MARS
Global Services
Laboratories

Shaping the food industry's future through laboratory risks mitigation

Andrei Sorin Bolocan

EU micro consultant and audit senior scientist

Mars Global Services

The world we want tomorrow starts with how we do business today

MARS

In the world we want tomorrow, our lab is a role model

EU Microbiology Regional Laboratory, Veghel, Netherlands

ISO17025 accredited

Everyday, MARS Makes Thousands Of Decision Based On Laboratory Data

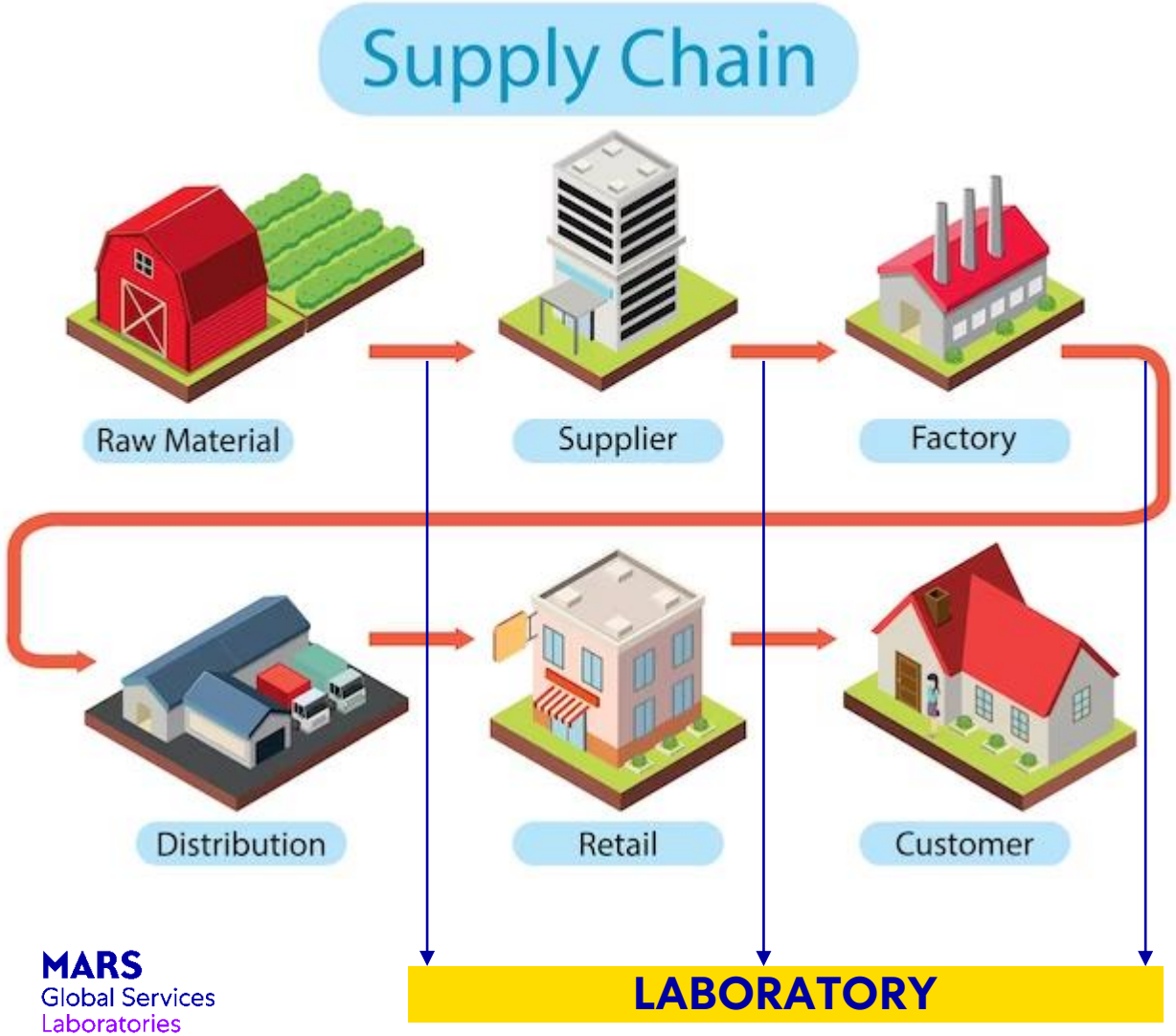
MARS
Global Services
Laboratories

Guaranteed access to trusted laboratory data & expertise

Standardizing regional testing capability and laboratory governance enhances our ability to provide trusted data to support both routine supply chain demands and incident response.

+ immeasurable value from minimizing negative reputational impact & protecting our freedom to operate

Supply chain
Food safety vs Food waste



MARS
Global Services
Laboratories

Food waste arises in every step of the food chain; thus, mitigation should be a common goal, and responsibility is shared among food-chain actors

Minimizing amount of waste and unnecessary usage of resources is among the principles of sustainable consumption

Food-safety regulations and guidelines issued by authorities are often regarded as too strict and contrasting to sustainability principles, though they primarily prioritize protection of human health

Officially ordered product recalls, withdrawals, and destruction of presumably hazardous batches often perceived as unnecessarily extreme measures and inspire various food-saving ideas.

