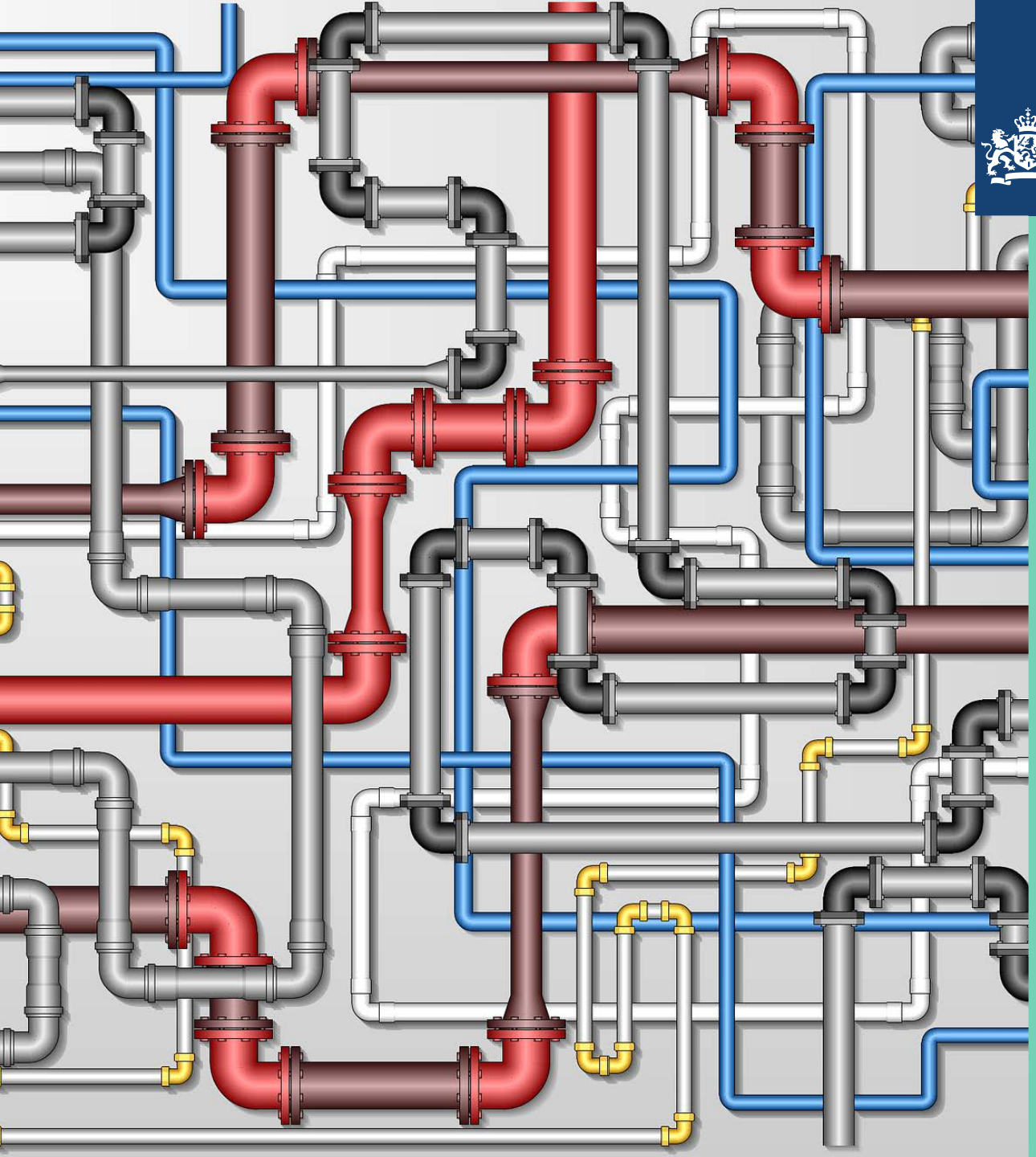


National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

The implementation of ISO accredited bioinformatic pipelines for AMR detection and how to report?

An interactive discussion

Maaike van den Beld, Maren Lanzl

RIVM

Interactive presentation

- Examples from the Netherlands
- Questions for discussion
- Colored cards

No answer is wrong or right!

Question 1

Does your lab have to adhere to a quality system?

