



# PFGE and serotyping Proficiency Testing Trial 2014

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**SEL Unit**

*[Salmonella - E.coli - Listeria]*

9<sup>th</sup> Workshop of the EURL for *Listeria monocytogenes*  
25-27 March 2015

# Participants- Timeline

- **32 NRLs registered**
  - **Conventional serotyping (CS) ⇒ 16 NRLs**
  - **Molecular serotyping (MS) ⇒ 25 NRLs**
  - **PFGE sub-typing ⇒ 25 NRLs**
- **12 NRLs registered for the 3 typing methods**
- **5 NRLs PFGE / CS or MS, 3 NRLs PFGE, 7 NRLs CS or MS**
- **Strain panel sent to participant on 12 October 2014**
- **Deadline for results: 12 December 2014**
- **Individual preliminary report: to come (April 2015)**



# Strain panel set up

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- **Eleven strains**
- **One duplicate (strain 3)**
- **Strain panel common to ECDC EQA (organized by SSI) and EURL PT trial**
- **Strain stability tested for serotyping and PFGE (x10 consecutive subcultures)**
- **Selected according *Lm* diversity**



# Strain panel

PT strain ID <sup>(2)</sup>	EURL Lm ID	Collection	Origin	Serotype	Molecular serogroup	<i>FlaA</i> PCR
1	05CEB583LM	EURL Lm	Salmon	1/2a	<u>IIa</u>	<u><i>FlaA</i>+</u>
2	06CEB435LM	EURL Lm	Spicy Sausage	1/2b	<u>IIb</u>	
3	06CEB476LM <sup>(*)</sup>	EURL Lm	Pizza	3a	<u>IIa</u>	<u><i>FlaA</i>+</u>
3	06CEB476LM	EURL Lm	Pizza	3a	<u>IIa</u>	<u><i>FlaA</i>+</u>
4	13CEB1022LM (List 9) <sup>(4)</sup>	SSI	Patient	1/2c	<u>IIc</u>	<u><i>FlaA</i>-</u>
5	13CEB1024LM (List 8) <sup>(4)</sup>	SSI	Patient	1/2a	<u>IIa</u>	<u><i>FlaA</i>+</u>
6	13CEB1031LM (List 1) <sup>(4)</sup>	SSI	Patient	4b	<u>IVb</u>	
7	TS21 <sup>(3)</sup> <b>E-1</b>	EURL Lm	Cheese	4b	<u>IVb</u>	
8	TS30 <sup>(3)</sup> <b>S-1</b>	EURL Lm	Patient	1/2a	<u>IIa</u>	<u><i>FlaA</i>+</u>
9	TS31 <sup>(3)</sup> <b>E-2</b>	EURL Lm	Patient	4b	<u>IVb</u>	
10	TS44 <sup>(3)</sup> <b>S-1</b>	EURL Lm	Patient	1/2a	<u>IIa</u>	<u><i>FlaA</i>+</u>

→ (WHO) international multicenter subtyping study (Bille and Rocourt, 1996 IJFM).

→ Strains repeated during ECDC EQA PT trials

→ Strains repeated during EURL PT trials

# EURL database larger groups

90% groups combined Apal Ascl	n	Serotypes	MLST clonal complex	PT/EQA strain panel + WHO multicentric study (Bille et al., 1996)
A	176	IIa	CC121	06CEB476M
B	82	IIa	CC8	TS44, TS30
C	71	IIc, IIa	CC9	List 9
D	53	IIa	CC121	
E	46	IIa	CC121	
F	29	IVb	CC2	List 1
G	26	IVb	CC6	
H	23	IIc	CC9	
I	20	IIa	CC155	
J	19	IIa	CC37	
Other groups (202) (n>1)	82			TS55, TS69
Other groups (126) (n=1)	126			List 8



## ECDC database larger groups

90% groups combined Ascl Apal	n	Serotypes	MLST	PT/EQA strain panel + WHO multicentric study (Bille et al., 1996)
A	58	IIa	CC8	TS30, TS44
B	48	IIa	CC8	
C	47	IVb	CC2	List1
D	41	IVb	CC1	TS69
E	40		-	
F	40	IVb	CC1	TS55
G	21	IIa	8	
H	16	-	-	
I	16	IIc	CC9	
J	15	-	CC1	
Other groups (151) (n>1)	649			
Other groups (297) (n=1)	297			



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# Results of conventional serotyping



# Results : conventional serotyping

Strains	Strain 1	Strain 2	Strain 3	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7	Strain 8	Strain 9	Strain 10
Lab code	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
1	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
4	1/2a	1/2b	3a	3a	1/2c	1/2a	4d	4b	1/2a	4b	1/2a
5	1/2a	1/2b	1/2a	1/2a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
6	1/2a	1/2b	3a?	3a?	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
10	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
11	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
15	1/2a	1/2b	N/A	N/A	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
16	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
19	1/2a	1/2b	3a	3a	1/2c	1/2a	4d	4d	1/2a	4d	1/2a
20	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4d	1/2a
21	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
22	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
26	1/2a	1/2b	1/2a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
30	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
31	1/2a	4d	3a	3c	1/2c	1/2a	4b	4d	1/2a	4b	1/2a
32	1/2a	1/2b	1/2a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
33	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a

17 NRLs

91.4%  
of results in  
agreement  
with EURL  
results

Expected results  
Deviation compared with the EURL results





# Results : conventional serotyping

Strains	Strain 1	Strain 2	Strain 3	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7	Strain 8	Strain 9	Strain 10
Lab code	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
1	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
4	1/2a	1/2b	3a	3a	1/2c	1/2a	4d	4b	1/2a	4b	1/2a
5	1/2a	1/2b	1/2a	1/2a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
6	1/2a	1/2b	3a?	3a?	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
10	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
11	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
15	1/2a	1/2b	N/A	N/A	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
16	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
19	1/2a	1/2b	3a	3a	1/2c	1/2a	4d	4d	1/2a	4d	1/2a
20	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4d	1/2a
21	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
22	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
26	1/2a	1/2b	1/2a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
30	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
31	1/2a	4d	3a	3c	1/2c	1/2a	4b	4d	1/2a	4b	1/2a
32	1/2a	1/2b	1/2a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
33	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a

17 NRLs

91.4%  
of results in  
agreement  
with EURL  
results

Strain 3 and duplicate : O:I/II, I+ (?) (II, IV+?) ; H: AB, A

Strain 3 duplicate : No clear agglutination

# Results : conventional serotyping

Strains	Strain 1	Strain 2	Strain 3	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7	Strain 8	Strain 9	Strain 10
Lab code	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
1	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
4	1/2a	1/2b	3a	3a	1/2c	1/2a	4d	4b	1/2a	4b	1/2a
5	1/2a	1/2b	1/2a	1/2a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
6	1/2a	1/2b	3a?	3a?	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
10	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
11	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
15	1/2a	1/2b	N/A	N/A	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
16	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
19	1/2a	1/2b	3a	3a	1/2c	1/2a	4d	4d	1/2a	4d	1/2a
20	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4d	1/2a
21	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
22	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
26	1/2a	1/2b	1/2a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
30	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
31	1/2a	4d	3a	3c	1/2c	1/2a	4b	4d	1/2a	4b	1/2a
32	1/2a	1/2b	1/2a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
33	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a

17 NRLs

91.4%  
of results in  
agreement  
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results

