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EUROFINS TECHNOLOGIES

Allergen Tests

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Types of Analytical Methods

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Polymerase Chain Reaction (DNA)

- PCR – Gel electrophoresis
- Real-Time PCR

Mass Spectrometry (Peptide)

- LC-MS

Immunoassays (Protein)

- Lateral Flow Device
- ELISA Test
- Biosensor chip
- Fluorescence


Lateral
Flow
Devices

LFD (Lateral Flow Devices)

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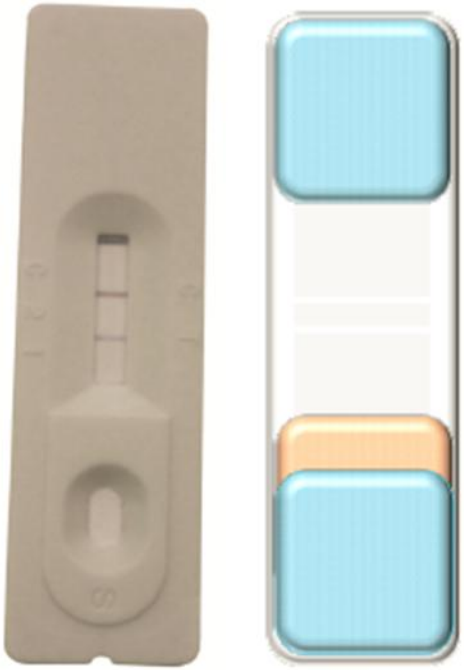

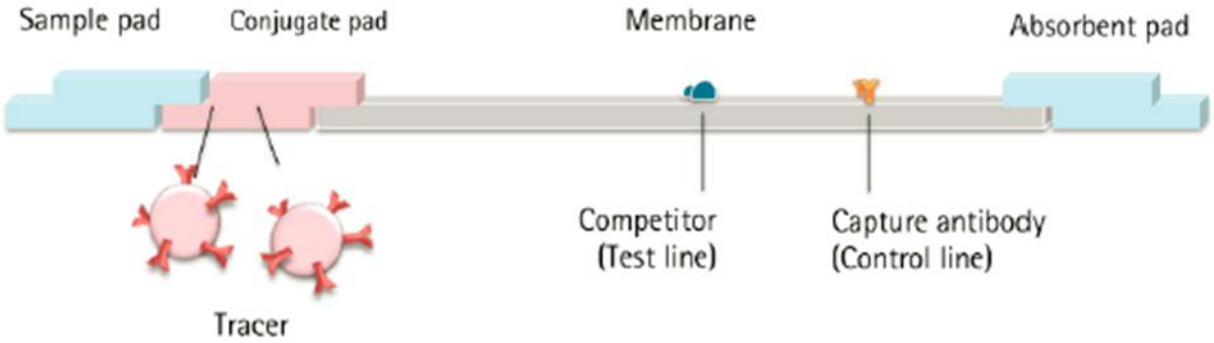

EPIstrip Fish

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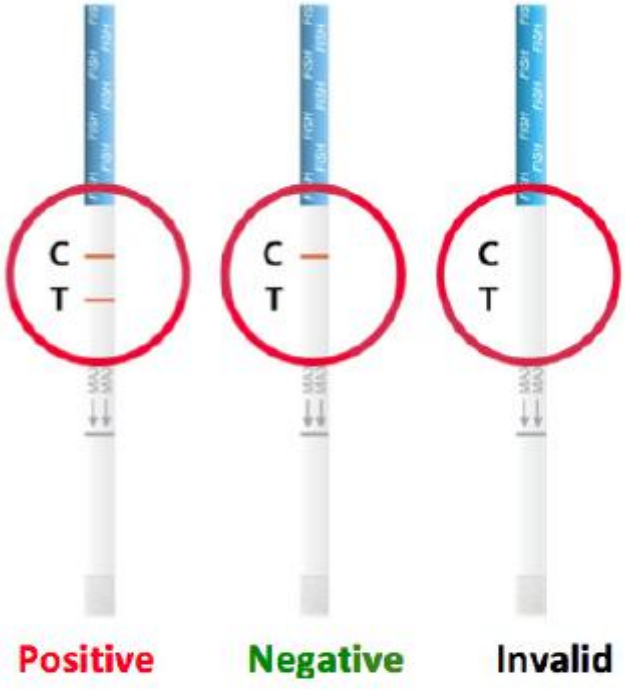
Environmental Swab Preparation

1. Transfer 500ul of buffer
2. Swab 10cm X 10cm area with wet swab
3. Insert into tube and agitate for 30s
4. Incubate at room temp for 1min

Assay

5. Insert test strip into the tube
6. Wait for 15 mins, read result



EPIstrip Peanut

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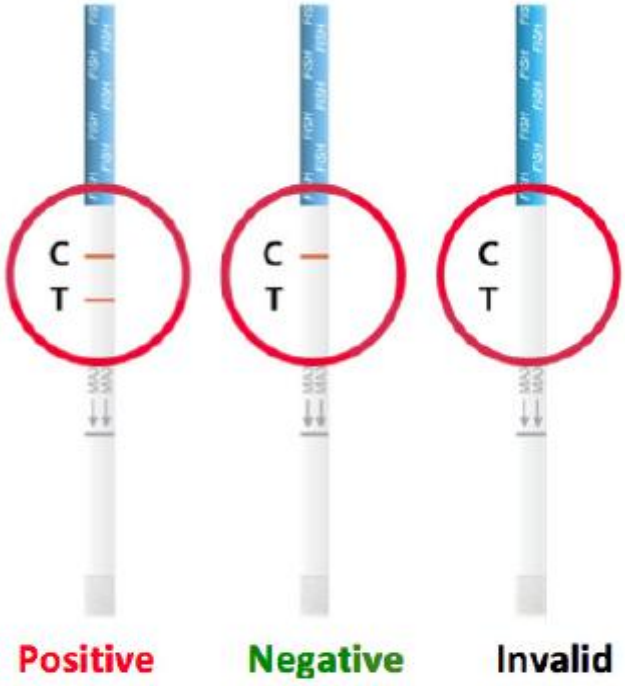
Solid Food Preparation

1. Weigh 0.30 g of finely ground sample with 3 mL buffer into 4 mL sample tube
2. Vortex/mix for 30 seconds. Leave sample mixture to settle for 1 min
3. Measure 500 µL of the supernatant into a 1.5 mL micro-centrifuge tube

Assay

4. Insert test strip into the tube
5. Wait for 15 mins, read result



Pros & Cons

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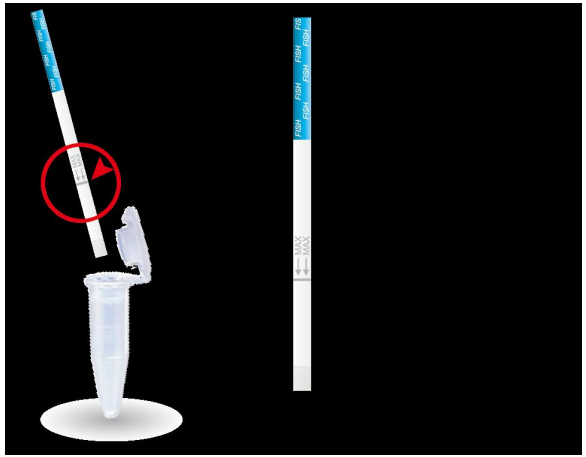
Advantage

- Extremely fast results
- Easy handling
- No equipment necessary
- LOD satisfactory
- Easy and fast or no sample preparation

Disadvantage

- Hook effect – additional dilution of samples necessary
- Not yet validated

LFD (Lateral Flow Devices)



LFD
Peanut
Casein
Shellfish
Almond
Fish
Soy
Egg
Gluten
β-lactoglobulin

LFD (Lateral Flow Devices)

