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

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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

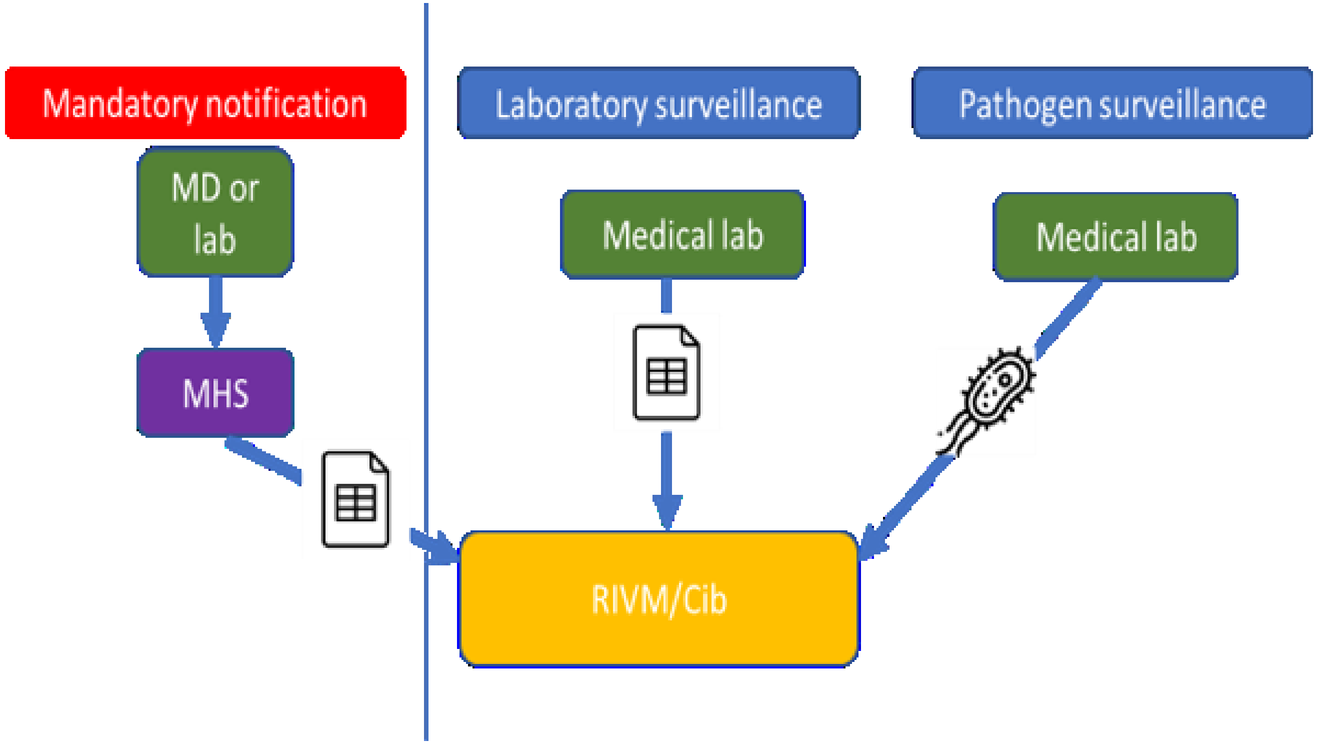
Does the quality system also apply to the bioinformatic pipelines- if used?

