



Rapid Methods from Oxoid, Thermo Fisher Scientific - Microbiology

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Introduction

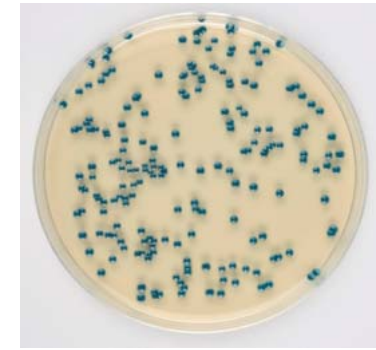
- What is a rapid method
- Rapid methods from Oxoid, Thermo Fisher Scientific-Microbiology
- Culture based rapid methods
 - Precis methods
 - Chromogenic media
- PCR based rapid methods
- Future of rapid methods
- Conclusions

What is a rapid method?

- A rapid method can be defined as:
- “Any method that reduces the time taken to obtain a result”
- Covers all methods:
 - Enumeration (quantitative)
 - Detection (qualitative)
 - Identification/confirmation

Rapid Methods from Oxoid, Thermo Fisher Scientific

- Listeria Precis
- Salmonella Precis
- Oxoid Listeria Rapid Test (OLRT)
- Oxoid Salmonella Rapid Test (OSRT)
- *Brilliance*TM Staph 24 Agar
- Identification and Confirmation kit-MicrobactTM, O.B.I.S and latex kits
- Western European, Canadian and Australian distributor of DuPont Qualicon BAX[®] System –molecular identification method



