



How do you validate a novel Universal Sample Prep Method that doesn't fit the conventional template for approvals?

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M A T R I X
M I C R O S C I E N C E



PRESENTATION OVERVIEW

- Overview of a novel re-circulating immuno-magnetic sample preparation technology
- Incorporation of post enrichment pooling as an effective screening strategy linked to sample prep
- What sample size do you validate e.g. 25g , 375g ?
- How do you validate a sample prep method like this ?

A vertical strip on the left side of the slide shows a microscopic view. At the top, there is a bright orange, rod-shaped bacterium with a yellowish, fibrous structure extending from it. Below this, there are several blue, rounded plant cells with visible cell walls.

OVERVIEW OF IMS

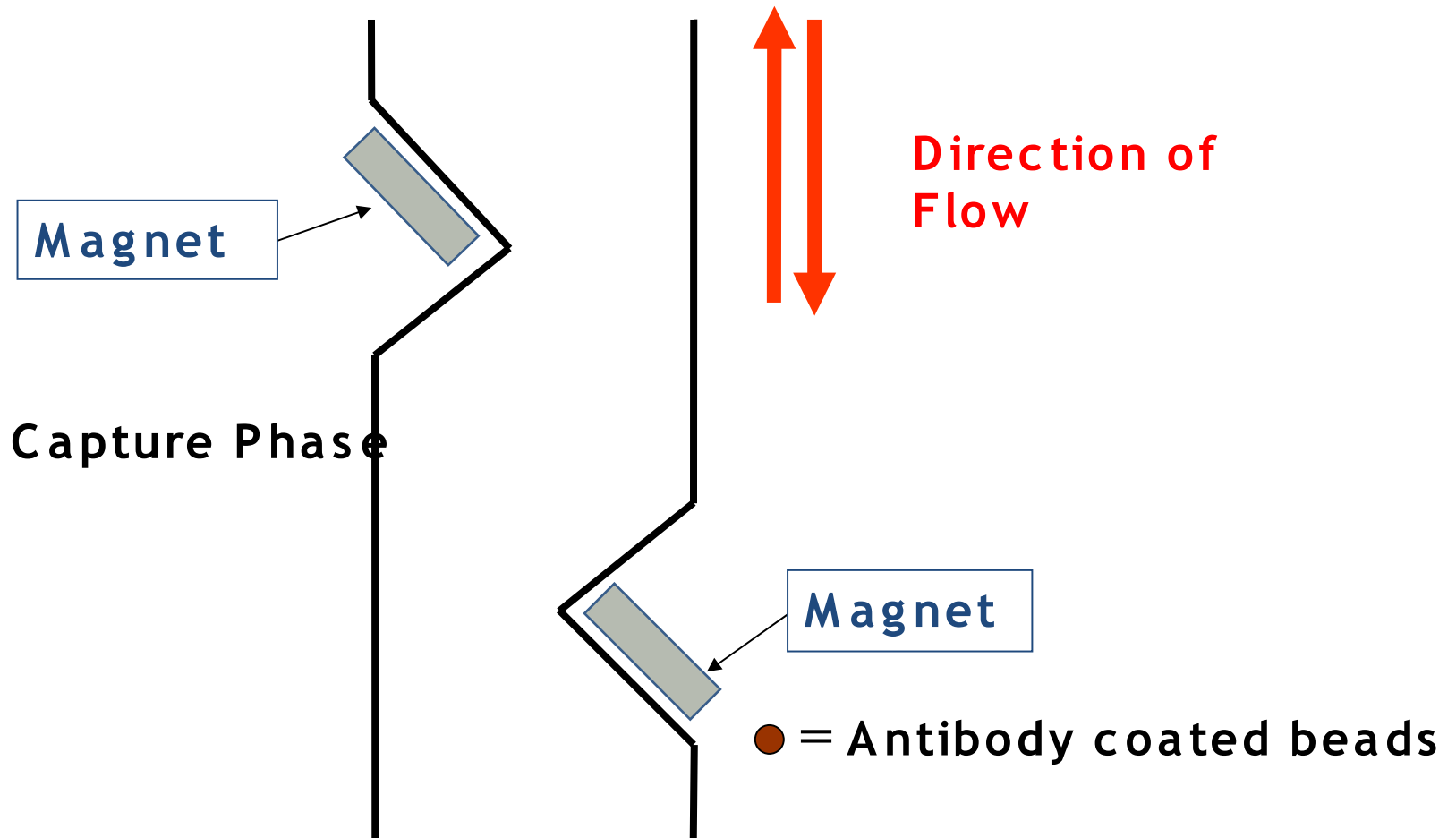
- The approach of using antibody coated magnetic particles in immuno-magnetic separation (IMS) techniques to aid the capture of pathogens from food has been well documented over the past 10 years
- Whilst IMS can be a useful tool for microbiologists there have been some drawbacks:
 - There are restrictions on sample volume that can be analyzed e.g. Typically < 1 ml
 - The passive nature of basic IMS systems leads to high levels of non-specific binding
 - Limited /inefficient low volume washes

