

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

Sacheli Rosalie, PhD

National Reference Center for *Vibrio cholerae* and *parahaemolyticus*-Clinical Microbiology unit- **CHU of Liège**

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

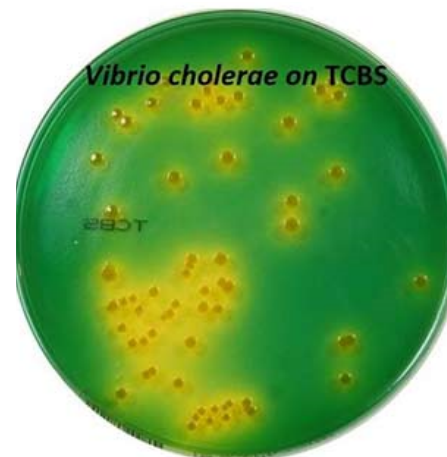
Upon reception : the strain is encoded

- ✓ Culture on blood agar/TCBS
- ✓ Maldi-Tof

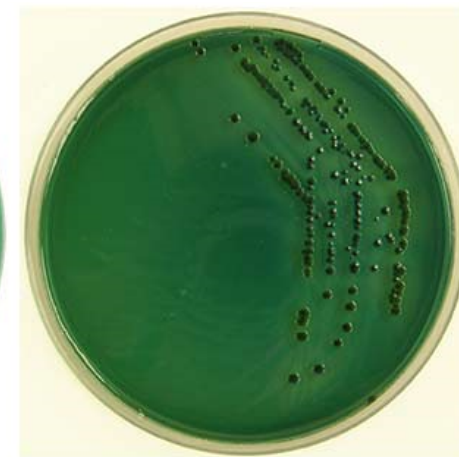
Culture TCBS (Thiosulfate bile sucrose)

