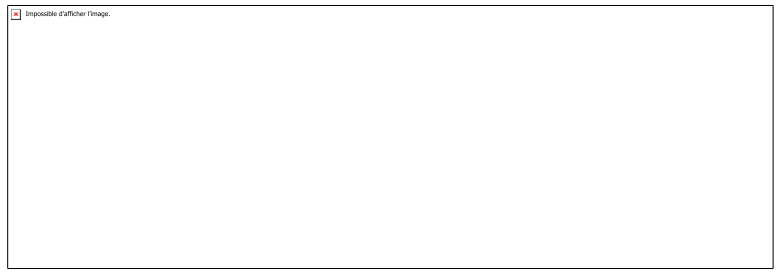
LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Identification of cholera: Rapid Diagnostic Tests

Nick Thomson

15th April 2019



Towards identification of cholera cases for: rapid outbreak responses & to guide long-term policies and interventions.

Culture = Recognised gold standard - 3 days to complete & requires laboratory.

Phenotypic methods.

- Serotyping/ELISA – based with specific antisera – most RDT's use this approach.
- Phage typing – collections of phage
- Multi-locus enzyme electrophoresis (MLEE)

Genotypic methods.

- DNA fragment length polymorphisms of restriction fragments (e.g. by ribotyping, pulsed-field gel electrophoresis).
- Amplified DNA fragments (e.g. by random amplification of polymorphic DNA, repetitive element-PCR) – e.g. RAPD PCR.
- Amplified DNA fragment length polymorphism (AFLP, essentially PFGE with amplification).
- Natural polymorphisms (e.g. by multi-locus sequence typing [MLVA], multiple-locus variable number tandem repeat analysis, DNA microarray [Array tube], whole genome sequencing [SNPs, MLST, cgMLST etc]).

GTFCC Surveillance
Laboratory Working Group

GLOBAL TASK FORCE ON
CHOLERA CONTROL

Interim Technical Note
The Use of Cholera Rapid Diagnostic Tests
November 2016

RESEARCH ARTICLE

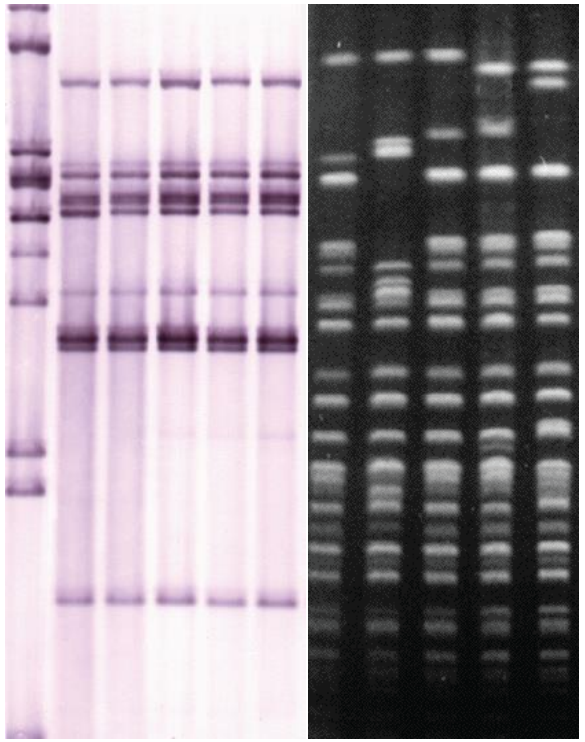
Laboratory evaluation of immunochromatographic rapid diagnostic tests for cholera in Haiti

Wilfredo R. Matias[1,2]*, Fabrice E. Julceus[3], Cademil Abelard[3], Leslie M. Mayo-Smith[4], Molly F. Franke[1], Jason B. Harris[4,5☯], Louise C. Ivers[1,2,6☯]*

