Confirmation methods proposed by the Belgian National Reference Center (NRC) for *Vibrio* when a suspected case is encountered

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General missions of the NRC

- To confirm the diagnostic of ***Vibrio*** serogroups **O1** and **O139**
- To confirm the diagnostic of ***Vibrio*** **non O1**, **non O139** and other *Vibrio* species
- To determine the production of **toxins**
- Monitor **circulating** strains
- Check the antibiotic **susceptibility**
- To participate in national surveillance
- Collaborate with the national and European food safety agencies

Specific mission

- Genome typing of all the virulent strains (**Diversilab**)

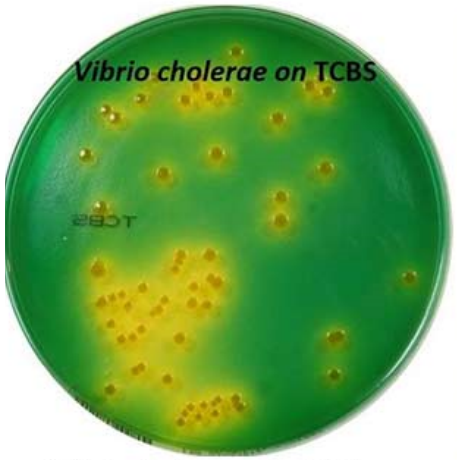
NRC algorithm

Upon reception : the strain is encoded
- ✓**Culture on blood agar/TCBS**
- ✓**Maldi-Tof**

Culture TCBS (Thiosulfate bile sucrose)

Most enteriobacteriacae from faeces are suppressed

S. faecalis can persist but distinguished by the colour and form

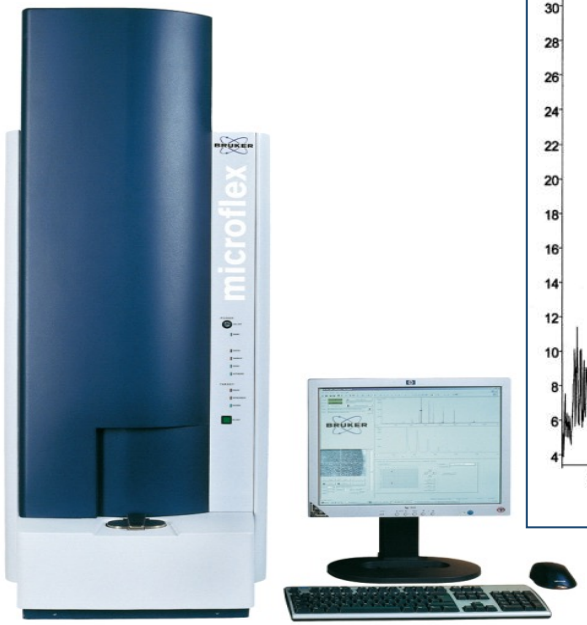


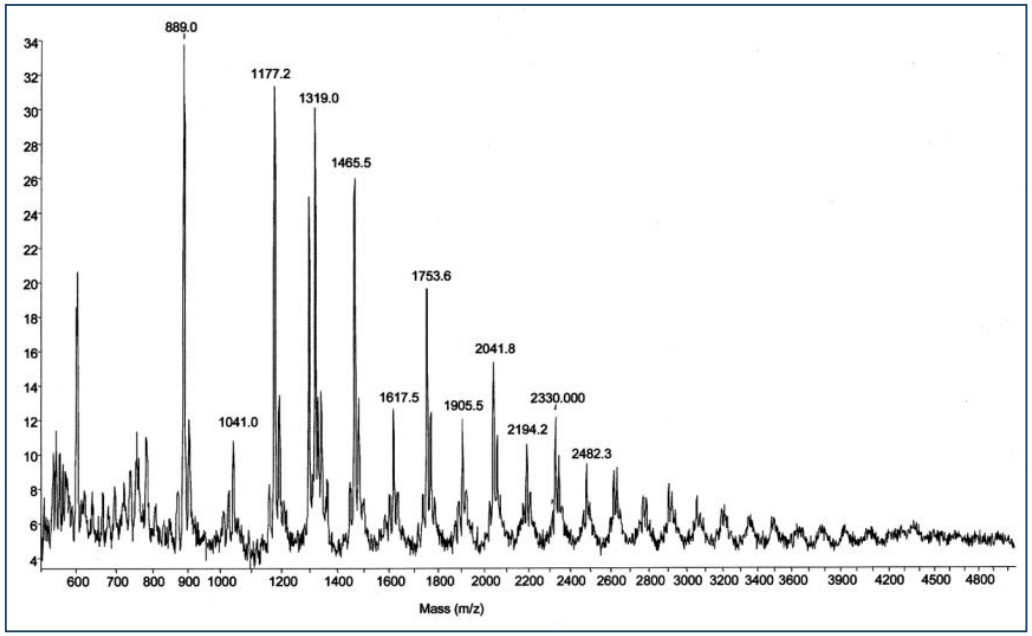
Vibrio cholerae on TCBS Agar

Vibrio parahaemolyticus on TCBS Agar

Identification

- Method: **Maldi-Tof**



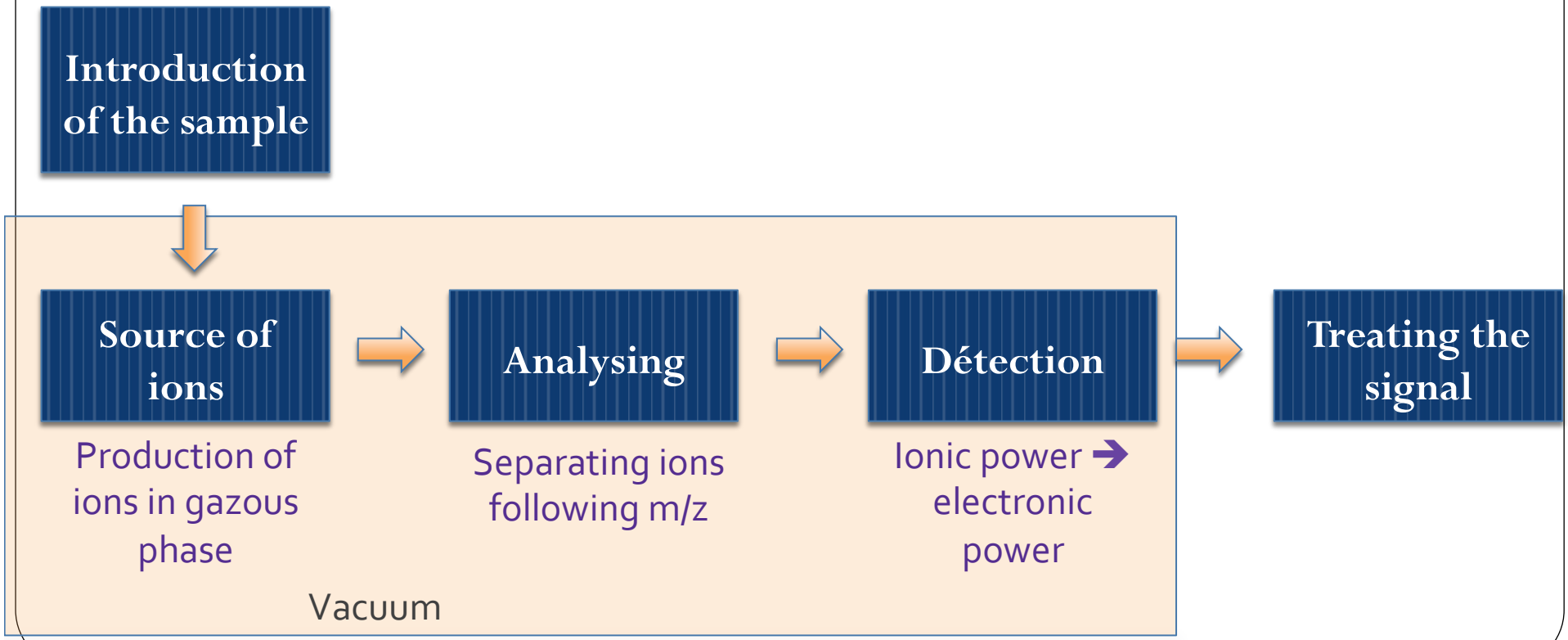


Microflex Bruker Daltonics

Mass spectrometry

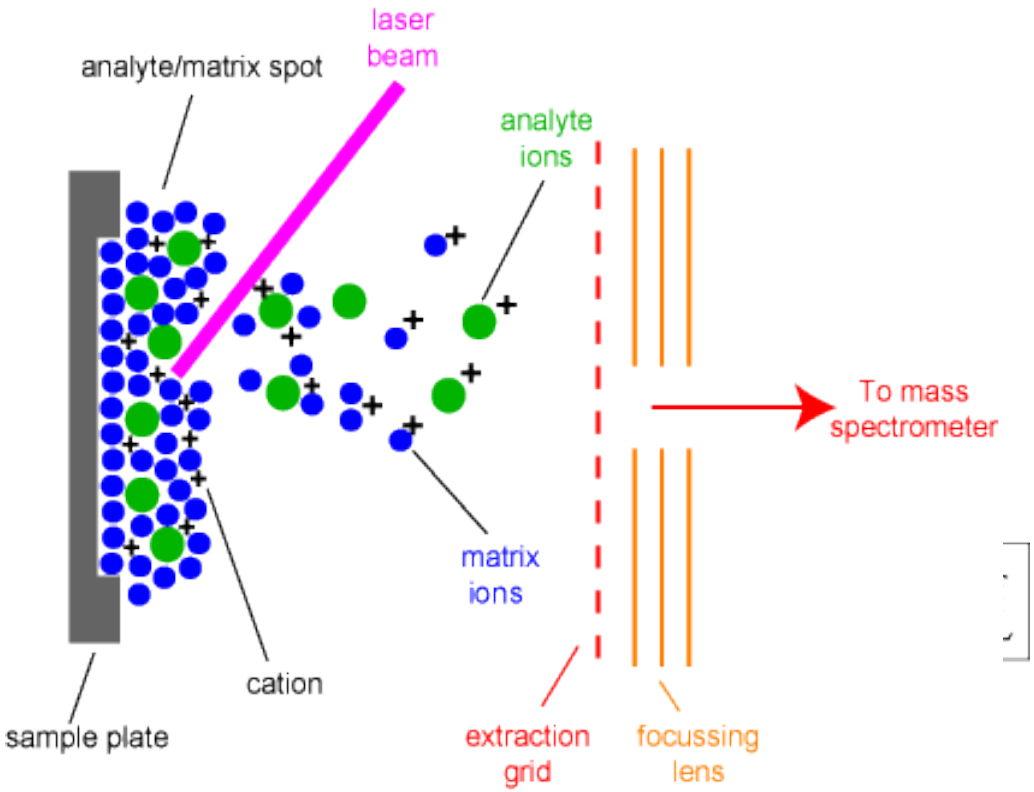
Principle:

Determine with **mass spectrometry** the molecular mass of free ions



Mass spectrometry MALDI-TOF

MALDI: **M**atrix **A**ssisted **L**aser **D**esorption/**I**onization



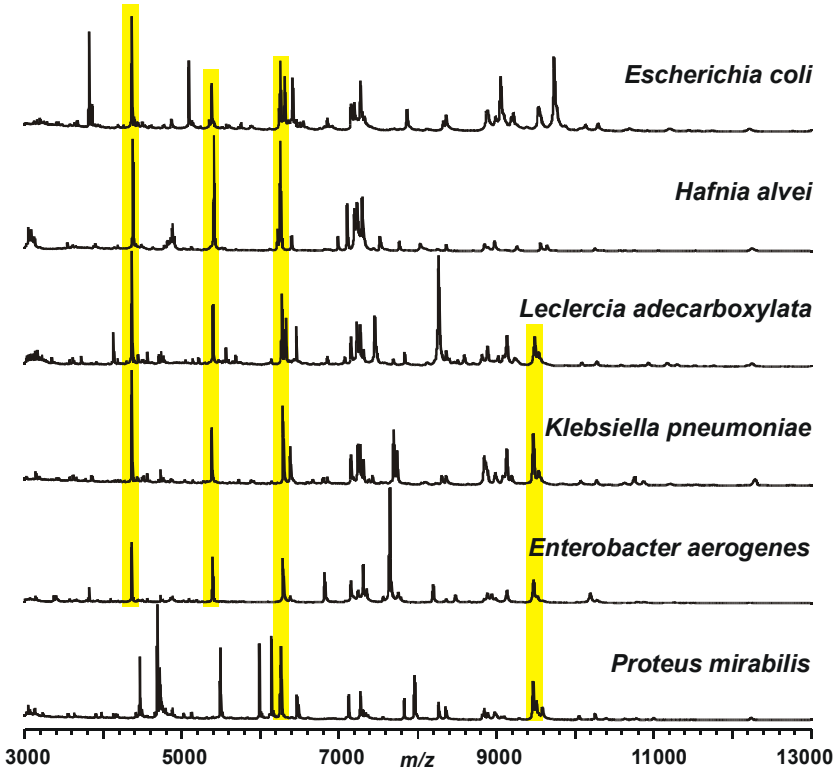
1. The sample is mixed with a matrix HCCA and then dried
2. The laser ionizes molecules of the matrix
3. Molecules of the samples are ionized by transfer from the matrix

$MH^+ + A \rightarrow M + AH^+$

Principle of ID

Detection of large proteins (1000 – 300000 Da)

- Spectra differing between microorganisms
- Reproducible spectra
- Peaks specific of the genus, species or sub species

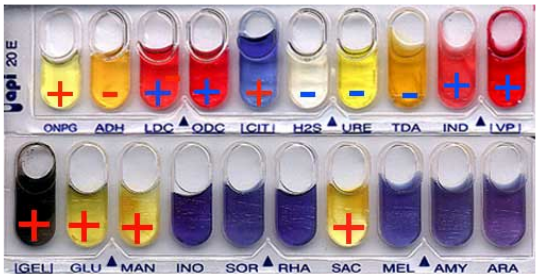


ID decision criteria

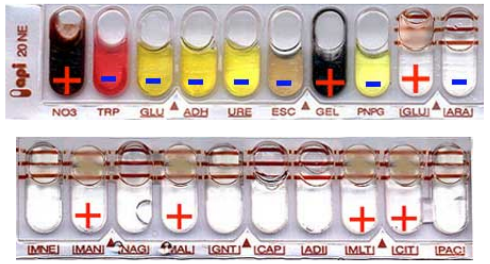
Meaning of Score Values

Range	Description
2.300 ... 3.000	highly probable species identification
2.000 ... 2.299	secure genus identification, probable species identificat
1.700 ... 1.999	probable genus identification
0.000 ... 1.699	no reliable identification

API System 20E



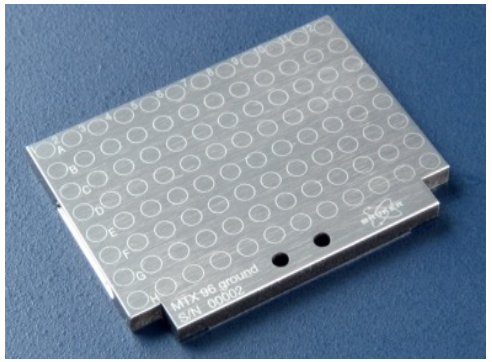
API System 20NE

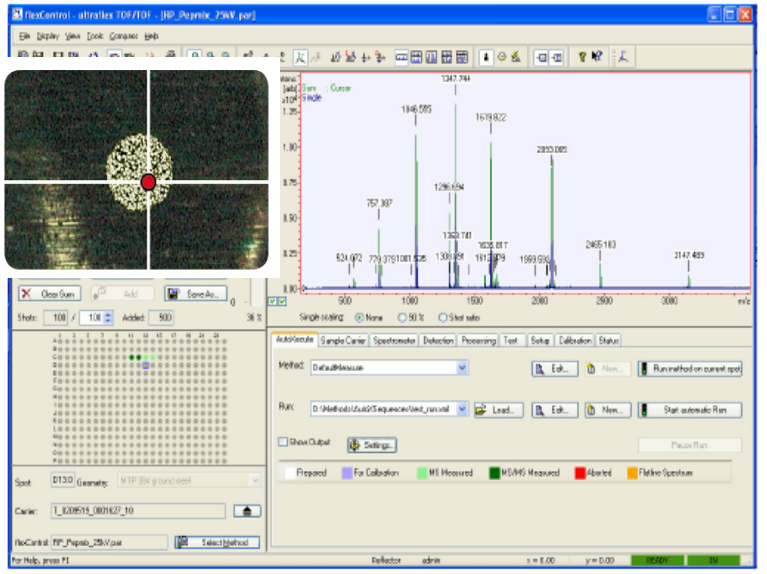


In the lab

Analysis of bacterial culture on agar

1. Direct deposit on the target
2. Add the matrix
3. Dry
4. Introduce in the spectrophotometer





First ID in less than 2 minutes!

ID of *V. cholerae*

Analyte2

BRUKER

Nom de l'échantillon:	G2
Description de l'échantillon:	
ID de l'échantillon:	160414-0051 V16-0005
Date/Heure de création de l'échantillon:	2016-04-18T09:20:23.143
Bibliothèque de MSP utilisée:	Bruker + SR Taxonomy (sans Shigella)
Arbre de taxonomie utilisé:	

Classement (Qualité)	Profil de référence	Score	Identifiant NCBI
1 (++)	Vibrio cholerae 6536	2.286	666
2 (++)	Vibrio cholerae 6550	2.253	666
3 (+)	Vibrio cholerae 71-89	1.863	666
4 (+)	Vibrio albensis LMG 4406T HAM	1.853	140100

ID of *V. parahaemolyticus*

Analyte2

BRUKER

Nom de l'échantillon: H2
Description de l'échantillon:
ID de l'échantillon: V16-026
Date/Heure de création de l'échantillon: 2016-10-04T14:02:10.771
Bibliothèque de MSP utilisée: Bruker + SR Taxonomy (sans Shigella)
Arbre de taxonomie utilisé:

Classement (Qualité)	Profil de référence	Score	Identifiant NCBI
1 (++)	Vibrio parahaemolyticus LMG 4423 LMG	2.152	670
2 (++)	Vibrio parahaemolyticus 4a IBS	2.13	670
3 (++)	Vibrio parahaemolyticus DSM 11058 DSM	2.117	670
4 (++)	Vibrio parahaemolyticus DSM 10027T DSM	2.108	670
5 (++)	Vibrio parahaemolyticus DSM 15416 DSM	2.102	670
6 (++)	Vibrio parahaemolyticus DSM 15477 DSM	2.06	670
7 (+)	Vibrio parahaemolyticus CCM 5937 CCM	1.927	670
8 (-)	Vibrio parahaemolyticus 7a IBS	1.691	670

Other *Vibrio* species

Description de l'échantillon:

ID de l'échantillon: 13061300380101

Date/Heure de création de l'échantillon: 2016-06-14T11:04:26.186

Bibliothèque de MSP utilisée: Bruker + SR Taxonomy (sans Shigella)

Arbre de taxonomie utilisé:

Classement (Qualité)	Profil de référence	Score	Identifiant NCBI
1 (++)	Vibrio fluvialis CCM 3689T CCM	2.117	676
2 (++)	Vibrio fluvialis CCM 3695 CCM	2.095	676

