5th annual workshop of the national reference laboratories for *E. coli* in the EU

STEC surveillance in France: official laboratory network and results of 2010 ring trial

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STEC surveillance in France:official laboratory network and results of 2010 ring trial

Plan

1- Official laboratory network

- Official controls organisation
- Networks creation/extension in 2009 and 2010
- Territorial distribution

2- Results of 2010 ring trial

STEC surveillance in France:official laboratory network and results of 2010 ring trial

Official controls organisation

Annual studies

(Directive EU 2003/99/CE; Regulation EU N° 882/2004)

Objective: Estimate STEC* prevalence in risk-associated food
(* *E. coli* possessing *eae* and *stx* genes and belonging to the 5 major EHEC-associated serogroupes *i.e*. O157, O26, O103, O111 and O145)

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Year	Matrix	Nb of samples screened	Nb of strains isolated	STEC * prevalence
2007	Ground beef batch (+4°C)	3605	11 STEC* (5 *eae*+ *E. coli*)	11/3605 0,3% CI_{95} [0,2 – 0,5%]
	Raw milk cheese	392	0	0/392 ≤ 0,9%
2008	Ground beef trimming (-20°C)	992 (4 units/samples)	10 STEC* (13 *eae*+ *E. coli*)	10/992 1% CI_{95} [0,6 – 1,8%]
2009	Minced beef at retail	1527	2 STEC* (3 *eae*+ *E. coli*)	2/1527 0,1% CI_{95} [0,04 – 0,5%]
	Raw milk cheese	1911	17 STEC* (14 *eae*+ *E. coli*)	17/1911 0,9% CI_{95} [0,6 – 1,4%]
2010	Minced beef at retail	1403 out of 2500	4 STEC* (*9 eae*+ *E. coli*)	3/1403

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Official controls organisation

Annual studies

⇨ Samples analyses

- Official method: CEN/ISO TS

« Microbiology of food and animal feeding stuffs - Horizontal method for the detection of STEC belonging to O157, O111, O26, O103 and O145 serogroups – Qualitative method »

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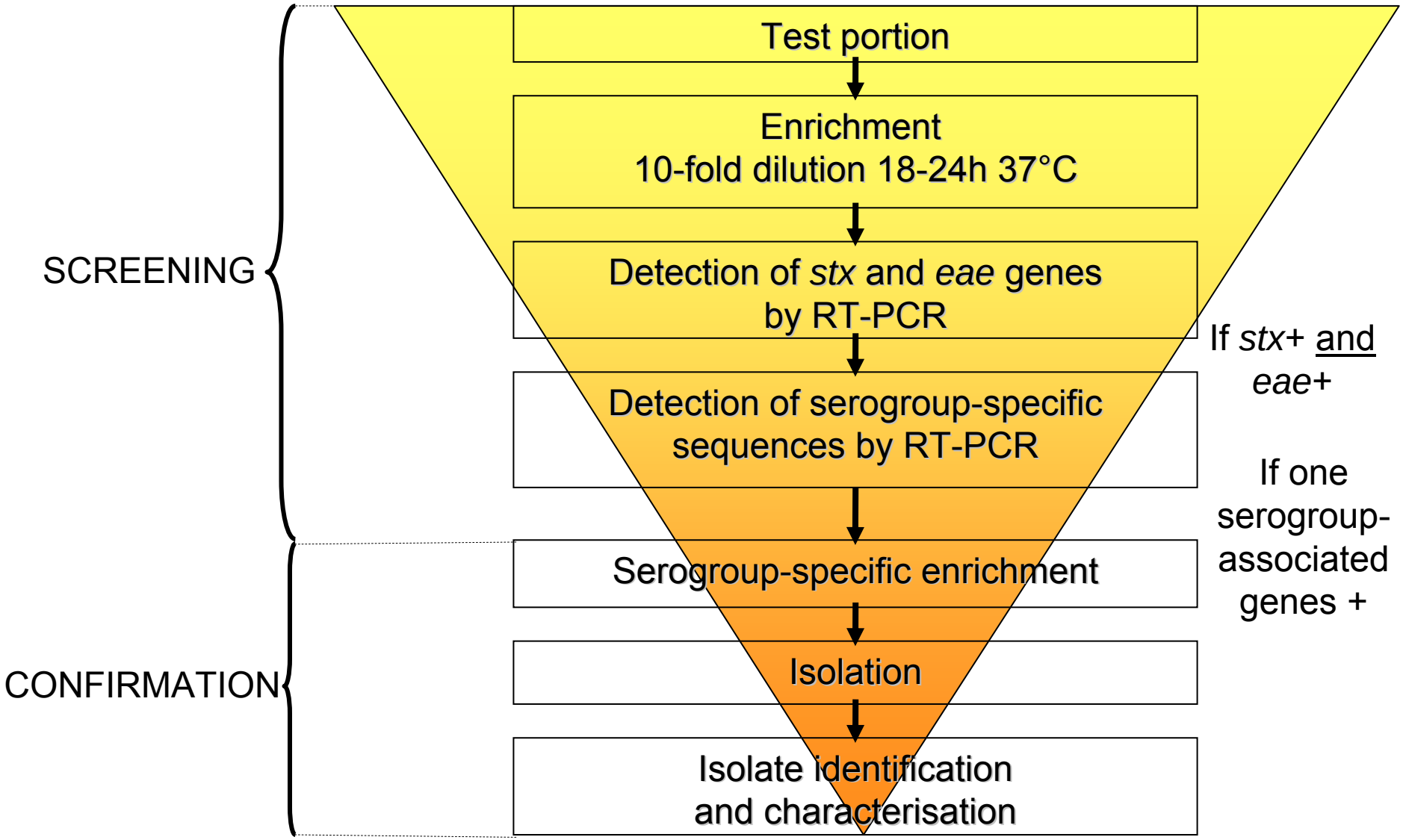

