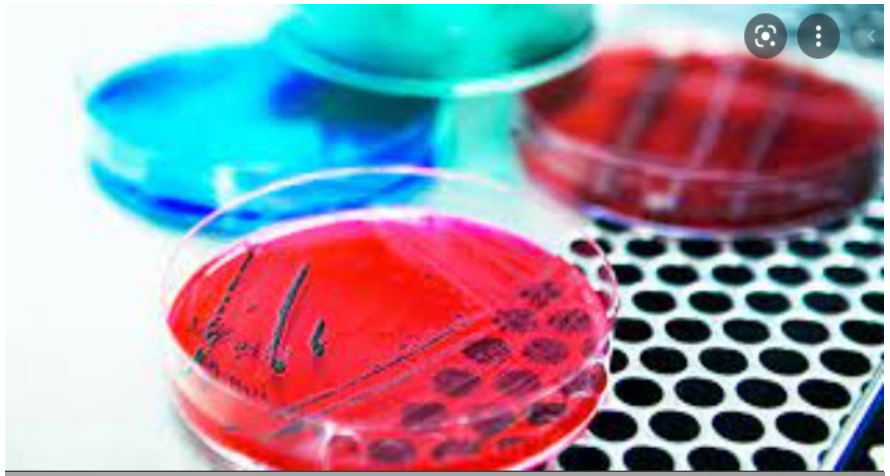
EURGen-RefLabCap Network Meeting 2021

Welcome

Thursday, 2 December 2021

09:00-12:30 CET

13:15-14:15 CET



European Antimicrobial Resistance Genes Surveillane Network Reference Laboratory Capacity (EURGen-RefLabCap)
EURGen-RefLabCap supports EU networking and capacity building with-in public health reference laboratory functions for antimicrobial resistance in priority healthcare-associated infections

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Virtual Housekeeping

Please **turn off your cameras and microphones** unless you're speaking – this will help with bandwidth and maximise audibility.

Do frequently **use the chat function** to share your views, comments and challenges. Keep the chat constructive, respectful and on topic!

If you wish to make a comment for e.g. the discussion, please use the **'Raise hand'** function.

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Meeting agenda of day 2

Thursday 2 December 2021

State-of-the-art in WGS and proposal on harmonised protocols for CRE/CCRE surveillance and outbreak detection

Time		Topic	Speaker/moderator
09:00	09:15	Good morning and re-cap of day 1	René S. Hendriksen
09:15	09:30	Highlights of existing WGS initiatives regarding CRE/CCRE	Ana Rita Rebelo
09:30	10:15	Recommendations and proposals to the harmonised protocols for molecular surveillance of CRE/CCRE and outbreak detection including questions	Ana Rita Rebelo
COFFEE BREAK (30')			

Harmonised protocols for CRE/CCRE surveillance and outbreak detection (part II)

10:50	11:00	Introduction to the break-out groups	René S. Hendriksen
11:00	11:45	Break-out groups on the recommendations and proposals to develop harmonised protocols for CRE/CCRE surveillance and outbreak detection	All
11:45	12:15	Feedback from the break-out groups	René S. Hendriksen
12:15	12:30	EURGen-RefLabCap: needs and wishes for collaboration and future exercises Thank you and goodbye	Anders Rhod Larsen
LUNCH BREAK for priority countries (45')			

Priority country workshop

13:15	13:30	Introduction to the development of the action plans for NRL capacity building	Anders Rhod Larsen
13:30	14:10	Concept of the action plan and presentation of a tool Questions	René S. Hendriksen
14:10	14:15	Where to go from here Thank you and goodbye	René S. Hendriksen

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René S. Hendriksen
rshe@food.dtu.dk

Re-cap of day 1

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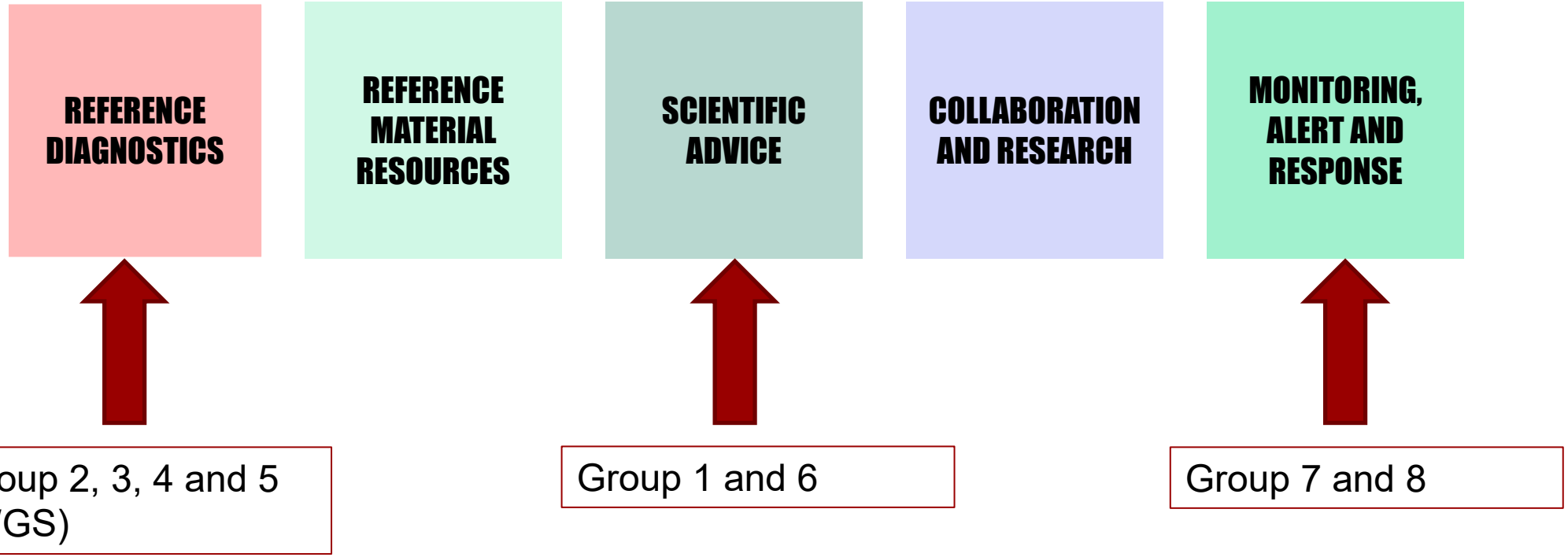
Re-cap of break-out session 1: NRL core functions



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Ana Rita Rebelo
anrire@food.dtu.dk

Highlights of existing WGS initiatives regarding CRE/CCRE

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CURRENT EUROPEAN UNION AND INTERNATIONAL GUIDANCE

2016: "ECDC Expert opinion on whole genome sequencing for public health surveillance"

2016: "ECDC roadmap for integration of molecular and genomic typing into European-level surveillance and epidemic preparedness"

2019: "ECDC strategic framework for the integration of molecular and genomic typing into European surveillance and multi-country outbreak investigations"

- Background supporting WGS as the method with higher phylogenetic resolution in outbreak investigation
- WGS potential for *in silico* prediction of AMR phenotypes and typing
- Importance of harmonising bioinformatics analysis is noted, with focus towards phylogenetic analysis
- Data storage and exchange requirements + integration of sequence data with epidemiological and clinical data
- Five strategies to ensure WGS can be implemented without compromising surveillance, outbreak investigation or risk assessments during the transition period
- Specific proposals for integrating molecular/genomic typing methods into EU-level surveillance and epidemic preparedness relating to 12 priority pathogens
- Oriented surveillance would include, analyses of high-risk clones using strategies such as cgMLST typing schemes, pMLST typing of plasmids and prediction of antimicrobial resistance genes (ARGs) through sequence analysis against reference gene databases

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CURRENT EUROPEAN UNION AND INTERNATIONAL GUIDANCE

2019: "Whole genome sequencing and metagenomics for outbreak investigation, source attribution and risk assessment of food-borne microorganisms"

- *"WGS offers, in comparison to conventional typing methodologies, a more detailed outcome and new possibilities for food-borne outbreak detection/investigation, source-attribution and hazard identification. […] The discriminatory power of WGS for pathogen characterisation is superior, compared to conventional molecular typing methods, leading to more robust case identification."*

2019: "EFSA and ECDC technical report on the collection and analysis of whole genome sequencing data from food-borne pathogens and other relevant microorganisms isolated from human, animal, food, feed and food/feed environmental samples in the joint ECDC-EFSA molecular typing database"

- Requirements of such database: grouped in data collection (submission, storage and sharing), data analysis (sequence read data quality, genome assembly, phylogenetic inference (further categorised into whole and core genome MLST, SNP analysis, k-mer-based distance estimates and comparing phylogenetic relationships), strain nomenclature and genome characterisation, general requirements and infrastructure.
- Each requirement was used to evaluate existing bioinformatics tools or initiatives, concluding that no existing tool was able to properly respect all requirements and that a combination of solutions should be considered.

2021: "EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain"

- Several recommendations can potentially be applied to public health: the suggestion to adapt a threshold of maximum 500 contigs for *de novo* assembled bacterial genomes, with a total size within 20% of deviation from the expected genome size for the respective species, and to perform detection of ARGs against at least two reference gene databases accepting a minimum of 80% identity and 70% coverage.

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CURRENT EUROPEAN UNION AND INTERNATIONAL GUIDANCE

2018: "Landscape paper on whole genome sequencing for foodborne disease surveillance"

2020: "Global Antimicrobial Resistance and Use Surveillance System (GLASS) document on whole-genome sequencing for surveillance of antimicrobial resistance"

- Essential to standardize protocols, data analysis pipelines and reporting guidelines before such an approach can be implemented at local, national or international level
- Ensuring that it is possible to compare new WGS data with older results acquired through different protocols.
- The proper infrastructure has to be developed both in terms of necessary physical locations, hardware and data management but also training of the respective professionals across the sectors of public, veterinary and human health. The organization of these sectors and communication with regulatory agencies should be reviewed.
- Legislation needs to be adapted to include acceptance of WGS results instead or in combination with the results of current methods used for surveillance and diagnostics, which also implies the creation and validation of normalized interpretative criteria for WGS results.

TBA: ISO/DIS 23418 standard "Microbiology of the food chain – Whole genome sequencing for typing and genomic characterization of foodborne bacteria – General requirements and guidance"

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CURRENT EUROPEAN UNION AND INTERNATIONAL GUIDANCE

Agreement on necessary requirements:

- prediction of clinically and epidemiologically relevant microbial phenotypes

antigenic profile, AMR and virulence, including identification of determinants encoded in the accessory genome and mobile genetic elements

- phylogenetic analysis
- well defined QC parameters
- integration of sequence data with epidemiological and clinical data
- database for the collection and analysis of WGS data + proper management

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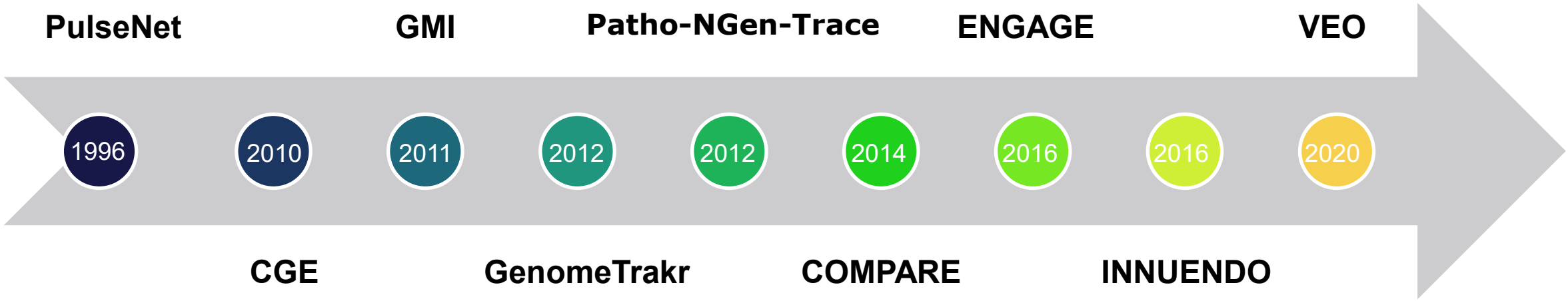
BIOINFORMATICS DEVELOPMENT INITIATIVES