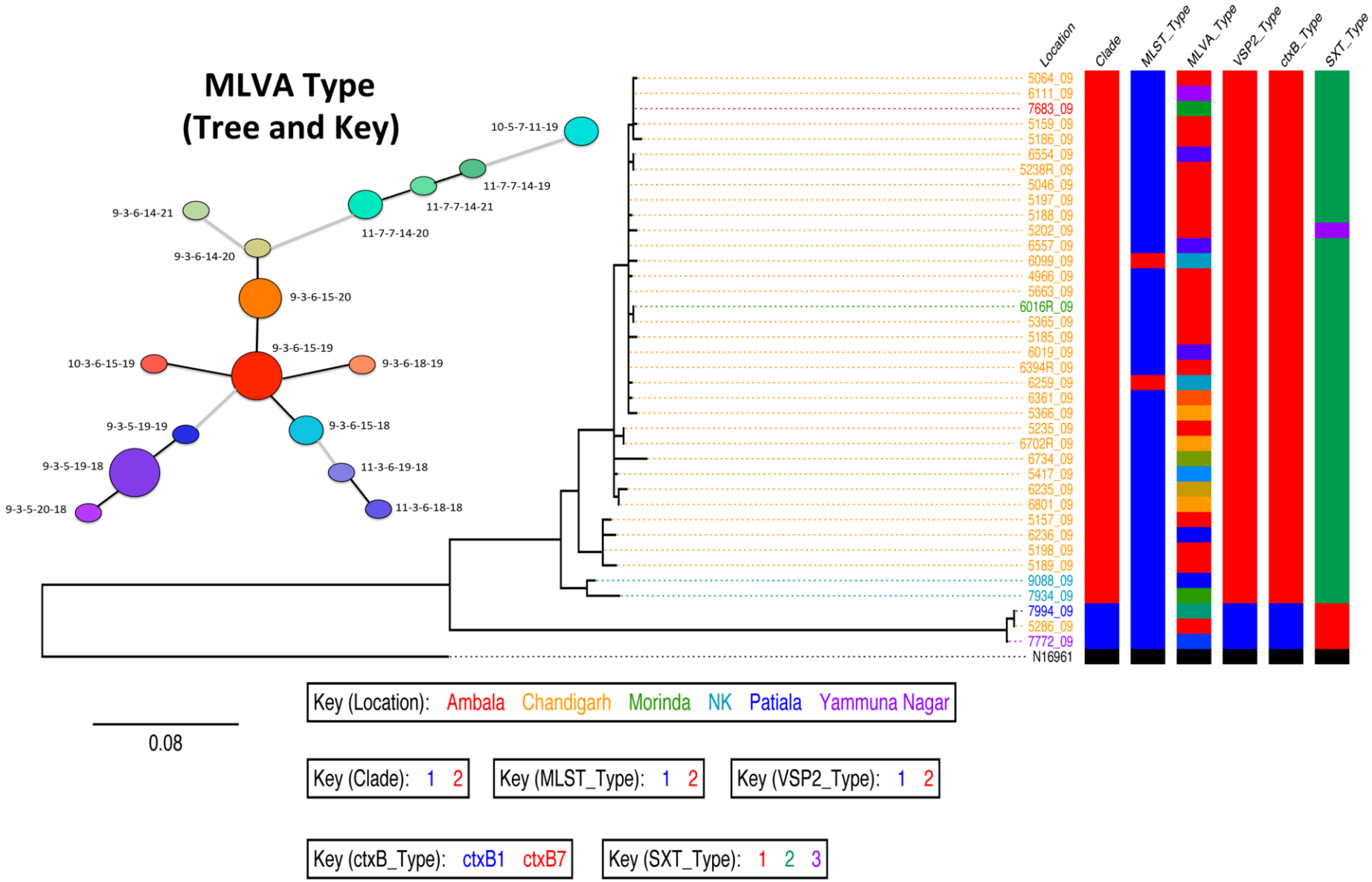
Citation: Matias WR, Julceus FE, Abelard C, Mayo-Smith LM, Franke MF, Harris JB, et al. (2017) Laboratory evaluation of immunochromatographic rapid diagnostic tests for cholera in Haiti. PLoS ONE 12(11): e0186710. https://doi.org/10.1371/journal.pone.0186710

Towards identification of cholera cases for: rapid outbreak responses & to guide long-term policies and interventions.

- O antigen serogrouping (O1, O139, O37, …)
- Biotyping (El Tor vs Classical)
- O1 serotyping (Ogawa, Inaba, Hikojima)
- Phage typing
- Multilocus enzyme electrophoresis (MLEE)
- Ribotyping
- Pulsed-field gel electrophoresis
- *ctxB* (B subunit of cholera toxin) RFLP or sequencing
- *tcpA* (toxin coregulated pilus A) sequencing
- Sequencing of other virulence genes …..
- Multiple loci VNTR analysis (MLVA)



A SNP based maximum likelihood phylogeny of the Chandigarh *V. cholerae*.



