

## Evidence-based Practice Center Technical Brief Protocol

### Project Title: Genetic Testing for Developmental and Intellectual Disabilities

#### I. Background and Objectives for the Technical Brief

Developmental disabilities (DDs) are a group of conditions due to an impairment in physical, learning, language, or behavior areas.<sup>1</sup> According to this definition, the Centers for Disease Control and Prevention (CDC) categorize a broad range of conditions as DDs, such as attention-deficit/hyperactivity disorder, autism spectrum disorders (ASD), cerebral palsy, fragile X syndrome, hearing loss, learning disability, intellectual disability (ID), Tourette syndrome, vision impairment, and others.<sup>1</sup>

DDs, such as ID and ASD, affect up to 3 percent of the U.S. population, respectively.<sup>1-3</sup> When including other developmental disabilities (e.g., ADHD, cerebral palsy, language disorders, learning disorders and others) the prevalence of having any developmental disorder increases to over 15% in children 3 to 17 years of age.<sup>4</sup> These disorders may have a profound impact on patients, families, and society, given the need for potentially lifelong individual and family support or treatment.

DDs can be caused by a variety of factors, including genetic causes, mother's health behaviors (e.g., smoking and drinking) and infections during pregnancy, premature delivery, complications during birth, and the exposure of the mother or child to environmental toxins.<sup>1</sup> The causes for some developmental disabilities (e.g., Down syndrome, fragile-X syndrome, fetal alcohol syndrome) have been well understood. However, the causes for many DDs are still unclear.

Some DDs (e.g., cerebral palsy, hearing loss, vision impairment) can be diagnosed based upon clinical symptoms or physical anomalies. However, for many patients with DDs who do not have dysmorphic or syndromic features or those who are too young for full expression of the condition, diagnosis is a challenge. Some of the clinical investigators and the key informants for this project observed that these patients may need to frequently visit their doctors and may sometimes receive a large number of clinical tests before a specific diagnosis or etiology can be established. This diagnostic odyssey may place a significant amount of stress and burden on patients, their families, the health care system, and the society as a whole.

#### *Advanced Genetic Tests for Diagnosing DDs*

Studies have suggested that patients with ASD and up to 40 percent of those with DDs/ID may have a genetic etiology for the disability.<sup>5,6</sup> The association between some DDs and genetic abnormalities such as Angelman syndrome, fragile X syndrome, Prader-Willi Syndrome has been established.<sup>7,8</sup> For these patients, genetic testing provides the opportunity to establish an

etiologic diagnosis during their early years in life, monitor for associated medical comorbidities, and provide genetic counseling to their families.

Conventional G-banded karyotyping has been used for many years to confirm the diagnosis of DDs (e.g., aneuploidies) with a well-defined genetic etiology. More recently, advanced genetic tests (e.g., microarray-based comparative genomic hybridization [aCGH] and sequencing) have been used to detect genetic abnormalities associated with DDs. These newer tests have a higher resolution and may show genetic abnormalities not seen on G-banded karyotyping. Proposed benefits of these advanced genetic tests include establishing an etiologic diagnosis in patients with neurodevelopmental manifestations but without syndromic features, ending the diagnostic odyssey of many visits to specialists, avoiding other forms of testing, improving understanding of prognosis and future medical needs, initiating treatment and surveillance earlier, and helping families in reproductive decision making.<sup>9-15</sup>

Due to these potential benefits, the use of advanced genetic tests is increasing at a rapid rate. Medical genetics groups now recommend chromosomal microarray analysis (CMA) as a first line genetic test to identify genetic mutations in children with multiple anomalies not specific to well-delineated syndromes, nonsyndromic DD/ID, and ASD.<sup>10</sup> Payers have seen a significant number of claims for genetic testing in children with alleged or proven DDs.<sup>16</sup> However, little evidence from controlled studies exists to directly link genetic testing to patient-centered outcomes.<sup>17</sup> Published studies have reported superior diagnostic yields of array-based genetic tests in identifying DD-related genetic abnormalities and some have identified the impact of the tests on medical management (e.g., medical referrals, diagnostic imaging, further laboratory testing).<sup>9-15</sup> However, these findings are not sufficient for drawing a conclusion that use of the tests will lead to improved health outcomes (further discussion on this issue is provided in a later section, *Establishing the Clinical Utility of Genetic Tests*).

The impact of increased utilization of advanced genetic tests, such as CMA, on healthcare costs is unclear. Advanced genetic tests are generally more expensive to perform than conventional G-banded karyotyping or other clinical tests.<sup>18</sup> Nominators of this topic noted that their average reimbursement was approximately \$1750.00 for microarray testing.<sup>16</sup> Identification of genetic abnormalities on germline cells also leads to genetic testing in patients' relatives, which further expands the pool of children for testing and magnifies the potential impact. Conversely, potentially increased diagnostic yield of advanced genetic tests may reduce the number of other clinical tests or services conducted to identify genetic etiologies for DDs. In addition to the uncertain clinical utility and concerns about economic impact, ethical issues, such as how to deal with genetic abnormalities unrelated to DD that are detected in genome-wide CMA, also remain controversial.<sup>19</sup>

### *Availability of Genetic Tests for DDs in the U.S.*

Currently, genetic tests become clinically available in the United States via one of two pathways. A genetic test may reach the market as a commercially distributed test kit approved or cleared by the U.S. Food and Drug Administration (FDA) or as a laboratory-developed test (LDT).<sup>20,21</sup> FDA-cleared or -approved test kits include all reagents and instructions needed to complete the test procedure and interpret the results. These test kits can be used in multiple laboratories. LDTs are developed in laboratories using either FDA-regulated or self-developed analyte-specific reagents and are intended for performance solely in the test developer's laboratory.