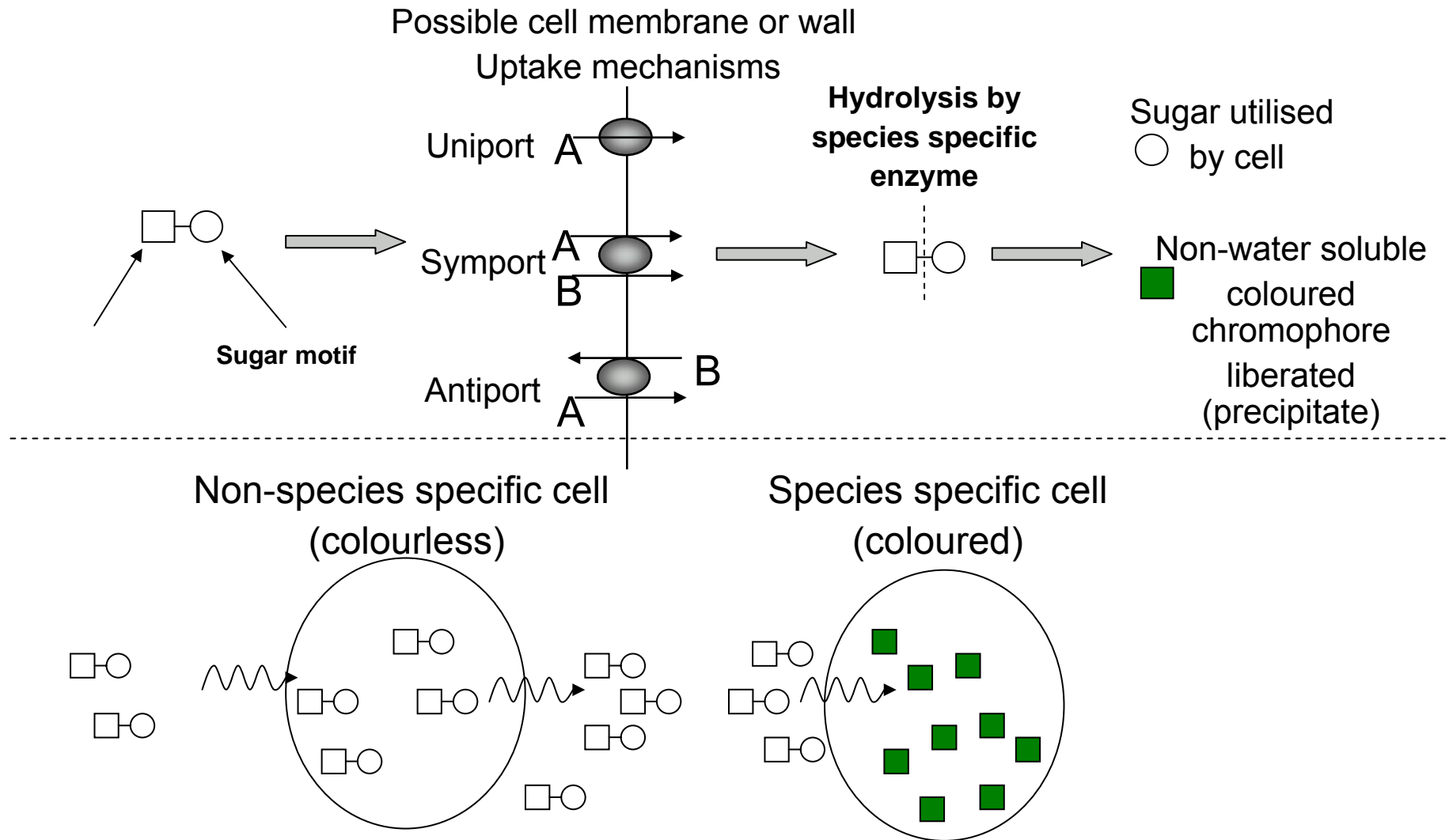
Culture based rapid methods: advantages

- Chromogens-can be thought of as an “upgrade” to traditional indicator systems (e.g. XLD Agar -H₂S production)
- Chromogens allow rapid presumptive identification of target organism
- Specific for a single biochemical pathway (form of biochemical ID)– reduces further confirmatory testing
- Highly coloured colonies easily visible & simpler to interpret when faced with mixed cultures
- Improved differentiation
- Simple, requires no training
