Validation of Multiplex Protein Methods

AEIC Protein Working Group

Fall meeting 2022

Team Members

- Co-chairs:
  - Simone Cummings - Syngenta
  - Kristen Kouba – Corteva
- Matt Cheever - BASF
- Jeff Shippar – Eurofins
- Yongcheng Wang – Bayer
- Yanfei Wang – Bayer
- Chris Ament - Eurofins

Goals

- Provide method validation guidance for protein-based technologies in Ag. Biotech
- Collaborate on validation parameters and gain agreement between workstream participants
- Outline standardized validation parameters for single-plex and multiplexed methods
- Describe the addition of a new trait to a validated multiplexed method
- Produce scientific literature that has maximum industry visibility

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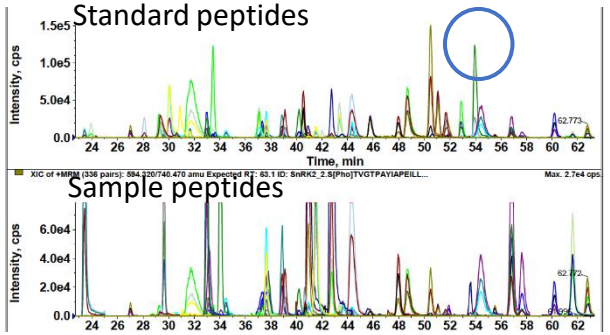
Technologies

- Multiplexed
  - Mass Spec
  - Luminex
  - MSD

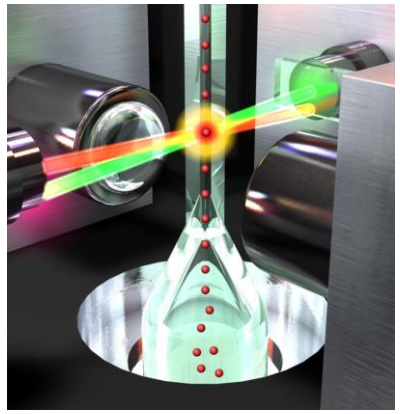
- Single-plexed
  - ELISA

Mass Spec

MRM Multiplexing Results



Luminex



MSD

Validation discussion areas

- Paper will briefly address:
  - method development experiments
  - Important considerations such as extract stability, tissue stability, assay re-validation
- Paper will detail validation parameters:
  - Specificity/selectivity
  - Standard curve, LOD, LLOQ, ULOQ
  - Accuracy
  - Precision
  - Dilutional linearity, parallelism, minimum required dilution
  - Extraction efficiency
  - Ruggedness

Progress

- Continue to review current literature landscape on analytical method validations
- We have monthly discussions about acceptance criteria/validation guidance and paper review
- First Draft in progress
  - Volunteers working all sections of the paper

Timelines

Validation parameter discussions on-going

Each team member working on sections of paper and reviewing each other's sections

Goal - AEIC and company draft reviews - Dec. 2022

This will move to first half of 2023

Appendix

Discussion

Dilutional Parallelism:
The purpose is to demonstrate that the sample dilution response curve is parallel to the standard calibrator response curve.

Parallelism - evaluated by serial dilution of a positive sample to allow measurement that span the range of the standard curve. The back calculated concentration of all dilutions is compared to the concentration of the undiluted or minimally diluted sample. A deviation of greater than 20% is considered a loss of parallelism. If there is no evidence of parallelism, then your minimum required dilution (MRD) needs to be reassessed.

- Can this approach be used as an option to determine/confirm MRD?
- Is this an efficient way to determine/confirm MRD?

Dilutional Linearity:

- Control spiked samples with a known concentration of analyte is used to demonstrate when an analyte is present in concentrations above the range of the assay, it can be diluted to bring the concentration into the validated range for analysis by the method
- Used to evaluate the hook effect or signal suppression caused by high concentration of analyte

