Immuno-detection of NNN Using Monoclonal Antibody (mAb)

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— FAMILY OF COMPANIES —

Background of Development

Business Need:

Proposed rule by FDA on product standards for NNN levels in smokeless tobacco*

Quick, easy, and on-site NNN detection method preferred

Objective:

Develop a mAb-based user-friendly and cost-effective NNN detection method for on-site sample evaluation/ screening with minimal sample prep requirements

e.g.:
- on-farm
- receiving station
- no LC or technical personnel

Small Molecule Detection Using mAb Examples

OPEN ACCESS

sensors

Review

Immunoanalysis Methods for the Detection of Dioxins and Related Chemicals

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METHOD 4025

SCREENING FOR POPYCHLORINATED DIBENZODIOXINS AND POLYCHLORINATED DIBENZOFURANS (PCDD/PCDFs) BY IMMUNOASSAY

Pribolab

Analytical Methods

Development and optimization of a multiplex lateral flow immunoassay for the simultaneous determination of three mycotoxins in corn. rice and peanut
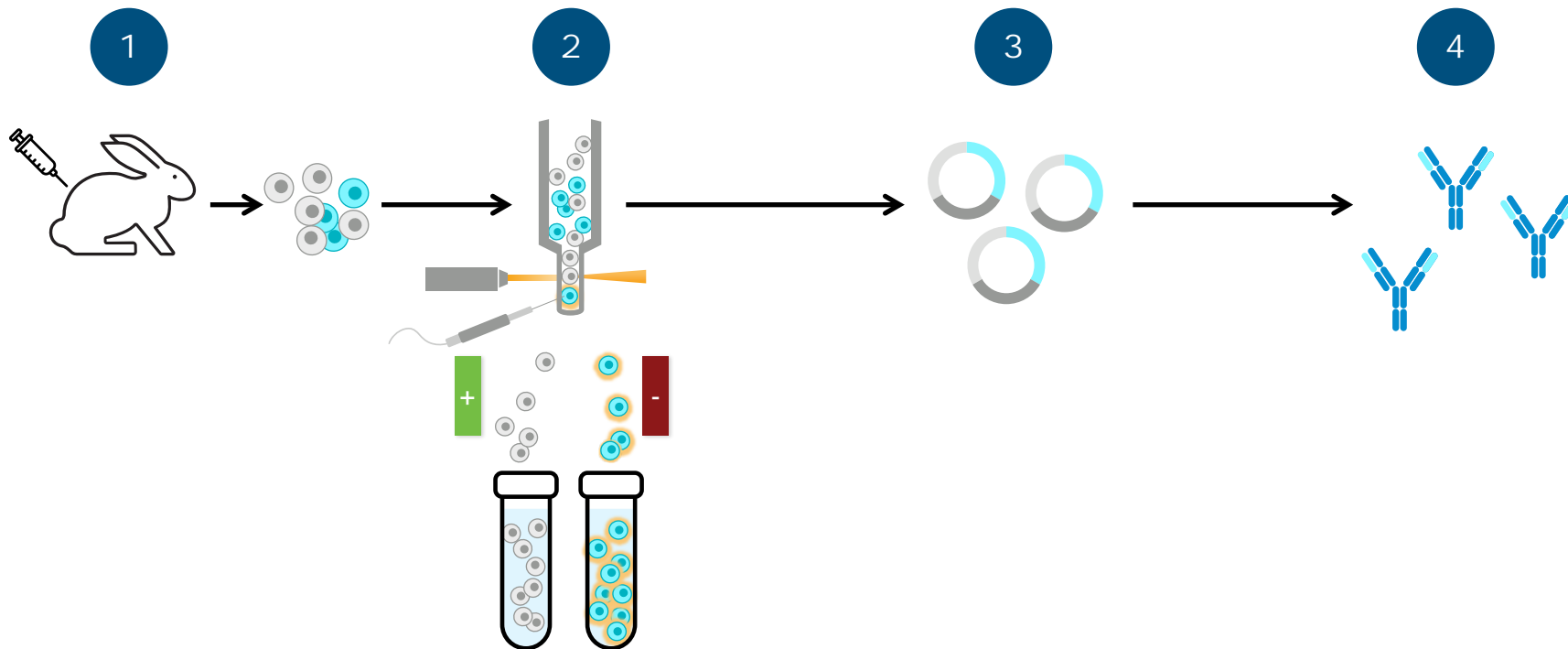
Antibody Clone Development and Selection

1 **2** **3** **4**

*Modified from Introduction to Recombinant Rabbit Monoclonal Antibodies (biossusa.com)

