



Enhanced Detection of *Listeria* spp. from Environmental Swab and Food Samples within 24 hours using Sample Pooling, Automated PATHATRIX[®] Re-circulating IMS linked to Real Time PCR

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PRESENTATION OVERVIEW

- Overview of a novel automated re-circulating immuno-magnetic sample preparation technology
- Description of method development and data
- Overview of study parameters
- Data from main study

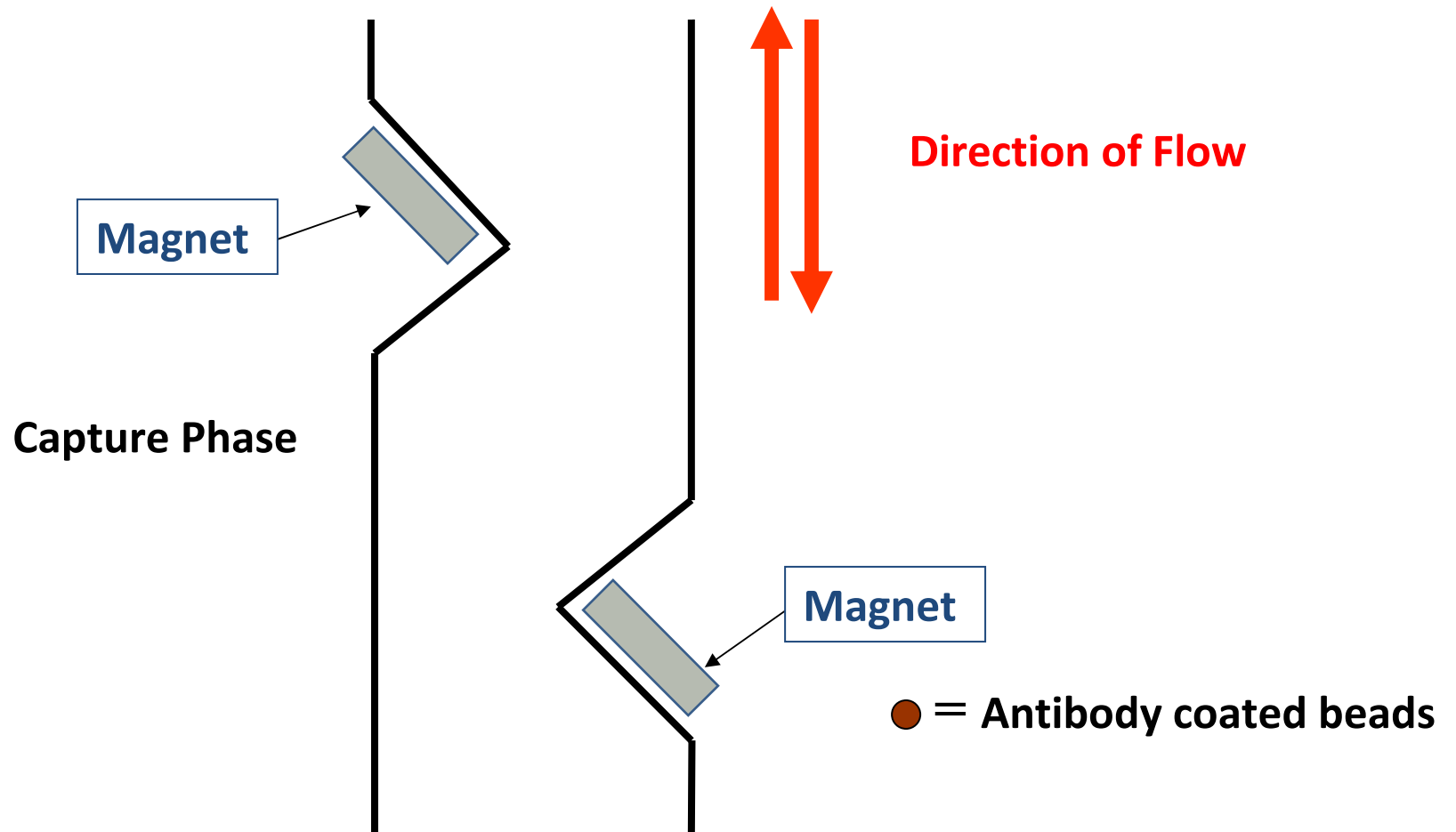


OVERVIEW OF IMS