Strain 3 duplicate : O:I/II, II, (IV+?); H: AB, A



# Results : conventional serotyping

Strains	Strain 1	Strain 2	Strain 3	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7	Strain 8	Strain 9	Strain 10
Lab code	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
1	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
4	1/2a	1/2b	3a	3a	1/2c	1/2a	4d	4b	1/2a	4b	1/2a
5	1/2a	1/2b	1/2a	1/2a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
6	1/2a	1/2b	3a?	3a?	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
10	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
11	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
15	1/2a	1/2b	N/A	N/A	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
16	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
19	1/2a	1/2b	3a	3a	1/2c	1/2a	4d	4d	1/2a	4d	1/2a
20	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4d	1/2a
21	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
22	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
26	1/2a	1/2b	1/2a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
30	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
31	1/2a	4d	3a	3c	1/2c	1/2a	4b	4d	1/2a	4b	1/2a
32	1/2a	1/2b	1/2a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a
33	1/2a	1/2b	3a	3a	1/2c	1/2a	4b	4b	1/2a	4b	1/2a

17 NRLs

91.4%  
of results in  
agreement  
with EURL  
results

Strain 6, 7 and 9 : O: V/VI+, VI+, VIII+ (?) ; H: ABC+

Antigenic formula not provided

Strain 6, 7 and 9 : O: V/VI+ (?), VI+ (?), VIII+ (?) ; H: ABC+

Serum quality ?

Culture medium ?

Agglutination method ?

## About the results

### Conventional serotyping 2010 vs 2009 vs 2014:

	Participating NRLs	Conventional serotyping results	Improvement 2009 2010 2014
2009	16	86.0%	84.6% (13 NRLs)
2010	20	91.0%	93.1% (13 NRLs)
2014	17	91.4%	93.5% (13 NRLs)

### Main causes of deviation:

- False positive or false negative agglutination ⇒ sera I, II, IV and VIII
  - ↳ Serum quality ?
  - ↳ Reading problem (clearness of the drop, medium use to revive and perform the agglutinations, etc...)?



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# Results of molecular serotyping



# Results: molecular serotyping

Strains	Strain 1	Strain 2	Strain 3	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7	Strain 8	Strain 9	Strain 10
Lab code	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
1	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
2	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
4	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
5	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
6	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
7	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
8	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
9	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
10	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
11	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
12	IIa (C+)	IIb (C+)	IIa (C+)	IIa (C+)	IIc (C+?)	IIa (C+)	IVb (C+)	IVb (C+)	IIa (C+)	IVb (C+)	IIa (C+)
15	IIa (C+?)	IIb	IIa (C+?)	IIa (C+?)	IIc (C+?)	IIa (C+?)	IVb	IVb	IIa (C+?)	IVb	IIa (C+?)
16	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
17	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
18	IIa (C+)	IIb (C+)	IIa (C+)	IIa (C+)	IIc (C+?)	IIa (C+)	IVb (C+)	IVb (C+)	IIa (C+)	IVb (C+)	IIa (C+)
19	IIa	IIb	IIa	IIa	IIc (C+?)	IIa	IVb	IVb	IIa	IVb	IIa
20	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
21	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
22	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
23	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
24	IIa (C+)	IIb	IIa (C+)	IIa (C+)	IIc (C+?)	IIa (C+)	IVb	IVb	IIa (C+)	IVb	IIa (C+)
28	IIa (C+)	IIb	IIa (C+)	IIa (C+)	IIc (C+?)	IIa (C+)	IVb	IVb	IIa (C+)	IVb	IIa (C+)
29	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
30	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
32	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
33	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa

26 NRLs

84.6%  
of results in  
agreement  
with EURL  
results

Results in agreement with EURL results

Deviation compared with the EURL results



EURL Lm  
European Union Reference Laboratory for  
*Listeria monocytogenes*



# Results: molecular serotyping

Strains	Strain 1	Strain 2	Strain 3	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7	Strain 8	Strain 9	Strain 10
Lab code	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
1	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
2	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
4	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
5	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
6	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
7	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
8	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
9	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
10	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
11	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
12	IIa (C+)	IIb (C+)	IIa (C+)	IIa (C+)	IIc (C+?)	IIa (C+)	IVb (C+)	IVb (C+)	IIa (C+)	IVb (C+)	IIa (C+)
15	IIa (C+?)	IIb	IIa (C+?)	IIa (C+?)	IIc (C+?)	IIa (C+?)	IVb	IVb	IIa (C+?)	IVb	IIa (C+?)
16	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
17	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
18	IIa (C+)	IIb (C+)	IIa (C+)	IIa (C+)	IIc (C+?)	IIa (C+)	IVb (C+)	IVb (C+)	IIa (C+)	IVb (C+)	IIa (C+)
19	IIa	IIb	IIa	IIa	IIc (C+?)	IIa	IVb	IVb	IIa	IVb	IIa
20	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
21	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
22	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
23	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
24	IIa (C+)	IIb	IIa (C+)	IIa (C+)	IIc (C+?)	IIa (C+)	IVb	IVb	IIa (C+)	IVb	IIa (C+)
28	IIa (C+)	IIb	IIa (C+)	IIa (C+)	IIc (C+?)	IIa (C+)	IVb	IVb	IIa (C+)	IVb	IIa (C+)
29	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
30	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
32	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
33	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa

26 NRLs

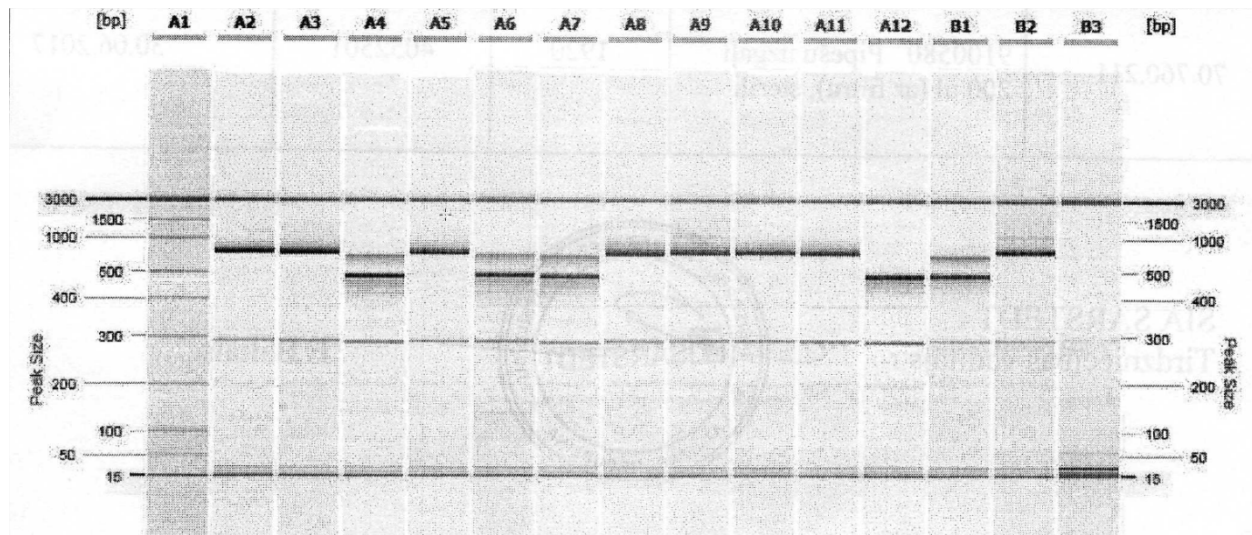
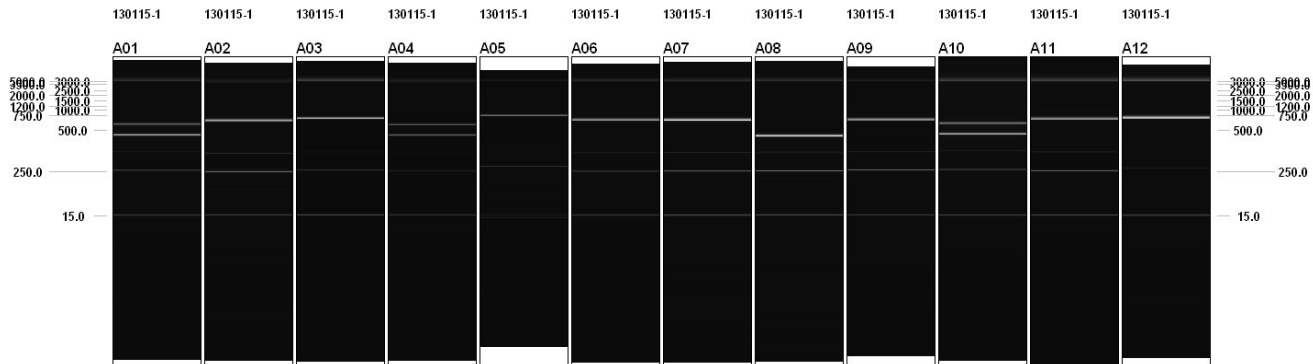
84.6%  
of results in  
agreement  
with EURL  
results