Most enterobacteriaceae from faeces are suppressed

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



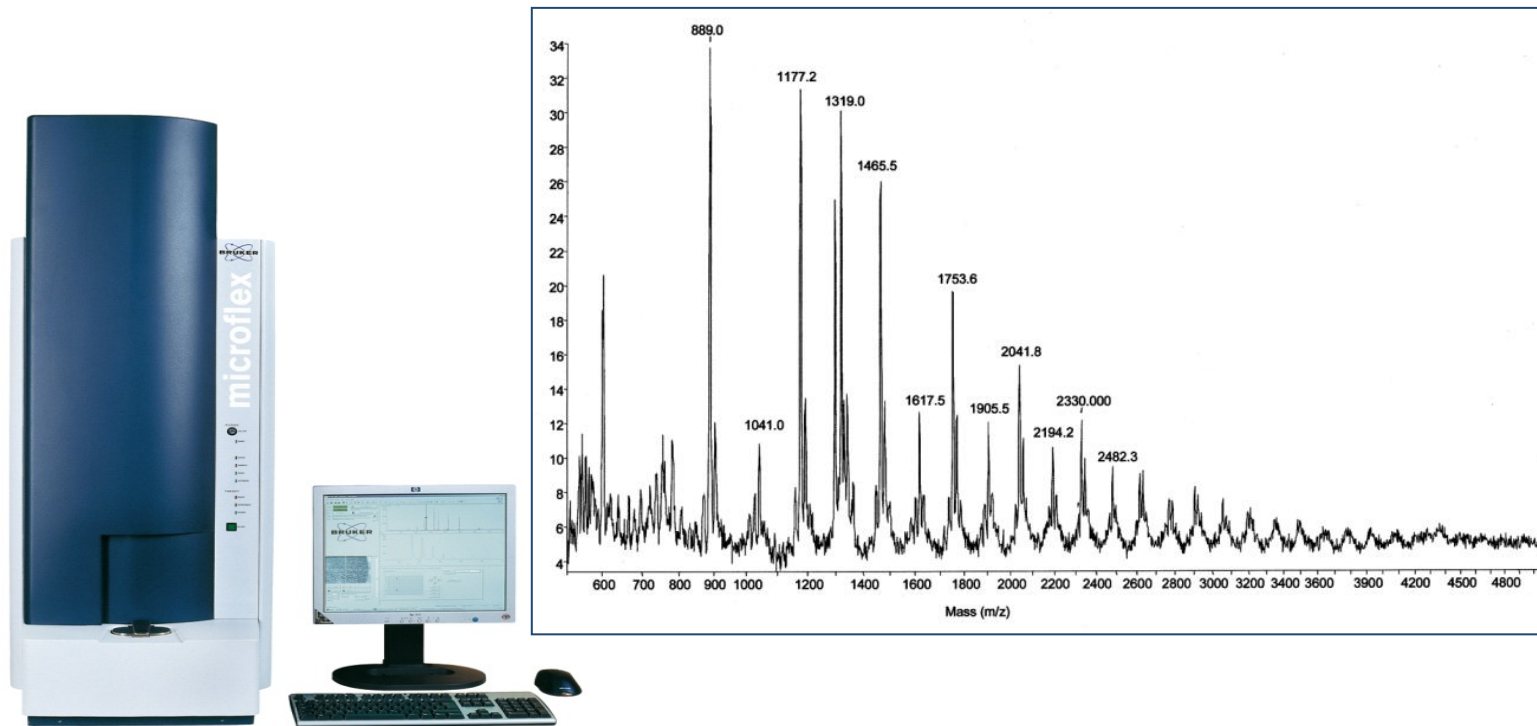
Vibrio cholerae on TCBS Agar



Vibrio parahaemolyticus on TCBS Agar

Identification

- Method: **Maldi-Tof**

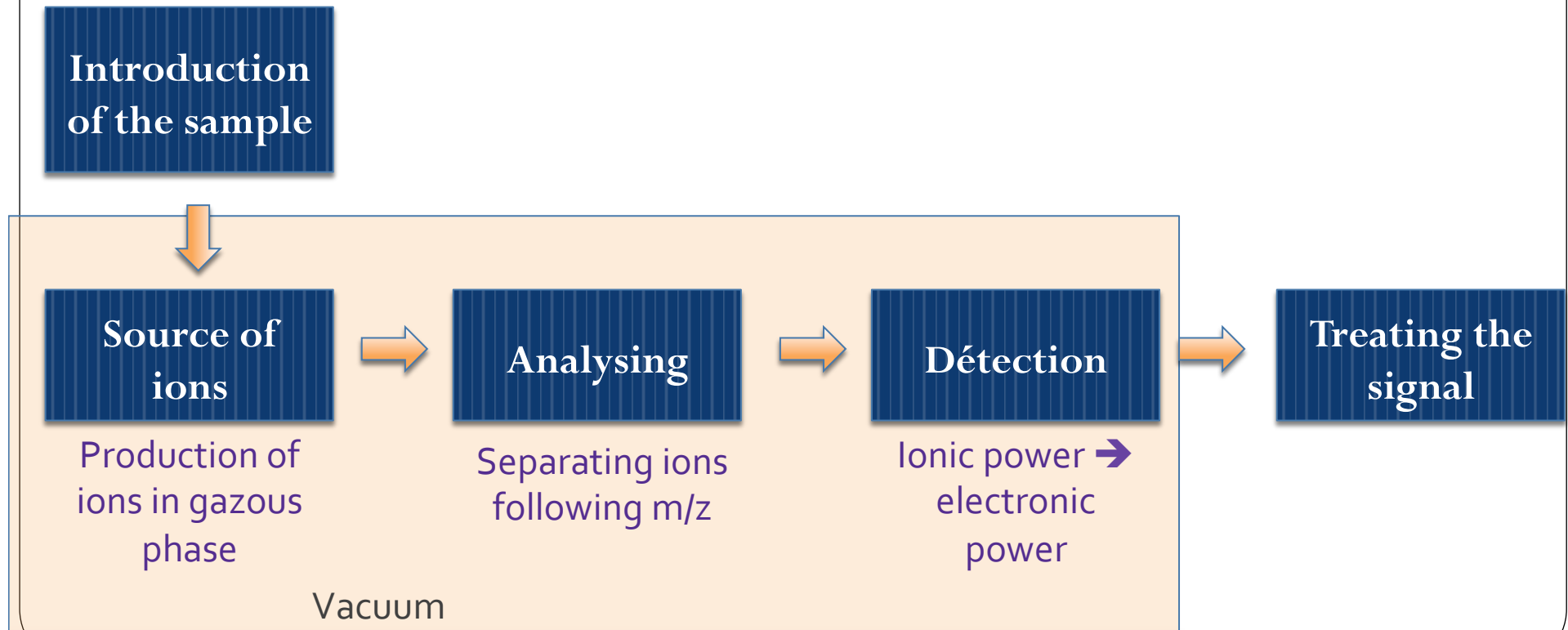


Microflex Bruker Daltonics

Mass spectrometry

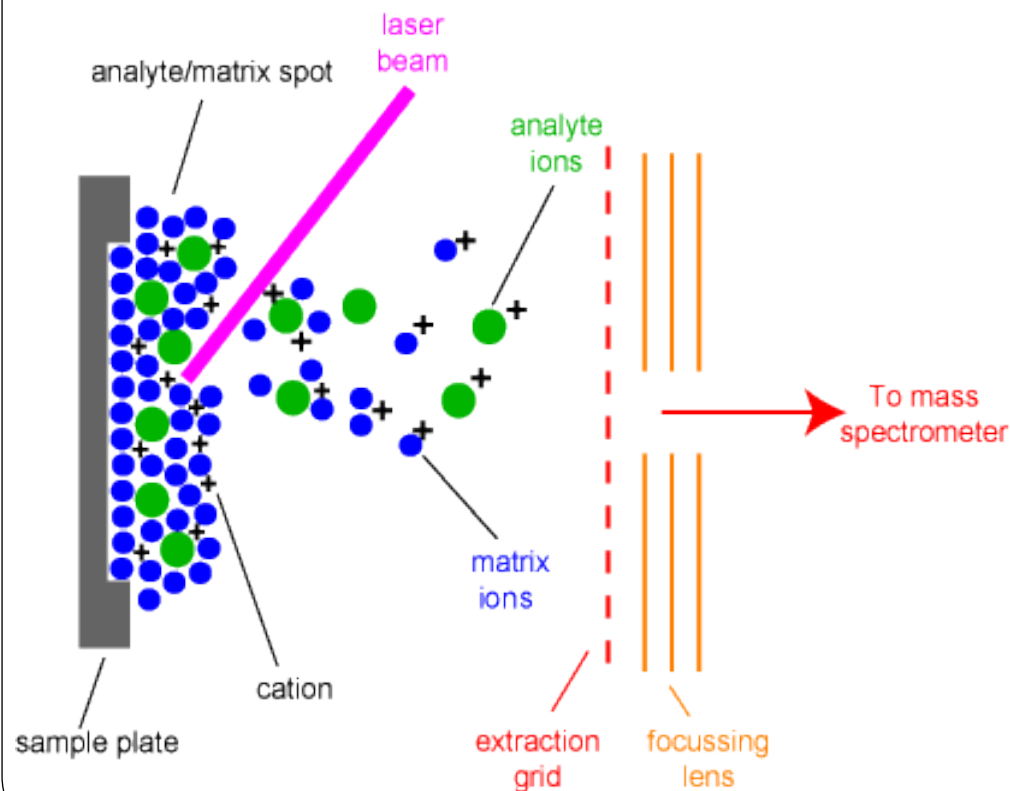
Principle:

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



Mass spectrometry MALDI-TOF

MALDI: Matrix Assisted Laser Desorption/Ionization



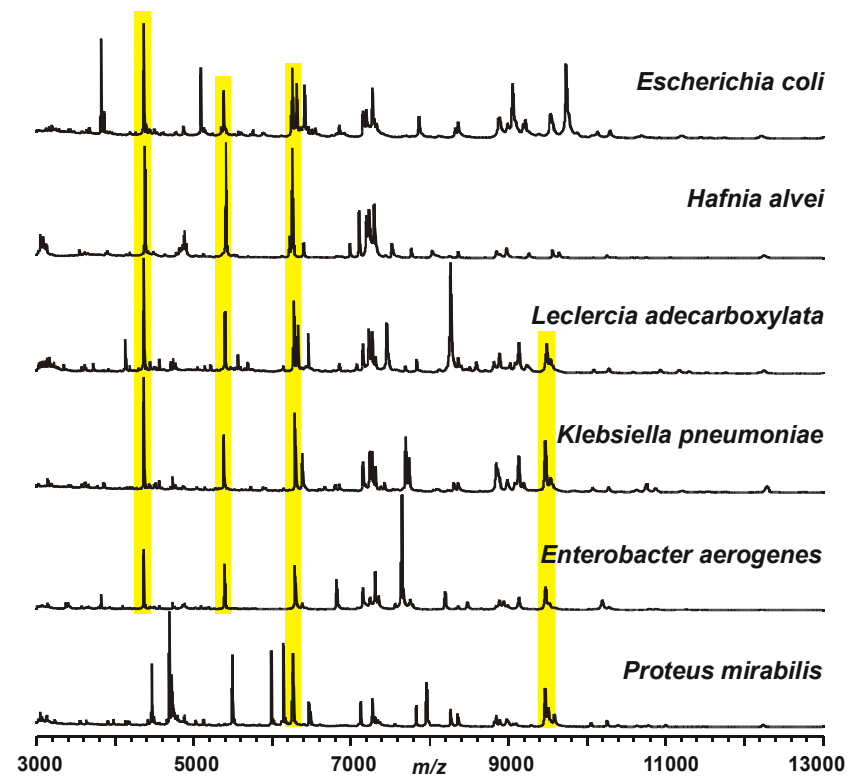
1. The sample is mixed with a matrix HCCA and then dried
2. The laser ionizes molecules of the matrix
3. Molecules of the samples are ionized by transfer from the matrix



Principle of ID

Detection of large proteins (1000 – 300000 Da)

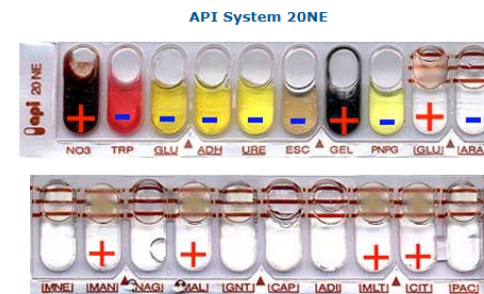
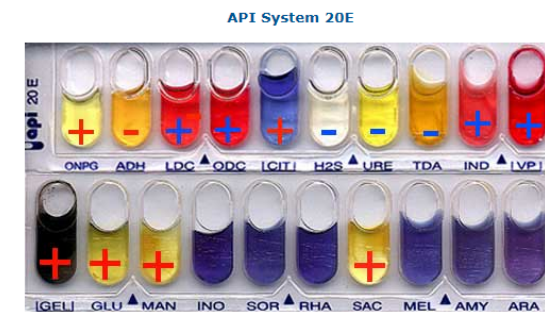
- Spectra differing between microorganisms
- Reproducible spectra
- Peaks specific of the genus, species or sub species



ID decision criteria

Meaning of Score Values

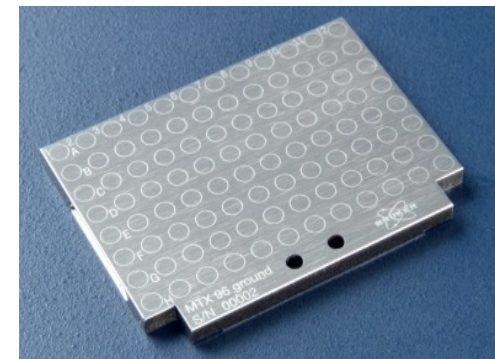
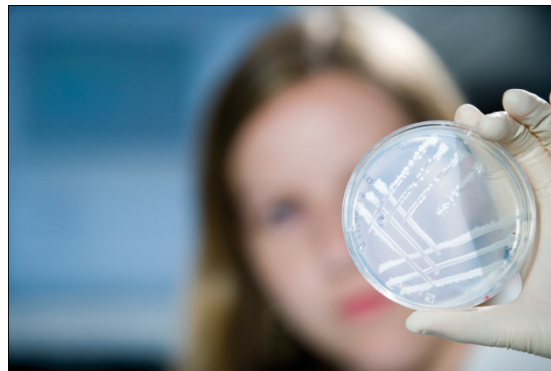
Range	Description
2.300 ... 3.000	highly probable species identification
2.000 ... 2.299	secure genus identification, probable species identification
1.700 ... 1.999	probable genus identification
0.000 ... 1.699	no reliable identification



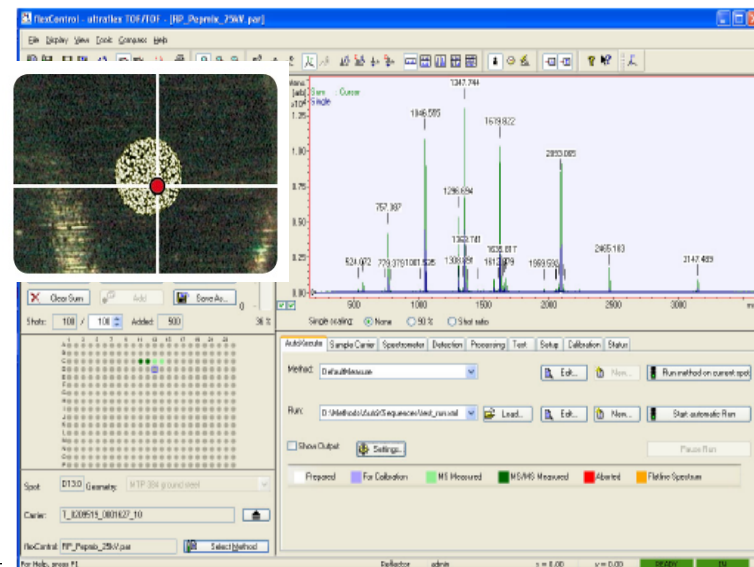
In the lab

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



Nom de l'échantillon: G2
Description de l'échantillon:
ID de l'échantillon: 160414-0051 *V16-005*
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 (++)	<i>Vibrio cholerae</i> 6536	2.286	<u>666</u>
2 (++)	<i>Vibrio cholerae</i> 6550	2.253	<u>666</u>
3 (+)	<i>Vibrio cholerae</i> 71-89	1.863	<u>666</u>
4 (+)	<i>Vibrio albensis</i> LMG 4406T HAM	1.853	<u>140100</u>