NNN mAb and Assay Development Route

Immunogen and Antigen Preparation

▶

Animal Immunization & Serum Screening

▶

Clone Library Prep and Screen

▶
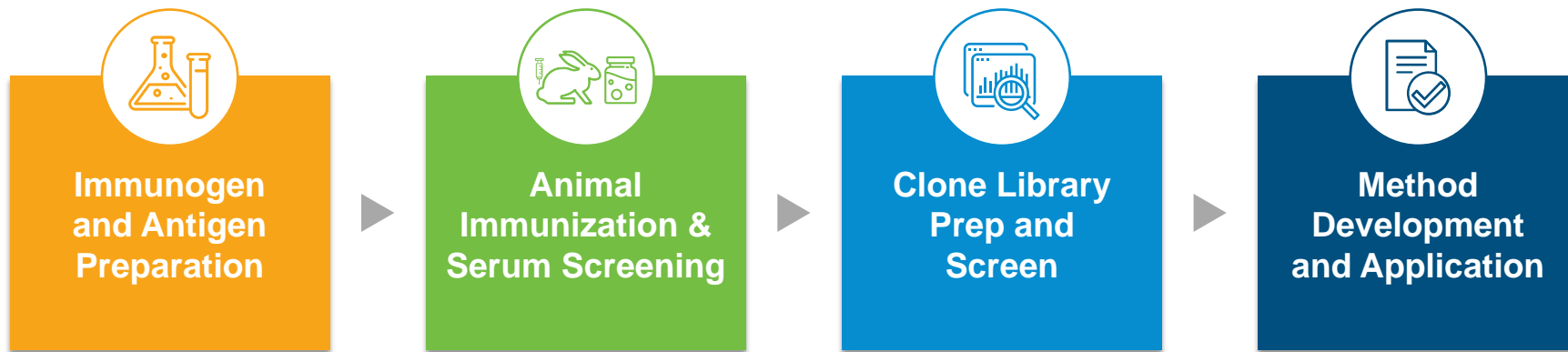
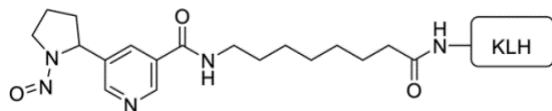**Method Development and Application**

Immunogen and Antigen Preparation

HAPTEN
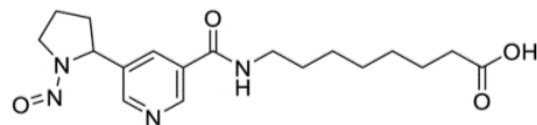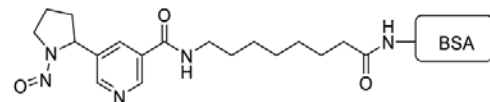

NNN-LINK1 (10 atoms linker)

IMMUNOGEN

NNN-LINK1-KLH

ANTIGEN

NNN-LINK1-BSA-Biotin

NNN Specific Immune Response Detected

● High Concentration Analytes

○ Low Concentration Analytes
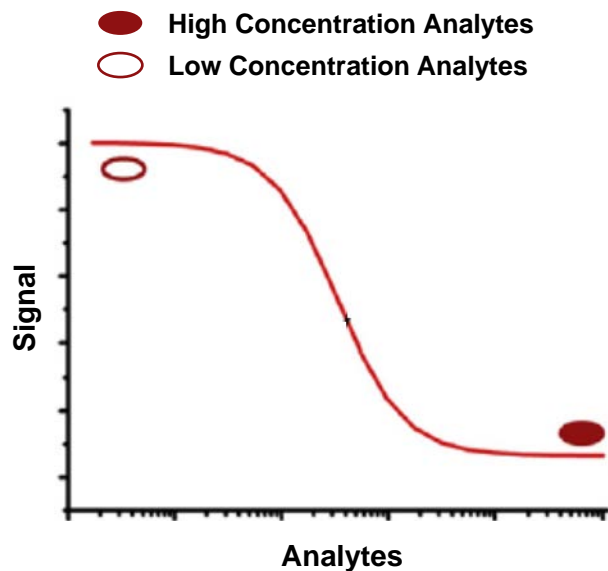
O.D.	Coat S-NNN-LINK1-BSA-Biotin at 1 µg/ml							
NNN (µg/ml)	12	6	3	1.5	0.8	0.4	0.2	0
23877 serum	1.732	1.654	1.657	1.814	1.852	1.863	1.827	1.935
23878 serum	1.826	1.978	1.850	1.997	1.997	1.903	1.971	1.948
23879 serum	2.108	1.997	2.092	2.039	1.971	2.133	2.018	2.147

NNN Specific mAb (D7) Identified

Coated NNN-LINK1-BSA (1ug/ml)

IC50 (1.56 ppm)

