# FOODBORNE PATHOGENS

Eurofins Technologies - @Argenta
05.10.2022

Ricardo Silva e Sousa

RicardoSilvaeSousa@eurofins.de

Jr. Product Manager

Agenda

- Why are we here? Statistics on Salmonella, Listeria & STEC E. coli

- Foodborne pathogens – regulation, methods & markets

- Portfolio overview – What is the BAC*Gene* product line

- Extra troubleshooting methods

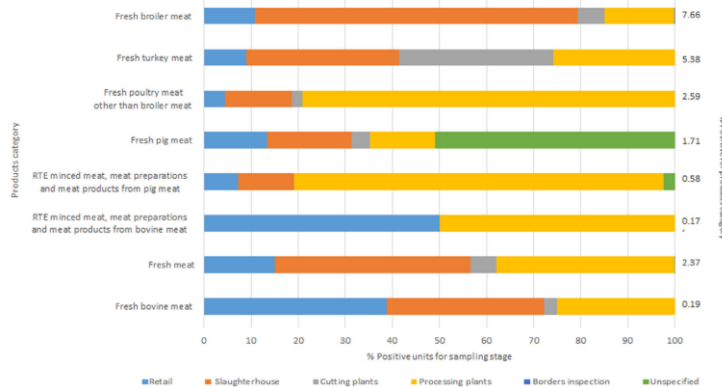- Available tools

- Technical overview

Why are we here?
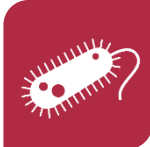
Salmonella

	2019	2018	2017	2016	2015	Data source
Humans						
Total number of confirmed cases	87,923	91,858	91,587	94,425	94,477	ECDC
Total number of confirmed cases/100,000 population (notification rates)	20.0	20.1	19.7	20.5	21.0	ECDC
Number of reporting MS	28	28	28	28	28	ECDC
Infection acquired in the EU	58,271	59,763	59,642	52,852	51,898	ECDC
Infection acquired outside the EU	6,343	6,376	6,001	6,466	6,830	ECDC
Unknown travel status or unknown country of infection	23,309	25,719	25,944	35,107	35,749	ECDC
Number of outbreak-related cases	9,169	11,631	9,607	11,428	8,531	EFSA
Total number of outbreaks	926	1,588	1,241	1,372	1,216	EFSA
Food						
Meat and meat products						
Number of sampling units	525,704	433,197	380,000	285,564	211,072	EFSA
Number of reporting countries	28	28	28	27	27	EFSA
Milk and milk products						
Number of sampling units	46,797	44,078	30,796	24,337	29,034	EFSA
Number of reporting countries	25	24	24	24	22	EFSA
Fish and fishery products						
Number of sampling units	14,010	17,123	13,507	12,287	11,373	EFSA
Number of reporting countries	24	22	22	21	22	EFSA
Eggs and egg products						
Number of sampling units	12,093	10,611	15,435	10,933	9,650	EFSA
Number of reporting countries	21	21	23	20	19	EFSA
Fruits and vegetables (and juices)						
Number of sampling units	17,068	10,888	7,579	7,515	6,797	EFSA
Number of reporting countries	22	22	25	20	22	EFSA

Summary of Salmonella statistics related to humans and major food categories, EU, 2015–2019

Summary of *Salmonella* monitoring results, by major meat and meat products categories and by sampling stage in the food chain, EU, 2019

Salmonella:

- Second most common zoonosis in humans in the EU

- poultry meats – where *Salmonella* was most frequently reported

- egg and eggs products ranked first of food vehicles

- Previous decreasing trend has stabilized

The fight is not being won. Rates of cases are flat.

Country	2019				2018		2017		2016		2015	
	National coverage[a]	Data format[a]	Total cases	Confirmed cases & rates		Confirmed cases & rates		Confirmed cases & rates		Confirmed cases & rates		Confirmed cases & rates
				Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate
Poland	Y	C	8,919	8,373	22.0	9,064	23.9	8,921	23.5	9,718	25.6	8,245	21.7

Reported human cases of salmonellosis and notification rates per 100,000 population in the EU/EFTA, by country and year, 2015–2019

EFSA Journal 2021;19(2):6406

Listeria

eurofins | Technologies

	2019	2018	2017	2016	2015	Data source
Humans						
Total number of confirmed cases	2,621	2,545	2,475	2,500	2,183	ECDC
Total number of confirmed cases/100,000 population (notification rates)	0.46	0.47	0.48	0.47	0.43	ECDC
Number of reporting MS	28	28	28	28	28	ECDC
Infection acquired in the EU	1,817	1,640	1,639	1,539	1,450	ECDC
Infection acquired outside the EU	12	8	4	6	7	ECDC
Unknown travel status or unknown country of infection	792	897	832	955	726	ECDC
Number of outbreak-related cases	349	159	39	27	233	ECDC
Total number of outbreaks	21	14	10	6	15	EFSA
RTE food categories[a]						
RTE milk and milk products	N = 62,019; 23 MS	N = 59,313; 23 MS	N = 56,428; 25 MS	N = 34,850; 26 MS	N = 45,996; 24 MS	EFSA
RTE meat and meat products	N = 64,666; 22 MS	N = 57,861; 22 MS	N = 45,219; 24 MS	N = 25,195; 21 MS	N = 25,396; 22 MS	EFSA
RTE fish and fishery products	N = 13,376; 22 MS	N = 14,081; 22 MS	N = 12,604; 24 MS	N = 6,601; 23 MS	N = 7,986; 25 MS	EFSA
Other RTE food products	N = 76,657; 24 MS	N = 25,179; 22 MS	N = 23,915; 23 MS	N = 21,085; 22 MS	N = 25,544; 23 MS	EFSA
RTE foods intended for infants and for special medical purposes	N = 1,721; 18 MS	N = 1,663; 18 MS	N = 1,462; 20 MS	N = 1,274; 16 MS	N = 1,754; 12 MS	EFSA

Summary statistics on human invasive *L. monocytogenes* infections and on sampled major RTE food categories in the EU, 2015–2019

Country	2019					2018		2017		2016		2015	
	National coverage[a]	Data format[a]	Total cases	Confirmed cases and rates		Confirmed cases and rates		Confirmed cases and rates		Confirmed cases and rates		Confirmed cases and rates	
				Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate
Poland	Y	C	121	121	0.32	128	0.34	116	0.31	101	0.27	70	0.18

Reported cases of human invasive listeriosis and notification rates per 100,000 population in the EU/EFTA, by country and year, 2015–2019

Listeria:

- The EU trend of confirmed listeriosis cases remained stable (flat) in 2015–2019 after a long period of an increasing trend.
- The overall EU case fatality was high (17.6%) - listeriosis one of the most serious food-borne diseases under EU surveillance

The battlefield is currently on the Ready To Eat products.
There was an increase of:
- 12% of the sampling units tested for 'RTE meat and meat products'
- 204% for 'other RTE food products'.
    - higher number of samples were tested for 'bakery products' (+75%),
    - 'broiler meat and meat products thereof' (+304%)
    - fruit and vegetables (+79%).