Other *Vibrio* species

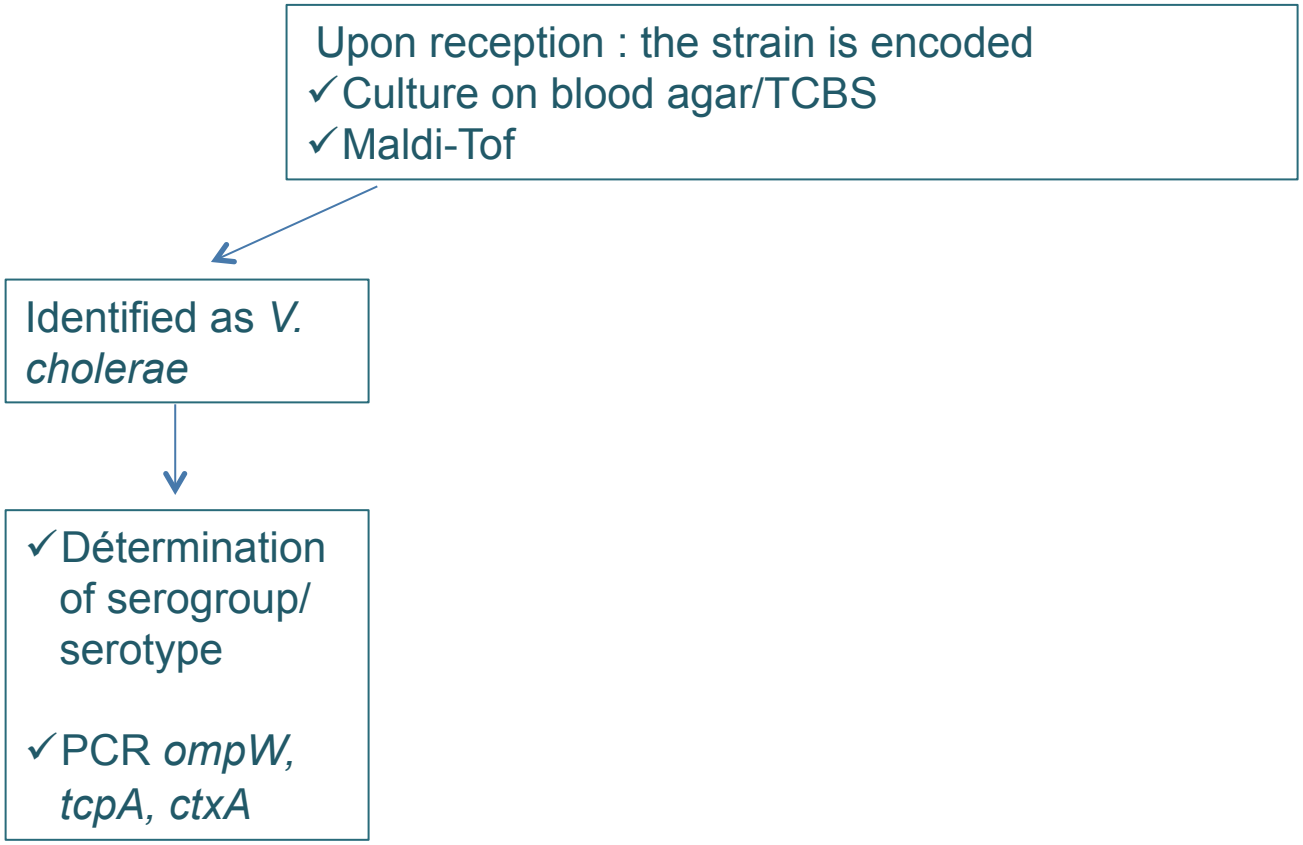
Analyte3

BRUKER

Nom de l'échantillon:	D3
Description de l'échantillon:	
ID de l'échantillon:	161006-0031 V16 - 0027
Date/Heure de création de l'échantillon:	2016-10-10T13:29:15.717
Bibliothèque de MSP utilisée:	Bruker + SR Taxonomy (sans Shigella)
Arbre de taxonomie utilisé:	

Classement (Qualité)	Profil de référence	Score	Identifiant NCBI
1 (+)	Vibrio alginolyticus CCM 7037 CCM	1.964	663
2 (+)	Vibrio alginolyticus CCM 2578T CCM	1.868	663
3 (+)	Vibrio alginolyticus DSM 2171T DSM	1.841	663
4 (+)	Vibrio alginolyticus CCM 5941 CCM	1.769	663

NRC algorithm



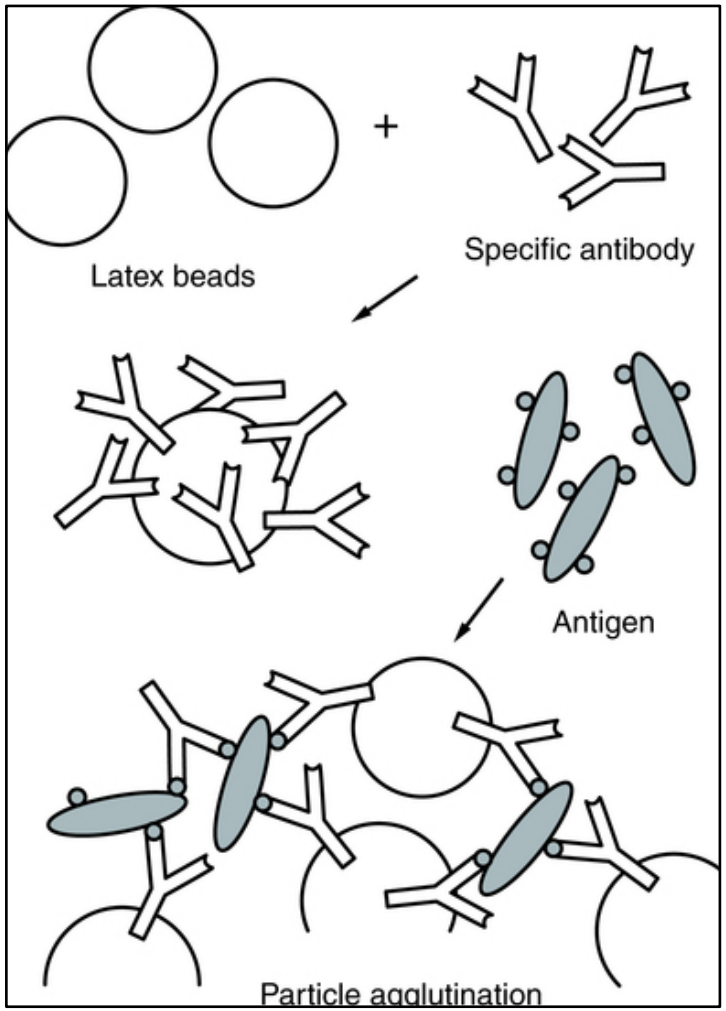
Serogroup/serotype determination

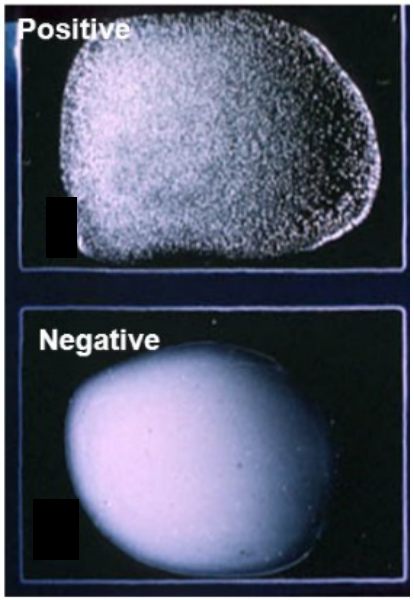
- Two common serotypes **Inaba, Ogawa, O1 (+Hikojima)**
- One less represented ; **Bengal, O139**

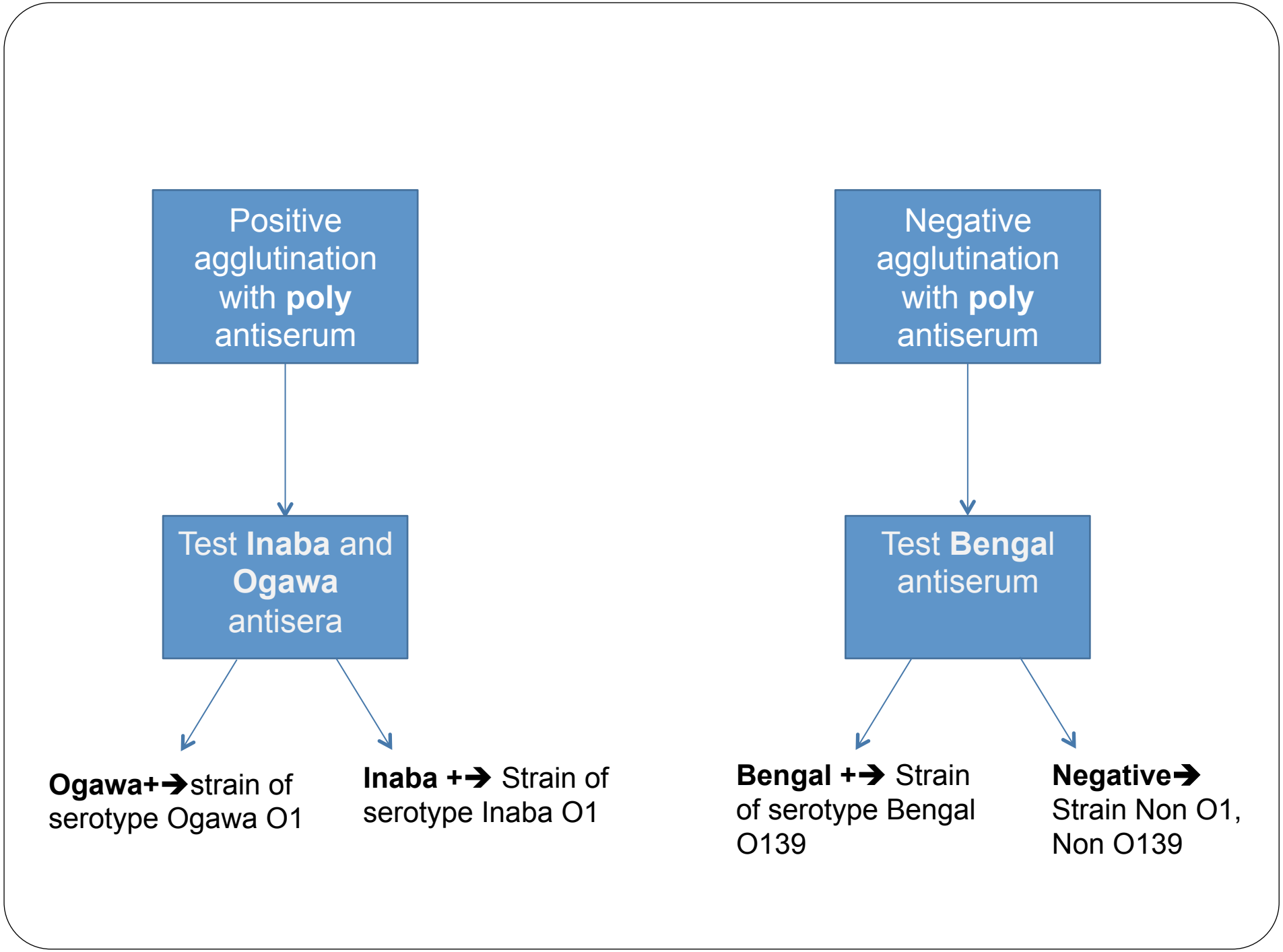
- These organisms may be identified by agglutination in O group 1- and O group139 specific antiserum directed against the lipopolysaccharide component of the cell wall