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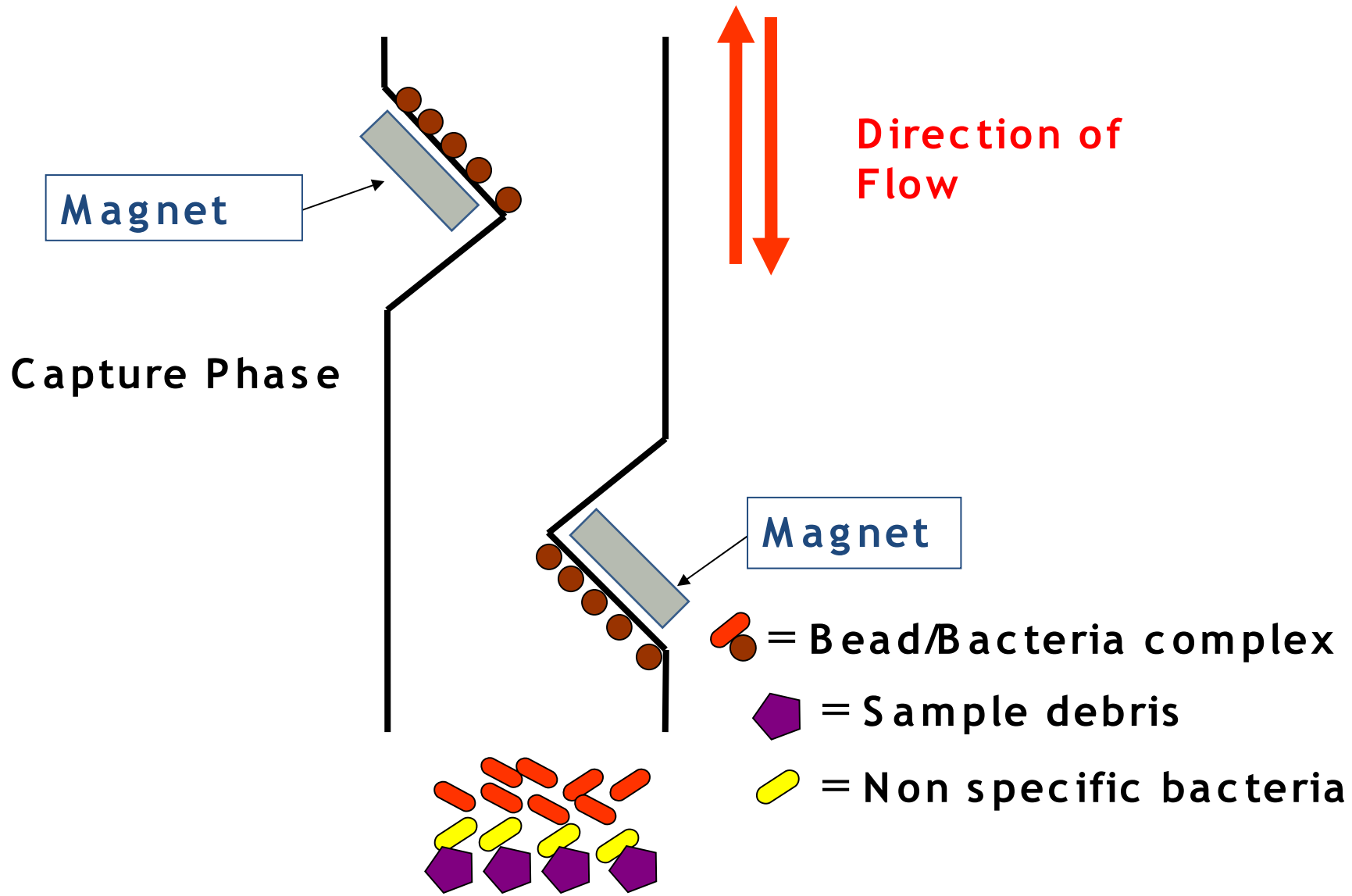
The Solution.... “PATHATRIX® AUTO”