23 million people in Europe are affected by foodborne illnesses each year

Bacillus cereus, L. monocytogenes,

Streptococcus, Staphylococus spp, Salmonella

Routine testing

Salmonella

NOT DETECTED

RELEASE THE PRODUCT

FALSE UNDETECTED

OUTBREAK RECALL INVESTIGATION

DETECTED

NOT RELEASE THE PRODUCT
RECALL DISTRIBUTION
INVESTIGATION

FACTORY

SCRAPE THE PRODUCT

INCONCLUSIVE

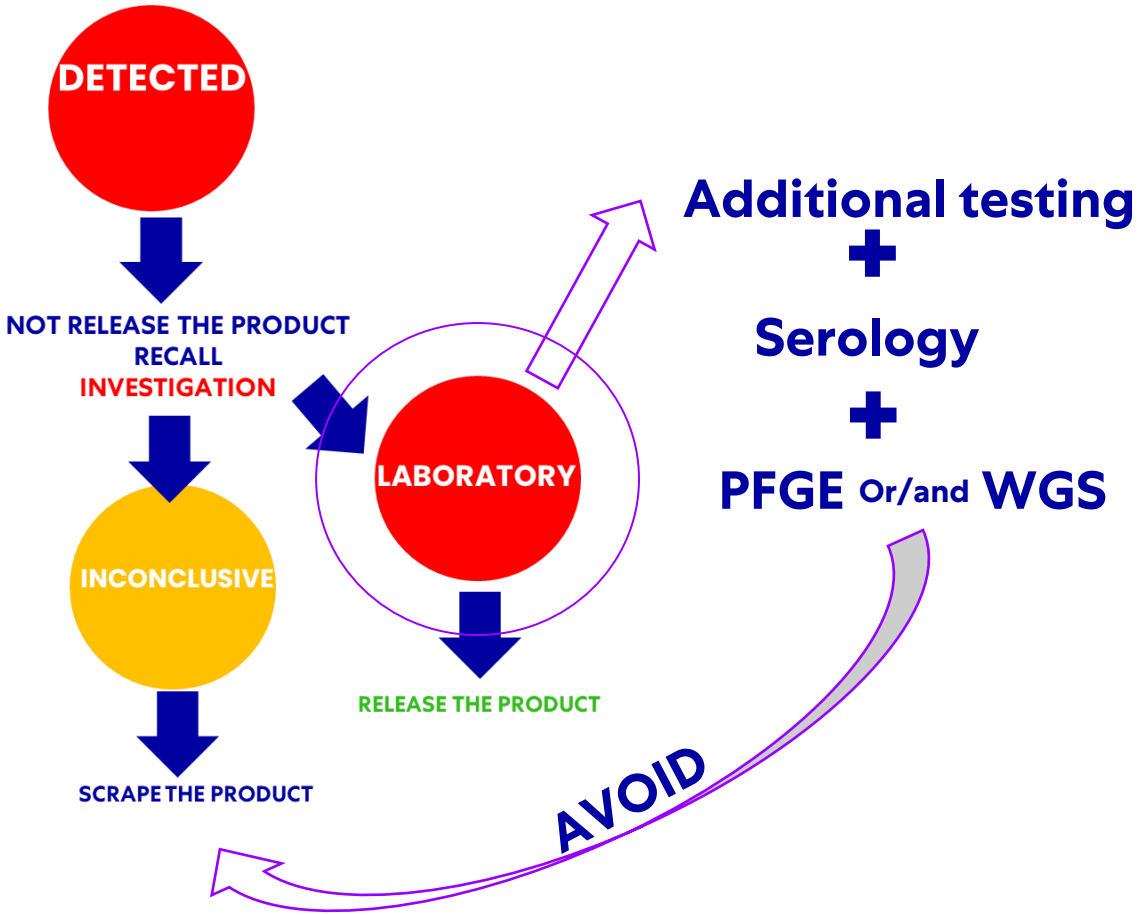
SCRAPE THE PRODUCT

LABORATORY

RELEASE THE PRODUCT

MARS
Global Services
Laboratories

Detected Salmonella



MARS
Global Services
Laboratories

Spot contamination at factory level & unedified source

Unedified source at laboratory level

Multiple samples, multiple isolates,

Improper root-cause analysis

MARS
Global Services
Laboratories

IS THE LABORATORY CROSS-CONTAMIANTION A REAL ISSUE?!

Case 1 -*Salmonella Rissen*

In April 2012, four containers of ready-to-eat chocolate bars were shipped from Belgium to the USA. Before they arrived in the USA, a sample of the chocolate tested positive for *Salmonella Rissen* in a Belgian accredited food laboratory using the ISO 6579 standard.

Salmonella Rissen (rare food serotype) was isolated from fish meal in the same food laboratory 7 weeks prior.

Salmonella Rissen absent from raw materials used for chocolate.

Rasschaert *et al. BMC Res Notes (2016) 9:156*
DOI 10.1186/s13104-016-1969-7

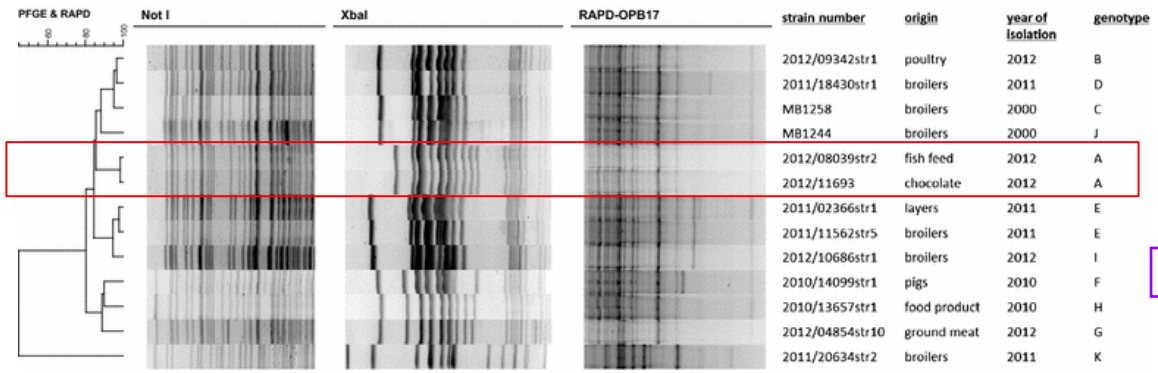
BMC Research Notes

RESEARCH ARTICLE Open Access

Case report of *Salmonella* cross-contamination in a food laboratory

Geertrui Rasschaert[1*], K. De Reu[1], M. Heyndrickx[1,2] and L. Herman[1]