CGE: Centre for Genomic Epidemiology
GMI: Global Microbial Identifier
VEO: Versatile Emerging Infectious Disease Observatory

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NETWORKS FOCUSING ON OR INCLUDING CRE/CCRE

Network	Organization	Sector	Main target audience	Type of guidance/support
EARS-Net	ECDC	🧍	National AMR surveillance laboratories and clinical microbiology laboratories	Monitoring of AMR, providing guidelines, designing recommendations
EURGen-Net	ECDC	🧍	NRLs for AMR	Monitoring of AMR, providing guidelines, designing recommendations
EpiPulse	ECDC	🧍	National PH authorities	Monitoring of AMR, providing guidelines, designing recommendations
EURL-AR	EC	🍽🐄	NRLs	Method development, laboratory and bioinformatics training, proficiency testing
EURL-VTEC	EC	🍽🐄	NRLs	Method development, laboratory and bioinformatics training, proficiency testing
ENGAGE	EC	🧍🍽🐄	All	Method development, networking, development of bioinformatics solutions, training
COMPARE	EC	🧍🍽🐄	All	Method development, networking, development of bioinformatics solutions, training, proficiency testing
VEO	EC	🧍🍽🐄	All	Method development, networking, development of bioinformatics solutions
NARMS	US CDC	🧍🍽🐄	PH laboratories and food authorities	Monitoring of AMR, providing guidelines, designing recommendations
AMD	US CDC	🧍🍽🐄	PH laboratories and food authorities	Method validation, networking, development of bioinformatics solutions
PulseNet	US CDC	🧍🍽🐄	PH laboratories and food authorities	Monitoring of AMR, providing guidelines, designing recommendations, laboratory and bioinformatics training
GenomeTrakr	US CDC	🍽🐄	PH laboratories and food authorities	Method development, networking, development of bioinformatics solutions
GMI	US CDC, DTU, FDA	🧍🍽🐄	All	Method development, networking, development of bioinformatics solutions, proficiency testing
GLASS	WHO	🧍	National AMR surveillance laboratories and clinical microbiology laboratories	Monitoring of AMR, providing guidelines, designing recommendations

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CONCLUSIONS

- Recommendations are similar regardless of setting (e.g. PH vs. food)
  - Importance of harmonization
  - Importance of data management infrastructures

- Several networks focused on training
  - Many training materials available
  - Potential to share and compare results with other national laboratories/other areas

- Standardization on the way
  - Almost agreement on QC parameters
  - Almost ISO standard (!! foodborne)
  - EURGen-RefLabCap + FWD AMR-RefLabCap

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Ana Rita Rebelo
anrire@food.dtu.dk

Recommendations and proposals to develop harmonised protocols for CRE/CCRE surveillance and outbreak detection

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RATIONALE – WHY WGS?

Advantages

- Only one protocol
- Very large amount of data
- Higher discriminatory power
- Harmonised and automatic analysis
- Direct comparison
- Ease of storage
- Retroactive screening

Why now?

- Increase in sequencing accuracy
- Decrease in cost
- Coordinated efforts throughout Europe

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RATIONALE – WHAT SHOULD WE BE ABLE TO DO?

harmonised protocols for CRE/CCRE surveillance and outbreak detection

Taxonomic analysis
Detection of AMR determinants

Serotyping
Virulence factors
Mobile genetic elements

Subtyping
Phylogenetic analysis
Integration of epidemiological data

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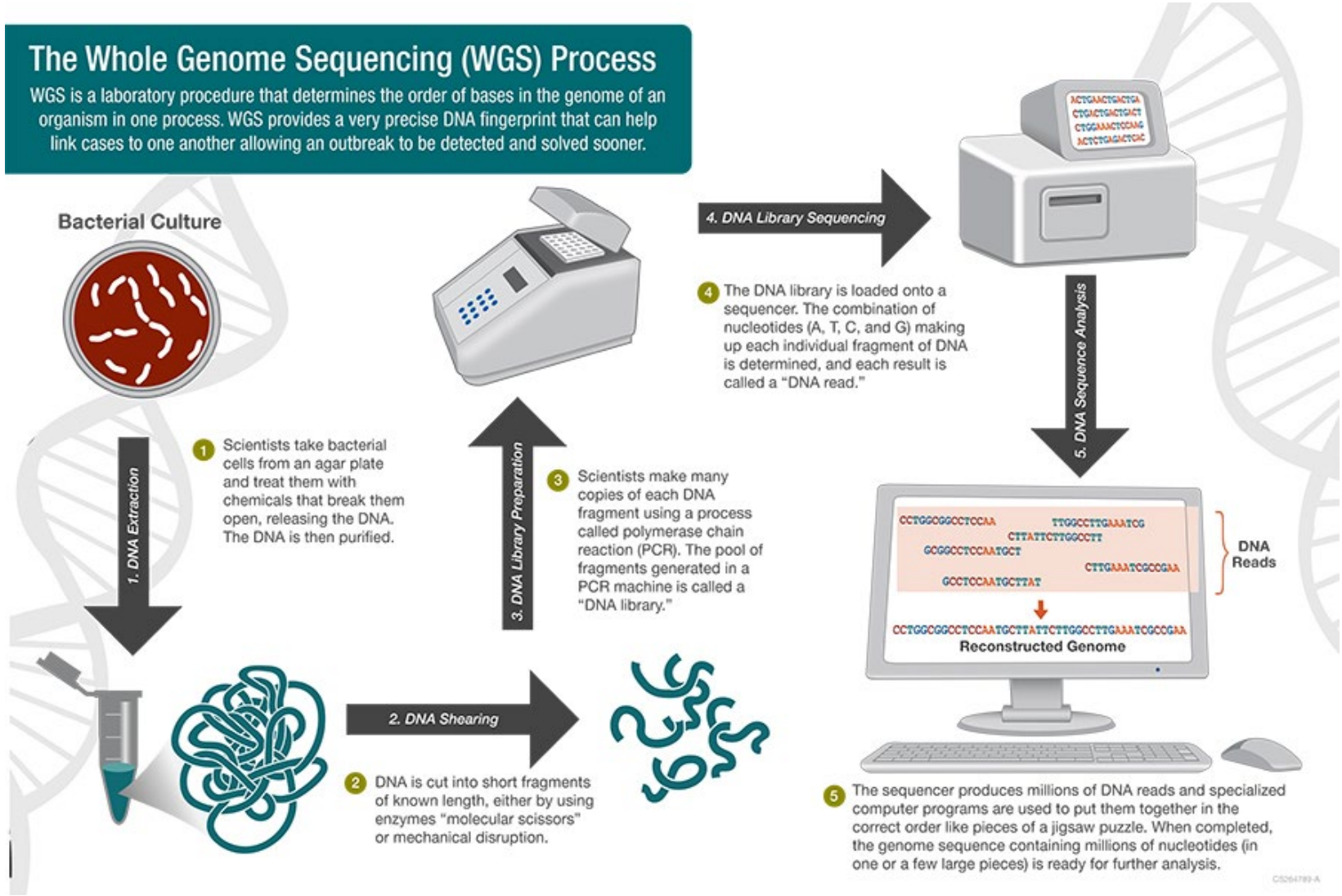
WGS-BASED ANALYSIS OF BACTERIA - OVERVIEW



https://www.cdc.gov/pulsenet/pathogens/protocol-images.html#wgs

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WGS-BASED ANALYSIS OF BACTERIA - REQUIREMENTS

- Expertise on DNA extraction methods
- Expertise on library preparation methods

Not too technically demanding
Ideally a dedicated room

- Access to sequencing platform
- Access and expertise on bioinformatics tools

Main challenges: cost, implementation

- Data management infrastructure

Main challenges: cost, compatibility

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EXAMPLE: A COMPLETE WGS WORKFLOW

Sample selection
- All

Sample prep
- Plate in blood agar
- Incubate ON
- Check for purity
- Subculture if needed

DNA extraction
- Easy DNA Kit

DNA QC
- Measure concentration and dilute with Qubit

Library preparation
- Illumina NexteraXT® DNA Library Prep

Sequencing
- Illumina NextSeq 500
- 96-192 libraries per run

Raw data QC
- FastQC on Danish Supercomputer for Lifesciences

SPN-based phylogenetic analysis
- CSI Phylogeny

Assembly
- SPAdes

Assembly QC
- FastQC
- Internal list of expected genome size

Taxonomic analysis
- KmerFinder
- rMLST

Subtyping
- MLST

AMR detection
- ResFinder

Detection of VFs and MGEs
- MobileElementFinder

+++ integrating metadata

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NOT THAT SIMPLE – TOO MANY OPTIONS

Tool	Reference database	Description and output
Tools for taxonomic analysis and typing		
KmerFinder [150,151]	KmerFinder	Provides hits of the query genome against whole reference genomes, the respective % of identity and % of coverage
SILVA [152]	SILVA	Collection of 16S rRNA genes, also possible to perform phylogenetic analysis and obtain phylogenetic trees
MLST [153]	PubMLST	MLST schemes, provides the sequence type
rMLST [126]	rMLST	rMLST schemes, provides the predicted species and respective allelic support metric
SerotypeFinder [154]	SerotypeFinder	Serotype, specific for E. coli
SeqSero [		
PneumoC		
Tools for		
cgMLST [		be used exclusively for typing but also clustering
CSIPhylog		through FastTree
Evergreen		
Tools for detection of antimicrobial resistance determinants		
ResFinder [129]	ResFinder, PointFinder	Provides hits against reference ARGs and PMs and the respective % of identity and % of coverage, position in genome and predicted phenotype
KmerResistance [150,151]	KmerResistance	Provides hits of the query genome against reference genomes, as well as the detected ARGs and respective % of identity and % of coverage
CARD/RGI [158]	CARD	Provides hits against reference ARGs and respective % of identity and % of coverage. Other options are possible and the service is highly focused on ontology and standardization
AMRFinder [159]; AMRFinderPlus [160]	NCBI RefSeq	Provides hits against reference ARGs and respective % of identity and % of coverage
ARIBA [161]	CARD, ResFinder, NCBI Bacterial AMR Reference Gene DB, ARG-ANNOT, MEGARes, PubMLST, others defined by user	Provides hits against reference ARGs and respective % of identity and % of coverage
Tools for detection of virulence factors		
VirulenceFinder [162]	VirulenceFinder	
Victors [131]	Victors	
Tools for detection and analysis of mobile genetic elements		
PlasmidFinder [163]	PlasmidFinder	
Platon [164]	Platon	
pMLST [153]	PubMLST	Plasmid typing schemes
MobileElementFinder [135]	MobileElementFinder	Provides type and reference sequences of MGEs, respective % of identity and % of coverage, as well as associated ARGs and VFs
Pipelines for extensive analyses		
NCBI Pathogen Detection	NCBI DBs	Detects ARGs and VFs, provides SNP-based phylogenetic analysis
Pathogenwatch [165]	Pathogenwatch, tools' DBs	Performs taxonomic analysis, determines MLST and cgMLST and provides cgMLST-based phylogenetic clustering
BIGSdb [166]	PubMLST BIGSdb	Performs annotation and taxonomic analysis, detects ARGs and plasmids, determines MLST, rMLST and cgMLST, provides phylogenetic and spatio-phylogenetic analysis
PATRIC [167]	PATRIC, but also includes others such as CARD, NDARO and VFDV	Performs assemblies, quality control, annotation and taxonomic analysis, detects ARGs and performs phenotype prediction, detects VFs and MGEs, provides phylogenetic analysis, variation analysis and genome alignments

This is just a subset….

….and just from tools for analysis .

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RECOMMENDATIONS FOR HARMONIZATION

i. Accounting for future priority pathogens and priority AMR profiles for surveillance.

ii. Choose well defined AMR genotypes for validation of the WGS approach.

iii. Establish the control parameters to be used.

iv. Establish the thresholds for the control parameters.

v. Define a set of bioinformatics tools and databases as potential candidates to be included in the harmonised approach.

vi. Consider the results of other tasks incurred in the context of this project.