- No capital outlay
- Definitive positive obtained

Chromogenic media

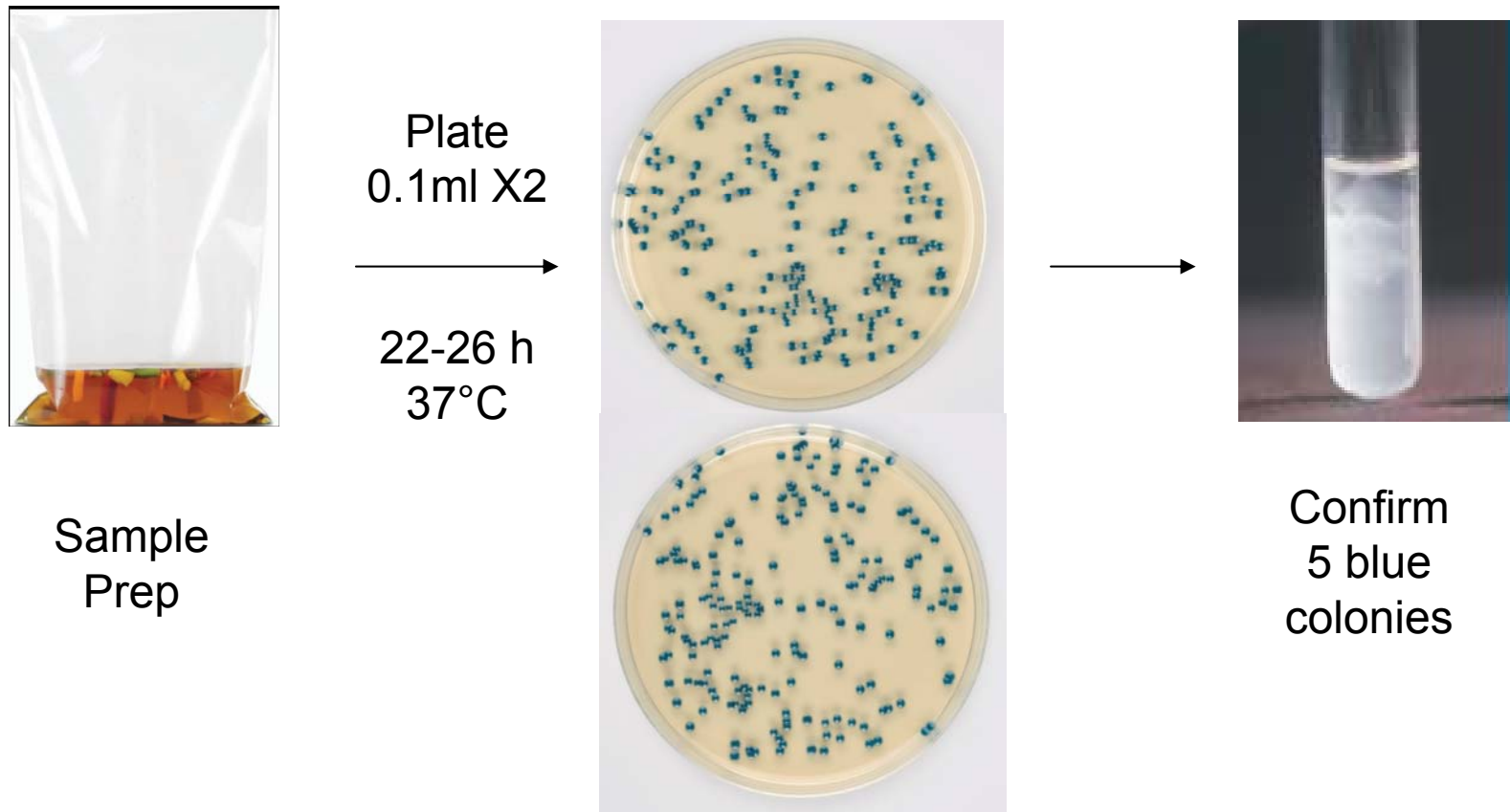
- Chromogens:
 - “compounds without colour, that can be transformed to a coloured product or which can react with another material to form a coloured compound”
- Four types of chromogenic reactions:
 - Oxidative step e.g. indoxyl substrates
 - Chelation with metal ions e.g. esculin
 - Addition of a developing reagent e.g. DMAC in the indole reaction
 - Require auxiliary agents, e.g. ALDOL™ substrates