Coated NNN-LINK1-BSA (0.1ug/ml)

$y = -11.292x + 2.35$
$R^2 = 0.9632$

D7 Reacted Minimally to Other Alkaloids
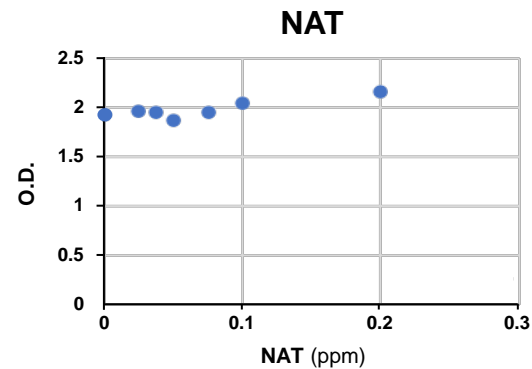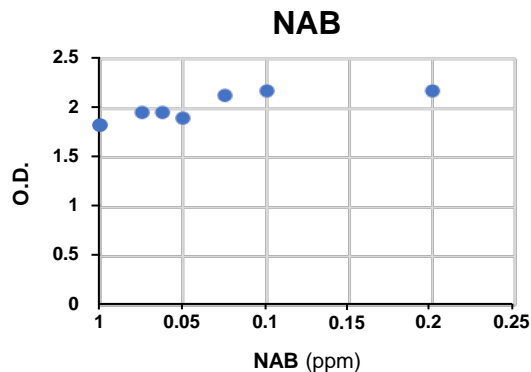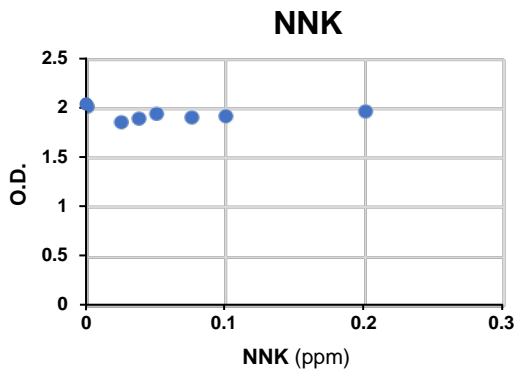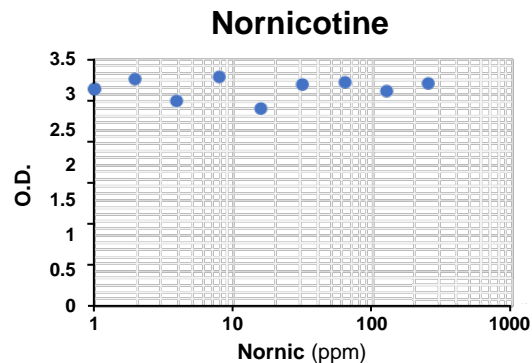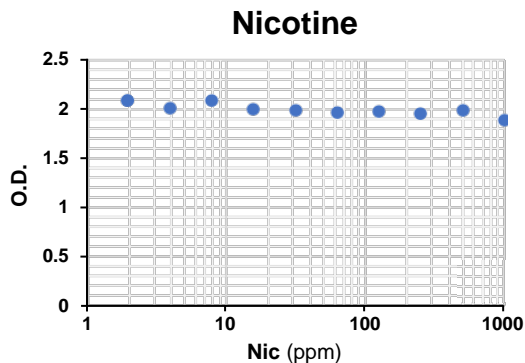
Tobacco Matrix Test Using the D7 Clone

Method Development and Application

Acetonitrile Extraction

$$y = -11.282x + 2.847$$
$$R^2 = 0.9479$$

X-axis: NNN (ppm)
Y-axis: O.D.
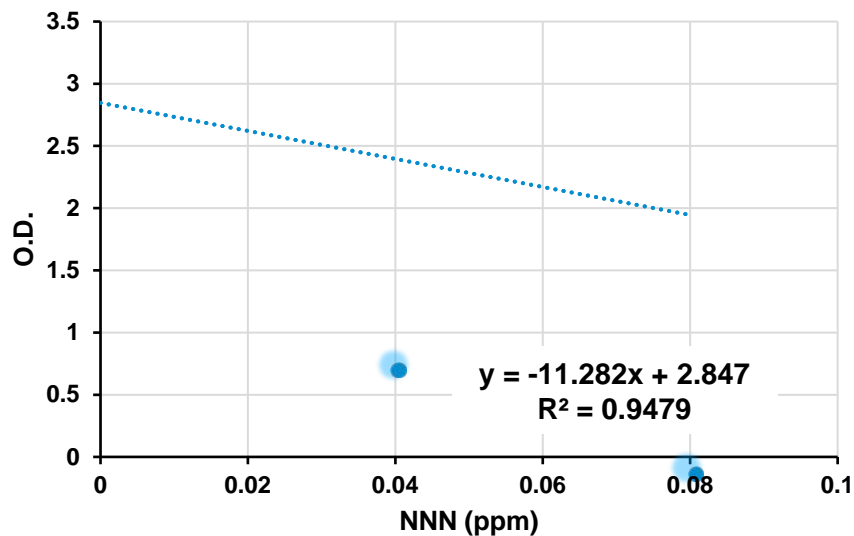
100X dilu.	O.D.	Cal. Valu (ppm)	Adjust
LowNNN1	3.04	2.5	
LowNNN2	2.75	3.6	3.4
LowNNN3	2.7	2.9	
LowNNN4	2.63	4.6	
1ppmSpike	2.26	5.2	1.8
2ppmSpike	2.07	6.9	3.5
3ppmSpike	1.94	8	4.6
4ppmSpike	1.95	8	4.6

