Innovation in Pathogen Detection

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Food Safety | Plant Disease

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ANSR

Amplified Nucleic Single-Temperature Reaction

- A new system for rapid detection of pathogens using a simple isothermic reaction

Simpler, Better, Faster

- Easy to implement - does not require expensive instrumentation
- *ANSR* is exceptionally easy to perform with minimal training
- Capable of producing results within a single shift – increases throughput

ANSR provides the fastest time to results

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Innovative Technology

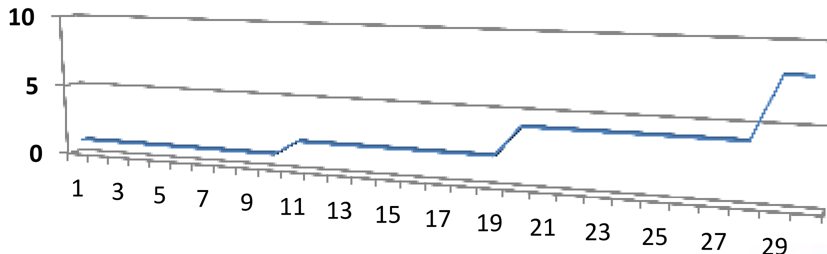
- Unique isothermal amplification method
  - No thermocycling
  - 10 minute assay time
- Selective detection of RNA or DNA sequences
  - Discrimination between target and closely related nontarget organisms
  - Reduced need for selective enrichment media
  - Reduced enrichment times
- Highly sensitive
  - Exponential amplification of target sequence
  - Small sample volume
  - Results in real time
- Technology is applicable to detection of any pathogenic organism
  - Specificity governed solely by differences in nucleic acid sequences

Thermocycling vs Continuous Replication

PCR

- A single or a few copies of a piece of DNA have to be amplified through thermo-cycling to generate thousands to millions of copies of a particular DNA sequence.
- Repeated cycles of denaturation and polymerisation are required.
- Exponential replication occurs once each cycle.

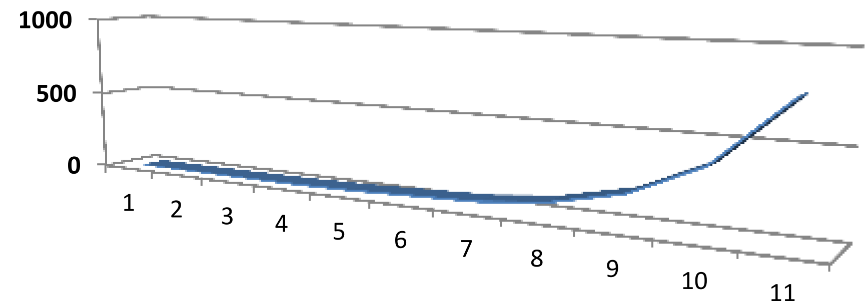
PCR Cycle Replication



Amplified Nucleic Single-Temperature Reaction - ANSR

- Exponential, continuous isothermal chain reaction in which the product of one reaction catalyses further reactions.
- Repeated thermo-cycling not required.