YES

NO

Situation Netherlands

- One National Centre for Infectious Disease control, Cib



With courtesy of Eelco Franz

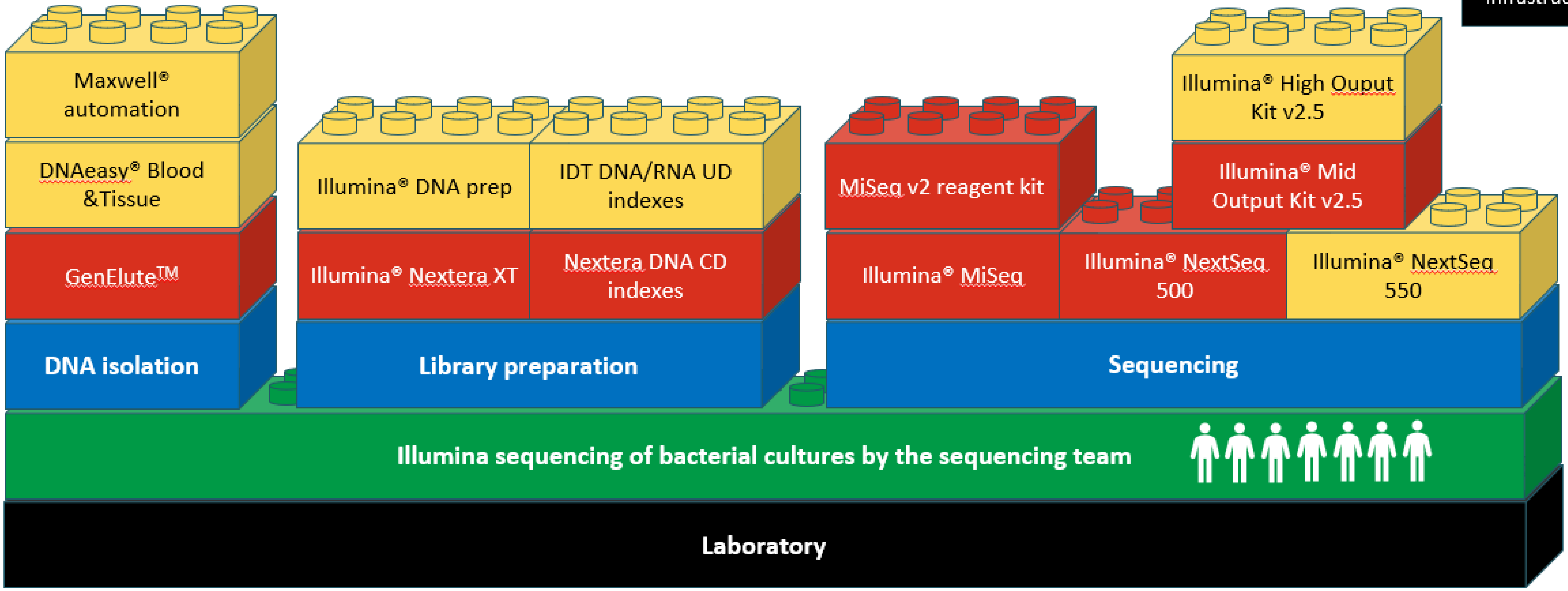
Pathogen surveillance Netherlands

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

Illumina sequencing at RIVM

- ~15,000 bacterial genomes/year

🟨 Additional implementation
🟥 Initial implementation
🟩 Team
⬛ Infrastructure



Maxwell® automation

DNAeasy® Blood &Tissue

GenElute™