Acetonitrile extract contains unknown interferences!

Method Development and Application

Solid Phase Extraction

y = -11.292x + 2.835
R² = 0.9632

(O.D. vs NNN (ppm) scatter plot)
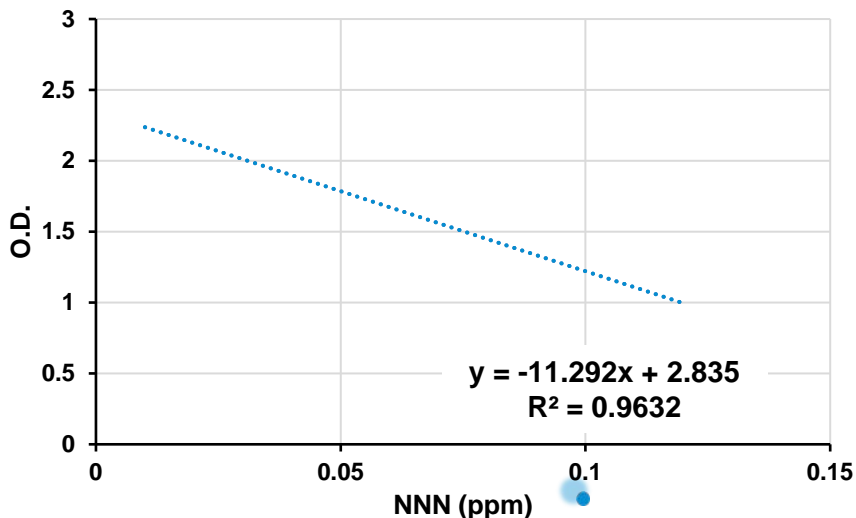
Molecular Imprinted Polymer Resin

100X dilu.	O.D.	Cal. Valu (ppm)	Adjust
LowNNNck	2.15	1.8	0
1ppmSpike	1.95	3.4	1.6
2ppmSpike	1.94	3.6	1.8
3ppmSpike	1.83	4.6	2.8

Polystyrene DVB Resin

100X dilu.	O.D.	Cal. Valu (ppm)	Adjust
LowNNNck	2.11	2.1	0
1ppmSpike	2.05	2.6	0.5
2ppmSpike	1.99	3.2	1.1
3ppmSpike	1.75	5.3	3.1

Conclusions

NNN specific mAb
was successfully generated

Sensitivity ranging from 1.56 ppm to 0.1ppm
can be obtained by adjusting coated antigen concentration

Extraction step was needed
to reduce leaf matrix interference or interference variations

Ongoing Work

✓

Optimizing/Exploring Extraction
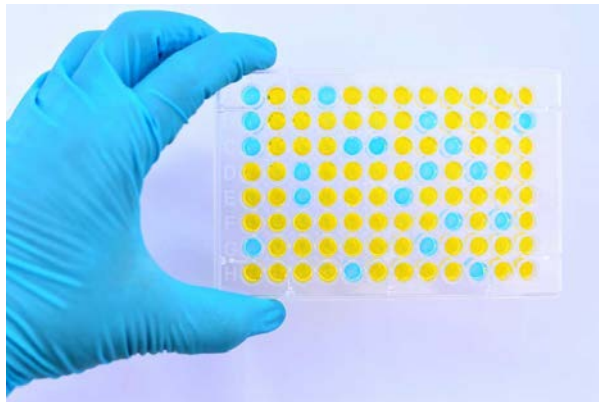
✓

Testing Other Relevant Tobacco Matrix

✓

Developing Alternative Methods

NNN Detection Using Monoclonal Antibody

Possible Formats of Application

ELISA-Based

Strip-Based

Thank you!