

# Interreg



UNIONE EUROPEA  
EVROPSKA UNIJA

## ITALIA-SLOVENIJA



### TRAIN

Progetto standard co-finanziato dal Fondo europeo di sviluppo regionale  
Standardni projekti sofinancira Evropski sklad za regionalni razvoj

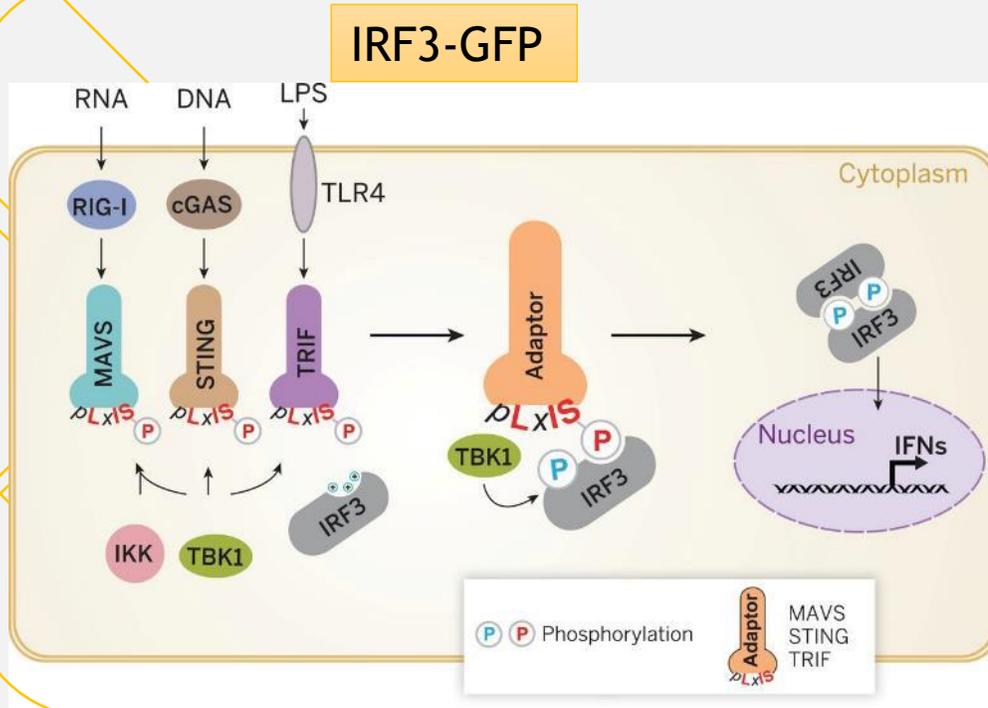
# EXPERTTEAM RESULTS

*Speaker: Francesca Bruno*

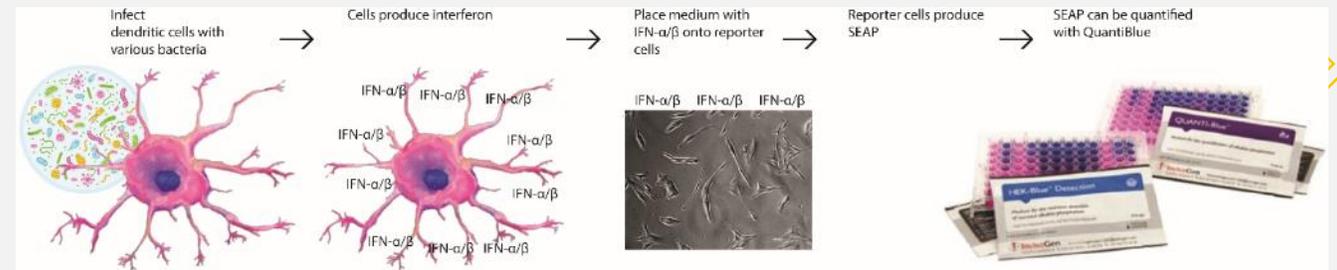


## Initial idea:

Test, by a molecular method, the presence or absence of one or more pathogens in food and verify the pathogen is live or dead, by High content analysis (HCA)