YES

NO

Question 2

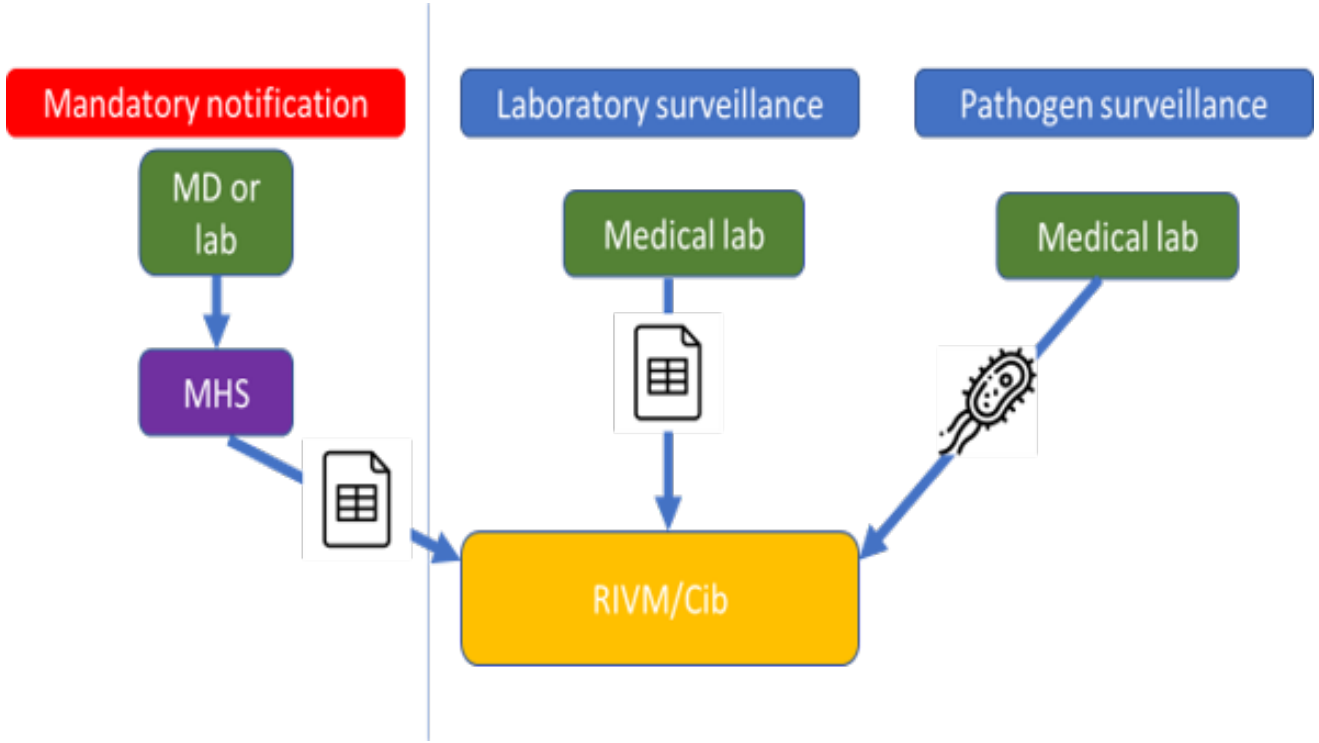
Does the quality system also apply to the bioinformatic pipelines- if used?

YES

NO

Situation Netherlands

- One National Centre for Infectious Disease control, Cib



Pathogen surveillance Netherlands

Pathogen	Notifications	Pathogen surveillance	Number per year (approx.)
STEC	X	X	900
Listeria monocytogenes	X	Only invasive isolates	100
Shigella spp	X	X	300
Yersinia spp		X	200
Salmonella		X	2500
Campylobacter		Sentinel	1200