- 15 minute cycle time e.g. 150 samples per hour per PATHATRIX® AUTO (based on 10 pooling)
- Small bench “footprint” only 50 cm wide x 28 cm deep
- Can handle sample volumes from 10 ml – 60 ml
- Incorporates multiple high volume wash steps
- Automation decreases the possibility for human error

Antibody coated beads immobilise onto the “Capture Phase”

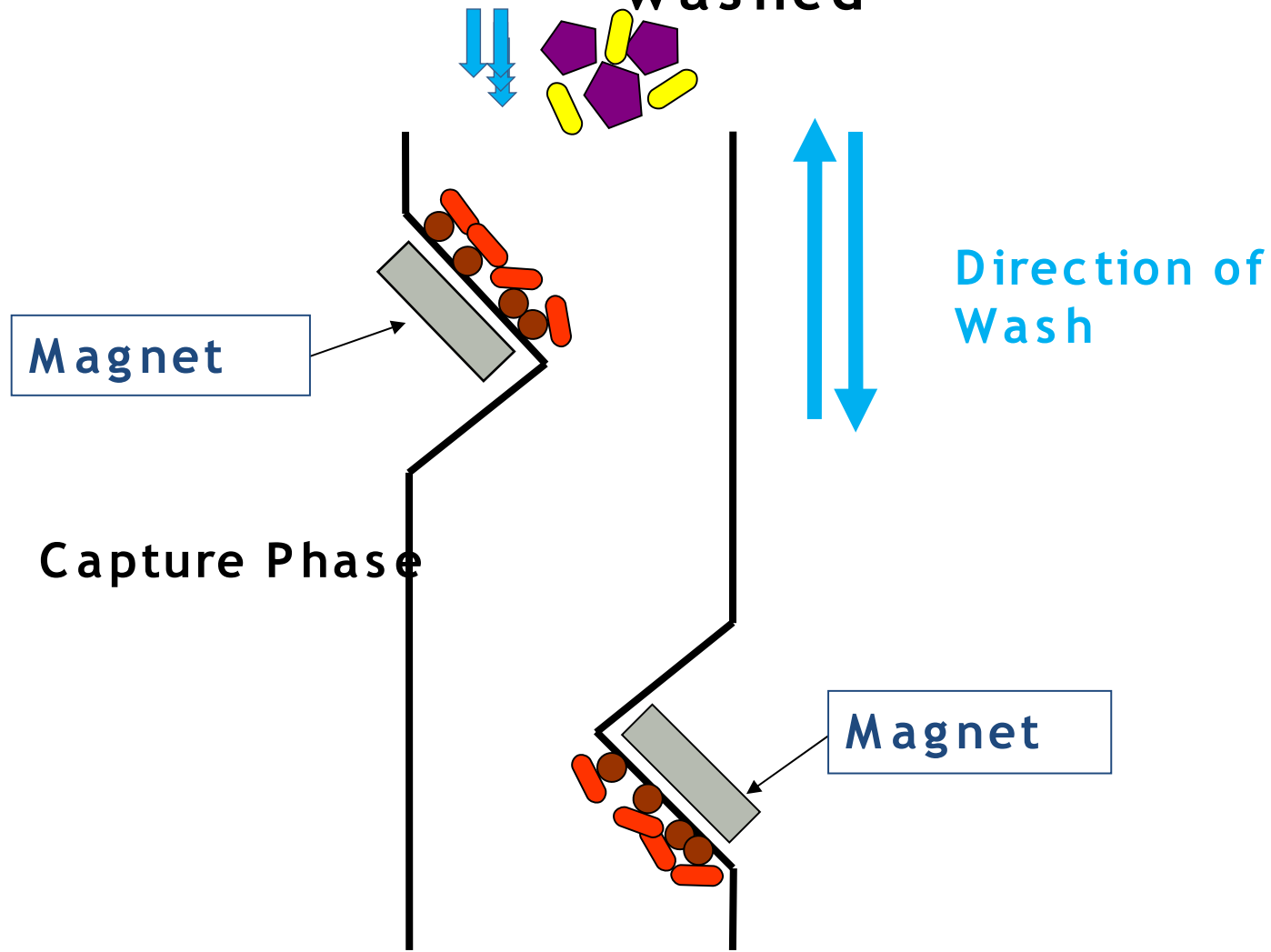