Cross-contamination?



strain number	origin	year of isolation	genotype
2012/09342str1	poultry	2012	B
2011/18430str1	broilers	2011	D
MB1258	broilers	2000	C
MB1244	broilers	2000	J
2012/08039str2	fish feed	2012	A
2012/11693	chocolate	2012	A
2011/02366str1	layers	2011	E
2011/11562str5	broilers	2011	E
2012/10686str1	broilers	2012	I
2010/14099str1	pigs	2010	F
2010/13657str1	food product	2010	H
2012/04854str10	ground meat	2012	G
2011/20634str2	broilers	2011	K

PFGE (NotI and XbaI) & RAPD-PCR (OPB-17) of 13 *Salmonella Rissen*; the Pearson correlation (optimization 1 %, tolerance 1 %), UPGMA algorithm

Confirmed!

Not enough segregation= fish meal, high risk & powder;

Inefficient air monitoring = contiguous circulating in the air or the laboratory

Improper handling of the isolate (slant tube)= cross contamination of the environment and improper disinfection

MARS
Global Services
Laboratories

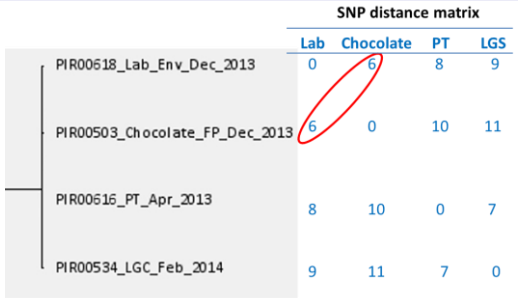
Personnel working in routine laboratories should always be aware of the possibility of cross-contamination, especially when enrichment is used in the microbiological analysis.

Case 2 -*Salmonella Hadar*

In 2013, during a routine laboratory analysis performed on food samples, one finished product from a European factory was tested positive for *Salmonella Hadar*.
At the same period, one environmental isolate in the same laboratory was serotyped *Salmonella Hadar*.
Prior to this event, the laboratory performed a proficiency testing involving a sample spiked with NCTC 9877 *Salmonella Hadar*.

Cross-contamination?

Isolate code	Origin of *S.* Hadar isolate	Isolation date
PIR00616	Proficiency test (PT) sample spiked with strain NCTC 9877	April 2013
PIR00618	Laboratory environmental sample (from the thermocouple in the incubator)	December 2013
PIR00503	Finished product sample (chocolate)	December 2013
PIR00534	Reference strain NCTC 9877, originally used in the PT	Acquired in February 2014

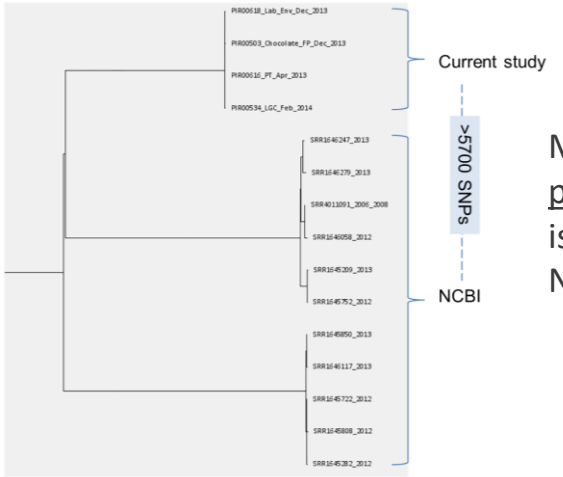


Confirmed!

International Journal of Food Microbiology
Volume 298, 2 June 2019, Pages 39-43

Whole genome sequencing used in an industrial context reveals a *Salmonella* laboratory cross-contamination

Katia Rouzeau-Szynalski [a], Caroline Barretto [a], Coralie Fournier [b], Deborah Moine [b], Johan Gimonet [a], Leen Baert [a]



SRR6159201 from Mullein leaves >44000 SNPs

Maximum-like lihood phylogenetic tree 4 isolates laboratory vs 11 NCBI *S.* Hadar.

Cross contamination PT isolate = improper handling PT sample during routine testing

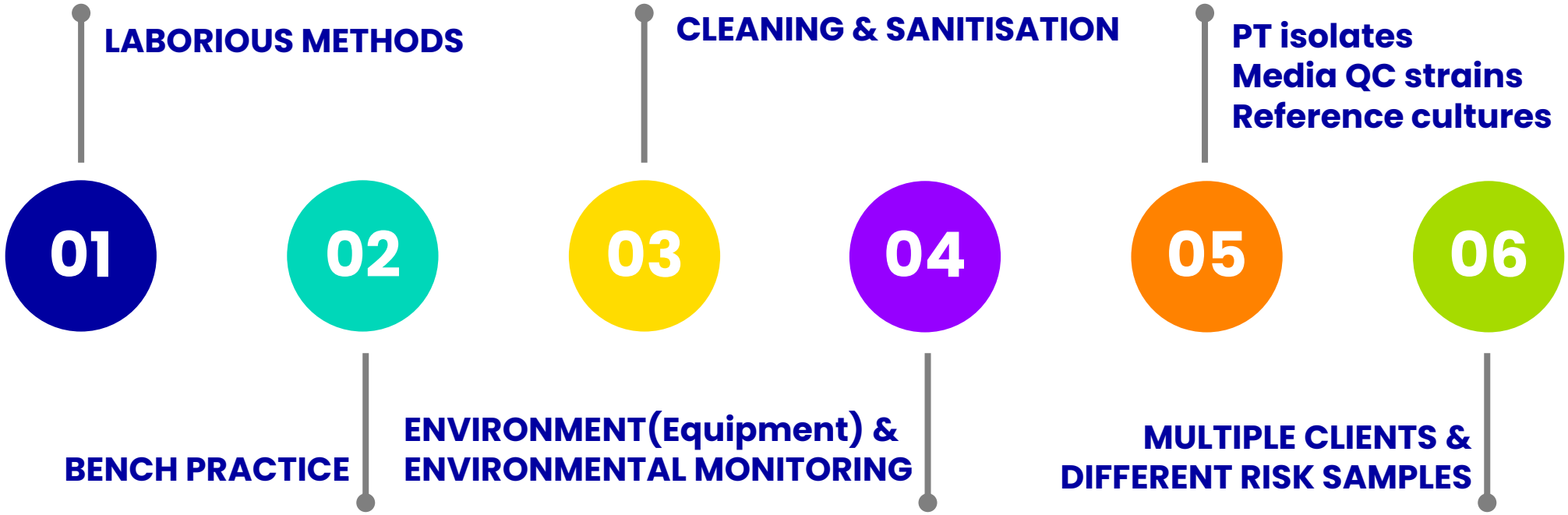
Cross contamination of the environment and improper disinfection