Strain 1-10 : No control identified on the gel



# Deviation

No controls identified on the gel and in the test report (gel map)





# Results: molecular serotyping

Strains	Strain 1	Strain 2	Strain 3	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7	Strain 8	Strain 9	Strain 10
Lab code	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
1	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
2	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
4	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
5	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
6	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
7	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
8	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
9	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
10	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
11	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
12	IIa (C+)	IIb (C+)	IIa (C+)	IIa (C+)	IIc (C+?)	IIa (C+)	IVb (C+)	IVb (C+)	IIa (C+)	IVb (C+)	IIa (C+)
15	IIa (C+?)	IIb	IIa (C+?)	IIa (C+?)	IIc (C+?)	IIa (C+?)	IVb	IVb	IIa (C+?)	IVb	IIa (C+?)
16	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
17	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
18	IIa (C+)	IIb (C+)	IIa (C+)	IIa (C+)	IIc (C+?)	IIa (C+)	IVb (C+)	IVb (C+)	IIa (C+)	IVb (C+)	IIa (C+)
19	IIa	IIb	IIa	IIa	IIc (C+?)	IIa	IVb	IVb	IIa	IVb	IIa
20	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
21	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
22	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
23	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
24	IIa (C+)	IIb	IIa (C+)	IIa (C+)	IIc (C+?)	IIa (C+)	IVb	IVb	IIa (C+)	IVb	IIa (C+)
28	IIa (C+)	IIb	IIa (C+)	IIa (C+)	IIc (C+?)	IIa (C+)	IVb	IVb	IIa (C+)	IVb	IIa (C+)
29	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
30	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
32	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
33	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa

26 NRLs

84.6%  
of results in  
agreement  
with EURL  
results

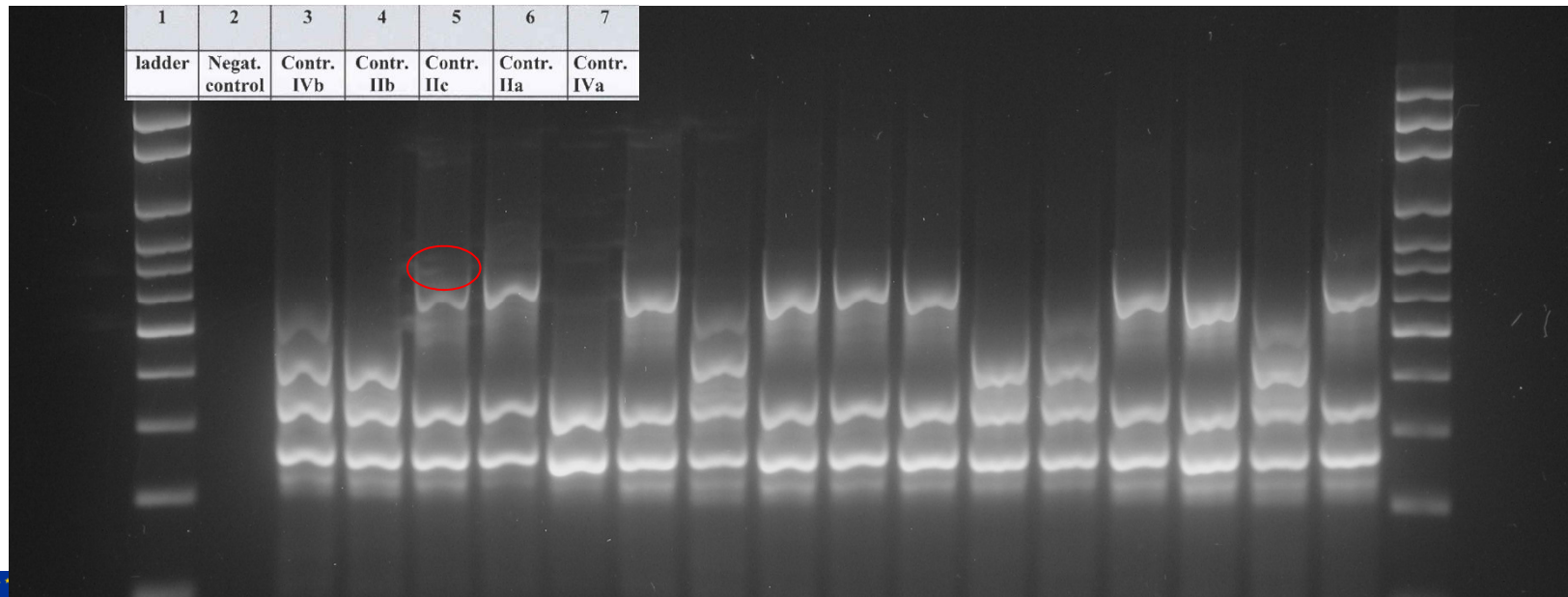
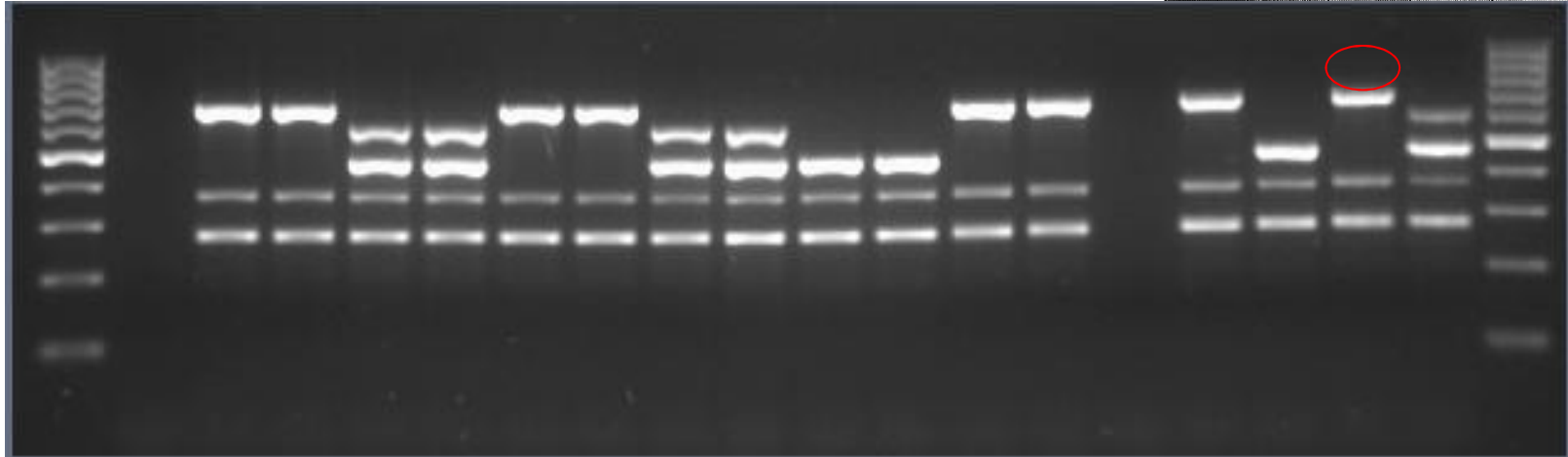
Strain 1-10 : Controls IIc and IIa no valid



