The Value of Rapid Methods Innovation for Food Safety and Security
Outline

• Neogen Corporation overview
• Corporate approach: food security and food safety
• Where will we be in 40 years?
• Food security and safety – flip sides of the same coin
• The value of rapid methods to the food producer and manufacturer
• Allergens
• Pathogen detection
• General microbiology
• What’s next
The mission of Neogen is to be the leading company in the development and marketing of solutions for food & animal safety.
A Leader in Food and Animal Safety

Food Safety & Animal Safety

Scale
- >200 million revenues
- >750 employees
- >110 countries

Capabilities
- Farm gate to dinner plate
- Innovative technologies
- Workflow and application expertise

NASDAQ: NEOG
Neogen Corporation Overview

Corporate approach: food security and food safety

Where will we be in 40 years?

Bringing food safety rapid methods to the masses

The value of rapid methods to the food producer and manufacturer

Allergens

General Microbiology

Pathogen detection
The food security continuum

From Inside the Farm Gate Intervention Products

Vet Instruments

Vaccines

Biosecurity & Disinfection

To Food on the Plate Diagnostic Products

Toxins, Allergens & Drug Residues

Bacterial & Sanitation

Dehydrated Culture Media

Genomic Tools
Food security

• **Food security is defined as:** “When all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life.”
  
  – UN Food and Agriculture Organization (FAO) 1996

• One out of every eight people in the world is chronically undernourished
  
  – UN FAO October 2012
“We need to strengthen research for efficiently produced, healthy food, while ensuring the availability of food at affordable prices. This includes improving logistics, infrastructure, and transportation systems to ensure those who need food are supplied with it.”

– Paul Bulcke, CEO of Nestlé

“Today we are seeing best practices in action. We know that, if scaled up with speed, these approaches could increase food production and improve livelihoods without damaging the environment. We need to create conditions for innovation and then invest so that innovation moves from the lab to the farmer’s fields.”

– Rachel Kyte, Vice President of the World Bank
Food security and food safety: Dairy Genomics Example

Select the right animals
Choose the best genes

Optimize inputs

Screen for disease

Manage output traits

Identify spoilage concerns

Product traceability

Detect foodborne pathogens

Brand value added products

The perfect cow
- Few metabolic disorders, maintains body condition
- Shows heat and conceives when bred
- Produces live calf without assistance
- High milk yield, correct composition, inexpensive ration, low maintenance costs
- Walks and stands comfortably, rarely needs trimming
- Resists mastitis, avoids injury

Neogen Corporation
One Sample : Many answers
SNP Chips - Informational Powerhouses
Single Nucleotide Polymorphisms

Fertility markers
Parentage
Milk Quality
Fawn Calf
GGP
The Power of the IGENITY® profile for Angus

1. Dry Matter Intake
2. Birth Weight
3. Mature Height
4. Mature Weight
5. Milk
6. Scrotal Circumference
7. Weaning Weight
8. Yearling Weight
9. Marbling
10. Ribeye Area
11. Fat Thickness
12. Carcass Weight
13. Tenderness
14. Percent Choice (quality grade)
15. Heifer Pregnancy
16. Maternal Calving Ease
17. Direct Calving Ease
18. Docility
19. Average Daily Gain
20. Feed Efficiency
21. Yearling Height

Also providing GE-EPDs for
Limousin
Red Angus
Hereford
Simmental
More pending
Molecular Confirmation with NeoSEEK™

Genomic profile for STEC determination

- DNA profiling:
  - Target genes compared to developed profiles
  - Detect and identify STECs for O26, O45, O103, O111, O121, O145, O157 using over 70 genetic markers. More markers = greater resolution.
  - From any enrichment culture or isolate
  - Next day turn around from receipt
Making the Link

ANSR technology can be utilized for ‘cow-side’ SNP testing
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Food and Animal safety ‘impede’ food security

- Foodborne Illness
- ‘Wastage’
- Regulation
- Lack of resources
- Global population
- Sustainability
Food safety is a growing concern

• “Approximately” one in six North Americans suffer from food poisoning every year, with over 48 million cases reported

• Of these, 128,000 people are hospitalized and 3,000 die
  • U.S. Centers for Disease Control & Prevention, 2011
Enough calories to go around – there are many opportunities to improve.