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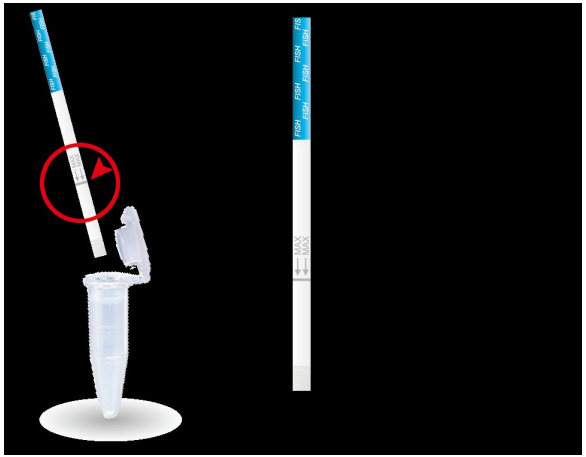
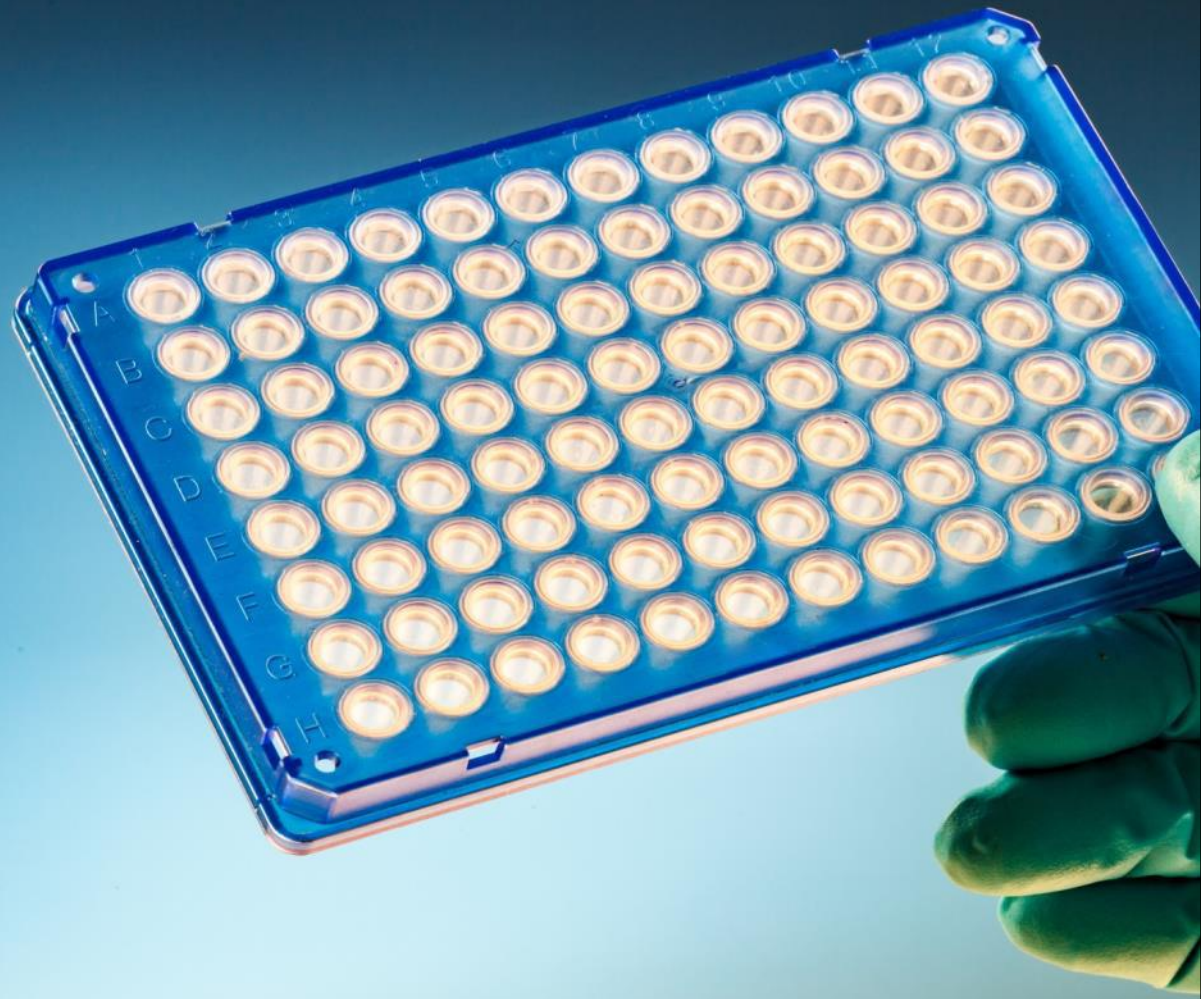
LFD	LOD	LOQ (finished product)	LOQ (surface)
Peanut	0.1 ppm	1 ppm	0.2 ppm
Casein	0.25 ppm	2.5 ppm	2.5 ppm
Shellfish	0.1 ppm	1 ppm	0.2 ppm
Almond	1 ppm	1 ppm	0.2 ppm
Fish	0.01 ppm	1 ppm	0.2 ppm
Soy	1 ppm	10 ppm	2 ppm
Egg	1.25 ppm	2.5 ppm	2.5 ppm
Gluten	1 ppm	2 ppm	2 ppm
β-lactoglobulin	0.25 ppm	2.5 ppm	2.5 ppm

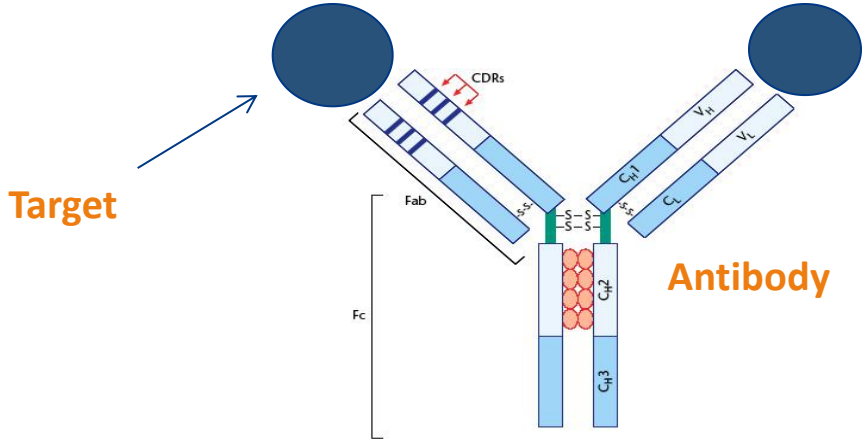


ELISA

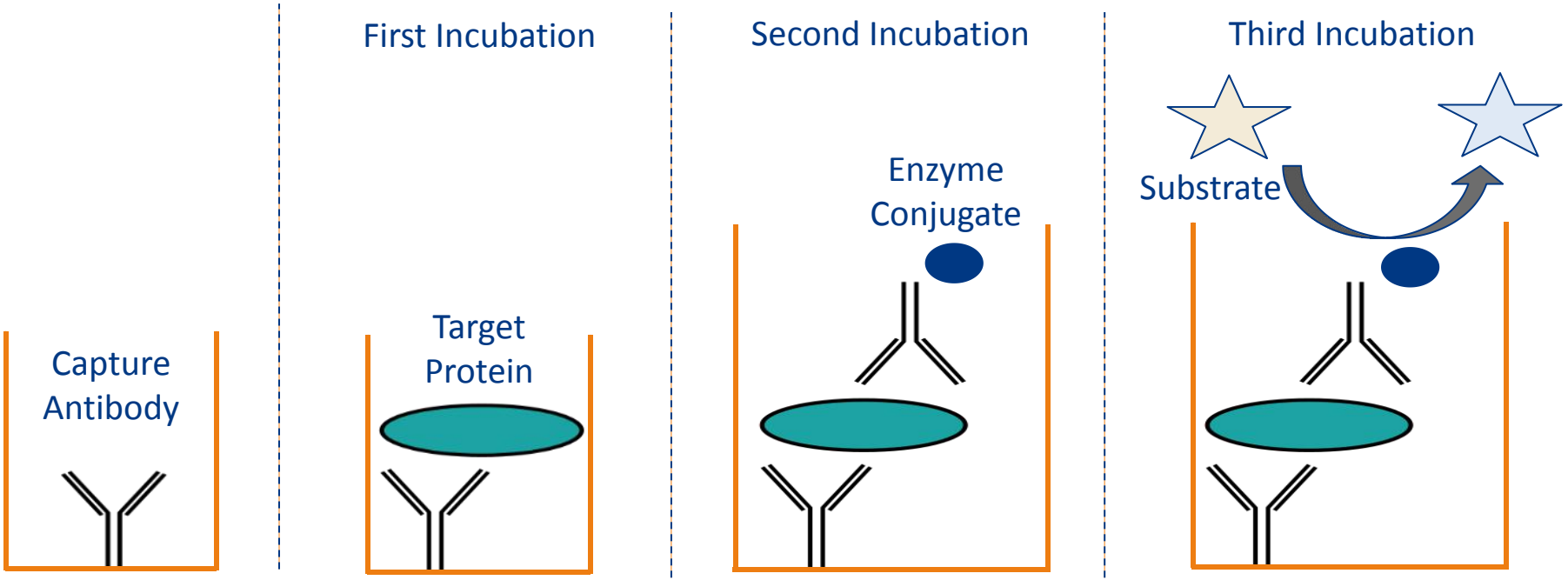


ELISA

- **E**nzyme-**L**inked **I**mmuno**S**orbent **A**ssay
- Uses binding abilities of antibodies
- Antibodies – blood protein produced during “alien substance invasion”
- Alien substance – allergen, mycotoxin, pathogen, virus, or any foreign substances