# Deviation

Amplification fragment I<sub>mo</sub>1118 is missing from IIc C+

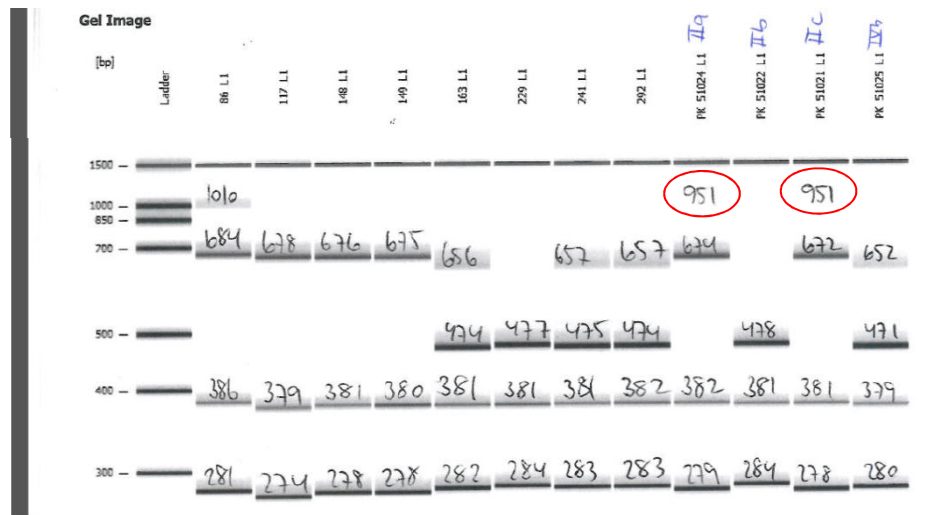
14	15	16	17	18
Ko+2a	Ko+2b	Ko+2c	Ko+4b	Ladder



# Deviation

Amplification fragment I<sub>mo</sub>1118 not visible but marked => IIc (strain control IIc)

Amplification fragment I<sub>mo</sub>1118 not visible but marked => IIc but strain control IIa ?



# About the results

## Molecular serotyping 2010 vs 2009 vs 2014:

	Participating NRLs	Molecular serotyping results	Improvement 2009 vs 2010 vs 2014
2009	14	93.5%	93.5% (14 NRLs)
2010	20	94.4%	96.8% (14 NRLs)
2014	26	84.6%	96,1% (14 NRLs)

## Main causes of deviation:

- Control not identified in the gel
- Band Im0737 not detected in the control





# Results of PFGE subtyping

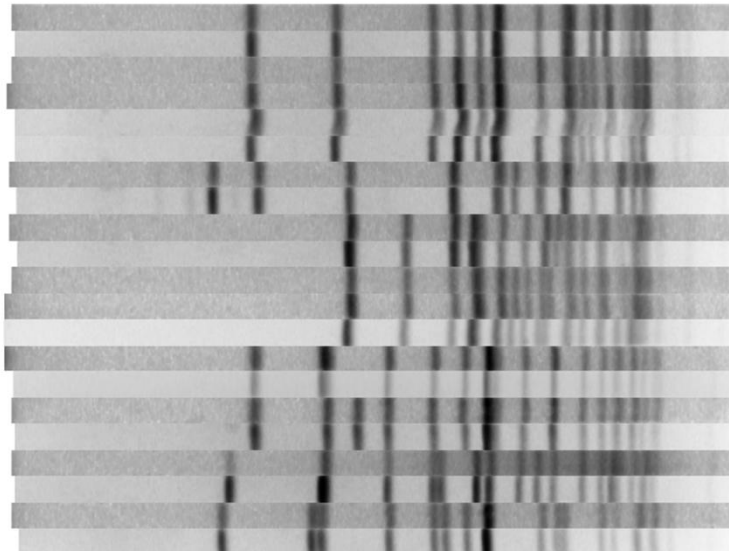
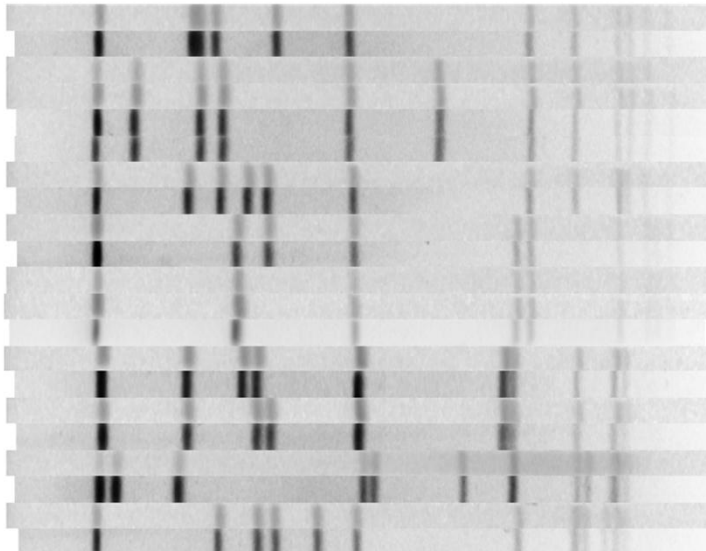


# Satisfactory results

PFGE-Ascl

PFGE-Apal

PT strain



245\_10\_5  
5  
165\_10\_10  
319\_10\_8  
8  
10  
266\_10\_4  
4  
283\_10\_1  
1  
36\_10\_3  
354\_10\_3  
3  
334\_10\_9  
9  
15\_10\_7  
7  
120\_10\_6  
6  
111\_10\_2  
2