ID of *V. parahaemolyticus*

Analyte2



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 (++)	<u><i>Vibrio parahaemolyticus</i> LMG 4423 LMG</u>	2.152	<u>670</u>
2 (++)	<u><i>Vibrio parahaemolyticus</i> 4a IBS</u>	2.13	<u>670</u>
3 (++)	<u><i>Vibrio parahaemolyticus</i> DSM 11058 DSM</u>	2.117	<u>670</u>
4 (++)	<u><i>Vibrio parahaemolyticus</i> DSM 10027T DSM</u>	2.108	<u>670</u>
5 (++)	<u><i>Vibrio parahaemolyticus</i> DSM 15416 DSM</u>	2.102	<u>670</u>
6 (++)	<u><i>Vibrio parahaemolyticus</i> DSM 15477 DSM</u>	2.06	<u>670</u>
7 (+)	<u><i>Vibrio parahaemolyticus</i> CCM 5937 CCM</u>	1.927	<u>670</u>
8 (-)	<u><i>Vibrio parahaemolyticus</i> 7a IBS</u>	1.691	<u>670</u>

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	<u>676</u>
2 (++)	Vibrio fluvialis CCM 3695 CCM	2.095	<u>676</u>

Other *Vibrio* species

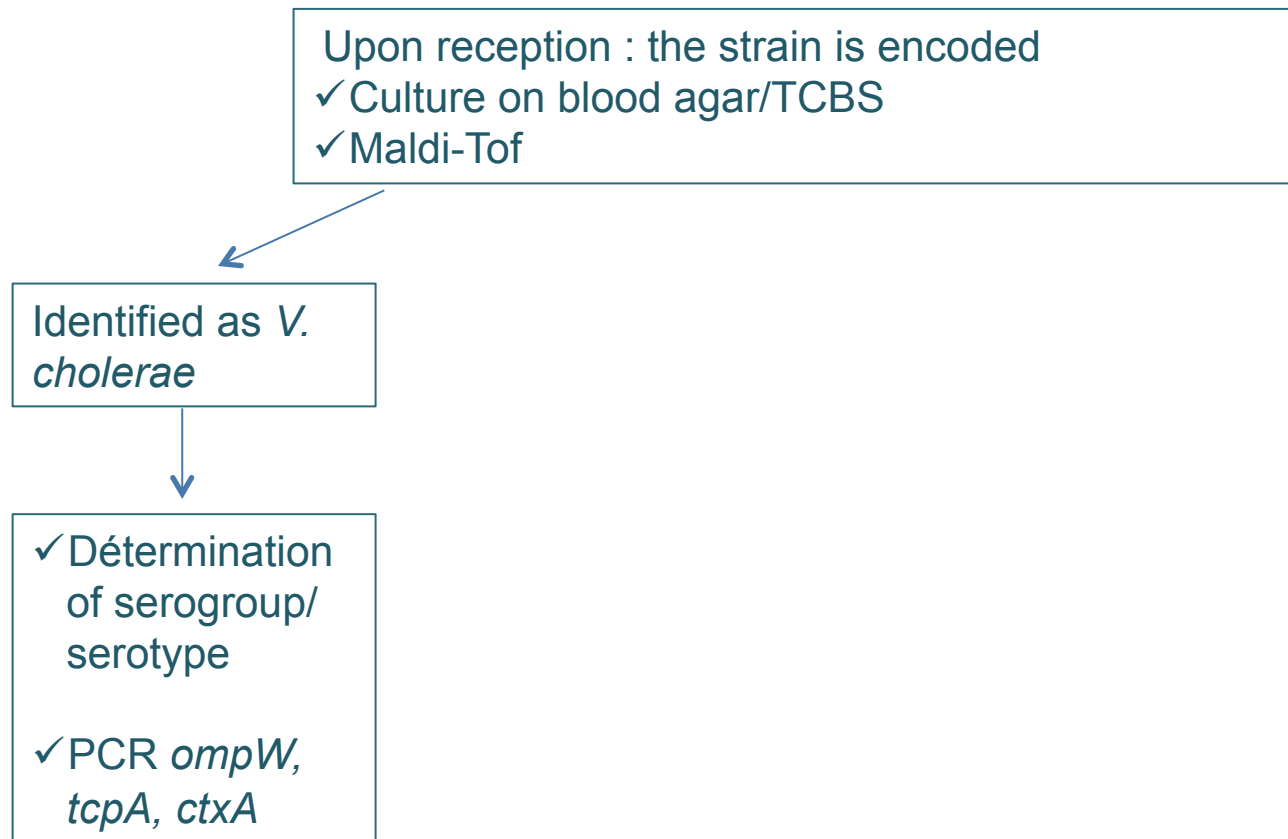
Analyte3



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 (+)	<u>Vibrio alginolyticus CCM 7037 CCM</u>	1.964	<u>663</u>
2 (+)	<u>Vibrio alginolyticus CCM 2578T CCM</u>	1.868	<u>663</u>
3 (+)	<u>Vibrio alginolyticus DSM 2171T DSM</u>	1.841	<u>663</u>
4 (+)	<u>Vibrio alginolyticus CCM 5941 CCM</u>	1.769	<u>663</u>

NRC algorithm

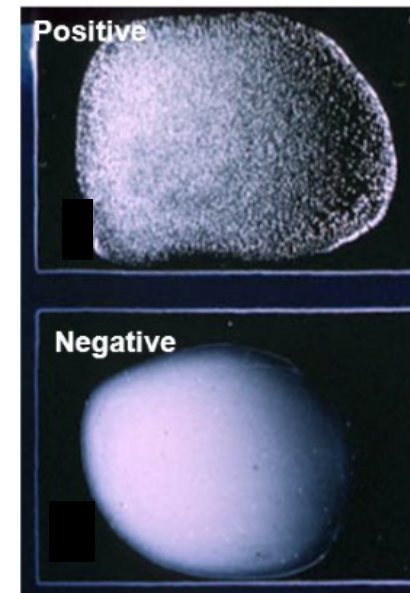
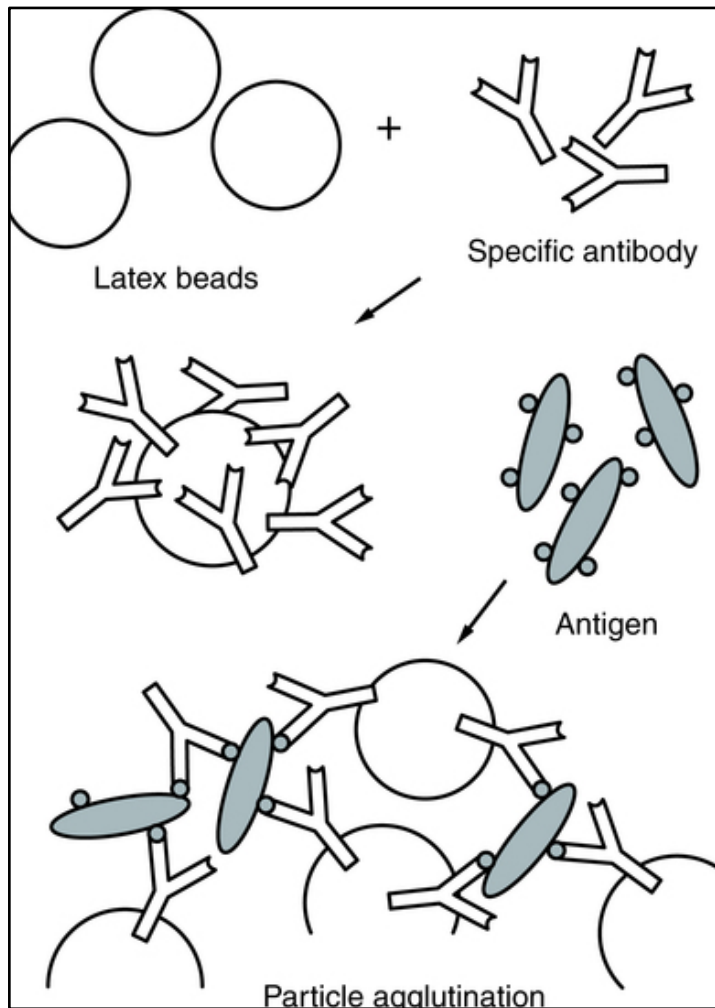