Abd El Ghany M, Chander J, Mutreja A, Rashid M, Hill-Cawthorne GA, et al. (2014

PLOS | NEGLECTED TROPICAL DISEASES

Molecular RDTs

PCR Targets,

<u>Species:</u>

16srRNA

<u>Serogroup:</u>

O1/O139

<u>Serotype:</u>

wbe

<u>*Virulence:*</u>

cholera toxin (*ctx*),

zonula occludens toxin (*zot*)

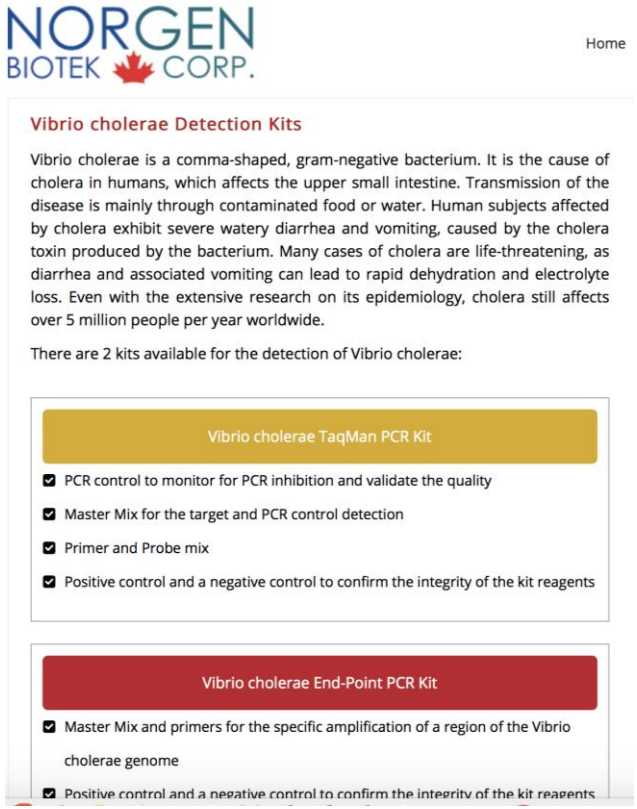
accessory cholera enterotoxin (*ace*)