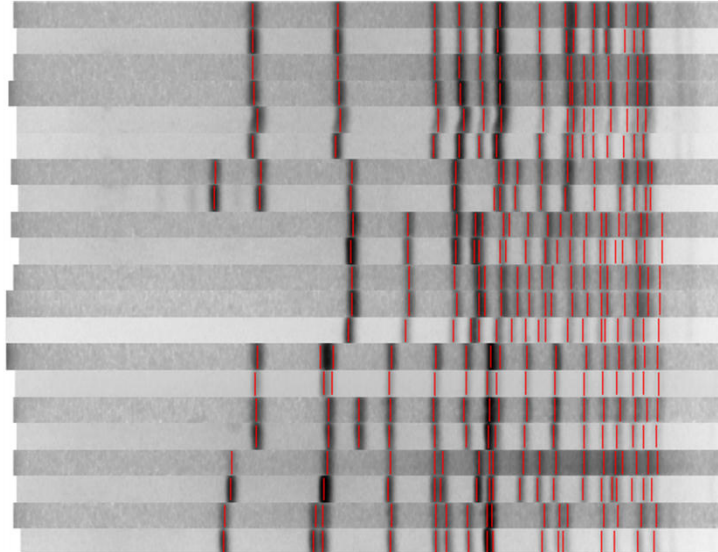
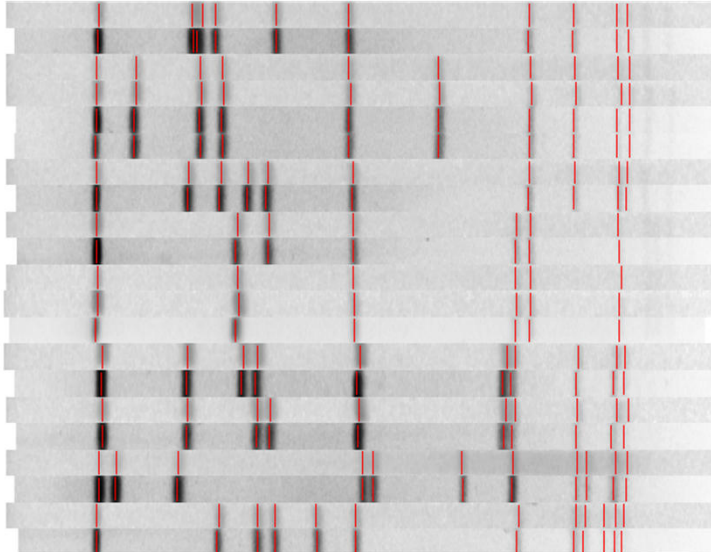
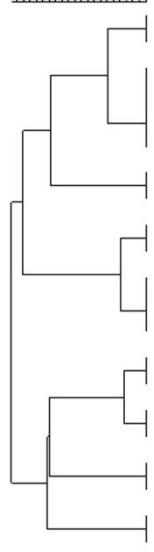
Apal-Ascl

PFGE-Ascl

PFGE-Apal

PT strain

100  
70  
40  
10



245\_10\_5  
5  
165\_10\_10  
319\_10\_8  
8  
10  
266\_10\_4  
4  
283\_10\_1  
1  
36\_10\_3  
354\_10\_3  
3  
334\_10\_9  
9  
15\_10\_7  
7  
120\_10\_6  
6  
111\_10\_2  
2








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# Analysis criterion

According to the EURL *Lm* Standard Operating Procedure

- Criterion: one band of difference (Barrett et al. 2006, FPD)
- Analysis down to 33 kb (Peters et al. 2003, *Eurosurveillance*)
- Deviations were divided in 5 categories

<ul style="list-style-type: none"><li>• Spotted gel</li><li>• Fuzzy image</li><li>• Inclined profile</li><li>• No reference system</li></ul>	<ul style="list-style-type: none"><li>• Extra band</li><li>• Band disappearance</li></ul>	<ul style="list-style-type: none"><li>• Distorted DNA migration profiles</li></ul>	<ul style="list-style-type: none"><li>• Oversaturated profiles</li><li>• Weakness of the low bands</li><li>• Weakness of the higher bands</li></ul>	<ul style="list-style-type: none"><li>• Abnormal band separation</li></ul>
Gel quality 	Incomplete restriction 	Migration distortion 	Band intensity 	Double band separation 

# PFGE Results: AscI

Strains	Strain 1	Strain 2	Strain 3	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7	Strain 8	Strain 9	Strain 10
Lab code	Ila	Iib	Ila	Ila	Iic	Ila	IVb	IVb	Ila	IVb	Ila
1	100	100	92,3	100	100	100	100	100	100	100	100
3	100	100	100	92,31	100	100	95,7	100	0	100	0
4	100	100	100	100	100	100	100	100	100	100	100
5	100	100	100	100	100	100	95,7	100	100	100	100
6	100	100	100	100	0	100	0	100	100	100	100
8	0	0	0	0	0	0	0	0	0	0	0
10	100	100	100	100	100	100	100	100	100	100	100
11	100	100	100	100	100	100	100	100	100	100	100
12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	100	96	100	92,31	83,3	80	0	100	76,9	100	0
16	100	100	100	100	100	100	100	100	100	100	100
17	100	100	100	100	100	100	100	100	100	100	100
19	100	100	100	100	100	100	100	100	100	100	100
20	0	100	0	0	100	100	100	100	0	100	0
21	100	100	100	100	100	100	100	100	100	100	100
23	100	100	100	100	100	100	100	100	100	100	100
25	100	100	100	100	100	100	100	100	100	100	100
29	100	100	100	100	100	100	0	100	100	100	100
30	100	100	100	100	94,2	100	100	100	100	100	100
33	100	100	100	100	100	100	100	100	100	100	100

Repeatability not satisfactory

Gel rejected : Poor Quality

84.3%  
of results in agreement with EURL  
results

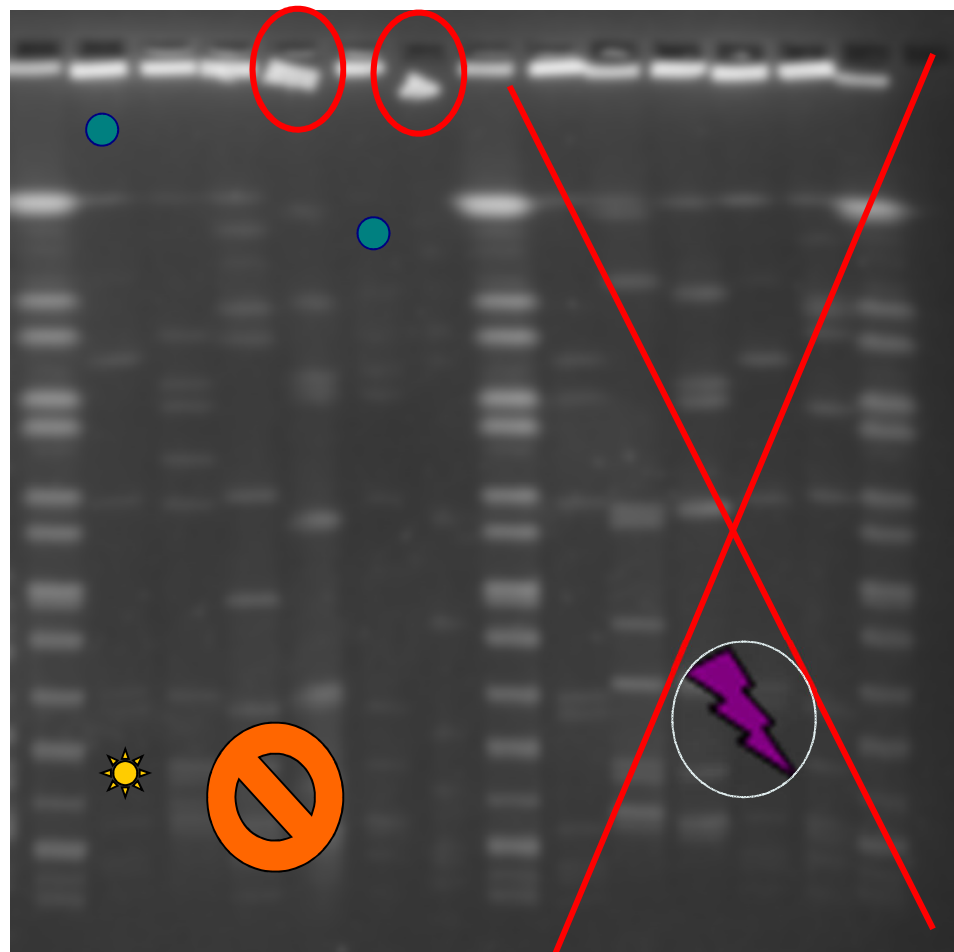


# PFGE *AscI* deviations

## Type of deviation

- Uncompleted restriction
- ⚡ Migration distortion
- ☀ Band intensity
- ⊘ Gel quality