The U.S. Centers for Medicare & Medicaid Services regulates laboratories that perform LDTs under the Clinical Laboratory Improvement Amendments of 1988 (CLIA).<sup>20,21</sup> Under the CLIA regulations, all facilities that perform tests on “materials derived from the human body for the purpose of providing information for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of, human beings” must obtain a certificate from the CLIA program. The requirements for CLIA certification are based on the complexity of the tests. Laboratories offering LDTs must be licensed as a high-complexity clinical laboratory under CLIA regulations.

LDTs compose the majority of the genetic tests that have become available to clinical practice.<sup>21</sup> A preliminary literature search that we performed and a technology assessment report<sup>17</sup> also suggested that genetic tests for diagnosing DDs are mainly available as LDTs. Some stakeholders have concerns about the quality, validity, and clinical utility of genetic LDTs due to the lack of active FDA regulation. However, there is no sufficient evidence demonstrating that FDA-regulated test kits perform better than LDTs.<sup>21</sup>

### *Establishing the Clinical Utility of Genetic Tests*

The clinical utility of a genetic test refers to how likely the test is to affect clinical decisions and ultimately improve patient outcomes. The ideal type of evidence for establishing the clinical utility is from high quality randomized controlled trials (RCTs) that compare use and no use of the test in clinical practice and analyze if any significant differences in health outcomes occur between the compared arms. In reality, however, this type of RCT is rarely conducted.<sup>20-22</sup> To answer the ultimate clinical utility question—whether use of the test will improve health outcomes, an inference-based chain of evidence often needs to be established.<sup>22,23</sup> Establishing this chain of evidence involves assessing the analytic validity and clinical validity of the test of interest, and establishing an indirect evidence link to clinical outcomes.

Analytic validity refers to how accurately and reliably the test measures the analyte of interest, such as a gene aberration. Analytic validity is a function of many factors such as analytic accuracy, precision, analytic sensitivity and specificity, reportable range of test results for the test system, reference range or normal values. The technical terms for analytic validity are defined in section V of this Technical Brief protocol, *Definition of Terms*.

Clinical validity, also known as diagnostic accuracy, refers to how accurately the test detects or predicts the clinical condition of interest. Clinical validity is usually described in terms of clinical sensitivity, clinical specificity, positive and negative predictive values, likelihood ratios, diagnostic odds ratios, and the area under a receiver operator characteristic (ROC) curve. These technical terms related to clinical validity are also defined in section V of this Technical Brief protocol.

To establish the chain of evidence, an evaluation framework for genetic tests is typically used. An evaluation framework is a conceptual approach to the evaluation of the tests and to organizing the relevant evidence. The framework is a tool for clarifying the scope of the questions to be addressed in health technology assessment and the nature of evidence necessary for answering the questions. Different stakeholders (e.g., patients, providers, payers, regulators, and test developers) may need somewhat different frameworks for their evaluation tasks. The framework presented in this document (Figure 1) takes the patients’ perspectives. We adapted this framework from a previous AHRQ methods report we authored on the evaluation of genetic

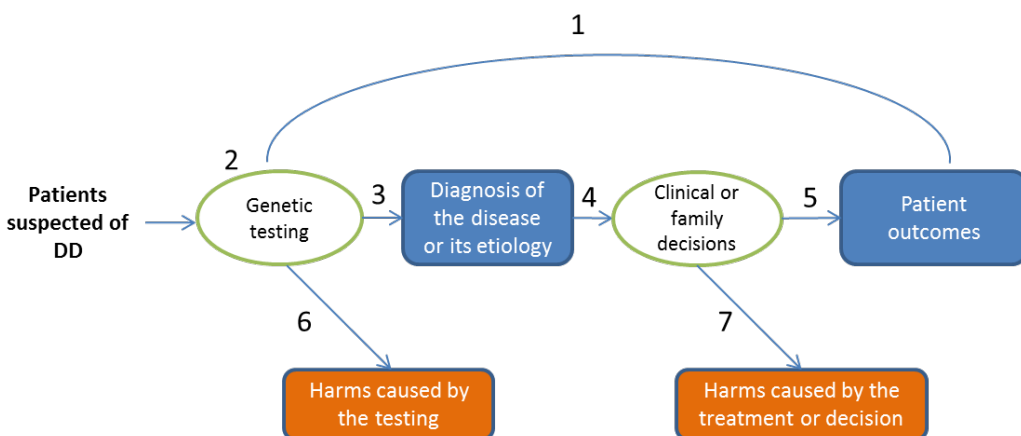
tests. The framework delineates the relationship between analytic validity, clinical validity and clinical utility and helps demonstrate areas where evidence is available or missing.<sup>22</sup>

Under this framework, a series of key research questions are asked and answered to establish the chain of evidence for clinical utility. These questions include:

- Question 1 (Overarching Question): Does use of a genetic test lead to improved health outcomes in patients with DDs compared to the standard-of-care diagnostic strategy?
- Question 2: Does the test have adequate analytic validity?
- Question 3: Does the test have adequate clinical validity?
- Question 4: Does use of the test have any impact on treatment decision making by clinicians or patients?
- Question 5: Does the altered treatment lead to improved patient outcomes?
- Question 6: Are there harms associated with use of the test?
- Question 7: Are there harms associated with the altered treatment?

