Highly Multiplexed Microbiology/MCM Molecular IVDs - Analytical Studies and Clinical Evaluation Design

FDA-INDUSTRY ROUNDTABLE

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Challenges for the Validation of Highly Multiplexed Dx Devices – CDRH Perspective

Clinical Challenges

- Availability of positive specimens
- Availability of sufficient sample volume
  - Determination of clinical truth (specificity)
  - Reference testing
- Appropriate design/selection of targets included in the assay menu
  - Intended Use of device
  - Specimen type
  - Relevance of targets (in context of Intended Use)

Challenges for the Validation of Highly Multiplexed Dx Devices – CDRH Perspective

Analytical Challenges

Variability of current approaches to accurately quantify infectious organisms.

- Extracted nucleic acid vs. culture isolates
- Molecular calibration of extracted stocks

- Cross-reactivity, within device competition, and interference increase with multiplex size

- Make use of the pre-submission process to customize your multiplexed device validation testing

Performance Validation Studies

Clinical Evaluation (multisite)

1. Precision/Reproducibility
2. Clinical Performance
   - Archived, retrospective samples to establish sensitivity (PPA)
   - Prospective evaluation of clinical specificity (NPA)

Analytical Studies (in-house)

1. Limit of Detection (LoD)
2. Cross-reactivity
3. Analytical Reactivity
   - Inclusivity
   - Exclusivity
4. Interference
   - Matrix evaluation
   - Interfering substances
5. Competition
   - Primer, probe, amplicon
   - Simultaneous target amplification

Multiplex Evaluation

Historical Evaluation (per analyte)

- Analytical evaluation establishes performance parameters and detection capability
- The magnitude of the clinical validation is driven by the prevalence of each analyte in a prospective “all-comers” study based on the intended use of the multiplex device menu
- Emphasis on clinical validation (comparative analysis) study to establish device performance in the end-user environment

Current Evaluation (multiplex)

- Emphasize analytical validation using alternative approaches to reduce testing load – panel approach, some *in silico* testing
- Validation through a modified clinical study (prospective, archived, retrospective, mock specimens)
- Use of multiplexed assay panels relevant to the proposed intended use (syndrome-based panels)
- Seek input from SME to structure panels by specimen type and relevance

Individual Validation vs. Multiplex Validation

	Multisite Validation Studies		Combined Analytical Studies	
	Single Analyte	20-plex	Single Analyte	20-plex
Current Validation (per analyte basis)	~700	>15,000	~300	~6,000
Proposed Validation Concept (20-plex)	**X**	**~3,000**	**X**	**~1,000**
Reduction **(%)**	X	~12,000 **(80% reduction)**	X	~5,000 **(84% reduction)**

The agreed upon CDRH concept for validation of highly multiplexed devices provides significant reduction in the development/validation burden for assay developers while providing the essential scientific elements to demonstrate device safety and performance.

Multiplex Clinical Evaluation

Device evaluation in the intended use environment and patient population

- **Sensitivity and specificity**
  - Prospective study with
  - Predetermined number of positive and negative samples
  - Format does not work for low prevalence or MCM targets
  - Not feasible for highly multiplexed devices
    - Sample volume
    - Comparator tests

- **Clinical truth is determined through the use of comparator tests**
  - Increase in targets = increase in sample volume required
  - Sample volume limits total number of comparator tests

Sensitivity

(Positive Percent Agreement - PPA)

Alternative Positive Specimens

- Prospective/Archived/Retrospective specimens reflecting intended patient population and clinically relevant range
  - Confirmation of archived/retrospective positive specimens
    - » Sample mix-up, degradation, etc.
  - Agreed upon prior to undertaking study
  - Low prevalence targets
    - » Biothreat agents
    - » Emerging pathogens
  - Minimum of 50 positive samples for each analyte
    - » Performance determined by pre-submission discussions
    - » Decrease in device performance triggers increase in required number of positive samples
  - Mock clinical specimens (select cases)
  - Use of processed nucleic acid remnants (modifying cleared device)
    - » Identical extraction methods, patient population, stability

Specificity

(Negative Percent Agreement - NPA)

Prospective Study Size

- Dictated by required comparator method (CM) to establish lower bound of 95% CI
  - Performance determined by pre-submission discussions
  - Decrease in device performance triggers increase in required number of true negative specimens
- Prospectively enrolled with common signs and symptoms of infection (e.g., GI, URI, etc.)

Comparator Methods (CM)

- Randomization of comparator assays established prior to study
- Specimen volume may drive comparator test towards molecular methods
  - Consider use of cleared/approved multiplexed devices
  - Targets without cleared/approved devices, a validated molecular approach
- Follow up all positives by subject device with comparator assay
  - Data should not bias sensitivity determination - included in a separate summary table

Modification of a Cleared Multiplex Device

There must be an effective way to modify an existing multiplexed device – performance will change!

- Validation based on the type of change
- Case-by-case basis – not a one size fits all
- Some existing methods are acceptable to CDRH to modify existing assays
  - Defined for less complex assay formats and modified for multiplexed devices
  - addition of new targets, masking, etc.

Successful Launch CDRH Multiplex Diagnostic Validation Concepts

- 2011 Public Workshop Concepts were implemented for various studies over extended period
- Draft Guidance published 2/13, Comments received
- Promoting Concepts through pre-submission communications – numerous sponsors
- Incoming submissions and those under review have adopted many of the Concepts
- Cleared multiplex assays to date (using Concepts at various levels) include:
  - Blood culture identification devices
  - Upper respiratory infection diagnostic devices
  - Gastroenteritis diagnostic devices

In Summary

- Multiplex Diagnostic Concept
  - Reduced the burden and sponsors came in
  - Continued outreach at early stages of development
  - Interdisciplinary approach to review - team effort
- Several already cleared for market – MANY on the horizon, with increasing complexity and detection capabilities
- Reporting multiplexed device results – not so simple
  - Information overload
  - Does highlight potential co-infections, secondary bact. infection
  - Colonization vs. infection
- Reimbursement of multiplex Dx
  - CMS/end users?