DNA isolation

Illumina® DNA prep

IDT DNA/RNA UD indexes

Illumina® Nextera XT

Nextera DNA CD indexes

Library preparation

MiSeq v2 reagent kit

Illumina® High Ouput Kit v2.5

Illumina® Mid Output Kit v2.5

Illumina® MiSeq

Illumina® NextSeq 500

Illumina® NextSeq 550

Sequencing

Illumina sequencing of bacterial cultures by the sequencing team

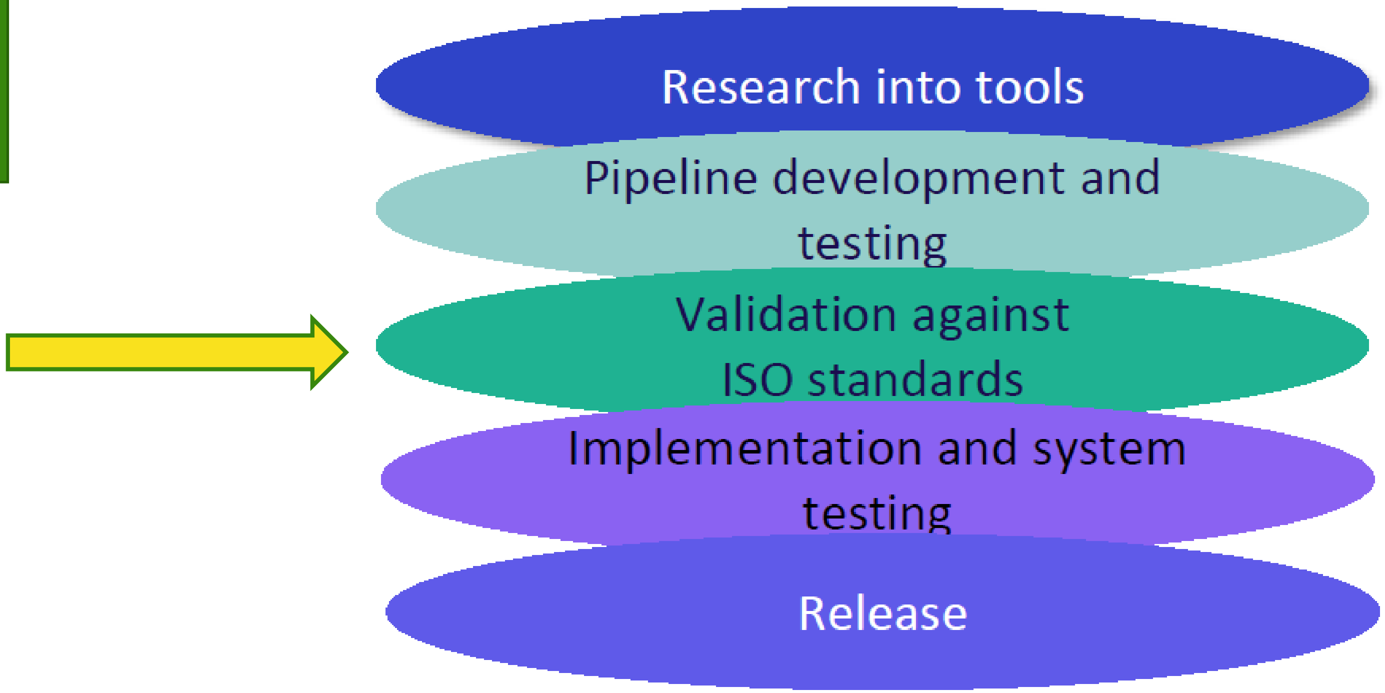
Laboratory

Question 3

How to validate the bioinformatic processes?