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RECOMMENDATION 1

Accounting for future priority pathogens and priority AMR profiles for surveillance.

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RECOMMENDATION 1

MAIN OBJECTIVE: the harmonised WGS approach is streamlined for surveillance and analyses of CRE/CCRE bacteria

BUT: should already account for the future integration of two additional epidemic-prone healthcare-associated antimicrobial-resistant bacterial pathogens of public health priority

BECAUSE: current European guidelines and Commission Implementing Decisions might at any time be reviewed if new epidemiological situations become established, changing the scope of organisms under surveillance

AND: we should avoid working in silos

EXAMPLES:

- selection of ARG databases that contain ARGs and point mutations for other microorganisms besides CRE/CCRE
- defining QC parameters at are not exclusive to *Enterobacterales*

  establishing the acceptable genome size deviation as a percentage of the expected genome size, and not as a numerical deviation from the expected 5 million base-pairs.

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RECOMMENDATION 2

Choose well defined AMR genotypes for validation of the WGS approach.

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RECOMMENDATION 2

MAIN OBJECTIVE: Predict AMR phenotypes (which are often regulated by a combination of several genotypic determinants)

BUT: many of the genetic mechanisms are currently not well defined and prove difficult to be detected through WGS approaches

SOLUTION: Defining a subset of well-studied ARGs and point mutations allows for comparison of sampling methods, laboratory protocols and bioinformatics workflows

EXAMPLE: Adequately dectecting *mcr*-genes in colistin-resistant *Enterobacterales* could correspond to the benchmark indicating an adequate approach, even knowing that other mechanisms of resistance (e.g. PMs) exist

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RECOMMENDATION 3 AND RECOMMENDATION 4

Establish the control parameters to be used.

Establish the thresholds for the control parameters.

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Recommendation 3 and Recommendation 4

Many different:

- DNA extraction kits
- Sequencing platforms
- Bioinformatics approaches
- Bioinformatics tools

Is harmonization feasible?

Well defined set of QC parameters

- For the raw data

  E.g. nr. and length of raw reads, depth of coverage

- For the assembled genomes

  E.g. N50, nr. of contigs, genome size

- For the performance of the tools

  E.g. accurately detect PMs and ARGs in sets of benchmarking data

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RECOMMENDATION 3 AND RECOMMENDATION 4

We propose two sets of QC parameters that will allow the users to maintain flexibility in their choice of WGS platform and bioinformatics tools:

- Sequencing QC parameters
- Data management QC parameters

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RECOMMENDATION 3 AND RECOMMENDATION 4

Sequencing QC parameters:

any sequencing platform, protocol and bioinformatics tool

↓

raw data and assembled genomes with quality equal or above limited thresholds for defined control parameters

Current general consensus:

- "number and length of raw reads"
- "depth of coverage"
- "number of contigs in the assembled genome"
- "N50 and deviation from expected genome size"

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RECOMMENDATION 3 AND RECOMMENDATION 4

Sequencing QC parameters:

- the depth of coverage (both of the raw data and also of the assembled genome) should be at least of 30 times (30X)

  15X has proven sufficient to an adequate detection of ARGs and point mutations in E. coli

- the number of contigs in the assembled genome must be lower than 1,000, and ideally lower than 500

  short-read technologies

- the size of the assembled genome is dependent on the target species. To account for genome plasticity and mobile genetic elements we suggest that a maximum threshold of 10% of variation in the number of base-pairs (BPs) should be adopted.

  CRE/CCRE: genome size 5 million BPs = a variation of plus/minus 0.5 million BPs would be acceptable

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RECOMMENDATION 3 AND RECOMMENDATION 4

Data management QC parameters:

Ensure that all workflows respect the same data management directions and there is:

-traceability of data and methods

-compatibility of data types and formats between different bioinformatics approaches

-comparability of results between settings

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RECOMMENDATION 3 AND RECOMMENDATION 4

Data management QC parameters:

Parameters:

?

ISO standard will soon become available and help guide us

Examples from ISO draft:

- defining the minimum metadata requirements and respective adequate descriptors
- describing the proper registry methods for the DNA extraction and sequencing protocols, WGS platforms and bioinformatics analyses.

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BENCHMARKING DATASETS

Data management QC parameters:

--upcoming ISO--

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BENCHMARKING DATASETS

European Commission's Joint Research Centre

2018: "The challenges of designing a benchmark strategy for bioinformatics pipelines in the identification of antimicrobial resistance determinants using next generation sequencing technologies"

2021: "A roadmap for the generation of benchmarking resources for antimicrobial resistance detection using next generation sequencing"

- Benchmarking approaches to validate sequencing and bioinformatics workflows
- Ensure that different pipelines can be used while at the same time adhering to the same minimum standards of performance

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BENCHMARKING DATASETS

HOW?

- creating platform-specific validation datasets
- using simulated data that complies with specific certifications (not biases towards the creating platform)
- accepting the *fastq* format as the standardized input for analysing the performance of bioinformatics tools
- **accuracy should be dependent on the ability of the workflow to identify the correct AMR determinants that are introduced in the dataset (and not on agreement with phenotypic results)**
- these AMR determinants should include species and mechanisms which are selected based on international priority lists

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RECOMMENDATION 5

Define a set of bioinformatics tools and databases as potential candidates to be included in the harmonised approach.

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BIOINFORMATICS TOOLS - OVERVIEW

Purpose

Quality control
Assembly
Taxonomic analysis
Phylogeny
Serotyping/Subtyping
Detection of AMR determinants
Detection of other determinants

Accessibility

Web-based
Command line
Local vs. server

Maintenance

Benchmarked
Curated

Data

Raw data as input
Assemblies as input
Integration of metadata

Cost

Open access
Subscription

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EXAMPLE: BIOINFORMATICS TOOLS FOR PREDICTION OF AMR

Tool	Target species	Reference database	Output	Comments
SRST2	All	CARD, PubMLST, or others defined by user	Reference sequences and respective % of coverage, depth	Also taxonomy, phylogeny, VFs, plasmids, other, depending on provided databases
ARIBA	All	CARD, ResFinder, ARG-ANNOT, MEGARes, NCBI Bacterial AMR Reference Gene Database, PubMLST, or others defined by user	Reference sequences and respective % of identity, % of coverage	Also phylogeny, VFs and plasmids, depending on provided databases (such as plasmidfinder, VFDB, VirulenceFinder)
KmerResistance	All	Own	Reference genomes, ARGs and respective % of identity, % of coverage	Also taxonomy
ResFinder	All	Own	ARGs and respective % of identity, % of coverage, position in genome, predicted phenotype	NA
PointFinder	Limited	Own	Mutated gene, protein translation, predicted phenotype	Included in ResFinder but can be used locally by itself. Currently under development for Klebsiella spp.
RGI	All	CARD	Reference sequences and respective % of identity, % of coverage, other options	Integrated in the Galaxy server; allows proteome analysis
AMRFinder; AMRFinderPlus	All (Limited PMs)	NCBI RefSeq	Reference sequences and respective % of identity, % of coverage	Included in NCBI Pathogen Detection
SSTAR	All	Own (created by merging ResFinder and ARG-ANNOT)	Reference sequences and respective % of coverage, depth	Can be used with other reference databases
ABRicate	All	CARD, ResFinder, ARG-ANNOT, MEGARES, NCBI AMRFinderPlus, or others defined by user	Reference sequences and respective % of identity, % of coverage	Also VFs and plasmids, depending on provided databases Also VFs and plasmids, depending on provided databases (such as plasmidfinder and VFDB)
CARD	All	Own	ARGs and point mutations, respective prevalence and predicted phenotype	Highly focused on ontology and standardization. VFs and mobile genetic elements currently being added

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EXAMPLE: BIOINFORMATICS PIPELINES FOR PREDICTION OF AMR

Tool	Target species	Reference database	Output
Pathogenwatch	Limited	Own, tools' databases	Taxonomy, MLST, cgMLST and clustering. Other functionalities for Klebsiella spp. derived from Kleborate
Enterobase	Limited	Tools' databases	Genome assembly and annotation, serotyping, MLST, cgMLST, rMLST, phylogenetic analysis
BIGSdb	All	PubMLST BIGSdb	Annotation, taxonomy, ARGs, plasmids, MLST, rMLST, cgMLST, phylogenetic and spatio-phylogenetic analysis, comparative genomics
NCBI Pathogen Detection	Limited	Own	ARGs, VFs, SNP-based phylogenetic analysis
PATRIC	All	Own, but also includes others such as CARD, NDARO and VFDV	Assemblies, QC, annotation, taxonomy, ARGs, phenotype prediction, VFs, mobile elements, phylogenetic analysis, variation analysis, genome alignments, comparative genomics, other options
Ridom SeqSphere+ €	All	Own, tools' databases; Includes NCBI AMRFinder and VFDB	Assemblies, QC, taxonomy, ARGs, VFs, MLST, cgMLST, phylogenetic analysis
Bionumerics €	All (wgMLST schemes available for limited species)	Own, tools' databases, others provided by user	Assemblies, QC, annotation, taxonomy, ARGs, plasmids, MLST, rMLST, cgMLST, wgMLST, phylogenetic analysis, comparative genomics, other options for E. coli (ARGs, PMs, VFs, plasmids, serotypes)

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RECOMMENDATION 5

- should be **open-access**, in order to respect probable budget limitations of certain users
- should be available as **online interfaces** (or be part of other interfaces) to avoid the need for expensive computing resources and specific professionals
- should also be **downloadable for local** usage for users with the resources and interest in doing so
- should be **benchmarked**, and should be transparently and continuously **curated**
- The owners should be available for **collaboration** with the European Commission, ECDC, and this provider to facilitate implementation and testing, and to potentially coordinate events such as suspension of updates during External Quality Assessments, and to provide scientific and technical support to the users

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RECOMMENDATION 6

Consider the results of other tasks incurred in the context of this project.

- WGS approaches currently used by the NRLs
- minimum conflict as possible with the ones currently used

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SOME CONSIDERATIONS

There's no one-size-fits-all approach
There's no perfect bioinformatics tool

Essential requirements:

QC
Benchmarking datasets → Ensuring accuracy of your approach

Knowing which database supports each tool
Understanding how the tool works → Knowing what data to provide
Knowing the limitations of your results

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Discussion and Questions

Input from NRLs with established WGS workflows

Positive and negative feedback regarding their specific approaches

Challenges so far

Input from NRLs with no-established systems

What part of the whole WGS workflow seems more demanding?

Specific fears (e.g. command-line seems too difficult)

To be discussed in break-out session → Very important comments now

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anrire@food.dtu.dk

Thank you!

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Coffee break

Back at 10:50.

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René S. Hendriksen
rshe@food.dtu.dk

Introduction to the break-out groups on the recommendations and proposals to develop harmonised protocols for CRE/CCRE surveillance and outbreak detection

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Purpose of the discussion

- To give the participants an opportunity to interact, inspire each other to address challenges and share best practices
  - important to identify common challenges and solutions and share good practice between the participating countries
    - divided into groups and assigned specific topics
    - each groups has an assigned moderator (chair), who will facilitate the active contribution of all participants in the group and clarify questions, if needed
    - each group has been assigned a rapporteur, who will summarize the key discussion item and present these in plenary
- The discussion within each group will be captured in a written summary, which will be shared with the EURGen-RefLabCap network after the meeting
  - to identify common challenges and solutions and share good practice between the participating countries

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Groups for break-out sessions

	Countries	Reporters (from the national coordinators' group)	Moderators (from the contractors' group)
Group 1	---	---	Jette Kjeldgaard
Group 2	---	---	Ana Rita Rebelo
Group 3	---	---	Camilla Wiuff Coia
Group 4	---	---	Berit Müller-Pebody
Group 5	---	---	René S. Hendriksen
Group 6	---	---	Anders Rhod Larsen
Group 7	---	---	Valeria Bortolaia
Group 8	---	---	Lina Cavaco

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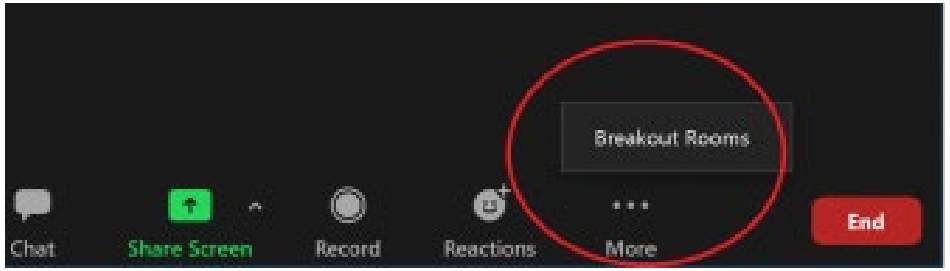
Break-out session 2



Please join your respective break-out session room now.