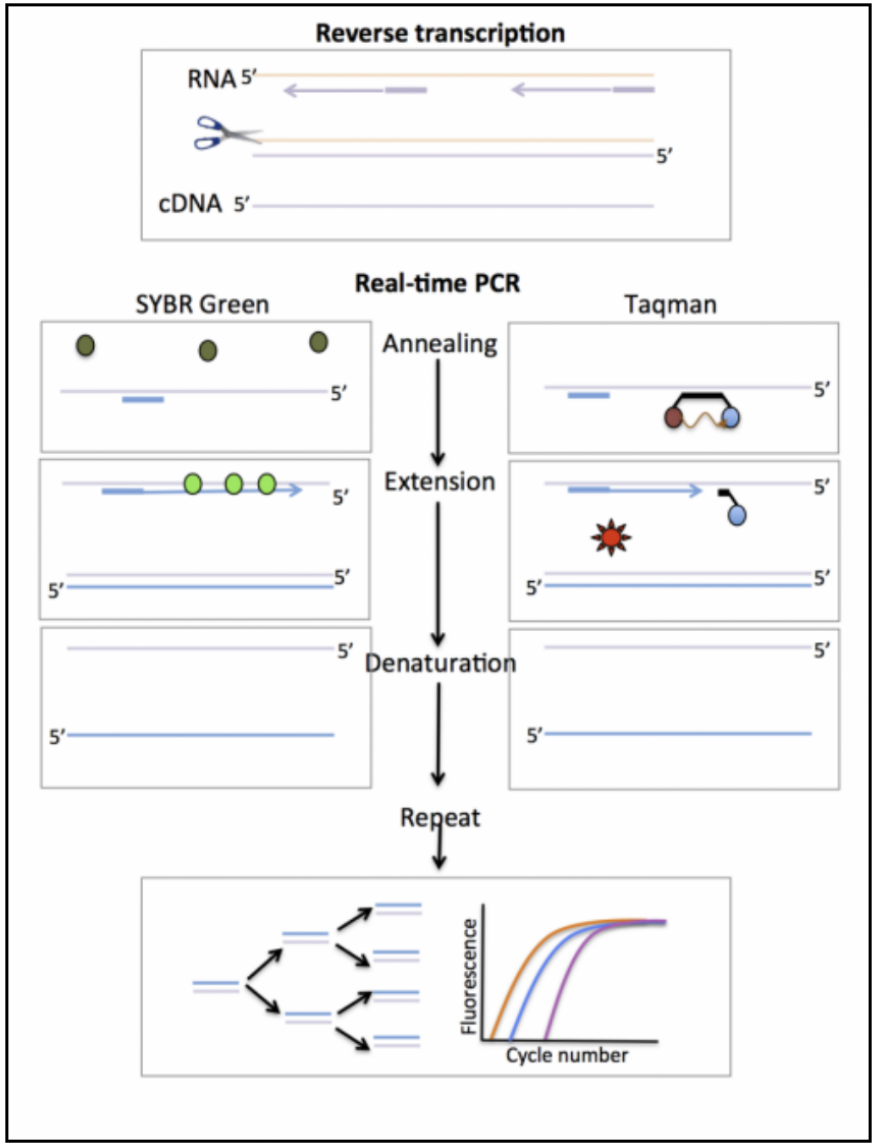
<u>Resistance:</u>

tetracycline resistance genotype (*tetA*).

<u>Geographically defined targets:</u>

WASA1 - Latin America 7th Pandemic Wave 1





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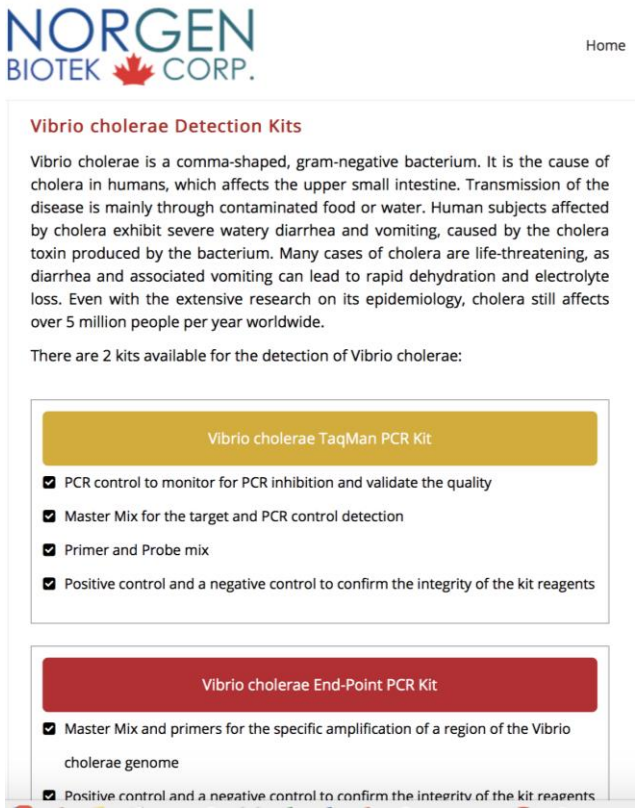


Real Time PCR

Plasmid design *ctxB* (cholera toxin) gen
and Outer membrane protein W (*ompW*
Genesig Easy detection kits for:
Toxigenic *Vibrio cholerae*.
All *V. cholerae* subspecies.