ANSR Continuous Replication



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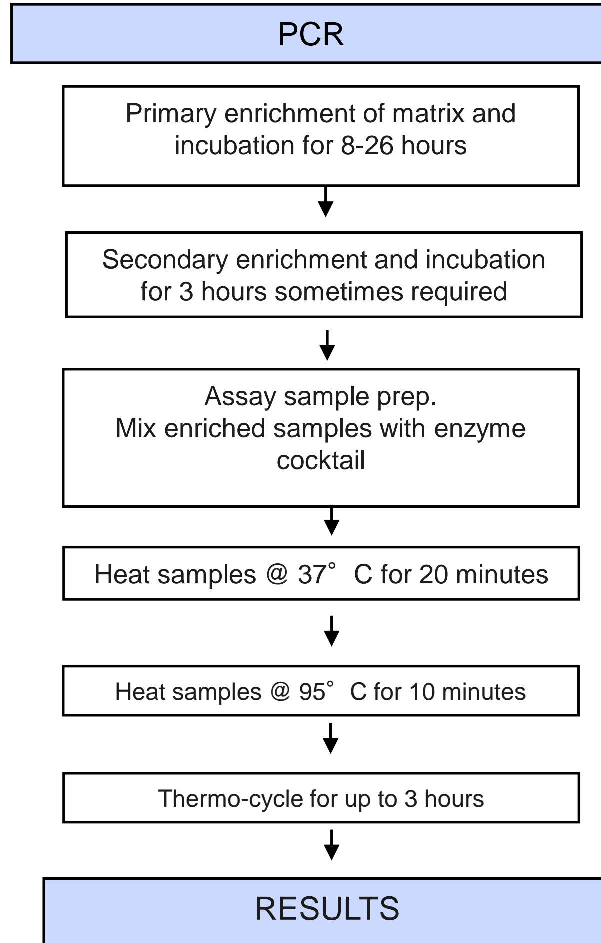
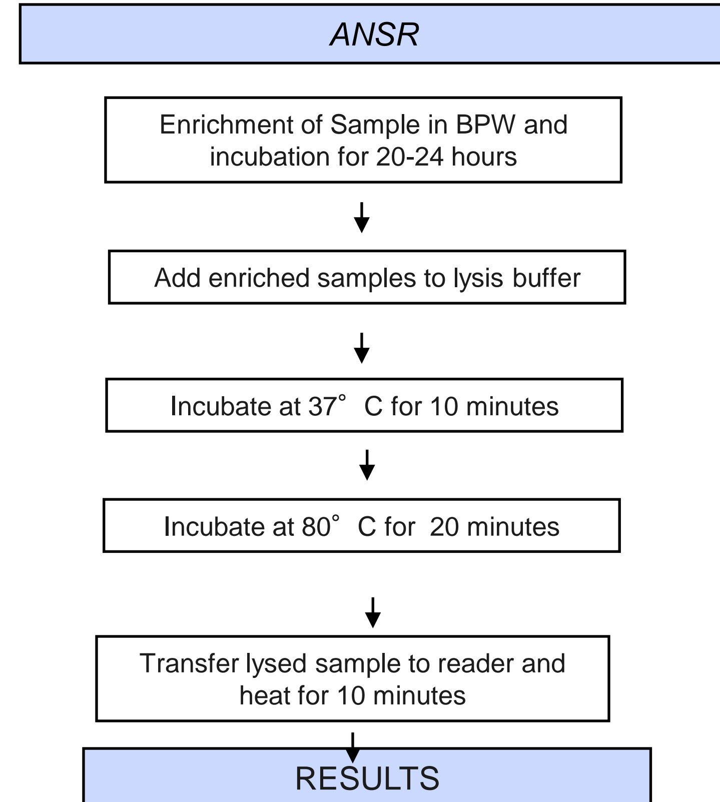
Comparative Workflow





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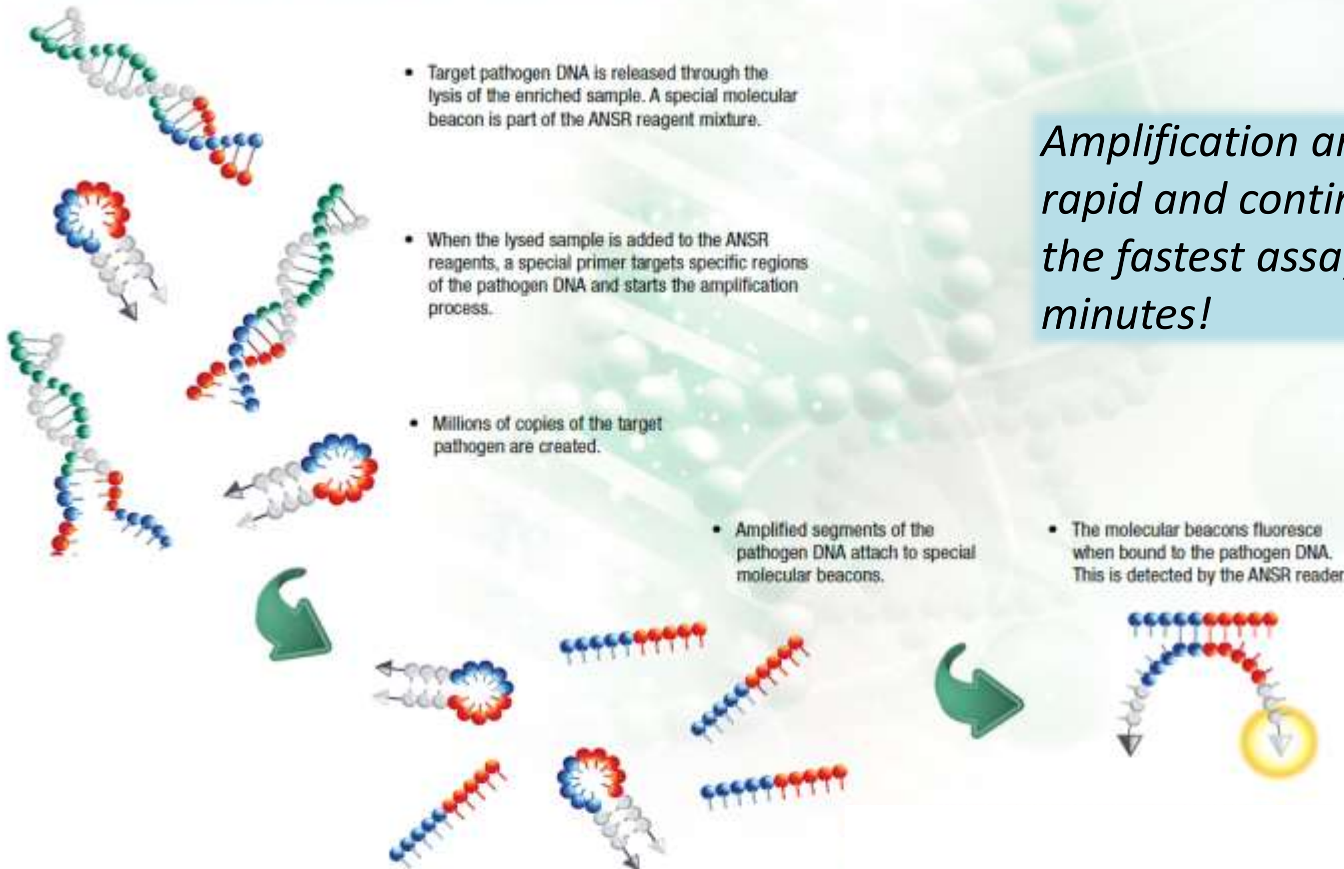
ANSR Chemistry