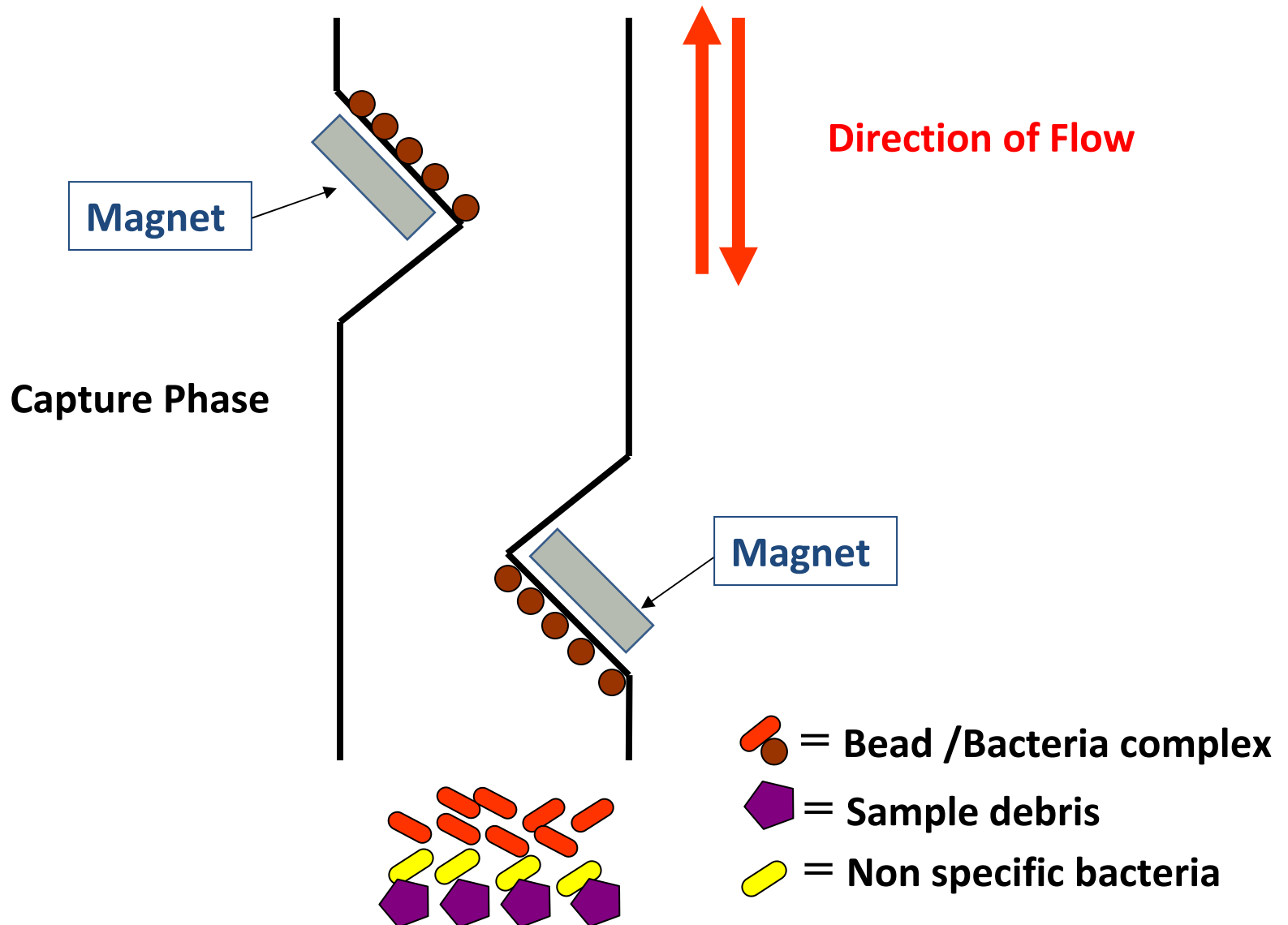
- The approach of using antibody coated magnetic particles in immuno-magnetic separation (IMS) techniques to capture pathogens from food has been well documented over the past 10 years
- Whilst IMS can be a useful tool for microbiologists there have been some drawbacks:
 - There are restrictions on sample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



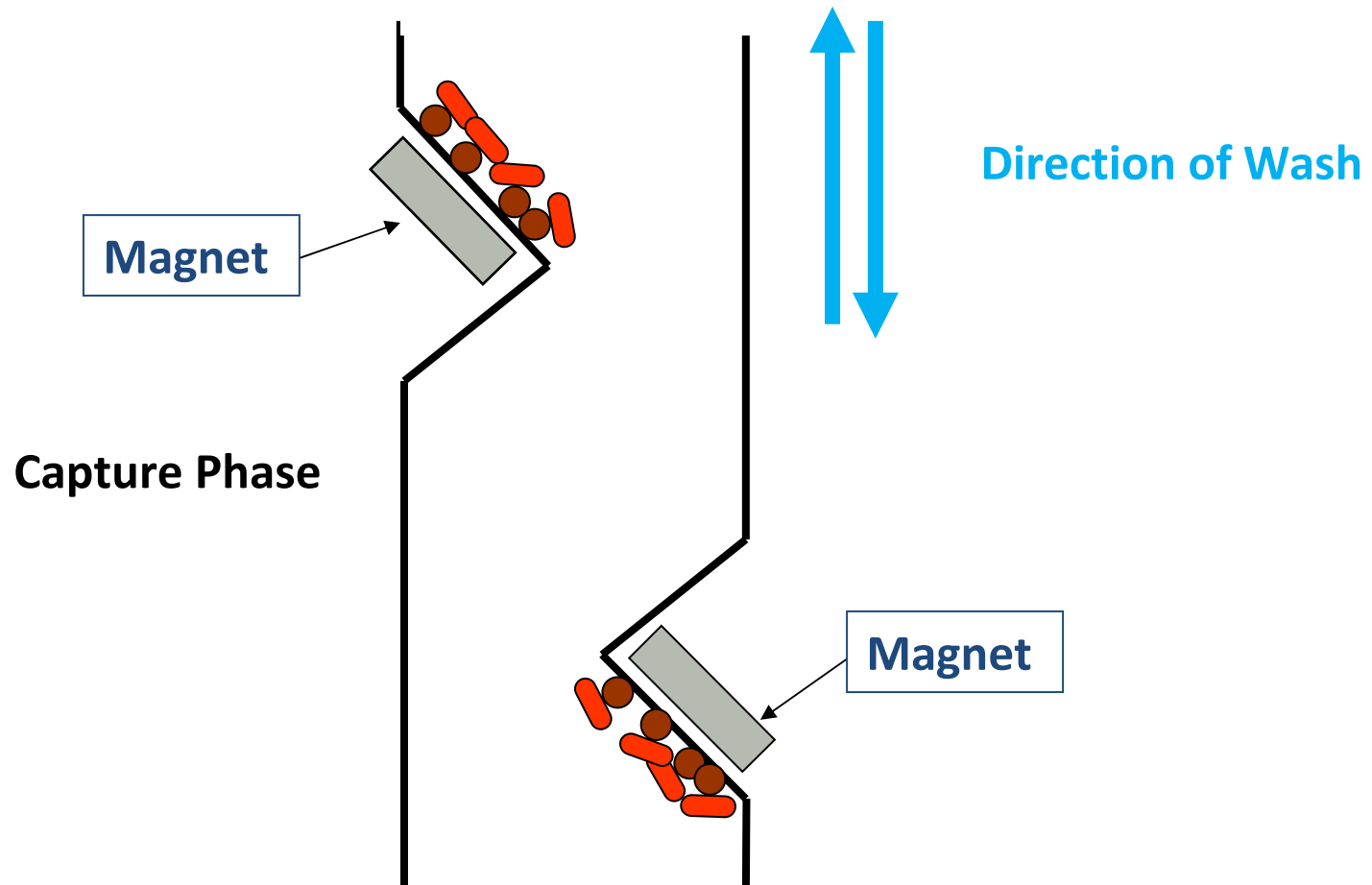
Antibody coated beads immobilise onto the "Capture Phase"