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Official controls organisation

Annual studies

⇨ Samples analyses

- First screening by official laboratories
- Confirmation by the NRL

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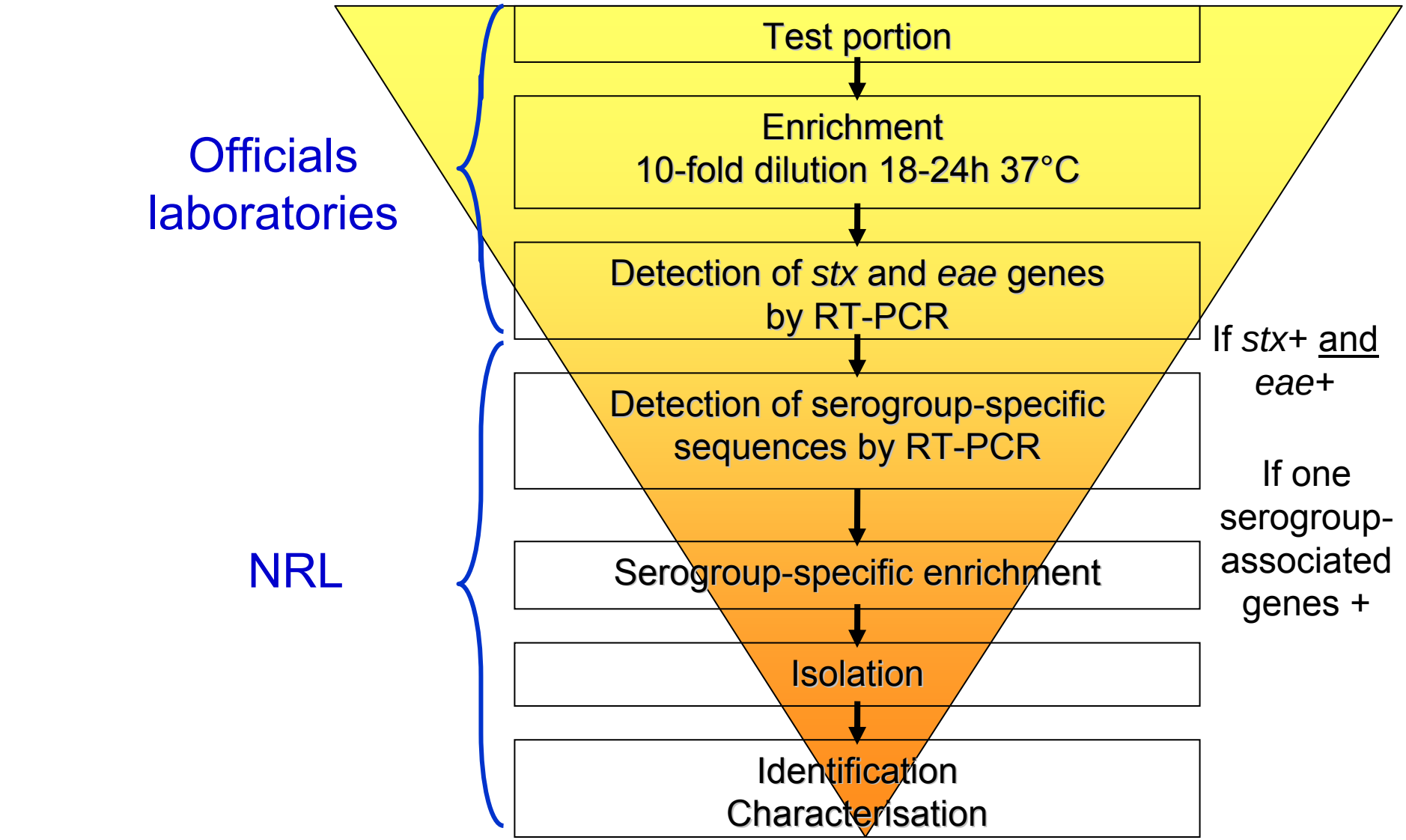

