REAL-TIME RT-PCR VS TRADITIONAL METHODS IN DRINKING WATER – A EUROPEAN STUDY

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Application of a real-time reverse transcription polymerase chain reaction for rapid detection of *Escherichia coli* in drinking water: an EU representative study

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CONTEXT & MOTIVATION

- EU Drinking Water Directive 2020/2184 requires zero *E. coli* per 100 mL
- Traditional detection: culture-based methods (21–24h)
- Need for rapid detection in case of contamination events, post-natural disasters scenarios and massive social gatherings

STUDY OBJECTIVE

- Validate a real-time RT-PCR method for *E. coli* detection on a pan-european scale
- Organised by the Joint Research Centre (JRC) of the European Commission
- Involved 19 labs across 11 EU Member-States

PARTICIPATING LABORATORIES

11 countries+Eur Comission (19 laboratories): AT, BE, HR, DE, FI, IT, NL, PL, PT, SI, SK, EC

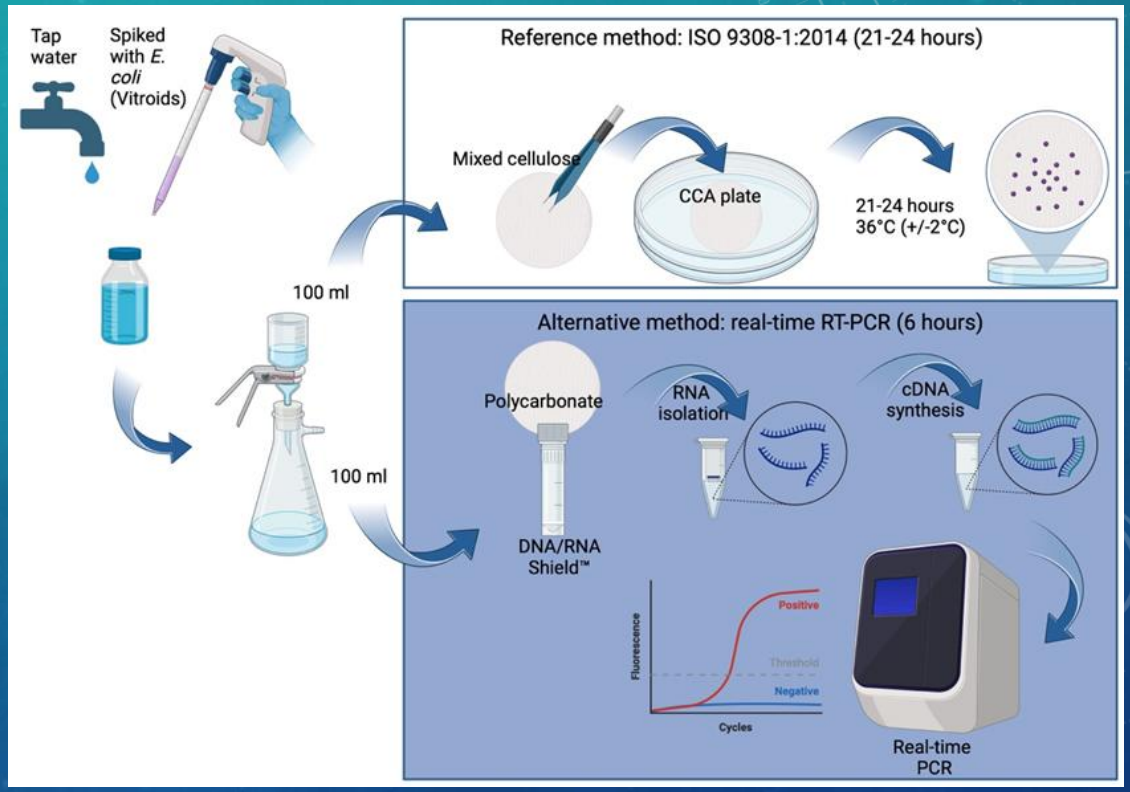
Laboratories participating in the interlaboratory studies. Member States (MS), organisation, laboratory and methods used for RNA extraction and real-time reverse transcription PCR (RT-PCR) are included. N/A, not applicable.