- Each step separately
- The whole pipeline as one

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


Research into tools

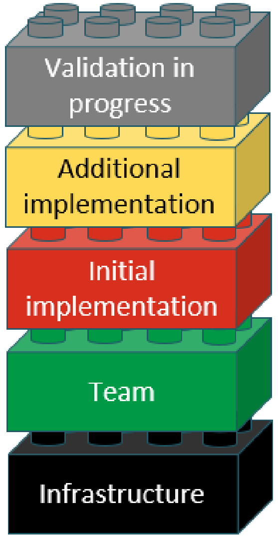
Pipeline development and testing

Validation against ISO standards

Implementation and system testing

Release

Pipeline validation at RIVM



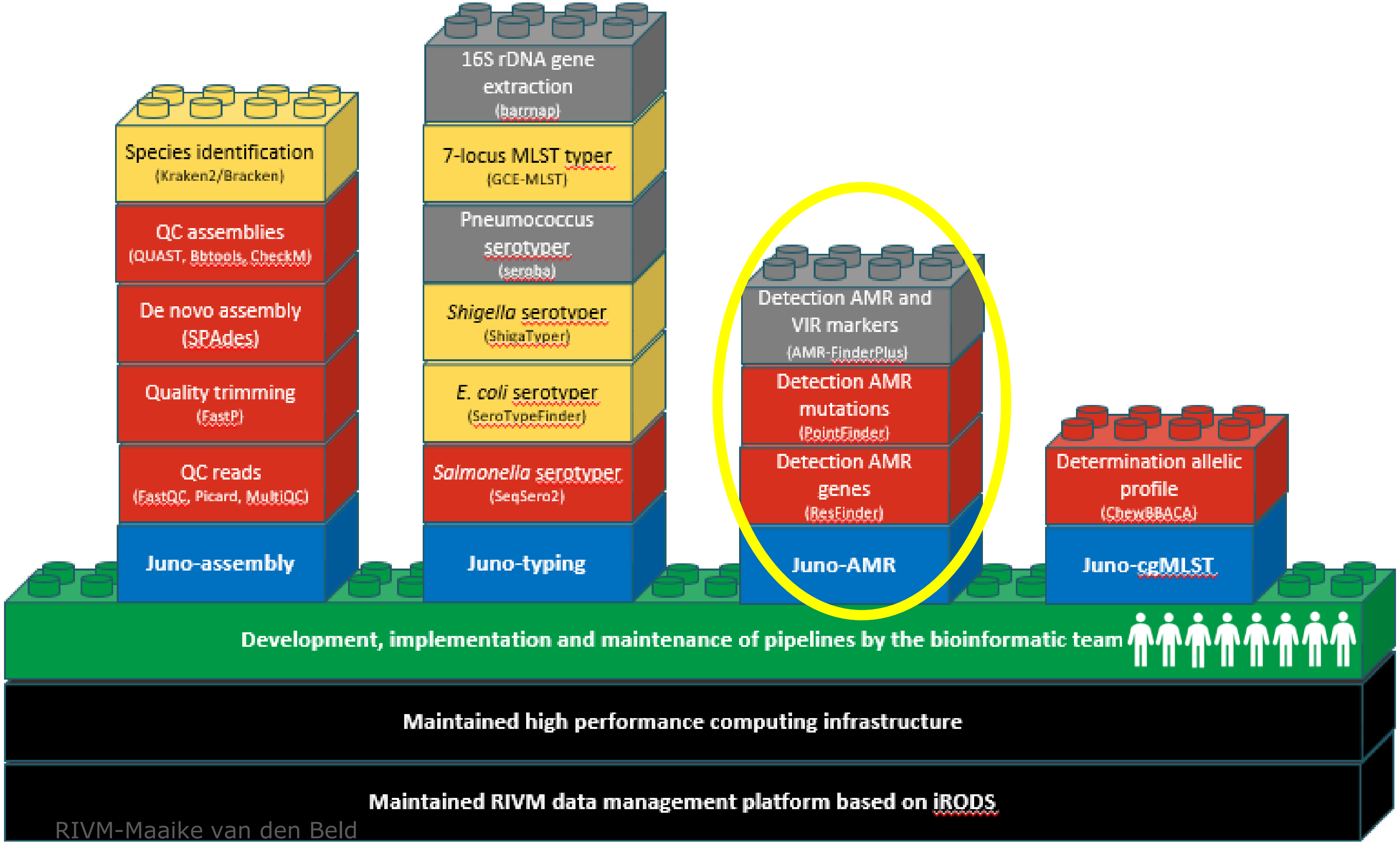
Validation in progress

Additional implementation

Initial implementation

Team

Infrastructure



Species identification (Kraken2/Bracken)

QC assemblies (QUAST, Bbtools, CheckM)

De novo assembly (SPAdes)

Quality trimming (FastP)

QC reads (FastQC, Picard, MultiQC)

Juno-assembly

16S rDNA gene extraction (barrnap)

7-locus MLST typer (GCE-MLST)

Pneumococcus serotyper (seroba)

Shigella serotyper (ShigaTyper)

E. coli serotyper (SeroTypeFinder)

Salmonella serotyper (SeqSero2)

Juno-typing

Detection AMR and VIR markers (AMR-FinderPlus)

Detection AMR mutations (PointFinder)

Detection AMR genes (ResFinder)

Juno-AMR

Determination allelic profile (ChewBBACA)

Juno-cgMLST

Development, implementation and maintenance of pipelines by the bioinformatic team

Maintained high performance computing infrastructure

Maintained RIVM data management platform based on iRODS

[redacted]

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

[redacted]

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

[redacted]

- Publication: June 2022

[redacted]

[redacted]

[redacted]

RIVM ISO 15189 validation Juno-AMR

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

- 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?