Sandwich ELISA



SENSISpec Fish

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Solid Food Preparation

1. Weigh/measure sample & homogenize
2. Mix in pre-diluted extraction and sample dilution buffer, incubate (60 ºC, 15 mins)
3. Centrifuge/filter

Assay

5. Add 100ul standards/samples into well, incubate (rtp, 20 mins), wash
6. Add 100ul conjugate, incubate (rtp, 20 mins), wash
7. Add 100ul of substrate, incubate (rtp, 20 mins, dark)
8. Add 100ul of stop solution
9. Measure in ELISA plate reader at 450 nm

SENSISpec Peanut

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All types preparation

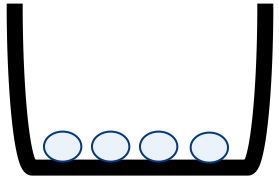
1. Weigh/measure sample & homogenize
2. Mix in pre-diluted extraction and sample dilution buffer, incubate (60 ºC, 15 mins)
3. Centrifuge/filter

Assay

5. Add 100ul standards/samples into well, incubate (rtp, 20 mins), wash
6. Add 100ul conjugate, incubate (rtp, 20 mins), wash
7. Add 100ul of substrate, incubate (rtp, 20 mins, dark)
8. Add 100ul of stop solution
9. Measure in ELISA plate reader at 450 nm

ELISA Kit Components

- ELISA Plate – Microtiter wells coated with antibody
- Enzyme Conjugate Solution – contains enzyme-linked secondary antibody
- Substrate Solution – Catalyst for oxidation
- Stop Solution – Acidic solution to stop oxidation reaction
- Washing Buffer – To wash out excess solution
- Standard Calibrators – To set a standard curve for every test



Antigen-Coated Microtiter well

What we offer

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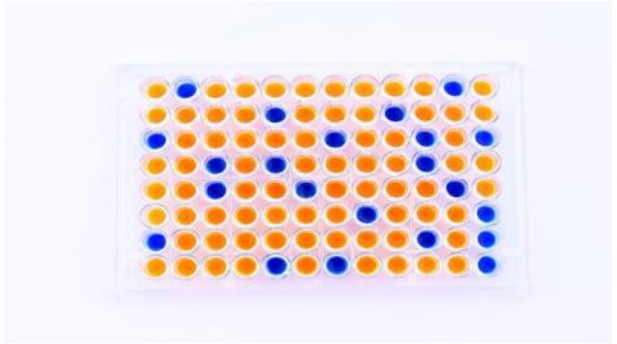
ELISA	
Gluten	Peanut
Crustaceans	Coconut
Fish	Milk
Egg white	Lysozyme
Casein	Lupin
β – Lactoglobulin	Almond
Cashew	Mollusc
Hazelnut	Ovalbumin
Walnut	Pistachio
Macadamia	Mustard
Brazil Nut	Sesame
Soy	

What we offer

ELISA kit	LOD	LOQ
Gluten	0.25 ppm (hydrolyzed) / 3 ppm (R5)	0.25 ppm (hydrolyzed) / 3 ppm (R5)
Crustaceans	0.9 ppb	20 ppb
Fish	1.4 ppm	4 ppm
Egg white	0.05 ppm	0.4 ppm
Casein	0.04 ppm	0.2 ppm
β – Lactoglobulin	1.5 ppb	0.01 ppm
Cashew	0.2 ppm	2 ppm
Hazelnut	0.3 ppm	1 ppm
Walnut	0.35 ppm	2 ppm
Macadamia	0.1 ppm	1 ppm
Brazil Nut	0.2 ppm	1 ppm
Soy	16 ppb	40 ppb



What we offer

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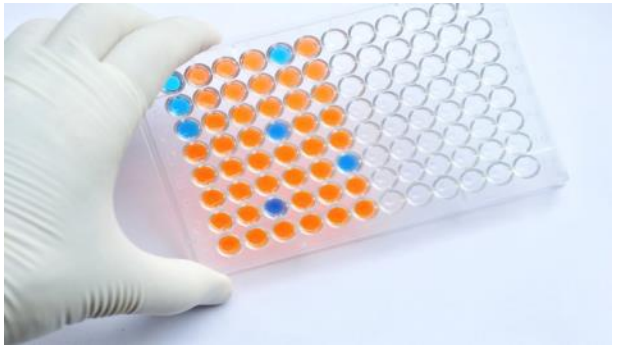
ELISA kit	LOD	LOQ
Peanut	0.1 ppm	1 ppm
Coconut	0.4 ppm	2 ppm
Milk	0.05 ppm	0.4 ppm
Lysozyme	2 ppb	25 ppb
Lupin	0.2 ppm	2 ppm
Almond	0.2 ppm	0.4 ppm
Molluscs	1.7 ppb	10 ppb
Ovalbumin	4 ppb	25 ppb
Pistachio	0.13 ppm	1 ppm
Mustard	1 ppm	2 ppm
Sesame	0.2 ppm	2 ppm
Pecan Nut	0.2 ppm	2 ppm



VIDEO


Automation

ELISA Automation

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Bolt

Thunderbolt

Bolt

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THE BOLT

Fluid Handling

Reader (EIA or EIA+CLIA)