Basic overview of how chromogens work



Culture based rapid methods

- Use either stand alone chromogenic agar plate or incorporate a chromogenic plate in the method.
- Stand alone: *Brilliance*[™] Staph 24 Agar



Salmonella Precis Method

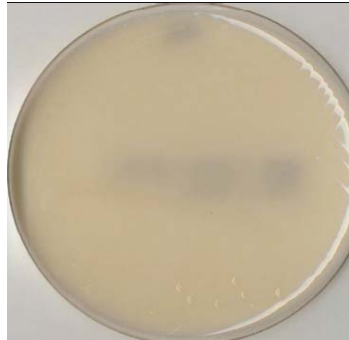
- Validated to ISO 16140:2003 for all human foods, animal feeds and environmental samples
- Traditional culture of 1 broth and 1 plate
- 18 hour enrichment
- Single sample transfer - broth to plate
- Chromogenic Plate contains Inhibigen™ Technology
 - Reduces false presumptive positives
- Simple confirmation method – Oxoid Salmonella latex test
- Time to result – 2 days

Culture based rapid methods

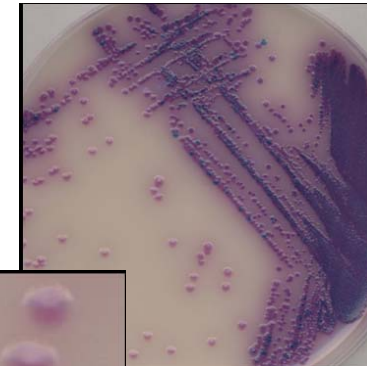
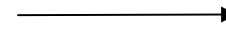
- Method based: PreciS methods. Salmonella PreciS



16-20 h
42°C



22-26 h
37°C



25g or 25 ml sample +
225ml ONE Broth
Salmonella

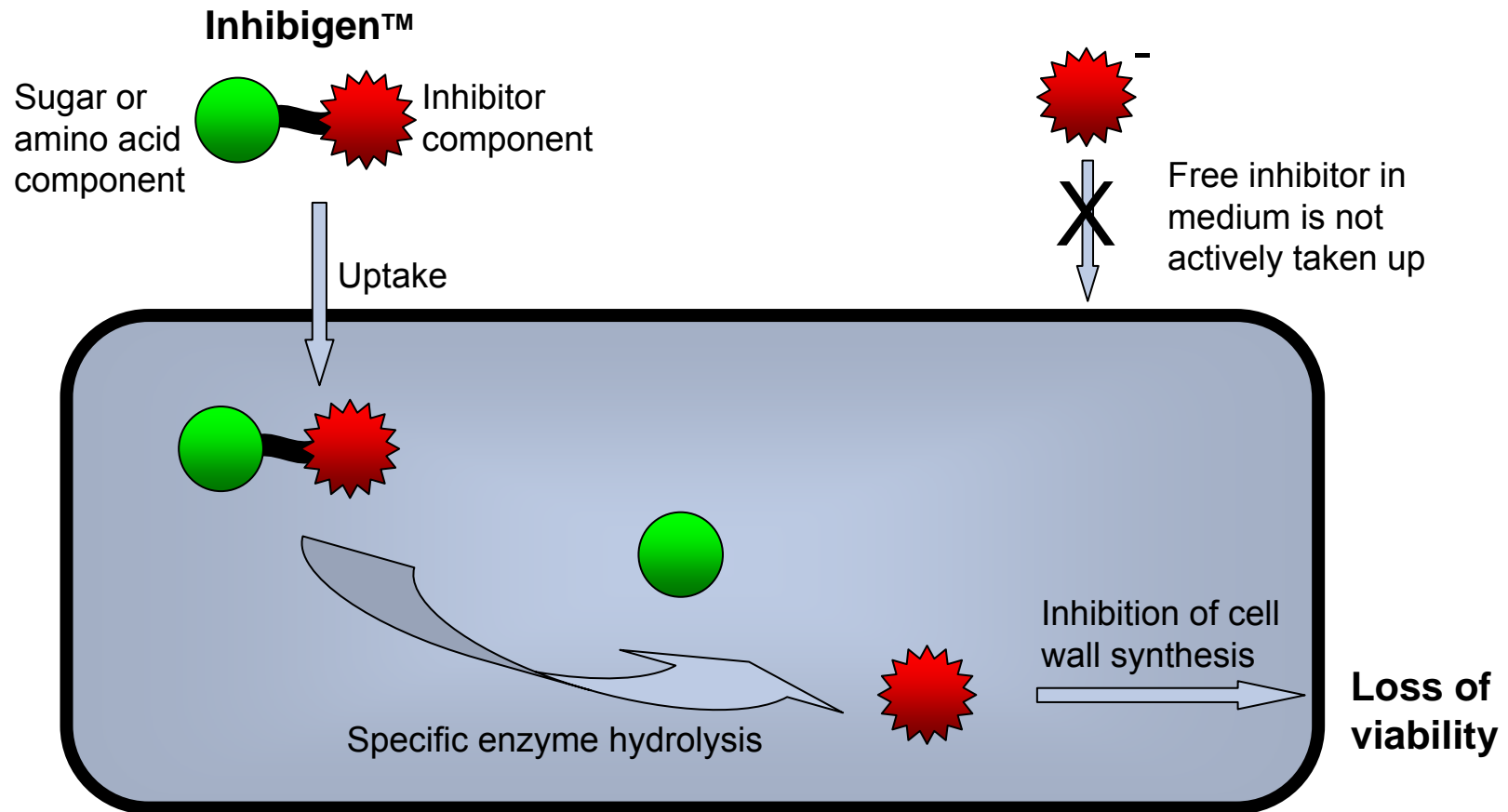
Plate 10µl onto single
Brilliance Salmonella plate