ATTRIBUTES definitions	DESIRED	ACCEPTABLE
Intended use of the test	Early detection, declaration, and monitoring of outbreak without need for cholera confirmation First intention test to be used on a predefined number of cholera suspect cases	Test to declare a cholera alert, to be confirmed by culture and/or PCR
Target molecule classic approach is based on LipoPolySaccharide (LPS) (O antigen), additional markers can be considered provided they show adequate performance	Biomarker for toxigenic *Vibrio cholerae* O1: LPS (O antigen) and cholera toxin marker (CT Monoclonal antibody)	Biomarker for *Vibrio cholerae* O1 and O139 (test will distinguish O1 from O139): LPS (O antigen)
Specimen type	Stool/Rectal swab Or Samples easier to collect (capillary blood if new markers are used)	Stool/Rectal swab
Analytical Sensitivity/Limit of Detection (Identification of positive reference material)	100%	≥ 95%
Clinical/Diagnostic Sensitivity (identification of clinical cases with toxigenic *Vibrio cholerae* only)	≥ 95% 95%CI (90 - 100)	≥ 90% 95%CI (85 – 95)
Analytical Specificity (assessment of cross reactivity with other pathogens)	100%	100%
Clinical /Diagnostic Specificity (identification of the cases not due to toxigenic vibrio cases)	≥ 98% 95%CI (95-100)	≥ 95% 95%CI (93 – 98)
Result output	Qualitative result	Qualitative result
Time to result	< 15 minutes	< 30 minutes
Throughput: number of tests to be performed in an hour	5-6	**4-3**
Intended users	Health worker from outbreak investigation team with dedicated training, present at community level And Health Community Worker at primary or secondary care levels for early detection of cholera transmission in patients presenting with compatible symptoms"	Non-laboratory trained health personnel

GLOBAL TASK FORCE ON
CHOLERA CONTROL

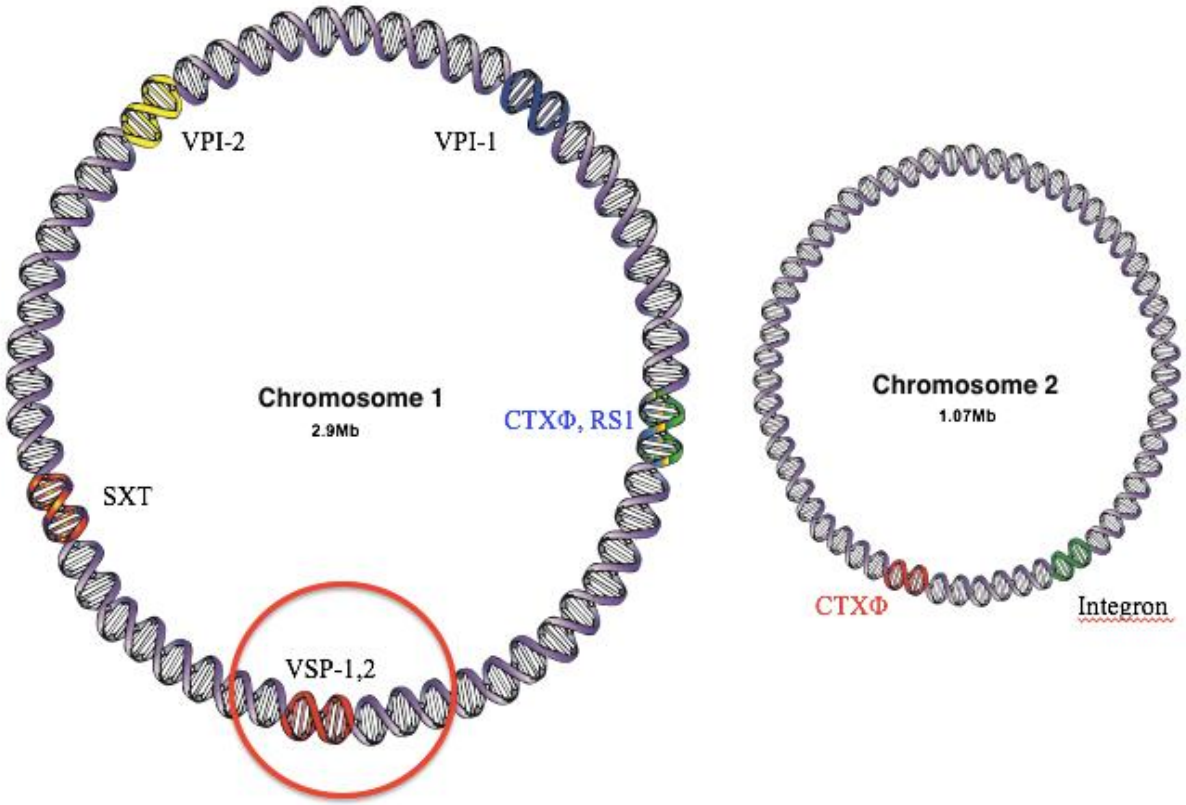
WHO – Global Task Force on Cholera Control