## IFN reporter system



Tested assays do not discriminate between pathogens and non-pathogens, and they are not sufficiently sensitive for our purpose

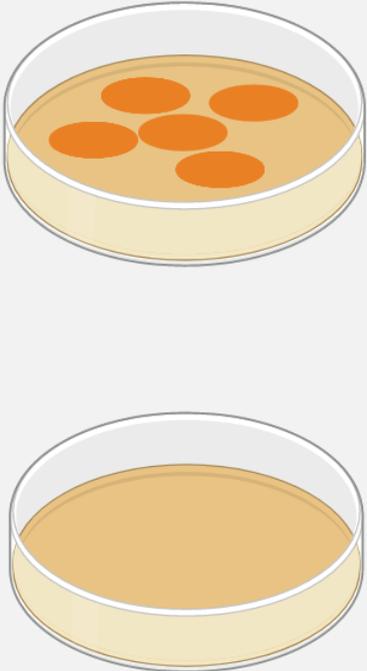
Quit these approaches

# Foodborne pathogen detection

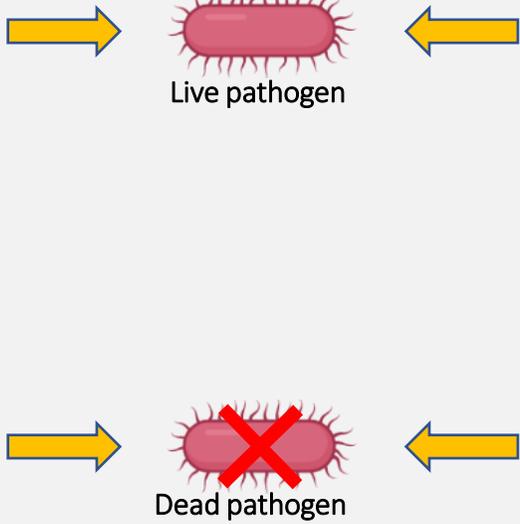
Illnesses resulting from the consumption of foods or water contaminated with pathogens and/or their toxins have a wide range of economic and public health impact worldwide

**CONVENTIONAL MICROBIOLOGICAL METHODS**

- Time-consuming
- Poor sensitive

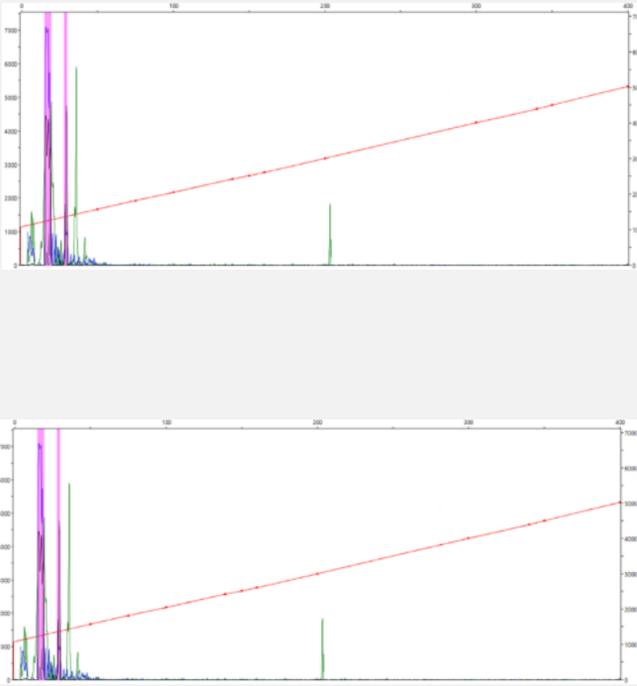


Different results



**PCR METHODS**

- Specific
- Sensitive
- Speed of detection



Same results

## New approach based on detection of m.o. using **mRNA**

mRNA has half-life on only few minutes (bacterial DNA is more stable than bacterial RNA)

