 2014.
[92] Quest Diagnostics. KIF6 Genotype Test. (No month) 2014. http://www.bhlinc.com/clinicians/test-descriptions/KIF6. Accessed December 1, 2014.
[93] Caris Life Sciences International. Caris Molecular Intelligence. October 2014. http://www.carislifesciences.com/oncology-molecular-intelligence. Accessed December 1, 2014.
[94] Von Hoff D, Stephenson JJ, Rosen P, et al. Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. *Journal Clin Oncol*. 2010;28(33):4877-83.
[95] Garber K. Ready or not: personal tumor profiling tests take off. *J Natl Cancer Inst*. 2011;103(2): 84-6.
[96] Doroshow JH. Selecting systemic cancer therapy one patient at a time: Is there a role for molecular profiling of individual patients with advanced solid tumors? *J Clin Oncol.* 2010;28(33):4869-71.
[97] National Cancer Institute. Surveillance, Epidemiology and End Results Program (SEER). SEER Stat Fact Sheets: Prostate Cancer. 2014. http://seer.cancer.gov/statfacts/html/prost.html. Accessed December 16, 2014.
[98] Rettner R. Prostate cancer: can new test reduce unnecessary treatment? May 31, 2013. http://www.livescience.com/37027-prostate-cancer-genetic-tests.html. Accessed December 1, 2014.
[99] Prolaris. 2014. http://www.prolaris.com/. Accessed December 1, 2014.
[100] Global NewsWire. Prolaris test predicts mortality risk in prostate cancer biopsy study. May 20, 2014. http://investor.myriad.com/releasedetail.cfm?releaseid=848939. Accessed December 1, 2014.
[101] Crawford ED, Scholz MC, Kar AJ, et al. Cell cycle progression score and treatment decisions in prostate cancer: results from an ongoing registry. *Current Medical Research & Opinion*. 2014;30(6):1025-31.
[102] Azad A, Tran B, Sengupta S, Lawrentschuk N. Gene expression profiling of localized prostate cancer: Getting answers to the questions that really matter. *Journal of Clinical Oncology*. 2013;31(26):3295-6.
[103] Lombardi VC, Ruscetti FW, Gupta JD, et al. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. *Science*. 2009;326(5952):585-9.
[104] VIPDx, a laboratory associated with the authors of the initial study, offered its LDT for $400 per test. http://phoenixrising.me/research-2/the-pathogens-in-chronic-fatigue-syndrome-mecfs/xmrv/xmrv-testing. Accessed November 12, 2014.

[105] Cooperative Diagnostics offered its LDT for $400 per test. http://www.fibromyalgia-symptoms.org/xmrv-virus.html. Accessed November 12, 2014.
[106] Clongen offered its LDT for $375. http://forums.phoenixrising.me/index.php?threads/clongen-lab-xmrv-test.1813/ Accessed November 12, 2014.
[107] Lo SC, Pripuzova N, Li B, et al. Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. *Proc Natl Acad Sci*. 2010;107(36):15874-9.
[108]Cooperative Diagnostics. Flaws in Mouse Virus Link to Chronic Fatigue Syndrome Known Before Blood Ban, States Cooperative Diagnostics. http://www.marketwired.com/press-release/flaws-mouse-virus-link-chronic-fatigue-syndrome-known-before-blood-ban-states-cooperative-1521452.htm. Accessed November 12, 2014.
[109] Stein R. Red Cross bars chronic fatigue patients from donating blood. *The Washington Post*. December 3, 2010. http://voices.washingtonpost.com/checkup/2010/12/red_cross_bars_chronic_fatique.html. Accessed November 12, 2014.
[110] (No Author). Living with chronic fatigue syndrome. December 4, 2010. http://livingwithchronicfatiguesyndrome.wordpress.com/2010/12/04/the-us-announces-cfs-patient-blood-donation-ban/. Accessed November 12, 2014.
[111] Lakham K. Cooperative Diagnostics pulls XMRV molecular test from clinical market. April 5, 2010. http://sacfs.asn.au/news//2010/04/04_10_cooperative_diagnostics_pulls_xmrv_molecular_test.htm. Accessed June 8, 2015.
[112] Switzer W, Jia H, Hohn O. Absence of evidence of xenotropic murine leukemia virus-related virus infection in persons with chronic fatigue syndrome and healthy controls in the United States. *Retrovirology*. 2010;7:57.