Target Product Profile (TPP) for the development of improved Cholera rapid diagnostic tests

June 2017

In use stability	≥ 1 hour after opening of individual pouch	≥ 30mn after opening of individual pouch
End-point stability (time window during which signal remain valid)	1 hour	≥ 30mn
Shelf-life	24 months	12 months
Storage conditions for test device (stability)	• 2°C - 40°C, relative humidity up to 98%, no cold chain required • Should be able to tolerate stress during transport (cycles of temperature of 30 to 50°C) without affecting the labelled expiry date	• Up to 35°C, no cold chain required • Should be able to tolerate stress during transport cycles of temperature of 30 to 50°C without affecting the labelled expiry date
Lot to lot variation - **Sensitivity** - **Specificity**	- SE: up to 50% in end-point sensitivities with all lots meeting the sensitivity specification - SP: no variation	Same
Reader to reader variation	90% of readers should detect a positive result near the limit of detection	Same

Vibrio cholerae genome



Banerjee, Das , Nair, Basak (2014) Infection, Genetics and Evolution 23, 32 - 41

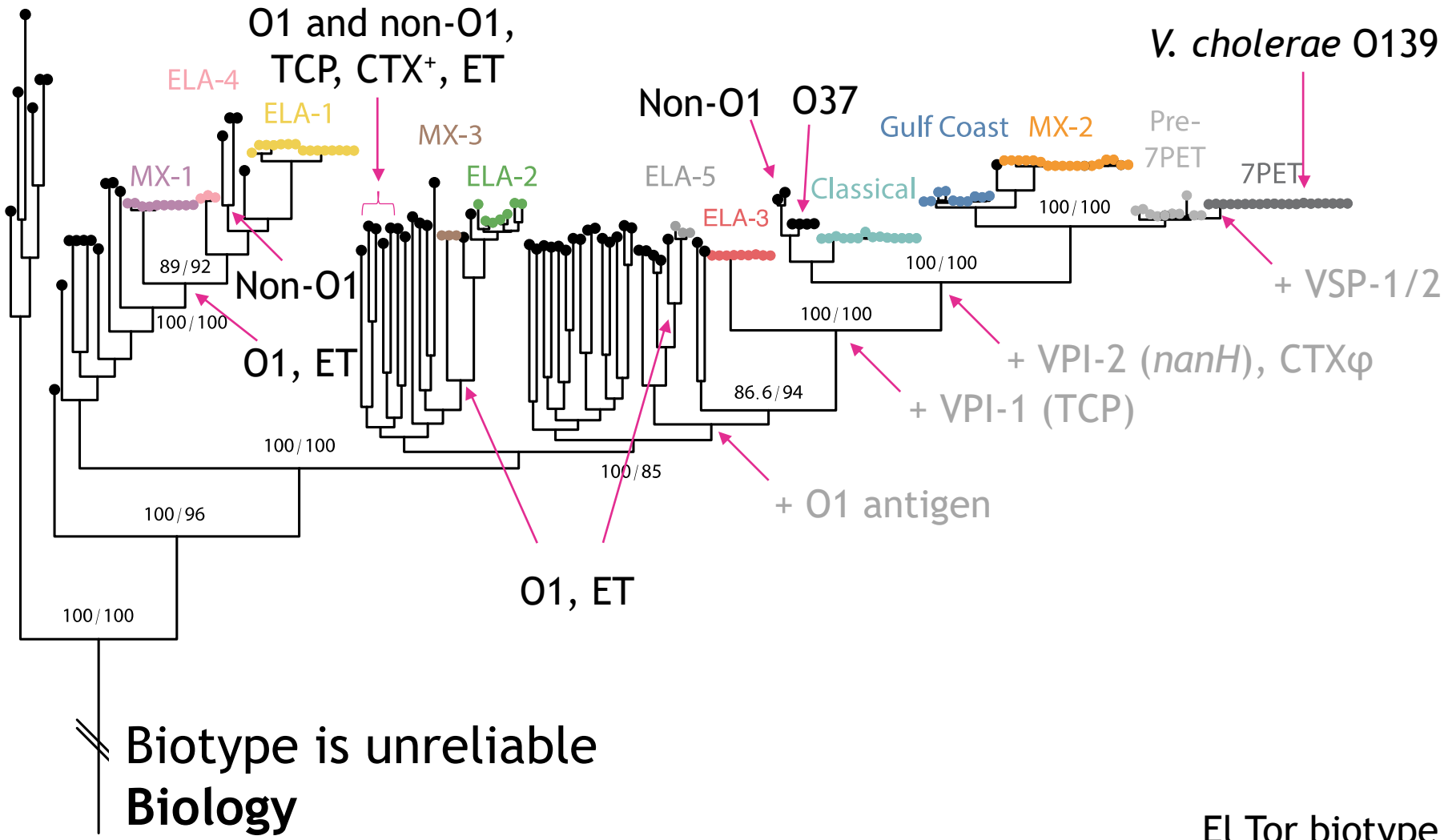
- RDTs
- Have limited usefulness for individual diagnosis among suspected cholera patients, unless the results of the test would influence on the immediate management of the case