MARS Global Services Laboratories

Measures to early detect cross-contamination are recommended, such as environmental monitoring and the selection of a rare serotype as a positive control strain.

Laboratory cross-contamination

5 WHY??

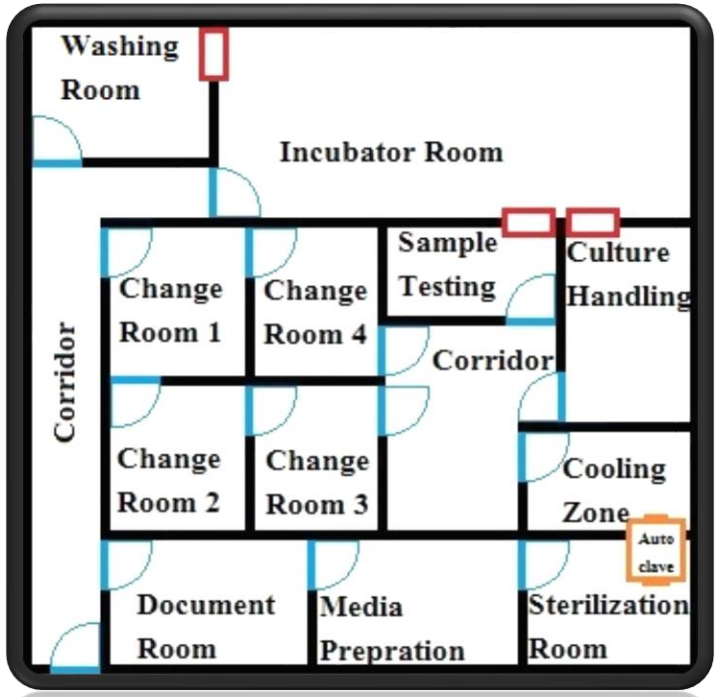


PREVENT!