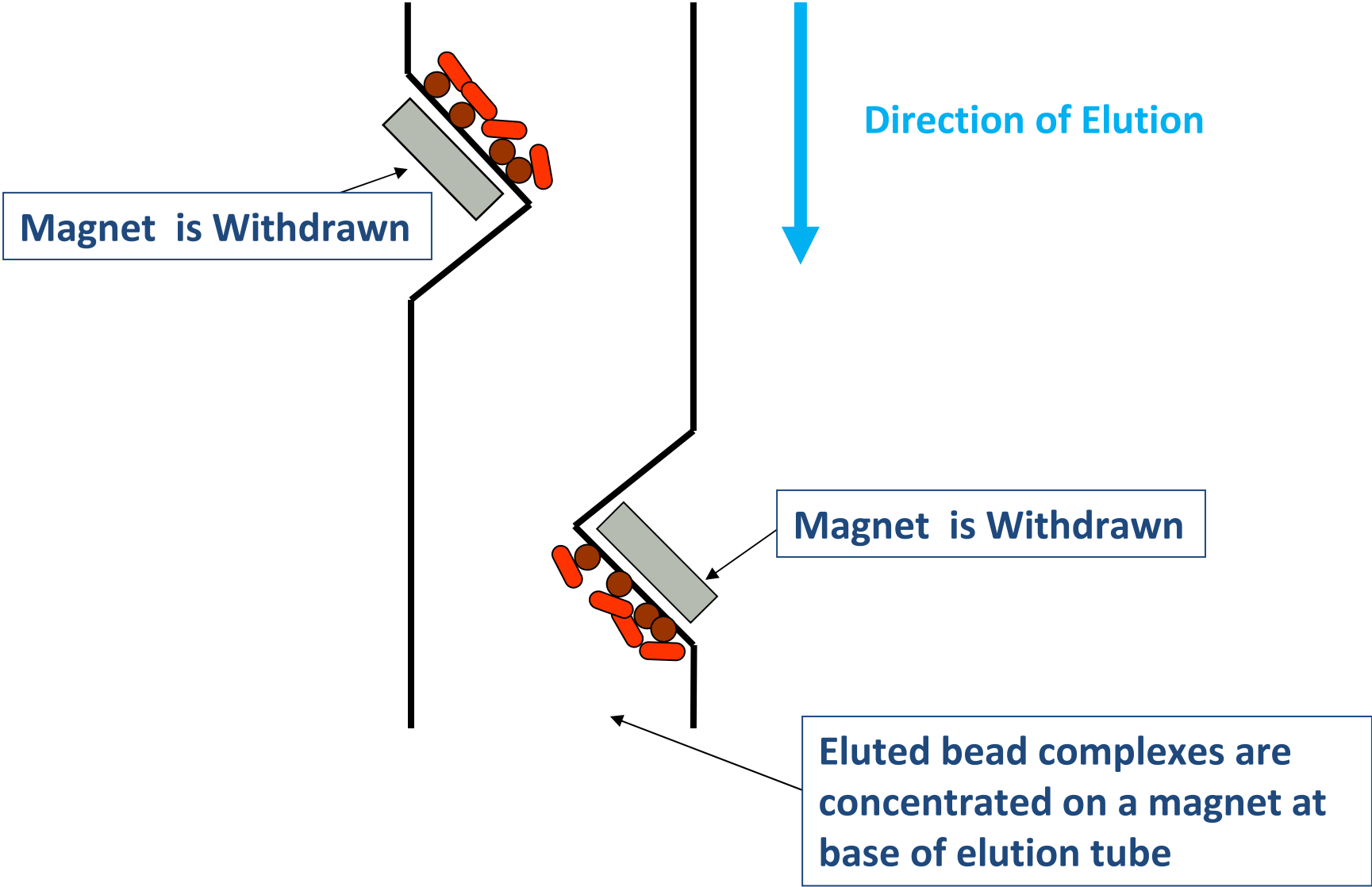
Specific capture of target onto the magnet from complex matrices