Serogroup/serotype determination

- 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 group 139 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

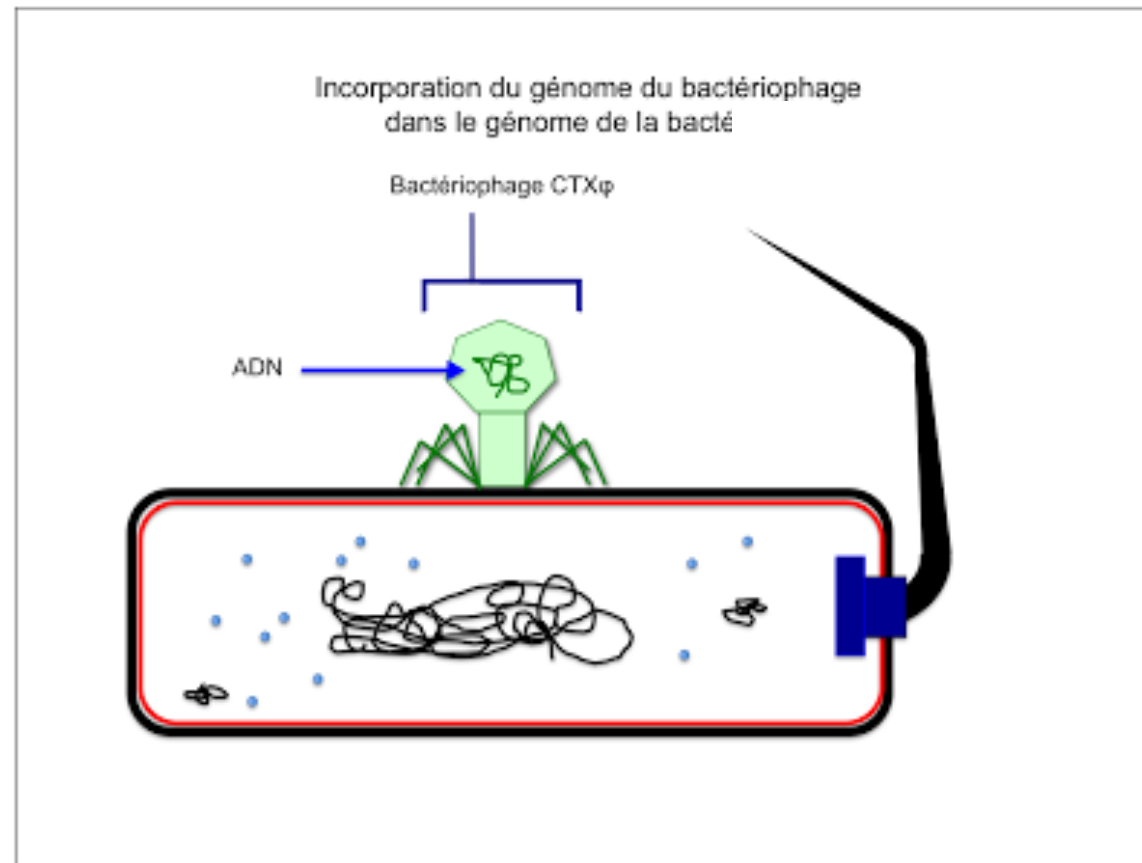
These genes are supposed to be associated with **clinical** strains of O1 and O139 serogroups

PCR *V. parahaemolyticus*

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

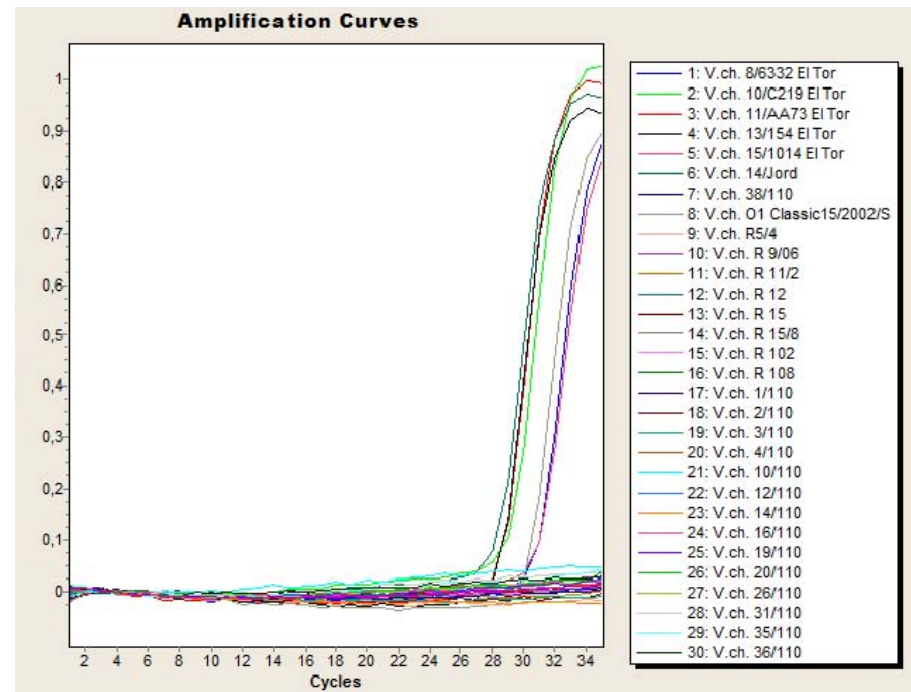
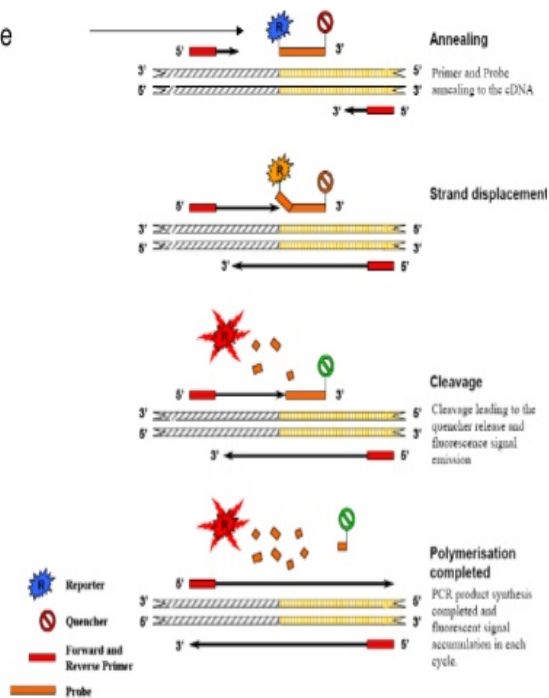
- ✓ ***Trh*** : gene coding for the Tdh related hemolysin

Production of toxins



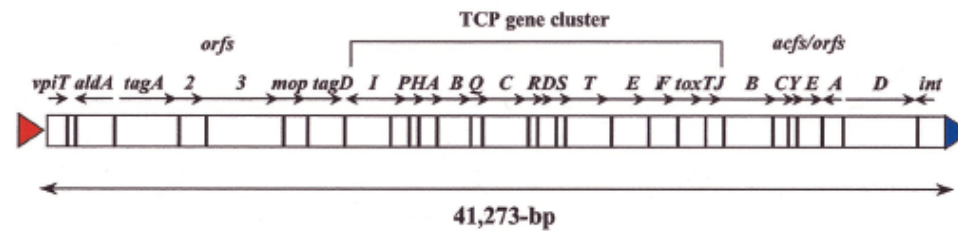
CtxA detection by PCR

Taqman probe

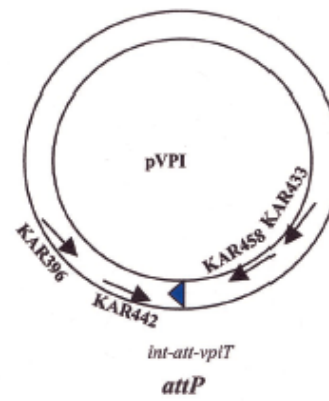


« Use of real time PCR assay for detection of the CtxA gene of *Vibrio cholerae* in an environmental survey of Mobile Bay » Blactson et al, 2006.