To address these key research questions, different types of evidence may be required. For example, to address the overarching clinical utility question, RCTs are most appropriate. To address question 3 regarding clinical validity, diagnostic cohort studies that use a gold-standard reference method are ideal.

**Figure 1. Evaluation Framework for Genetic Tests for Diagnosing DDs**



In early March 2014, we conducted a preliminary search of MEDLINE and EMBASE using controlled vocabulary terms relevant to DDs/ID/ASDs and genetic testing. Our preliminary search did not identify any study that directly linked testing and patient-centered outcomes. Most studies reported a test's diagnostic yield and none investigated a test's diagnostic accuracy (i.e., sensitivity, specificity, positive predictive value, and negative predictive value). Table 1 summarizes these studies identified.

**Table 1. Preliminary Literature Search Summary**

Publication Type	Number of Publications	References
Systematic reviews, meta-analysis, technology assessments	7	8,9,17,24-27
Guidelines	3	7,28,29
Cost-effectiveness analyses	6	30-35
Studies that directly linked a test to patient-centered outcomes	0	
Studies that address a test's analytic validity	4	36-39
Studies that report a test's diagnostic yield	47	12,40-85
Studies that explored the association between phenotypes and genetic abnormalities	12	86-97
Studies that explored the feasibility or validity of a newly developed test or an algorithm	22	98-119
Studies that addressed ethical issues, family opinions on testing, use of the testing in practice, or patient selection for testing	4	19,120-122
Narrative reviews	21	123-143

## *Objectives of the Technical Brief*

This Technical Brief is intended to provide an overview of advanced genetic tests for diagnosing DDs or determining the etiology of DDs that are clinically available in the United States. We will collect and analyze following information on tests identified: testing technique used (e.g., aCGH, PCR-based, sequencing) and how it works, proposed use (e.g., population, indications, timing, settings), targeted DDs, targeted gene or chromosomal regions, theoretical advantage over other diagnostic methods, potential harms, whether it is a FDA-cleared or approved commercial test kit or a LDT, vendors/laboratories providing the test (including the CLIA certification status of the laboratory), whether it is recommended by clinical guidelines, information on diffusion of the test in clinical practice, and important ethical, privacy, equity or cost considerations.

Given the rapid diffusion of advanced genetic tests for diagnosing DDs or determining etiology of DDs, it is important to understand the clinical utility of these tests versus other diagnostic options. This Technical Brief will identify existing evidence addressing the clinical utility of genetic tests for DDs. We will create an evidence map to summarize information about what types of studies have been completed or are underway for the tests and what questions they can answer. The map will depict evidence gaps for each test and provide guidance for future research.

Because of the rapid pace of change in genetic technologies, genetic tests currently used in clinical practice may be replaced by more advanced technologies in the near future. In this Technical Brief, we will discuss and provide a summary on emerging genetic tests that may significantly affect the management of DDs.

## *Scope of Work*

The scope of work for this Technical Brief is described below by the population, interventions, comparators, and outcomes of interest. This scope reflects the ECRI Institute-Penn Medicine EPC team's current thinking and incorporated the input from AHRQ, the stakeholders, (e.g., the topic nominators), and Key Informants of the Brief.

**Population:** Children with DDs (e.g., ID and ASD) and their families (e.g., their siblings who may also suffer from the same disorder)

This Technical Brief will particularly focus on patients with idiopathic or unexplained DDs. These patients have functional manifestations of DDs, IDs or ASDs but may not have shown any distinct dysmorphic or syndromic features. Differential diagnoses for these patients can be difficult based on clinical manifestations or conventional G-banded karyotyping. Patients with DDs characterized by distinct syndromic features (such as Down syndrome) that are typically diagnosed based on clinical manifestations or conventional G-banded karyotyping are beyond the scope of the Technical Brief.

**Interventions:** Genetic tests for diagnosing DDs

This Technical Brief only includes tests that are available in the U.S., either as FDA-cleared or –approved test kits or as an LDT provided by a CLIA-certified laboratory. We will focus on CMA (including aCGH and single nucleotide polymorphism [SNP] assays). CMA is widely used in clinical practice. Medical genetics groups now recommend CMA as a first line genetic test to identify genetic mutations in children with multiple anomalies not specific to well-delineated syndromes, nonsyndromic DD/ID, and ASD.<sup>10</sup> CMA is also the most studied type of test identified by our preliminary literature search.

Other types of genetic tests within the scope of work include polymerase chain reaction (PCR)-based tests (e.g., quantitative PCR), multiplex ligation-dependent probe amplification, Southern blot, sequencing, high-resolution G-banded karyotyping, fluorescence in situ hybridization (FISH, including subtelomeric FISH [StFISH]), and tests used for methylation analysis, deletion/duplication analysis, and uniparental disomy study.