EFSA Journal 2021;19(2):6406

Why are we here?

STEC *E. coli*

	2019	2018	2017	2016	2015	Data source
Humans						
Total number of confirmed cases	7,775	8,161	5,958	6,474	5,929	ECDC
Total number of confirmed cases/100,000 population (notification rates)	2.21	2.28	1.67	1.79	1.65	ECDC
Number of reporting MS	27	28	28	28	28	ECDC
Infection acquired in the EU	4,835	5,783	4,747	4,037	3,991	ECDC
Infection acquired outside the EU	750	693	525	339	532	ECDC
Unknown travel status or unknown country of infection	2,190	1,685	686	2,098	1,406	ECDC
Number of food-borne outbreak-related cases	273	390	260	737	676	EFSA
Total number of food-borne outbreaks	42	50	48	43	70	EFSA
Food						
All						
Number of sampling units	25,030	20,498	19,351	17,977	13,777	EFSA
Number of reporting MS	22	20	22	17	17	EFSA
Meat and meat products						
Number of sampling units	14,110	9,250	10,706	8,771	7,865	EFSA
Number of reporting MS	20	17	18	17	15	EFSA
Milk and milk products						
Number of sampling units	5,479	5,339	3,485	3,773	3,005	EFSA
Number of reporting MS	13	14	10	11	8	EFSA
Fruits and vegetables (and juices)						
Number of sampling units	2,658	3,339	2,295	1,475	1,384	EFSA
Number of reporting MS	13	13	15	11	10	EFSA

Summary of STEC statistics related to humans and to major food categories, EU, 2015–2019

Reported human cases of STEC infections and notification rates per 100,000 population in the EU/EFTA, by country and year, 2015–2019

Country	2019							2018		2017		2016		2015	
	National coverage[a]	Data format[a]	Total cases	Confirmed cases and rates				Confirmed cases and rates		Confirmed cases and rates		Confirmed cases and rates		Confirmed cases and rates	
				Cases	Rate			Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate
Poland	Y	C	17	14	0.04			6	0.01	4	0.01	4	0.01	0	0.00

STEC *E. coli*:

- The EU/EEA trend has been increasing from 2015 to 2019.
- STEC - 3rd most frequent bacterial agent detected in food-borne outbreaks in the EU:
  - 42 outbreaks, 273 cases, 50 hospitalisations and 1 death (2019)
- Sprouted - tested by six MS with no positive STEC results from 331 official samples.
  - An EU regulation has been in force since 2013.

- Overall, STEC was most commonly found in:
  - meat of different types derived from different animal species (4.1% STEC-positive),
  - followed by 'milk and dairy products' (2.1%)
  - while 'fruits and vegetables' was the least contaminated category (0.1%).

EFSA Journal 2021;19(2):6406

EU Regulation

Food category	Micro-organisms/their toxins, metabolites
1.1. Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes [4]	Listeria monocytogenes
1.2. Ready-to-eat foods able to support the growth of L. monocytogenes, other than those intended for infants and for special medical purposes	Listeria monocytogenes
1.3. Ready-to-eat foods unable to support the growth of L. monocytogenes, other than those intended for infants and for special medical purposes [4] [8]	Listeria monocytogenes
1.4. Minced meat and meat preparations intended to be eaten raw	Salmonella
1.5. Minced meat and meat preparations made from poultry meat intended to be eaten cooked	Salmonella
1.6. Minced meat and meat preparations made from other species than poultry intended to be eaten cooked	Salmonella
1.7. Mechanically separated meat (MSM) [9]	Salmonella
1.8. Meat products intended to be eaten raw, excluding products where the manufacturing process or the composition of the product will eliminate the salmonella risk	Salmonella

Food category	Micro-organisms/their toxins, metabolites
1.9. Meat products made from poultry meat intended to be eaten cooked	Salmonella
1.10. Gelatine and collagen	Salmonella
1.11. Cheeses, butter and cream made from raw milk or milk that has undergone a lower heat treatment than pasteurisation [10]	Salmonella
1.12. Milk powder and whey powder [10]	Salmonella
1.13. Ice cream [11], excluding products where the manufacturing process or the composition of the product will eliminate the salmonella risk	Salmonella
1.14. Egg products, excluding products where the manufacturing process or the composition of the product will eliminate the salmonella risk	Salmonella
1.15. Ready-to-eat foods containing raw egg, excluding products where the manufacturing process or the composition of the product will eliminate the salmonella risk	Salmonella
1.16. Cooked crustaceans and molluscan shellfish	Salmonella
1.17. Live bivalve molluscs and live echinoderms, tunicates and gastropods	Salmonella

| Food category	Micro-organisms/their toxins, metabolites
1.18. Sprouted seeds (ready-to-eat) [12]	Salmonella
1.19. Pre-cut fruit and vegetables (ready-to-eat)	Salmonella
1.20. Unpasteurised fruit and vegetable juices (ready-to-eat)	Salmonella
1.21. Cheeses, milk powder and whey powder as referred to in the coagulase-positive staphylococci criteria in Chapter 2.2 of this Annex	Staphylococcal enterotoxins
1.22. Dried infant formulae and dried dietary foods for special medical purposes intended for infants below six months of age, as referred to in the Enterobacteriaceae criterion in Chapter 2.2 of this Annex	Salmonella
1.23. Dried infant formulae and dried dietary foods for special medical purposes intended for infants below six months of age, as referred to in the Enterobacteriaceae criterion in Chapter 2.2 of this Annex	Enterobacter sakazakii
1.24. Live bivalve molluscs and live echinoderms, tunicates and gastropods	E.coli [14]
1.25. Fishery products from fish species associated with a high amount of histidine [5]	Histamine

Regulation:

- EC no 2073/2005 is the most relevant document towards EU regulation

- Most of the regulation requires absence in 10/25g of either *Salmonella* or *Listeria monocytogenes*.