Incubator

Shaker

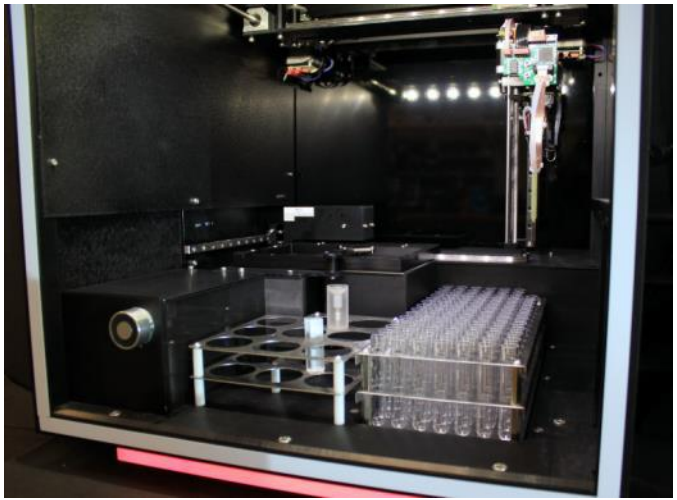
1 plate/96 samples

Robust Software

Bolt

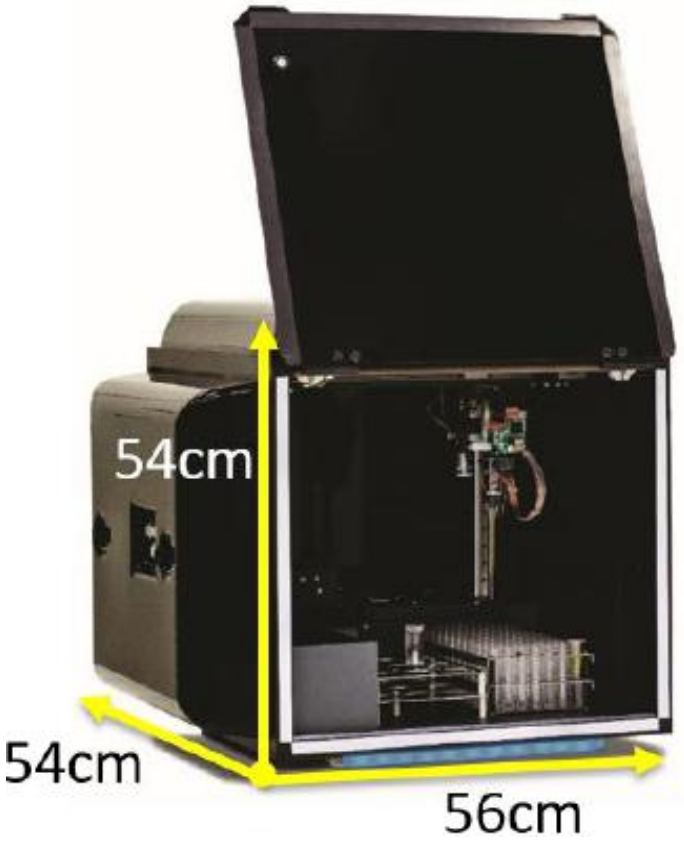
- Fully automated
- Single plate – 96 wells
- Compact
- ELISA + CLIA tests
- Pipette, washer, incubator, shaker, reader
- High precision micro syringe
- Extensive configuration
- Point to point, linear regression, cubic spline, 4PL, 5 PL, Lin-Lin, Lin-Log, and Log-Log representation
- 405, 450, 490, 550 and 630 nm
- 96 wells
- Simple but robust software
- Minimal consumables

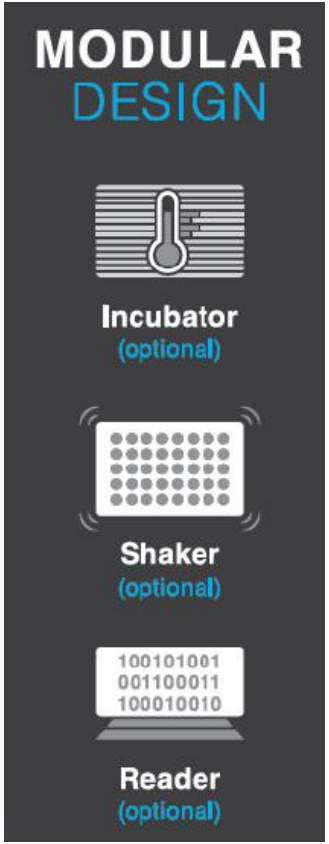
THE BOLT



Bolt – Specification

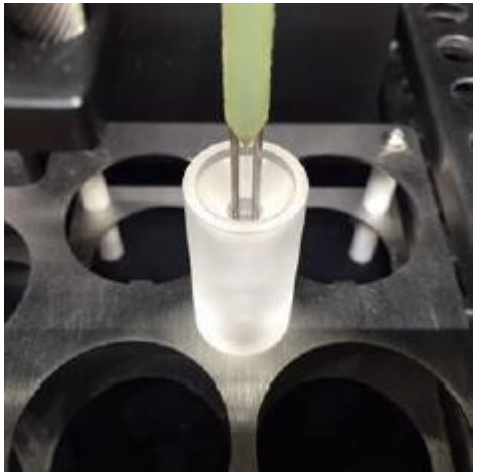
- Optional Incubator, Shaker, Reader (EIA only or EIA+CLIA)
- Utilize components/reagents as needed
- Suitable for any size laboratory
  - 56cm x 54cm x 54cm
  - 20kg
- User-friendly software

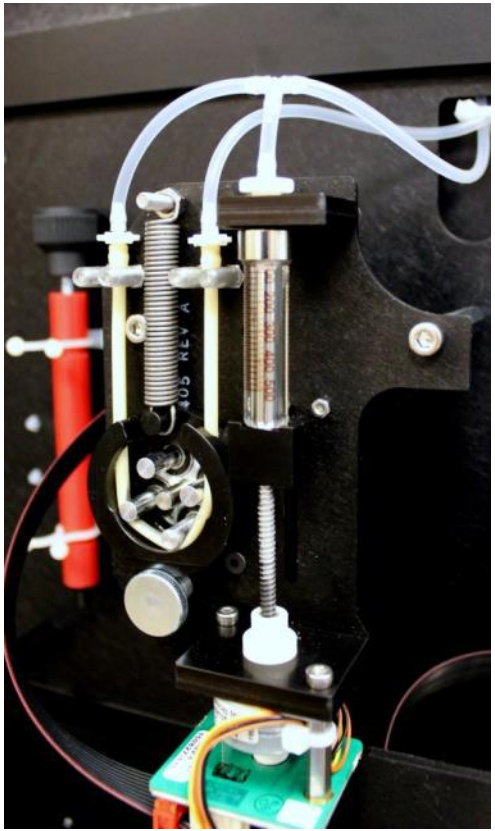




Bolt - Hardware

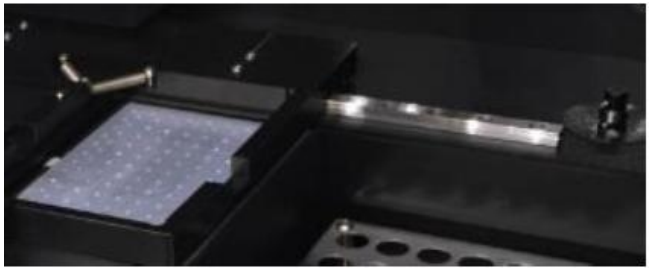
- Minute volume minimizes carry over
- 1:101 dilution directly into reaction wells
- Two-step dilutions up to 1:30,000
- Aspirate 1µl with less than 3% CV
- Single probe – dual needle
  - Liquid detection/level sensing via conductivity
  - Minimizes dead volume
  - Clog detection mechanisms
- Minimal consumable costs – no disposable tips

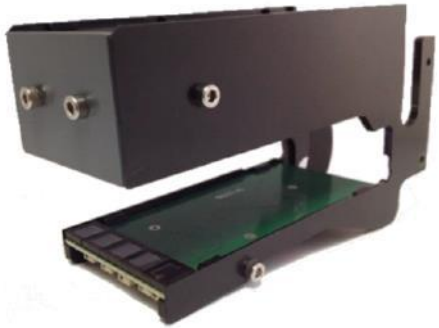




Bolt - Hardware

- Linear shaker
  - Up to 900 RPM
  - Keeps fluids in the wells
  - Dispense and/or incubate while shaking
- Forced convection incubator
  - Even heating of each well
  - Rapid heating
- On-board readers
  - Accurate and efficient
  - 405, 450, 490, 550 and 630 nm
  - EIA: LED technology; energy efficient; low heat; increased bulb life by 100x (compared to incandescent)
  - CLIA: adjustable integration time