If present, confirm isolated
purple colonies using
Oxid Salmonella Latex
Test.



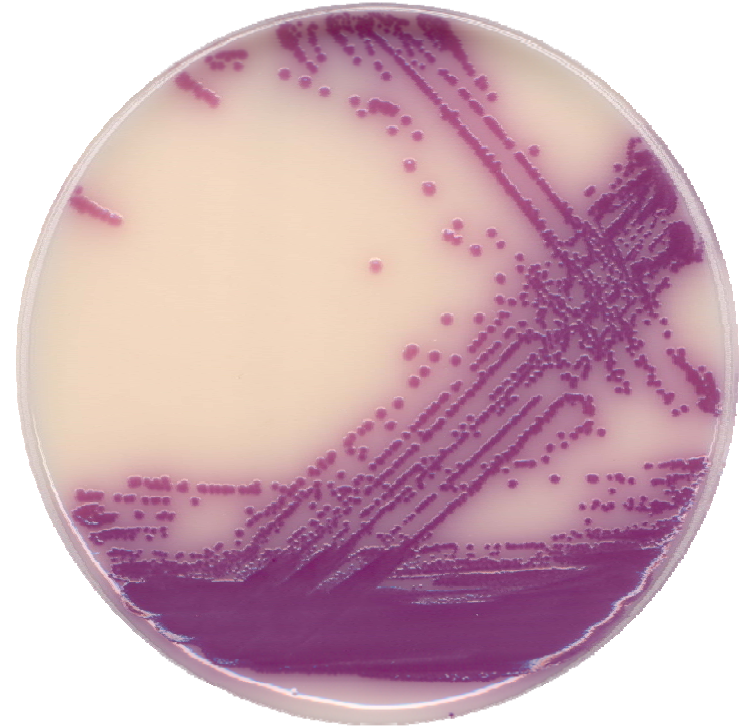
Inhibigen™ technology: *Brilliance* Salmonella Agar

Novel inhibitors based on enzyme specificity



Inhibigen™ technology: *Brilliance* Salmonella Agar

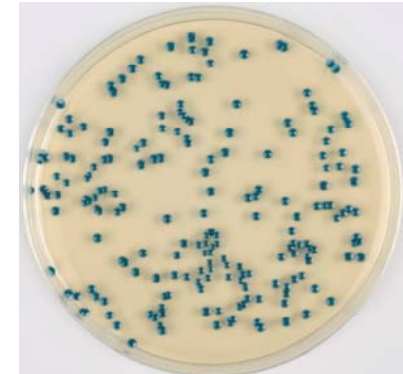
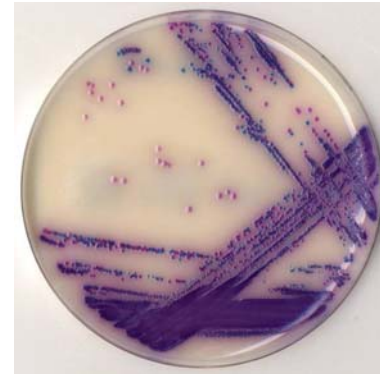
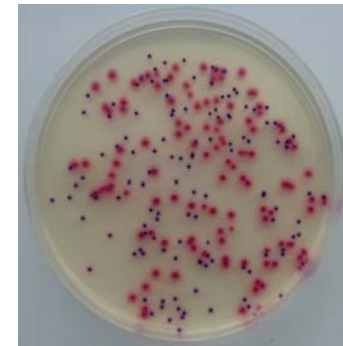
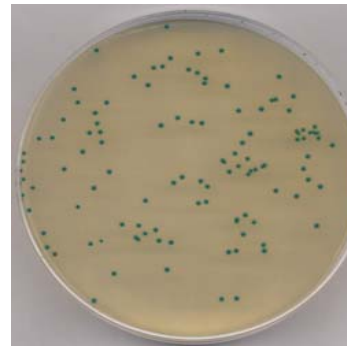
- Most sophisticated medium on the market
- Incorporates chromogens for differentiation
 - Clear, easy to interpret results
 - High sensitivity
- Inhibigen included for improved selectivity
 - Inhibits growth of “false-positives”
 - High specificity