- There is almost no EU regulation for STEC (addendum for sprouts since 2013)

- *Campylobacter* also added for broiler carcases (Chapter 2 of Annex I)

- Other testing results from local regulations or market pressure – **bottom up approach**.

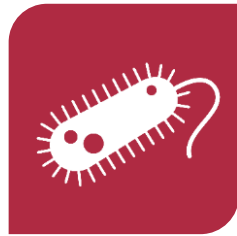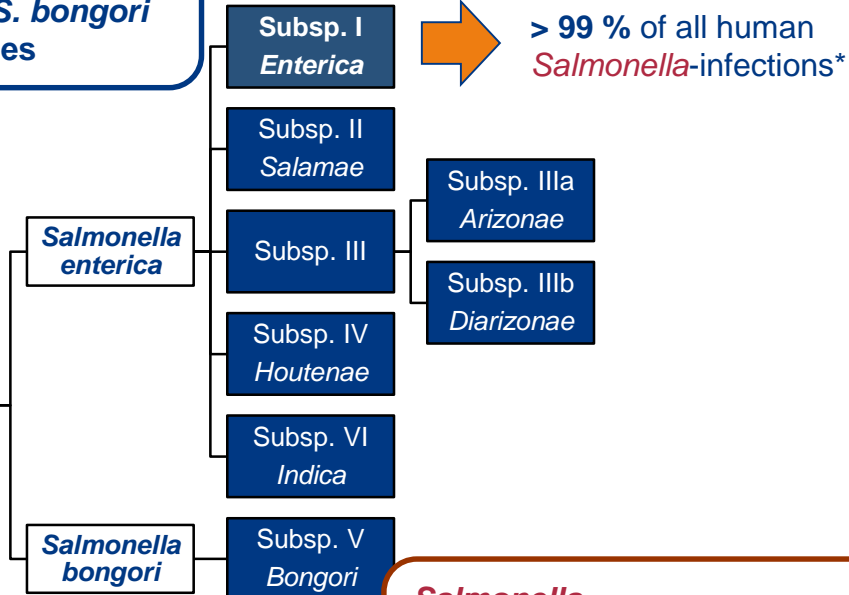# Food Pathogen Testing: Target pathogens
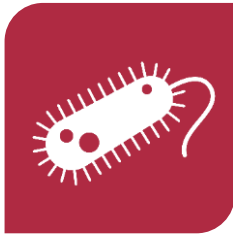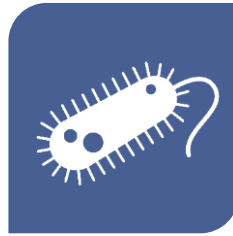
Salmonella

Listeria
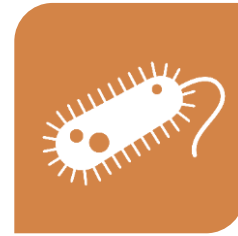
STEC E coli

Background Information: *Salmonella*

- **Gram negative**
- ***Enterobacteriaceae***
- **2 species: *S. enterica* & *S. bongori***
- **Six *S. enterica* sub-species**

Salmonella

Salmonella enterica

Salmonella bongori

Subsp. I
Enterica

➡ **> 99 %** of all human *Salmonella*-infections*

Subsp. II
Salamae

Subsp. III

Subsp. IIIa
Arizonae

Subsp. IIIb
Diarizonae

Subsp. IV
Houtenae

Subsp. VI
Indica

Subsp. V
Bongori

Warm blooded animals

Environment & cold blooded animals

Salmonella

- **Symptoms include diarrhea, fever and stomach cramps**
- **Symptoms start within 6 - 72 hours and last between 4 - 7 days**

Background Information: *Listeria*

- **Gram positive**
- **Belonging to the phylum Firmicutes**
- **Resistant to stress (temp, salt, pH)**
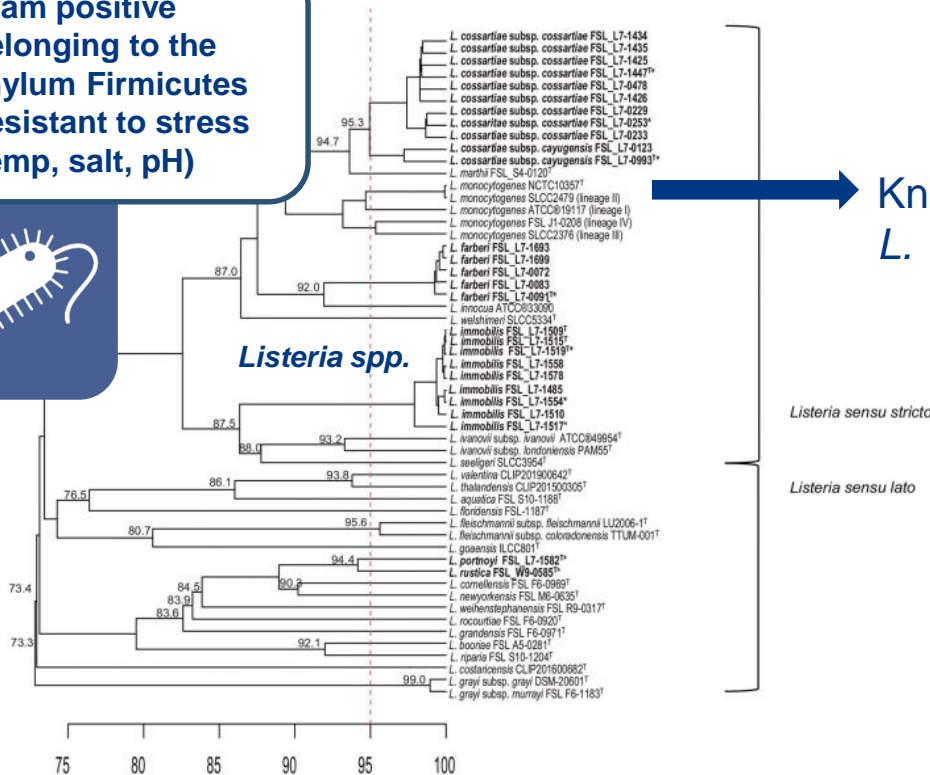
Listeria spp.