Member State	Organisation	Laboratory	Extraction method	Real-time RT-PCR
Austria (AT)[a]	AGES Austrian Agency for Health and Food Safety	Reference Laboratory for *E. coli* including VTEC	ZymoBIOMICS DNA/RNA Mini kit	SensiMIX II Probe kit Roche, LightCycler 480
Belgium (BE)	Pidpa	N/A	NucliSens extraction Biomerieux (no bead beating)	One-step mix - Evoscript Roche Biorad CFX96
	De Watergroep, Heverlee[2]	N/A	Qiagen RNeasy Powerwater (50) Lot 172037190	BioRad CFX96 Deep Well Real-Time System + C1000 Touch Thermal Cycler
Croatia (HR)	IPH Institute for Public Health in the Primorje-Gorski Kotar County	N/A	ZymoBIOMICS DNA/RNA Mini kit	Meridian bioscience Appliedbiosystems Quantstudio 5
	IOF Institute of Oceanography and Fisheries	Laboratory of Microbiology	ZymoBIOMICS DNA/RNA Miniprep Kit	SensiMIX II Probe kit Roche, LightCycler 480
Germany(DE)	NLGA Public Health agency of Lower Saxony	N/A	ZymoBIOMICS DNA/RNA Miniprep Kit	SensiMIX II Probe kit Bio-RAD CFX96
Finland (FI)	Finnish Institute for Health and Welfare	Laboratory of Water Microbiology, Microbiology Unit	ZymoBIOMICS DNA/RNA Miniprep Kit Modifications to the RNA extraction protocol. Total nucleic acid extraction, followed by TURBO DNA-free™ Kit to remove DNA to obtain pure RNA.	SensiMIX II Probe kit QuantStudio™ 6 Flex Real-Time PCR System, 96-well (Applied Biosystems)
Italy (IT)	Italian National Institute of Health	National Center For Water Safety	ZymoBIOMICS DNA/RNA Miniprep Kit	SensiMIX II Probe kit Bio-RAD CFX96 C1000 Touch
The Netherlands (NL)	WLN	N/A	ZymoBIOMICS DNA/RNA Miniprep Kit	HawkZ05 Fast One-step RT-PCR Kit Quantstudio 5 Thermo Fisher Scientific
	Vitens N.V. Water Expertise Centre	N/A	NucliSens extraction Biomerieux	SensiMIX II Probe kit CFX96 Touch Real-Time PCR
	AQZ	N/A	NucliSens extraction Biomerieux	SensiMix II Probe kit QuantStudio™
	Het Waterlaboratorium	N/A	NucliSens extraction Biomerieux Filters and RNA shield fluid were transferred into Lysisbuffer after which our own protocol was followed.	Roche EvoScript RNA Probes Master Bio-Rad CFX96 C1000 Touch
	KWR Water Research Institute	N/A	NucliSens extraction Biomerieux	Roche EvoScript RNA Probes Master Bio-Rad CFX Opus 96 Real-Time PCR System
Poland (PL)	Voivodship Sanitary and Epidemiological Station, Unit of the State Sanitary Inspection	Interdisciplinary Laboratory of Molecular Diagnostics in Katowice	ZymoBIOMICS DNA/RNA Miniprep Kit	SensiMIX II Probe kit LightCycler 480 II
		Laboratory of Microbiological Analysis in Bydgoszcz	ZymoBIOMICS DNA/RNA Miniprep Kit	SensiMIX II Probe kit Rotor-Gene® Q MDx CE
		Laboratory of Environmental and Food Analysis in Olsztyn	ZymoBIOMICS DNA/RNA Miniprep Kit	SensiMIX II Probe kit Roche LightCycler96
Portugal (PT)	National Institute of Health Doutor Ricardo Jorge	Department of Environmental Health	ZymoBIOMICS DNA/RNA Miniprep Kit	SensiMIX II Probe kit Bio-Rad CFX96
Slovakia (SK)	WRI Water Research Institute	National Water Reference Laboratory in Slovakia	ZymoBIOMICS DNA/RNA Miniprep Kit	SensiMIX II Probe kit Brilliant III Ultra-Fast QPCR Master Mix AriaMX, Agilent, real-time PCR
Slovenia (SI)	NLZOH National Laboratory of Health, Environment and Food	N/A	ZymoBIOMICS DNA/RNA Miniprep Kit	SensiMIX II Probe kit Bio-Rad CFX96
European Commission (EC)[b]	Joint Research Centre (JRC)	Laboratory of Molecular Ecology; Water and Marine Resources	ZymoBIOMICS DNA/RNA Miniprep Kit	SensiMIX II Probe kit Applied Biosystems 7900 HT

[a] Participated only in the first interlaboratory study.
[b] Participated only in the second interlaboratory study.