**Comparators:** Standard-of-care diagnostic methods, including no genetic testing or using other clinical tests for diagnosing DDs

Clinical tests considered as comparators may vary across DDs. For example, for ASD, comparators may include Autism Behavior Checklist, Autism Diagnostic Interview-Revised, Autism Observation Scale for Infants, Checklist for Autism in Toddlers, Childhood Autism Rating Scale, Gilliam Autism Rating Scale-2nd Edition, Autism Diagnostic Observation Schedule-Generic, and Autism Diagnostic Observation Schedule-Toddler Module.<sup>16</sup> For Angelman syndrome, comparators may include Wechsler Preschool and Primary Scale of Intelligence, Wechsler Intelligence Scales for Children, Stanford-Binet Intelligence Scale, Kaufman Assessment Battery for Children, McCarthy Scales of Children's Abilities, Differential Abilities Scales, Leiter International Performance (tests non-verbal abilities), Inventory for Client and Agency Planning, Scales of Independent Behavior, and Vineland Adaptive Behavior Scales.<sup>16</sup>

**Outcomes:** Patient-centered health outcomes, changes in clinical or family decisions, diagnostic accuracy (e.g., sensitivity, specificity, positive and negative predict values), and parameters for measuring the analytic validity of a test.

## II. Guiding Questions

We have developed a series of questions to guide our efforts in collecting appropriate information for this Technical Brief. These include:

1. Description of genetic tests for diagnosing DDs or determining the etiology of DDs



- a. What genetic tests for diagnosing DDs or determining the etiology of DDs are currently available for clinical practice in the United States?
  - b. What genetic techniques or analysis methods (e.g., CMA, aCGH, StFISH) are used in these tests? How do these types of techniques or methods work?
2. Context in which genetic tests are used for diagnosing DDs or determining the etiology of DDs:
  - a. What is the current regulatory status (i.e., FDA clearance or approval status, CLIA certification of the test provider) of the tests?
  - b. What kinds of credentials (i.e., trainings, certification) are required for interpreting the results of the tests?
  - c. Who are the providers ordering the tests and using the test results?
3. State of the evidence on genetic tests for diagnosing DDs or determining the etiology of DDs
  - a. What are the patient inclusion criteria in studies of these tests?
  - b. What are the study designs utilized?
  - c. What outcomes are reported?
    - i. What data have been reported in the literature about the analytic validity of the tests?
    - ii. What data have been reported in the literature about the clinical validity of the tests?
    - iii. What data have been reported in the literature about the clinical utility of the tests?
    - iv. What are the potential safety issues or harms related to the tests?
4. What are the important issues raised by genetic tests for diagnosing DDs or determining the etiology of DDs?
  - a. What are the proposed advantages and disadvantages of these tests compared to standard-of-care diagnostic methods?
  - b. What recommendations do clinical practice guidelines include regarding the use of the tests?
  - c. Given the current evidence status, what are the implications of the tests in terms of ethics, privacy, equity, cost, or economic efficiency?
  - d. What are the current evidence gaps and potential areas of future research?
  - e. What are the ongoing clinical trials for the clinical utility of the tests?
  - f. What genetic tests or testing methods currently under research may become clinically available for diagnosing DDs in the near future?

### III. Methods

We describe below the methods for addressing the guiding questions previously defined.

## *Data Collection*

### Discussions with Key Informants

Key Informants are particularly important in this Technical Brief, because the area of genetic testing for DDs is dynamic and published data for addressing some of the guiding questions (e.g., those regarding identifying LDTs and addressing the analytic validity of genetic tests) are often unavailable. KIs helped identify relevant data sources and contributed to a better understanding of how advanced genetic tests work, the tests' role in clinical practice, and potential advantages or harms.

KIs who participated on this project include clinicians who treat patients with a DD, experts on genetic testing, patient advocates, medical directors from Medicaid programs, and individuals representing professional societies. Discussions with these KIs allowed us to identify important issues from different perspectives.

OMB clearance will not be required as we limited our standardized questions to no more than nine nongovernment-associated individuals.

After review and approval of the completed Disclosure of Interest forms for proposed KIs by (AHRQ), we have held interviews with eight selected KIs. The interviews were held with small groups of KIs based on availability and concordance of perspectives. Each interview was summarized in writing. KIs' input has been considered as we defined the scope of work and prepare the draft report for this project. Section VII of this document provides additional information on how KIs serve as a resource for AHRQ reports.

### Grey Literature Search

A main objective of this Technical Brief is collecting information on genetic tests for diagnosing DDs or determining the etiology of DDs. As previously discussed, the majority of these tests are available as LDTs. Identifying all LDTs within the scope of this Technical Brief will be a significant challenge and will require a multi-faceted approach, including a comprehensive search of peer-reviewed and grey literature. Based on our experience in developing an EPC horizon scan report on molecular LDTs,<sup>21</sup> we believe grey literature sources will be particularly helpful.

For this Technical Brief, we will use the National Center for Biotechnology Information (NCBI) Genetic Testing Registry (GTR) (<https://www.ncbi.nlm.nih.gov/gtr/>) as the primary source for identifying tests of interest. NCBI is a division of the National Library of Medicine at the National Institutes of Health. GTR is a central location for genetic test information voluntarily submitted by providers. The submitted information includes the test's purpose, methodology, validity, evidence of the test's usefulness, and laboratory contacts and credentials. We anticipate identifying a large number of tests of interest from GTR. For example, our preliminary search of the GTR database identified 207 clinical tests linked to "Angelman syndrome," 155 tests linked to "fragile X syndrome," and 142 tests linked to "Prader-Willi syndrome." Most of these tests are LDTs.