Known pathogenic species:
L. monocytogenes (13 serovars)

Listeria monocytogenes

- **Listeriosis is a serious infection (fever – flu-like symptoms)**
- **Most likely to sicken pregnant women and their newborns, elderly and immunocompromised people**

Background Information: *Escherichia coli*

- **Gram negative**
- ***Enterobacteriaceae***
- **Common gut flora**
- **Most non-pathogenic**

O-Group
O157
O145
O111
O121
O103
O45
O26
Top 7

O157:H7
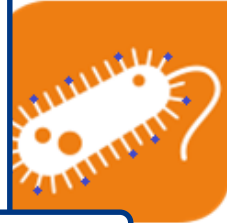
Shiga toxins
(*stx*1 & *stx*2)

Intimin
(*eae*)
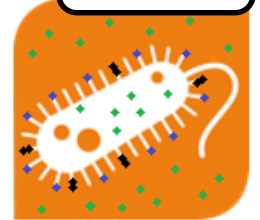
STEC – Shiga toxin-producing *E. coli*

- **Symptoms: bloody diarrhoea, cramps, vomiting**

- **Severe diseases such as haemolytic uremic syndrome (HUS) or haemorrhagic colitis (HC)**

- **Highly infectious (infection dose: <10 cells)**

Food Pathogen Testing: Target pathogens

eurofins | Technologies

Overview of numbers of tests run per target pathogen

Test in Millions

Bar chart showing test counts (in millions) per target pathogen across four regions: NA, EU, Asia, ROW. Y-axis scaled 0 to 60. EU region is highlighted with a box.

- **NA:** Salmonella ~51, Listeria ~50, O157/STEC ~9, Campylobacter ~1, Other ~1
- **EU:** Salmonella ~36, Listeria ~41, O157/STEC ~3.5, Campylobacter ~1, Other ~3.5
- **Asia:** Salmonella ~48, Listeria ~41, O157/STEC ~11.5, Campylobacter ~3.5, Other ~11.5
- **ROW:** Salmonella ~21, Listeria ~20, O157/STEC ~3.5, Campylobacter ~1, Other ~3.5

Legend: ■ Salmonella ■ Listeria ■ O157/STEC ■ Campylobacter ■ Other

Target pathogens:

- Majority of testing target towards *Salmonella* and *Listeria*.

- Despite *Campylobacter* being the most common cause for gastroenteritis in the EU is one of the least tested.

- Honorable mention to *Cronobacter* which is very relevant for the infant formula industry

Food Pathogen Testing: Target Markets

pathogen testing in millions

144 (40%)   **148 (41%)**

40 (11%)   **29 (8%)**

■ Protein   ■ Dairy   ■ Fruit/Veg   ■ Processed
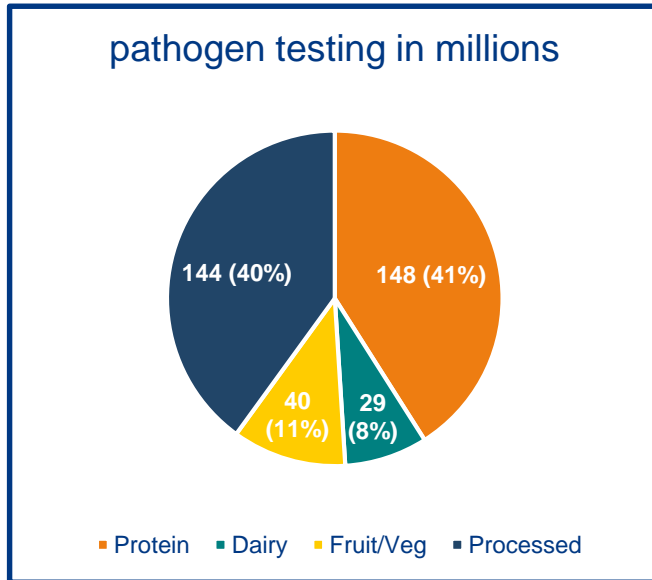
- Protein segment includes
  - Meat, fish, poultry and eggs
- Processed food highest variety
- Fruits/vegetables ranges third
- Lowest dairy

Food Pathogen Testing: Test methods

Tests in millions

Bar chart legend: ■ immunoassay ■ Molecular ■ Traditional media ■ chromogenic

Categories: NA, EU, Asia, ROW
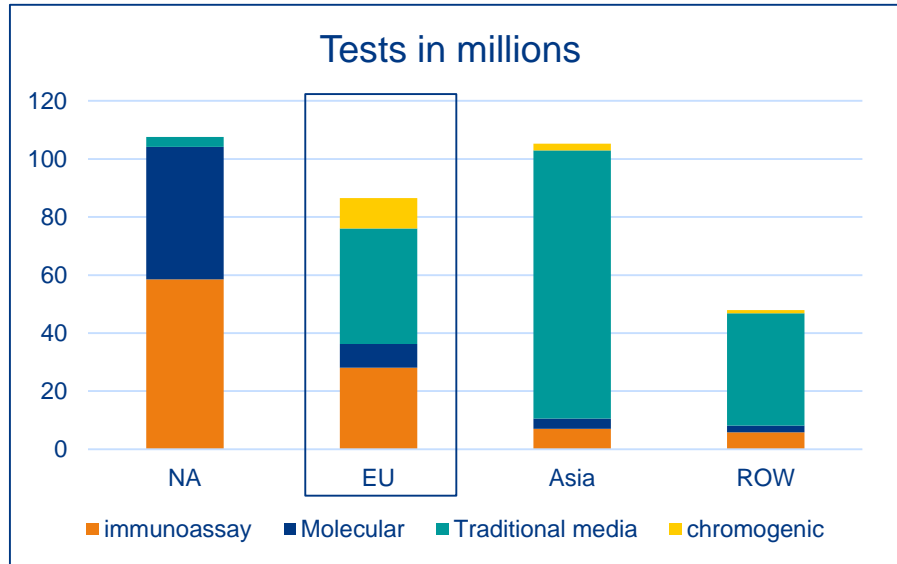
Testing methods:

- Molecular methods with high growth potential by entering other markets.