- ➔ **Latex agglutination kit (Mast assure)**

Latex agglutination: principle






Positive agglutination with **poly** antiserum
Test **Inaba** and **Ogawa** antisera
Ogawa+→strain of serotype Ogawa O1
Inaba +→ Strain of serotype Inaba O1
Negative agglutination with **poly** antiserum
Test **Bengal** antiserum
Bengal +→ Strain of serotype Bengal O139
Negative→ Strain Non O1, Non O139

PCR on *Vibrio sp.*

PCR *Vibrio cholerae*

- **Identification gene**
  - ✓ ***ompW***→ External protein of the membrane → Specific to *V. cholerae*

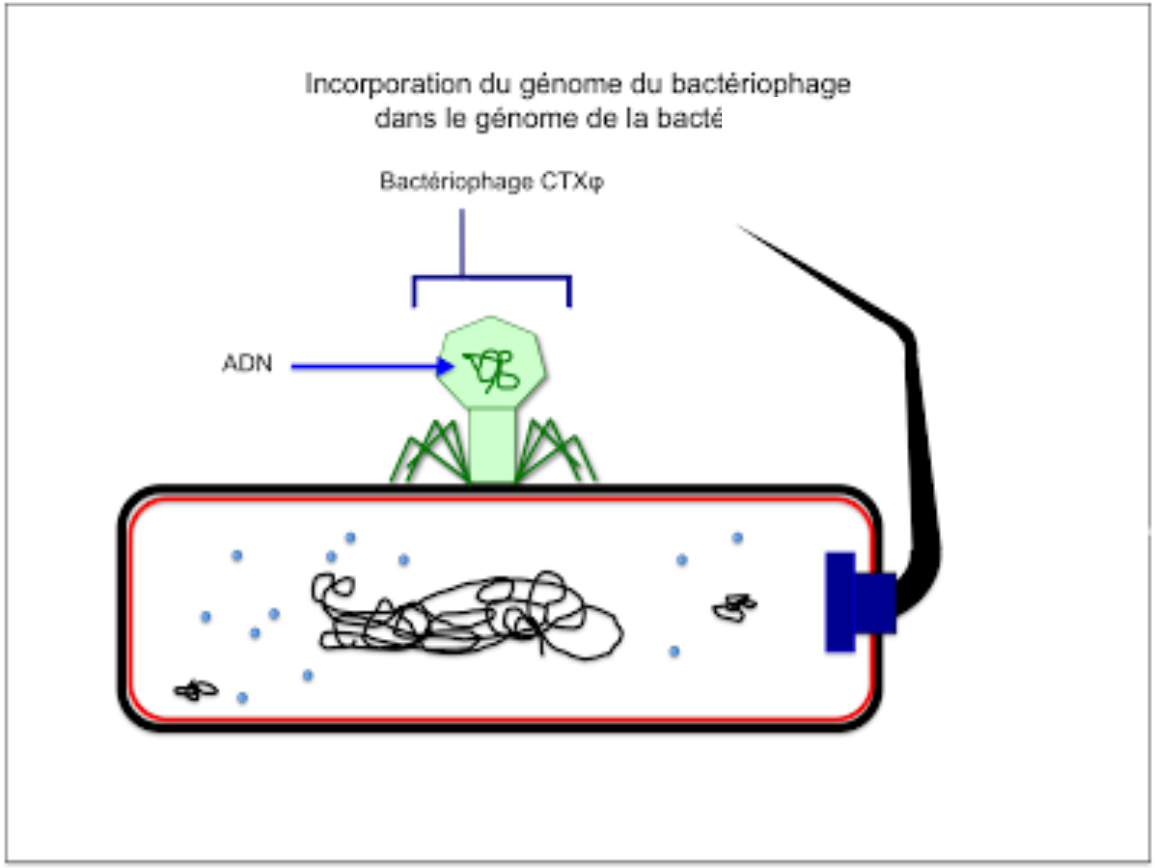
- **Genes associated with virulence**
  - ✓ ***ctxA***→ Gene coding the A subunit of the cholera toxin
  - ✓ ***tcpA (toxin coregulated pilus)*** → Gene coding for a pilus coregulating the cholera toxin → Favor colonisation of the intestinal tract

These genes are supposed to be associated with **clinical** strains of O1 and O139 serogroups

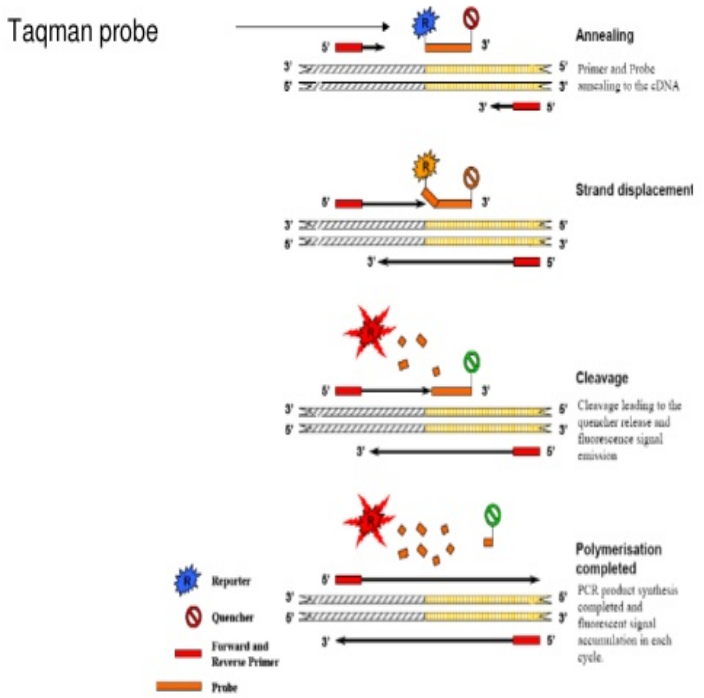
PCR *V. parahaemolyticus*

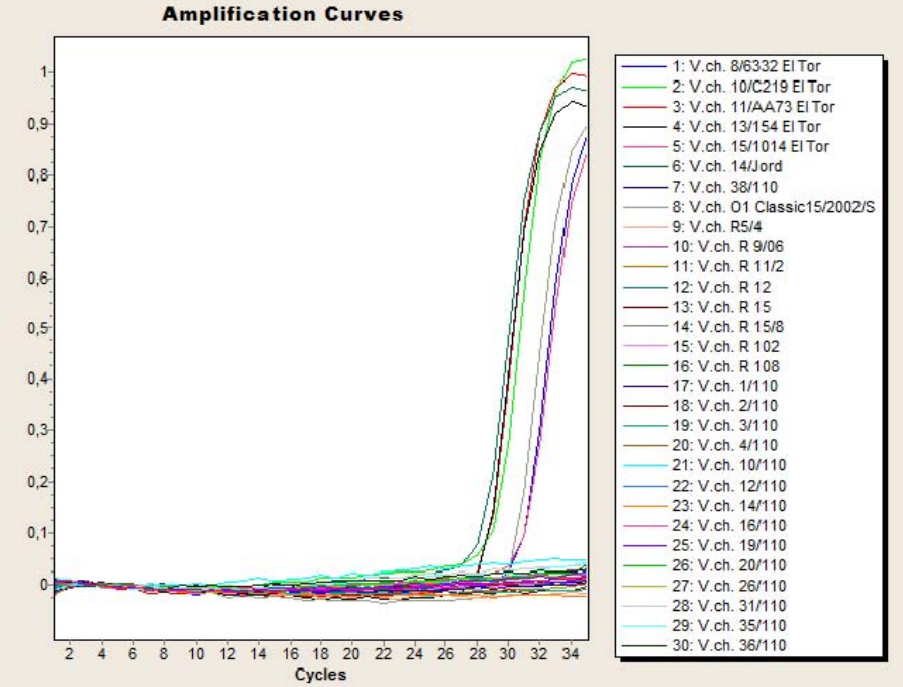
- ✓ ***Tdh*** : gene coding for the thermostable direct hemolysin
- ✓ ***Trh*** : gene coding for the Tdh related hemolysin

Production of toxins



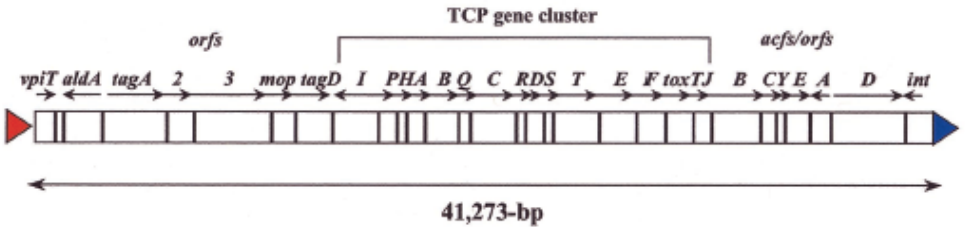
CtxA detection by PCR



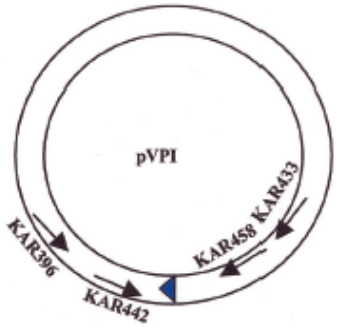


« Use of real time PCR assay for detection of the CtxA gene of *Vibrio cholerae in an environmental survey of Mobile Bay » Blactsone et al, 2006.*