In the U.S. it is estimated that up to 25 percent of fruits and vegetables are thrown away, at a cost of $35 billion a year.
Regulating North American food Supply

INTERNATIONAL REGISTERED PLANTS

Grower ➔ Manufacturer/Processor ➔ Warehouse ➔ Importer

>250,000 Facilities

Grower ➔ Manufacturer/Processor ➔ Warehouse ➔ Distributor ➔ Retail ➔ Food Service

>2 million farms

>170,000 North American Facilities

>114,000 Facilities

>900,000 Facilities
Resource management

- Dramatic increases in yields during 1970s, 1980s
- Soil now depleted, resulting in leveling off or dropping yields
- 6% of crop land in India now useless
Growing Global Demand

- **Currently** 6.9 billion
- **By 2050** 9.0 billion

30% more people
Emerging middle class seeking higher quality food

Globally livestock production is growing faster than any other sector
Sustainable Agriculture Goals

- Environmental Health
- Economic Profitability
- Social and Economic Equity
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Food security & safety – one mirrors the other

• Less waste & greater process efficiency

• Better access to resources & less variable supply chain

• Often similar technologies

• Consistency throughout process & international standards conformity

• Integration of supply & good manufacturing practices
The Next Green Revolution?

- Biotechnology helps farmers produce higher yields on less land

- Technology allows us to have less impact on soil erosion, biodiversity, wildlife, forests, and grasslands

- Bioinformatics lets us uncover variables that previously were not available

Norman Borlaug Nobel Peace Prize
Continuing pressure on food production

50:100:70 rule*

70% of this will come from innovation

Key Data

In 50 years, the world population will require 100% more food, and

Source: Food Economics and Consumer Choice (Jeff Simmons, 2008)
Dairy example: increased security and safety

- While different in management practices and vertical integration (vs. beef) - it does illustrates how powerful innovation can be to solve food security and safety issues

- Compared to 1944 – to produce 1 gallon of milk it takes
  - 65% less water
  - 90% less land
  - 76% less manure

- In the last 40 years – 22% of the Holstein genome has been altered by human intervention
Dairy example: milk production technological advances

- Dairy herd genetics and management
- Regulatory standards
- Producer controls
- Raw milk quality improvement
- Improved supply chain management
- Transportation controls
- Manufacturing controls
- Increased shelf-life
- ESL – UHT and Microfiltration
- More stringent microbiological specifications
- New requirements for process monitoring
Significant spoilage organisms (and pathogens) are zoonotic pathogens that are essentially part of the gut microbiome food animals.

Coliforms

Pseudomonas
• **Two areas**
  - National Animal ID
  - Linking product backwards in the supply chain

• **Even with massive investment in supply chains - this is a huge challenge for the industry**
  - Human health
  - Business reactivity

• **With enough HD genotyping you could trace raw milk back to an individual cow**
  - HD genotypes for somatic cell / mastitis discovery
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Rapid methods

- A diagnostic analytical method that moves an answer or measurement closer to real time
- Often represents an improvement in sensitivity and specificity
- Validations of methods through Health Canada’s Compendium of microbiological methods, AOAC, AFNOR, etc
- Technology often applied to food after clinical R & D
- Food matrix often has impact on the rapid method: allergen recovery, microbiological inhibition, sanitizer interference
- Ease of use critical for commercial food safety rapid method
Assessing the value of rapid methods

• More and better information
• Usually much faster
• New actions possible that one couldn’t consider before
• Eliminates variables/roadblocks
• Consistency of methodology
• Trace-back to deviation > now we can go much further
Rapid methods for process efficiency

- Faster reaction to upstream deviations
- Often much more sensitive than previously possible
- Faster reaction to process deviations
- Less waste
- Greater diagnostic ability
- Coming bioinformatics wave
- Movement toward integration of systems to handle complicated data
Assessing the value of rapid methods

Actionable data

• Identification of a deviation
• Determination of root cause
• Corrective action
Assessing the value of rapid methods

• Software
  – Big improvements coming to manage complicated problems
  – Beginning to integrate results for
    • Pathogens
    • Indicators
    • Allergens
    • Sanitation
    • Toxins
    • Bioinformatics
    • Date, time, location, lot code, historical information, trace-back information?
    • Automatic deviation detection across specifications, deviation past normal, etc.
Food safety diagnostics  the STEC Problem

• No longer just concerned about O157:H7
  – Big 7 – (STEC E.coli – “the big 6”)
• A single marker won’t work for even one O-group
• Apply ‘genome wide’ tools to pathogen detection
Example: NeoSeek™ Platform Summary

• > 70 independent Sequenom® targets
  – O-groups
    • 26, 45, 103, 111, 121, 145, 157
  – H-types