- Company size/volumes must reach a threshold to consider an in-house laboratory.

- Chromogenic is sizeable in EU. Highly used in the UK market which is very price sensitive.

Immunoassay = ELISA-based Antibody systems
Molecular = real-time PCR
Traditional media = plate counts
Chromogenic = targeted visualization of microcolonies

Traditional media vs ELISA vs qPCR
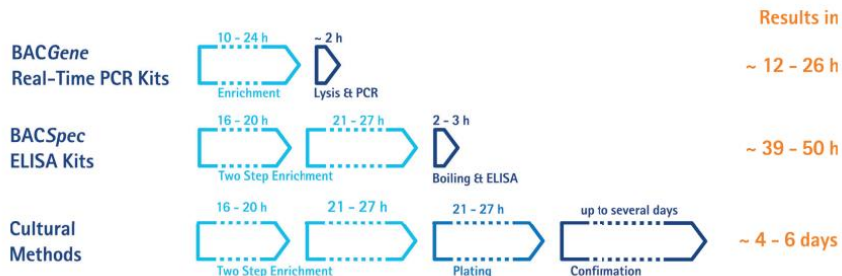
Testing methods:

In the end not every customer can use molecular methods.

There is a market and a place for every method.

- Traditional media
  - Inexpensive
  - Long TTR
  - Requires a trained eye from an experienced technician.

- ELISA
  - Easy to use and interpret especially with the automated methods available (robots and readers)
  - Suboptimal specificity

- qPCR
  - Fastest by a large margin
  - Increasingly simple
  - Very high specificity

PCR **BAC**_Gene_

Overview

Overview BAC*Gene* portfolio

eurofins | Technologies

BAC*Gene*: Real-time PCR based detection of pathogens in food, feed and environmental samples

Short Time to Results
- **Short** enrichment & **Absence** of secondary enrichment step for standard matrices
- **Simple** DNA preparation

High Reliability
- Probe based assay => excellent **specificity**
- Positive and negative PCR controls => PCR target detection and **absence of contamination**
- **Internal Positive Control** (IPC) for verification of PCR performance
- **PR*Eraser* –** certified free-DNA removal & **UNG –** cross contamination prevention (STEC only)

Proven performance
- Certified by **independent** organisations (AFNOR, AOAC)
- **Automated** data analysis (Excel Evaluation Sheet)

High Flexibility
- **Open system**: certified on AriaMX and Bio-Rad CFX96
- One PCR program for **all** BAC*Gene* kits

Overview BAC*Gene* portfolio

BACGene			Extras
Salmonella spp. *Salmonella spp.*	**Listeria spp.** *Listeria spp.*	**E. coli O157:H7** *E. coli* serotype O157:H7	**PREraser BACGene** (Elimination Free-DNA)
	Listeria monocytogenes *Listeria monocytogenes*	**E. coli STEC Multiplex** STEC virulence genes stx1, stx2 & eae	
	Listeria Multiplex *Listeria monocytogenes* and *Listeria spp.*	**E. coli Serotype 1** STEC serotypes O111, O103 & O26	**Salmonella Tranoroa** (Cross Contamination Troubleshooting)
		E. coli Serotype 2 STEC serotypes O145, O121 & O45	

The BAC*Gene* Solution
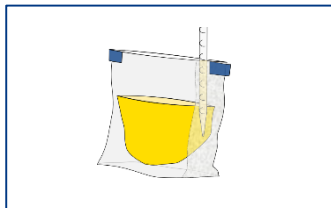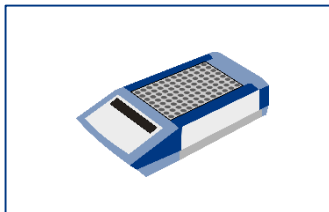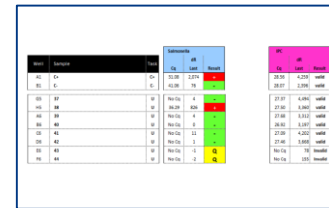
Enrichment	Lysis	PCR	Thermocycler	Readout

BAC*Gene* kits

Evaluation sheets

Media Development Ongoing

Agreement Agilent (Aria MX)

Enrichment

	Salmonella	Listeria	E.coli
Media	BPW	Actero	mTSB
Sample size	25 g 375 g	25 g	25 g 375 g
Dilutions	1:10	1:10	1:3 (meat) 1:5 (leafy greens)
Subbing	Chocolate (NF milk, not BPW) -> BHI		

BACGene *Salmonella* spp. - AFNOR Certified Protocols

Sample / Matrix	Milk and dairy (25 g)	All foods (25 g)	Feed (25 g)	Pet food (up to 375 g)	Milk powders & infant formula (up to 375 g)	Milk powders & infant formula + probiotics (up to 375 g)	Environmentals
Media	1:10 BPW			1:10 BPW pre-warmed		1:10 BPW² pre-warmed	Swab (10 mL)/sponge (100 mL)/wipe/cloth (225 mL)/dust, process water (1:10) - BPW
T °C	41,5 ± 1°C	37 ± 1°C					
Time	18 - 24 h	16 - 24 h	18 - 24 h				

BACGene *Listeria* spp., *monocytogenes* & Multiplex - AFNOR Certified Protocols

Sample / Matrix	All foods (25 g)	Dust & process water (25 g/mL)	Environmentals
Media	1:10 Actero™ pre-warmed		Swab (10 mL)/Sponge (100 mL)/wipe/cloth (225 mL) Actero™ pre-warmed
T °C	37 ± 1°C		
Time	18 - 24 h		

Lysis

	Salmonella	Listeria	E.coli
Lysis Buffer	Lysis buffer I	Lysis buffer L	Lysis buffer S
Enzyme	Proteinase K	Proteinase K + Lysozyme	Proteinase K
Incubation	37±2°C - 20 min + 95±5°C - 10 min		
Equipment needed	Heating block		

PCR & Thermocyclers

	Salmonella	Listeria	E.coli
Run time	~ 1:30 h:min		
Thermal profile	Same		
Thermo Cycler	Agilent AriaMX™ Bio-rad CFX 96 touch™ Standard Bio-rad CFX 96 touch™ Deep Well		
Certification	Afnor & AOAC	Afnor & AOAC	Afnor (O157:H7) & AOAC

Data Interpretation
Example - BACGene Salmonella spp.