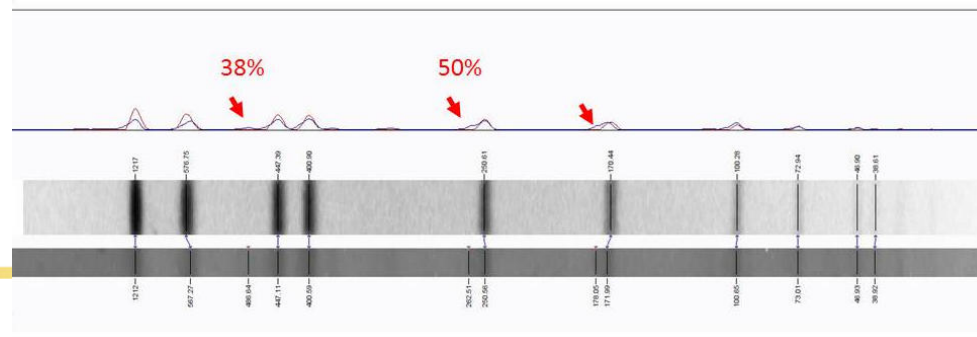
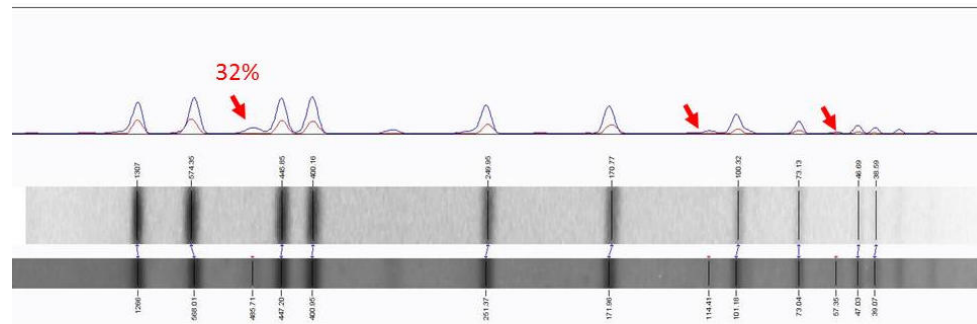
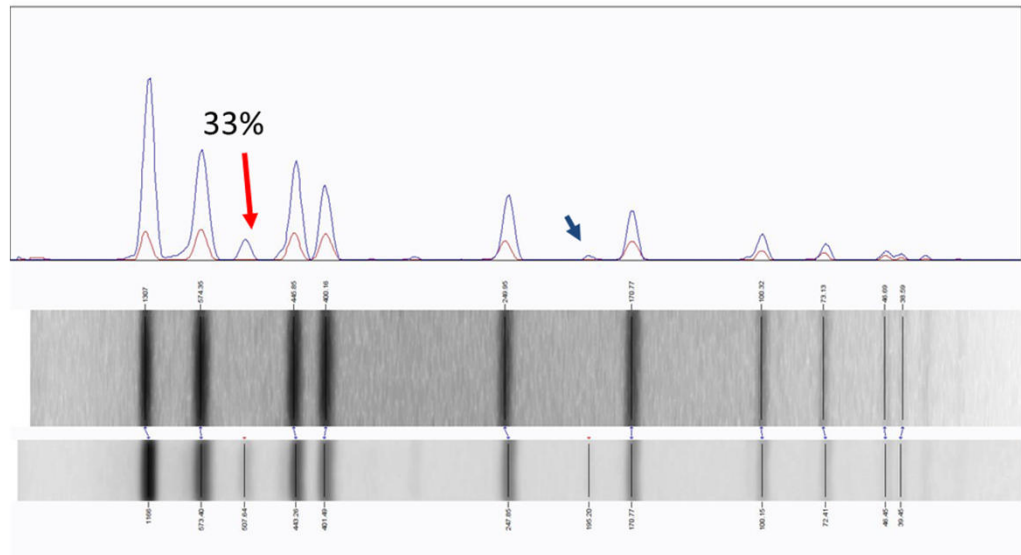
Gel rejected