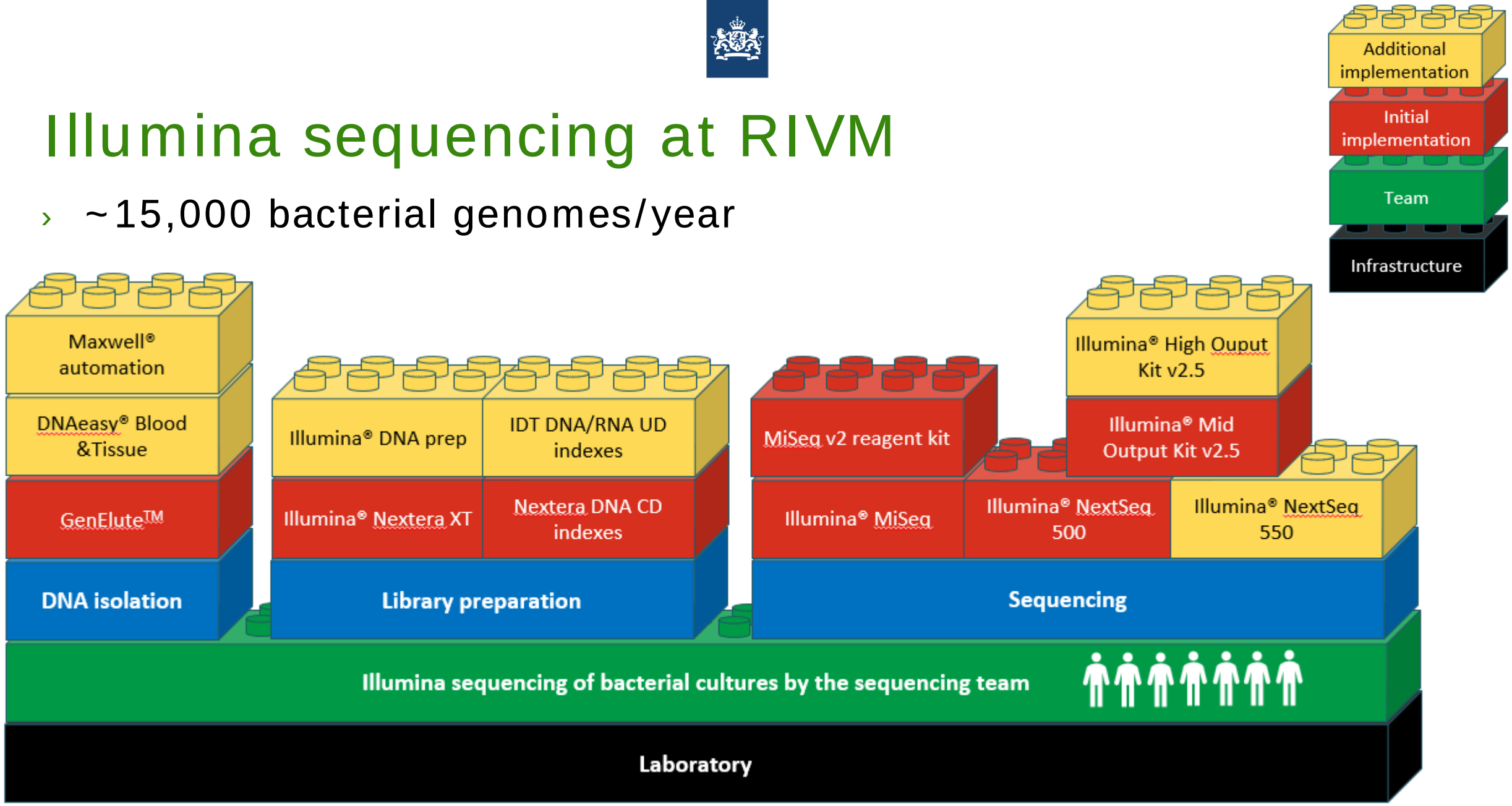
Gradual transition from 2016

ISO 15189
ACCREDITATION
MEDICAL LABORATORY

JUNO
BACTERIAL GENOMICS PIPELINES

Illumina sequencing at RIVM

- ~15,000 bacterial genomes/year



Question 3

How to validate the bioinformatic processes?