YES

NO

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

Reproducibility
Multiple generations of same sequences, compare SHA1 hashes

PASS

Accuracy
Comparison with "gold standard"

Trueness
Sequences of ATCC strains with known phenotypic resistance

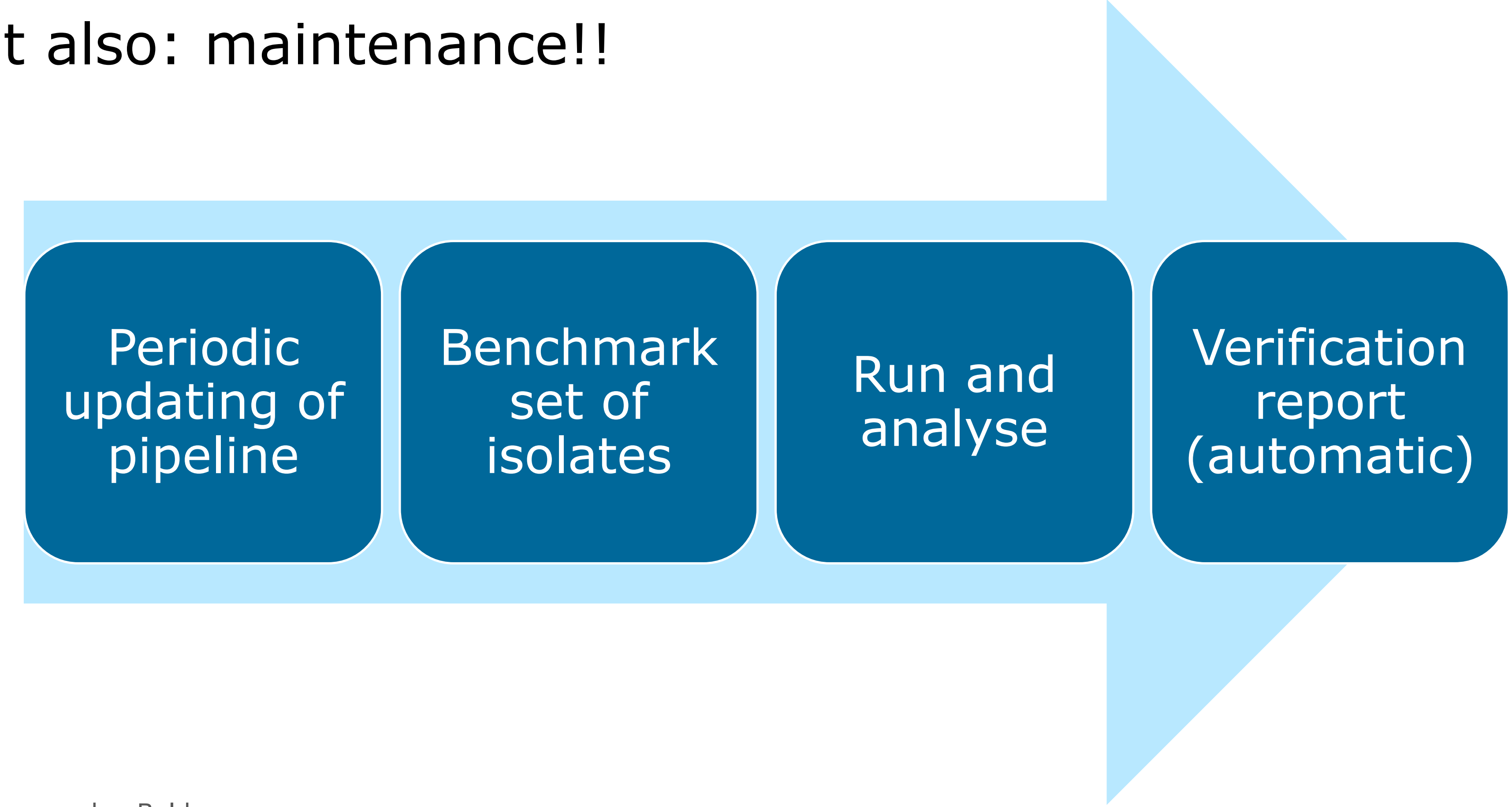
PASS

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!!