Bolt - Software

- Many assays have already been programmed
- Infinite design limits with complete control over every protocol setting
- Data reductions
  - Index Calculation
  - Point to Point
  - Cubic Spline
  - 4PL/5PL Curve Fit
  - Regression
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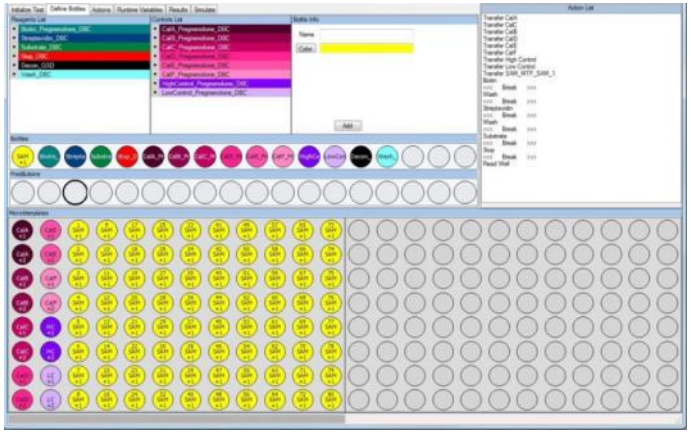
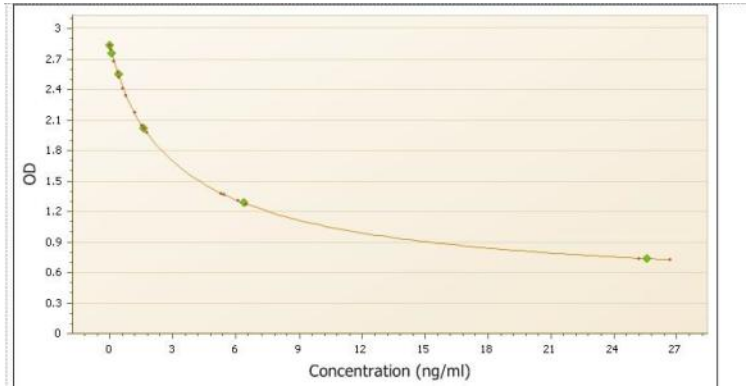
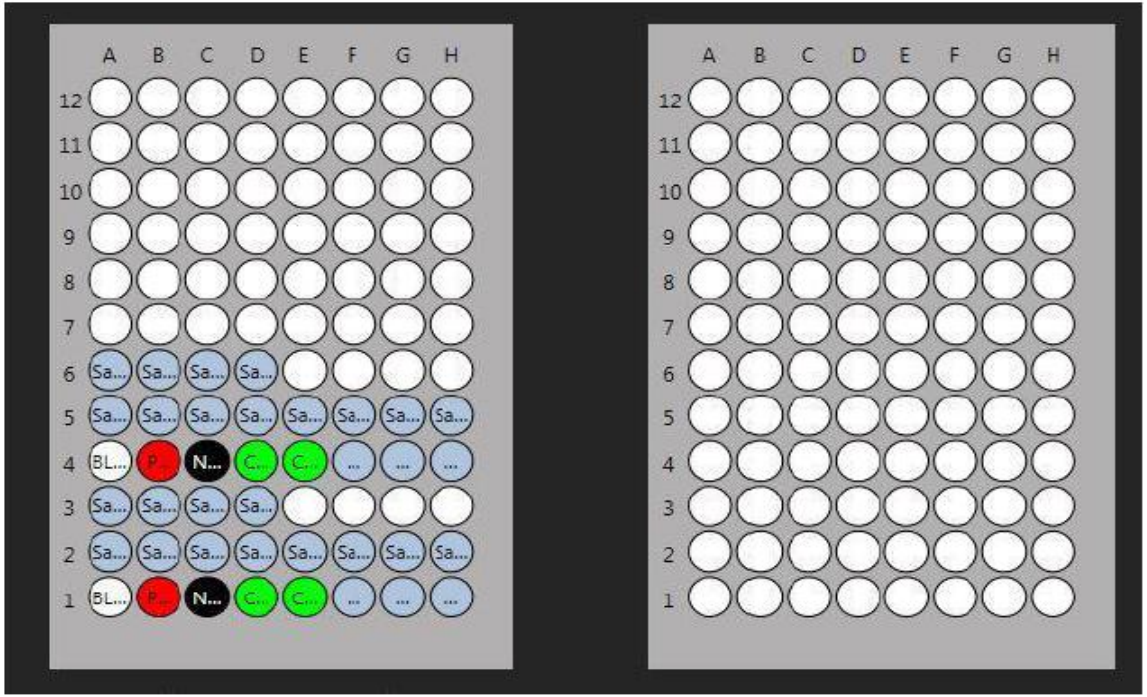




Bolt - Software

- Individual well tracking
  - Individually time incubation and processing steps
  - Consistent results regardless of position



Thunderbolt

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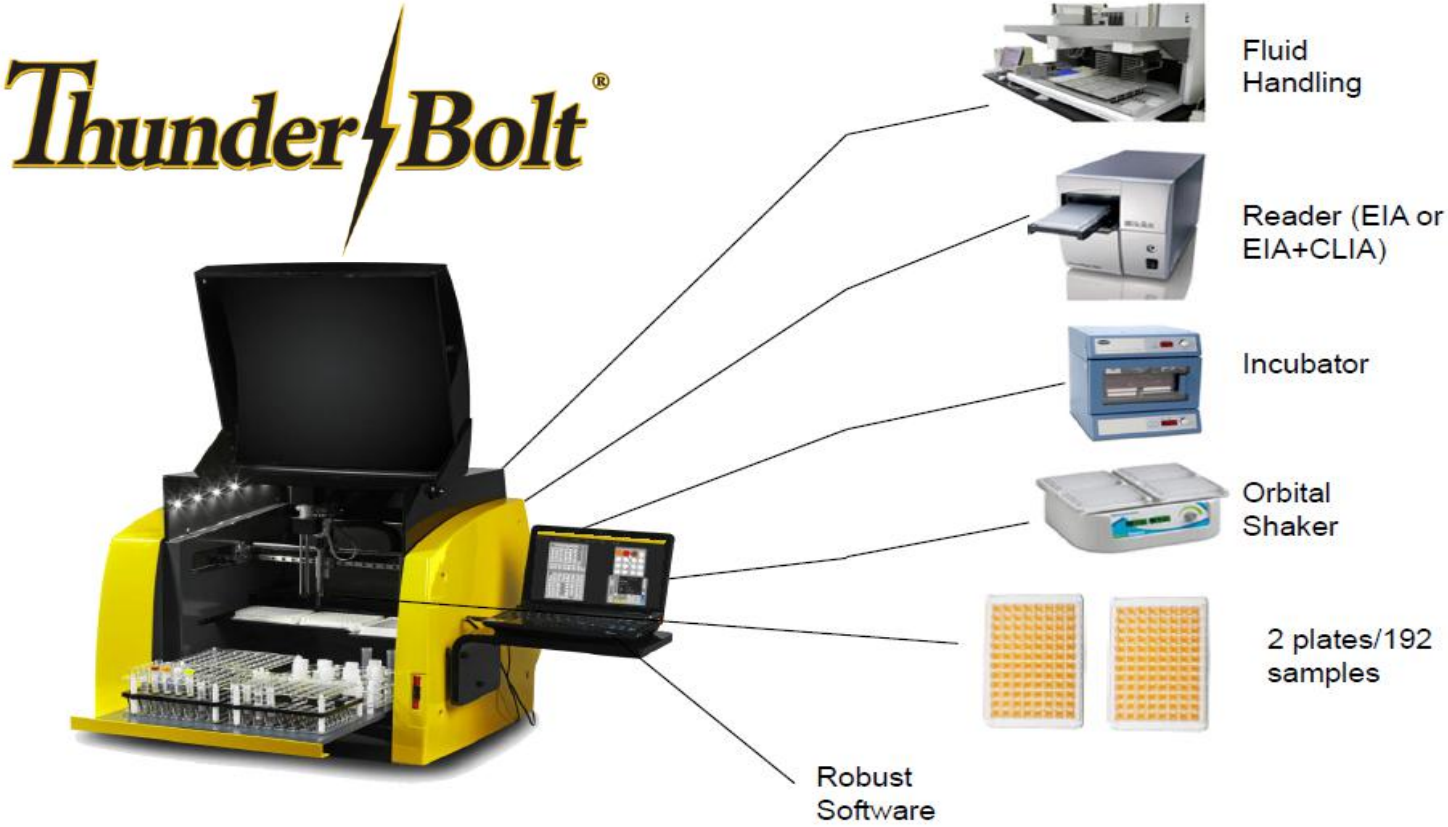