• *Brilliance*™ Salmonella Agar

Oxoid *Brilliance* Agar: Industrial range



- *B. cereus*
- *C. jejuni* and *C. coli*
- *Cronobacter sakazakii*
- *E.coli*/coliforms
- *Listeria* spp.
- Salmonella
- Coagulase-positive Staphylococci



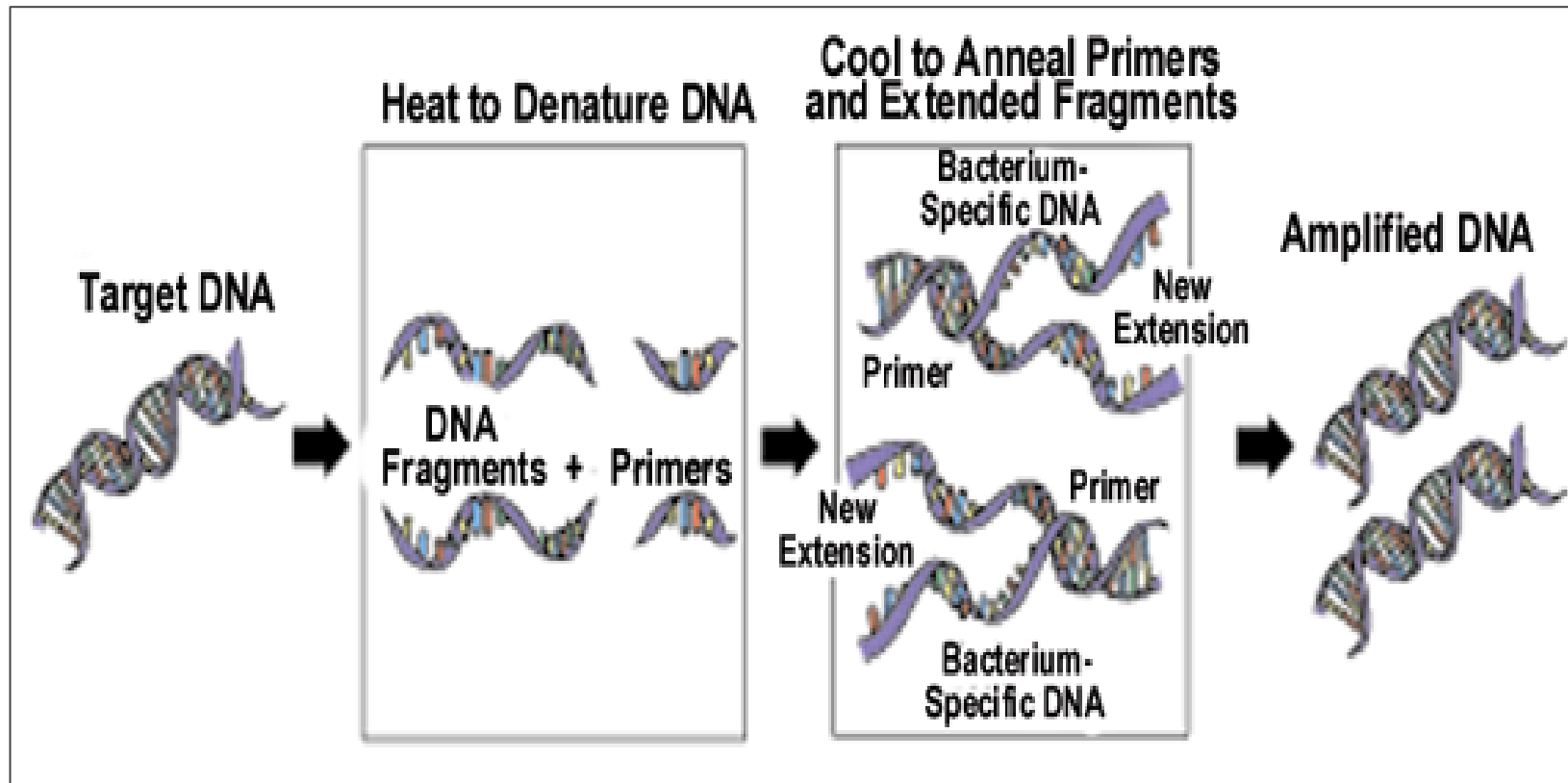
PCR based rapid methods

- DuPont Qualicon BAX[®] System



- Highly specific-targeted DNA sequence
- Proven, accurate technique
- Fast time to result-same day or next day
- Simple to use-tableted reagents
- Automated result interpretation  
- BAX tests for: *Listeria monocytogenes*, *Listeria* spp. *E. coli* O157,
- *Salmonella*, *Campylobacter*, *Staph. aureus*, Yeast and moulds, *Vibrio* spp., *Cronobacter sakazakii*

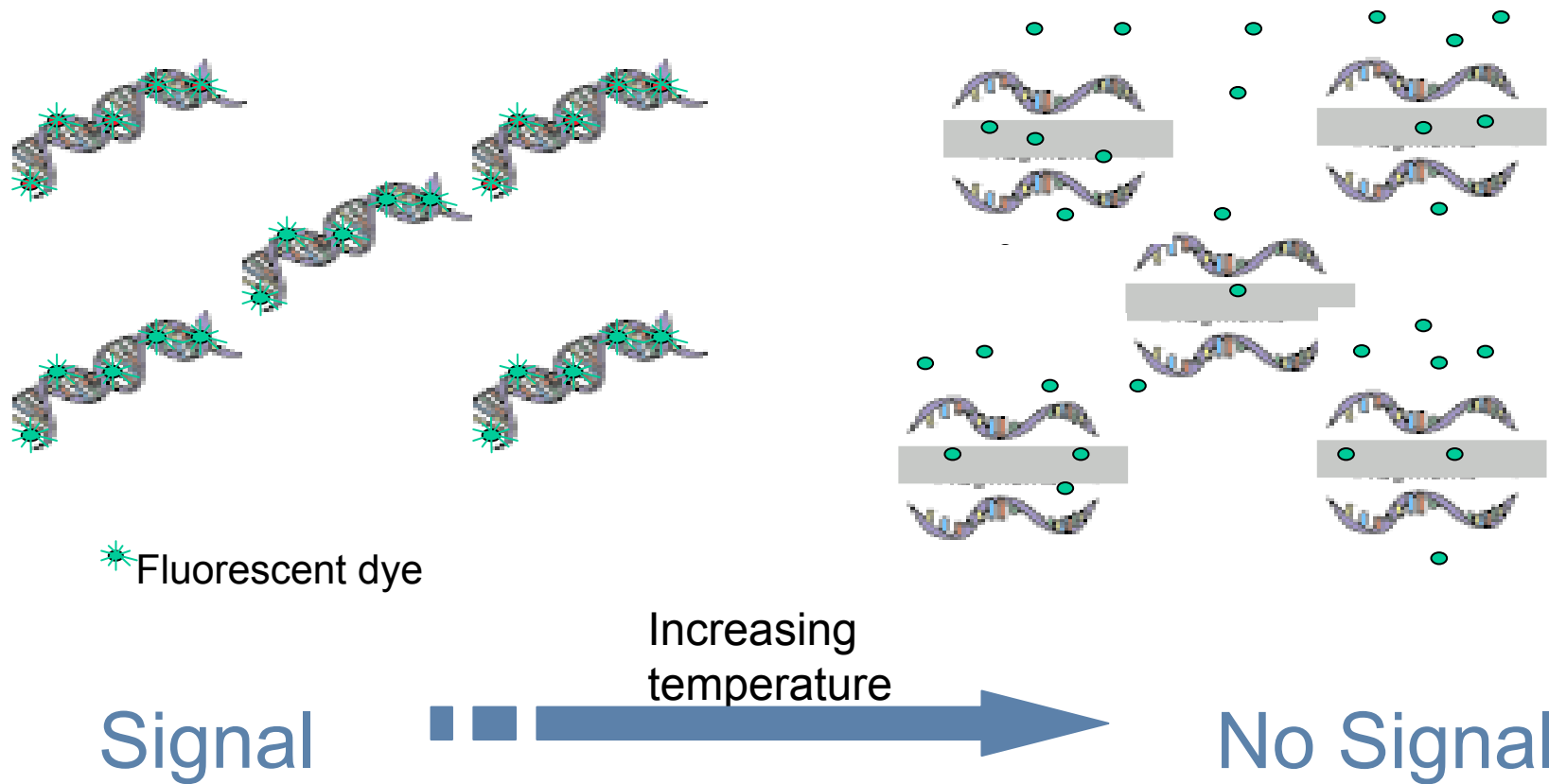
PCR process



Standard BAX[®] PCR (1)