The session ends at 11:45.

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René S. Hendriksen
rshe@food.dtu.dk

Feedback from the break-out groups

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Questions for group 2 and 5

1. What have been the main challenges preventing the implementation of WGS in your NRL?

For example: lack of scientific evidence supporting the replacement of routine methods with WGS; national legislation or policies demanding specific surveillance protocols; national legislation or policies demanding that harmonized protocols are used throughout the country, etc.

2. What is the most important step to begin implementation of WGS-based analysis and surveillance in your NRL?

For example: organize coordination with other laboratories or agencies which own sequencing platforms; secure budget to buy a sequencing platform; ensure that personnel in your laboratory is trained in WGS and/or bioinformatics analysis, etc.

3. When WGS is implemented in your laboratory, which specific aim will you be more interested in developing?

For example: performing WGS-based typing; performing WGS-based phylogenetic analysis if there are suspicions of an outbreak; developing a national monitoring and alert system based on WGS data and automated bioinformatics pipelines, etc.

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Questions for group 1, 3 and 4

1. What specific aims do you routinely use WGS for?

For example: performing WGS-based typing; performing WGS-based phylogenetic analysis if there are suspicions of an outbreak; developing a national monitoring and alert system based on WGS data and automated bioinformatics pipelines, etc.

2. What have been the challenges to using WGS-based analysis for other purposes, and which specific aim are you most interested in developing further?

For example: you already perform in silico typing of CRE/CCRE but do not use WGS data for outbreak investigations because you lack the computing power to perform large-scale phylogenetic analysis; you perform WGS-based monitoring of carbapenemases with a web-based bioinformatics tool but would like that the results are automatically integrated into a national database; etc.

3. Have you identified any important weaknesses in the bioinformatics tools you currently use for outbreak analysis, AMR detection, bacterial typing and phylogenetic analysis? Can you envision how being part of the EURGen-RefLabCap network can help your laboratory to overcome those weaknesses?

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Questions for group 6, 7 and 8

1. Have you identified any important weaknesses in the bioinformatics tools you currently use for outbreak analysis, AMR detection, bacterial typing and phylogenetic analysis? Can you envision how being part of the EURGen-RefLabCap network can help your laboratory to overcome those weaknesses?

2. Do you think any of the steps in your WGS protocol and bioinformatics pipeline should be incorporated in the harmonized protocol being developed by the EURGen-RefLabCap project? If yes, please elaborate on which steps and why.

3. What is the most challenging part of your WGS protocol and bioinformatics pipeline?

For example: reliance on web-based bioinformatics interfaces, which increases the time necessary to analyze several isolates; not having access to enough computing power to be able to perform batch analysis of hundreds/thousands of isolates simultaneously; lack of long-term data storage solutions, etc.

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Anders Rhod Larsen
arl@ssi.dk

Needs and wishes for collaboration and future exercises

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Training plan including activities for all network members

- Physical technical training workshop on WGS based AMR detection
- Virtual multidisciplinary training to resolve a CCRE outbreak scenario using simulation exercises
- Webinars on scientific topics of interest and/or country presentations on CCRE
- Others….

- Finalized **June 2022**

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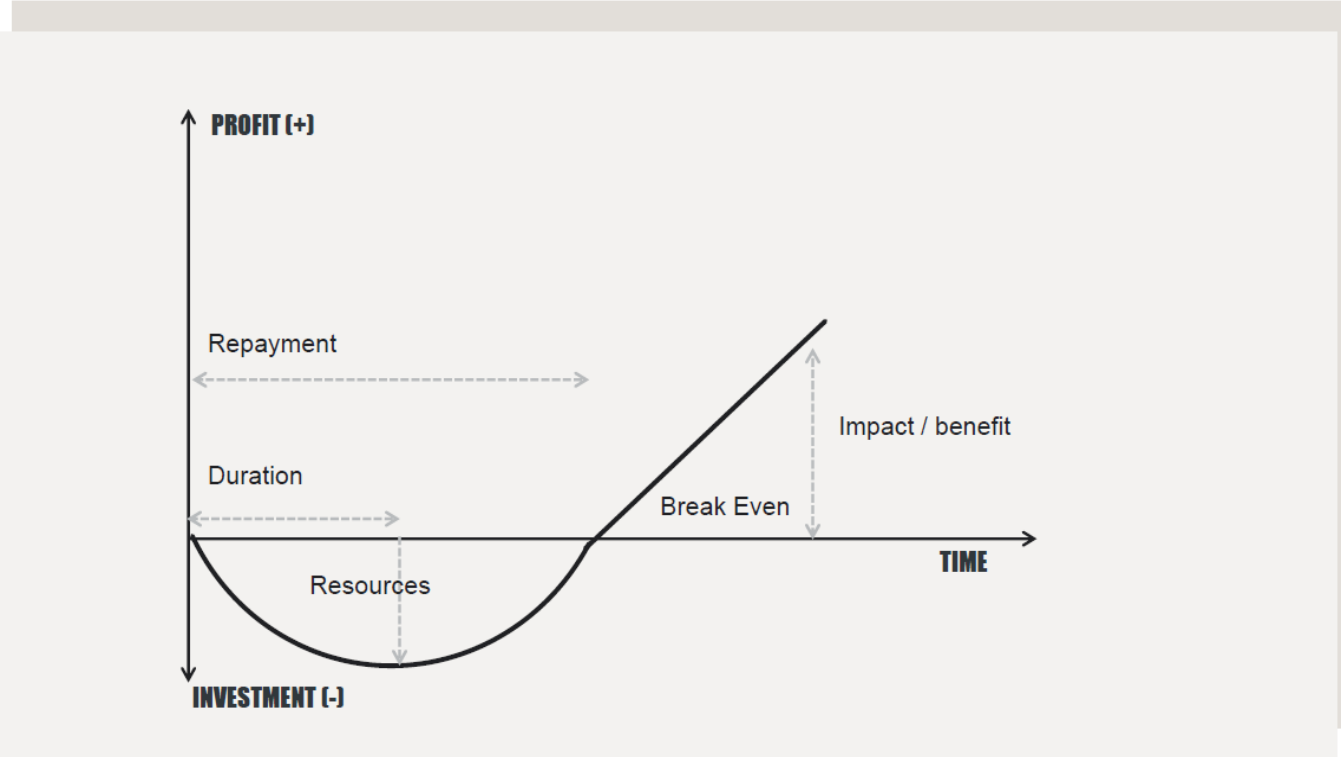
Inspiration from the break out sessions Yesterday



- How to convince stakeholders/ funds to invest in WGS/ infrastruture?
- Make common business case?- include as training and exercise?

WGS benefits Grp 2,3,4,5

1. Please outline a strategic aim to the provision of WGS-based reference laboratory services.

- Describe “WHY” we need to implement WGS at the NRL.

1. Improve quality of the ref. testing which will feed into the strengthening surveillance and outbreak detection.
2. Investigation of AMR mechanisms, characterization and infection control/ AMR
3. Better and timely (early warning sys/ prediction of the next outbreak clone) identification of outbreak and AMR
4. Better tracing of transmission routes to improve control at hospital outbreaks (investigation of the origin of the outbreak)
5. One technology for all markers but beyond also AMR and for other purposes e.g. virus (multi-functional approach)
6. Standardization and sharing of data for PH purposes

WGS challenges

1. Funding is a huge issue for implementation
2. Lack of skilled staff for WGS
3. Need for re-training of current staff for molecular biology
4. A need to understand the technology (bioinformatics) and principles (the steps)
   – Expert knowledge is needed
5. Lack of explanation/ interpretation (e.g. when is a phylo-tree correct) - what does the data means
6. **Need to have epi and bioinformaticians to work together**

<u>The desired output and impacts</u>

- The approach must provide a full overview of the national/local situation, meaning that the **results from WGS analysis need to be integrated with epidemiological and clinical data** → this was the most important point.

How can we adress this issue in exercises/ best prectice examples?

One size doesn´t fit all

– Grp.8, Grp.6

Endemic situation vs low prevalence, size of network and complexity very different

- Differences in number of submitted isolates
- Differences in lab-work procedures, outbreak investigations, communication etc?

Governance/ coordination

- **GROUP 7**
- strengthen coordination at all levels, professionals, health centres like hospitals, but also between health institutions
- The main obstacles are related to the outbreaks being officially reported, some of therm are studied and managed within health centres without official communication to health authorities, and others are not studied in depth. Much depends on the individual implication of professionals and managers of health centres.

- Specific mapping of stakeholders and dataflow to propose more agilent coordination of
  - Surveillance
  - Outbreak investigations

EURGen-RefLabCap@food.dtu.dk

Thank you on behalf of the EURGen-RefLabCap team

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Lunch break

Priority Countries back at 13:15.

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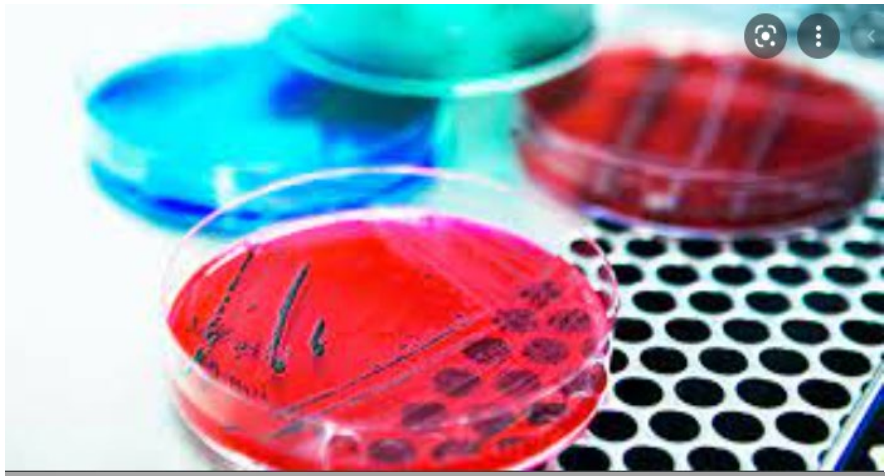
EURGen-RefLabCap Network Meeting 2021

Priority country workshop

Thursday, 2 December 2021

13:15-14:15 CET



European Antimicrobial Resistance Genes Surveillane Network Reference Laboratory Capacity (EURGen-RefLabCap)
EURGen-RefLabCap supports EU networking and capacity building with-in public health reference laboratory functions for antimicrobial resistance in priority healthcare-associated infections

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Anders Rhod Larsen
arl@ssi.dk

Introduction to the development of the action plans for NRL capacity building

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René S. Hendriksen
rshe@food.dtu.dk

Concept of the action plan and presentation of a tool inc. questions

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Purpose of the action plan

To strengthen laboratories' capabilities and capacities to detect and effectively prevent and control spread of CRE and CCRE in healthcare settings at local and national level, across Europe and globally