[113] Shin CH, Bateman L, Schlaberg R, et al. Absence of XMRV and other MLV-related viruses in patients with Chronic Fatigue Syndrome. *Journal of Virology*. 2011;85(14):7195-202.
[114] Simmons G, Glynn SA, Komaroff AL, et al. for the Blood XMRV Scientific research working group. Failure to confirm XMRV/MLVs in the blood of patients with chronic fatigue syndrome: a multi-laboratory study. *Science*. 2011;332:814-7.
[115] Wren K. Science: More doubts on the XMRV-Chronic Fatigue connection. AAAS News. September 22, 2011. http://www.aaas.org/news/science-more-doubts-xmrv-chronic-fatigue-connection. Accessed December 1, 2014.
[116] Alberts B. Retraction of Lombardi et al. *Science*. 2011;334(6063):1636.
[117] ScienceDaily. Chronic fatigue syndrome not related to XMRV retrovirus, comprehensive study finds. May 4, 2011. www.sciencedaily.com/releases/2011/05/110504151337.htm. Accessed November 21, 2014.
[118] Reust CE. Common adverse effects of antiretroviral therapy for HIV disease. *Am Fam Physician*. 2011;83(12):1443-51.
[119] Centers for Disease Control and Prevention. Autism Spectrum Disorder. March 2014. http://www.cdc.gov/ncbddd/autism/signs.html. Accessed December 1, 2014.
[120] Myers SM, Johnson CP, the Council on Children with Disabilities. From the American Academy of Pediatrics: Management of children with autism spectrum disorders. *Pediatrics*.2007;120(5):1162-82. http://pediatrics.aappublications.org/content/120/5/1162.full. Accessed May 28, 2015.
[121] Barrett S. The rise and fall of CARE Clinics and the Center for Autistic Spectrum Disorders (CASD). Autism Watch. January 3, 2013. http://www.autism-watch.org/reports/casd/overview.shtml. Accessed December 1, 2014.
[122] U.S. Food and Drug Administration. FDA issues warnings to marketers of unapproved "chelation" products. October 14, 2010. www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm229320.htm. Accessed December 1, 2014.
[123] Croen LA, Najjar DV, Ray T, Lotspeich L, Bernal P. A comparison of health care utilization and costs of children with and without autism spectrum disorders in a large group-model health plan. *Pediatrics*. 2006;118:e1203-e1211.
[124] Montes G, Halterman JS. Association of childhood autism spectrum disorders and loss of family income. Pediatrics. 2008;821-6.
[125] Michigan CR. Financing Special Education: Analyses and Challenges. March 2012. Livonia, MI. http://maase.org/Files/CRC_Financing_SE_March_2012_rpt378.pdf. Accessed May 26, 2015.

[126] Warniment C, Tsang K, Galazka SS. Lead poisoning in children. *American Family Physician*. 2010;81(6):751-7. http://www.aafp.org/afp/2010/0315/p751.html. Accessed May 28, 2015.
[127] World Health Organization. Elemental Mercury and Inorganic Mercury Compounds: Human Health Aspects. Geneva, 2003. http://www.who.int/ipcs/publications/cicad/en/cicad50.pdf. Accessed December 1, 2014.
[128] Kathuria P, Rowden AK, O'Malley RN. Lead Toxicity. *Medscape*. January 28, 2014. http://emedicine.medscape.com/article/1174752-overview. Accessed December 1, 2014.
[129] Centers for Disease Control and Prevention. Case definition: mercury (organic). May 2013. http://emergency.cdc.gov/agent/mercury/mercorgcasedef.asp. Accessed December 1, 2014.
[130] Centers for Disease Control and Prevention. Case definition: mercury (inorganic). May 2013. http://emergency.cdc.gov/agent/mercury/mercinorgcasedef.asp. Accessed December 1, 2014.
[131] Centers for Disease Control and Prevention. Managing elevated blood lead levels among young children: Recommendations from the Advisory Committee on Childhood Lead Poisoning Prevention. March 2002. http://www.cdc.gov/nceh/lead/CaseManagement/caseManage_main.htm. Accessed December 17, 2014.