The detection of mRNA shall be a good indicator of viability of cells

Detection of LIVE pathogens cells in food by a TaqMan-based quantitative reverse Transcriptase Real-Time PCR (RT-qPCR)



# Salmonella enterica:

*Salmonella enterica* contamination in various foods is a significant public health concern, domestically and internationally.

invA gene investigated for the presence of Salmonella

| Color      | Name  | Type    | Ct    |
|------------|---|---------|-------|
| Black      | Salmonella A strain Live cDNA (1 Dnase treatment) qRT-PCR | Unknown | 23,48 |
| Light Blue | Salmonella A strain Live RNA (1Dnase treatment) qPCR      | Unknown | 30,45 |

$$\Delta C_t(\text{qRT-PCR}) - (\text{RT-PCR}) > 4$$

| Color | Name  | Type    | Ct    |
|-------|---|---------|-------|
| Red   | Salmonella A strain Dead cDNA (1 Dnase treatment) qRT-PCR | Unknown | 23,53 |
| Blue  | Salmonella A strain Dead RNA (1Dnase treatment) qPCR      | Unknown | 23,81 |

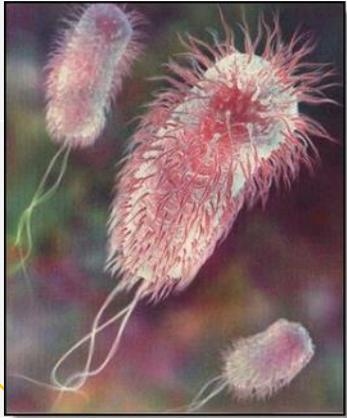
$$\Delta C_t(\text{qRT-PCR}) - (\text{RT-PCR}) < 4 \rightarrow \text{No RNA in sample}$$

| Color  | Name  | Type    | Ct    |
|--------|---|---------|-------|
| Yellow | Salmonella B strain Live cDNA (1 Dnase treatment) qRT-PCR | Unknown | 23,41 |
| Purple | Salmonella B strain Live RNA (1Dnase treatment) qPCR      | Unknown | 30,05 |

$$\Delta C_t(\text{qRT-PCR}) - (\text{RT-PCR}) > 4$$

| Color | Name  | Type    | Ct    |
|-------|---|---------|-------|
| Green | Salmonella B strain Dead cDNA (1 Dnase treatment) qRT-PCR | Unknown | 25,78 |
| Pink  | Salmonella B strain Dead RNA (1Dnase treatment) qPCR      | Unknown | 26,61 |

$$\Delta C_t(\text{qRT-PCR}) - (\text{RT-PCR}) < 4 \rightarrow \text{No RNA in sample}$$



# ESCHERICHIA COLI O157:H7

- E. coli has a lot of major virulence genes (stx1, stx2, fliC, eaeA, rfbE, uidA, hlyA)
- Not all genes are suitable targets for detecting viable E.coli

**rfbE** gene investigated for the presence of Escherichia, it encodes the O157 antigen

| Color   | Name  | Type    | Ct    |
|---|---|---------|-------|
|  | Escherichia coli Live cDNA (2 DNase treatment)      | Unknown | 27,84 |
|  | Escherichia coli Live RNA (non reverse-transcribed) | Unknown | 32,12 |
|  | Escherichia coli Dead cDNA (2 DNase treatment)      | Unknown | 22,15 |
|  | Escherichia coli Dead RNA (non reverse-transcribed) | Unknown | 23,43 |