# PFGE *AscI* deviations

- Extra band
- Band disappearance

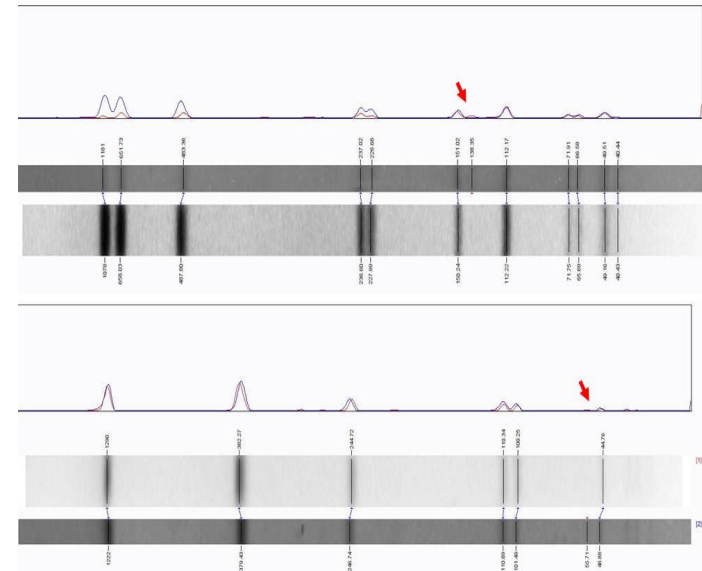
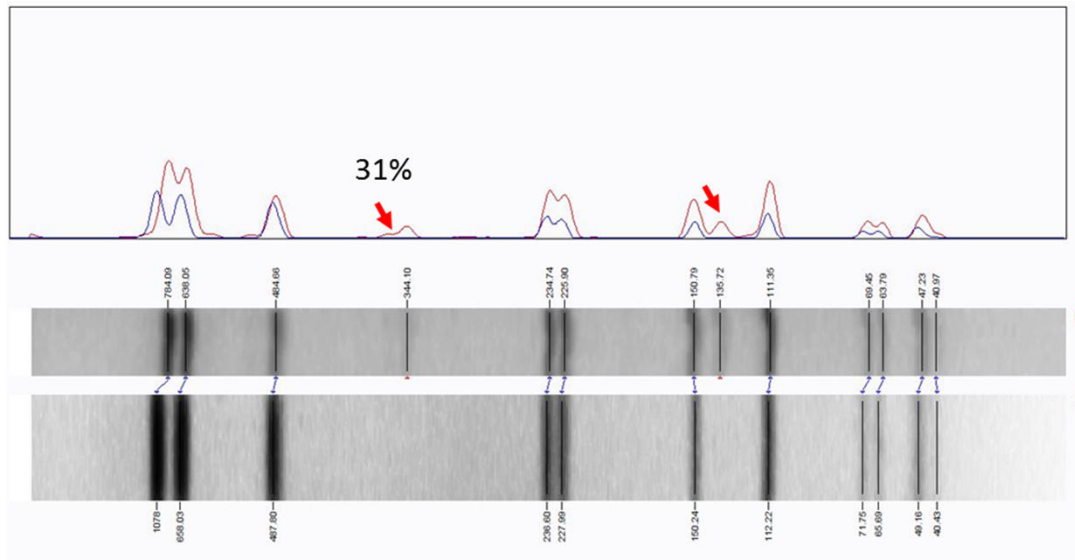
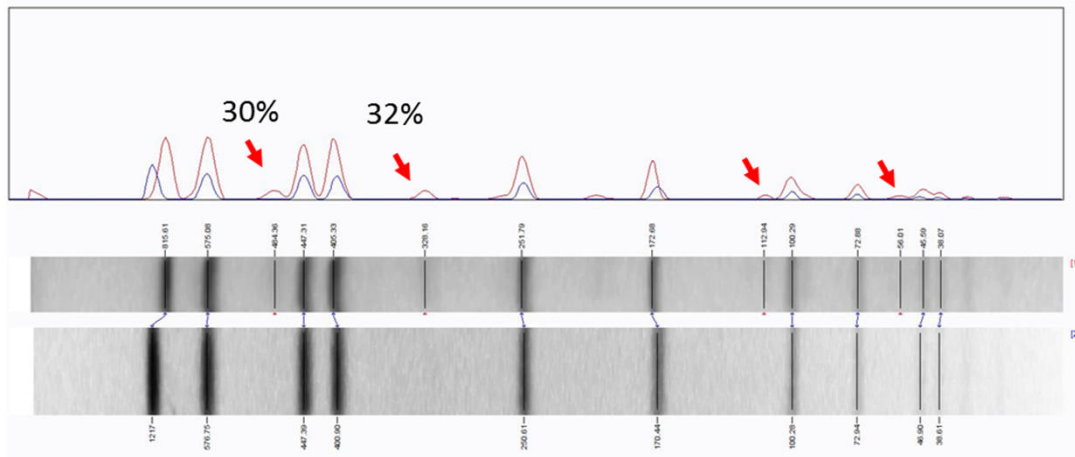
Incomplete restriction




# PFGE *AscI* deviations

- Extra band
- Band disappearance

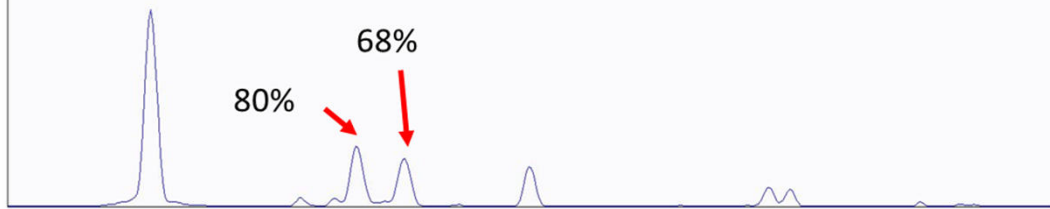
Incomplete restriction



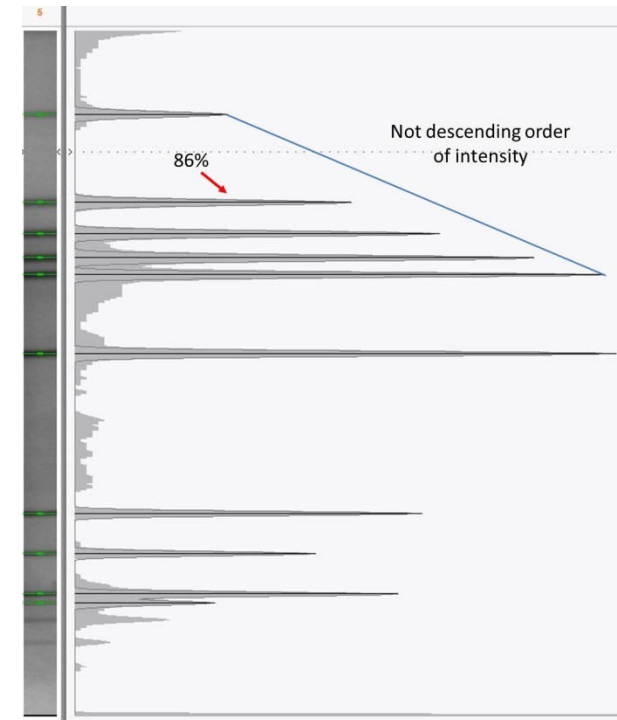
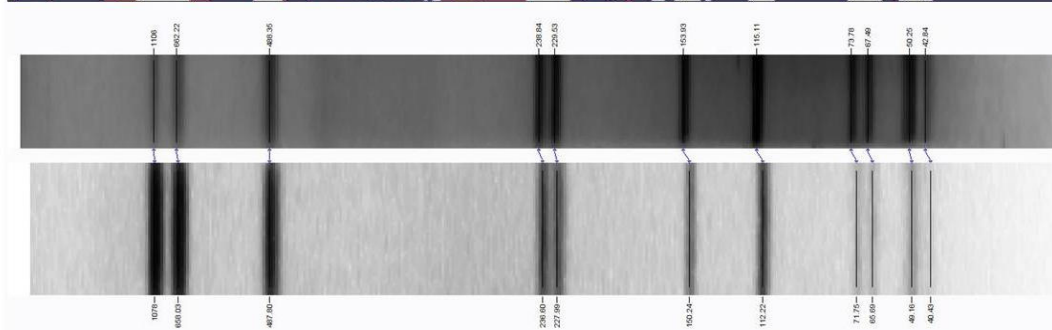
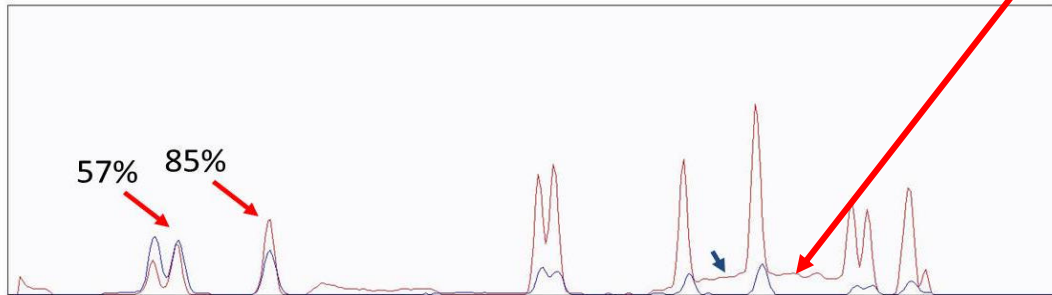
# PFGE *AscI* deviations

- Oversaturated profiles
  - Weakness of the low bands
  - Weakness of the higher bands
- Band intensity** 

Not assigned <30% < bd intensity < 100% ⇒ profile rejected (above 200kbp)




smear



Above 200kbp relative intensity of band >100% (average of bands intensity of the profile)