TcpA



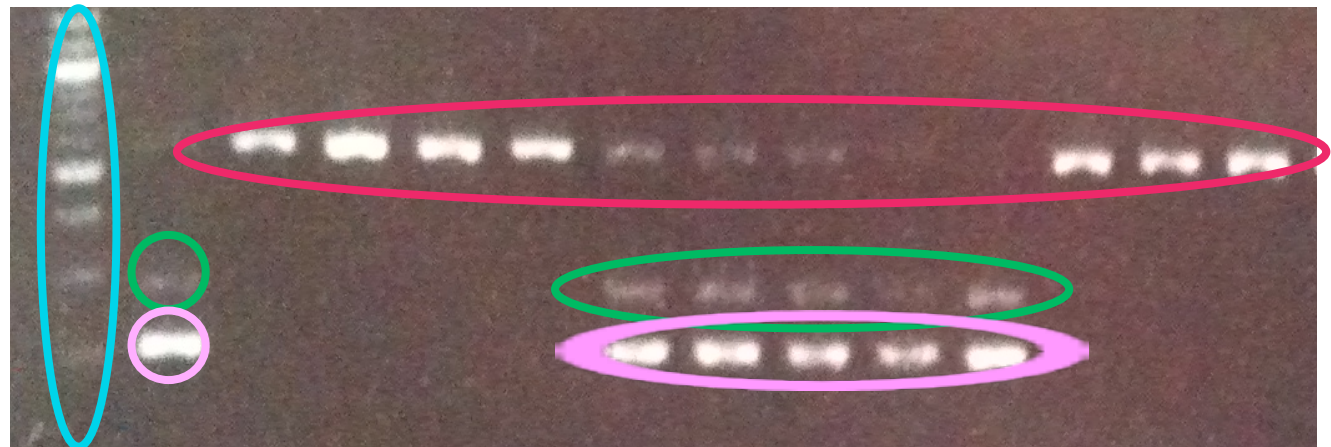
VPI



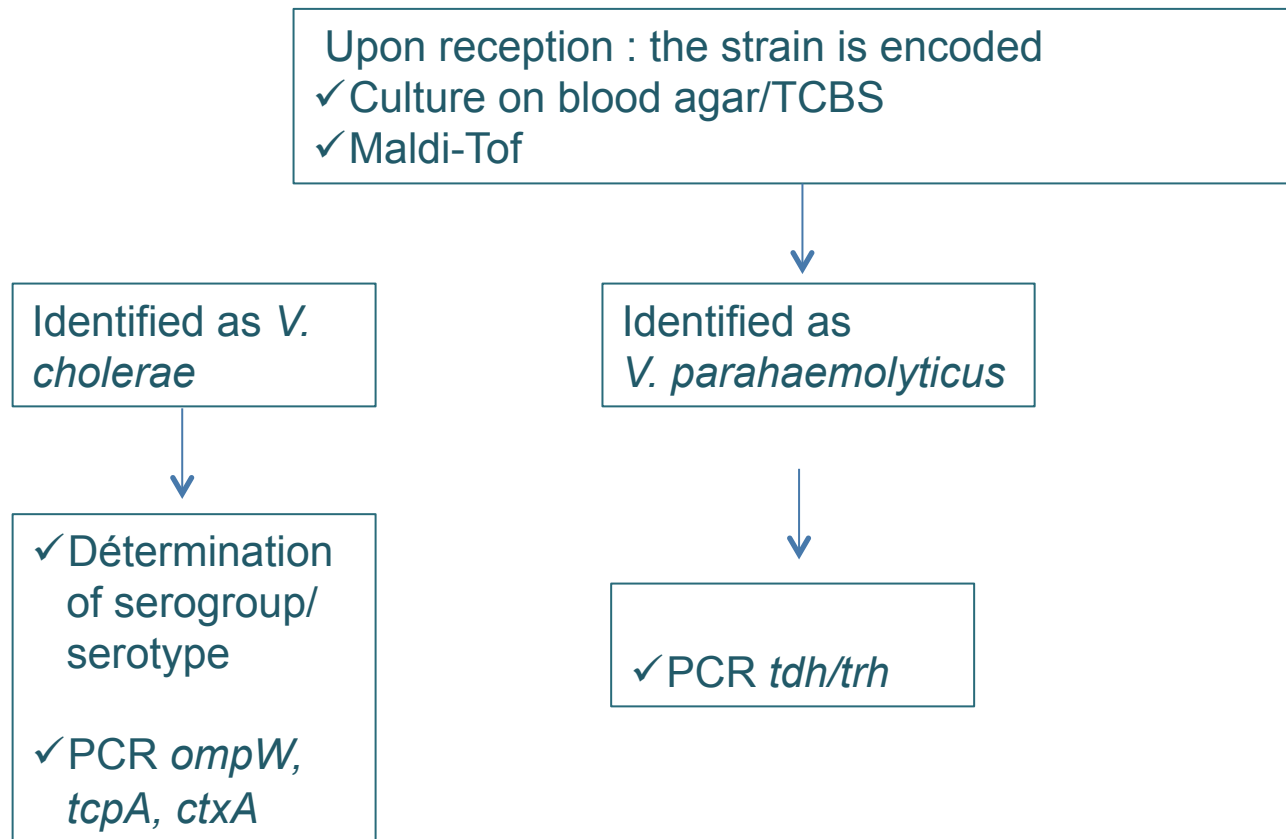
TcpA detection

Multiplex Qualitative PCR

- Molecular weight marker
- Gène *ompW* (588pb)
- Gène *tcpA* (297pb)
- Gène *ctxA* (219pb)