[132] Oregon Public Health Division Office of Environmental Public Health. Heavy metals and your health: frequently asked questions about testing, treatment and prevention. https://public.health.oregon.gov/HealthyEnvironments/HealthyNeighborhoods/LeadPoisoning/MedicalProvidersLaboratories/Documents/HeavyMetals.pdf. Accessed December 1, 2014.
[133] Ruha AM. Recommendations for provoked challenge urine testing. *J Med Toxicol*. 2013;9(4):318-25. http://www.ncbi.nlm.nih.gov/pubmed/24113861. Accessed May 28, 2015.
[134] Brown MJ, Willis T, Omalu B, Leiker R. Deaths resulting from hypocalcemia after administration of edetate disodium: 2003-2005. *Pediatrics*. 2006;188(2)e534-6.
[135] ChemGenex Pharmaceuticals, Inc. FDA Briefing Book: Oncology Drugs Advisory Committee Meeting, NDA 22-374 Omapro™. February 10, 2010. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM204345.pdf. Accessed November 12, 2014.
[136] U.S. Food and Drug Administration. Summary Minutes of the Oncologic Drugs Advisory Committee. March 22, 2010. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM207638.pdf. Accessed December 9, 2014.
[137] Teva Pharmaceutical Industries Ltd. Press Release: Teva receives approval for SYNRIBOTM (Omapro Mepesuccinate) for injection. Reuters. October 26, 2012. http://www.reuters.com/article/2012/10/26/idUS161284+26-Oct-2012+BW20121026. Accessed July 3, 2015.
[138] Teva Pharmaceutical Industries Ltd. Synribo is intended to be used in patients whose cancer has progressed, despite treatment with at least two tyrosine kinase inhibitors. http://www.tevapharm.com/Media/News/Pages/2012/1750668.aspx. Accessed November 24, 2014.
[139] U.S. Food and Drug Administration. Press Release. Omacetaxine Mepesuccinate October 2012. http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm325990.htm. Accessed November 20, 2014.
[140] Potti A, Dressman HK, Bild A, et al. Genomic signatures to guide the use of chemotherapeutics. *Nature Medicine*. 2006;12:1294-1300. (Retracted January 2011)
[141] Kolata G. How bright promise in cancer testing fell apart. *The New York* Times. July 7, 2011. http://www.nytimes.com/2011/07/08/health/research/08genes.html. Accessed December 1, 2014.
[142] Coombes KR, Wang J, Baggerly KA. Supplementary Reports 0-9. *Nature Med*. 2007;13:1276-77. http://www.nature.com/nm/journal/v13/n11/suppinfo/nm1107-1276b_S1.html. Accessed December 1, 2014.
[143] Coombes KR, Wang J, Baggerly KA. Microarrays: retracing steps. *Nat Med*. 2007;13:1276-77. http://www.nature.com/nm/journal/v13/n11/full/nm1107-1276b.html. Accessed December 1, 2014.
[144] Baggerly K, Coombes K. Deriving chemosensitivity from cell lines: Forensic bioinformatics and reproducible research in high-throughput biology. *Annals of Applied Statistics*. 2009;3(4):1309-34.
[145] Institute of Medicine. Evolution of Translational Omics: Lessons Learned and the Path Forward. The National Academies Press (US). 2012. http://www.nap.edu/catalog/13297/evolution-of-translational-omics-lessons-learned-and-the-path-forward. Accessed December 1, 2014.

[146] Institute of Medicine. Evolution of Translational Omics: Lessons Learned and the Path Forward. Section 3, page 67-8. The National Academies Press (US). 2012. http://www.nap.edu/catalog/13297/evolution-of-translational-omics-lessons-learned-and-the-path-forward. Accessed December 1, 2014.
[147] 80 Federal Register 69230. November 9, 2015.
[148] Shanmugalingam T, Crawley D, Bosco C, et al. Obesity and cancer: The role of vitamin D. *BMC Cancer*. 2014;14:712.
[149] Welles CC, Whooley MA, Karumanchi SA, et al. Vitamin D deficiency and cardiovascular events in patients with coronary heart disease: data from the Heart and Soul Study. *Am J Epidemiol*. 2014;179(11):1279-87.
[150] Pollack A. Quest acknowledges errors in Vitamin D tests. *The New York Times*. January 7, 2009. http://www.nytimes.com/2009/01/08/business/08labtest.html?_r=0. Accessed December 1, 2014.