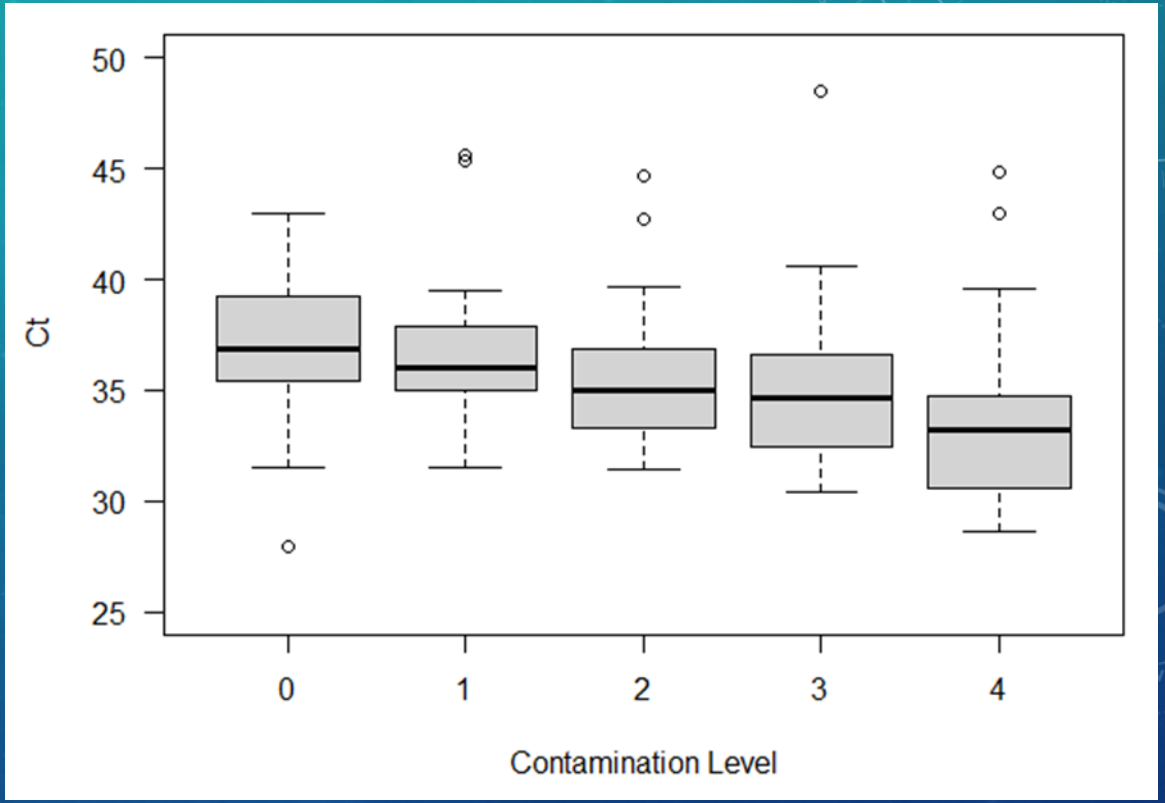
METHODS OVERVIEW (1ST STUDY -2021)

- The JRC prepared and shipped artificially contaminated water samples (0–10 CFU/100 mL).
- The participating laboratories analysed the samples upon arrival using both the reference and alternative methods