Captured Bead/Bacteria Complexes are “washed”

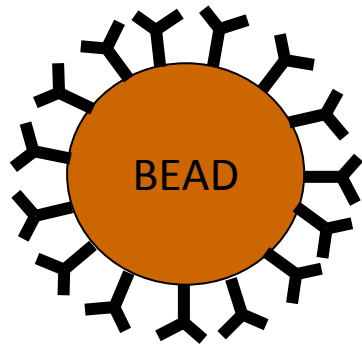


Bead / Bacteria complexes are Eluted from Magnet

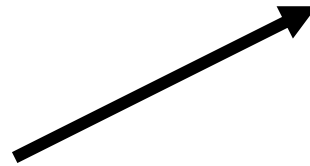




DETECTION OPTIONS



POST PATHATRIX
COMPLEX



PCR



EIA



Agar Plate



Y = Specific Antibody



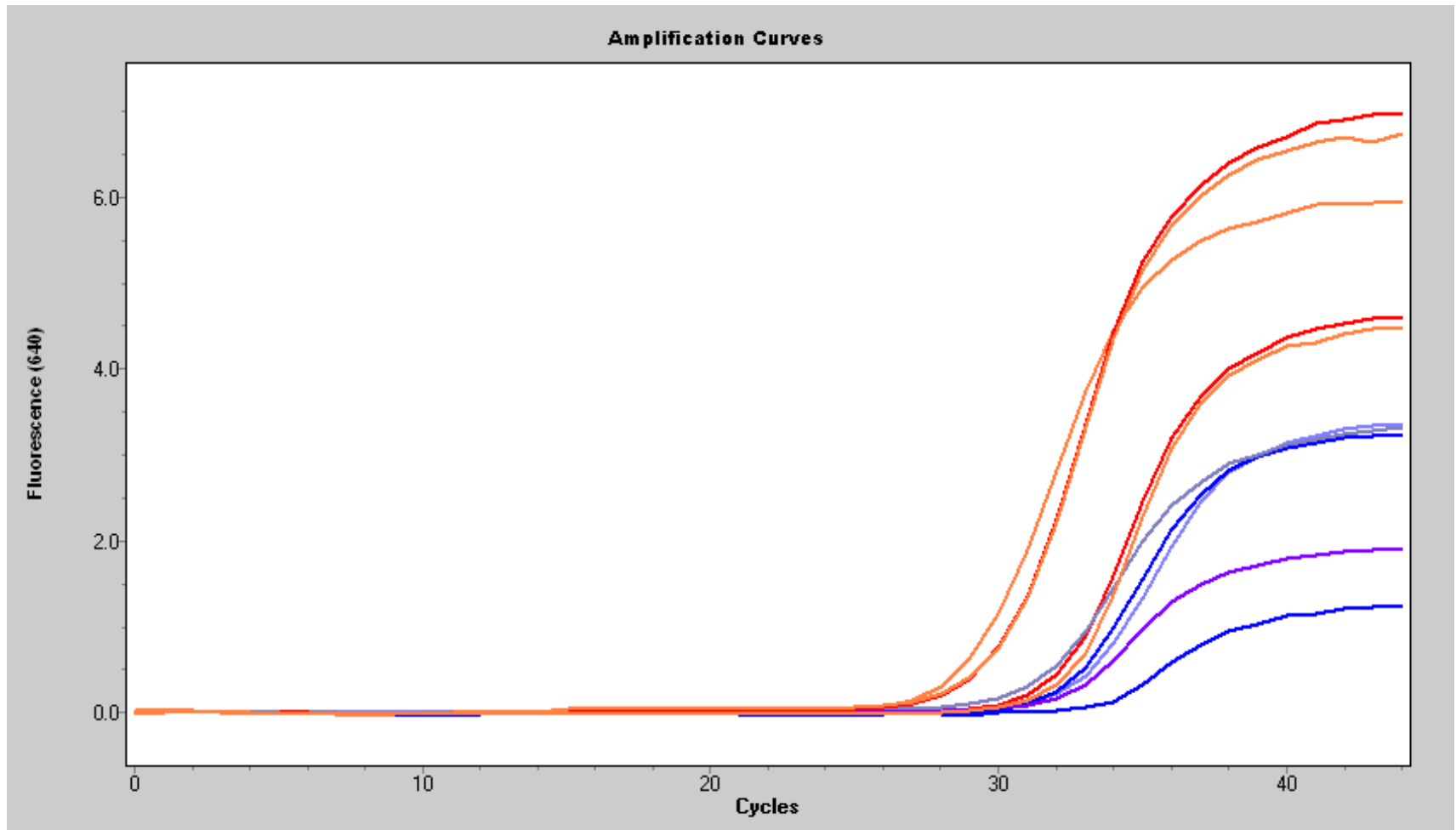
Method Development

Focused on several key areas:

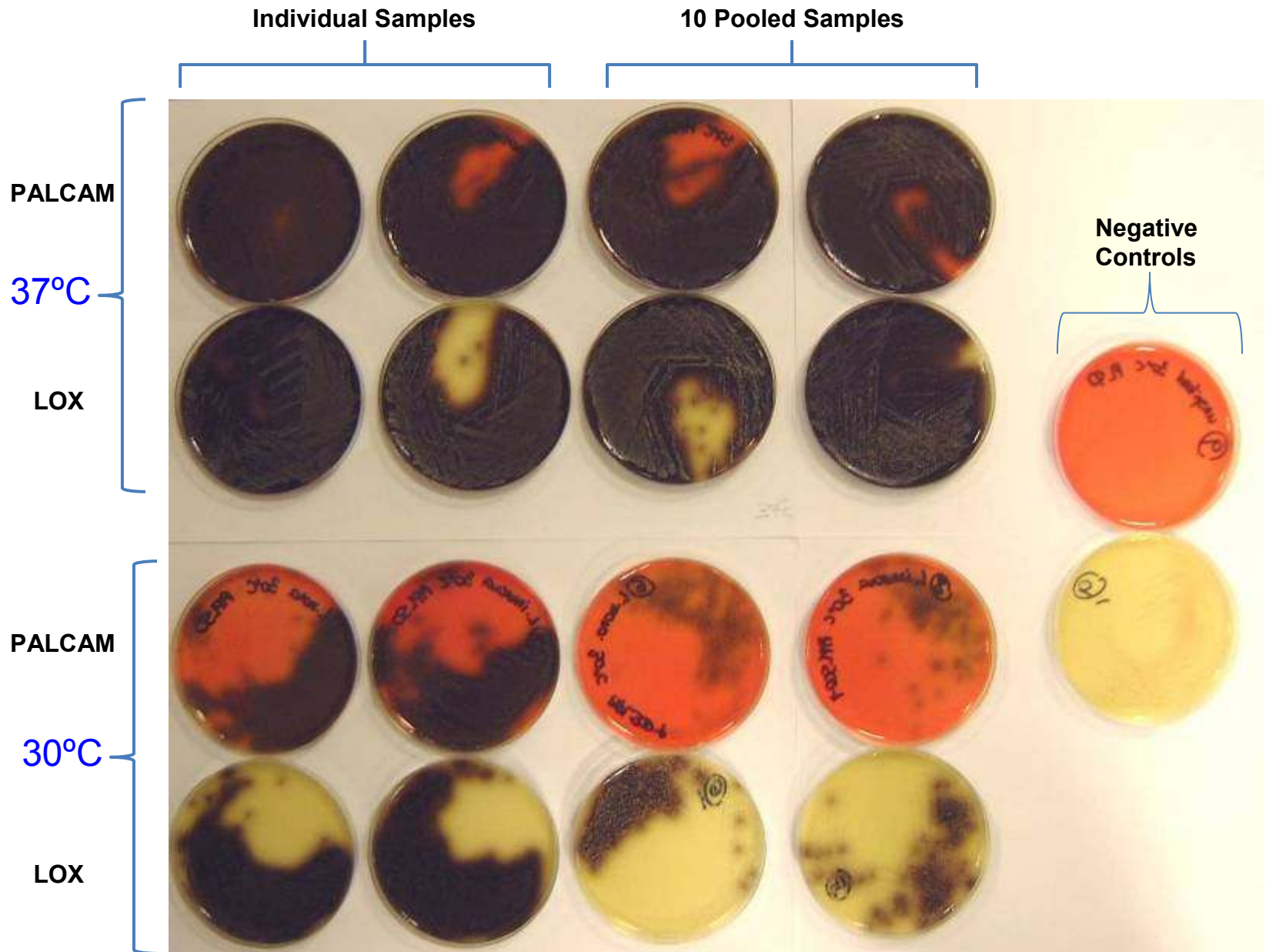
- **Enrichment Temperature**
- **Enrichment Time**
- **Pooling Strategy**
- **Linking to PCR**