RDTs may be used for:

- early outbreak detection,
- as a tool for an initial alert
- monitoring of outbreaks
- monitoring of seasonal peaks in highly endemic areas

Areas where WGS are more appropriate (combined with good Epi data)

- Low incidence and inter-outbreak periods
- Defining routes and patterns of long distance transmission
- Connecting hotspots and understanding repeat patterns of disease
- Fine mapping routes of transmission
- Understanding carriage



Biotype is unreliable

Biology

O1 and CTX are necessary but not sufficient

El Tor biotype

Matthew Dorman



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Whole Genome Sequence Resource

Nick Thomson

15th April 2019

wellcome sanger institute

Centre for Genomic Pathogen Surveillance

Pathogenwatch

A global platform for genomic surveillance.

- Fast predictions of resistant genotypes and clustering.
- Real-time analytics and genomic epidemiology.
- Facilitates processing, clustering and exploration of whole genome assemblies.

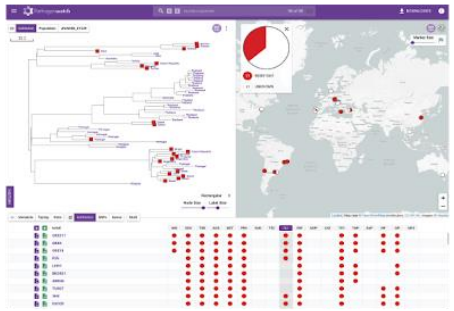
http://pathogen.watch

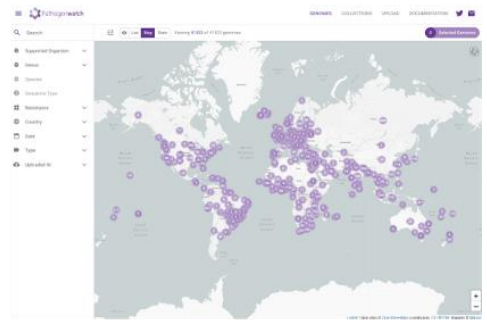
Analyse data from anywhere

or

linked epi metadata

Pathogenwatch





Genome Report
Risk Markers (eg AMR)
Typing

Collections
Risk Markers (eg AMR)
Genome Neighbors

Global analytics
Trends

Individual

Population

1

2 3

3

Use Cases

Drag and drop files to begin.

Genomic Data

One or more **assemblies** in multi-FASTA format with one of the following extensions:

.fa, .fas, .fna, .ffn, .faa, .frn, .fasta, .genome, .contig, .dna

Please ensure that there is **one file per genome**.

Settings

Enable Compression

Recommended for slow connections.

Upload Files Individually

Recommended for unstable connections.

Metadata

Files in CSV format with the extension **.csv**.

Files should contain a column **filename** containing the names of genome files uploaded at the same time.

To make full use of metadata, we strongly recommend including the following columns:

latitude, longitude, year, month, day

When providing a date, month and day are optional. Additional metadata may be included and will be saved.

CSV Templates

General

Salmonella Typhi

Bacterial Genomes: Accessing and Analysing Microbial Genome Data

04 February - 09 June 2019

Online, FutureLearn platform

Use computational tools to investigate microbial genomes