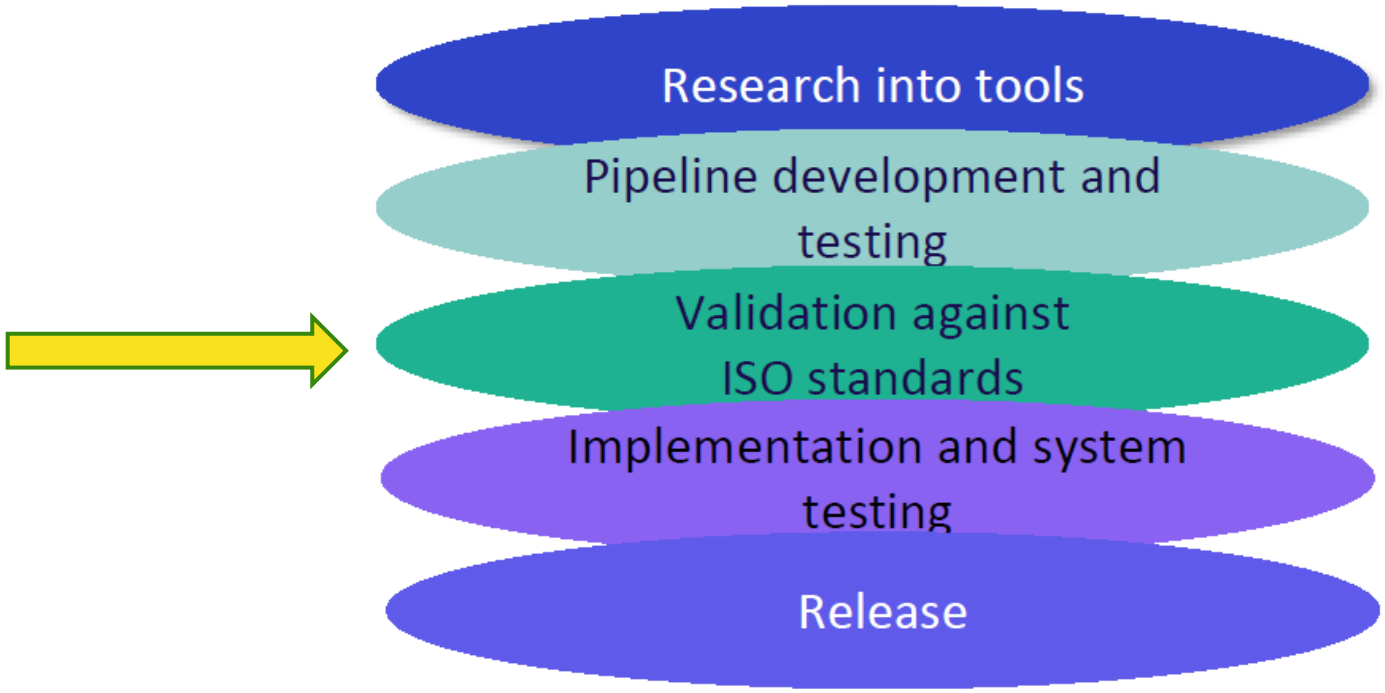
Each step separately

The whole pipeline as one

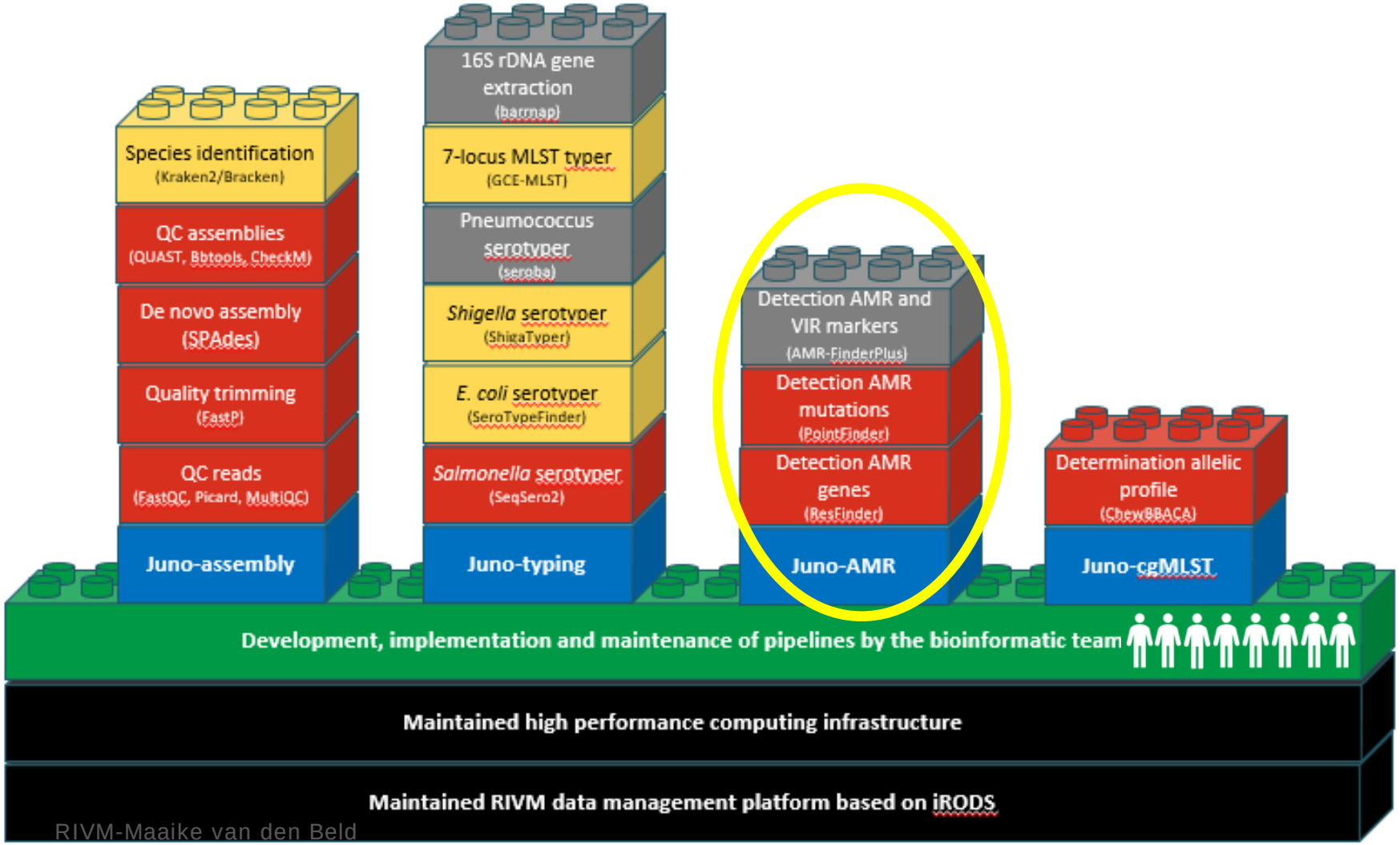
JUNO

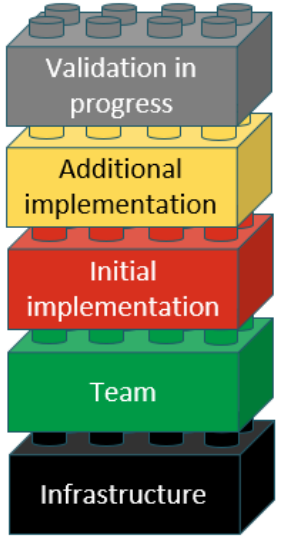
BACTERIAL GENOMICS PIPELINES

- Juno-assembly
- Juno-typing
- Juno-cgMLST
- Juno-AMR



Pipeline validation at RIVM





Juno-AMR

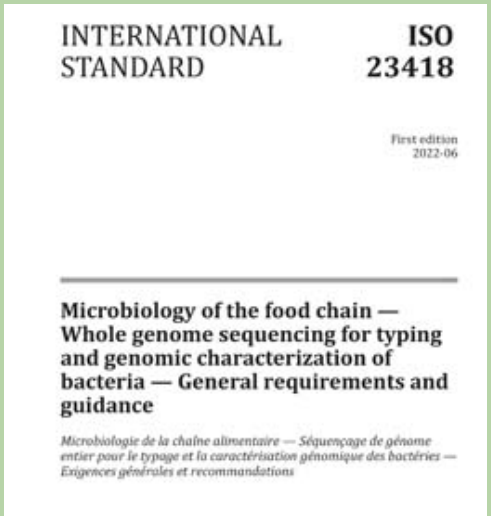
- Technical validation of pipeline: bioinformatics
- ISO validated part: ResFinder, PointFinder
- To be validated part: AMRFinderPlus
- Problem with validation: before use of WGS, only phenotypic resistance was determined. What to compare pipelines with??

Validation of bioinformatic pipelines

ISO 15189 ACCREDITATION

MEDICAL LABORATORY

- Made for laboratory tests
- Used by RIVM for validation of bioinformatic pipelines


INTERNATIONAL STANDARD

ISO 23418

First edition
2022-06

Microbiology of the food chain — Whole genome sequencing for typing and genomic characterization of bacteria — General requirements and guidance

Microbiologie de la chaîne alimentaire — Séquençage de génome entier pour le typage et la caractérisation génomique des bactéries — Exigences générales et recommandations

- Publication: June 2022

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Validation stage	Repeatability (accuracy/precision)	Reproducibility (accuracy/precision)	Agreement with other methods (accuracy/trueness)
4. Bioinformatics pipeline	Demonstrate identical results from same data set at least twice on same computer/IT infrastructure, using the same version of the software with the same options/parameters.	Demonstrate comparable results from same data set at least twice on different computers such as local Linux/Unix/any OSX workstations or computing clusters or supercomputing nodes using the same version of the software with the same options/parameters. Use of a workflow management system is recommended for such testing on different platforms.	Demonstrate results are comparable with other pipelines for the same application and specify any known differences between pipelines that can affect the outcome (e.g. built-in reference databases). If no such pipeline is available, then simulated data, where the evolutionary relationships of the isolates are known and reflect variability expected in real data, should be used to demonstrate the pipeline's ability to produce the correct answer.