Effect of enrichment temperature upon recovery

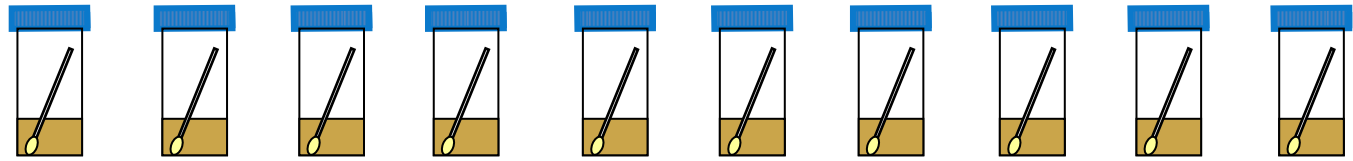


Effect of enrichment temperature upon recovery





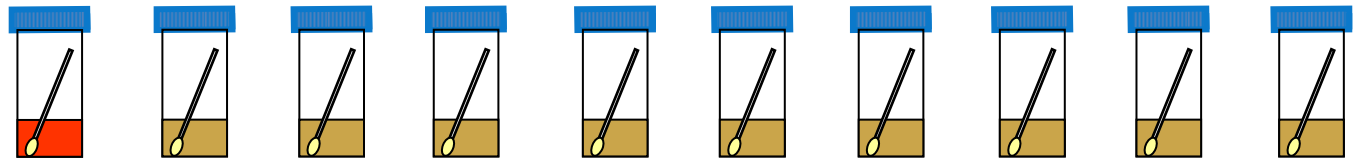
10 x Individual Swabs



Individual Enrichment of Swabs



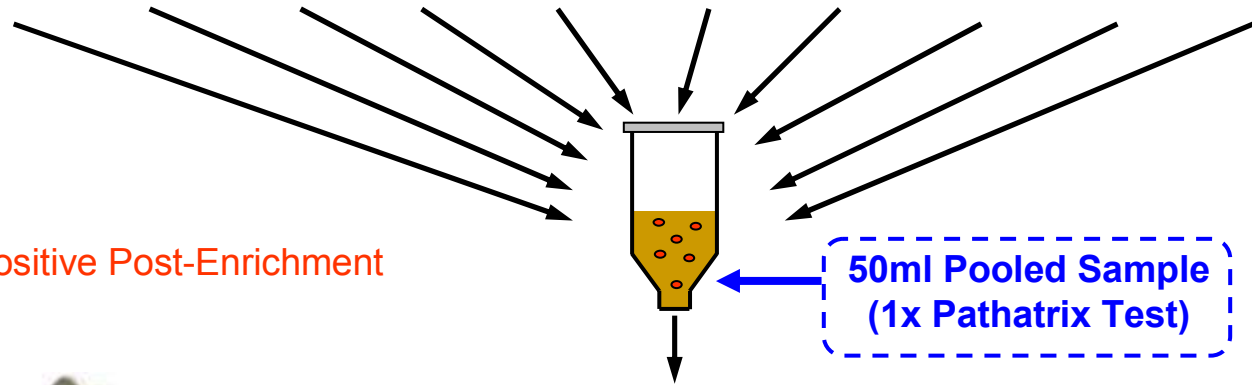
Post-Enriched Swabs



Pooling of Samples



= Positive Post-Enrichment



PCR



PATHATRIX[®] AUTO



Agar Plate

If Pooled Swabs are -ve; then all 10 Individual Swabs are -ve

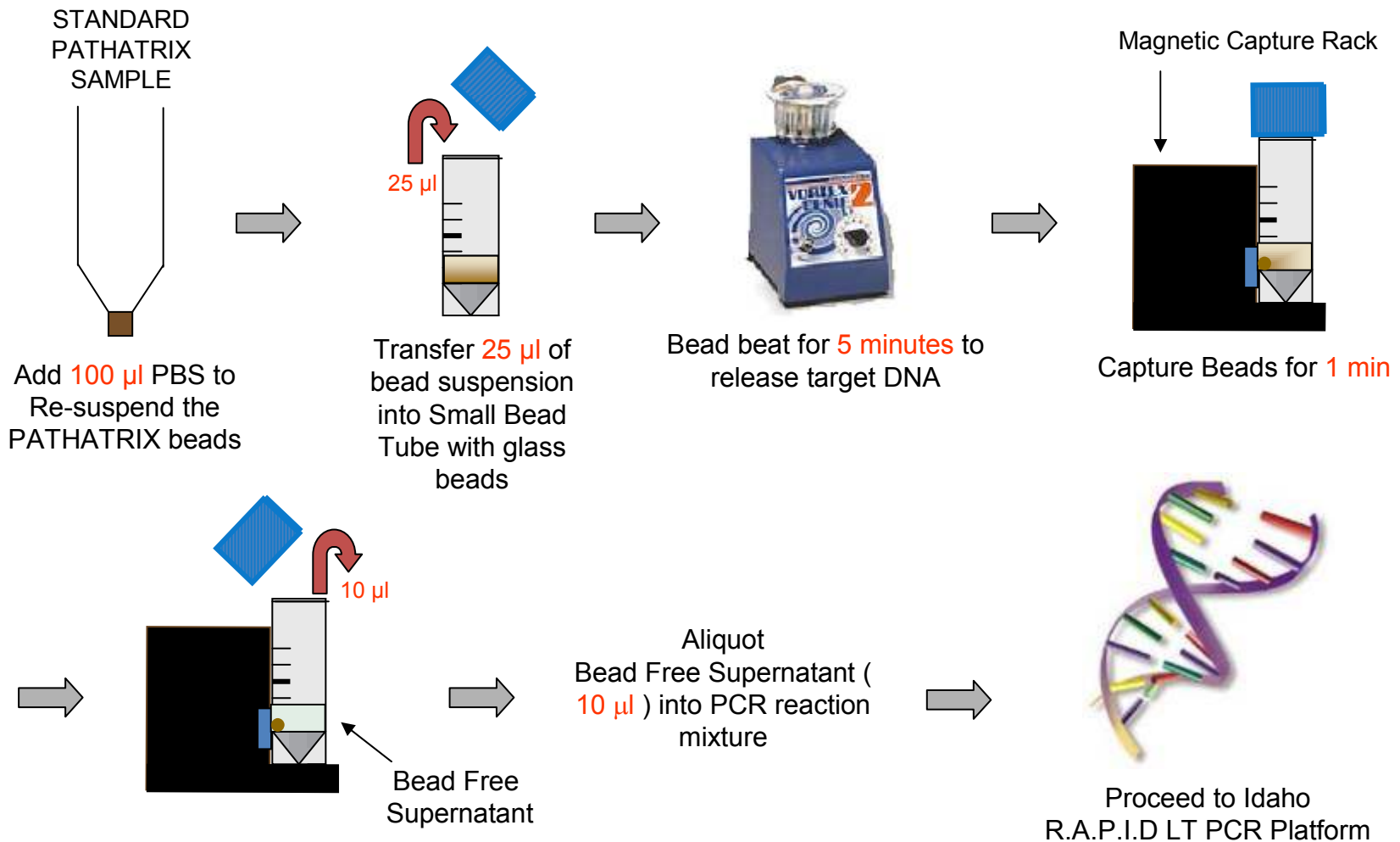
If Pooled Swabs are +ve; then Re-Test all 10 Individual Swabs Post-Enrichment