Specific capture of target onto the magnet from complex matrices

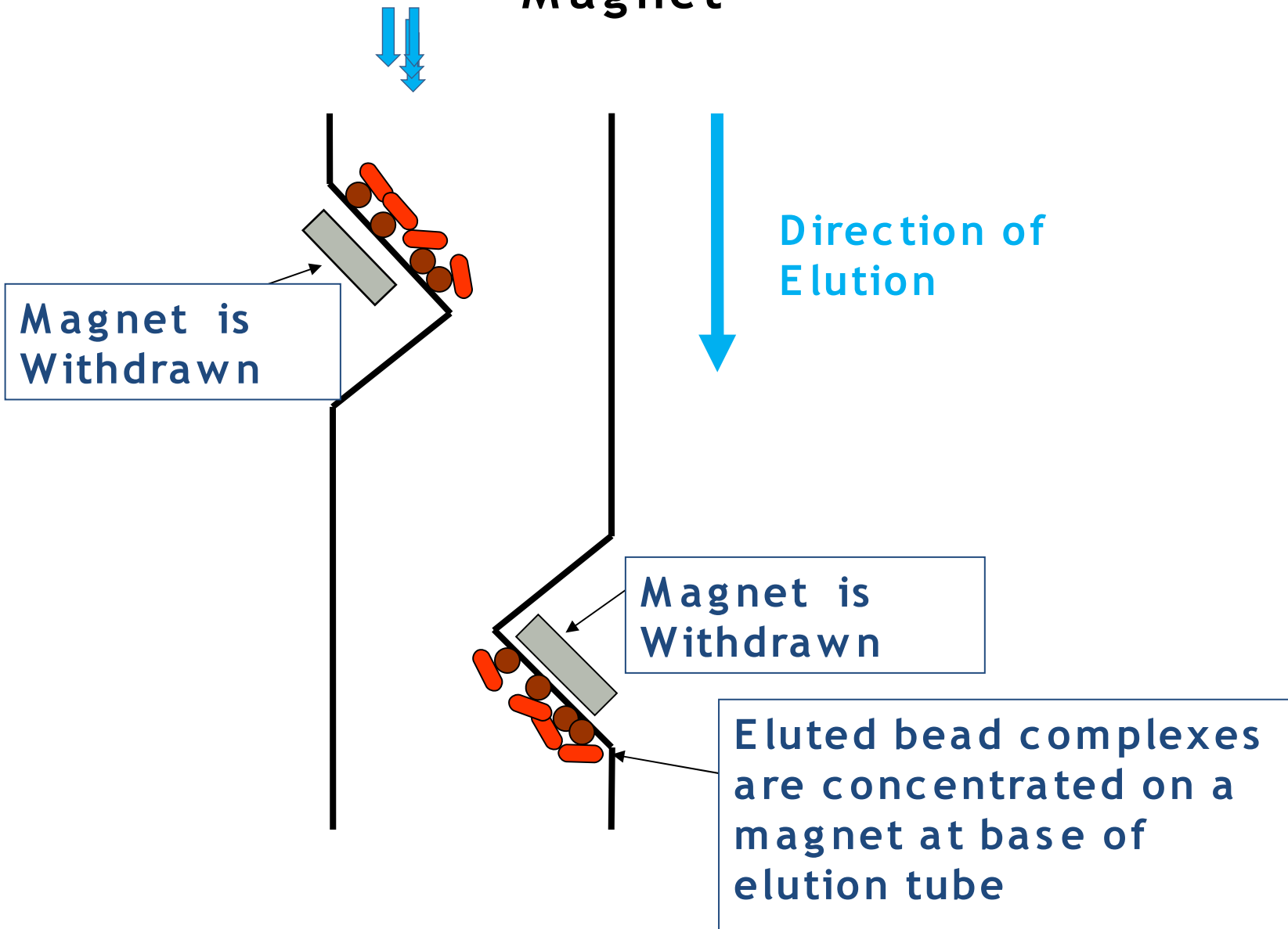


Captured Bead/Bacteria Complexes are

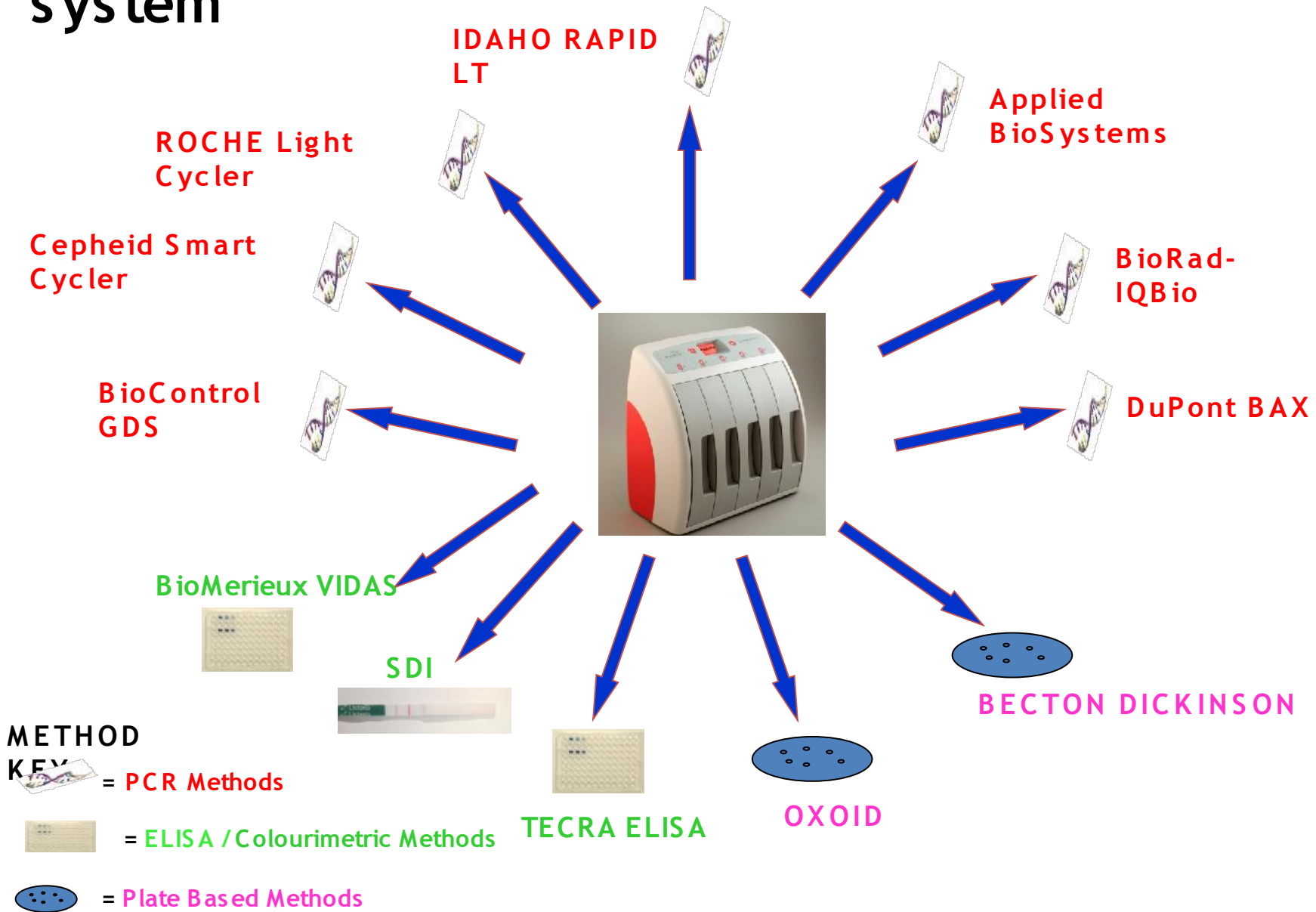
“washed”