Thunderbolt

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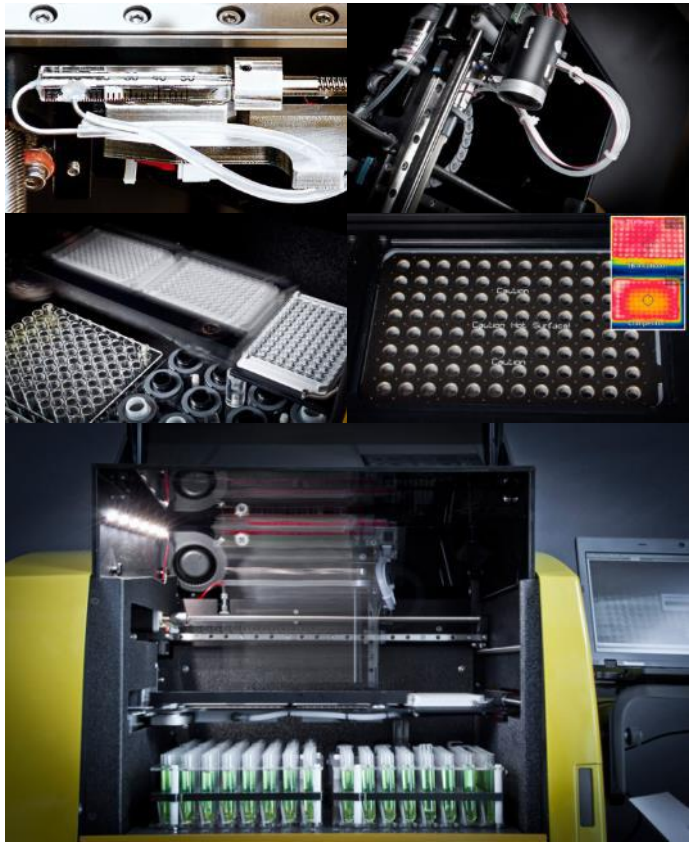
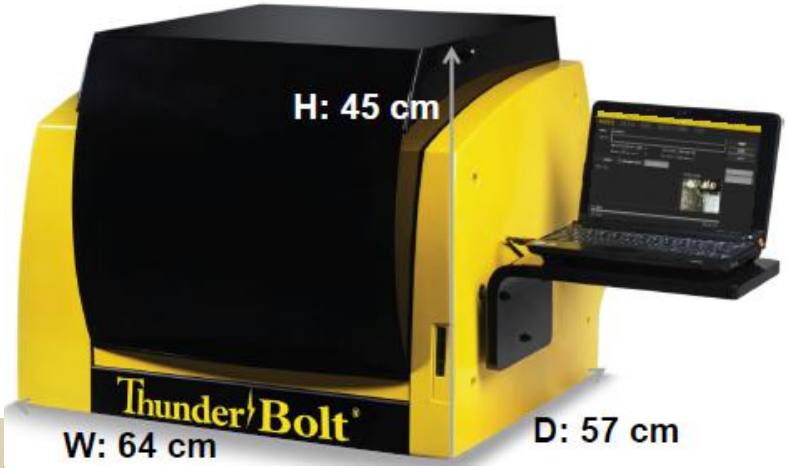
- Fully automated
- **2-plate – 192 wells**
- Compact
- ELISA + CLIA tests
- Pipette, washer, incubator, **orbital shaker**, reader
- High precision micro syringe
- Extensive configuration **– up to 8 protocols in same batch**
- Extensive data reduction functions
- 405, 450, 490, 550 and 630 nm
- **Internal camera – can be viewed remotely**
- **Multi-language**
- Simple but robust software
- Minimal consumables

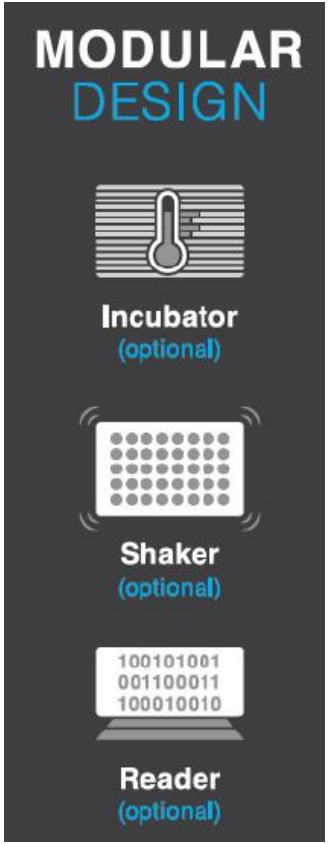


Thunderbolt – Specification

eurofins | Technologies

- Optional Incubator, Shaker, Reader (EIA only or EIA+CLIA)
- Utilize components/reagents as needed
- Suitable for any size laboratory
  - 64cm x 57cm x 45cm
  - 28kg
- User-friendly software





Thunderbolt - Hardware

- Slide-out sample and reagent racks
- 3 Intelligent Sample Racks
  - 192 patient sample capacity
  - Racks sense and record sample location
  - Track sample ID
- Reagent Rack
  - 16 positions
  - Various reagent adapters to fit 22 to 35 mm bottles
  - No reagent transfer required
- Integrated Barcode Scanner
  - Supports virtually all barcode types

Thunderbolt - Hardware

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- Minute volume minimizes carry over
- 1:101 dilution directly into reaction wells
- Two-step dilutions up to 1:30,000
- Aspirate 1µl with less than 3% CV
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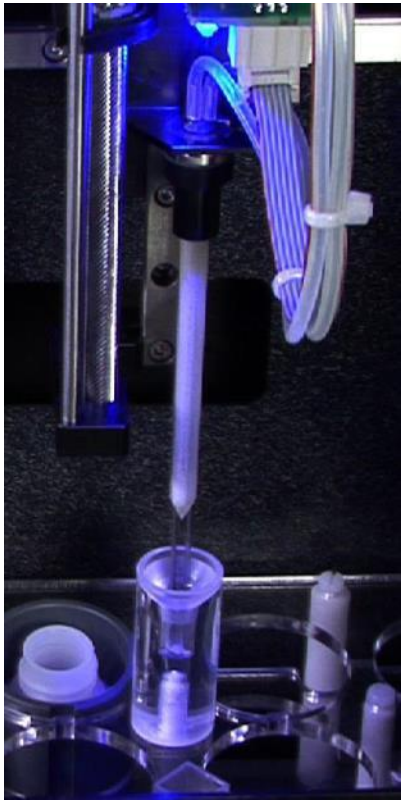

Thunderbolt - Hardware

- Orbital shaker
  - Up to 900 RPM
  - Keeps fluids in the wells
  - Dispense and/or incubate while shaking
- Forced convection incubator
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  - Rapid heating
- On-board readers
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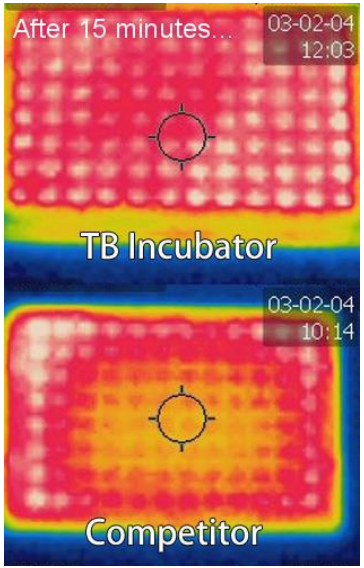
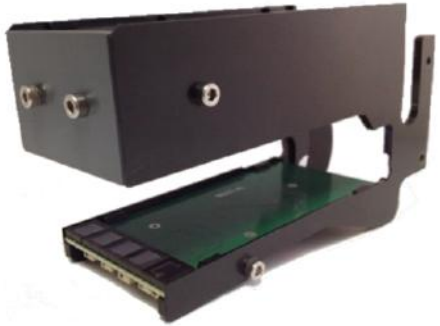
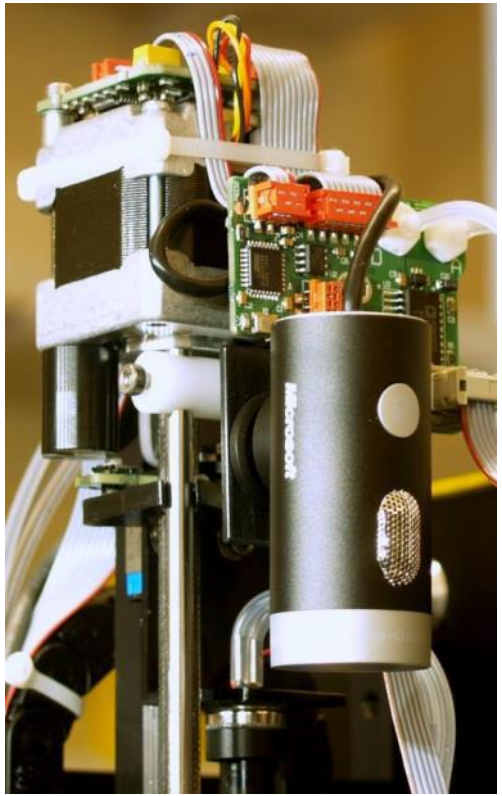




Thunderbolt - Hardware

- On-board camera
  - Adjustable LED lighting for clear view of probe operation
  - Process monitoring while lid is closed
  - Remote troubleshooting
  - Minimize instrument downtime