Salmonella	Internal Positive Control	Final results	
Reaction positive	Valid/Invalid (*Not significant*)	Positive	123 **+**
Reaction negative	Valid	Negative	456 **-**
Reaction negative	Invalid	Questionable*	789 **Q**

 * Refer to troubleshooting section in the BAC*Gene Salmonella* spp. manual

PCR **BAC** *Gene*

troubleshooting

Products for "troubleshooting"

Questions:

- Does a positive result comes from a cross contamination?
  - **BAC*Gene Salmonella* Tranoroa**

- Does a positive result comes from viable or non-viable pathogen?
  - **PR*Eraser* BAC*Gene***

Products for "troubleshooting"

Questions:

- Does a positive result comes from a cross contamination?
  - **BAC*Gene Salmonella* Tranoroa**

- Does a positive result comes from viable or non-viable pathogen?
  - PR*Eraser* BAC*Gene*

BAC*Gene Salmonella* Tranoroa

Ideal routine positive control strain

- Not naturally occurring in food matrices
- Easily and quickly discriminated from other serotypes
- Same growth conditions as food related serotypes

⬇

Salmonella enterica serovar Tranoroa

BAC*Gene Salmonella Tranoroa* a rapid test <u>from the enrichment broth</u>

Can be run after BAC*Gene Salmonella* spp. from the same lysate to **confirm absence of S. Tranoroa**

Evaluation sheets BAC*Gene* includes *Salmonella* Tranoroa

BAC*Gene Salmonella* Tranoroa workflow

Integration of BAC*Gene Salmonella* Tranoroa (enrichment positive control)
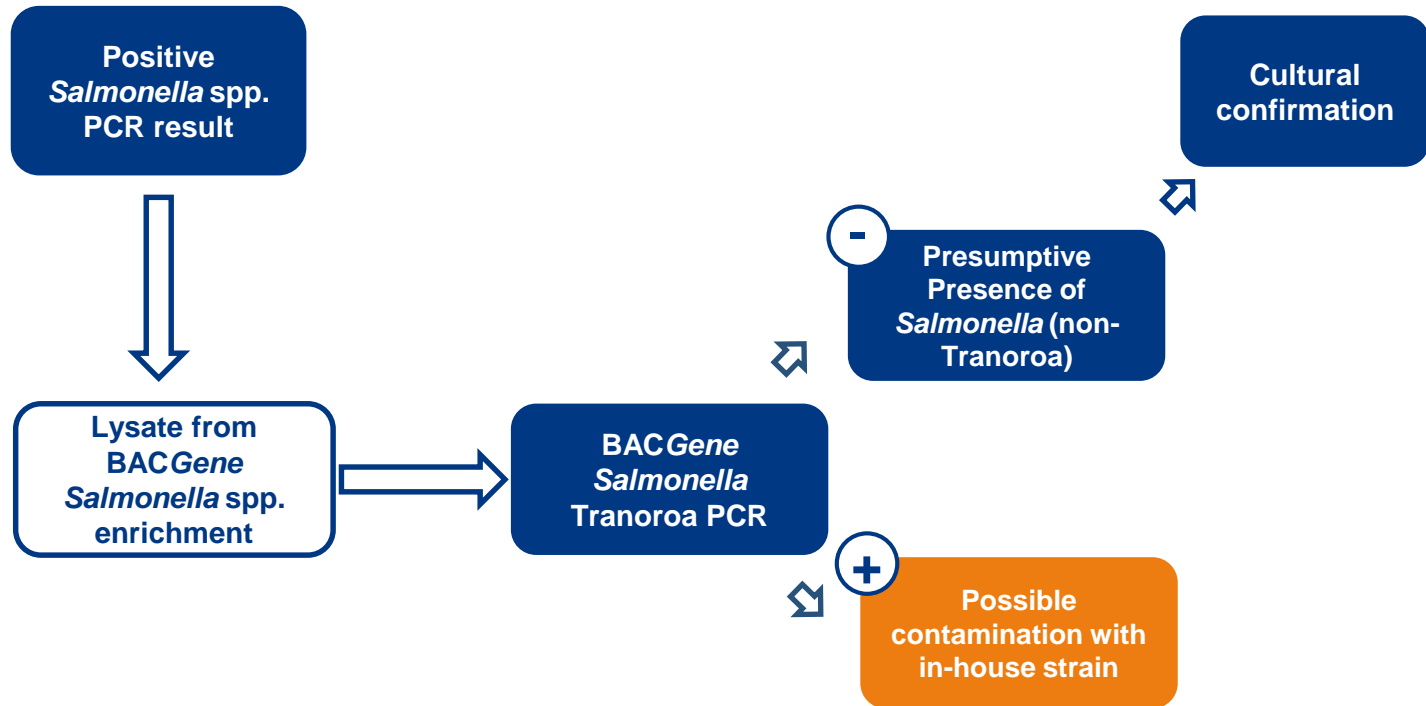
Products for "troubleshooting"

Questions:

- Does a positive result comes from a cross contamination?
  - **BAC*Gene Salmonella* Tranoroa**

- Does a positive result comes from viable or non-viable pathogen?
  - **PR*Eraser* BAC*Gene***