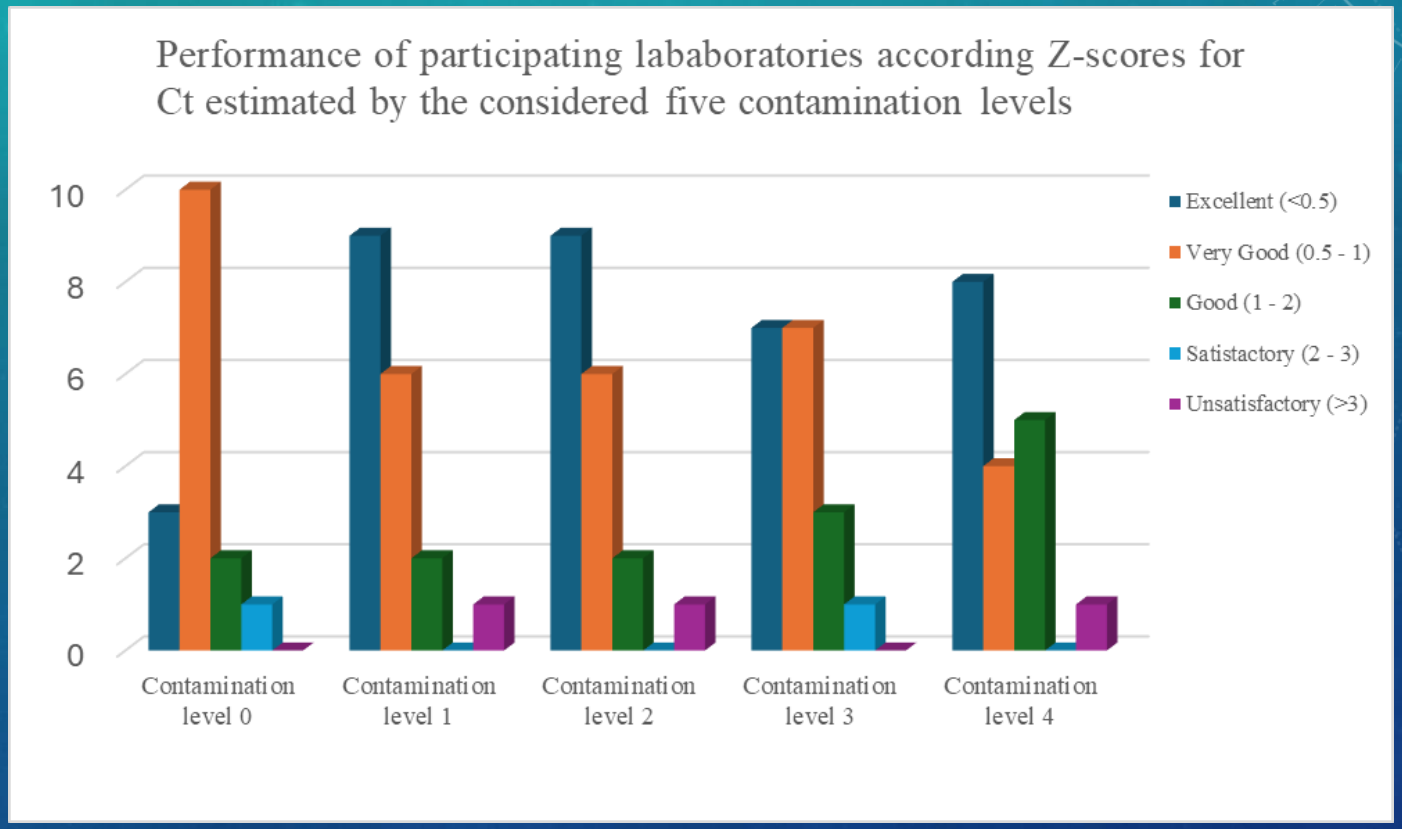
RESULTS (1ST STUDY)

Boxplot of the obtained Ct values by participating laboratories for each contamination level (0, 1, 3, 5 and 10 CFU/100 mL)



Circles indicate outliers according to the R programming language for Statistical Computing. Outliers are defined by the interquartile interval (Q3 - Q1) as values outside the range [Q1 - k (Q3 - Q1), Q3 + k (Q3 - Q1)] where k=1.5, Q1=25th percentile and Q3=75th percentile of data