PATHATRIX® AUTO

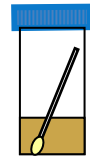


PATHATRIX to IDAHO: Bead Beating Protocol



BEAD BEATING LYSIS

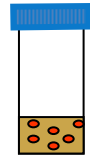
Listeria spp. 10 Pooling “Swab” Protocol



Add swab to 10 ml
of Half Fraser Broth



Incubate swabs for
22-24 hrs at 37°C



Pool 10 x 1 ml and run on
PATHATRIX AUTO +
IDAHO RAPID LT



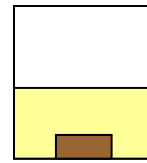
Test Pooled sample
with and re-test
individuals if positive

~ 23 hrs to a negative PCR result

~ 24 hrs to a positive PCR result



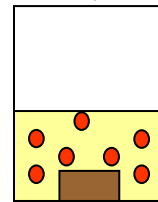
Listeria spp. 10 Pooling “Food” Protocol



Add 25 g food
sample to 225 ml of
Half Fraser Broth



Incubate samples for
22-24 hrs at 37°C



Pool 10 x 5 ml and run on
PATHATRIX AUTO +
IDAHO RAPID LT



Test Pooled sample
with and re-test
individuals if positive

~ 23 hrs to a negative PCR result

~ 24 hrs to a positive PCR result





***Listeria* spp. strains used in this study**

- ***Listeria monocytogenes* ATCC 7644**
- ***Listeria ivanovii* NCTC 11846**
- ***Listeria innocua* NCTC 11288**
- ***Listeria seeligeri* NCTC 10889**
- ***Listeria welshimeri* NCTC 11857**

All strains were inoculated onto the surfaces at 1-10 cfu / 100 cm² and into food samples at 1-10 cfu / 25 g