Reference system

- Spotted gel
  - Fuzzy image
  - Inclined profile
  - No reference system
- Gel quality 

5 wide wells

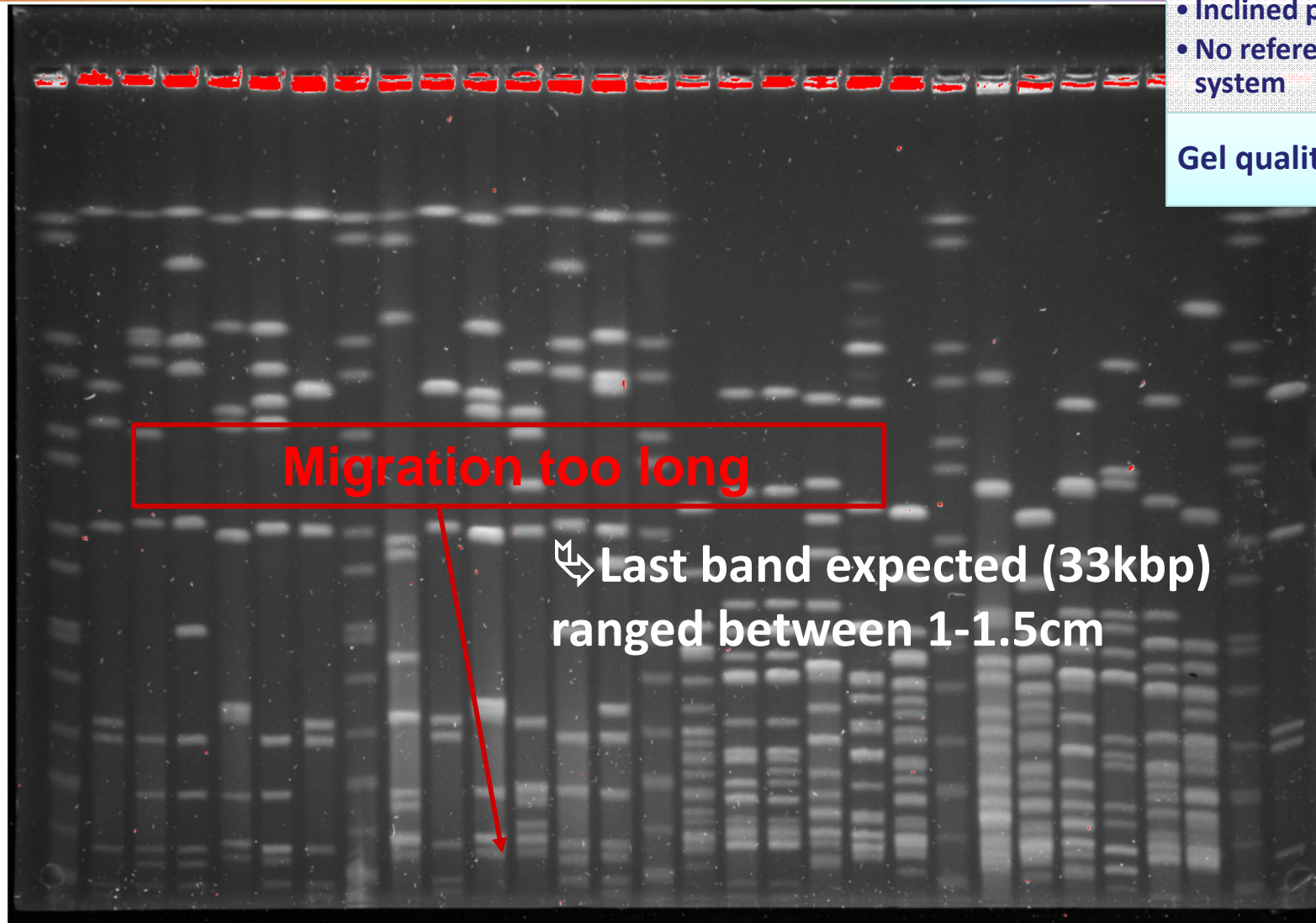
For wide wells ⇒ Not exceed 4  
Narrow wells ⇒ Not exceed 6

Not well-normalized

## PFGE *AscI* deviations

- Spotted gel
- Fuzzy image
- Inclined profile
- No reference system

Gel quality



# PFGE Results: *Apal*

Strains	Strain 1	Strain 2	Strain 3	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7	Strain 8	Strain 9	Strain 10
Lab code	IIa	IIb	IIa	IIa	IIc	IIa	IVb	IVb	IIa	IVb	IIa
1	100	100	100	100	100	100	100	100	100	100	100
3	100	100	100	100	100	100	100	100	100	100	100
4	100	100	100	100	100	100	100	100	100	100	100
5	100	100	100	100	100	100	100	100	100	100	100
6	100	100	100	100	100	100	100	100	100	100	100
8	100	100	100	100	100	0	100	100	100	100	100
10	100	100	100	100	100	100	100	100	100	100	100
11	100	100	100	100	100	100	100	100	100	100	100
12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	100	100	100	100	100	100	100	100	100	100	100
16	100	100	100	100	100	100	100	100	100	100	100
17	100	100	100	100	100	100	100	100	100	100	100
19	100	100	100	100	100	100	100	100	100	100	100
20	100	100	100	100	100	100	100	100	100	100	100
21	100	100	100	100	100	100	100	100	100	100	100
23	100	100	100	100	100	100	100	100	100	100	100
25	100	100	100	100	100	100	100	100	100	100	100
29	100	97	100	100	100	100	100	100	100	100	100
30	100	100	100	100	100	100	100	100	100	100	100
33	100	100	100	100	100	100	100	100	100	100	100

- Extra band
- Band disappearance

Incomplete restriction



- Oversaturated profiles
- Weakness of the low bands
- Weakness of the higher bands

Band intensity



99.0%  
of results in agreement with EURL results



## About the results

	Participating NRLs	PFGE results
2009	15 (Ascl) / 14 (Apal)	83.0% (Ascl) / 81.0% (Apal)
2010	17 (Ascl) / 17 (Apal)	94.8% (Ascl) / 83.0% (Apal)
2012	18 (Ascl) / 18 (Apal)	82.8% (Ascl) / 77.8% (Apal)
2014	19 (Ascl) / 19 (Apal)	84.3% (Ascl) / 99.0% (Apal)





# Deviations and improvement

## Main causes of deviation 2014

1. Uncompleted restriction
2. Band intensity



## Main improvement in 2014 compare to 2012

1. Migration distortion (only one gel)



**Area for Improvement ⇒ incomplete restriction**



## Follow-up to the PT trial

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All undistinguishable profiles = « Good » 52.6% of participating NRLs

Only one deviation (*Apal* or *Ascl*) = « Satisfactory » 15.7% of participating NRLs

More than one deviation = « Unsatisfactory » 31.6% of participating NRLs

**You are invited to perform again your analysis after implementing corrective measures**



**Participant interpretation (XML file assessment)**

**⇒ Satisfactory**

# Acknowledgement to EURL Lm/SSI/NRL network



**SEL unit *Listeria monocytogenes* team**

**Sophie Roussel (Team leader)**

**Benjamin Félix**

**Léna Barre**

**Jean François Mariet**

**Trinh Tam Dao**

**Karol Romero**

**Emilie Brasseur**



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**Marie-Christine Salvi**

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**Jonas Larsson**

**Susanne Shjørring**

**Eva Møller-Nielsen**



**EQA3**

**The NRL network**



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