RESULTS (1ST STUDY): Z-SCORES OF THE ALTERNATIVE METHOD



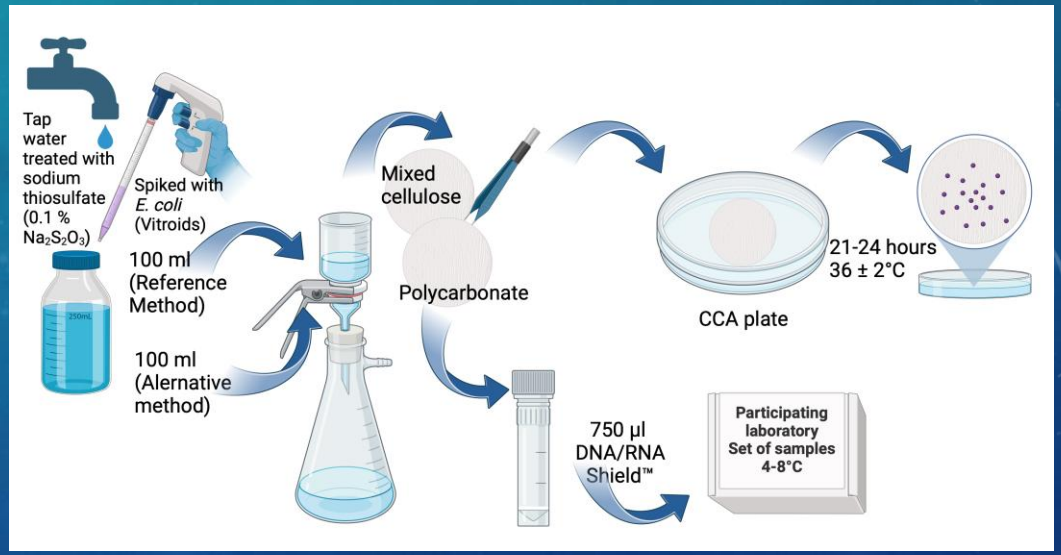
KEY RESULTS (1ST STUDY)

- 18 data sets produced by the participating laboratories.
- 4/18 laboratories reported problems during RNA extraction step and were excluded from the analysis.
- Sensitivity analysis showed lower sensitivity (65.4%) compared to the reference method (90.4%).
- Participating laboratories using polycarbonate filters instead of mixed cellulose filters achieved higher sensitivity of the alternative method (89.2%) compared to the reference method (87.7%).

METHODS OVERVIEW (2ND STUDY -2023)

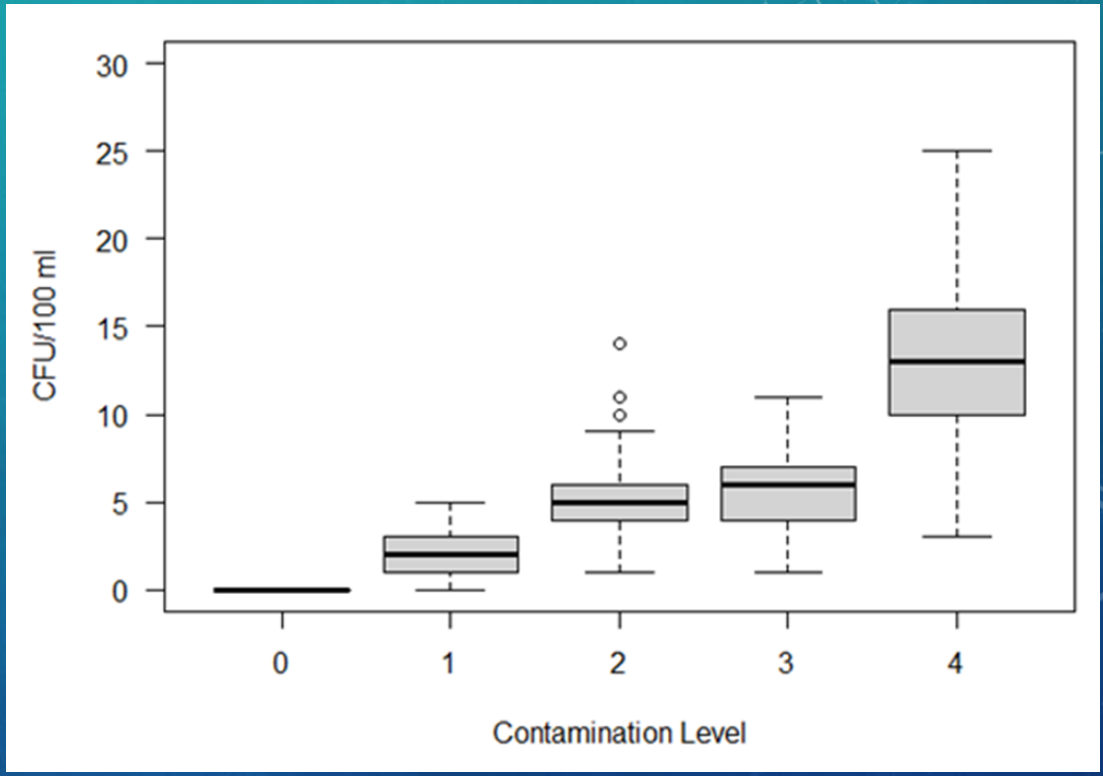
Artificially contaminated water samples (0–10 CFU/100 mL) were filtered and sent out to the participating laboratories