Amplification and detection are rapid and continuous resulting in the fastest assay time of only 10 minutes!

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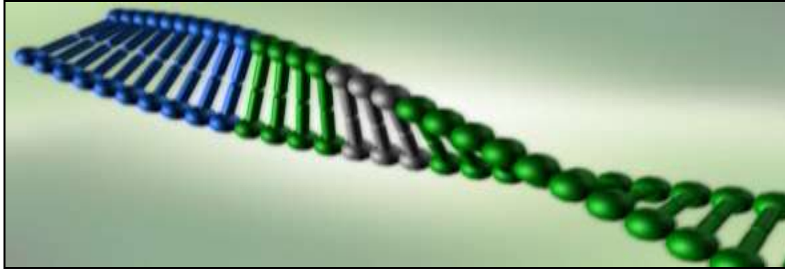
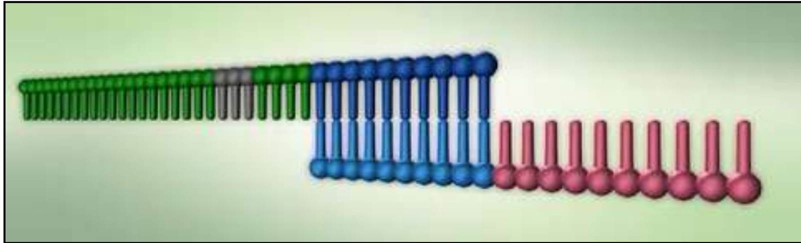
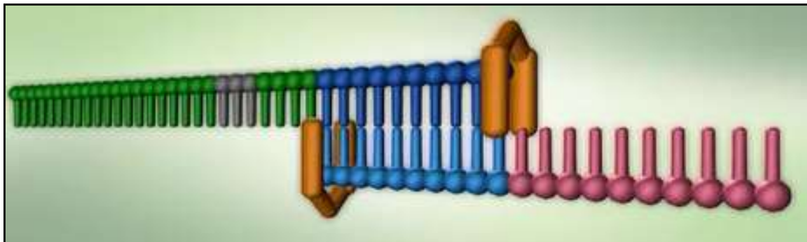
The ANSR Reaction



DNA is released through lysis of the enriched sample



A small primer binds to the complementary section of the pathogen DNA



A DNA polymerase recognizes the overhang as being damaged and extends the nucleotides along the strand creating a new piece of DNA

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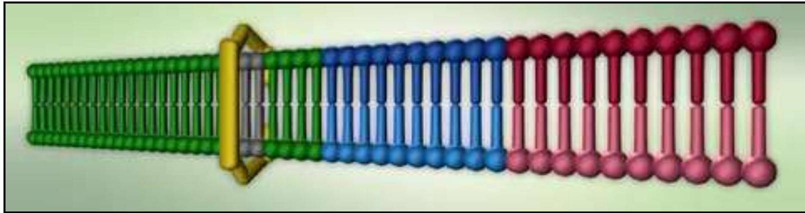
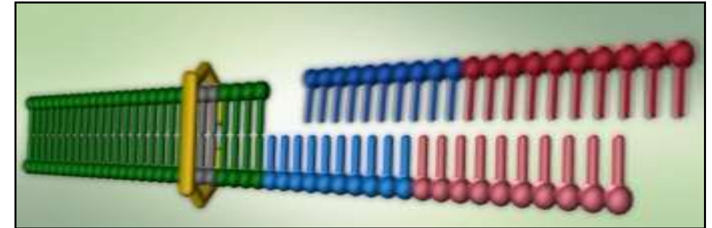
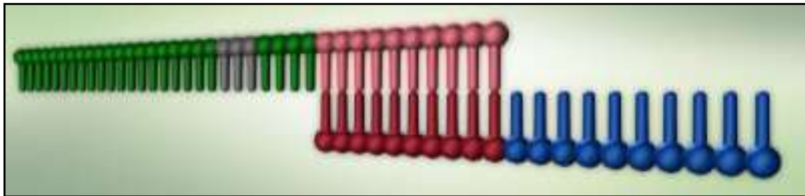
The ANSR Reaction