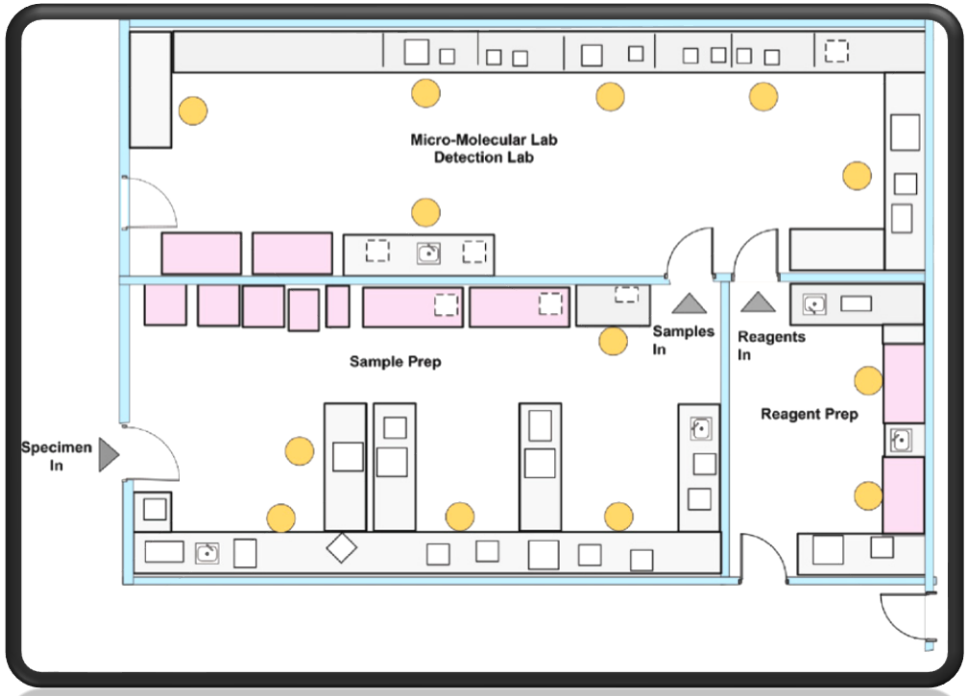
Other clients samples

MARS
Global Services
Laboratories

LABORATORY FACILITY/SEGREGATION



Microbiology

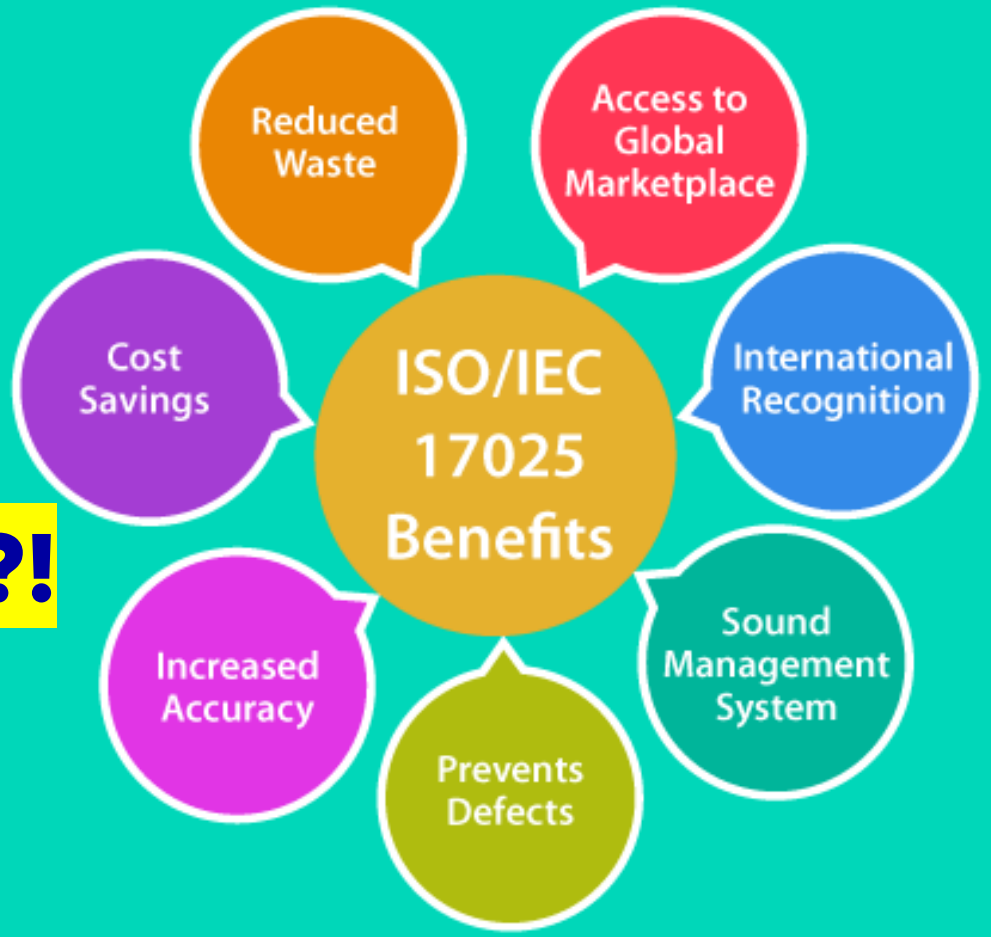


Molecular biology

MARS
Global Services
Laboratories

Quality System

IS IT ENOUGH?!