$\Delta C_t(qRT-PCR)-(RT-PCR) > 4$

$\Delta C_t(qRT-PCR)-(RT-PCR) < 4$

→ No RNA in sample

# Multiplex detection of both pathogenes cDNA

| Color        | Name   | Type     | Ct    | Given Conc (copies/reaction) | Calc Conc (copies/reaction) |
|--------------|--|----------|-------|------------------------------|-----------------------------|
| Black        | Multiplex Detection Mix (Escherichia coli 10 <sup>5</sup> copies/reaction) | Standard | 24,52 | 1,00E+05                     | 9,80E+04                    |
| Cyan         | Multiplex Detection Mix (Escherichia coli 10 <sup>5</sup> copies/reaction) | Standard | 24,35 | 1,00E+05                     | 1,09E+05                    |
| Gold         | Multiplex Detection Mix (Escherichia coli 10 <sup>4</sup> copies/reaction) | Standard | 28,17 | 1,00E+04                     | 9,99E+03                    |
| Green        | Multiplex Detection Mix (Escherichia coli 10 <sup>4</sup> copies/reaction) | Standard | 28,14 | 1,00E+04                     | 1,02E+04                    |
| Light Green  | Multiplex Detection Mix (Escherichia coli 10 <sup>3</sup> copies/reaction) | Standard | 31,97 | 1,00E+03                     | 9,28E+02                    |
| Blue         | Multiplex Detection Mix (Escherichia coli 10 <sup>3</sup> copies/reaction) | Standard | 32,09 | 1,00E+03                     | 8,61E+02                    |
| Purple       | Multiplex Detection Mix (Escherichia coli 10 <sup>2</sup> copies/reaction) | Standard | 34,85 | 1,00E+02                     | 1,54E+02                    |
| Light Purple | Multiplex Detection Mix (Escherichia coli 10 <sup>2</sup> copies/reaction) | Standard | 35,99 | 1,00E+02                     | 7,52E+01                    |
| Pink         | Multiplex Detection Mix (Escherichia coli Live cDNA+ Salmonella Live cDNA) | Unknown  | 28,83 |                              | 6,63E+03                    |

| Color        | Name  | Type     | Ct    | Given Conc (copies/reaction) | Calc Conc (copies/reaction) |
|--------------|---|----------|-------|------------------------------|-----------------------------|
| Red          | Multiplex Detection Mix (Salmonella enterica 10 <sup>5</sup> copies/reaction) | Standard | 24,74 | 1,00E+05                     | 1,12E+05                    |
| Yellow       | Multiplex Detection Mix (Salmonella enterica 10 <sup>5</sup> copies/reaction) | Standard | 24,51 | 1,00E+05                     | 1,31E+05                    |
| Blue         | Multiplex Detection Mix (Salmonella enterica 10 <sup>4</sup> copies/reaction) | Standard | 28,40 | 1,00E+04                     | 1,04E+04                    |
| Purple       | Multiplex Detection Mix (Salmonella enterica 10 <sup>4</sup> copies/reaction) | Standard | 28,82 | 1,00E+04                     | 7,90E+03                    |
| Pink         | Multiplex Detection Mix (Salmonella enterica 10 <sup>3</sup> copies/reaction) | Standard | 32,26 | 1,00E+03                     | 8,43E+02                    |
| Blue         | Multiplex Detection Mix (Salmonella enterica 10 <sup>3</sup> copies/reaction) | Standard | 32,24 | 1,00E+03                     | 8,50E+02                    |
| Teal         | Multiplex Detection Mix (Salmonella enterica 10 <sup>2</sup> copies/reaction) | Standard | 36,31 | 1,00E+02                     | 6,05E+01                    |
| Light Red    | Multiplex Detection Mix (Salmonella enterica 10 <sup>2</sup> copies/reaction) | Standard | 35,16 | 1,00E+02                     | 1,27E+02                    |
| Green        | Multiplex Detection Mix (Salmonella enterica 10 copies/reaction)              | Standard | 39,10 | 1,00E+01                     | 9,80E+00                    |
| Pink         | Multiplex Detection Mix (Salmonella enterica 10 copies/reaction)              | Standard | 38,40 | 1,00E+01                     | 1,54E+01                    |
| Light Purple | Multiplex Detection Mix (Salmonella Live cDNA+ Escherichia coli Live cDNA)    | Unknown  | 28,58 |                              | 9,23E+03                    |

## Conclusions:

- Detect foodborne pathogens and discriminate between live and dead pathogen that can prevent to waste food
- With the same q-PCR cycle we can detect 2 (at moment) different foodborne pathogens (salmonella and E. coli), developing a method that can permit to distinguish live and dead cells amplifying mRNA of the m.o