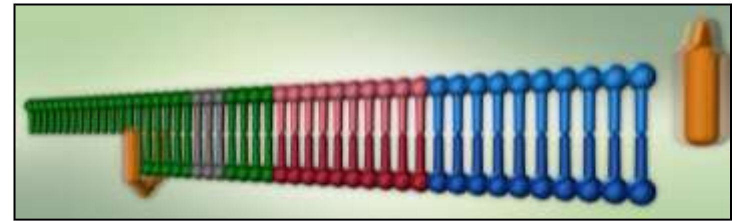


A nicking enzyme “cuts” through one strand of the DNA releasing the fragment



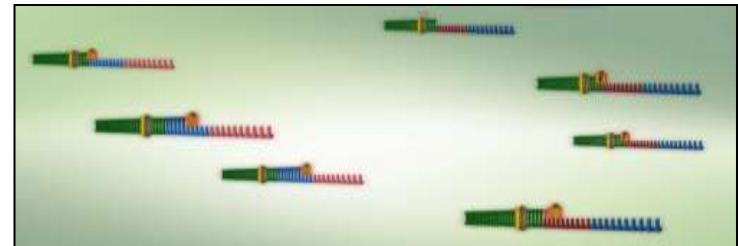


The product from the first reaction binds with a second primer



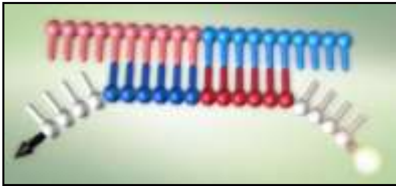
A DNA polymerase again extends the nucleotides creating a new piece of DNA