Doesn’t focus on day-to-day management

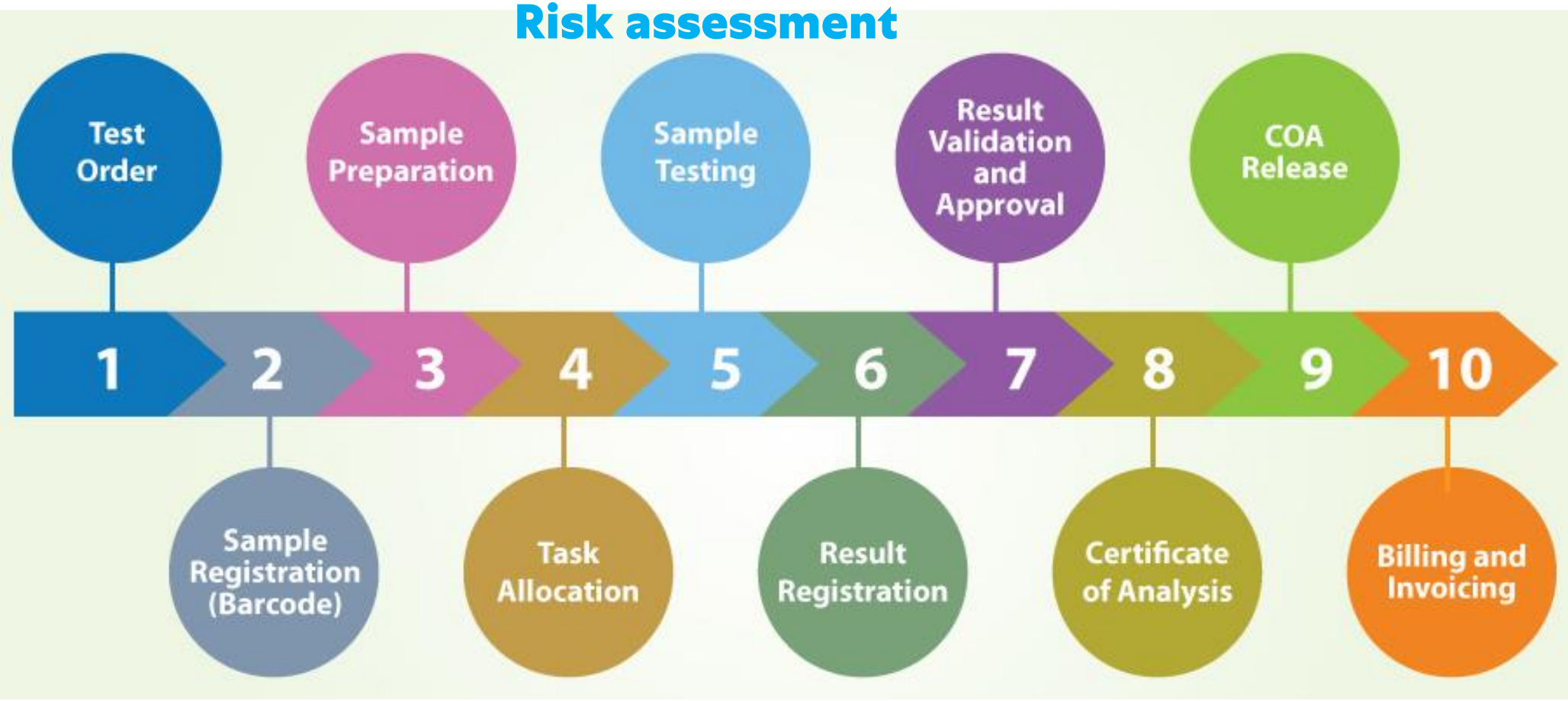
More for external laboratories

Challenging to maintain it

Expensive

MARS

Each step = risk of inaccurate results



MARS
Global Services
Laboratories

Multiple Clients & Different Risk Samples

Finish product

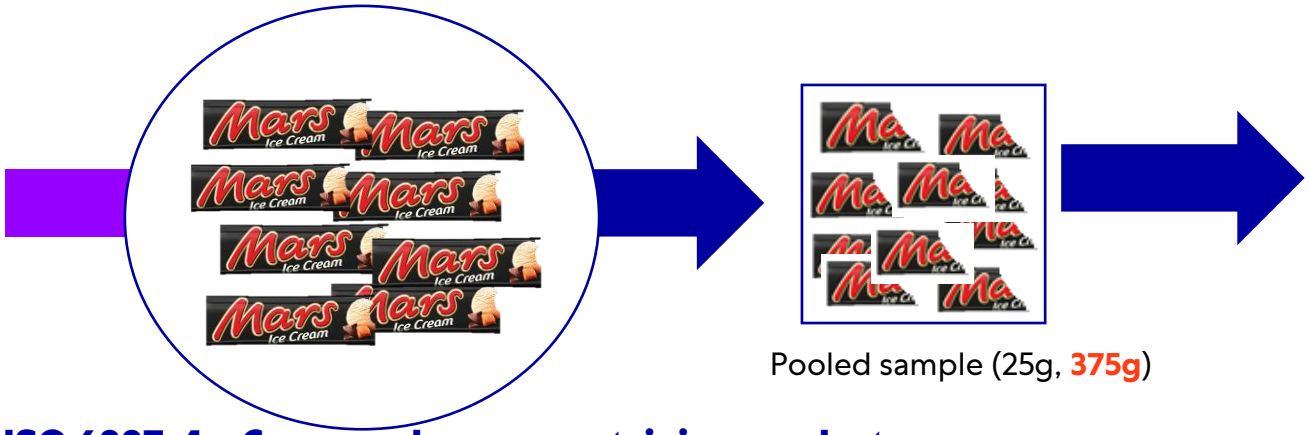
Raw materials

Environmental samples

Separate handling in each processing step

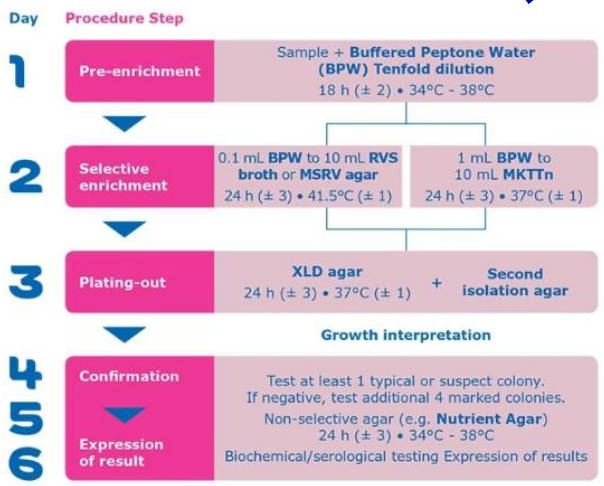
MARS
Global Services
Laboratories

STANDARD VS ALTERNATIVE METHODS



Pooled sample (25g, **375g**)

ISO 6887-4 – Cocoa and cocoa-containing products
Sample



Procedure for Salmonella detection according to EN ISO 6579-1

Skim milk

STANDARD (ISO) = LABORIOUS METHODS

3 days negative / 5 days positive

AUTOMATIZATION & ALTERNATIVE METHODS

Culture methods

1 days negative / 3 days positive

SALMA One Day
Salmonella PRECIS

Molecular methods

1days negative / 3 days positive

QIAGEN mericon Salmonella spp
BAX System Real-Time PCR Assay
BAC*Gene Salmonella* spp
GENE-UP *Salmonella 2*

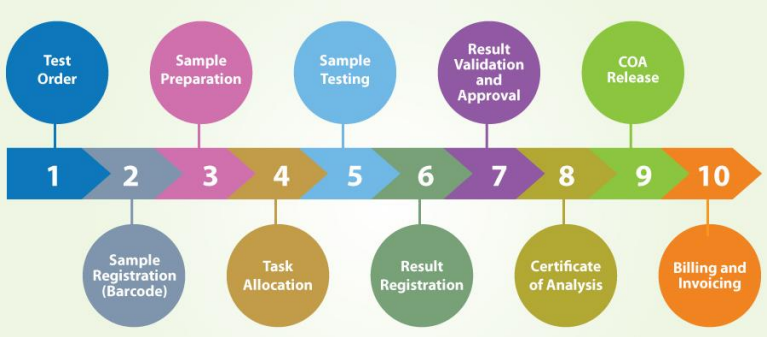
Immunological tests

2 days negative / 4 days positive

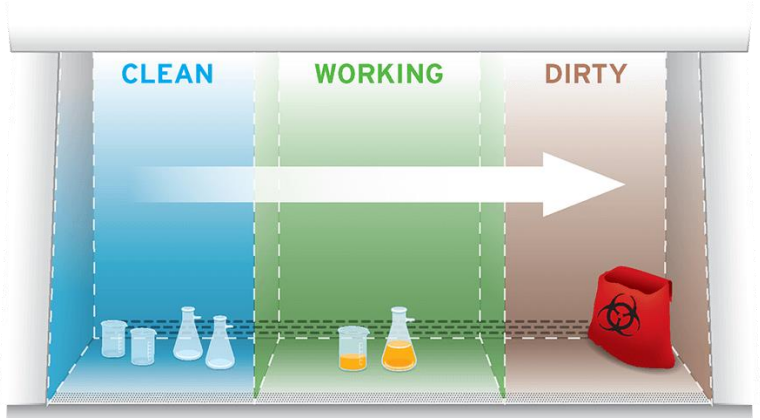
Solus *Salmonella* ELISA
VIDAS *Salmonella*
BACSpec *Salmonella* 2