Surfaces and food samples tested in the study

*Surfaces**

- Sealed concrete flooring
- Stainless steel sink drain
- Floor Vinyl
- Tiles

Foods

- Prawns
- Salad
- Soft Cheese

* Area swabbed = 100 cm²

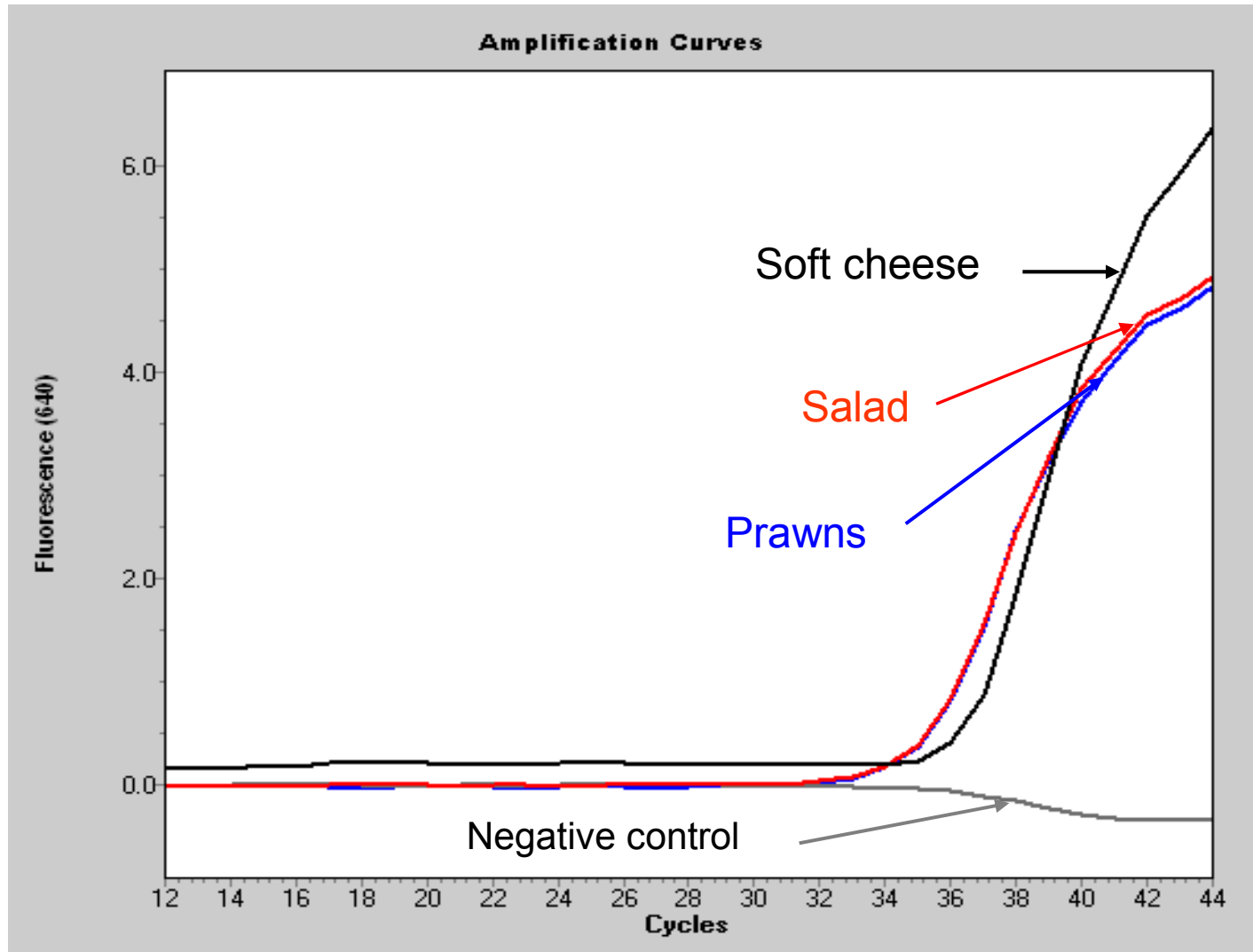


PATHATRIX 10 pooling swab & food sample protocol

- **30 samples from each food or surface type were tested individually**
 - **3 x 10^{*} pooled samples were created and tested within each food and swab surface type.**
 - **4 x 10^{*} for swabs and 3 x 10^{*} food pooled samples were randomly created by mixing food/swab surface types**
 - **All samples were analysed using the specific protocol developed for the study**
- * Only 1 sample in each 10 pooled sample was inoculated to provide the greatest challenge***

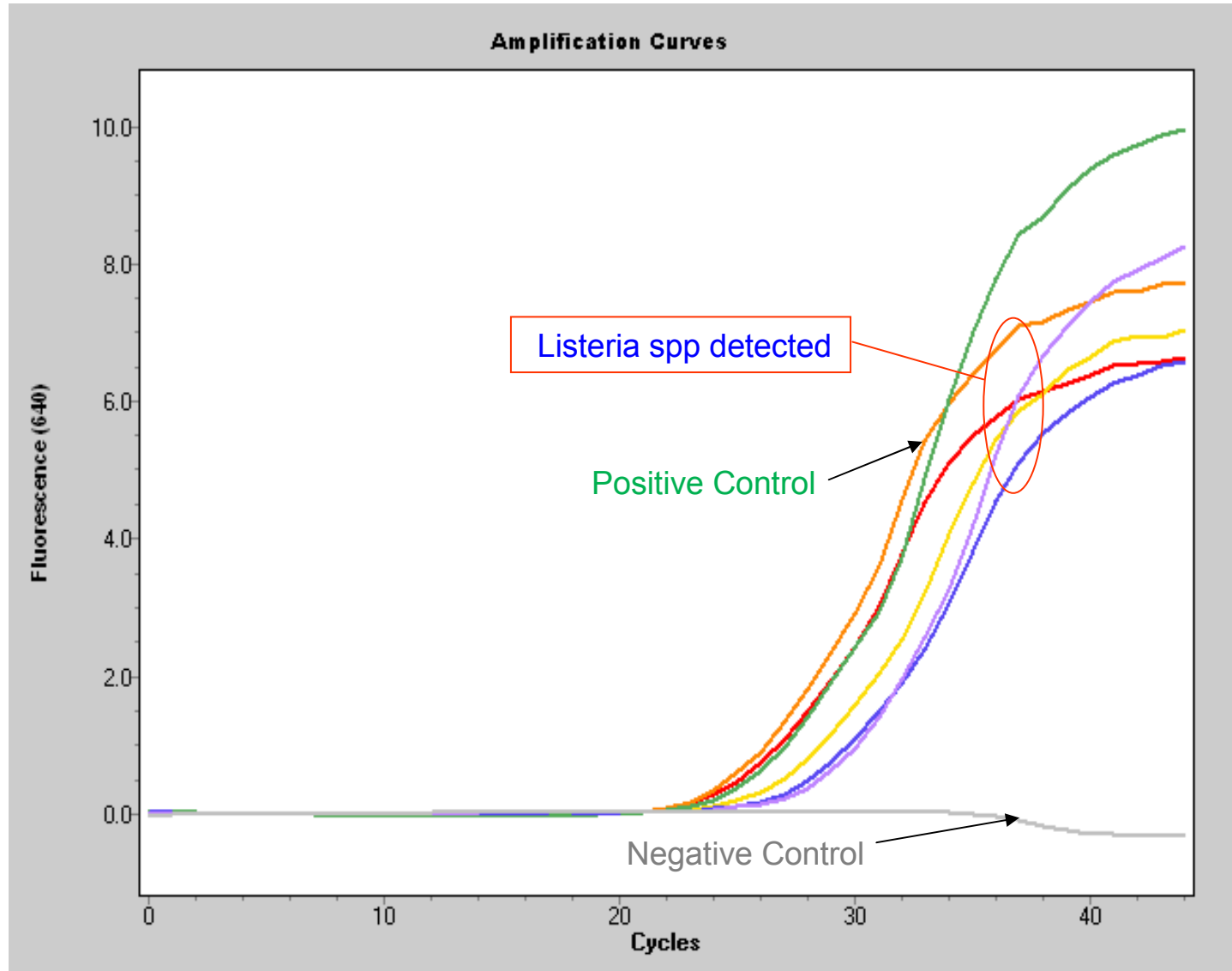


Typical Results for 10 pooled Food Samples





Typical Results for 10 pooled Swab Samples





Summary of Results

- **All 30 individual food samples and surface type swabs that were inoculated were detected**
- **All inoculated pooled samples from within each food and surface type were positive**
- **All inoculated “Cross matrix” – surface swabs and cross food type samples that were randomly pooled samples were positive**

Conclusion

- **The protocol developed enables *Listeria* spp. to be detected at low level inoculation levels (1-10 cfu) from food/swab samples, in less than 24 hrs (including re-testing of positives)**
- **The use of the 10 pooling strategy on post enriched samples is very cost effective, as costs are reduced by approximately 80-90%**





Acknowledgements:

- Dr John Murray
- Nicole Prentice
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