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Network creation/extension in 2009 and 2010

➡ **French regulation**
(Regulation EU N° 882/2004)

- Publication of a call for application
- General requirements

(accreditation/information exchange)

- Specific requirements

(technical accreditation/material/training/ring trial)

➡ 2 networks created (O157 and STEC)

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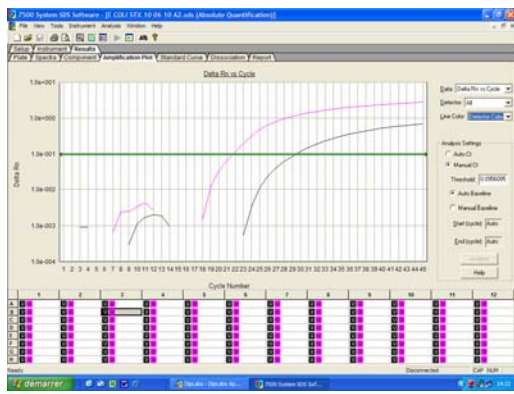
Network creation/extension in 2009 and 2010

Role of the NRL

- Provide an official method
- Provide technical support for the call and for the analyses of the laboratories' files
- Organize trainings
- Organize a ring trial



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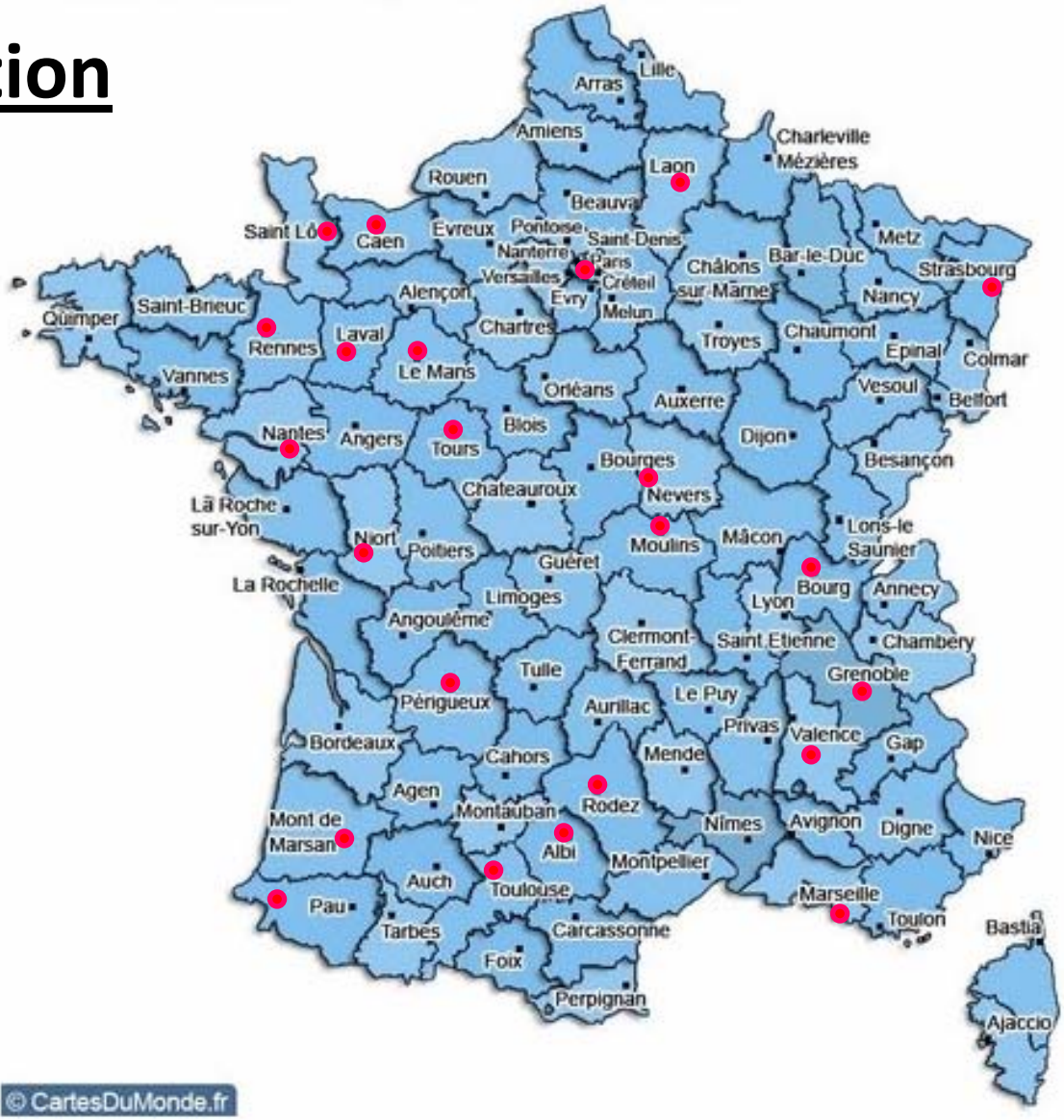
Territorial distribution