We will also search two other U.S.-focused online sources—McKesson Diagnostics Exchange and GeneTests.org—to complement and confirm the information collected from GTR. McKesson Diagnostics Exchange (<https://app.mckessonindex.com>) is an online registry of molecular diagnostic tests. GeneTests.org (<http://www.genetests.org>) is a medical genetics



information resource including a directory of international laboratories offering genetic testing. Both McKesson Diagnostics Exchange and GeneTests.org are proprietary but accessible to the public. Additional grey data sources we will consider include (e.g., GeneReviews [<http://www.ncbi.nlm.nih.gov/books/NBK11116/>], the Association for Molecular Pathology Test Directory [<http://www.amptestdirectory.org/index.cfm>], NCBI's Online Mendelian Inheritance in Man (OMIM) database [<http://omim.org/>], and EuroGentest [<http://www.eurogentest.org/>]).

We will also search other grey literature sources, such as government and specialty society Web sites, clinical trial databases, AHRQ's Healthcare Horizon Scanning System, trade publications, and meeting abstracts. From these sources, we may be able to identify data addressing the analytic validity, clinical validity, and clinical utility, as well as professional society consensus statements regarding use of genetic tests for DDs, and new technologies or tests under development.

## Published Literature Search

We will use a variety of databases to search the peer-reviewed literature. These include Medline and Embase (Embase.com), PreMedline and PubMed in process subset (PubMed), PsycINFO (OVID) and the Cochrane library (including the Central Register of Controlled Trials, the Cochrane Database of Methodology Reviews, and the Cochrane Database of Systematic Reviews, the Database of Abstracts of Reviews of Effects, the Health Technology Assessment Database, and the U.K. National Health Service Economic Evaluation Database). The National Guideline Clearinghouse (NGC) will be searched for relevant clinical practice guidelines. The searches will use a combination of controlled vocabulary terms and free text words and will be limited to English language studies published since 2000. A detailed literature search strategy is provided in Section IX of this protocol.

## *Data Organization and Presentation:*

### Information Management

Because of the broad scope of this Technical Brief (multiple DDs, multiple genetic tests, and multiple aspects regarding the tests' performance—analytic validity, clinical validity and utility), we expect to screen and review an extremely large body of literature. Reviewing full-text articles to address all the guiding questions is not feasible within the timeframe of the project. It is most likely that we will have to collect a significant portion of data via review of the abstracts. Given the type of data we intend to collect for this Technical Brief (refer to Table 2 and Table 3), we anticipate that abstract review will suffice for data collection in most cases. A preliminary literature search and abstract review (refer to Table 1) suggested that the vast majority of clinical studies in the area are case series that reported a test's diagnostic yield or the prevalence of a genetic aberration among certain patient populations. For this type of study, we can identify the study design (i.e., case series) and the reported outcome (e.g., diagnostic yield) at the abstract level with confidence. By design, these diagnostic yield case series do not report outcome measures for analytic or clinical validities (e.g., testing sensitivity, specificity, positive or negative predictive values). In rare cases in which a diagnostic yield study also address a clinical utility issue (e.g., reporting on the impact of the test on clinical or family decisions), we think it is reasonable to expect the authors of the study to report or, at least, mention this important issue in the abstract.

In any case in which we determine the information in the abstracts is insufficient, we will retrieve and review full-text articles to abstract data. For example, some case series may not report the study sample size in the abstract; other studies (e.g., a study validating a test's analytic validity or a diagnostic cohort study) may not report all outcome measures in the abstract. We will retrieve full-text articles for these studies to collect data.

We will collect data only from the articles/abstracts that meet the population, interventions, comparators, and outcomes criteria defined in the *Scope of Work* section of this document. In addition, we will collect data only from articles/abstracts published in English

We will use the DistillerSR<sup>®</sup> (Evidence Partners, Inc., Ottawa, Ontario, Canada) Web-based systematic review software for abstract screening. Two researchers will extract data onto the standardized forms (refer to Table 2 and Table 3). Each team member's data extraction will be reviewed by one other team member. We will resolve all discrepancies through discussion. Multiple publications of the same study will be identified by examining author affiliations, study designs, enrollment criteria, and enrollment dates.

Table 2 and Table 3 are data forms that we have developed to capture the key information that address the guiding questions. We will fill out the forms for each genetic test identified. In the forms, we will provide detailed guidance to data abstractors about what information needs to be collected.

**Table 2. Data Form for Each Test Identified**

<b>Data Items</b>	<b>Data Collected</b>	<b>Data Source</b>
<b>Test</b>	Describe the full name of the test, e.g.,	Provide reference numbers or links to online data sources
<b>Disease/Disorder</b>	Describe the disease or disorder that the test is used to diagnose, e.g., Prader-Willi syndrome, Angelman syndrome	See above
<b>Targeted Gene or Chromosomal Area</b>	Describe the gene the test targets, e.g., MECP2, 15q11.2-q13	See above
<b>Analysis Method Used</b>	Describe the genetic methods, platform, system that test uses, e.g., deletion/duplication analysis, sequencing, aCGH, SNP assay, StFISH	See above
<b>Test Provider</b>	Describe the manufacturer (in the cases of FDA-cleared or – approved commercial test kits) or the laboratory (in the cases of LDTs) providing the test	See above
<b>Regulatory Status</b>	Describe the regulatory status of the test, e.g., when it is FDA-cleared or -approved (in the cases of commercial test kit) or the laboratory's CLIA certification status (in the cases of LDTs)	See above
<b>Detailed Indications</b>	Describe the FDA-cleared or –approved (in the cases of commercial test kit) or the laboratory-proposed (in the cases of LDTs) indication for the test, e.g., population, timing, and settings	See above
<b>Advantages Proposed</b>	Describe potential advantages of the tests over standard-of-care diagnose strategies that the manufacture, the laboratory, or published studies suggest	See above
<b>Disadvantages Proposed</b>	Describe potential harms or any disadvantages of the tests that the manufacture, the laboratory, or published studies suggest	See above
<b>Analytic Validity Studies</b>	Describe if any studies are available that address the analytic validity of the test.	See above
<b>Clinical Validity Studies</b>	Describe if any studies are available that address the clinical validity diagnostic accuracy of the test.	See above