MARS
Global Services
Laboratories

Bench Practice



Personnel



CLEAN to DIRTHY BENCH ORGANISATION

TRAINING → **HABILITATION** → **ONGOING MONITORING** → **PT**
SPIKED SAMPLES
BENCH PRACTICE AUDIT

DISINFECTION
UNDERSTAND THE RISKS/CROSS-CONAMINATION

RECOMMENDATION: ISO7218

MARS
Global Services
Laboratories

CLEANING & SANITISATION

CLEANING SCEDULE → ADEQUATE CLEANING FREQUENCY → PROPER CEONCENTRATION & TIME OF ACTION → VALIDATE & ONGOING MONITORING

PROPER ACTIONS FOR OUT OF SPEC RESULTS

Disinfectants	Active against							Inactivated by					Toxicity		
	Fungi	Bacteria		Myco-bacteria	Spores	Lipid viruses	Non-lipid viruses	Protein	Natural materials	Synthetic materials	Hard water	Detergent	Skin	Eyes	Lungs
		Gram positive	Gram negative												
Hypochlorites	+	+++	+++	++	++	+	+	+++	+	+	+	C	+	+	+
Alcohols	–	+++	+++	+++	–	+	V	+	+	+	+	–		+	
Formaldehyde	+++	+++	+++	+++	+++ [a]	+	+	+	+	+	+	–	+	+	+
Glutaraldehyde	+++	+++	+++	+++	+++ [b]	+	+	NA	+	+	+	NA	+++	+++	+++
Iodophors	+++	+++	+++	+++	+	+	+	+++	+	+	+	A	+	+	–

ENVIRONMENT & ENVIRONMENTAL MONITORING

ADEQUATE ENVIROMNETAL MONITORING → **ADEQUATE METHODS & FREQUENCY** → **After Cleaning** / **DURING SAMPLES PROCESSING** → **PROPER ACTIONS FOR OUT OF SPEC RESULTS**

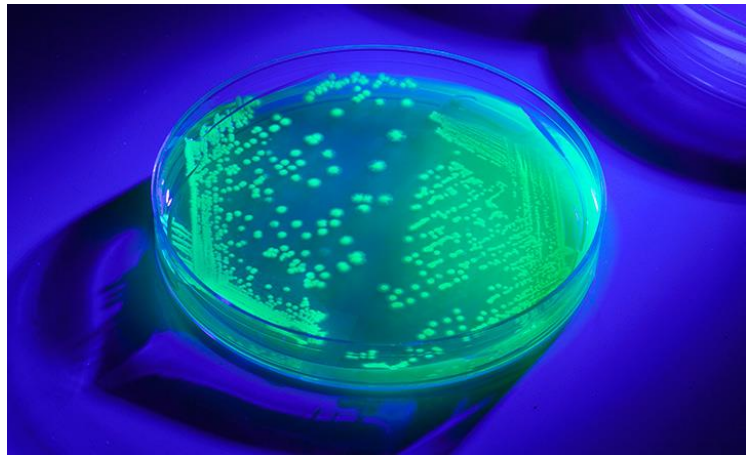
SPOTS (pipettes, filter pipettes, floor, bench, hands ….

Do you ever check for DNA contamination?

Laboratory strains and isolates

Daily Positive Control

Rare Serotype
UV-BioTAG™ GFP microoganism

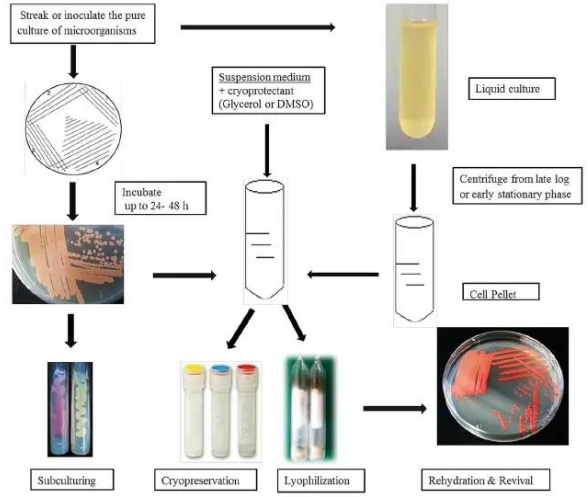


Media QC strains

Separate media QC process ISO11133
No result without media QC!
Sterility, Productivity, Selectivity, Specificity

Laboratory isolates/ reference culture

High Risk zone for handling (BSC)
Maintenance and Preservation process



MARS
Global Services
Laboratories

Waste Disposal

The Bucket List

Getting rid of lab waste? Here's how to dispose of it.