TcpA



VPI



attP

TcpA detection

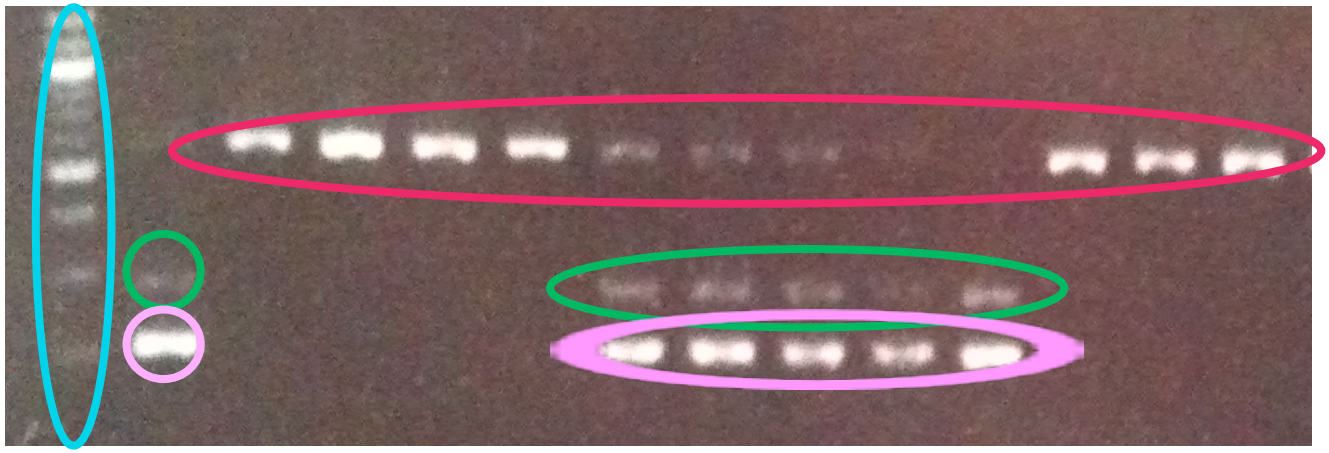
Multiplex Qualitative PCR

- Molecular weight marker

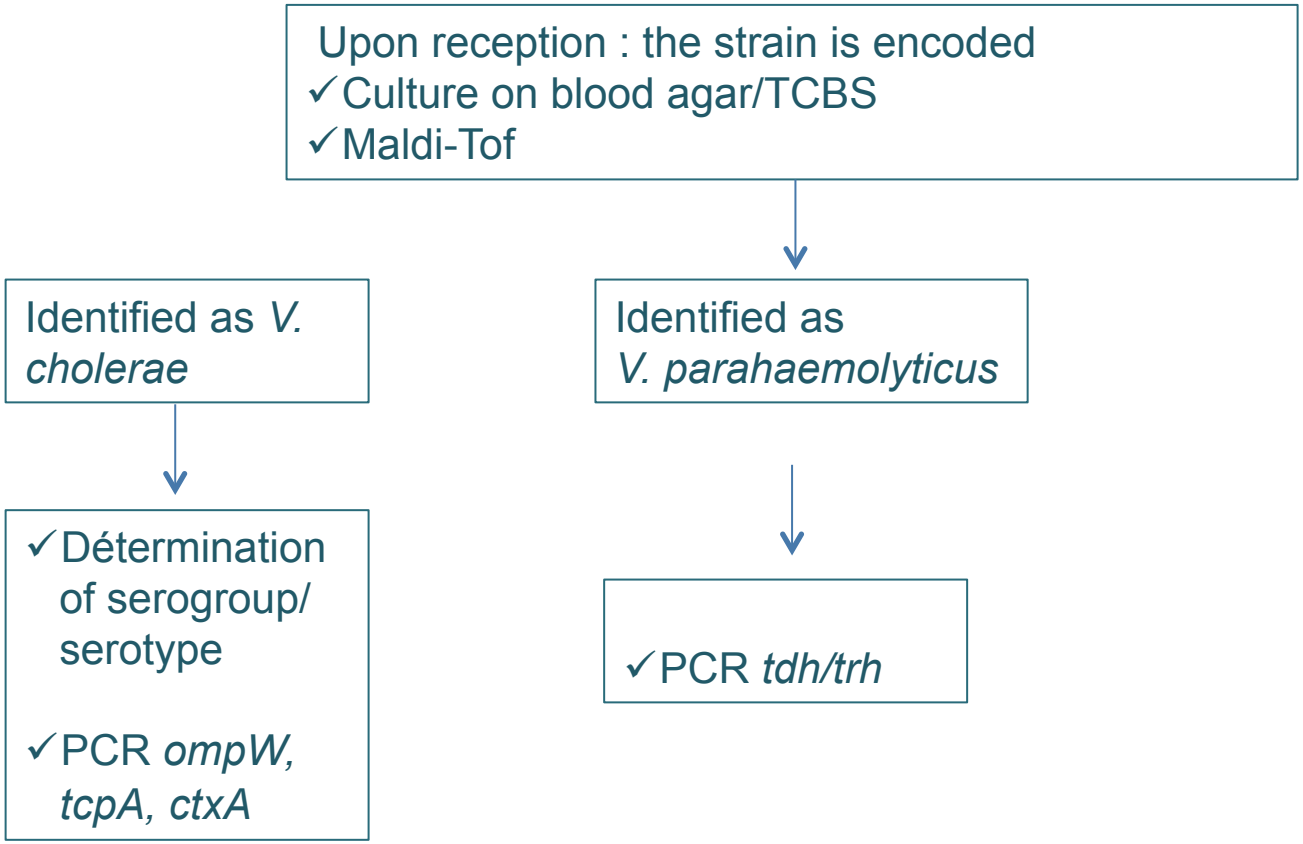
→ Gène *ompW* (588pb)

→ Gène *tcpA* (297pb)

→ Gène *ctxA* (219pb)



NRC algorithm

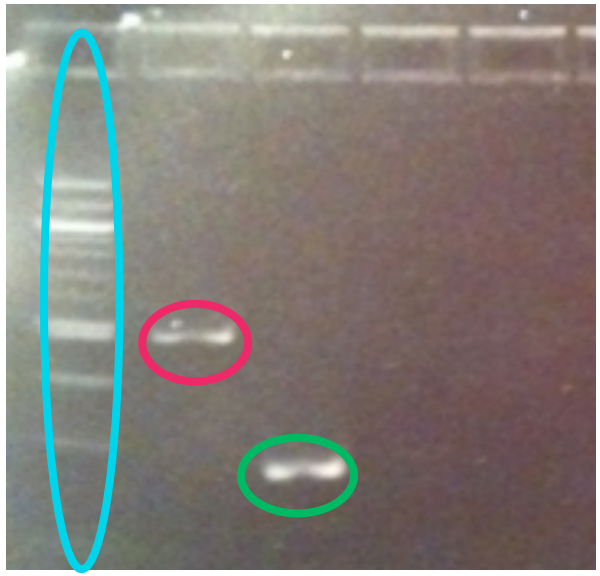


V. parahaemolyticus

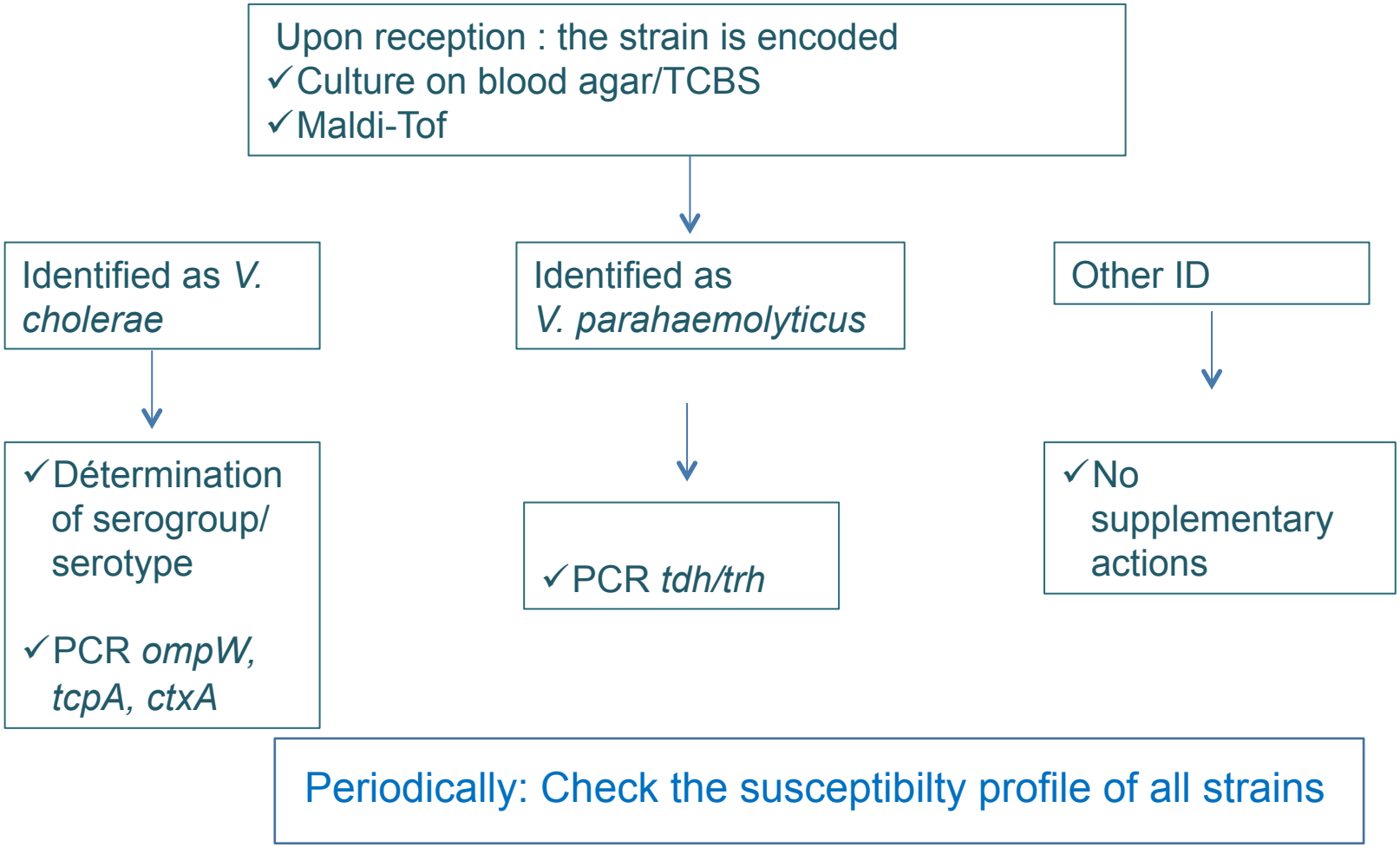
Detection of hemolysins

- **Multiplex Qualitative PCR**
- TDH: Thermostable direct hemolysin
- TRH:TDH related hemolysin