Periodic updating of pipeline

Benchmark set of isolates

Run and analyse

Verification report (automatic)

Question 5

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

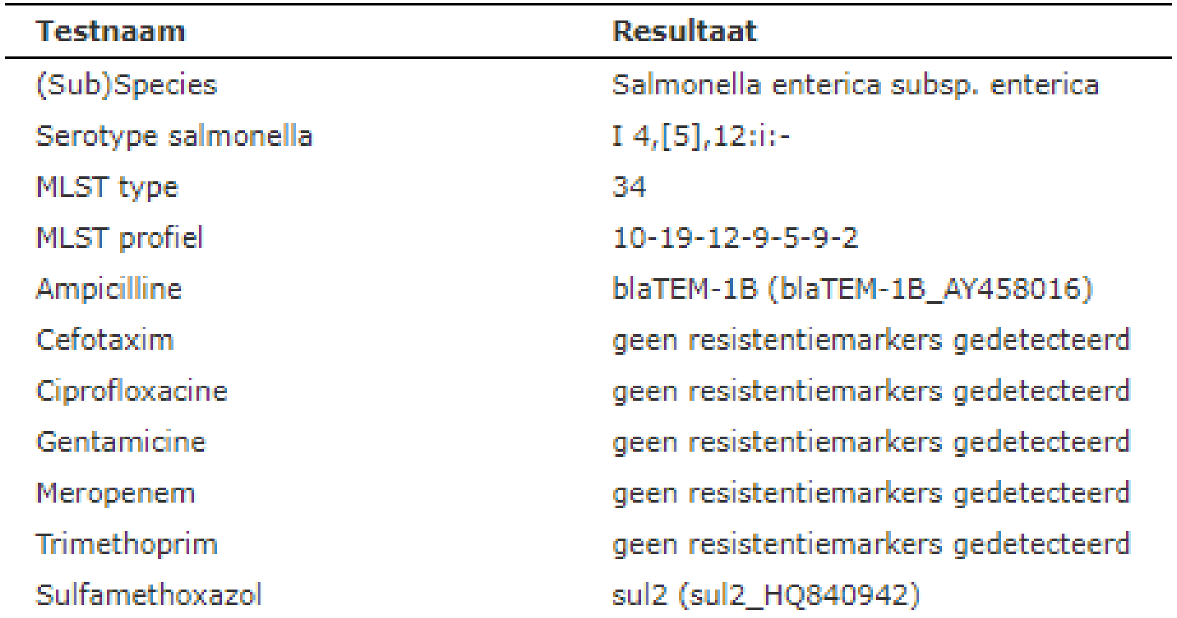
- YES
- NO

Question 6

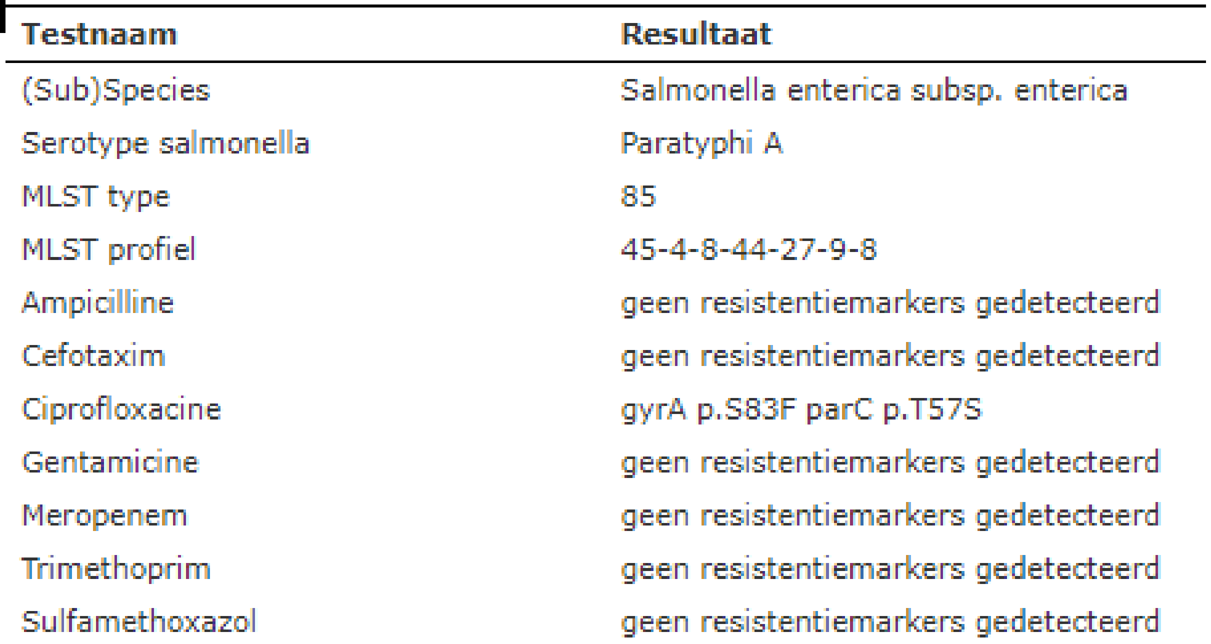
Do/would you report results of detection of genetic AMR markers?