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<th>True Positive (NeoSEEK vs MLG 5B.01)</th>
<th>True Negative (NeoSEEK vs MLG 5B.01)</th>
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<tr>
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<td>43</td>
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<td>5</td>
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<tr>
<td>Total</td>
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<td>48</td>
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<td>Relative Sensitivity</td>
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<td>Relative Specificity</td>
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<td>100%</td>
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– Epidemiology studies
– Prevalence studies
– Risk assessment

USDA Letter of No Objection
NeoSeek™ Platform Summary

Old Way:
• Mixed microflora, some selective agents
• Immunomagnetic separation
• Differentiate colonies on chromagenic plates
• Picking colonies, etc

New Way:
• Characterize sample directly from enrichment
• Composition of target microflora within sample
• What trait belongs to which microbe
• Discern presence of microbes possessing troublesome gene combinations
• Much more inclusive – pushes back to farm gate
1. Mix sample with lysis buffer. Heat for 10 min at 37°C and 20 minutes at 80°C

2. Add sample to reagents in reader

3. Seal the tubes, start the reader. Results available in 10 minutes

- Same technology can be utilized for ‘cow-side’ SNP testing
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Verification tools for environmental food allergen and general sanitation

BCFPA
November 5th, 2013
The role of ATP monitoring

• Robust hygiene and general sanitation test “Did I clean as well today as yesterday?“
• Acceptable for routine sanitation monitoring
• Not specific or sensitive enough for allergen validation or verification
• ATP varies greatly in foods, and some foods (ie eggs) contain very little ATP
ATP = Adenosine Triphosphate

ATP is the energy source for all living organisms

...a by-product of all living things
The bioluminescent reaction that produces light

The substrate/enzyme complex combines with ATP to produce light.

Luciferin/Luciferase + ATP = Light
What Are Food Allergens?

- Naturally occurring proteins
- Heat and processing resistant
- Resistant to extremes in pH
- Usually major proteins in food
- Foods can have 1 or many allergens
Allergens of Concern

U.S. i.e., Big 8

- Peanuts
- Soy
- Milk
- Eggs
- Fish
- Shellfish
- Tree Nuts
- gluten

Canada

- Peanuts
- Soy
- Milk
- Eggs
- Seafood (fish, crustaceans, shellfish)
- Mustard
- Tree Nuts
- gluten
- Sesame Seeds
- Sulfites
Allergens of Concern

...and any ingredient that contains protein that is derived from The Big 8

- Edible oils (except highly refined oils)
- Hydrolyzed proteins
- Lecithin
- Lactose
- Starch
- Gelatin
- Lysozyme
- Lactoferrin
- Soy sauce
- Worcestershire sauce
- Natural colors
- Natural flavors
- Vinegar
- Enzyme modified egg yolk
- Xanthan gum
Allergen control plan

Main Objectives:
- Prevent cross-contact
- Insure label accuracy
- Insure accurate and adequate documentation
- Identify key team members
- Avoid regulatory issues
Key Elements of an Allergen Control Plan

- Allergen Risk Assessment
- Ingredients/Raw Materials
- Scheduling
- Operations/Processing
- Process Controls
- Maintenance
- Labeling/Packaging
- Sanitation/Change-over Cleaning
- Consumer Complaint Systems
- Training
- Validation
Allergen cleaning validation*

• Purpose: confirm that specifics of cleaning process are effective, sufficient, implemented and will produce the same results...every time
• Where multiple allergens in use, validate most difficult to clean allergen (milk; cooked egg) or allergen in highest concentration.
• Where allergens are in particulate or chunk, sampling plan may have to be more rigorous.
• Current standards require validation on “Big 8” allergens. Make sure referencing most current list
• Finished product testing by itself is not sufficient to validate cleaning due to dilution effect.
• For dry products, “Best Practice” is to conduct 3 product flushes

* Allergen Cleaning Validation, October 21, 2011, v 2.4.
Allergen validation example

1. Identify corrective action plan before any testing is done
2. Run a positive-control sample to ensure test can detect allergen of concern
3. Identify all equipment and surfaces that come in contact with allergen(s)
4. Swab at multiple sites with different swabs; area of coverage does not matter
5. Record whether there is visual allergen present; may allow for support of “Visually Clean” standard
6. Once swabs are negative/BLQ, proceed to first-off/push through product testing; product should be held until negative/BLQ results
The Role of Rapid Test Kits