Chemical Waste Pail

- Designate and label for lab specific use
- Ethidium Bromide gels
- Contaminated solids including plastics and glass
- No sharps (needles)
- Provided by EPS

Radioactive Solid Waste Container

- Contaminated plastics and solids
- Ensure tag provided is completed before pickup
- No liquid scintillation vials
- Provided by EPS

Radioactive Liquid Waste Container

- Radioactive aqueous liquid waste
- No liquid scintillation vial contents
- Green tag: half-life <30 days
- Blue tag: half-life >30 days & <90 days
- Yellow tag: half-life >90 days
- Provided by EPS

Biohazard Waste Pail

- Risk Group 2 biologically contaminated solids
- No liquids, sharps, Risk Group 1 biologicals or animal anatomical waste
- Provided by EPS
- (Some locations receive pails that are lined)

Biohazard Bag

- Biologically contaminated solids only
- No sharps
- Risk Group 1 solids should be in bags with no biohazardous symbol
- Purchased by lab

Sharps Container (CSA Approved)

- Needles, syringes, lancets, blades, etc.
- Designate, separate and Label as Biological, Chemical or Radioactive waste
- Purchased by lab

Animal Anatomical Waste Pail

- All animal anatomical waste
- All materials contaminated with toxins requiring incineration
- Biobags, provided by DCM can be used to transport tissues to DCM
- Cytotoxic waste
- No biologically or chemically contaminated bedding
- Provided by EPS

Paper Recycling Bin

- Uncontaminated paper
- Boxboard
- Catalogues
- No Cardboard. Recycle separately
- Call Recycling for larger toters for office/ lab clean outs
- Provided by REC

Regular Garbage

- Uncontaminated refuse (paper towels, pipet wrappers, etc.)
- Decontaminated Risk Group 1 biological solids
- Provided by Caretaking

Amber Laboratory Glass Tote

- Uncontaminated coloured glass (TRIPLE RINSED)
- No hazardous materials, garbage or gloves
- No clear glass
- Provided by REC

Teal Laboratory Glass Tote

- Uncontaminated Clear glass (TRIPLE RINSED)
- No hazardous materials, garbage or gloves
- No coloured glass
- Provided by REC

Orange Laboratory Plastic Tote

- Uncontaminated laboratory plastics (TRIPLE RINSED)
- No hazardous materials, garbage or gloves
- Provided by REC

MARS Global Services Laboratories

UNIVERSITY OF TORONTO

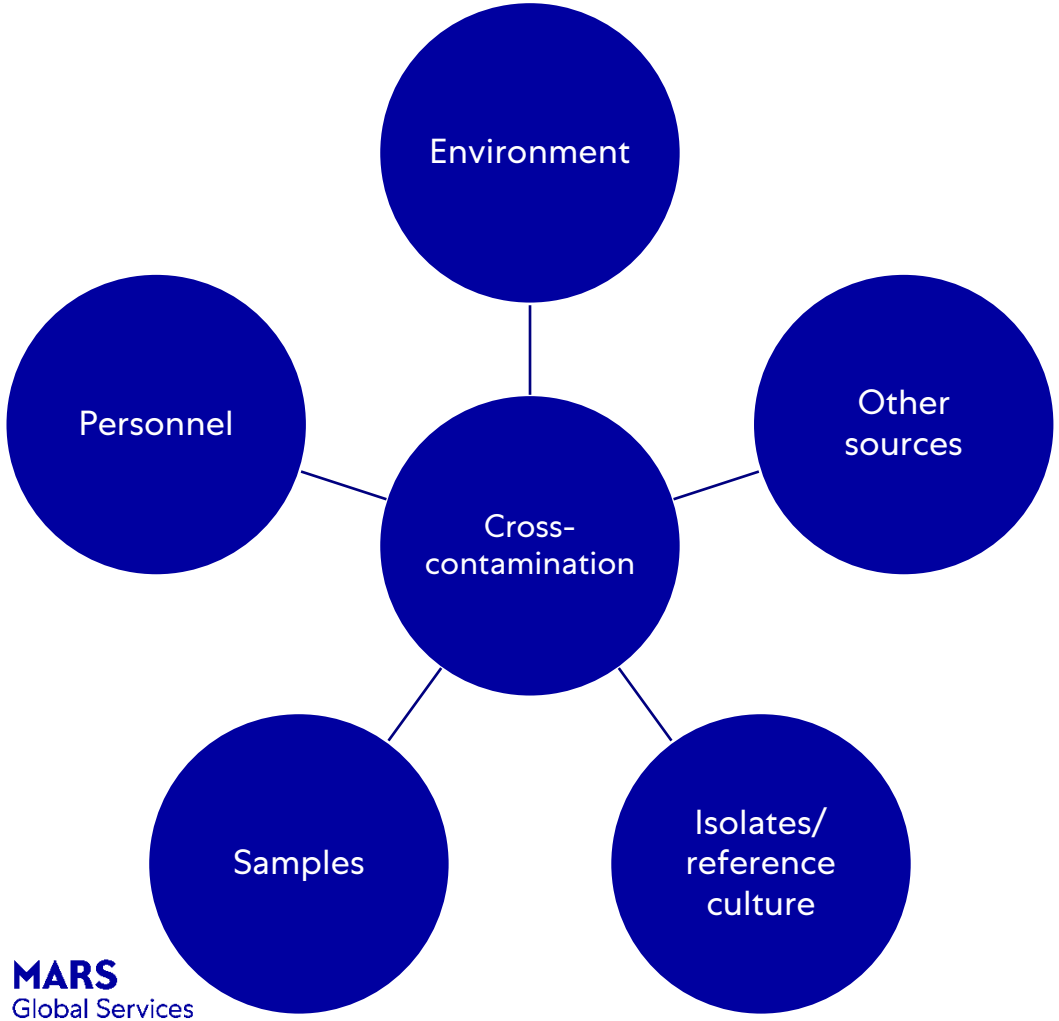
Environmental Protection Services
www.fs.utoronto.ca

F&S: Facilities & Services Departments
EPS: Environmental Protection Services (416-946-3473)
CAR: Caretaking (416-978-6252)
REC: Recycling (416-946-5711)

Version 2 – June 2021

Conclusions

Microbiological cross contamination can be an issue for food testing laboratories.



MARS
Global Services
Laboratories

Laboratory investigations can often be inconclusive, or not end fast enough to support the release of the products.

Retrospective analyses are supporting to conclude the investigations, but not useful for release of the product and prevent food waste.

Finding the source of microbial deviations early enough will result in a significant cost and waste reduction.

Prevention though an optimized and maintained quality system can mitigate the risk of cross contamination in food testing labs.

MARS
Global Services
Laboratories

The Future starts today!

Thank You!!