RIVM ISO 15189 validation Juno-AMR

Recovery
Added sequences of ARG to WGS of pathogens

Trueness
Sequences with known genetic resistance from literature

Trueness
Sequences of ATCC strains with known phenotypic resistance

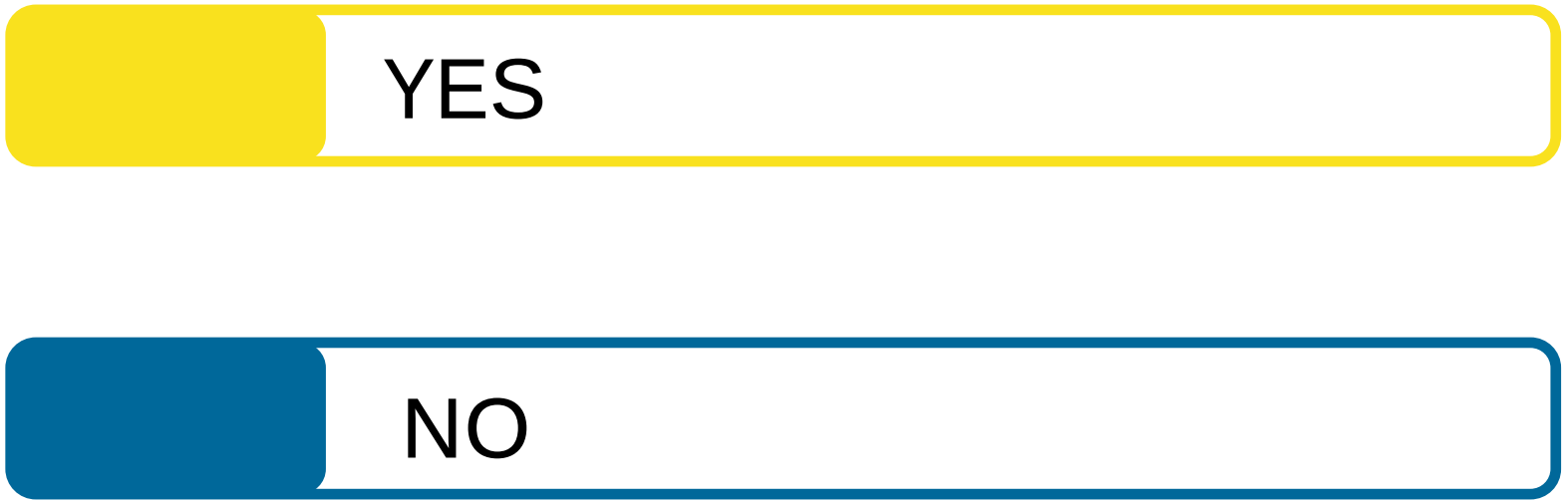
Reproducibility
Multiple generations of same sequences, compare SHA1 hashes

Accuracy
Comparison with "gold standard"

- Sensitivity $= \frac{TP}{TP+FN}$
- Specificity $= \frac{TN}{TN+FP}$
- Positive predictive value $= \frac{TP}{TP+FP}$
- Negative predictive value $= \frac{TN}{TN+FN}$

Question 4

Any ideas about additional parameters to validate?



Question 4

Any ideas about additional parameters to validate?

Recovery
Added sequences of ARG to WGS of pathogens

Trueness
Sequences with known genetic resistance from literature

Reproducibility
Multiple generations of same sequences, compare SHA1 hashes

Accuracy
Comparison with “gold standard”