Standard PCR assays

Fluorescent signal drops with heat to generate a melt curve

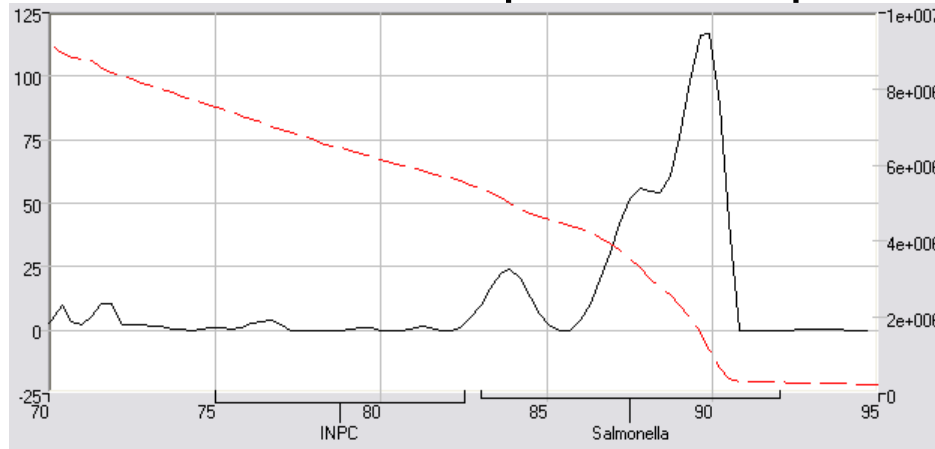


Standard BAX[®] PCR (2)

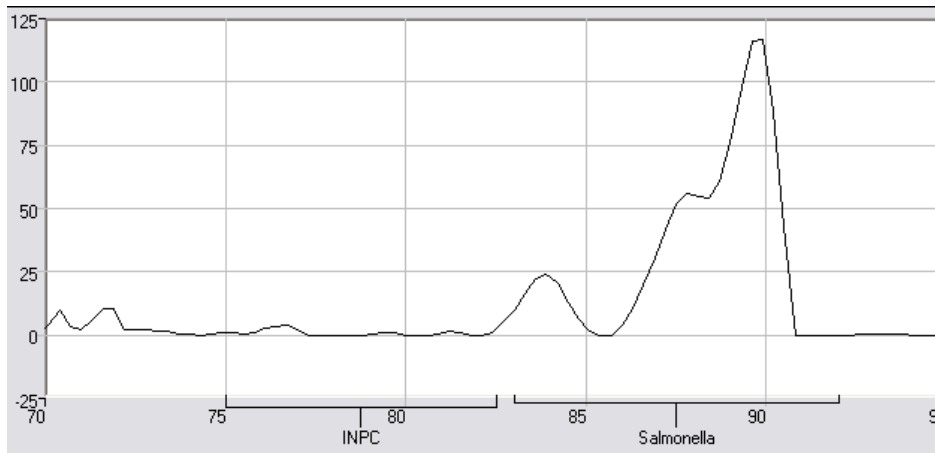
Standard PCR assays

Algorithms convert raw melt curve to processed peaks

Raw
Data



Processed
Data

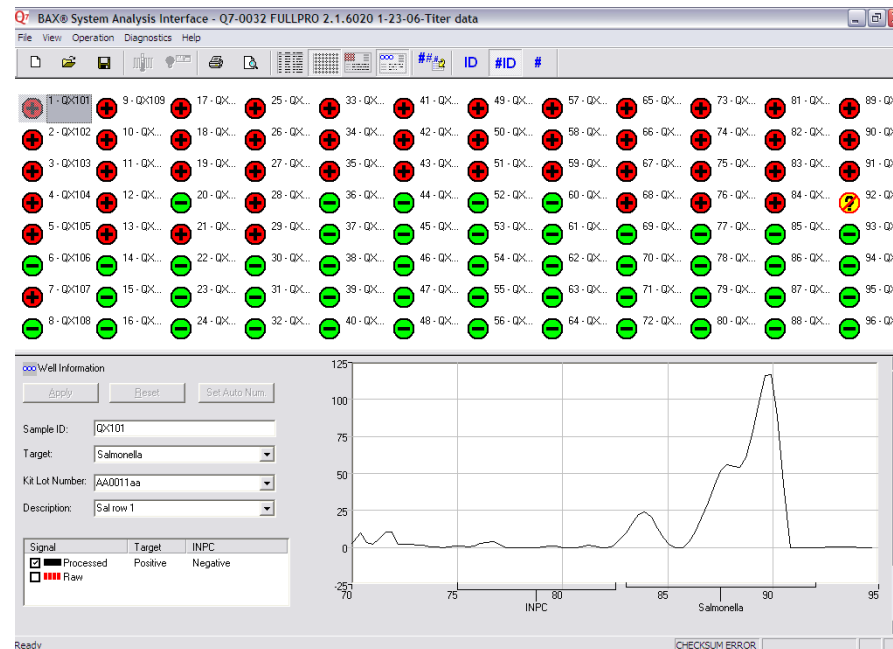


Standard BAX[®] PCR (3)