- YES
- NO

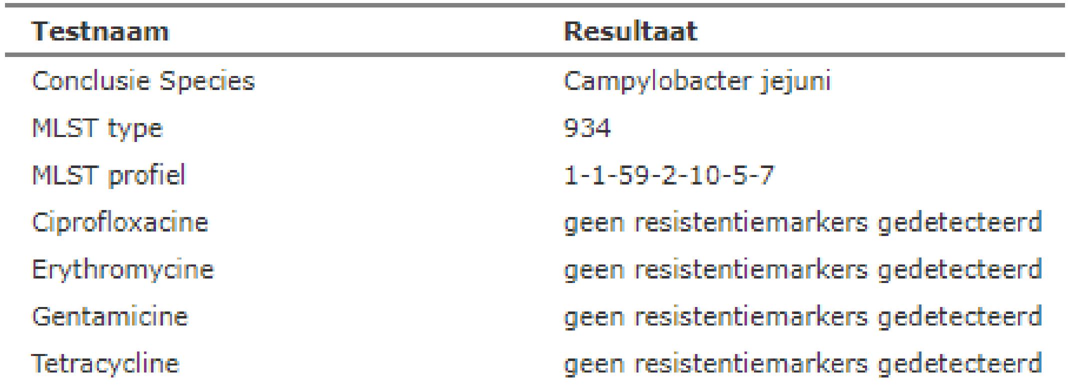
Reporting RIVM



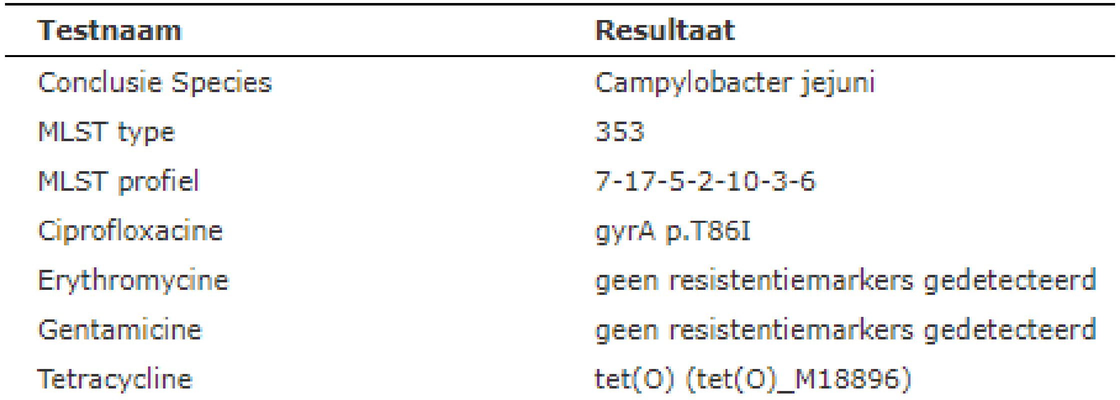
Testnaam	Resultaat
(Sub)Species	Salmonella enterica subsp. enterica
Serotype salmonella	I 4,[5],12:i:-
MLST type	34
MLST profiel	10-19-12-9-5-9-2
Ampicilline	blaTEM-1B (blaTEM-1B_AY458016)
Cefotaxim	geen resistentiemarkers gedetecteerd
Ciprofloxacine	geen resistentiemarkers gedetecteerd
Gentamicine	geen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



Testnaam	Resultaat
(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