NRC algorithm

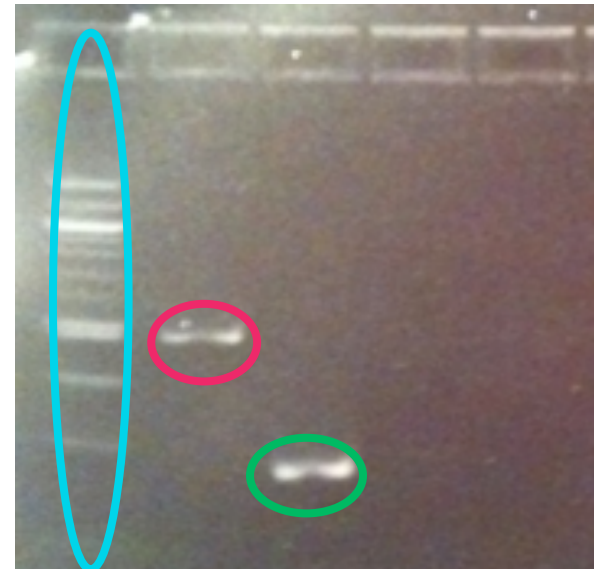


V. parahaemolyticus

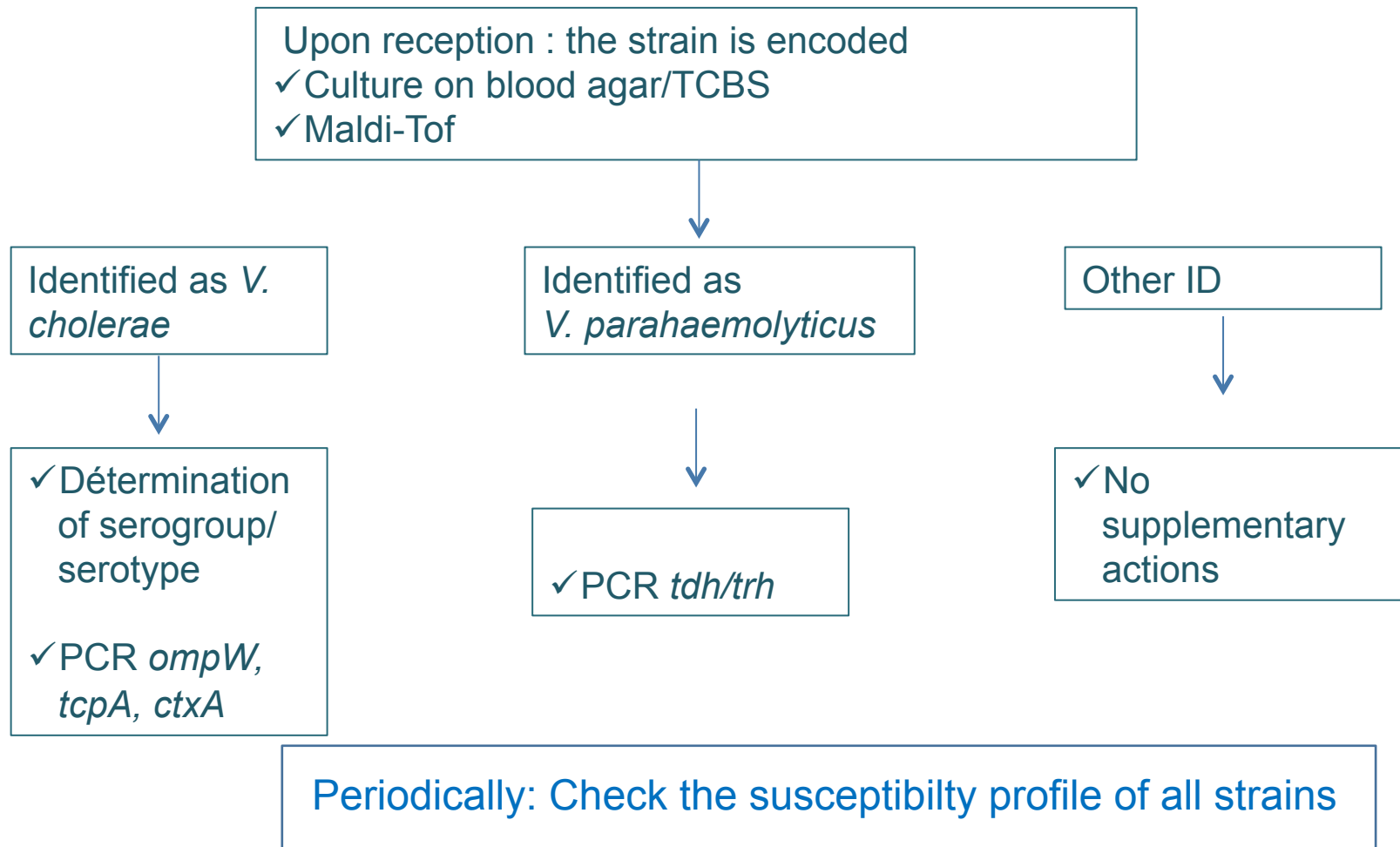
Detection of hemolysins

- **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

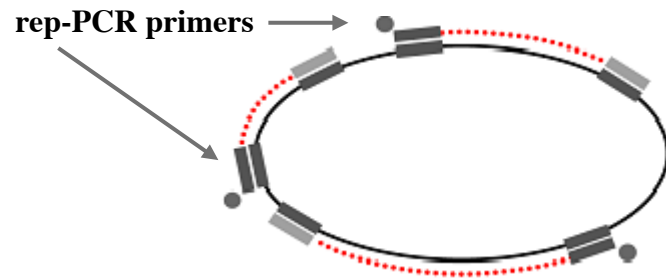
- **Genome typing of all virulent strains (Diversilab)**



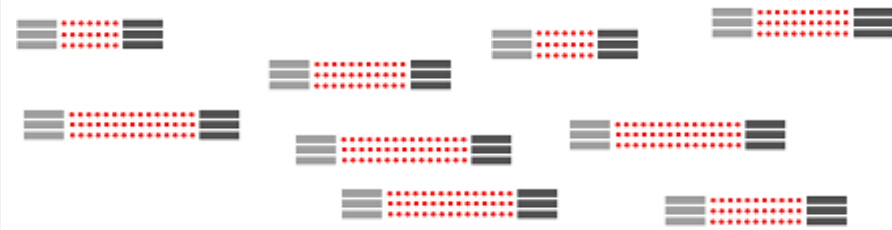
Diversilab Method



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



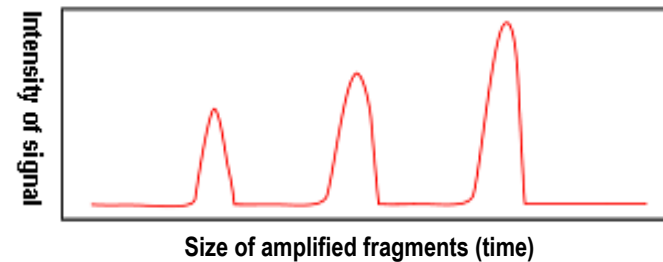
2. Multiple fragments of various lengths are amplified