A nicking enzyme “cuts” through one strand of the DNA releasing the fragment

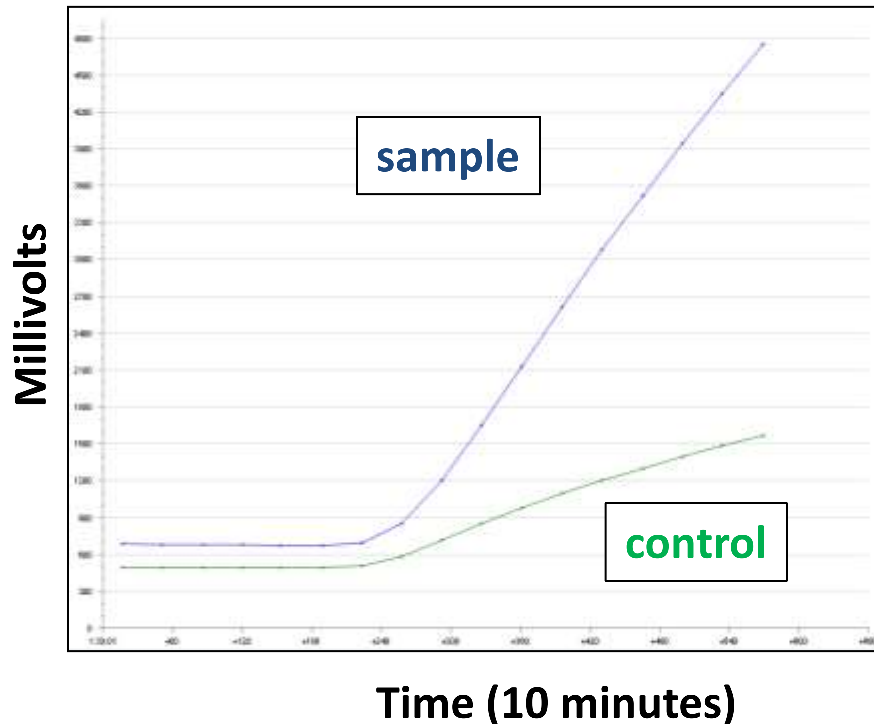


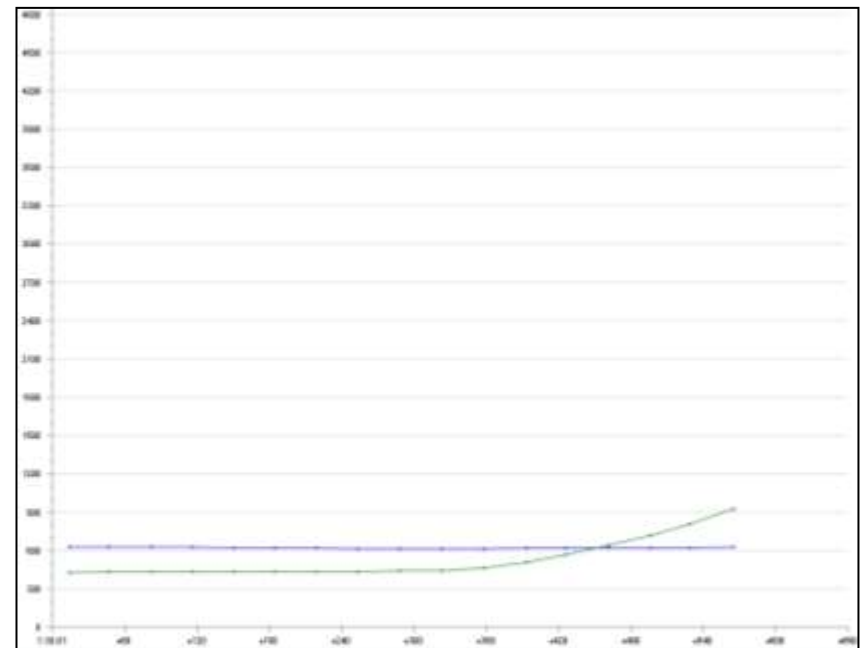
The process proceeds in rapid succession until all reaction components are exhausted

Viewing Results in 10 Minutes



The reader measures the fluorescence generated from the molecular beacon.





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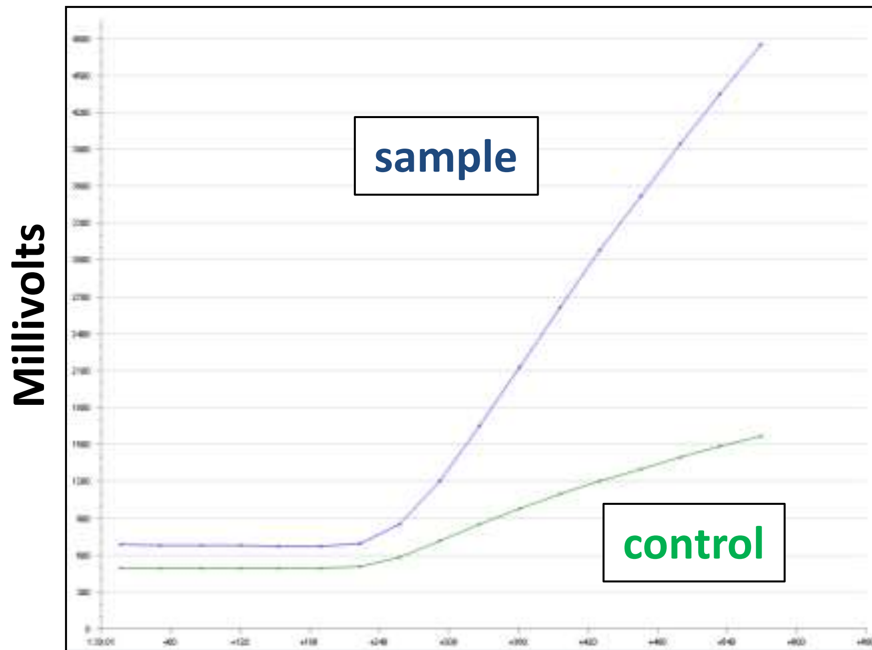
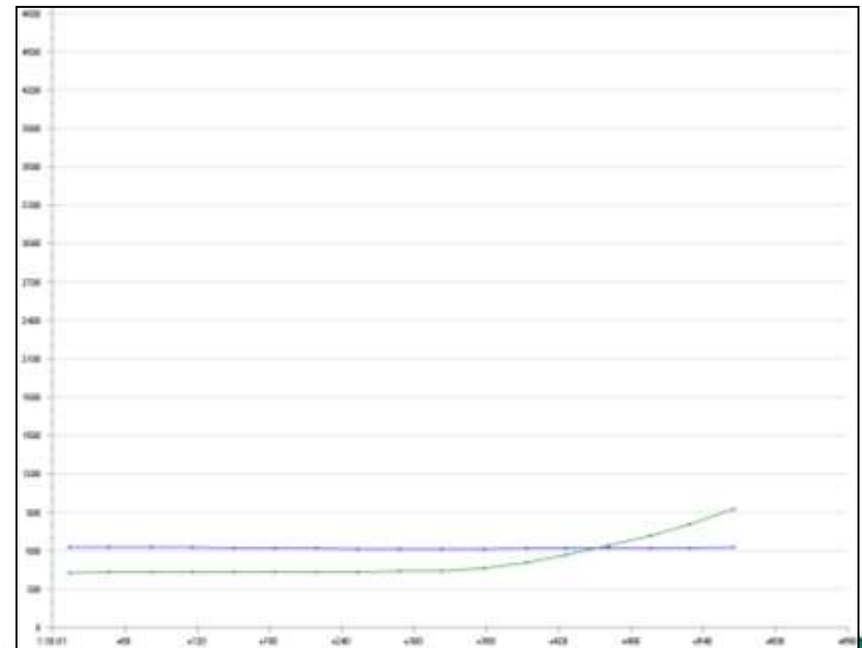
How Does the Control Work?