➡ **O157 network:**
23 laboratories (•)



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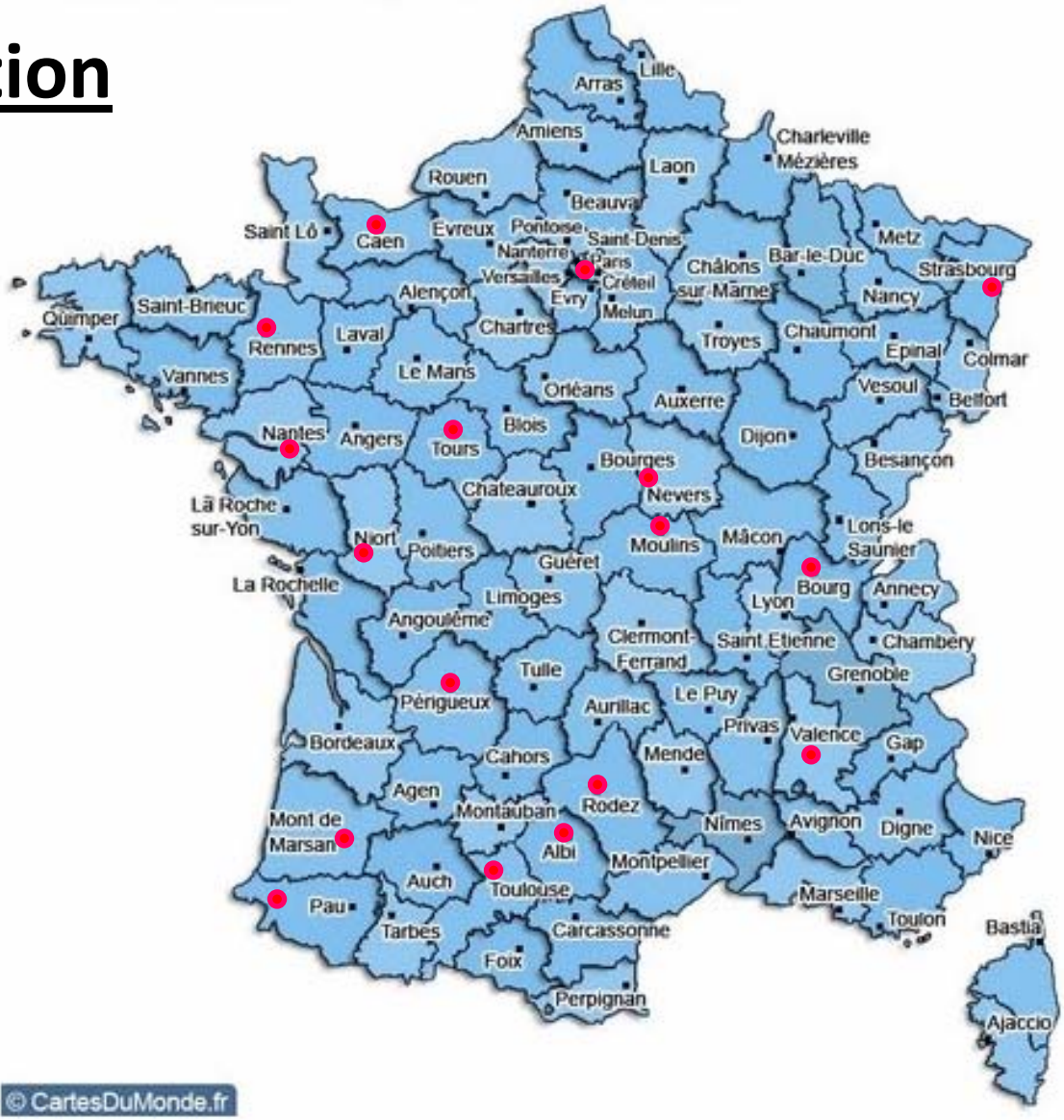
Territorial distribution