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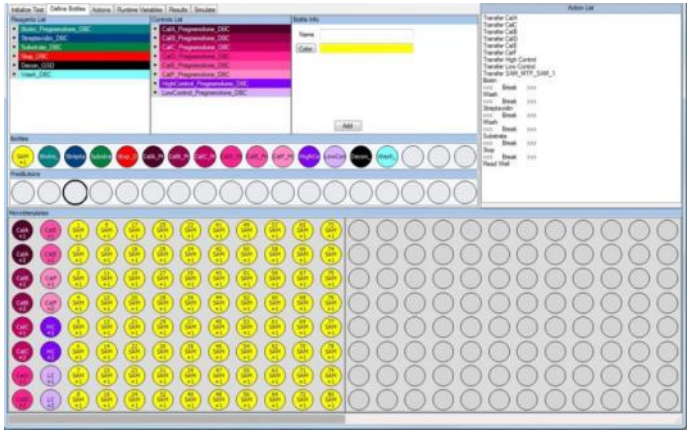

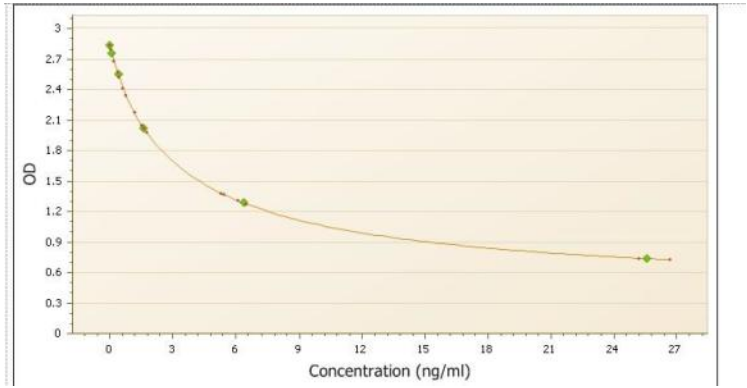

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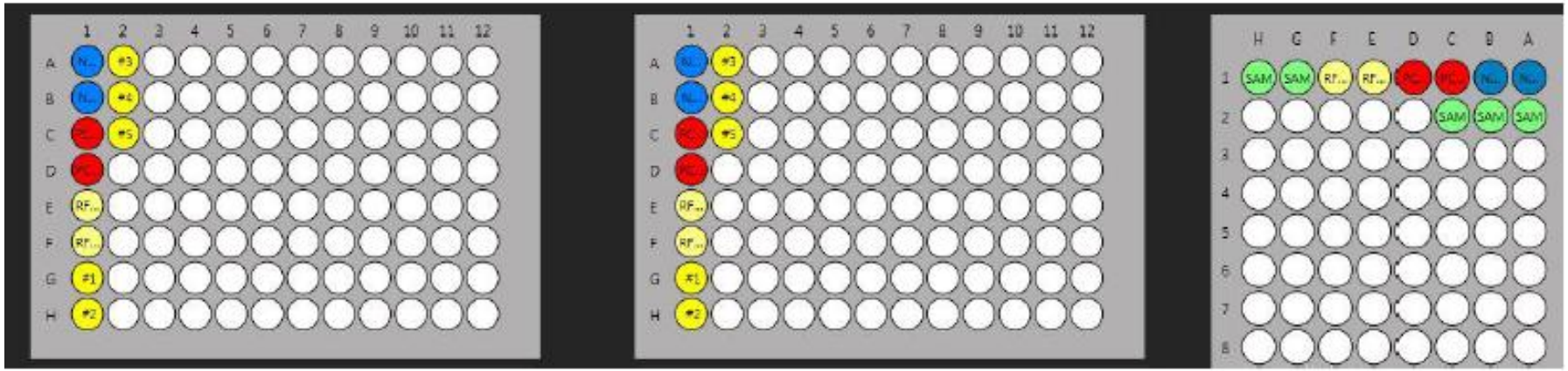

Pros & Cons

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Advantage

- Automated, inexpensive
- Objective
- Small quantities required
- Class specific antibodies measurable
- No hook effect

Disadvantage

- Dependent on pipetting skills
- Variable sensitivity / specificity of variable tests
- Matrix interference

ALTERNATIVES

Polymerase Chain Reaction

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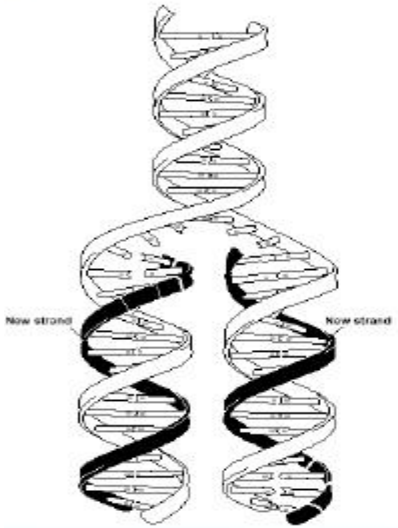
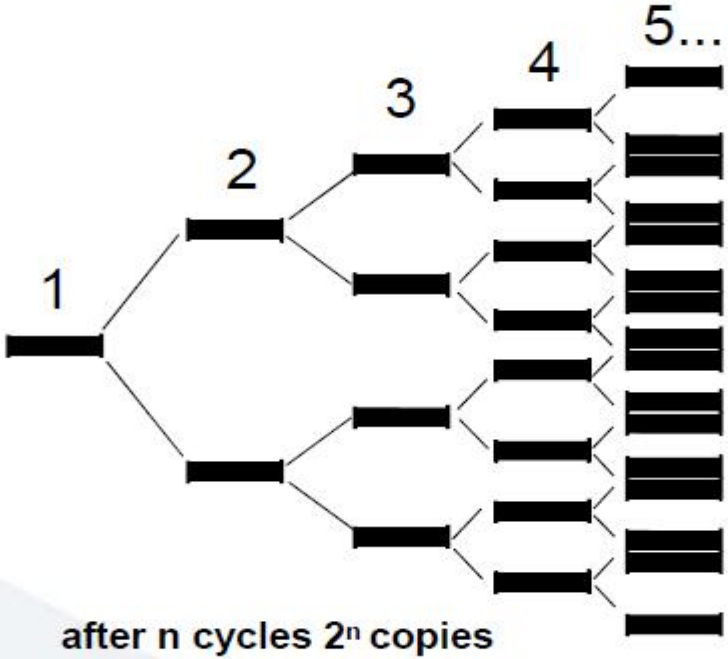
DNA-Extraction

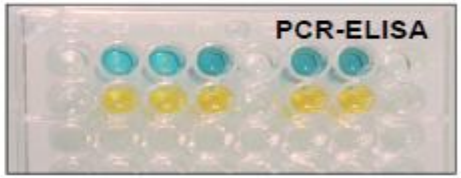


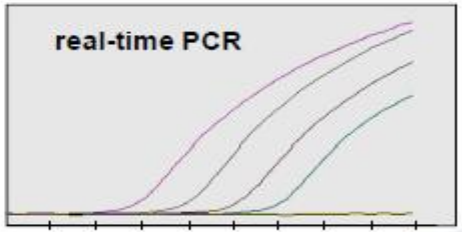
PCR-Amplification



after n cycles 2^n copies

Detection





Polymerase Chain Reaction

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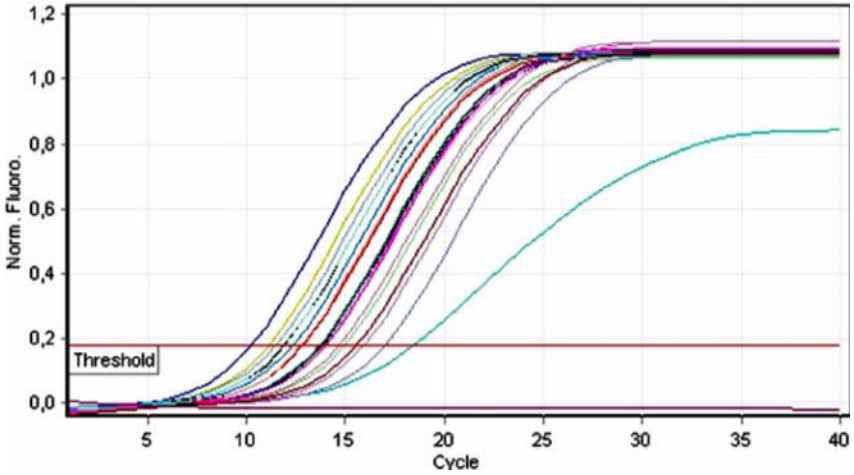
Advantage