- There can be binding of the templates to each other (since they are complimentary) creating double-stranded DNA.
- A dye called SYTO 82 inserts itself into the double-stranded DNA giving off a signal at a different wavelength than the beacon
- The signal is often stronger in positive samples because there are more chances for the templates and products to bind to each other

Positive Result



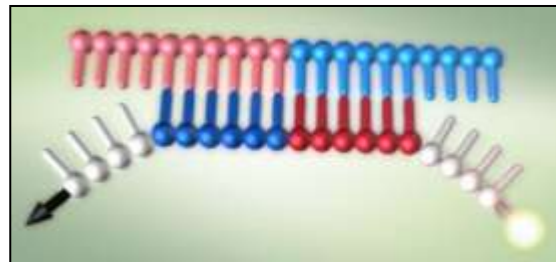
Negative Result



Time (10 minutes)

How is ANSR Different?

- ANSR utilizes an isothermal reaction: the amplification process takes place at a single temperature
  - 56°C
- A single temperature reactions eliminates the cycling used in traditional PCR thus drastically shortening the amplification time.
  - 10 minutes (ANSR) vs. 3.5 hrs (PCR assay)
- Detection is by fluorescence using a molecular beacon



2 step lysis-Dual Selectivity

- 37°C – 10 min
  - Most effective temperature for the enzymes to function.
  - Proteinase K is used for the destruction of proteins and for the release of nucleic acids, since it very effectively inactivates DNases and Rnases
  - Lysozyme and mutanolysin break down cell wall protein

- 80°C – 20 min
  - Lysis of the sample
  - Deactivation of the enzymes

ANSR Salmonella

- Target gene common to all species and serovars of *Salmonella*
- No known cross-reactivity with other organisms
- Simplified, single-step enrichment protocols
- Reduced time-to-result
  - Raw meats 20-24 hours in supplemented BPW
  - Processed foods and environmental samples 20-24 hours in BPW
- Applicable to food & environmental sample matrices

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ANSR *Salmonella* Enrichment Example Protocol

- Weigh 25g sample in a Stomacher-type bag.
- Add 225 mls BPW to the bag. Homogenize (Stomacher, etc.) the sample as appropriate for the sample type.
- Incubate the sample at 37 °C (+/-1) for 20-24 hours.
- Test with ANSR