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Literature

Alarcon et al	Journal of Agriculture and Food Chemistry	Application of DNA and Protein Based Detection Methods in Agricultural Biotechnology
Boja et al	Clinical Chemistry	The Journey to Regulation of Protein-Based Multiplex Quantitative Assays
CAC/GL 74-2010	Codex Alimentarius Commission	Guidelines on Performance Criteria and Validation Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods
DeSilva	Pharmaceutical Research	Recommendations for the Bioanalytical Method Validation of Ligand-binding Assays to Support Pharmokinetic Assessments of Macromolecules
EFSA (Paraskevopoulos Et al)	EFSA Technical Note	Explanatory note on the determination of newly expressed protein levels in the context of genetically modified plant applications for EU market authorisation
EMA	European Medicines Agency	Guideline on bioanalytical method validation
ENGL	Guidance document from the European Network of GMO laboratories	Verification of analytical methods for GMO testing when implementing interlaboratory validated methods
FDA	report	Bioanalytical Method Validation - Guidance for Industry
Grothaus et al	Journal AOAC International	Immunoassay as an analytical tool in agriculture biotechnology
Jani et al	AAPS Journal	Recommendations for Use and Fit-for-Purpose Validation fo Biomarker Multiplex Ligand Binding Assays in Drug Development
ICH	International Conference on Harmonisation - Harmonised Tripartite Guideline	Validation of Analytical Procedures: Text and Methodology Q2(R1)
ISO	International standard	ISO 21572 - Food stuffs - molecular bio-marker analysis - protein based methods
Kapoor	www.cambridgebiomedical.com	Challenges of Multiplex Assay Validation
Leligdowicz et al	PLOS one	Validation of two multiplex platforms to quantify circulating markers of inflammation and endothelial injury in sever infection
Lipp, Anklam	Journal AOAC International	Validation of an Immunoassay for Detection and Quantitation of a Genetically Modified Soybean in Food and Food Fractions Using Reference Materials: Interlaboratory Study
Lipton et al	Food and Agricultural Immunology	Guidelines for the Validation and Use of Immunoassays for Determinination of Introduced Proetins in Biotechnology Enhanced Crops and Derived Foods
Meso Scale Discovery	MSD Validation Report	The Proinflammatory Panel 1 (mouse) V-PLEX™ Kit: A summary Report of Development and Validation Studies Demonstrating High Performance and Lot-to-Lot Reproducibility
Rodriguez et al	Clinical Chemistry	Analytical Validation of Protein-Based Multiplex Assays: a workshop report by the NCI-FDA interagency oncology task force on molecular diagnostics
Settlage et al	Food Analytical Methods	Validation Parameterss for Quantitating Specific Proteins Using ELISA or LC-MS/MS:Survey results
Schmidt, Alarcon	Immunoassays in Agriculture Biotechnology book	Immunoassay Method Validation, Chapter 6
Shillito, Currier	Immunoassays in Agriculture Biotechnology book	Immunoassays as a GM Dection Method in International Trade, Chapter 15
Tighe et al	Proteomics	ELISA in the multiplex era: Potentials and pitfalls
Anneli et al	Analytica Chimica Acta	Tutorial review on validation of LCMS
Gupta	www.cambridgebiomedical.com	development, optimization and validation of Luminex based cytokine assays

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Relevant parties

Company	Participant
Romer Labs	Christy Swoboda
Knoell (Critical Path)	Pat DeLisio
Charles River	Sheri Lordi
Simplot	Ashely Fisher
Luminex	Stephen Angeloni
EMD Millipore	Reeti Makeshwari
MSD	Jill Clampit
Intrexon/Okanagen (Oxitec)	Nathan Rose
Eurofins/Covance	Jeff Shippar
BASF	Matt Chever
Bayer/Monsanto	Andy Deffenbaugh, Yongcheng Wang, Yanfei Wang, Lucy Liu
DowDuPont/Corteva	Norma Houston, Kristen Kouba
Syngenta	Simone Cummings

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Information collection

- Survey relevant parties
  - Simple survey to gauge interest
  - Detailed follow-up survey to gather information
- Literature search
- Guidance documents
  - Example – new EFSA guidance documents

- Group discussion around documents we should reference?

Simple survey results

Respondent	Using Multiplexing?	Which Technologies	Plans for new multiplexing?	New Technologies?	Share validation parameters?	Participate in AEIC workstream?
Eurofins/Covance	Yes	LCMSMS - Surrogate peptide	NA	NA	Yes	Yes
Romer Labs	No	NA	NA	NA	Yes	No
Intrexon/Okanagen (Oxitec)	Yes	Western Blot	Yes	Mass Spec	Yes	No
Charles River	Yes	Luminex and MSD	NA	NA	Yes	Yes
Syngenta	Yes	Mass Spec	NA	NA	Yes	Yes
Corteva	Yes	Mass Spec and MSD	Yes	NA	Yes	Yes
Bayer	Yes	Mass Spec, Luminex, MSD	Yes	Mass Spec	Yes	Yes
BASF	Yes					
MSD	not interested, but did share a validation summary					
Knoell (Critical Path)	no response					
Simplot	no response					
Luminex	no response					
EMD Millipore	no response					

Validation Parameters

- Completed
  - ~~Standard Curve~~
  - ~~Accuracy~~
  - ~~Quantitative range~~
  - ~~Repeatability precision~~
  - ~~Intermediate precision~~
  - ~~Extract stability (long and short term, freeze thaw)~~
  - ~~Appropriate controls for all technological platforms~~
  - ~~Limit of detection~~

- To be discussed
  - ~~Minimum required dilution~~
  - ~~Linearity (dilutional)~~
  - Extraction efficiency
  - Specificity

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