- Marqueur de poids moléculaire
- Souche de *Vibrio parahaemolyticus*
  → Gène *trh* (500pb)
- Souche de *Vibrio parahaemolyticus*
  → Gène *tdh* (269pb)



NRC algorithm



Antibiotic susceptibility

- Several reports have documented **tetracycline- and fluoroquinolone-resistant *V. cholerae*** and multidrug resistance is increasing.

➔Importance of following drug susceptibility profile

Methods

Vitek

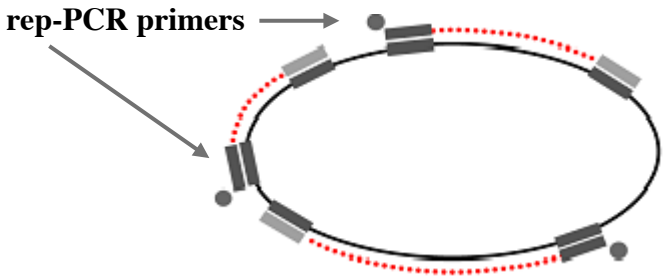
Etest

Specific mission of the NRC

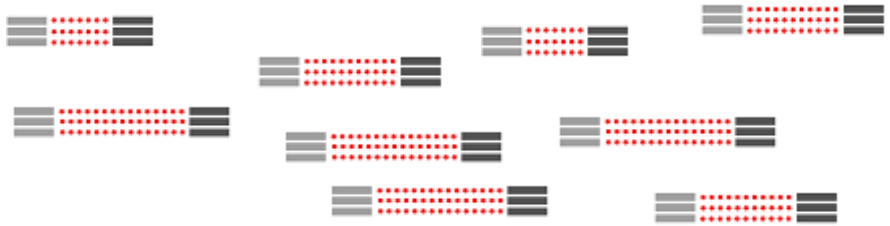
- **Genome typing** of all virulent strains (**Diversilab**)

Diversilab Method

1. rep-PCR primers bind to **many** specific repetitive sequences interspersed throughout the genome

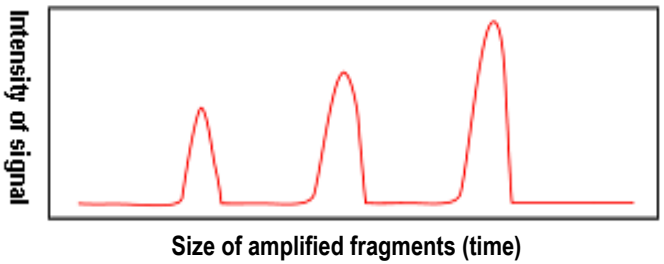


2. Multiple fragments of various lengths are amplified



3. Fragments can be separated by mass and charge via electrophoresis

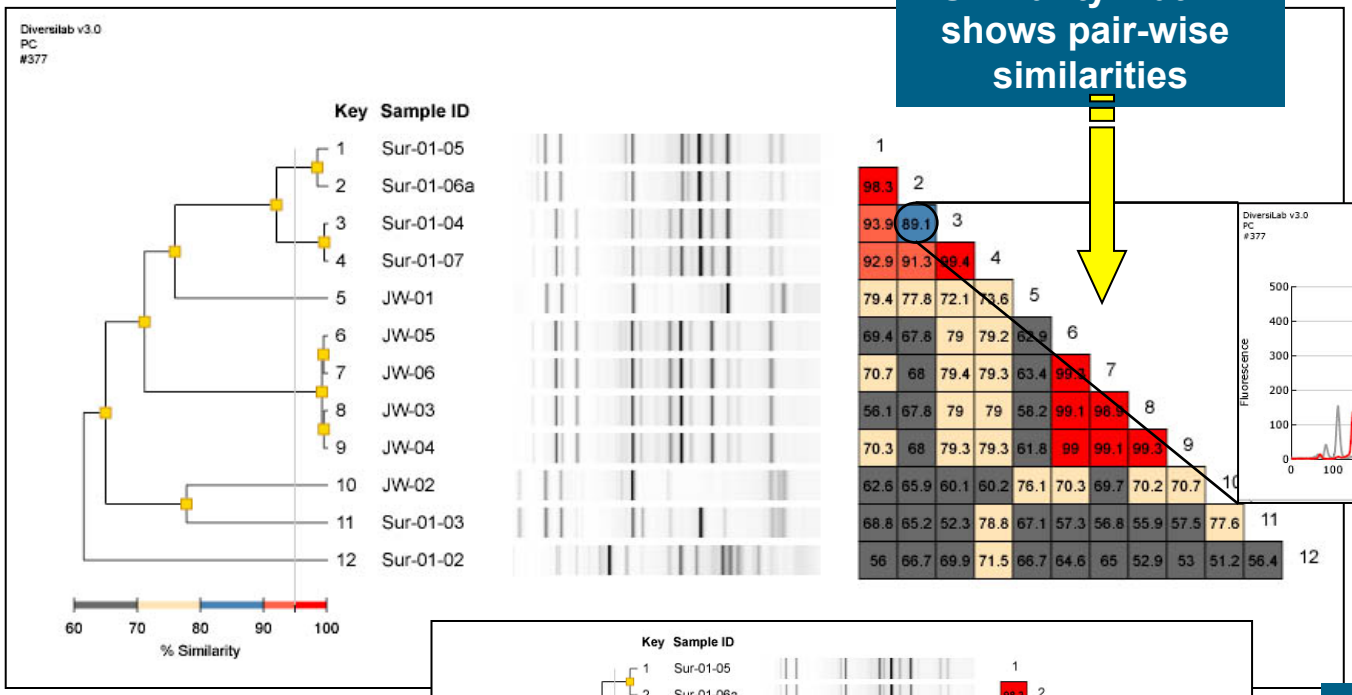
4. A **unique rep-PCR DNA fingerprint profile** is created with multiple bands of varying intensity

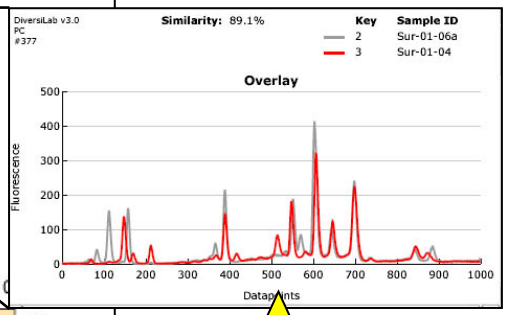


Analysis tools

> 95% similarity
< than 2 differences in peaks

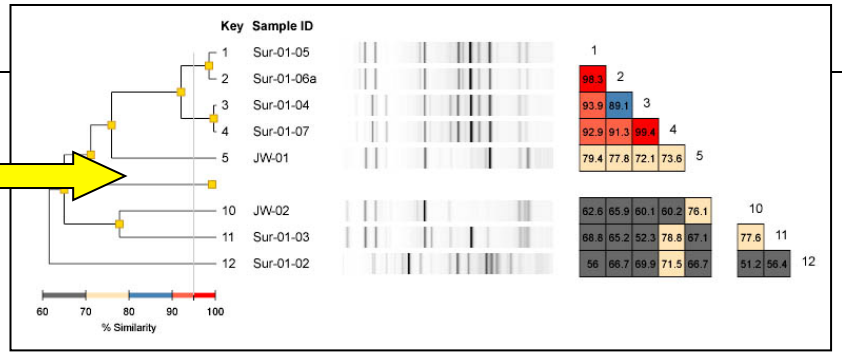
Similarity matrix shows pair-wise similarities