ANSR Salmonella Lysis Protocol

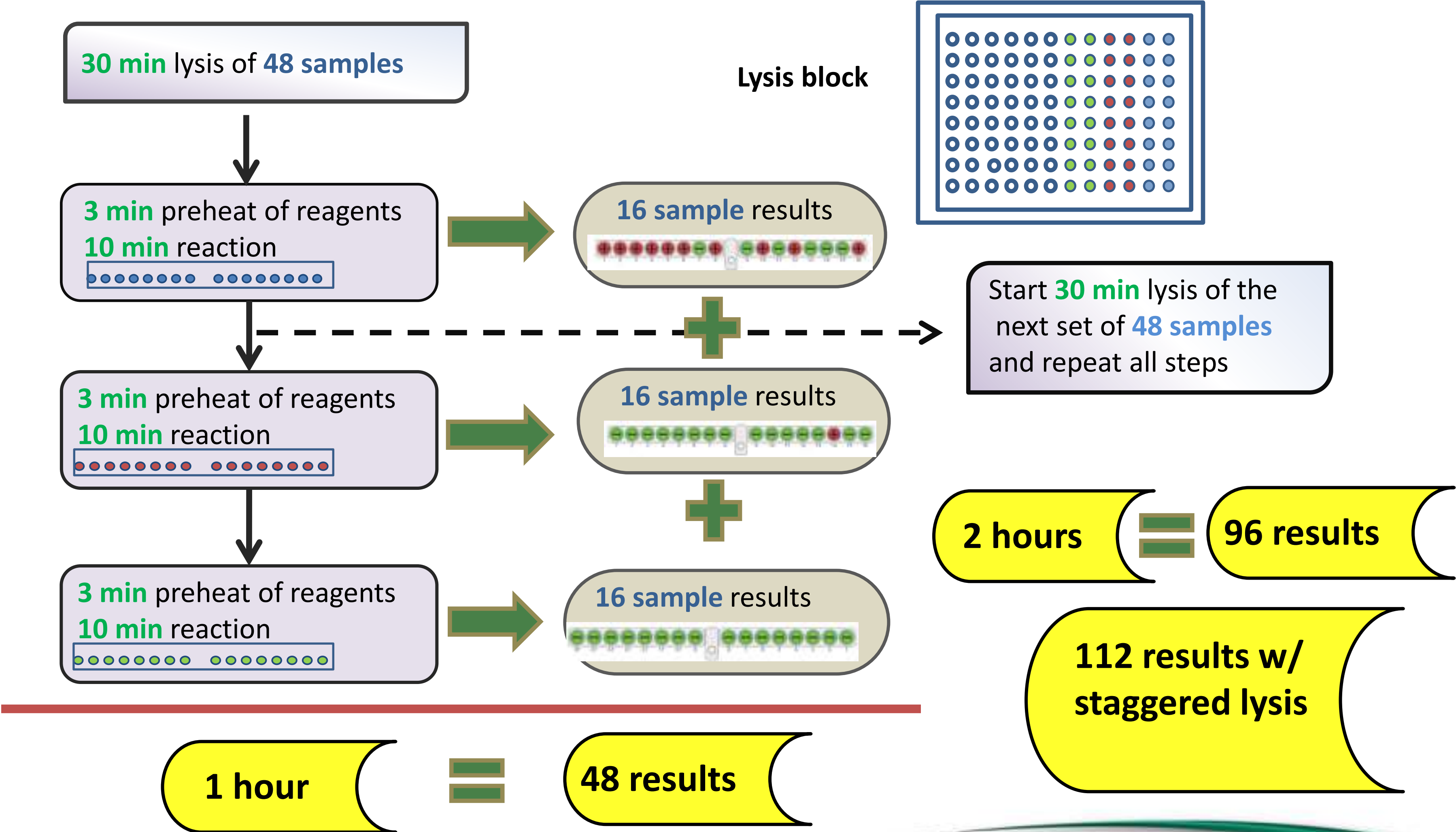
- Mix enriched sample with lysis buffer heat to 37°C heat block for 10 minutes

- Transfer cluster tubes to 80°C for 20 minutes

- Mix lysed samples with reagents in the reader.

- Seal the tubes, start the reader. Results available in <u>10 minutes.</u>

Throughput With One 16-well Incubator / Reader



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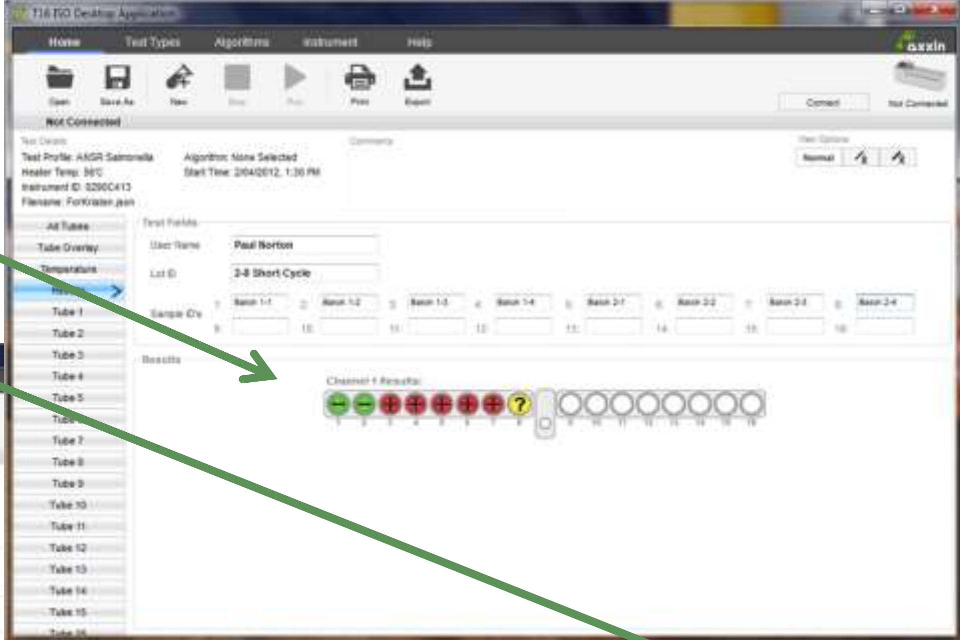
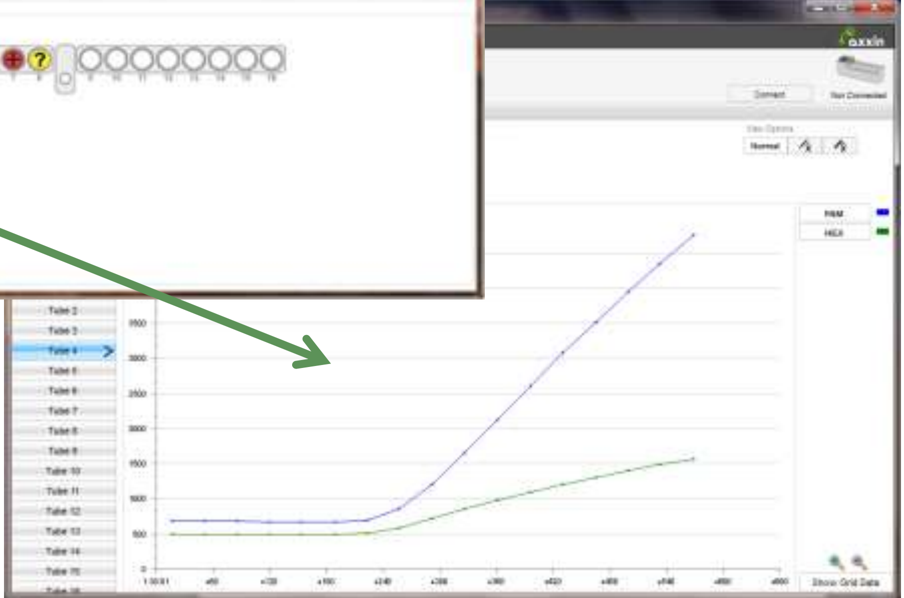
No Unambiguous Results – Simple and Clear