<b><i>Clinical Utility Studies</i></b>	Describe if any studies are available that address the clinical utility of the test.	See above
<b><i>Recommendations in Clinical Guidelines</i></b>	Describe any relevant recommendations in clinical practice guidelines regarding use of the test	See above
<b><i>Non-clinical Implications</i></b>	Describe significant ethics, privacy, equity, or, cost implications discussed in literature	See above
<b><i>Ongoing Clinical Trials</i></b>	Describe any ongoing clinical trials for the clinical utility of the tests	See above

aCGH—microarray-based comparative genomic hybridization; SNP—single nucleotide polymorphism; StFISH—subtelomeric fluorescence in situ hybridization; CLIA—the Clinical Laboratory Improvement Amendments of 1988; FDA—the U.S. Food and Drug Administration; LDT—laboratory-developed test

**Table 3. Data Form for Clinical Studies Available for Each Test Identified**

Authors/ Year/ Reference	Genetic Test or Analysis Method Studied	DD Studied	Outcomes Reported	Study Design	Comparators if applicable	Study Population	Sample Size	Study Period/ Followup
	e.g., aCGH, SNP array, sequencing, PCR, etc.	e.g., ASD, ID, etc.	For analytic validity studies, analytic sensitivity, specificity, repeatability, reported range, etc.  For clinical validity studies, diagnostic sensitivity, specificity, positive and negative predictive values, etc.  For clinical utility studies, health outcomes, impact on clinical or family decisions, harms, etc.	For analytic validity studies, case-controlled studies, validation studies, etc.  For clinical validity studies, diagnostic cohort studies, case-controlled studies, etc.  For clinical utility studies, randomized controlled trials, non-randomized clinical trials, observational studies, surveys, etc.	For clinical validity studies, gold-standard or other reference used  For clinical utility studies, standard-of-care diagnosing strategies, other genetic tests, etc.			

aCGH—array comparative genomic hybridization; ASD—Autism spectrum disorder; DD—developmental disability; ID—intellectual disability; PCR—polymerase chain reaction; SNP—single nucleotide polymorphism

## Data Presentation

Collected data on each individual test using Table 2 and Table 3 will be compiled as an appendix in the Technical Brief. We will also summarize data across the tests to help clinicians and policy makers understand the landscape of genetic testing for diagnosing DDs. Table 4 demonstrates how we intend to summarize data across the tests. Table 5 is an evidence map previously described. This map will help clinicians, policy makers and researchers identify existing evidence gaps and directions for future research. In addition, we will provide a section to discuss any ongoing trials that address the clinical utility and any tests under development that may come to clinical practice in the future. We will further write a section to provide an overview of the genetic analysis methods that are commonly used in the tests we identified in this Technical Brief. These analysis methods may include CMA (including aCGH), PRC-based methods, and sequencing.

**Table 4. The Landscape of Genetic Testing for Diagnosing DDs**

Clinical Condition	Test Available	Test Provider/Regulatory Status	Targeted Gene or Chromosomal Area	Analysis Methods Used	Recommendations in Clinical Guidelines
Describe the name of the DD (including ID and ASD) here	Describe the genetic test identified in the search	Describe the name of the manufacturer or laboratory providing the tests and whether it is a FDA-cleared or approved commercial kit or a LDT	Describe the gene targeted by the test, e.g., MECP2, 15q11.2-q13	Describe the genetic methods, platform, system that test uses, e.g., deletion/duplication analysis, sequencing, aCGH, SNP assay, StFISH	Describe any relevant recommendations in clinical practice guidelines regarding use of the test

DD—developmental disability; ID—intellectual disability; ASD—autism spectrum disorder; LDT—laboratory-developed test

**Table 5. Evidence Map for Genetic Tests for Diagnosing DDs**

Clinical Condition	Test Identified	Analytic Validity Studies Identified	Clinical Validity Studies Identified	Clinical Validity Studies Identified	Other Relevant Studies
Describe the name of the DD (including ID and ASD) here	Describe the genetic test identified in the search, including the information about the test provider, the targeted gene or chromosomal area, and testing method used	Describe the number of studies identified (including the citations), the analytic parameters reported (e.g., analytic sensitivity, reported range), and the study design and sample size for each individual study	Describe the number of studies identified (including the citations), the diagnostic accuracy measures reported (e.g., diagnostic sensitivity, positive predictive value), and the study design and sample size for each individual study	Describe the number of studies identified (including the citations), the outcome measures reported, and the study design and sample size for each individual study	Describe the number of other types of clinical studies (e.g., diagnostic yield studies) relevant to the test, the outcome measures reported, and the study design and sample size for each individual study

DD—developmental disability; ID—intellectual disability; ASD—autism spectrum disorder

## IV. References

1. National Center on Birth Defects and Developmental Disabilities (NCBDDD). Facts about developmental disabilities. [internet]. Atlanta (GA): Centers for Disease Control and Prevention (CDC); 2013 Dec 26 [accessed 2014 Apr 1]. [4 p]. Available: <http://www.cdc.gov/ncbddd/developmentaldisabilities/facts.html>.
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## V. Definition of Terms

The key terms used in this report are defined in this section. The definitions are from an AHRQ EPC method report we authored, *Addressing Challenges in Genetic Test Evaluation. Evaluation Frameworks and Assessment of Analytic Validity*.<sup>22</sup>

**Analytic accuracy:** refers to the closeness of the agreement between the result of a measurement and a true value of the measurand.