Overlay graphs can be seen for any sample pair

Collapse by clicking yellow node

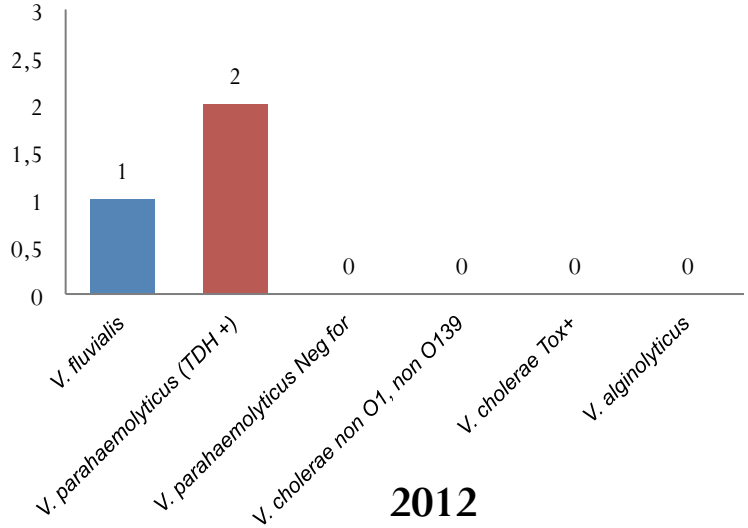


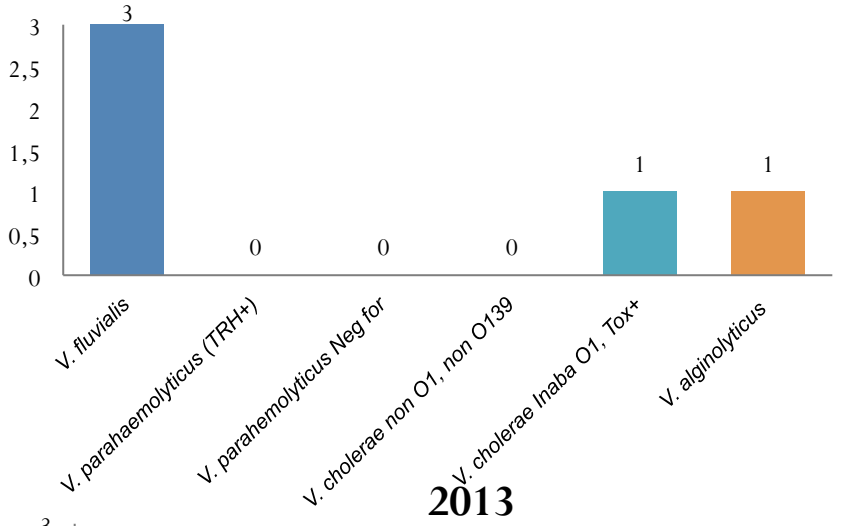
Some statistics from the Belgian NRC

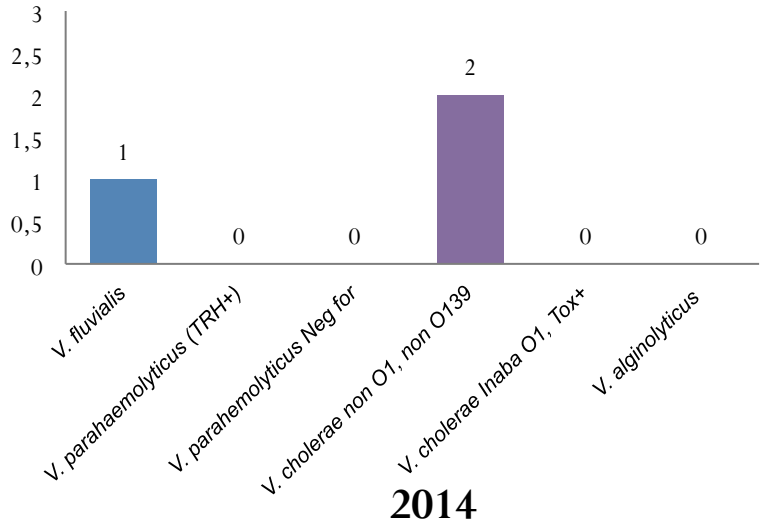
Period 2012-2016

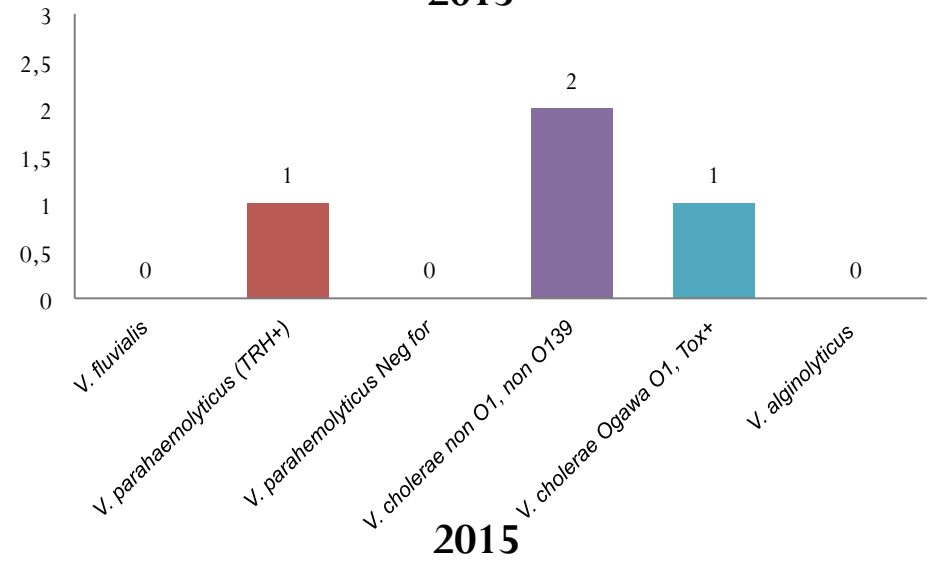
Some statistics for Belgium (NRC data)

Clinical strains





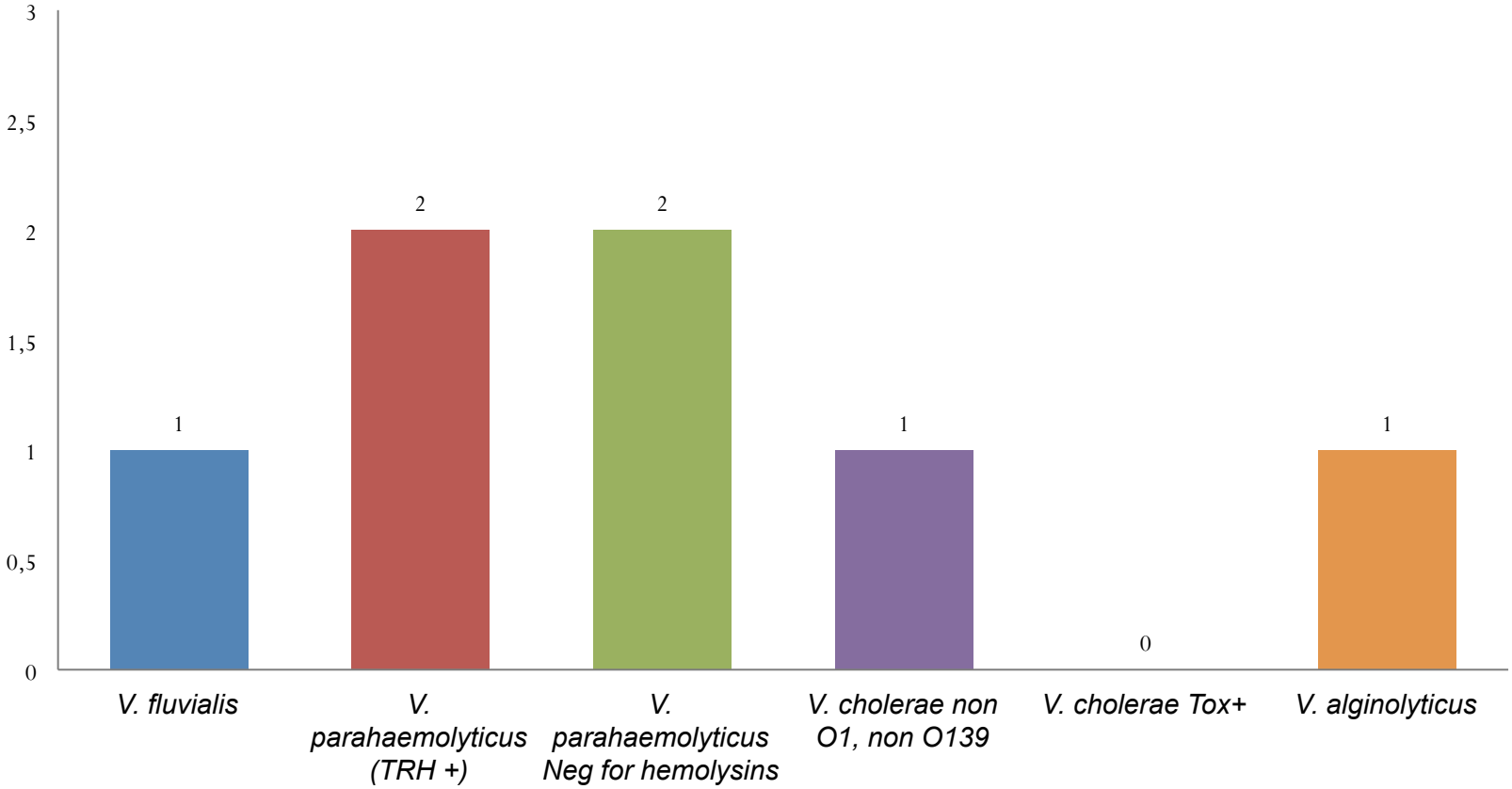




Some statistics for Belgium (NRC data)