Software Screens:
Results

Individual Samples



Negative Result



Positive Result

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ANSR Reader

- Modes of operation
  - Stand alone
  - With PC
- Scalability
  - Small footprint and quick time assay
- Capacity
  - 16 samples per reader
- Maximum throughput
  - 48 samples / hour single reader
  - 112 samples / 2 hours with staggered lysis.

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Validated Matrices

MEAT & POULTRY
- Chicken carcass rinse
- Raw ground turkey
- Raw ground beef
- Hot dogs
- Chorizo
- Dried pasteurized egg

DAIRY
- Ice cream
- Non-fat dry milk
- Raw milk
- Grade A pasteurized milk
- Butter
- Mozzarella cheese
- Shredded chedder cheese

NUTS
- Almonds
- Pistachios
- Cashew cluster
- Raw & roasted peanuts

CONFECTIONARY PRODUCTS
- Cocoa powder
- Peanut butter
- Dark chocolate
- Milk chocolate

PRODUCE
- Whole cantaloupe
- Sliced cantaloupe
- Sprout rinse
- Raw spinach
- Orange juice
- Apple juice
- Dried fruit
- Baby spinach
- Romaine lettuce
- Fresh mango

SEAFOOD
- Raw shrimp
- Breaded fish sticks

SPICES & CONDIMENTS
- Tomato bouillon
- Parsley flakes
- Black pepper
- Ground red pepper
- Cracked coriander
- Ground paprika
- Cracked cumin seed
- Cracked white pepper
- Cracked green pepper
- Whole savory
- Whole basil
- Whole cilantro

PET FOODS
- Dog biscuits
- Jerky style dog treats
- Lamb meal
- Wet dog food
- Dry pet food, 375g

GRAINS
- Oat cereal
- Soy flour
- Wheat flour
- Rice

OTHER PROCESSED FOODS
- Potato flakes
- Cookie dough
- Corn kernels
- Hummus
- Infant formula

NUTRITIONAL PRODUCTS
- Nutritional shakes

MISC
- Probiotic product
- Yeast
- Chicken fat
- Palatant flavoring
- Poultry feed
- Yeast fermentation product

BOTANICALS
- Olive oil
- St. John's Wort
- Oil garlic bulk extract
- Rehmannia raw herb
- Maca, gelatinized
- Rhodioloa root
- Ginger extract

ANSR Assay Development

Assay	Status
***Salmonella* spp.** **AOAC and Afnor Validation**	Available • Environmentals • Raw meats, seafood, poultry, chicken carcass rinse • Produce, vegetables and seafood • Processed foods including deli meats, dairy, eggs, nuts, flour, spices, chocolate, dry pet food
***Listeria* spp.** **AOAC Validation**	Available
Listeria Monocytogenes	In Development
Non-O157 STECs (CDC top 7) **Campylobacter**	In Development In Development

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Thank you

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