Development and Validation of In Vitro Diagnostic Tests

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Validated Diagnostic Test should:

“Provides test results that identify positive or negative for an analyse or process (e.g. antibody, antigen or induration at skin test site) with a degree of statistical certainty”

“Principles of Validation of Diagnostic Assays for Infectious Diseases”, R.H. Jacobson, New York State College of Veterinary Medicine
Outline:
1) Analytical Validation
2) Clinical Validation
3) Regulatory Perspectives
4) Summary
Analytical Validation
Development Stage:

1. Feasibility Studies
   - determine or identify a method for a particular use
   - determine if the selected reagents and protocol have the capacity to distinguish between a range of antibody concentrations to an infectious agent while providing minimal background activity
   - Give initial estimates of repeatability, and of analytical sensitivity and specificity
Analytical Validation

Development/Validation Stage:

2. Assay Development and Standardization
   - Selection of optimal reagent concentrations
   - Determine the optimal temporal, chemical, and physical variables in the protocol,
     - e.g. incubation temperatures and durations; pH, molarity of diluent, washing, and blocking buffers
   - Repeatability - preliminary estimates (agreement between replicates within and between runs)
   - Determination of analytical sensitivity (the smallest detectable amount of the analyte) and specificity (the degree to which the assay does not cross-react with other analytes)
Validation Stage:

3. Determining Assay Performance Characteristics
   - Determine diagnostic sensitivity and specificity
   - Precision - a measure of dispersion of results for a repeatedly tested samples
   - Repeatability - the amount of agreement between replicates
   - Reproducibility - the amount of agreement between results of samples tested in different laboratories
   - Accuracy - the amount of agreement between a test value and the expected value for an analyte in a standard sample of known activity (clinical samples; compare to cleared or gold standard method)
   - Selection of the cut-off/reporting threshold/LOD for qualitative/LOQ for quantitative
Validation - Other Parameters to Consider:

- Potential Interferences
- Sample preparation / conditions (Clinical samples should be used if possible)
- Performance around the cut-off
- Potential for carryover or cross-hybridization
- Assay Limitations
- Confidence Intervals
- Software
Clinical Validation
Considerations:

- **Purpose** - assess clinical sensitivity, specificity and reproducibility

- **Studies** should be performed in a representative sample of the intended use population

- **Common components** - Precision, Reproducibility, and non-specificity of the investigational assay
Clinical Validation

Clinical Validation Considerations:

- Precision studies - assess the coefficient of variation for the test results for each sample and for various lots tested.

- Operators Proficiency testing - a panel of 10 or more samples including low reactives should be tested by a number of operators on multiple days, at all clinical testing sites.

- Reproducibility and Precision - assess variability intra- and inter-site, intra- and inter-assay and intra- and inter-lot, as well as total variability for both qualitative and quantitative assays. Assay precision may be established by performing multiple tests using multiple operators and multiple kit lots on a panel of specimens. Testing may be performed in-house and at least one clinical site.
Clinical Validation

Clinical Validation Considerations:

- **Instrumentation** - Instrumentation effects on product performance should be evaluated using the sample panel employed for reproducibility testing and a minimum of three different machines.

- **Non-specificity studies**
  - The presence of non-specific reactivity (i.e., response of blank sample) and the impact of potential interfering factors on assay performance should be assessed.
How FDA regulate diagnostic tests?

Regulate by:

• Formal Regulatory Approval, e.g., 510(k), pre-market approval
• Approved for limited use, e.g., Research Use Only, Investigation Use Only
• Certification of Laboratories e.g., Lab Developed Tests (CLIA)
FDA guidelines - FFDCA Part 201(h)

Devices

- The term “device” (...) means an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including any component, part, or accessory, which is—

  1. recognized in the official National Formulary, or the United States Pharmacopeia, or any supplement to them,
  2. intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or
  3. intended to affect the structure or any function of the body of man or other animals, and which does not achieve its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of its primary intended purposes.
FDA guidelines - 21 CFR 809.3(a)

- *In vitro diagnostic products* are those reagents, instruments, and systems intended for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. Such products are intended for use in the collection, preparation, and examination of specimens taken from the human body. These products are devices as defined in section 201(h) of the Federal Food, Drug, and Cosmetic Act (the act), and may also be biological products subject to section 351 of the Public Health Service Act.
Regulation by Risk

- Risk defined by possible harm to patient of unrecognized incorrect result
- Three classes from low to high risk (Class I, II & III) - Regulatory control increases from Class I to Class III
  - Class I - most exempt from Premarket Notification 510(k)
  - Class II - most require Premarket Notification 510(k)
  - Class III - most require Premarket Approval (PMA)
- A 510(k) is a premarketing submission made to FDA to demonstrate that the device to be marketed is as safe and effective, that is, substantially equivalent (SE), to a legally marketed device that is not subject to premarket approval (PMA).