➡ **STEC network:**
17 laboratories (•)

…Probably
19 laboratories in 2011



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Results of 2010 ring trial

Objectives:

- Quality assurance
- Extension of the STEC network in 2010

(Participation is mandatory in the call for application of French ministry of agriculture)

Results of 2010 ring trial

Method:

- Target: Detection of *eae* and *stx* genes in minced beef
- Official method:

Detection of *stx* and *eae* genes by RT-PCR with internal control

(1 method provided by the NRL adapted from CEN/ISO TS)

Results of 2010 ring trial

- **Participants:**
  - Food inspections laboratories in France
  - Only candidates that were first selected by the French ministry of Agriculture

(general and specific requirements indicated in the call)

  - 19 laboratories participate + the NRL

Results of 2010 ring trial

Sample preparation:

- Minced meat (10 kg)

(collected at retail)

- Enrichment performed by the NRL (37°C – 20h)

(enrichment broth checked for the absence of *stx* and *eae* genes)

- Artificial contamination (or not) of enrichment broth

(level of contamination checked before and after contamination)

- 8 samples (5ml) of enrichment broth

(same test portion for each participant)

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Results of 2010 ring trial

Characteristics of the 8 samples sent

Strains /samples	A	B	C	D	E	F	G	H
***E. coli* O157:[H7] *stx*$_1$+, *stx*$_2$+, *eae*+**	$5x10^4$ CFU/ml						$5x10^4$ CFU/ml	
***E. coli* O157:[H7] *stx*$_1$-, *stx*$_2$+, *eae*+**		10^5 CFU/ml						
***E. coli* O26:[H11] *stx*$_1$-, *stx*$_2$-, *eae*+**				10^5 CFU/ml				
***E. coli* O26:[H11] *stx*$_1$+, *stx*$_2$-, *eae*+**					10^5 CFU/ml			
***E. coli* O139:H – *stx*$_1$- *stx*$_2$+, *eae*-**			10^5 CFU/ml					

stx+ or *eae* + samples: Detectability

stx- or *eae* - samples: Specificity

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Results of 2010 ring trial

➡ Detection of *stx* genes by RT-PCR

Détection des gènes	Echantillons	Valeur attendue	Laboratoires participants																		
			L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20
stx	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
	D	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	F	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Detectability (samples A, B, C, E, G)

19 laboratories do detect all the *stx*+ samples

1 laboratory fails to detect *stx* gene in sample C (internal control OK)

Specificity (samples D, F, H)

19 laboratories get expected results (*stx*-)

1 laboratory gets false positive results (cross contamination?)

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Results of 2010 ring trial

➡ Detection of *eae* genes by RT-PCR

Détection des gènes	Echantillons	Valeur attendue	Laboratoires participants																		
			L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20
eae	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
	D	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	F	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
	G	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Detectability (samples A, B, D, E, G)

All laboratories (20) do detect all the *eae* + samples

Specificity (samples C, F, H)

19 laboratories get expected results (*eae*-)

1 laboratory gets false positive results (cross contamination ?)

Results of 2010 ring trial

Conclusion

- Number of samples screened and statistical interpretation!

Ex: Detectability (evaluation with 5 samples)
If 5 expected results/5, discrepancy frequency CI_{95} [0,48 – 1]
If 4 expected results/5, discrepancy frequency CI_{95} [0,28 – 0,99]

Lamy *et al*., Simple table for estimating confidence interval of discrepancy frequencies in microbiological safety evaluation. Journal of microbiology methods (2004) 137-139.

Results of 2010 ring trial

➡ **Conclusion**

- 18 out of 20 laboratories get all the expected results
- 2 laboratories failed

➡ New ring trial next week!

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Fanny Savoye

Stéphane Miszczycha

Sarah Ganet

Marion Bouvier

Audrey Gleizal

Franck Ferre

And Manuelle Neto, Cécile Dutrieux, Françoise Clavel, Patrice Zurcher!!

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