PR*Eraser* BAC*Gene* Workflow
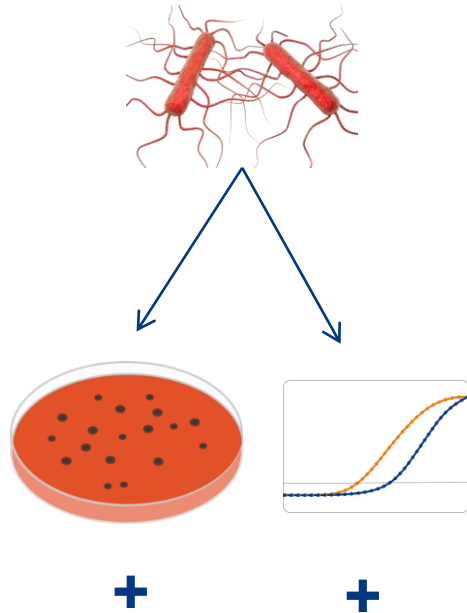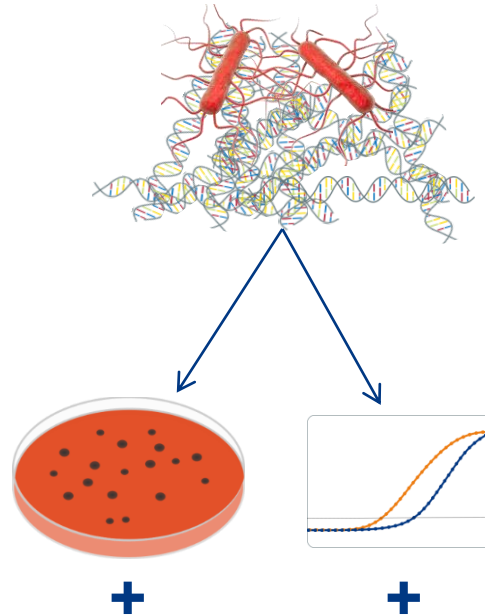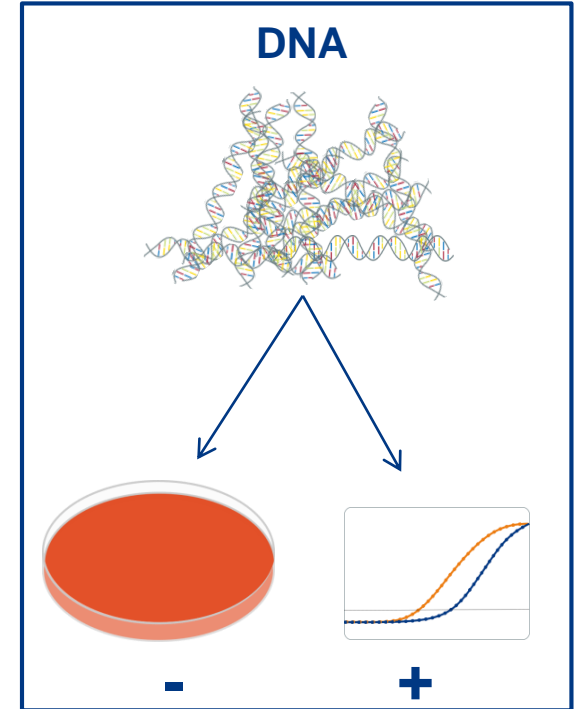
- **Enzymatic pre-treatment prior to lysis**
- **In case of high rate of not confirmable PCR positive results due to free DNA**

Prepare working solution by adding complete volume of diluent to stock solution

Distribute working solution into reaction vials

Add sample aliquot

Homogenize by vortexing/pipetting

Spin down

Proceed with normal protocol

Spin down

Cool down @ RT

10min @ 95 ± 1°C

30min @37 ± 1°C

PCR **BAC** *Gene*

Tools available

Quick Reference Guide

PCR **BAC** *Gene*

Technical

Excellent specificity

BAC*Gene Salmonella* spp.*:*

- The amplified target fragment is:

  ✓ Detected with **FAM**™ fluorescence-labelled hybridization probe

  ✓ Quenched by non-fluorescent Tide Quencher™ 2 (TQ2)

- An internal positive control (IPC) is included in the MasterMix and amplified in parallel:

  ✓ Detected with **Cy5**™ fluorescence-labelled hybridisation probe

  ✓ Quenched by non-fluorescent Tide Quencher™ 3 (TQ3)

  ✓ IPC detection indicates the proper functioning of the PCR

https://www.biosearchtech.com/qpcr-multiplex-spectral-overlay-tool

High reliability
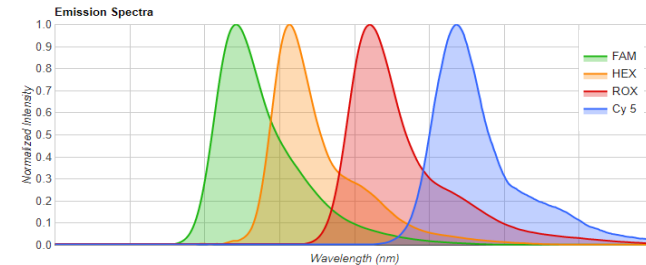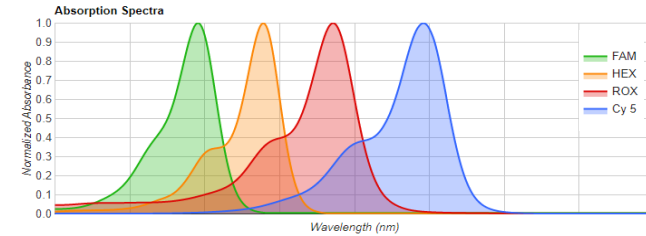
- For every PCR, it is _necessary_ to prepare a positive (C+) and a negative (C-) control reaction.

- Preparation of a positive (E+) and a negative (E-) enrichment controls are _recommended_.

- Different BAC*Gene* detection kits can be analyzed in one run

Example of simultaneous RT-PCR run for BAC*Gene Salmonella* spp
and BAC*Gene Listeria Monocytogenes*:

	1	2	3	4	5	6	7	8	9	10	11	12
A	C+	7	15							C+	7	15
B	C-	8	16		BAC*Gene*					C-	8	16
C	1	9	17		*Salmonella* spp					1	9	17
D	2	10	18							2	10	18
E	3	11	19							3	11	19
F	4	12	20		BAC*Gene Listeria*					4	12	20
G	5	13	E-		*Monocytogenes*					5	13	E-
H	6	14	E+							6	14	E+

C+ = positive control; **C-** = negative control; **E-** = negative enrichment control;
E+ = positive enrichment control

Kit components:
BACGene Salmonella spp.