- Focus on the five core NRL fuctions

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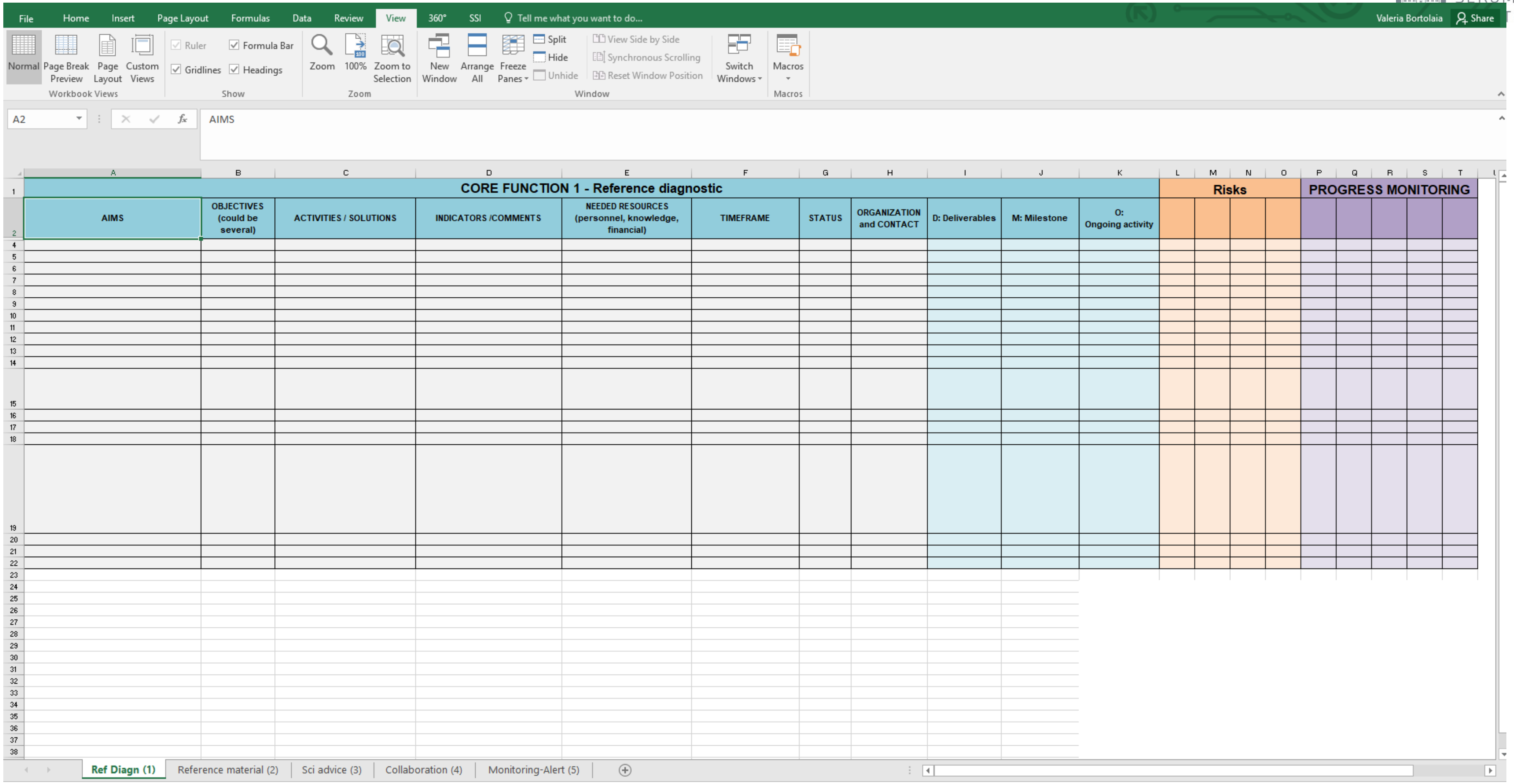

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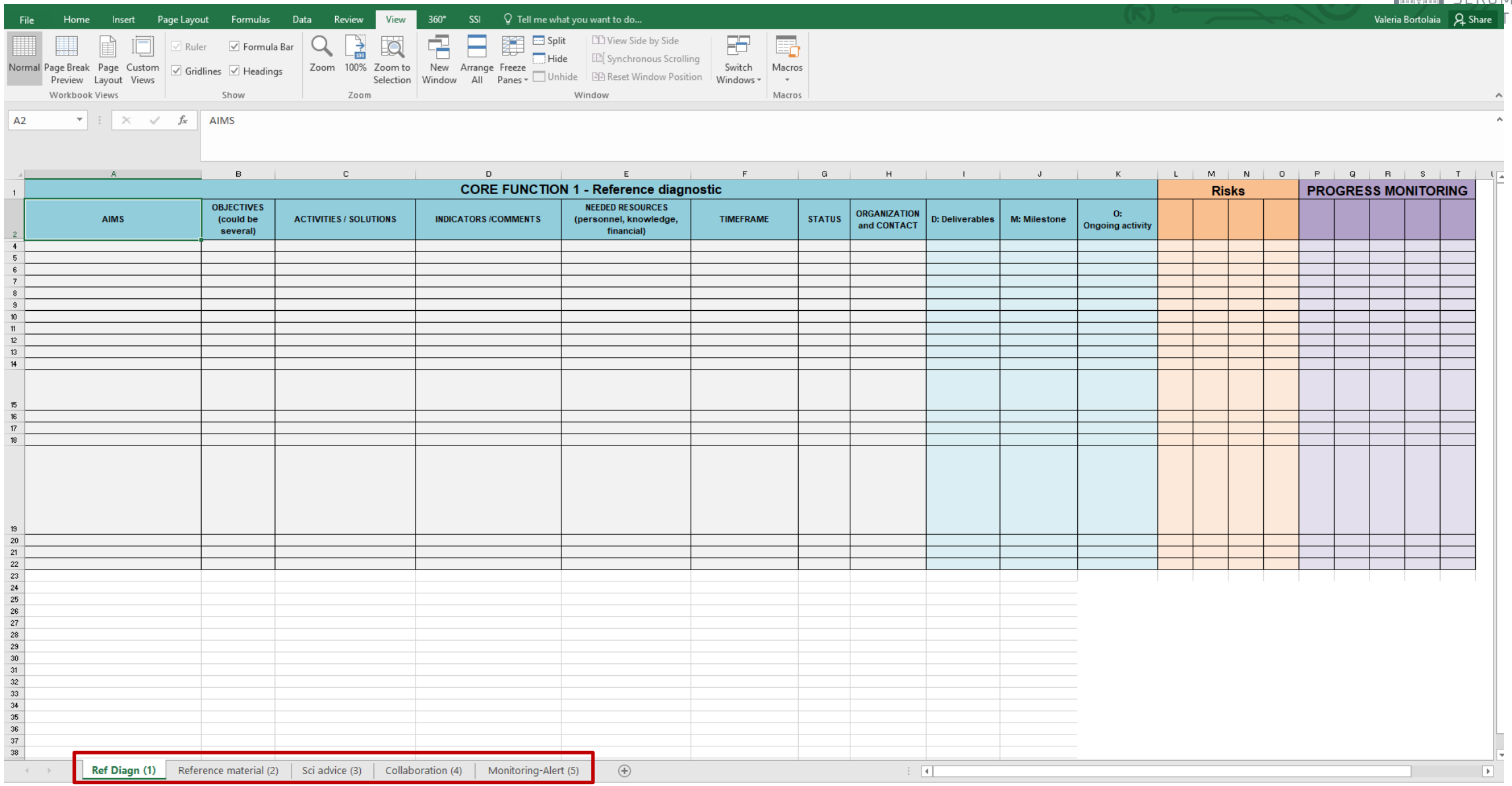

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A2 AIMS

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	CORE FUNCTION 1 - Reference diagnostic											Risks				PROGRESS MONITORING				
2	AIMS	OBJECTIVES (could be several)	ACTIVITIES / SOLUTIONS	INDICATORS /COMMENTS	NEEDED RESOURCES (personnel, knowledge, financial)	TIMEFRAME	STATUS	ORGANIZATION and CONTACT	D: Deliverables	M: Milestone	O: Ongoing activity									
4–18																				
19																				
20–22																				

Ref Diagn (1) | Reference material (2) | Sci advice (3) | Collaboration (4) | Monitoring-Alert (5)

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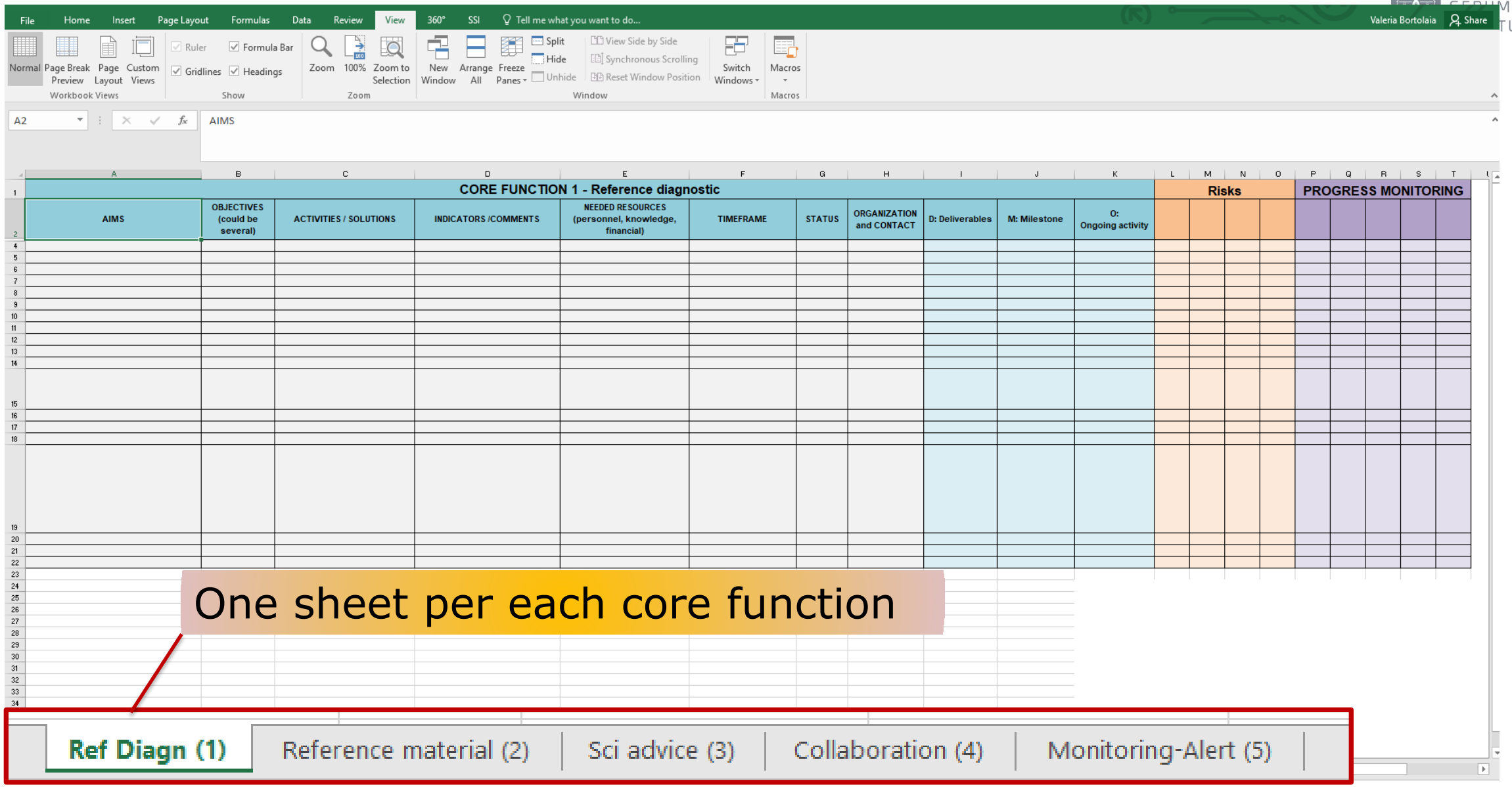