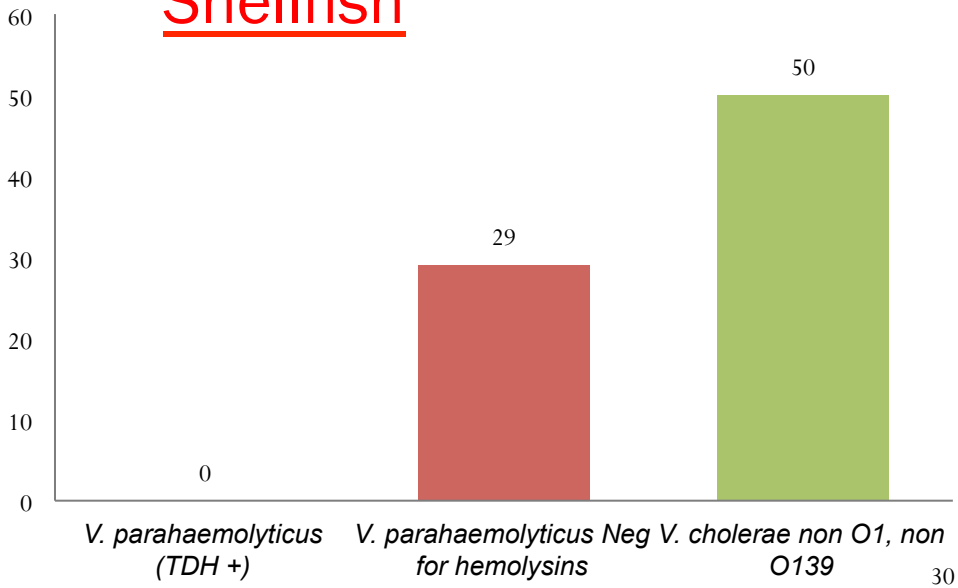
Clinical strains

- 2016

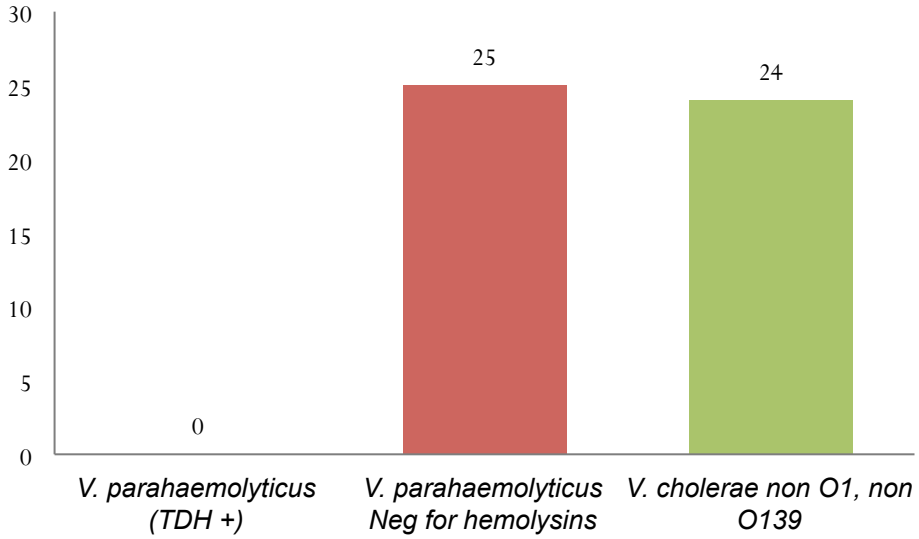


Some statistics for Belgium (NRC data)

Shellfish



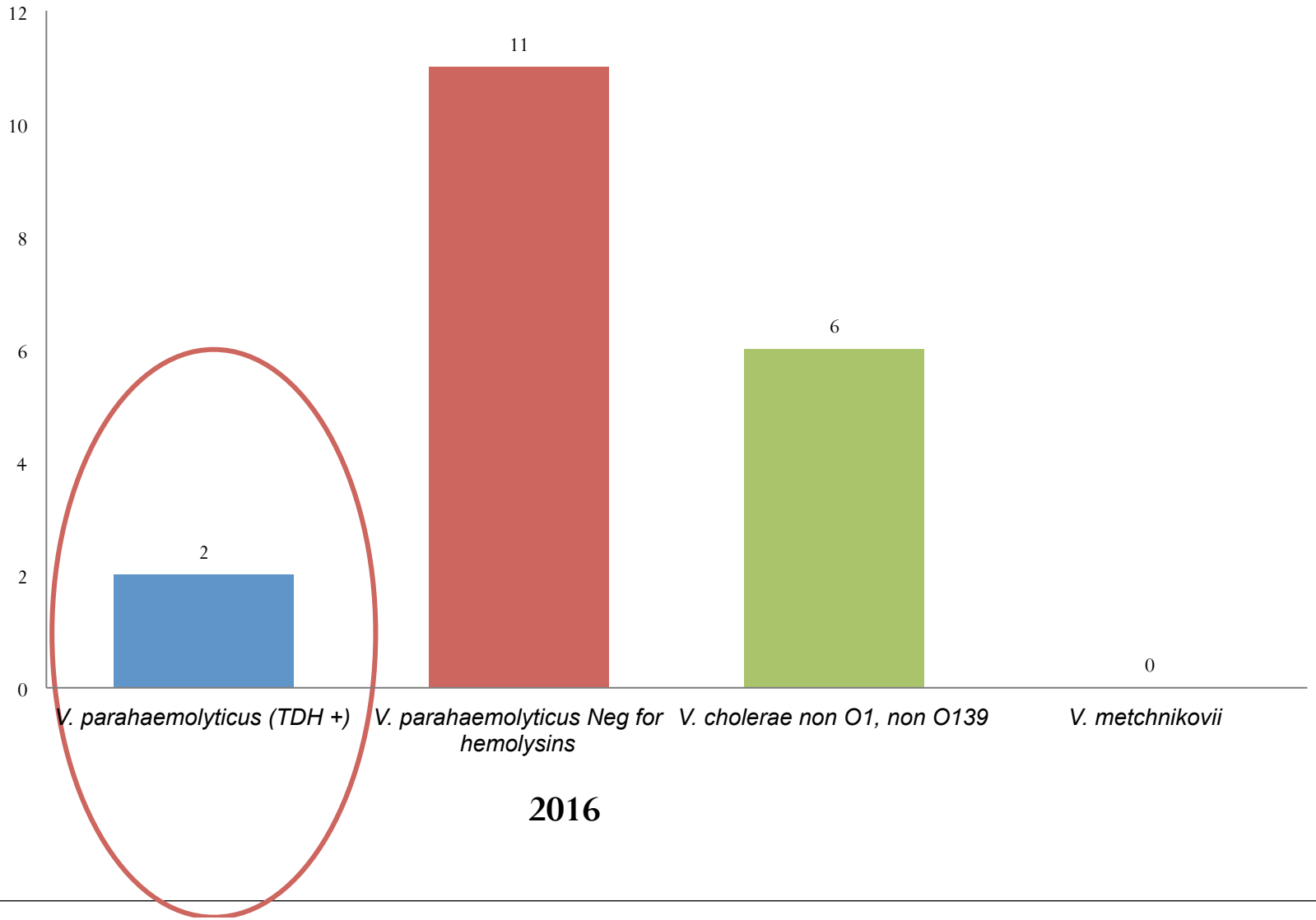
2013



2014

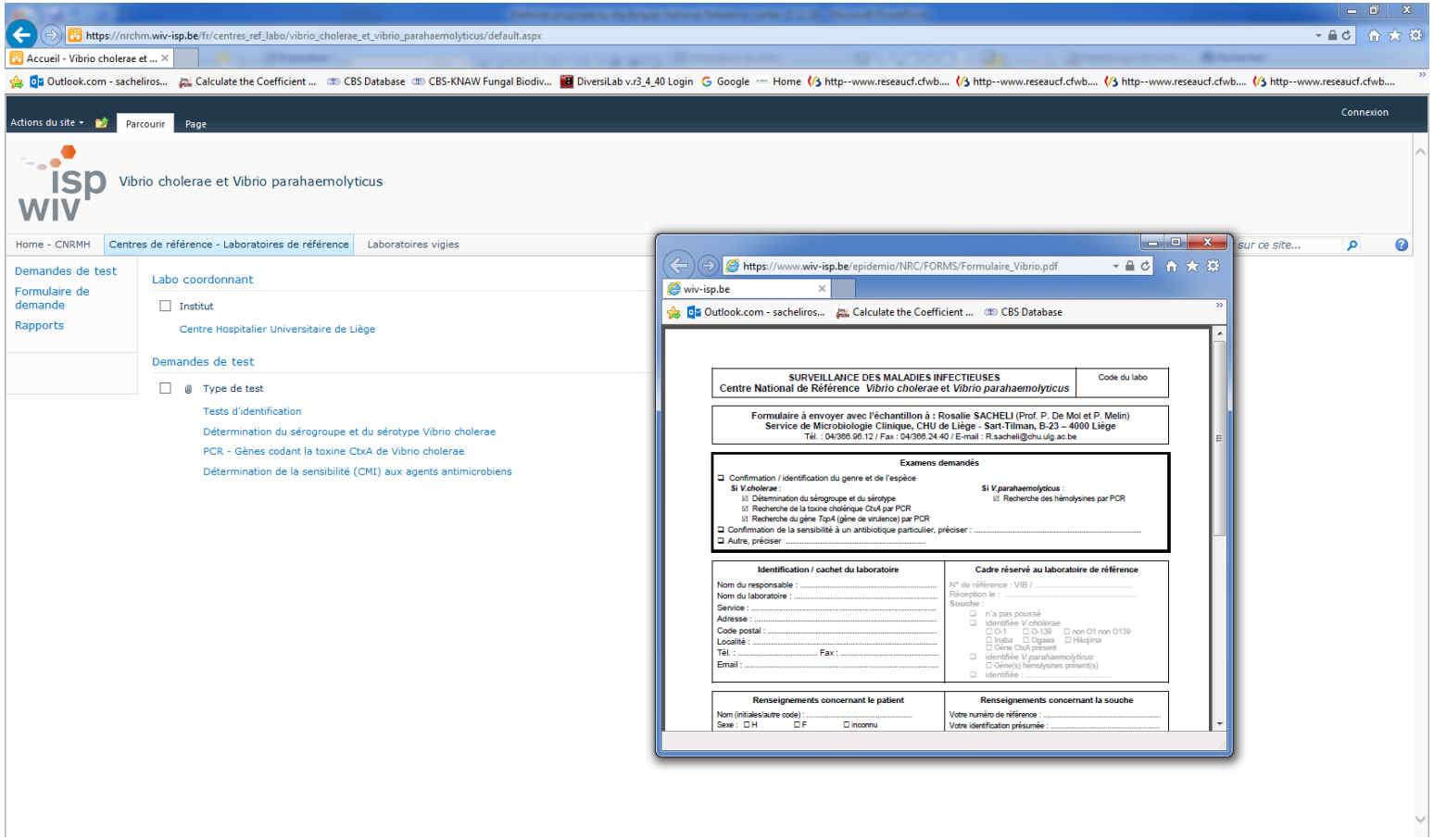
Some statistics for Belgium (NRC data)

Shellfish



2016

NRC form



https://nrchm.wiv-isp.be/fr/centres_ref_labo/default.aspx

Thank you for your attention!