Standard PCR assays

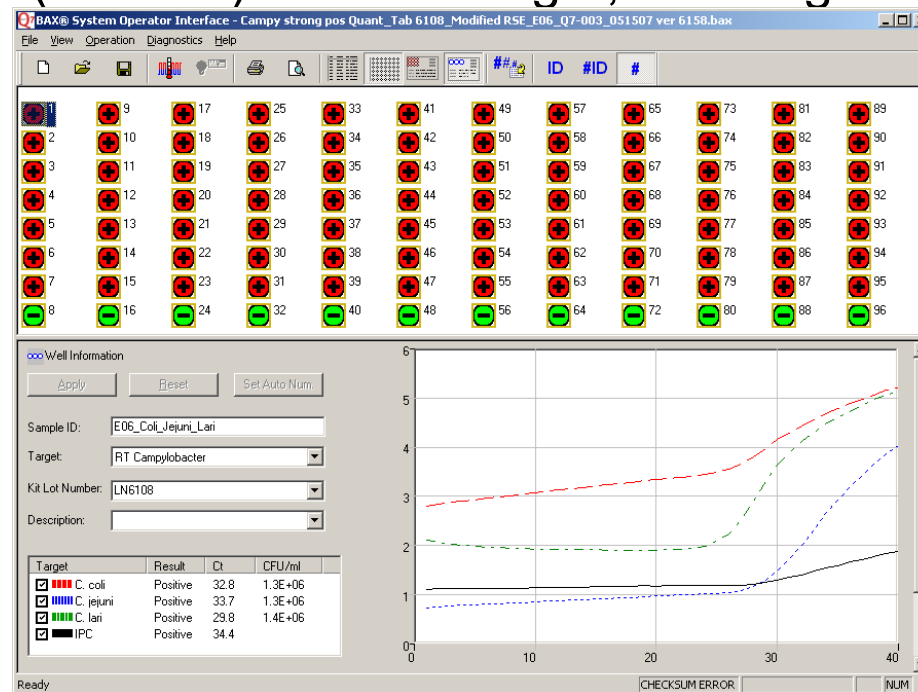
Analysis reports positive or negative result

- After detection, raw data is smoothed and transformed automatically
- Transformed data is analysed
- Output is a positive or negative value



BAX[®] Real Time assays

- Detection integrated with amplification – 90 minutes processing
- When fluorescent signal reaches the detectable threshold (Ct), the amplification curve begins to rise
- Qualitative output is positive or negative
- Quantitative output (CFU/ml) for each target, referring to concentration going into lysis



BAX[®] Reverse Transcriptase PCR (Listeria-environmental samples)

- Reverse transcriptase PCR utilises the multiple RNA copies present to provide a “jump-start” to the DNA PCR based method
- Ribosomal RNA (rRNA) from the sample is combined with Reverse-Transcriptase (RNA-dependent DNA polymerase), DNA polymerase, nucleotides and specific primers for a given sequence
- The mixture then goes through a series of timed heating and cooling cycles and primers anneal (bind) to the many copies of rRNA sequences
- Increasing the temperature activates Reverse Transcriptase, which synthesizes single strands of complementary DNA
- The temperature is then raised higher to stop the RNA reactions and activate the thermostable Taq-polymerase
- During the cooling cycle, primers then bind to the single strands of complimentary DNA
- Taq polymerase uses nucleotides to extend the primers, creating double-stranded DNA, and the reaction proceeds as in standard PCR

Future of Rapid Methods

- Culture:
 - More novel chromogens to improve detection of current and additional target organisms
 - Improved selectivity of existing culture media using Inhibigen™ Technology
 - Improved media formats to help laboratory workflow
- Molecular:
 - Increased availability of real time assays
 - Multiplex PCR assays to allow detection of multiple targets
 - Simpler methods of analysis

Conclusions

- Thermo Fisher Scientific-Microbiology, provide a wide range of traditional culture media and rapid culture based methods under the Oxoid and Remel brands

remel



- Chromogenic and method based rapid methods available are simple traditional culture based methods for rapid detection of food pathogens
- Molecular detection available using DuPont Qualicon BAX[®] system
- Rapid alternative methods only option for industry as reference methods slow, expensive and/or difficult

**Thank you for listening
any questions?**