• Accessible technology
• Become “Standard of Care” in food industry
• A valuable tool, but know what to do with results.
• ELISA tests are required in most auditing programs
• Can help justify the use of a “Visually Clean” standard.
• Can help determine use of precautionary labeling.
Industry Usage of Test Kits

High Frequency
- Manufacturing issue diagnosis
- Sanitation validation

Medium Frequency
- Allergenic ingredient determination
- Consumer complaint sample

Low Frequency
- Routine finished product testing
- Supplier ingredient verification
Limitations of Test Kits
All antibody-based tests

• Hydrolyzed proteins
  – Example: HVP, hydrolyzed egg protein
• Fermentation substrates
  – Examples: xanthan gums, starter cultures, soy sauce
• Processing aids
  – Examples: lecithin, enzymes

Proteins from these products are generally not detectable on the test kits. However, allergenicity can remain.
Know What You’re Detecting

• ELISA tests are designed to detect *whole, in-tact* proteins

• Milk/Dairy: some detect casein, some detect whey, some detect both

• Soy: raw soybeans easy to detect; highly processed soy ingredients much more difficult

• “Gluten Free”: prohibited grains = wheat, rye, barley
Lateral Flow Products – Testing for Allergens used to be Difficult!
Allergen Specific Tests

- Almond
- Casein
- Egg
- Gluten
- Hazelnut
- Peanut
- Crustacea
- Soy
Test Kit Components

Components provided
(from top left to bottom right):
• Extraction buffer sachet
• Sample tube & cap
• Reveal 3-D test device
• swab
Reveal 3-D Procedure

Surface swabs

- Ensure samples are as homogenous as possible
- Avoid cross contamination during sample preparation
- Ensure assay steps are followed as outlined in insert
Result Interpretation

From Left to Right:
Negative; Positive; High Positive

HIGH POSITIVE
No line at O, faint or line absent at T (Overload)

POSITIVE
Any intensity of line at T
- Above detection limit

NEGATIVE
No line at T
- Undetectable
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Pathogen Detection

**Amplified Nucleic Single-Temperature Reaction**

- A system for rapid detection of pathogens using a simple isothermic reaction
- Easy to implement - does not require expensive instrumentation
- Easy to perform with minimal training
- Capable of producing results within a single shift – increases throughput
- Fastest time to results
Pathogen Detection

• **Unique isothermal amplification method**
  – No thermo-cycling
  – Minimal sample prep-less hands on time
  – 10 minute assay time for *Salmonella* and 18 minutes for *Listeria*

• **Selective detection of DNA sequences**
  – Discrimination between target and closely related non-target organisms
  – Reduced need for secondary enrichment media
  – Reduced enrichment times

• **Highly sensitive**
  – Exponential amplification of target sequence
  – Small sample volume
  – Recovery of 1 injured cell per sample
    • $10^4$ cfu/ml post enrichment-*Salmonella*
    • $10^2$ cfu/ml post enrichment-*Listeria*
  – Real time
Pathogen Detection

How does ANSR work?

• Target pathogen nucleic acid is released through the lysis of the enriched sample.

• When the lysed samples is added to the ANSR reagents, a special primer targets specific regions of the pathogen DNA and starts the amplification process. A special molecular beacon is part of the ANSR reagent mixture.

• Millions of copies of the target pathogen DNA are created in minutes.

• Amplified segments of the pathogen DNA attach to special molecular beacons.

• The molecular beacons fluoresce when bound to the pathogen DNA. This is detected by the ANSR reader.
ANSR Protocol

1. Add 50 µL of enriched sample to the cluster tube.

2. Add 450 µL of lysis reagent solution to the sample.

3. Transfer sample tubes to 37°C heat block and incubate for 10 minutes. Then transfer the lysis tubes to the 80°C heat block and incubate for 20 minutes.

4. Transfer 50 µL of the lysed sample to preheated lyophilized reagents (56°C) in the reader. Close reader’s lid and click START in the ANSR software to begin assay.