Bead / Bacteria complexes are Eluted from Magnet

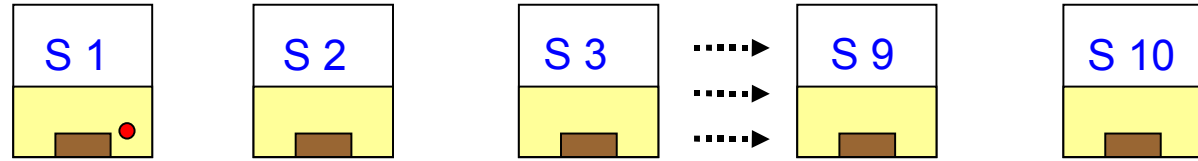


PATHATRIX[®] AUTO is a Universal Sample Prep system

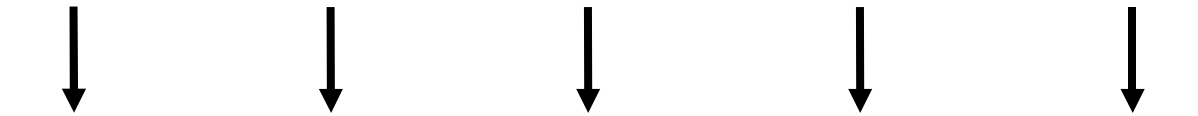


PATHATRIX® AUTO “10” POOLING STRATEGY

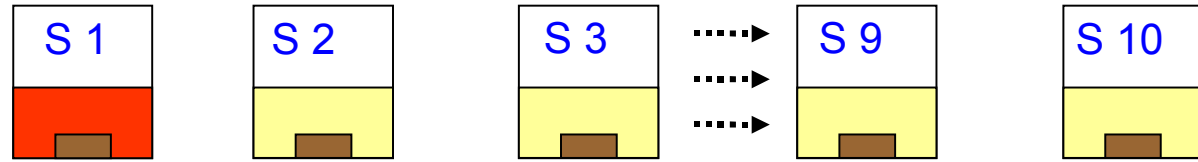
10 x 25-375g
Samples



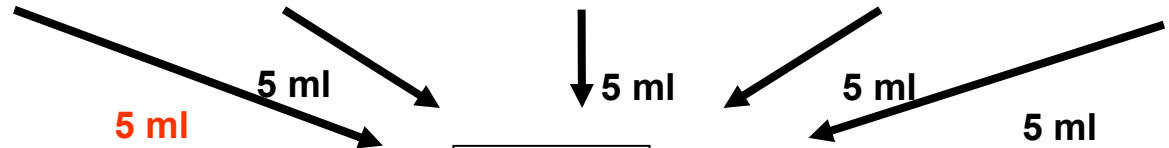
Individual
Enrichment
of Samples



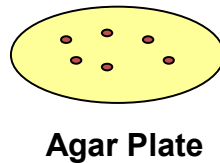
Post
-enriched
Samples



Pooling of
Samples to
create 1
Pooled Batch
Sample



 = Positive post-enrichment



Agar Plate

PATHATRIX



PCR

If Pooled Sample is Negative; then all 10 Individual Samples are Negative

If Pooled Sample is Positive; then Re-Test all 10 Individual Post-Enrichment



How do you validate sample prep methods?

PATHATRIX® AUTO provides several challenges in the validation process:

- It is sample prep method that is technically prior to detection
- Validation Programmes are focused on “detection” based methods
- Validation Programmes have not previously had to deal with post enrichment pooling
- How do we choose the “detection” method to couple this to, as validation programmes all require this?

A vertical strip on the left side of the slide shows a microscopic view of a bacterium, likely Salmonella, with a red, rod-shaped body and yellowish, hair-like flagella extending from one end. The background is dark blue.

MATRIX had to make choices !

Sample size choices?

We tried to pick appropriate for Pathogen and food type:

- *Salmonella spp* – multiple food types using 25g standard samples and 325g for cooked meats and 375g for peanut butter
- *E.coli* O157 – validated 25g samples (EU customers) and 375g (US customers)
- *Listeria spp* – validated multiple food types mainly with 25g samples

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MATRIX had to make choices !

Post Enrichment Pooling choices?

- *Salmonella spp* – Validated single, 5 pooled and 10 pooled samples for multiple food types
- *E.coli* O157 – Validated single, 5 pooled and 10 pooled samples for raw ground beef
- *Listeria spp* – Validated single and 5 pooled samples for multiple food types



MATRIX had to make choices !

Which Validation Body & Which type of Validation?

AOAC

- Chosen as they are widely accepted by multinational customers
- Used AOAC-RI performance tested methods programme as is the most cost effective, and rapid route to get an approved method
- Have 8 AOAC-RI approved products

MicroVal

- Currently pursuing an ISO 16140 Validation for PATHATRIX to plate and PCR to satisfy European customers

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Life After Validation?

- *Having a validation certificate is Not the end of the story!*
- Merely enables discussions to be opened with companies about use of the technology
- Despite having a validated method customers still insist on validation of their products and will do their own in-house trials
- Diagnostic Companies need, large amounts of money to pay for validation, a lot of patience, as it takes a long time to get validation, and then hope that they sell products to pay for it all!



Thanks for listening
any questions ?