- A PMA is an application submitted to FDA to request approval to market, or continue marketing, a class III medical device. PMA approval is based on scientific evidence providing a reasonable assurance that the device is safe and effective for its intended use or uses. PMA process is more involved and includes the submission of clinical data to support claims made for the device.
Regulation Notes (continue)

- Compliance with general controls
  - Registration/listing, GMPs, premarket review, postmarket controls, etc
- Labeling
  - Instructions for use, etc
Problems commonly seen with In Vitro Diagnostic Tests

- Inappropriate sample size
- Over-fit data
- Bias
- Tests not independently validated
- Lack of control mechanisms
  - Reagents
  - Processes
  - Samples
Lab Developed Tests (CLIA test)

- **Currently Status:**
  - Self-defined by labs
  - No way to know what’s out there; No registry of Lab Developed Tests exists
  - No regulatory definition of Lab Developed Tests
  - More-or-less blank approach by FDA

- **Proposed changes:**
  - Blanket approach no longer appropriate
    - Risk-based approach to regulation
  - Need to see what is being offered - Registry of Lab Develop Tests
  - Need an approach to regulation that serves public health
FDA announces Public Meeting (FDA-2010-N-0274)

- Public meeting on July 19-20, 2010
- Intent to establish risk-based oversight framework for all tests
- General expectations at this time
  - Registration and listing period will be needed
  - Risk-based phase in (highest risk first)
  - Avoid disruption to access
    - Low or no bar for certain tests (rare disease, etc)
FDA announces Public Meeting (FDA-2010-N-0274)

Seeking input on:

- Risk assessment paradigm
- Oversight mechanisms for Lab Develop Tests
  - Evaluation of necessary info to assure safety and effectiveness
  - Encourage innovation
  - Avoid total disruption of marketplace
  - Provide fair field of play
- Timing
CLIA regulates laboratory testing and requires that clinical laboratories obtain a certificate before accepting materials derived from the human body for the purpose of providing information for the diagnosis, prevention, or treatment of any disease or the impairment of, or assessment of the health of human beings. The type of CLIA certificate a laboratory obtains depends upon the complexity of the tests it performs.
Clinical Laboratory Improvement Amendments (CLIA)

- Categorization bases on:
  - Required scientific & technical knowledge
  - Training & experience
  - Stability and reliability of reagents & materials
  - Characteristics of operation steps - automatically or close monitoring needed
  - Calibration, quality control & proficiency testing materials
  - Test system troubleshooting and equipment maintenance
  - Interpretation & judgment
Under CLIA, laboratories performing only waived tests are subject to minimal regulation. Laboratories performing moderate or high complexity tests are subject to specific laboratory standards governing certification, personnel, proficiency testing, patient test management, quality assurance, quality control, and inspections.

http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/IVDRegulatoryAssistance/ucm124208.htm
Clinical Laboratory Improvement Amendments (CLIA)

Waived tests
- simple lab examinations and procedures that are cleared by the FDA for home use
- Methodologies are simple and accurate
- No reasonable risk of harm to the patient if the test is performed incorrectly
- Examples:
  - Urine pregnancy tests - visual color comparison tests
  - Blood glucose by glucose monitoring devices cleared by FDA for home use
RUO and IUO are IVDs in different stages of development.

- FDA considers:
  
  • RUO are in the laboratory research phase of development, that is, either basic research or the initial search for potential clinical utility, and not represented as an effective in vitro diagnostic product. During this phase, the focus of manufacturer-initiated studies is typically to evaluate limited-scale performance and potential clinical or informational usefulness of the test.

  • IUO are in the clinical investigation phase of development. During this phase, the safety and effectiveness of the product are being studied; i.e., the clinical performance characteristics and expected values are being determined in the intended patient population(s).
Thank you

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