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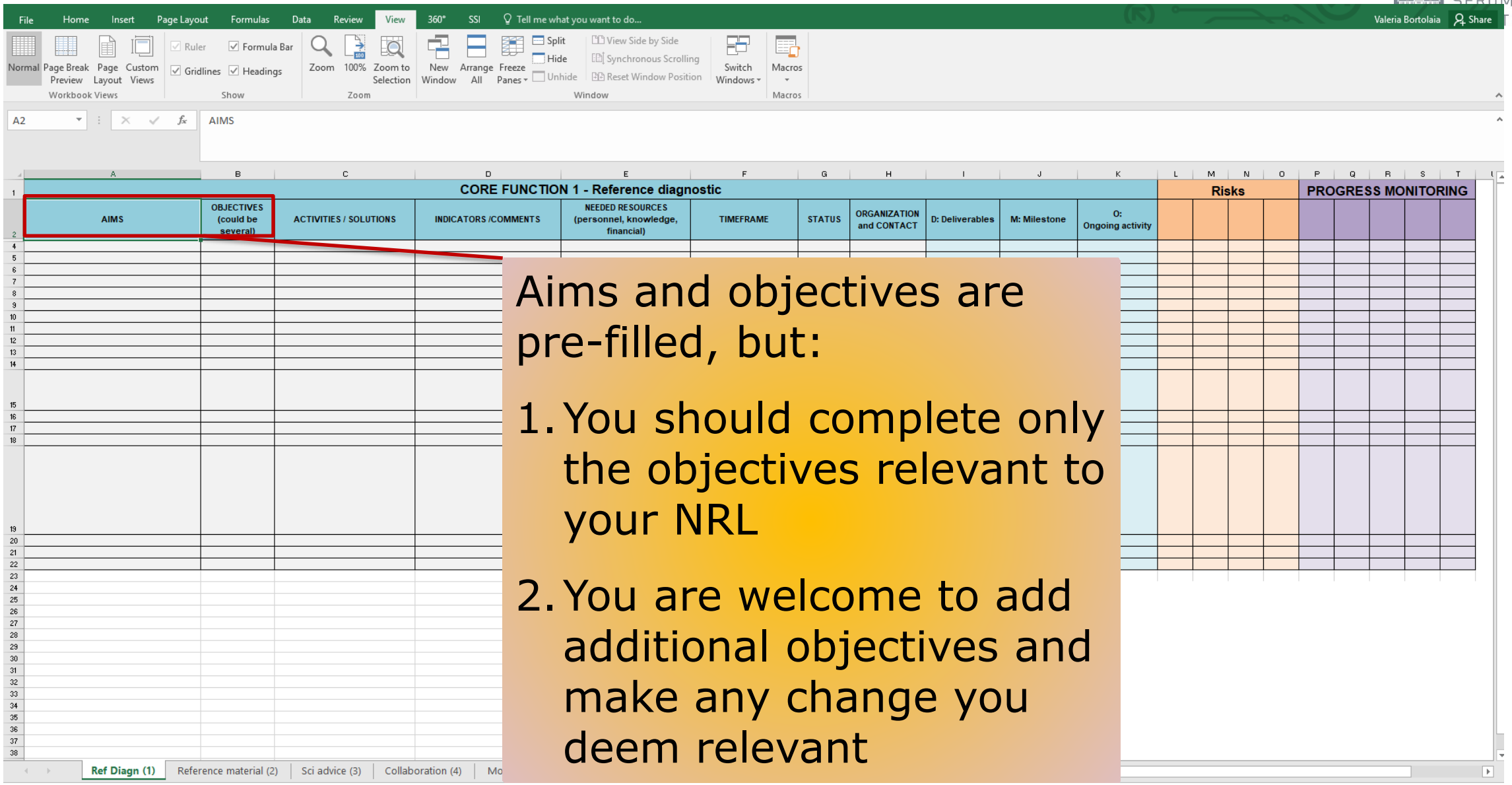

Aims and objectives are pre-filled, but:

1. You should complete only the objectives relevant to your NRL
2. You are welcome to add additional objectives and make any change you deem relevant

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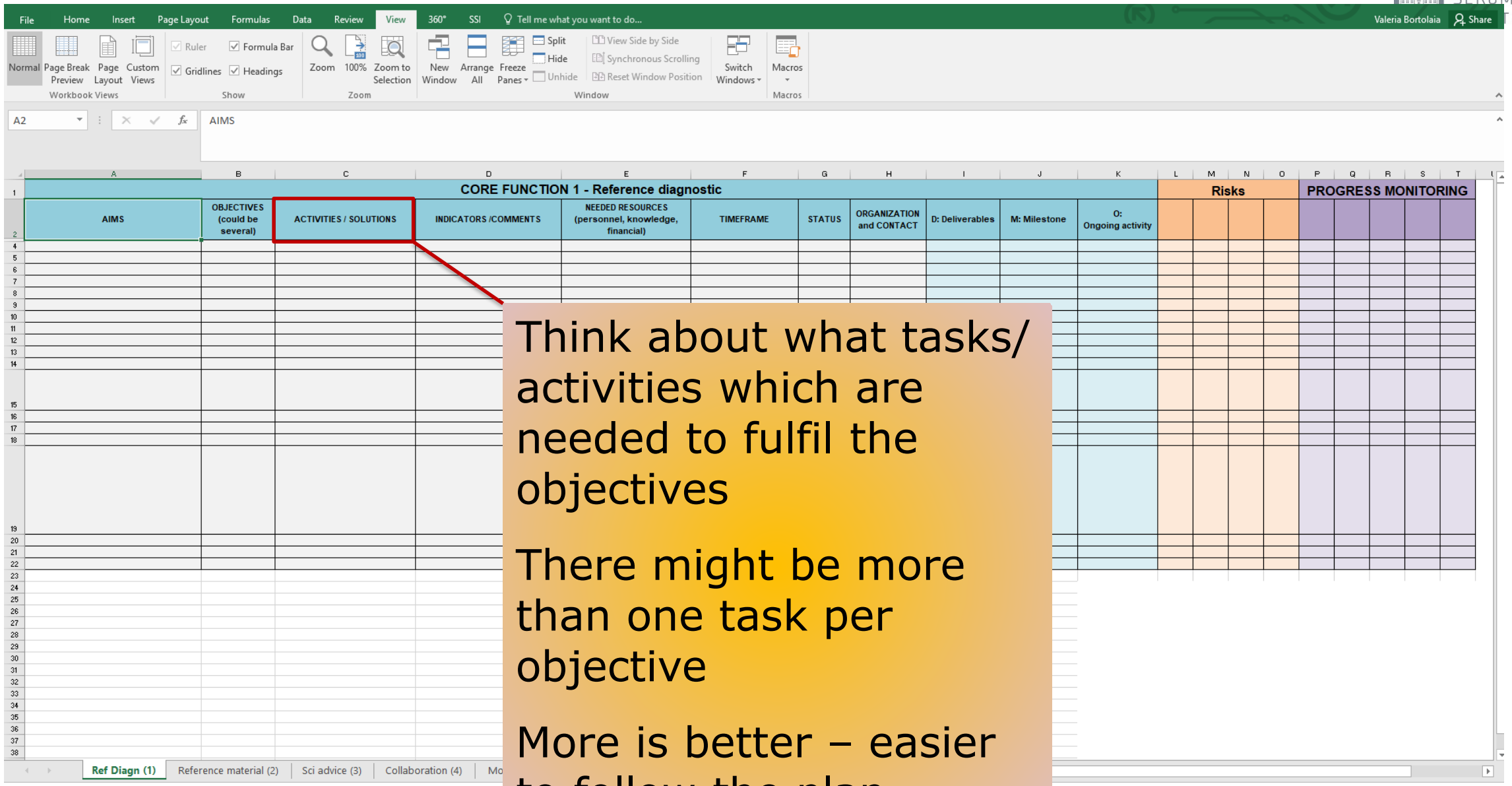

Think about what tasks/ activities which are needed to fulfil the objectives

There might be more than one task per objective

More is better – easier to follow the plan

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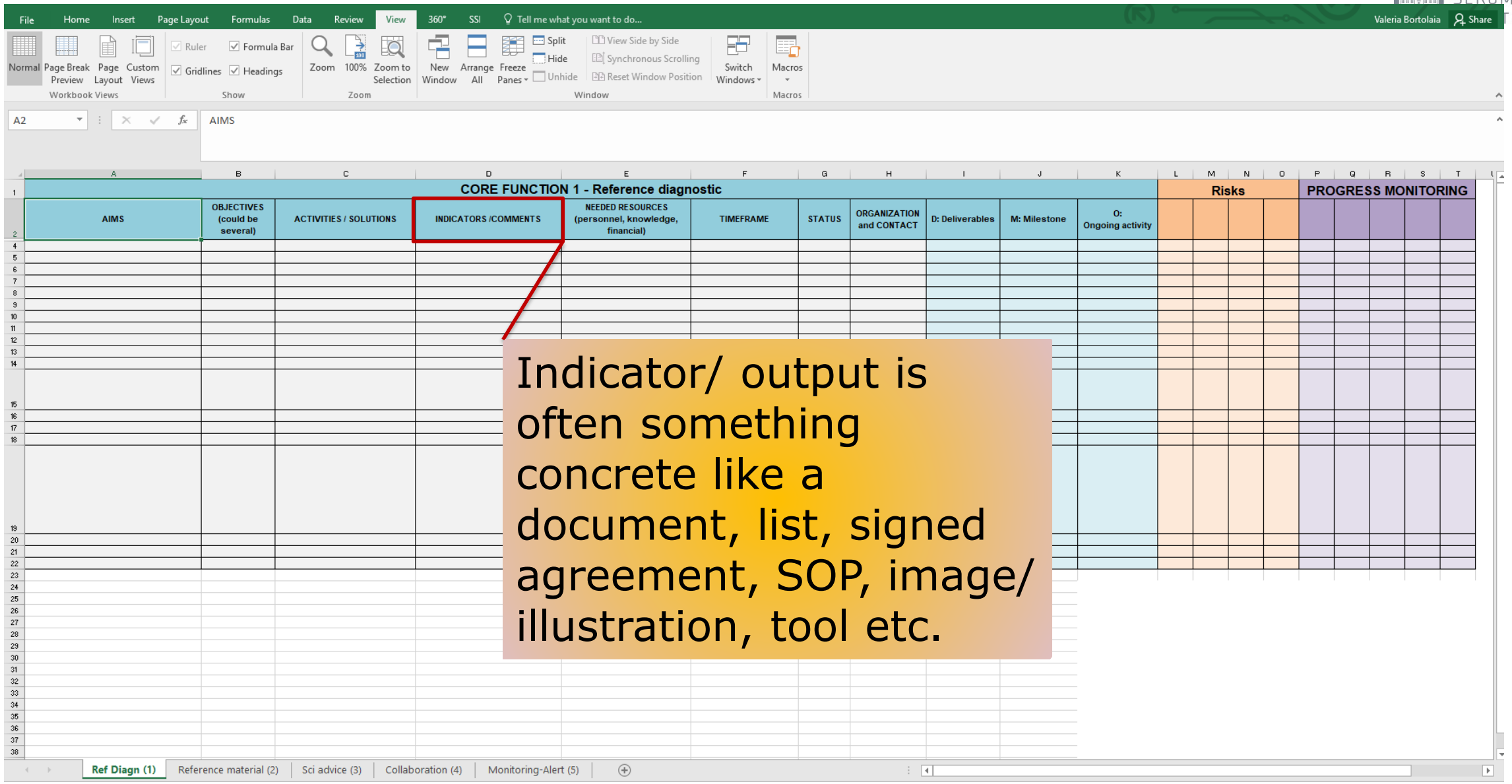