Trueness
Sequences of ATCC strains with known phenotypic resistance

YES

NO

Results ISO 15189 validation Juno-AMR

Recovery
Added sequences of ARG to WGS of pathogens

PASS

Trueness
Sequences with known genetic resistance from literature

PASS

Trueness
Sequences of ATCC strains with known phenotypic resistance

PASS

Reproducibility
Multiple generations of same sequences, compare SHA1 hashes

PASS

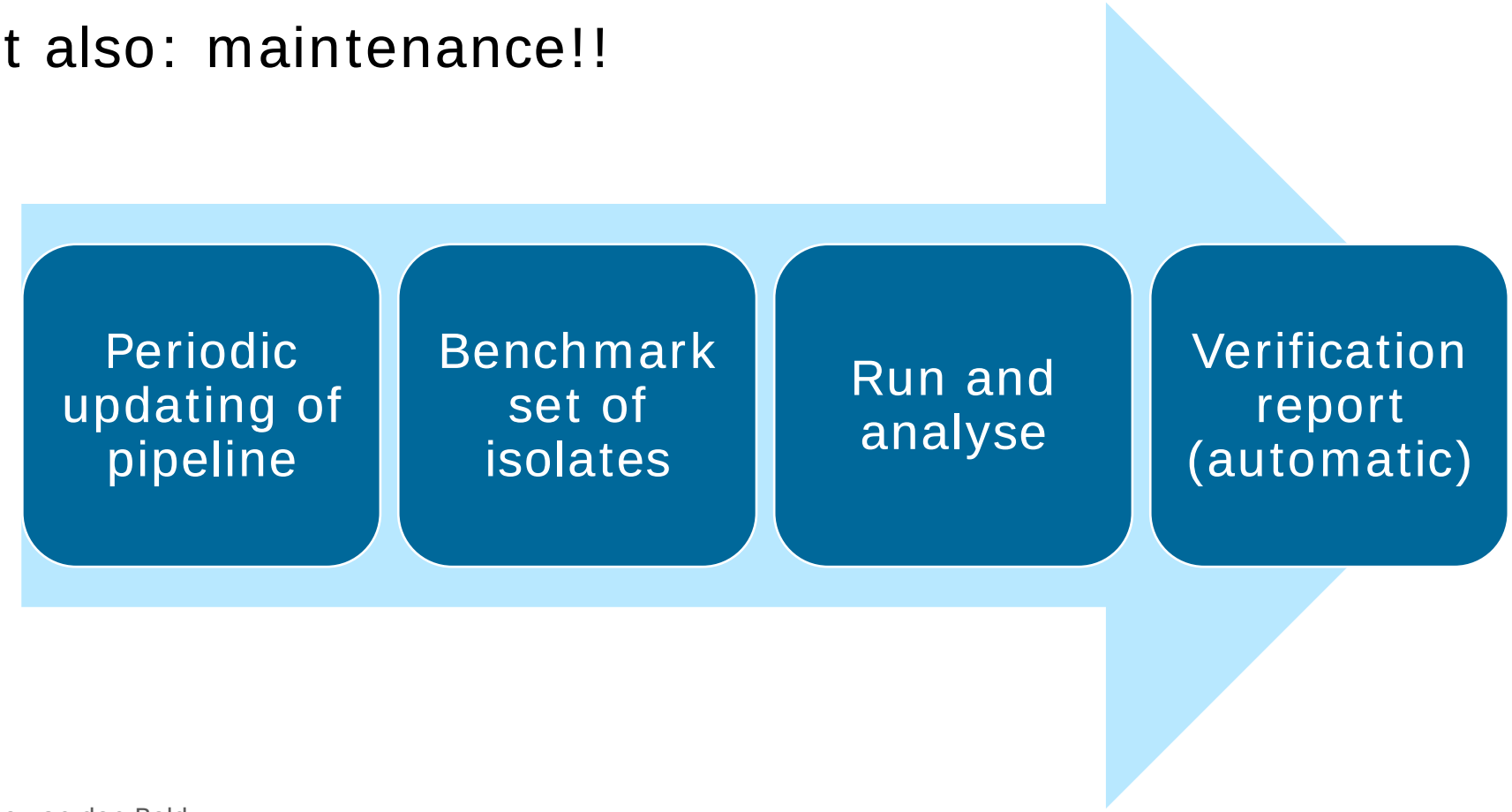
Accuracy
Comparison with “gold standard”

Results accuracy (compared to phenotypic)

SalmShigEcol	n R/S	Accuracy	Sensitivity	Specificity	NPV	PPV
Ampicillin	139/260	98.7	97.8	99.2	98.9	98.6
Ciprofloxacin	45/294	99.7	100	99.7	100	97.8
Cefotaxim	14/385	100	100	100	100	100
Meropenem	15/397	100	100	100	100	100
Gentamicin	18/283	94.7	11.1	100	94.6	100
Sulfamethoxazol	167/173	99.4	100	99.3	100	96.3
Trimethoprim	26/135	99.1	100	98.3	100	98.2
Campy						
Ciprofloxacin	182/223	98.5	97.8	99.1	98.2	98.9
Erythromycin	10/395	100	100	100	100	100
Tetracyclin	99/306	97.5	99.0	97.1	99.7	91.6
Gentamicin	2/403	100	100	100	100	100