5. Results will be reported in 10 minutes and displayed as positive, negative or invalid.
Pathogen Detection

Software Screens:
- Results
- Individual Samples

Negative Result

Positive Result
ANSR Norovirus

• Molecular assay for the detection of Norovirus in seafood and produce
• Developed in conjunction with the University of Guelph
  – Dr. Mansel Griffiths
• Development continues
**Linking Screening & Confirmation**

- Screen for STECs using conventional or rapid methods
- Most rapid assays have two parts: 1) stx and eae 2) O-groups.
  - The results are only indicative that the sample possibly contains an STEC but should not be interpreted that it is a pathogenic STEC
  - Also, positive results but PCR screening does not necessarily indicate whether they (stx, eae, and O group) are in the same cell
Linking Screening & Confirmation: NeoSEEK

- **Bacterial strain ID via DNA profiling**
  - Mass-spec based multiplexing
  - Look for the presence/absence pattern of a particular set of target genes compared to developed profiles
  - Detect and identify STECs from O26, O45, O103, O111, O121, O145, O157

- **Send a colony isolate or enrichment aliquot**
  - Results in 24 h

- **USDA Letter of No Objection received on 9/12/**
- **ISO 17025 accredited from A2LA**
How is NeoSEEK being used?

• Importing requirements to the U.S.A.
  – Definitive results, reducing risk of false positives from alternative methods

• Validation of processes
  – Validation that new or current interventions sufficiently reduce non-O157 STECs per a HACCP plan

• Verify positive results using NeoSEEK
  – Prevent making incorrect product dispositions from presumptive or potential positives
Confirmatory Application

• Develop method for identification of colony picks from selective/differential agar media.
• Pursuing AOAC OMA approval and inclusion in BAM and MLG
• AOAC committee meeting in December

Protocol
1. Pick colony from plate using inoculating loop or needle
2. Resuspend in 0.5 mL PBS
3. Run ANSR assay using one-step lysis protocol
Enrichment Free Assays

• Most assays require an enrichment to grow the target to a detectable level
• Some assays, such as Campylobacter testing from rinse samples, don’t require an enrichment
• What if you could test for Listeria right now?
  – Swab an area
  – Add swab to lysis solution and heat
  – Run the assay
  – Results in 18 minutes
## Assay Development Timelines & Approvals

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Microbiology...

...at the Speed of Light

Change in color = microorganism growth
Target Spoilage Organisms

Bacteria:
- Aerobic count
- Coliforms
- E. coli
- Alicyclobacillus
- Staphylococcus
- Lactics
- Thermophiles
- Pseudomonas

Yeast and mold
- Low acid yeast
- High acid yeast
- Osmophilic – 72 hr. negative results
- 48 hr. negative results
**Soleris Sterility Applications**

**Deviation monitoring:**
- Event testing
- In process testing

**Finished product testing:**
- Problems identified quickly
- Reduced inventory management

**Environmental monitoring**

**Real time detections**
- Majority of low acid positives in 4 - 18 hours
- Majority of high acid positives in 12-24 hours

**Products validated:**
- Soups, dairy, soy, nut, muscle milk, infant formula, dietary supplements, juices, teas, syrups, juice concentrates, meal replacements, etc.
Aseptic Sample Handling

**Syringe**
- Easily maintains sterility
- Appropriate in some conditions
- Special cap with septum
- 5ml sample size

**Pipette**
- 1-5 ml
- Laboratory setting

**Maintaining sterility**
- Piercing
- Punching
- Opening
- Swab with alcohol
- Vacutainers

**5ml sample size**
- Increased sensitivity
Deviation monitoring:
• Time to detection
• Microbial growth rates
• Real time results
• Event testing
• Cleaning related spore germination

Aerobic testing (and thermal process control):
• Contamination issues captured
• Supplements thermal control data

Objective Results:
• Easy to interpret growth curves
• Comparative data analysis
• LIMS/SAP/Process Pro compatible
• Confirm directly out of vial
Reporting

Many reporting options

Multiple fields

Networked with remote viewing
  • Live viewing and reporting
### Detailed Reporting

Many reporting formats

---

**Detailed Report**

[Image of detailed report]

---

**Table:**

<table>
<thead>
<tr>
<th>Product</th>
<th>Test</th>
<th>Sample</th>
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**Documentation:**

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<td>Nutraceutical</td>
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**Appendix:**

- Nutraceutical 020-000010 Lactic Red
- Nutraceutical 020-000010 Lactic Red
- Nutraceutical 020-000010 Lactic Red
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What’s next – opportunities for incremental improvement

- More definitive tests of product spoilage
- Better shelf-life prediction through bioinformatics
- Faster pathogen detection
- Simpler, more available methods
- Less cumbersome compliance to standards, like allergen tests
- More integrated software
- Harmonized methods
- Reduction of risk through genomic management
- Push-back to farm
- Food safety and food security will be strongly linked
- Research collaborations
- Technological advances lead to incremental improvements
Thank You!

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