**Assay linearity:** is defined as the ability (within a given range) to provide results that are directly proportional to the concentration (amount) of the analyte in the test sample. Linearity of tests is established by testing a dilution series of a positive sample.

**Analytic sensitivity:** Analytic sensitivity describes how effectively a test can detect all true positive specimens, as determined by a reference method. As it is more often used, this term is used for tests that yield a qualitative result.

**Analytic specificity:** is defined as the ability of a measurement procedure to measure solely the analyte of interest. Two important aspects of analytic specificity are interference by endogenous or exogenous substances other than the analyte of interest and cross-reactivity of the analytic system with substances other than the intended analyte of interest.

**Analytic validity:** simply refers to how well a test performs in the laboratory—how well does the test measure the properties or characteristic it is intended to measure (e.g., a gene mutation)?

**Clinical validity:** (also known as diagnostic accuracy) refers to the accuracy with which a test predicts the presence or absence of a clinical condition or predisposition.

**Clinical utility:** refers to the usefulness of the test and the value of information to medical practice. If a test has utility, it means that the results of the test can be used to seek effective treatment or provide other concrete benefit.

**Cross-reactivity:** refers to the reaction that an assay may have with analytes other than the ones it is designed to measure.

**Diagnostic accuracy:** is also known as clinical validity (see definition of *clinical validity*).

**Diagnostic sensitivity:** refers to the probability of a positive test result when disease is present.

**Diagnostic specificity:** refers to the probability of a negative test result when disease is absent.

**Grey literature:** consists of reports, studies, articles, and monographs produced by federal and local government agencies, private organizations, educational facilities, consulting

firms, and corporations. These documents do not appear in the peer-reviewed journal literature.

**Health outcomes:** are symptoms and conditions that patients can feel or experience, such as visual impairment, pain, dyspnea, impaired functional status or quality of life, and death.

**Interference:** may result from contamination, admixture, or presence of exogenous substances in samples, which can occur for a variety of reasons such as poor sampling, lack of sample stabilizer (where appropriate), cross-contamination during sample processing, inclusion of normal, non-diseased tissue with the diseased tissue of interest, tissue from a source additional to the desired sample (e.g., maternal cells obtained during fetal specimen collection), or failure to remove exogenous substances (e.g., anticoagulants used during blood collection, residual reagents used during sample processing).

**Intermediate outcomes:** are pathologic and physiologic measures that may precede or lead to health outcomes. For example, elevated blood cholesterol level is an intermediate outcome for coronary artery disease.

**Precision:** is defined as the closeness of agreement between independent results of measurements obtained under stipulated conditions. Precision is commonly determined by assessing repeatability (also defined in this Appendix) and reproducibility (also defined in this Appendix).

**Recovery:** as a term in the area of analytic validity, refers to the measurable increase in analyte concentration or activity in a sample after adding a known amount of that analyte to the sample.

**Reportable range of test results:** is defined as the span of test result values over which the laboratory can establish or verify the accuracy of the instrument or test system measurement response.

**Reference range:** also known as reference interval or normal values, is the range of test values expected for a designated population of persons (e.g., 95% of persons that are presumed to be healthy [or normal]).

**Repeatability:** replication of results when the assay is performed multiple times on a single specimen. Repeatability is also referred to as precision (in the term's narrow sense) when the test result is expressed quantitatively.

**Reproducibility:** refers to the closeness of agreement between independent results of measurements obtained with the same assay method when as many known variables as possible (e.g., operators, instruments, reagent lots, day of the week, sites/laboratories) are tested for their effect on the assay result.



**Robustness:** refers to the ability of a method to remain unaffected by small fluctuations in assay parameters; it is often assessed through inter-laboratory comparison studies or by varying parameters such as temperature and relative humidity to determine the operating range of the method.

**Traceability:** refers to a property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons, all having stated uncertainties.

**Uncertainty:** refers to a parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand; it is a formal quantitative statement of the confidence in the result of an assay.

## **VI. Summary of Protocol Amendments**

In the event of protocol amendments, the date of each amendment will be accompanied by a description of the change and the rationale.

## **VII. Key Informants**

Within the Technical Brief process, Key Informants serve as a resource to offer insight into the clinical context of the technology/intervention, how it works, how it is currently used or might be used, and which features may be important from a patient or policy standpoint. The Key Informants for this Technical Brief include clinical experts, patients, researchers, and payers. Differing viewpoints are expected, and all statements will be crosschecked against available literature and statements from other Key Informants. Information gained from Key Informant interviews is identified as such in the report. Key Informants do not do analysis of any kind nor contribute to the writing of the report and have not reviewed the report, except as given the opportunity to do so through the public review mechanism.

Key Informants must disclose any financial conflicts of interest greater than \$10,000 and any other relevant business or professional conflicts of interest. Because of their unique clinical or content expertise, individuals are invited to serve as Key Informants and those who present with potential conflicts may be retained. The TOO and the EPC work to balance, manage, or mitigate any potential conflicts of interest identified.

## **VIII. Peer Reviewers**

In a later phase of the project, ECRI Institute-Penn Medicine EPC will submit a list of potential peer reviewers to AHRQ for review and approval.