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

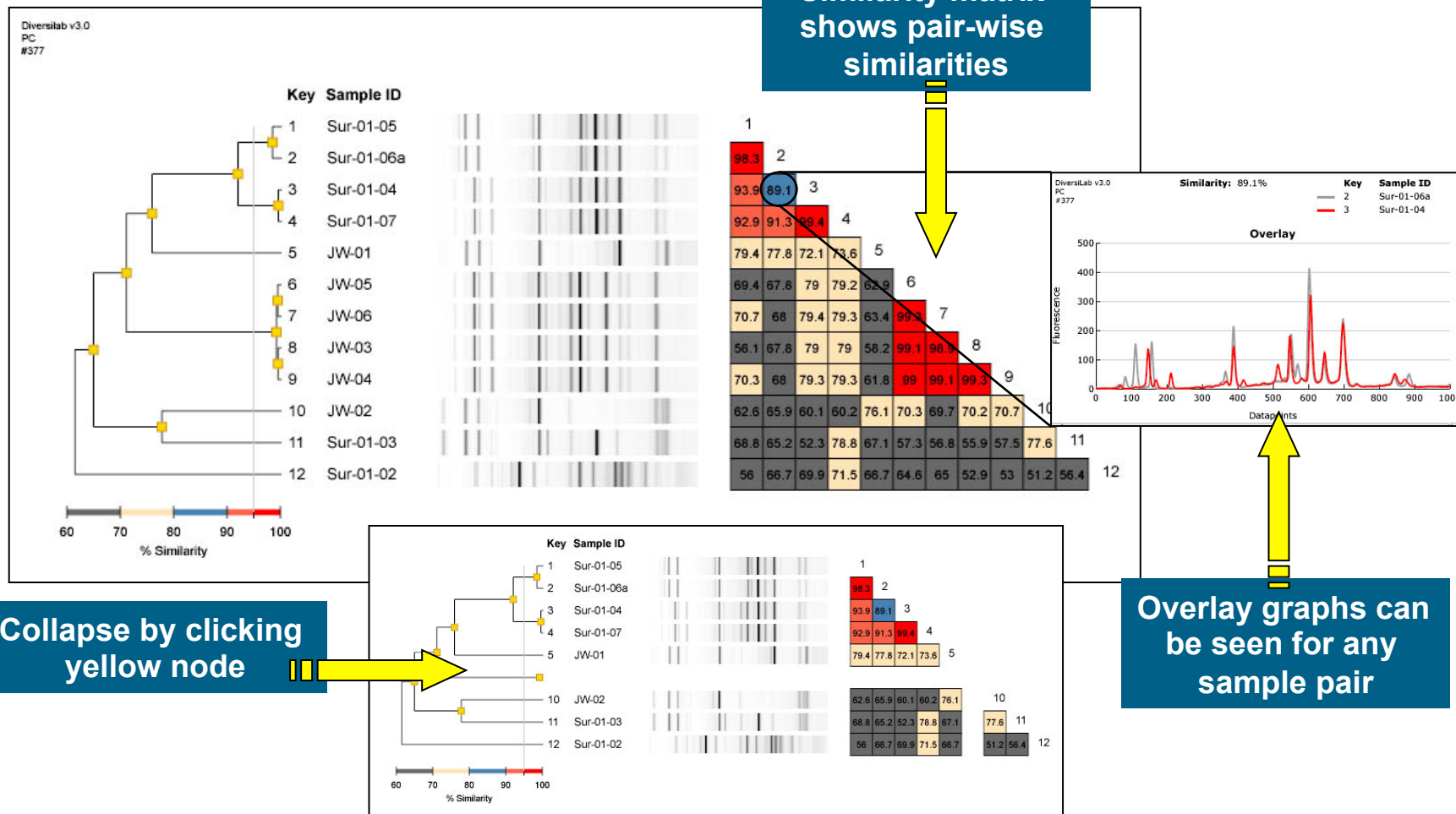


4. A unique rep-PCR DNA fingerprint profile is created with multiple bands of varying intensity



Analysis tools

> 95% similarity
< than 2 differences in peaks

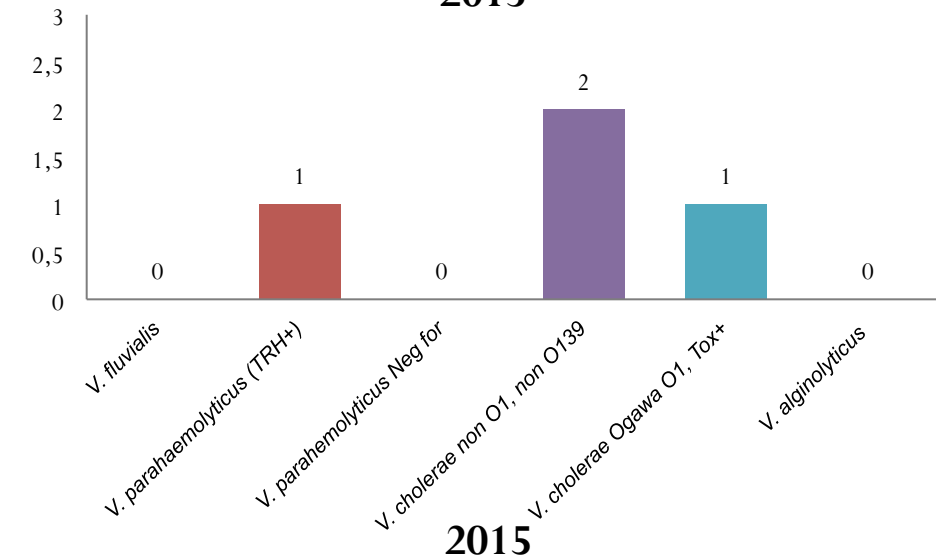
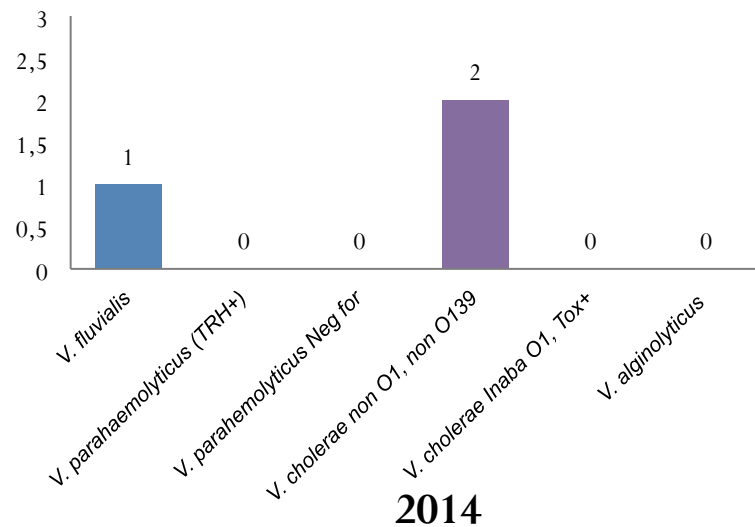
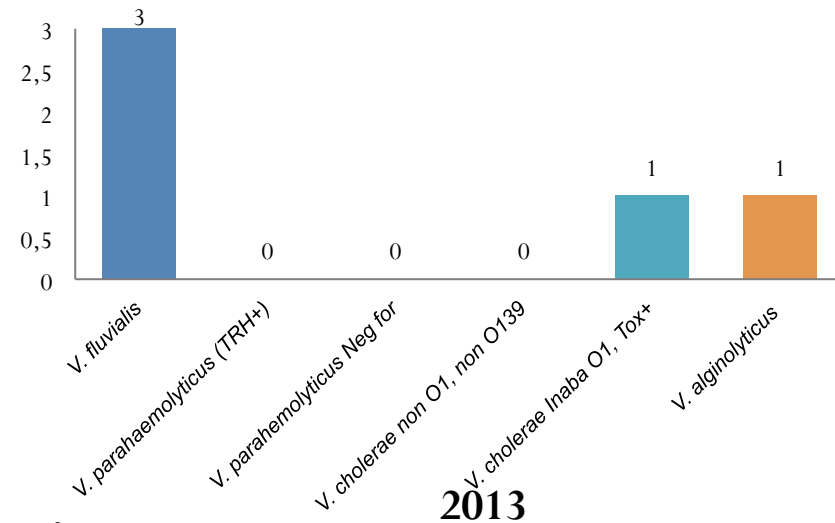
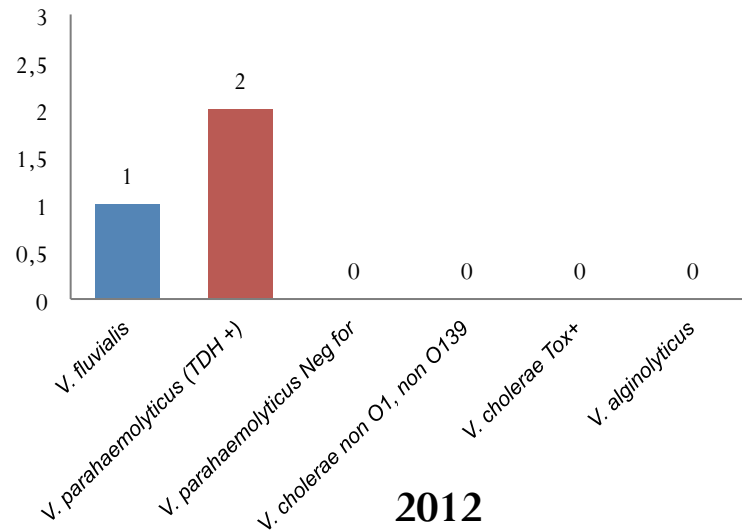


Some statistics from the Belgian NRC

Period 2012-2016

Some statistics for Belgium (NRC data)

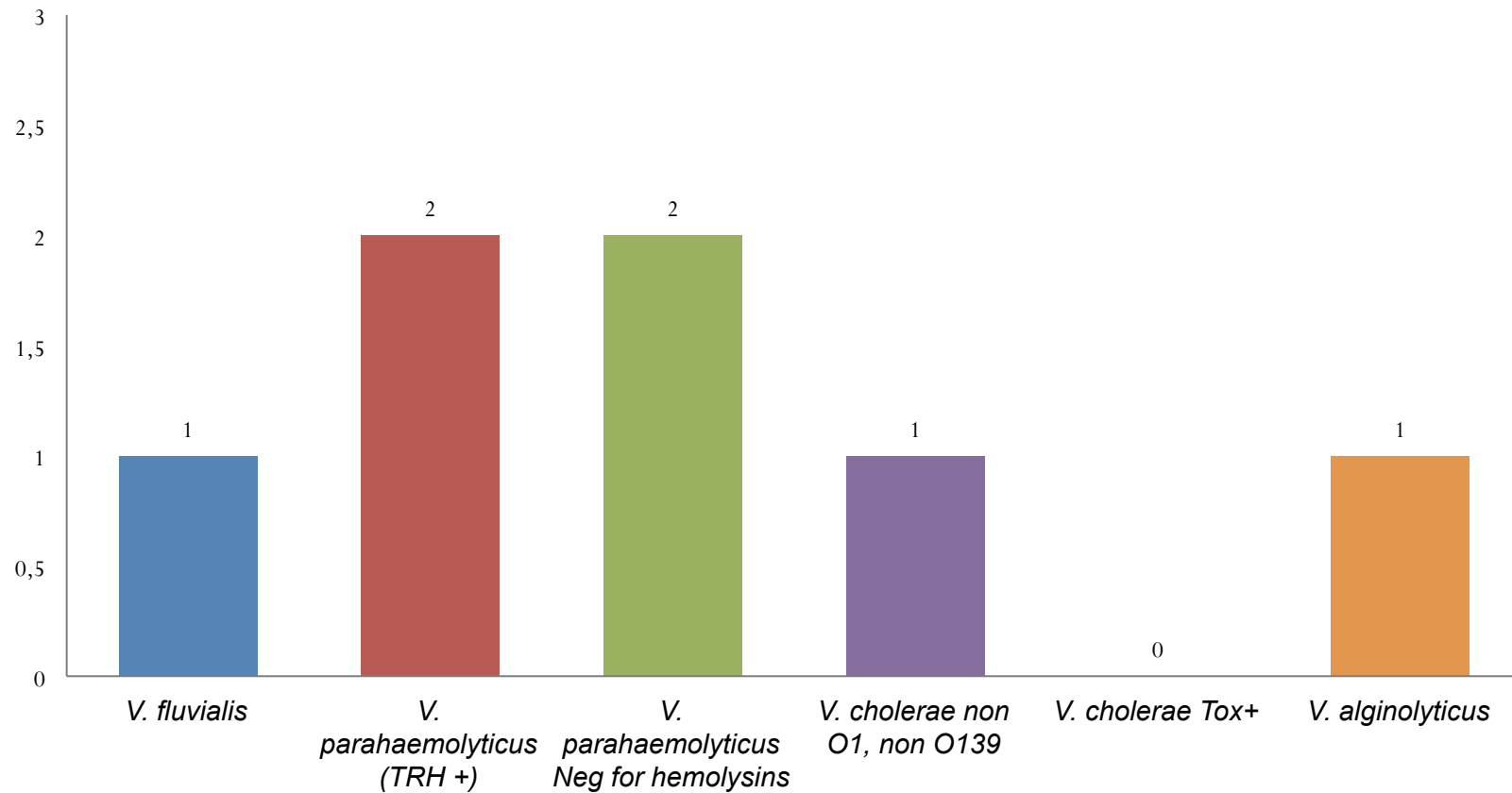
Clinical strains



Some statistics for Belgium (NRC data)