[151] Mayo Clinic. What is vitamin D toxicity, and should I worry about it since I take supplements? http://www.mayoclinic.org/healthy-living/nutrition-and-healthy-eating/expert-answers/vitamin-d-toxicity/faq-20058108. Accessed December 1, 2014.
[152] Quest Diagnostics Insight Newsletter. New video and web site highlight the importance of vitamin D testing. July 2009. http://www.examone.com/news-media/insight-newsletter/insight-newsletter-july-1-2009. Accessed December 1, 2014.
[153] GL Group. Quest notifies clients of inaccurate vitamin D tests. January 20, 2009. http://www.investorvillage.com/mbthread.asp?mb=10853&tid=6510198&showall=1. Accessed December 1, 2014.
[154] SEER Cancer Statistics Factsheets: Breast Cancer. National Cancer Institute. Bethesda, MD. November 25, 2014. http://www.cancer.gov/cancertopics/pdq/treatment/breast/Patient#_136. Accessed December 18, 2014.
[155] Aston CE, Ralph DA, Lalo DP, et al. Oligogenic combinations associated with breast cancer risk in women under 53 years of age. *Hum Genet.* 2005; 116(3):208-21.
[156] Pharoah PD, Antoniou AC, Easton DF, et al. Polygenes, risk prediction, and targeted prevention of breast cancer. *N Engl J Med*. 2008;358(26):2796-803.
[157]Progressive Medical Enterprises LLC. OncoVue (R) for Women. 2012. http://progressivemedical.org/oncovue%C2%AE-for-women. Accessed December 1, 2014.
[158] Dalessandri KM, Miike R, Wrensch MR. Validation of OncoVue, a new individualized breast cancer risk estimator in the Marin County, California adolescent risk study. 2008 San Antonio Breast Cancer Symposium; Texas, U.S.A. Abstract 502.
[159] Dalessandri KM, Miike R, Wrensch MR. Breast cancer risk assessment in the high risk Marin County population using OncoVue compared to SNPs from genome wide association studies. 2009 San Antonio Breast Cancer Symposium; Texas, U.S.A. Abstract 3057.
[160] Premera. Medical policy: Use of common genetic variants (single nucleotide polymorphisms) to predict risk of non-familial breast cancer. June 9, 2014. https://www.premera.com/medicalpolicies/cmi_126901.htm. Accessed December 1, 2014.
[161] Reeves GK, Travis RC, Green J, et al. Incidence of breast cancer and its subtypes in relation to individual and multiple low-penetrance genetic susceptibility loci. *JAMA*. 2010;304(4):426-34.
[162] Peres J. Experts question new DNA test for breast cancer risk. *Chicago Tribune*. October 22, 2006. http://articles.chicagotribune.com/2006-10-22/news/0610220424_1_oncovue-breast-cancer-tamoxifen. Accessed December 1, 2014.
[163] Genentech. BRAF in metastatic melanoma. 2014. http://www.biooncology.com/research-education/braf/metastatic-melanoma. Accessed December 1, 2014.
[164] Ascierto PA, Kirkwood JM, Grob JJ, et al. The role of BRAF V600 mutation in melanoma. *J Translat Med*. 2012;10:85.
[165] Genentech. Vemurafenib (Zelboraf) drug label. October 25, 2013. https://www.pharmgkb.org/download.do?objCls=Attachment&objId=Vemurafenib_10_17_13.pdf. Accessed December 1, 2014.
[166] U.S. Food and Drug Administration. Medical Devices: Cobas 4800 BRAF V600 Mutation Test. August 17, 2011. http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/ucm268836.htm. Accessed December 1, 2014.

[167] NeoGenomics Laboratories. BRAF V600E, K, & D Mutations: Identify 10-15% more patients who may respond to vemurafenib. 2014. http://www.neogenomics.com/braf-v600e-melanoma.htm. Accessed December 1, 2014.
[168] Molecular Response. BRAF V600E Mutation Test. 2014. http://molecularresponse.com/braf/. Accessed December 1, 2014.
[169] Ray T. Quest study finds Sanger-based BRAFT mutation test more sensitive than Roche's Cobas test. GenomeWeb. June 13, 2012. https://www.genomeweb.com/mdx/quest-study-finds-sanger-based-braf-mutation-test-more-sensitive-roches-cobas-te. Accessed December 18, 2014.