Cat. no. 5123221801 of BAC*Gene Salmonella* **spp. (96 reactions)**
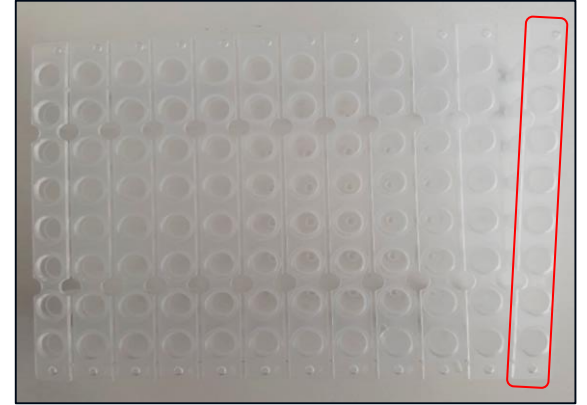
For Lysis:

1x Lysis plate for sample preparation, empty, rippable (high profile)

1x Domed caps, for use with Lysis plate, set of 12 strips

2x Lysis buffer I, 4 mL, store at -20 °C ± 2°C

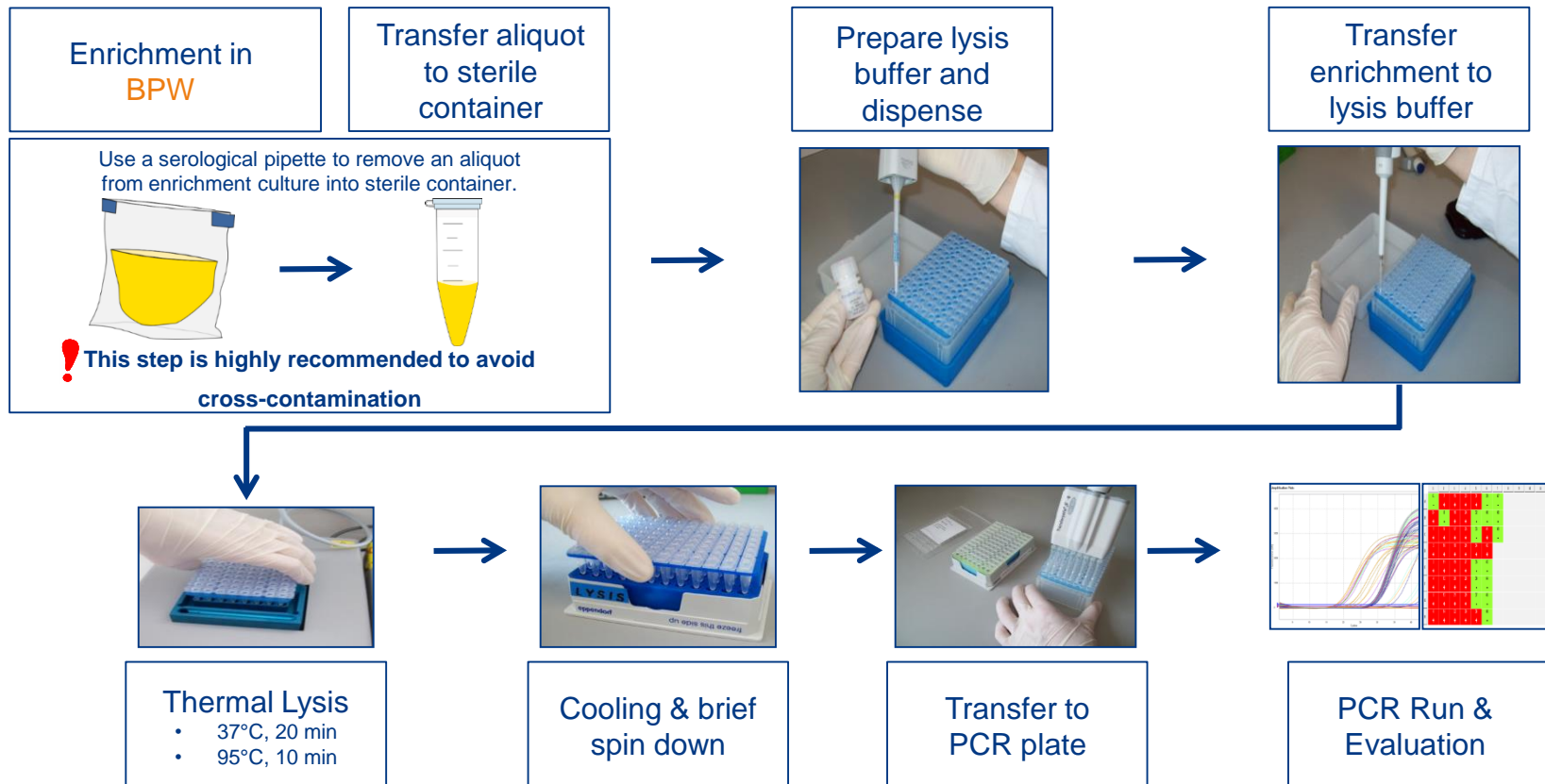**2x Proteinase K**, vials with blue cap, each with 500 µL, store at -20°C ± 2°C

For PCR:

1x BACGene Salmonella spp. PCR plate, with pre-dispensed MasterMix and PCR support grid. Store light protected at -20°C ± 2°C

1x Optical caps, for use with PCR plate (1 bag with 120 strips)

2x Salmonella positive control plasmid DNA, vial with yellow cap, 50 µL, store at -20°C ± 2°C. Do not freeze/thaw more than 6 times

Overview: BACGene Salmonella spp.

Enrichment in BPW	Transfer aliquot to sterile container	Prepare lysis buffer and dispense	Transfer enrichment to lysis buffer

Use a serological pipette to remove an aliquot from enrichment culture into sterile container.

! **This step is highly recommended to avoid cross-contamination**

Thermal Lysis • 37°C, 20 min • 95°C, 10 min	Cooling & brief spin down	Transfer to PCR plate	PCR Run & Evaluation

Technical aspects - summary

- **Certified assays** by independent organisations **(AFNOR, AOAC)**

- **No spectral overlapping**: Spectra from Salmonella target sequence (FAM) and the internal control IPC (CY5) do not overlap

- **No internal passive reference** necessary (ROX)

- **Enriched samples** can be stored up to **72 h at 4 °C**

- **Simple lysis**: Thermal enzymatic incubation

- **Final lysis buffer** can be stored for **2 weeks at 4 °C or 2 months at -20 °C**

- **Easy handling**: Very few pipetting steps

- **Prefilled RT-PCR plate** with **ready-to-use Mastermix where IPC is included**

- **Rippable strips**; individual wells can be used

- **Simultaneous RT-PCR run**: Different pathogens can be analyzed in one run

- **Ready to use RT-PCR run templates**

- **Simple evaluation**: possible on different computers

- **Colored evaluation result display**

Q&A