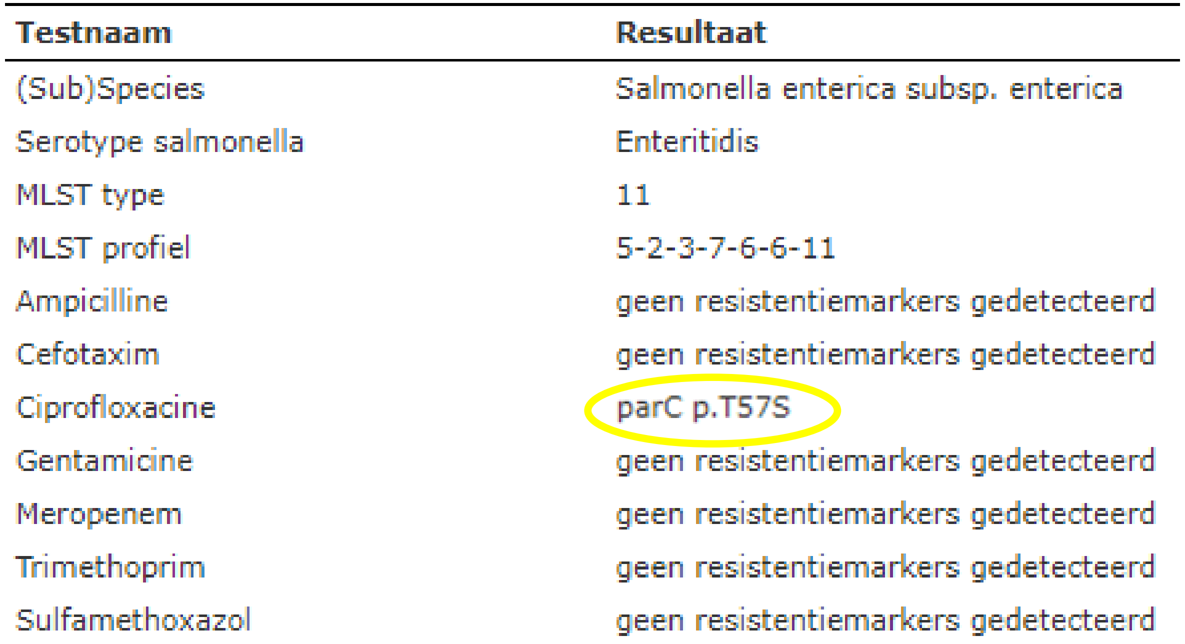


Testnaam	Resultaat
Conclusie Species	Campylobacter jejuni
MLST type	934
MLST profiel	1-1-59-2-10-5-7
Ciprofloxacine	geen resistentiemarkers gedetecteerd
Erythromycine	geen resistentiemarkers gedetecteerd
Gentamicine	geen resistentiemarkers gedetecteerd
Tetracycline	geen resistentiemarkers gedetecteerd



Testnaam	Resultaat
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?

YES

NO

Thank you for the great discussion!

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