- Highly specific
- Very sensitive
- DNA is relatively stable
- DNA is not affected by environmental factors
- Qualitative to quantitative results



Disadvantage

- Results cannot be linked to protein content
- Highly sensitive

Mass Spectrometry

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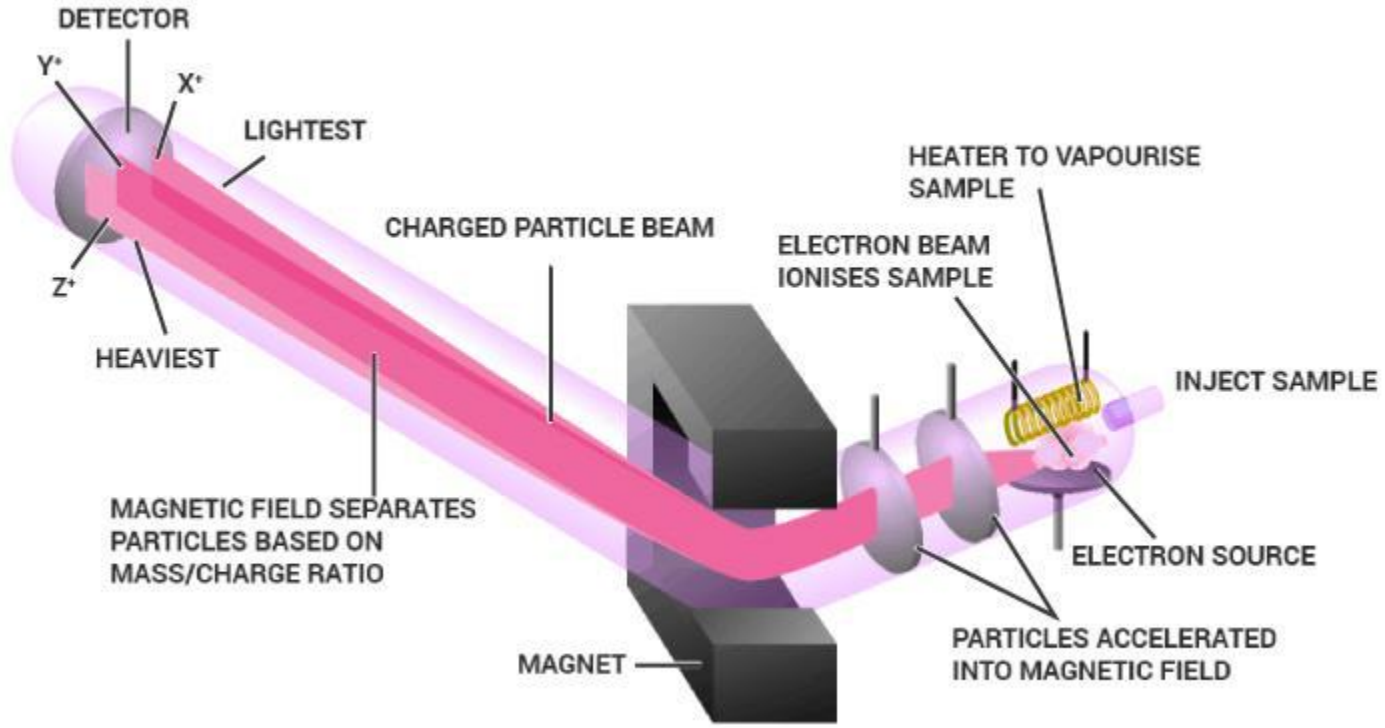

Mass Spectrometry

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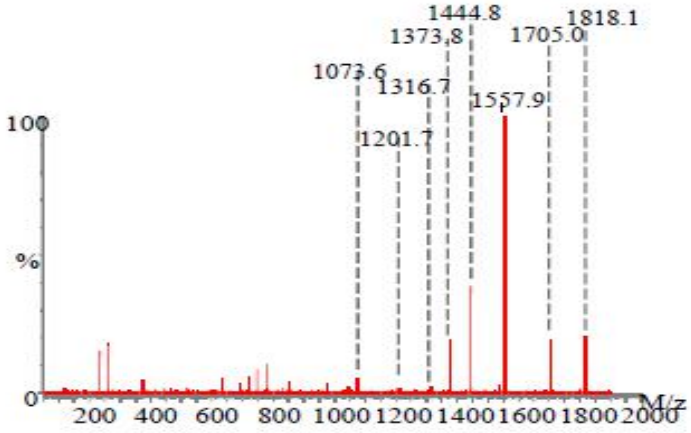
Advantage

- Highly specific
- High throughput
- High degree of automation
- Processing effects might not be as relevant

Disadvantage

- Complex matrices are problematic
- No validated protocols
- Expensive
- Well trained staff required
- Appliance to appliance variation



Food Allergen Analysis

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Method	Principle	Advantage	Application
PCR	DNA based	Fast, sensitive, high-throughput	Comparison, confirmation
Immunoassay	Protein based	Fast, sensitive, high-throughput	Screening and validation
Mass Spectrometry	Peptide based	Multi-screening, high-throughput	Laboratory service based


Note

Pipetting Techniques

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- Use the correct pipette that is within the range suggested by manufacturer
- Confirm tip is firmly seated on the pipette
- Confirm there are no air bubbles while pipetting
- Change tips between each standard, sample, or reagent
- Use different reservoirs for each reagent
- Pipette sample into the side of wells to avoid splashing
- Run samples/standards in duplicates

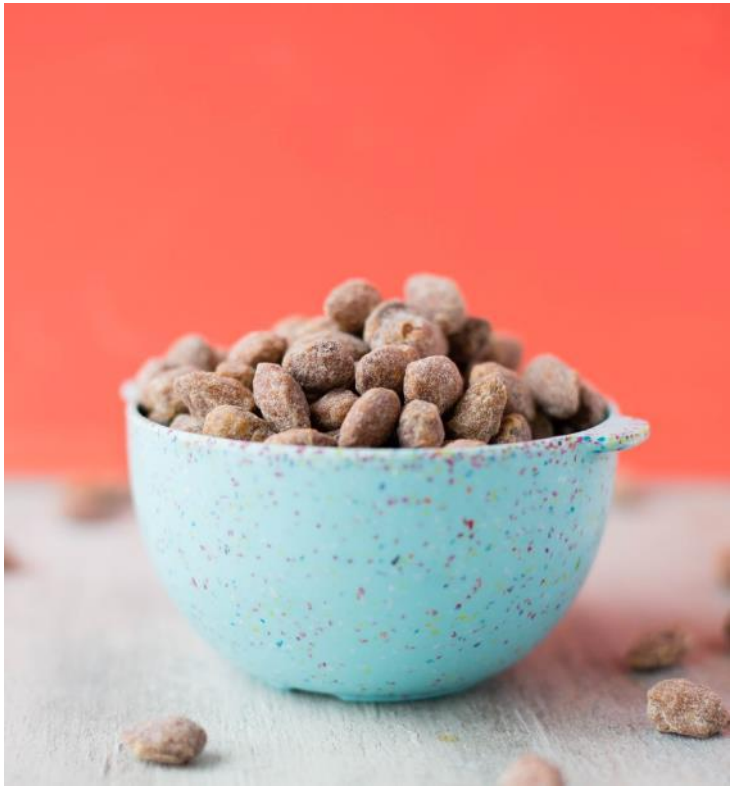
Obtaining reproducible results?

- Make sure all reagents are at room temperature before running the assay
- Store kits properly (fridge)
- Keep proper timing
- Standardize washing conditions
- Calibrate and check pipettes regularly
- Check room temperature
- Do not allow the wells to dry
- Follow instructions to the letter


Conclusion

Conclusion

- 3 main techniques
- LFD qualitative screening
- ELISA quantitative evaluation
- Proteins are the causes of allergies
- We provide a wide range of solutions
- Automated ELISA robots available
- Proper lab techniques required to minimize CV%



Thank you!