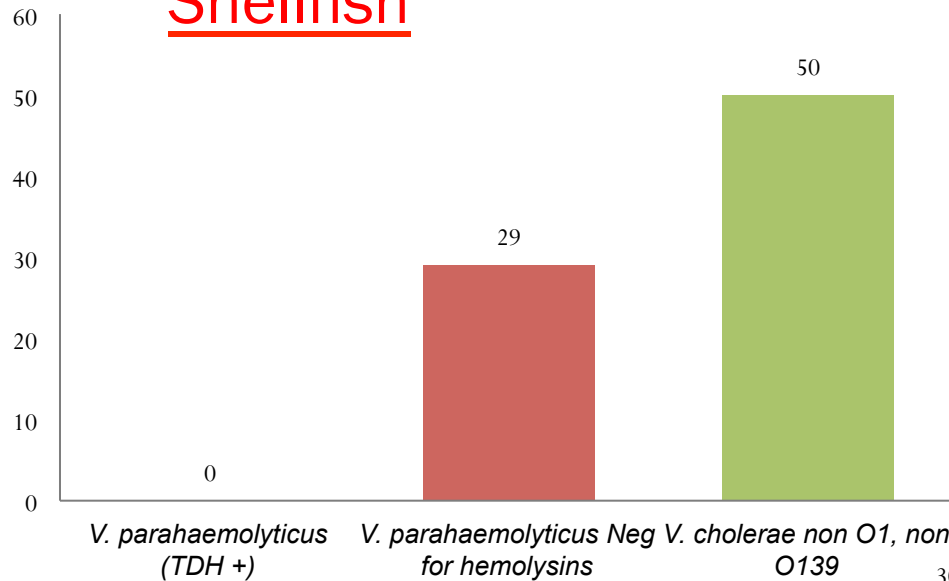
Clinical strains

• 2016

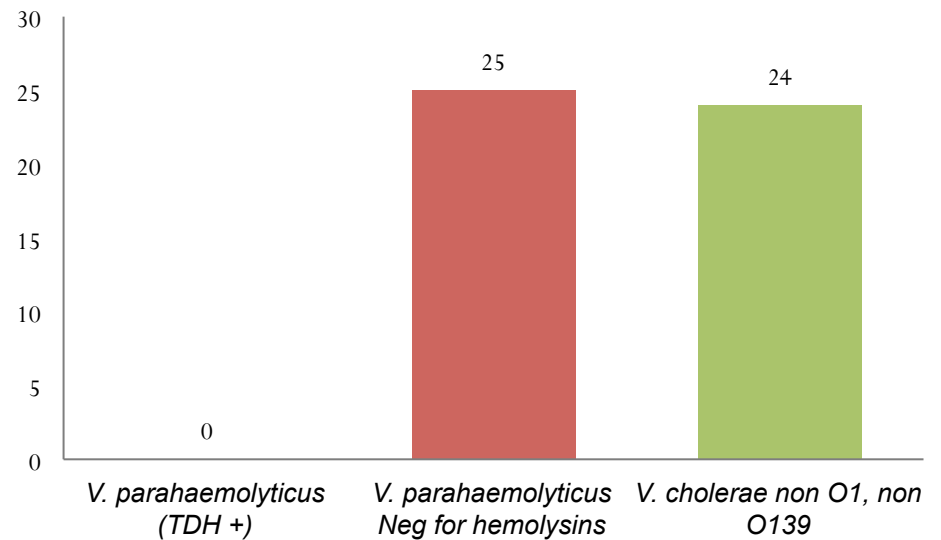


Some statistics for Belgium (NRC data)

Shellfish



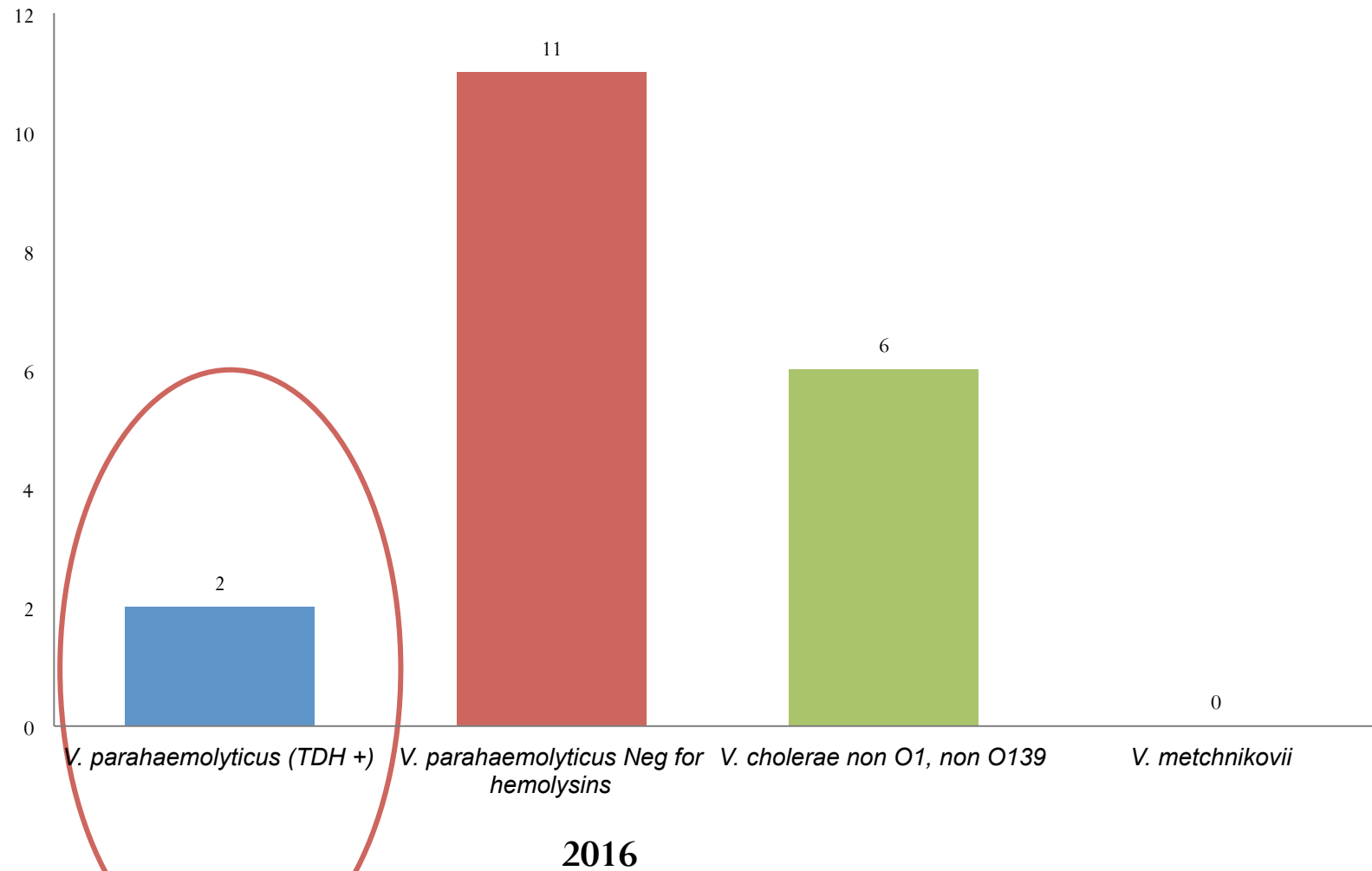
2013



2014

Some statistics for Belgium (NRC data)

Shellfish



NRC form

The image shows a screenshot of a web browser displaying the NRC form website. The browser address bar shows the URL: https://nrchm.wiv-isp.be/fr/centres_ref_lab/vibrio_cholerae_et_vibrio_paraahaemolyticus/default.aspx. The website header includes the logo for 'isp wiv' and the text 'Vibrio cholerae et Vibrio paraahaemolyticus'. The main content area is titled 'Centres de référence - Laboratoires de référence' and lists 'Laboratoires vigies'. A sidebar on the left contains navigation links for 'Demandes de test', 'Formulaire de demande', and 'Rapports'. A PDF form titled 'Formulaire_Vibrio.pdf' is overlaid on the website, showing the following content:

SURVEILLANCE DES MALADIES INFECTIEUSES
Centre National de Référence *Vibrio cholerae* et *Vibrio paraahaemolyticus* Code du labo

Formulaire à envoyer avec l'échantillon à : Rosalie SACHELI (Prof. P. De Mol et P. Melin)
Service de Microbiologie Clinique, CHU de Liège - Sart Tilman, B-23 - 4000 Liège
Tél. : 04366.96.12 / Fax : 04366.24.40 / E-mail : R.sachel@chu.ulg.ac.be

Examens demandés

Confirmation / identification du genre et de l'espèce

Si *V.cholerae* :
 Détermination du sérotype et du sérotype
 Recherche de la toxine cholérique CtxA par PCR
 Recherche du gène ToxA (gène de virulence) par PCR

Si *V.paraahaemolyticus* :
 Recherche des hémolysines par PCR

Confirmation de la sensibilité à un antibiotique particulier, préciser :

Autre, précisez :

Identification / cachet du laboratoire

Nom du responsable :
Nom du laboratoire :
Service :
Adresse :
Code postal :
Localité :
Tél. : Fax :

Cadre réservé au laboratoire de référence

N° de référence : VIB /
Réception le :
Souche :
 n'a pas poussé
 identifiée *V.cholerae*
 O-1 O-139 non O1 non O139
 Inaba Ogawa Hikojima
 Ome CtxA présent
 identifiée *V.paraahaemolyticus*
 Ome(h) hémolysines présentes
 identifiée :

Renseignements concernant le patient

Nom (initiales/autre code) :
Sexe : H F inconnu

Renseignements concernant la souche

Votre numéro de référence :
Votre identification présumée :

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

**Thank you for your
attention!**