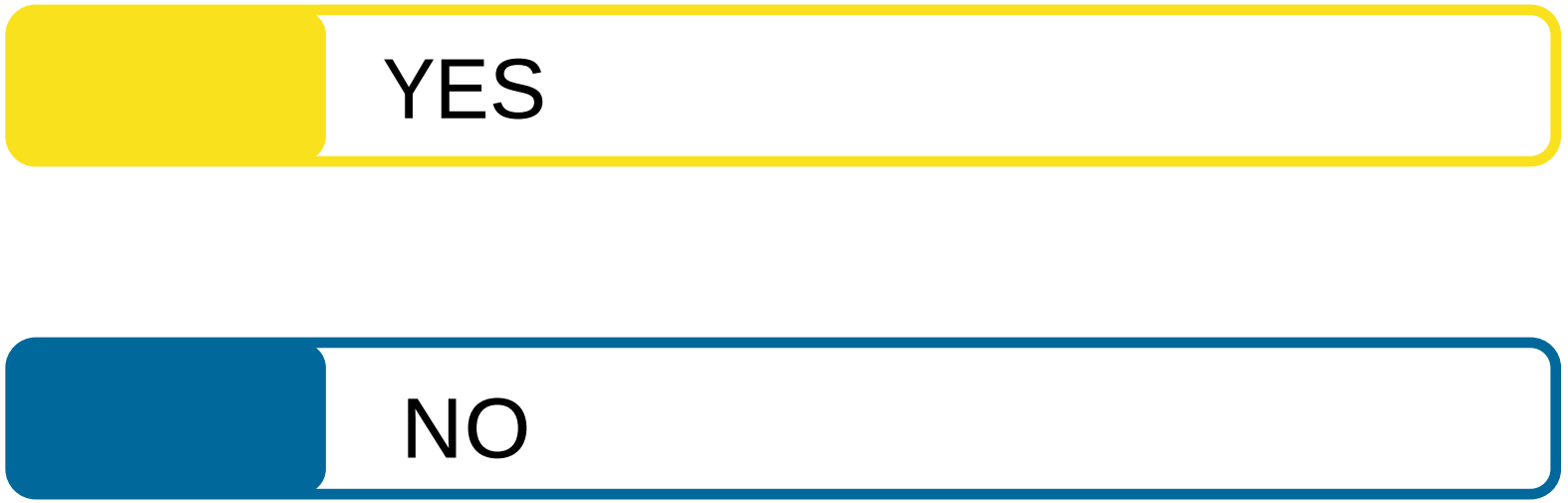
Validation and maintenance

- Juno-AMR ISO validated using the parameters as shown
- But also: maintenance!!



Question 5

Any other important parts for the quality of pipelines, next to validation and maintenance?



Question 6

Do/would you report results of detection of genetic AMR markers?

Reporting RIVM

Testnaam	Resultaat
(Sub)Species	Salmonella enterica subsp. enterica
Serotype salmonella	I 4,[5],12:i:-
MLST type	34
MLST profiel	10-19-12-9-5-9-2
Ampicilline	blaTEM-1B (blaTEM-1B_AY458016)
Cefotaxim	geen resistentiemarkers gedetecteerd
Ciprofloxacine	geen resistentiemarkers gedetecteerd
Gentamicine	geen resistentiemarkers gedetecteerd
Meropenem	geen resistentiemarkers gedetecteerd
Trimethoprim	geen resistentiemarkers gedetecteerd
Sulfamethoxazol	sul2 (sul2_HQ840942)

Testnaam	Resultaat
Conclusie Species	Campylobacter jejuni
MLST type	934
MLST profiel	1-1-59-2-10-5-7
Ciprofloxacine	geen resistentiemarkers gedetecteerd
Erythromycine	geen resistentiemarkers gedetecteerd
Gentamicine	geen resistentiemarkers gedetecteerd
Tetracycline	geen resistentiemarkers gedetecteerd

Testnaam	Resultaat
(Sub)Species	Salmonella enterica subsp. enterica
Serotype salmonella	Paratyphi A
MLST type	85
MLST profiel	45-4-8-44-27-9-8
Ampicilline	geen resistentiemarkers gedetecteerd
Cefotaxim	geen resistentiemarkers gedetecteerd
Ciprofloxacine	gyrA p.S83F parC p.T57S
Gentamicine	geen resistentiemarkers gedetecteerd
Meropenem	geen resistentiemarkers gedetecteerd
Trimethoprim	geen resistentiemarkers gedetecteerd
Sulfamethoxazol	geen resistentiemarkers gedetecteerd

Testnaam	Resultaat
Conclusie Species	Campylobacter jejuni
MLST type	353
MLST profiel	7-17-5-2-10-3-6
Ciprofloxacine	gyrA p.T86I
Erythromycine	geen resistentiemarkers gedetecteerd
Gentamicine	geen resistentiemarkers gedetecteerd
Tetracycline	tet(O) (tet(O)_M18896)

Reporting RIVM

Testnaam	Resultaat
(Sub)Species	Salmonella enterica subsp. enterica
Serotype salmonella	Enteritidis
MLST type	11
MLST profiel	5-2-3-7-6-6-11
Ampicilline	geen resistentiemarkers gedetecteerd
Cefotaxim	geen resistentiemarkers gedetecteerd
Ciprofloxacine	parC p.T57S
Gentamicine	geen resistentiemarkers gedetecteerd
Meropenem	geen resistentiemarkers gedetecteerd
Trimethoprim	geen resistentiemarkers gedetecteerd
Sulfamethoxazol	geen resistentiemarkers gedetecteerd

Question 7

Other ideas about how to report results of detection of genetic AMR markers?

Thank you for the great discussion!

National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

NPHRL Foodborne Infections (IDS)
Epidemiology of intestinal infections and zoonoses (EPI)
Team genomics (IDS)
Team bioinformatics (IDS)
Bioinformatics core team RIVM

WAGENINGEN
UNIVERSITY & RESEARCH

Department Bacteriology, Host Pathogen Interaction and Diagnostics Development

And special thanks to all the medical laboratories!