Peer reviewers are invited to provide written comments on the draft report based on their clinical, content, or methodologic expertise. Peer review comments on the preliminary draft of the report will be considered in preparation of the final draft of the report. Peer reviewers do not participate in writing or editing of the final report or other products. The synthesis of the scientific literature presented in the final report does not necessarily represent the views of individual reviewers. The dispositions of the peer review comments are documented and will be published three months after the publication of the Evidence report.

Potential Reviewers must disclose any financial conflicts of interest greater than \$10,000 and any other relevant business or professional conflicts of interest. Invited Peer Reviewers may not have any financial conflict of interest greater than \$10,000. Peer reviewers who disclose potential business or professional conflicts of interest may submit comments on draft reports through the public comment mechanism.

## IX. Detailed Literature Search Strategy

The following table summarizes the detailed literature search strategy, including the concepts and key terms used for the search. The search is limited to studies on human and published in English.

Set Number	Concept	Search Statement
1	Genetic testing	'Chromosome aberration'/exp or (chromosom* NEAR/2 (duplicat* or deletion or 'copy number' or insertion))
2		'microarray analysis':de or 'nucleic acid analysis'/exp or 'molecular diagnosis':de or 'genetic screening':de or 'genetic procedures'/exp or 'array cgh' or 'aCGH' or 'CMA' or 'comparative genomic hybridization' or 'array genomic hybridization' or microarray or (molecular NEAR/2 diagnos*) or snp or 'single nucleotide polymorphism array' or (genetic NEAR/2 test*)
3		(exome:de OR genome:de) and 'gene sequencing':de
4		('whole exome' or 'whole genome') NEAR/3 sequencing
5		'next generation sequencing' or 'NGS'
6		'gene expression assay'/exp or 'gene chips' or 'cDNA array' or 'cDNA microarray' or 'genome imprinting':de or imprinting
7		Methylation or 'epigenetics':de or epigenetic*
8		#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7
9	Conditions	Development* NEAR/2 (delay* or disabilit*)
10		'mental deficiency'/exp or (mental* NEAR/2 retard*) or (intellect* NEAR/2 (disabilit* or delay*))
11		(Neurocognitive NEAR/2 impair*) or 'cognitive defect':de or 'intellectual impairment':de
12		'Fragile X' or 'fragile-x' or 'mental retardation malformation syndrome'/exp
13		'autism'/exp or autistic* or autism or Asperger*:ti,ab or 'asd':ti,ab or 'rett syndrome' or 'pervasive developmental disorder' or 'PDD'
14	Specific syndromes (original)	'angelman syndrome'/exp OR 'happy puppet' OR 'prader-willi'/exp OR 'rubinstein-taybi'/exp OR 'smith magenis'/exp OR 'velocardiofacial syndrome'/exp OR 'digeorge syndrome'/exp OR 'shprintzen syndrome' OR 'conotruncal anomaly face syndrome' OR 'williams syndrome'/exp OR 'williams-beuren syndrome'/exp
15	Specific syndromes – KI suggested	'kleefstra syndrome' OR 'miller-dieker syndrome' OR 'koolen-de vries syndomre' OR 'wagr syndrome' OR 'langer gideon syndrome' OR 'cri du chat syndrome' OR 'wolf-hirschorn syndrome' OR 'jacobsen syndrome' OR 'alagille syndrome' OR '1p36 deletion syndrome' OR '9q deletion syndrome' OR '17q21.31 deletion syndrome' OR '18p minus syndrome' OR '18q minus syndrome' OR 'sry deletion' OR 'pten deletion' OR 'charcot-marie-tooth syndrome'
16	Specific genes	ube3a OR fmr1 OR mecp2 OR cdk15 OR foxg1 OR crebbp OR ep300
17	Combine sets	#9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16
18	Removing rodents J	#17 NOT (mouse*:ti OR mice:ti OR murine:ti OR rat:ti OR rodent:ti)
19	Diagnosis	'diagnostic test accuracy':de OR 'diagnosis':lnk OR 'receiver operating characteristic':de OR 'roc curve'/exp OR 'roc curve' OR 'sensitivity and specificity':de OR 'sensitivity' OR 'specficity' OR 'accuracy':de OR 'precision'/exp OR precision OR 'prediction and forecasting'/exp OR 'prediction and forecasting' OR 'diagnostic error'/exp OR 'diagnostic error' OR 'maximum likelihood method':de OR 'likelihood' OR 'predictive value'/exp OR 'predictive value' OR ppv OR (false OR true) NEAR/1 (positive OR negative)
20	Combine sets	#18 AND #19
21	Limit by keywords	#18 AND (idiopathic or (clinical NEAR/2 (valid* or util* or relevanc*)))

Set Number	Concept	Search Statement
22	Combine sets	#20 OR #21
23	Limits	#22 NOT (prenatal:ti or maternal:ti)
24	Limit by publication and study type	#23 AND ('clinical article'/de OR 'clinical trial'/de OR 'cohort analysis'/de OR 'comparative study'/de OR 'controlled study'/de OR 'diagnostic test accuracy study'/de OR 'intermethod comparison'/de OR 'major clinical study'/de OR 'medical record review'/de OR 'practice guideline'/de OR 'prospective study'/de OR 'retrospective study'/de OR 'validation study'/de) AND ('Article'/it OR 'Article in Press'/it OR 'Conference Abstract'/it OR 'Conference Paper'/it OR 'Review'/it)