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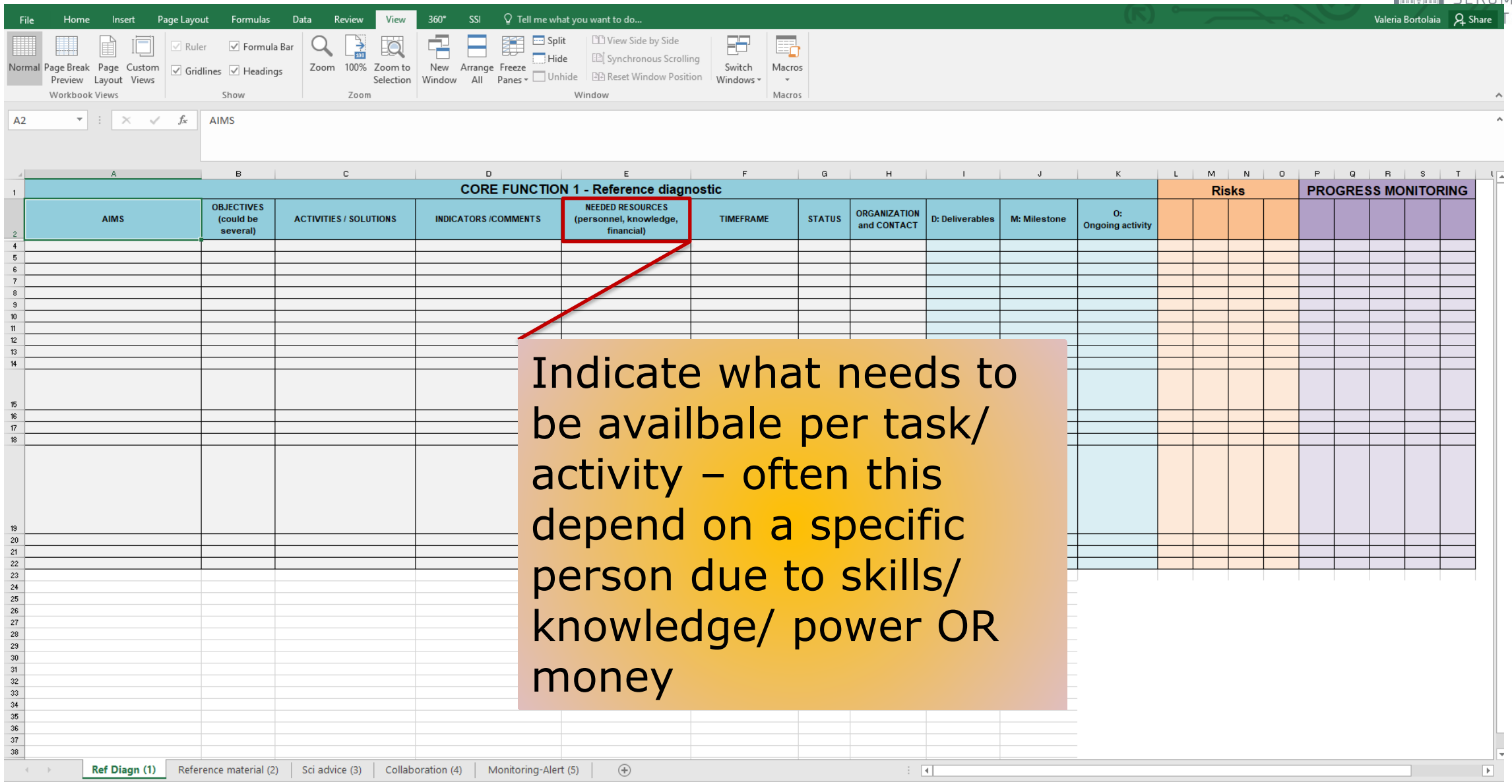

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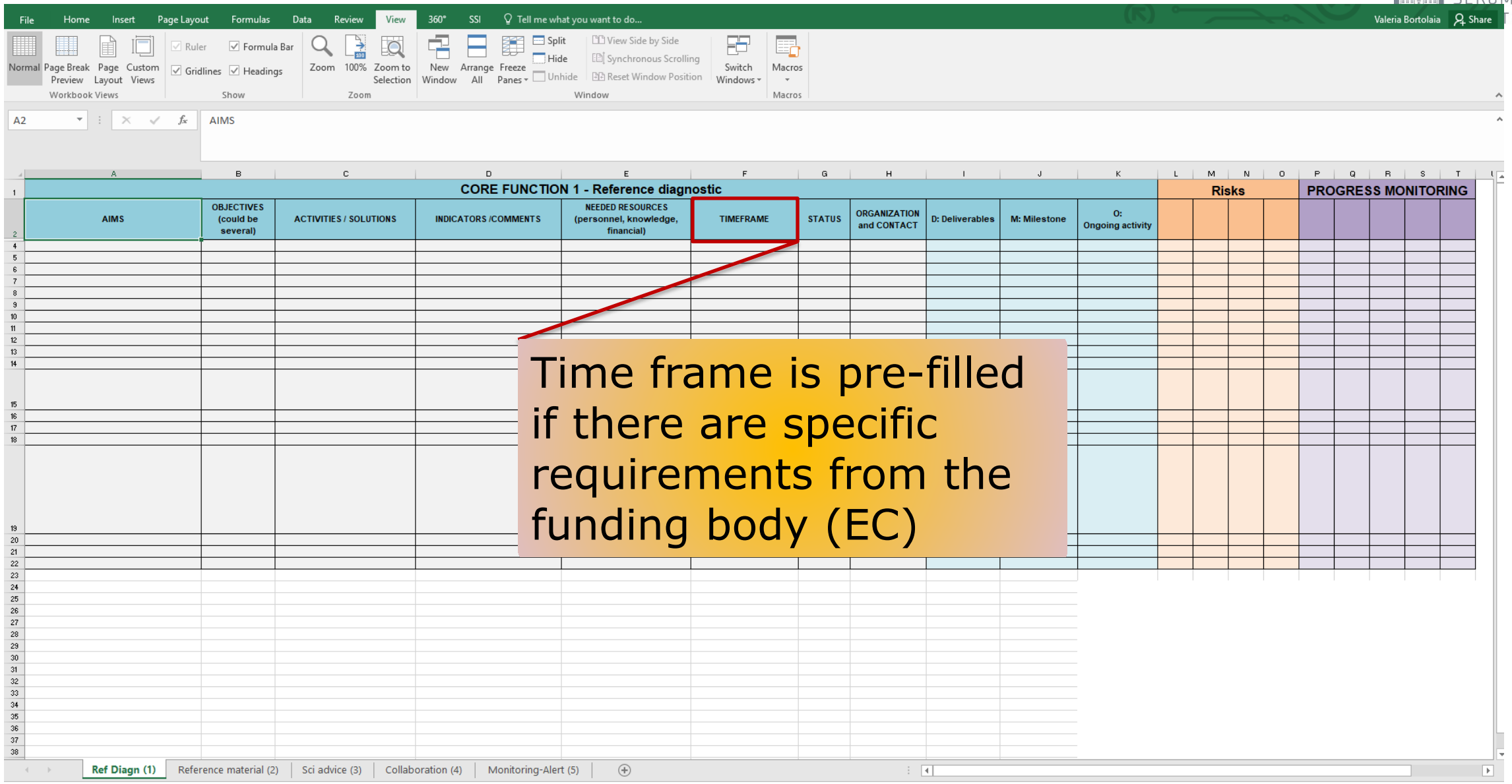

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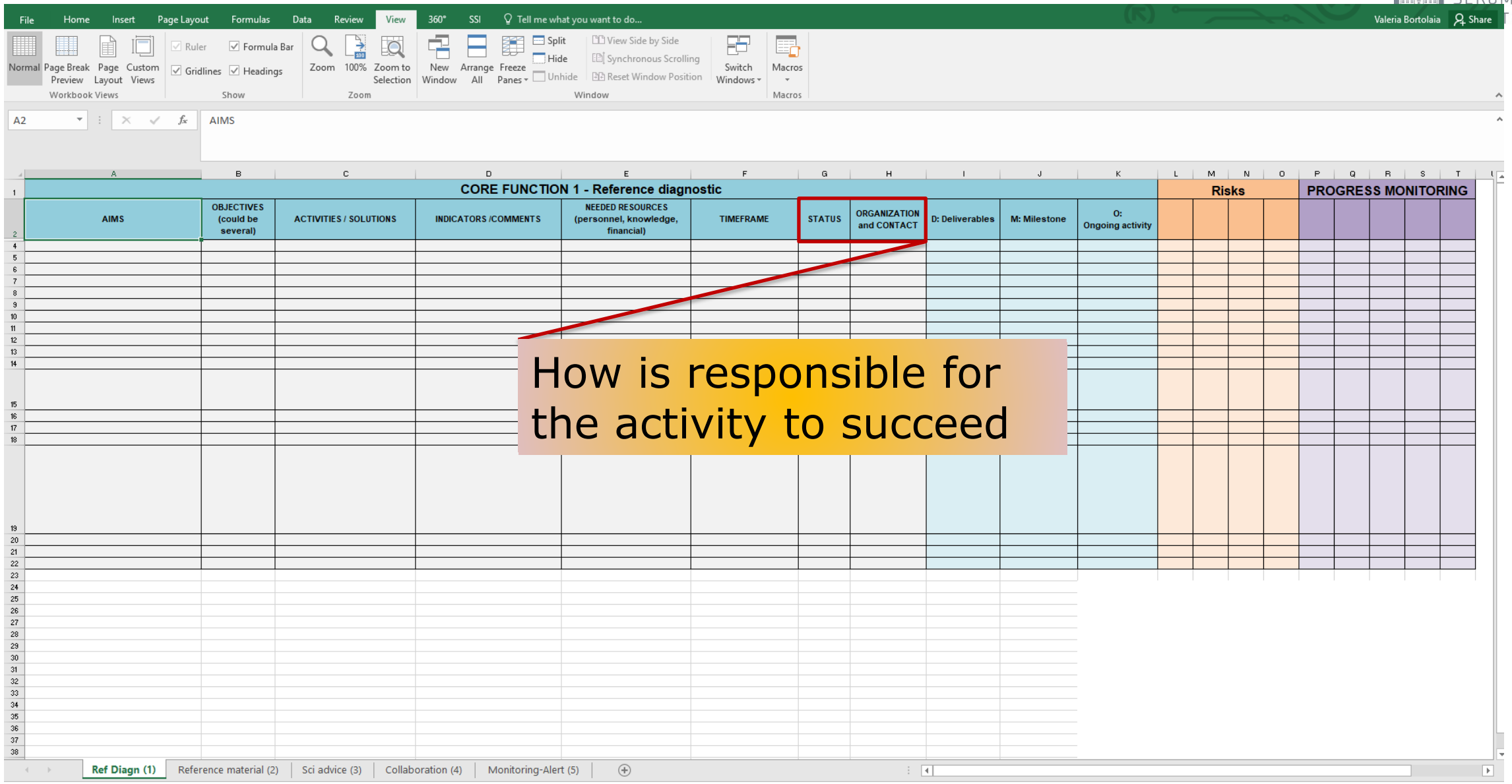

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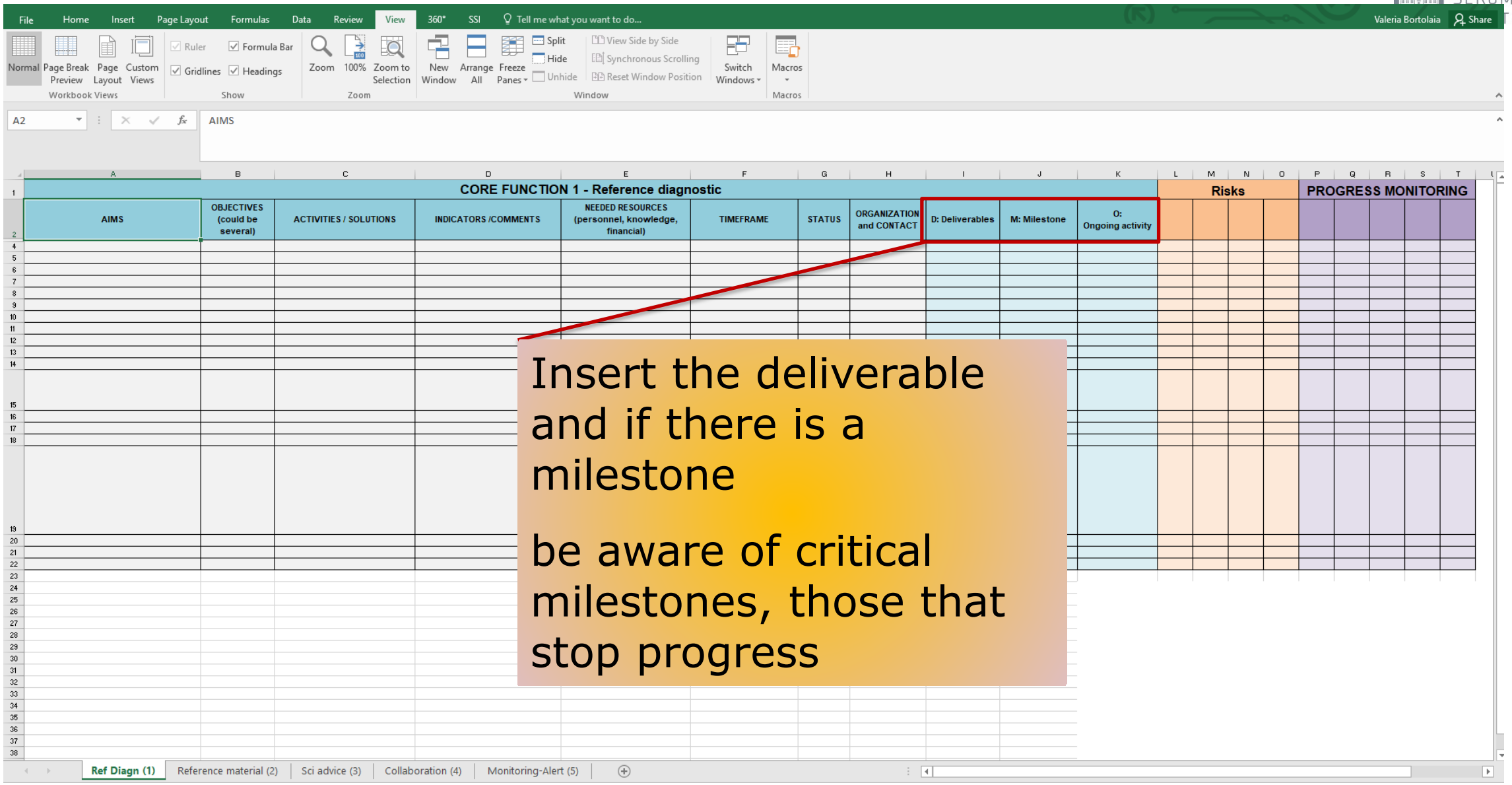

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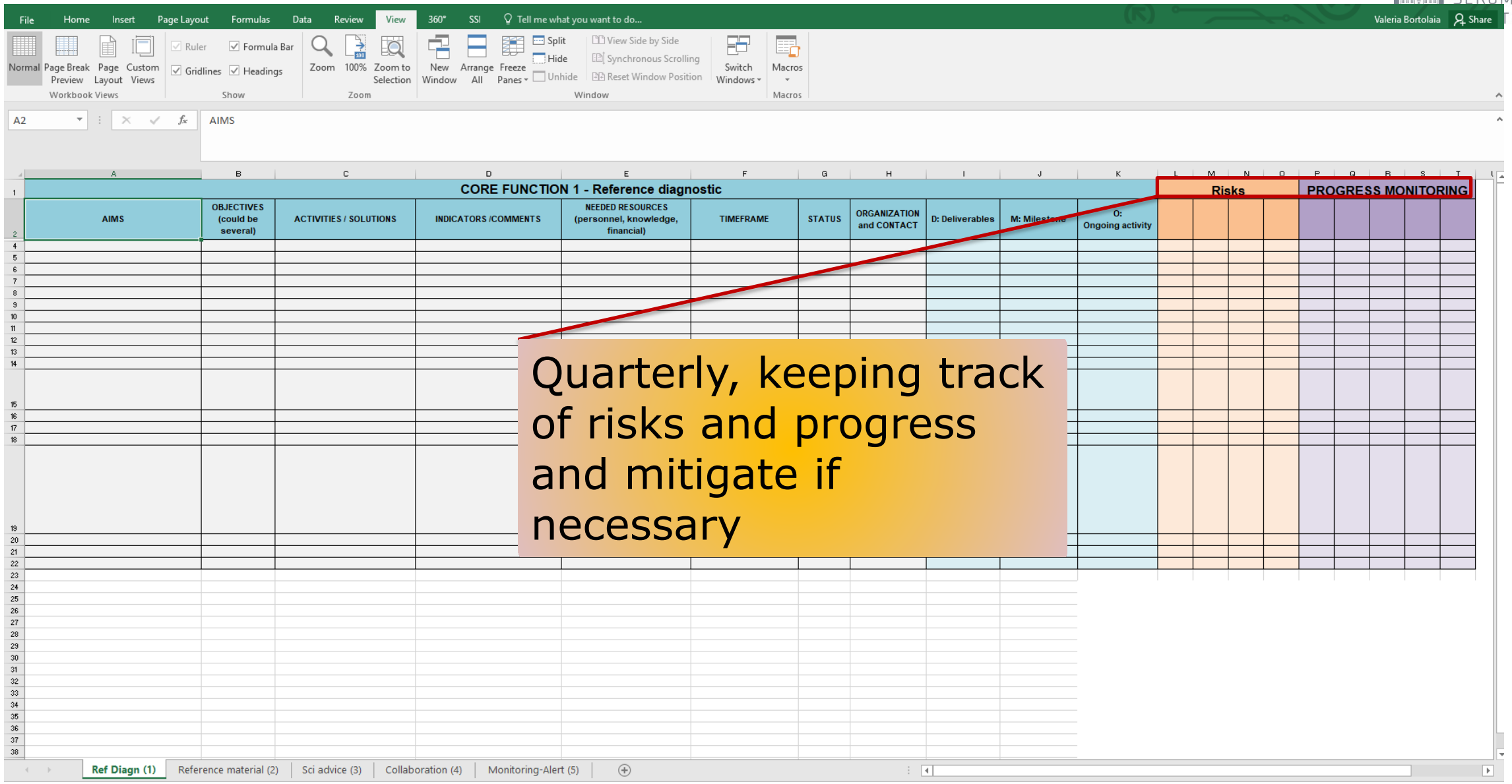

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An example of an action plan!

AIMS	OBJECTIVES (could be several)	ACTIVITIES / SOLUTIONS	INDICATORS /COMMENTS	TIME-FRAME	D: Deliverables	M: Milestone
The NRL provides support and data to surveillance systems by contributing to national recommendation for public health notification of CRE/CCRE	**Establish harmonised criteria for selection of CRE/CCRE for referral to the NRL**	Identify and invite relevant stakeholders to propose and reach an agreement on harmonised criteria for CRE/CCRE referral	Documentation (minutes from meetings, report of questionnaire, etc.) that the NRL has consulted the clinical laboratories and other relevant stakeholders to propose and reach agreement on the harmonised criteria for CRE/CCRE referral			The NRL has identified the stakeholders that should participate to drafting the proposal and reach the agreement
		Share the established harmonised criteria for CRE/CCRE referral	Documentation (E-mail, minutes from meetings, etc.) that the NRL has informed all clinical laboratories (and also public health authorities) on the harmonised criteria for CRE/CCRE referral	1 month from the agreement on harmonised criteria		All relevant people have received information on the harmonised criteria for CRE/CCRE referral
		Verify sustainability of harmonised referral criteria	Documentation (e.g. contact list of people that have 'read and understood' the harmonised criteria) that at least two people in each reporting clinical laboratory are familiar with the harmonised criteria and consider them feasible Each reporting laboratory has a documented internal system explaining the harmonised criteria for referral to the NRL	3 months from the establishment of harmonised criteria	Written "chapter" to be included in national guidance for CRE/CCRE surveillance	All relevant people have "read and understood" the harmonised criteria for CRE/CCRE referral

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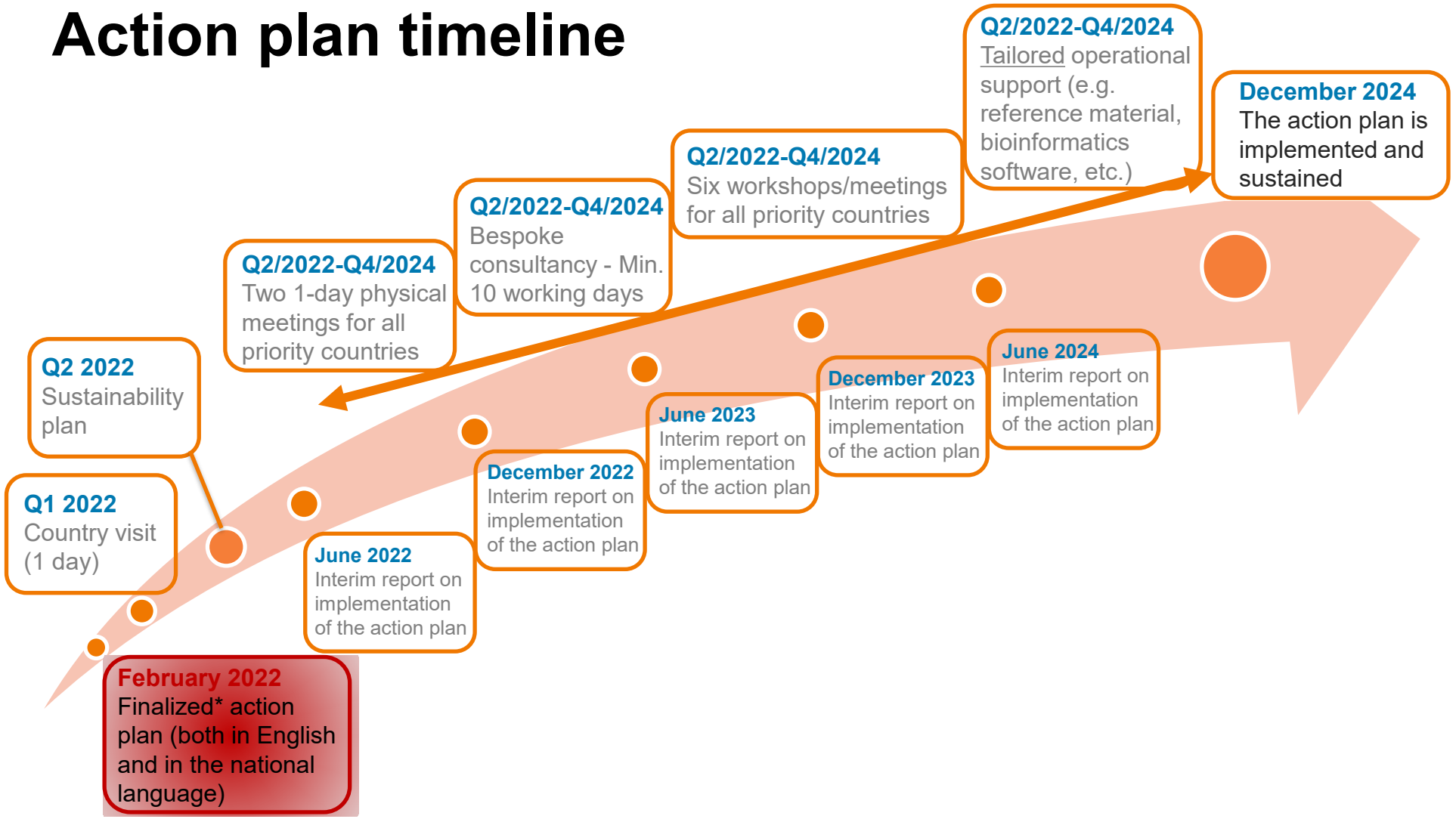
Action plan timeline



*Important! It will be possible to revise and reassess the action plan during the project

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Thank you for your attention

Prof. Rene S. Hendriksen, PhD

Head of Unit, Research Group Global Capacity Building

WHO Collaborating Centre for Antimicrobial Resistance in Food borne Pathogens and Genomics

European Union Reference Laboratory for Antimicrobial Resistance

FAO Reference Laboratory for Antimicrobial Resistance

National Food Institute, Technical University of Denmark

rshe@food.dtu.dk

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René S. Hendriksen
rshe@food.dtu.dk

Where to go from here

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Thank you on behalf of the EURGen-RefLabCap team

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