- The JRC prepared the samples and carried out all filtrations.
- A set of already filtered samples was sent to the participating laboratories that analysed them by the alternative method.
- The JRC analysed all samples by the reference method.



RESULTS (2ND STUDY)

Variability of the water sample analysis by the reference method: boxplot CFU/ 100 mL by contamination level (0, 1, 3, 5 and 10 CFU/100 mL)



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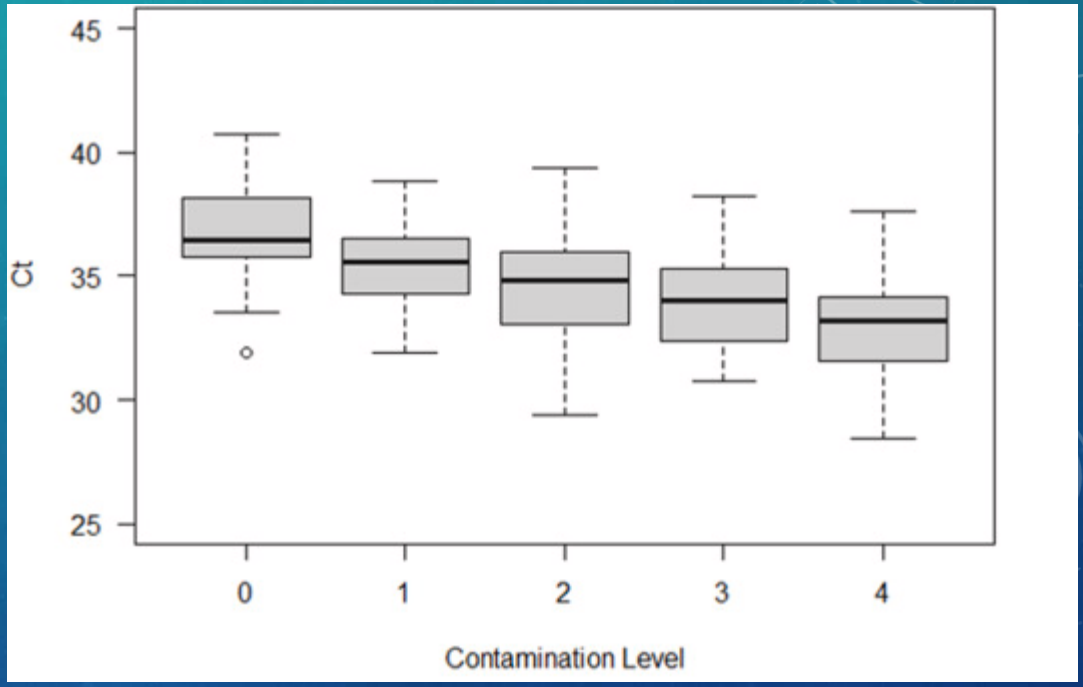
RESULTS (2ND STUDY)

Boxplot of cycle threshold (Ct) values by participating laboratories for each contamination level (0, 1, 3, 5 and 10 CFU/100 mL)



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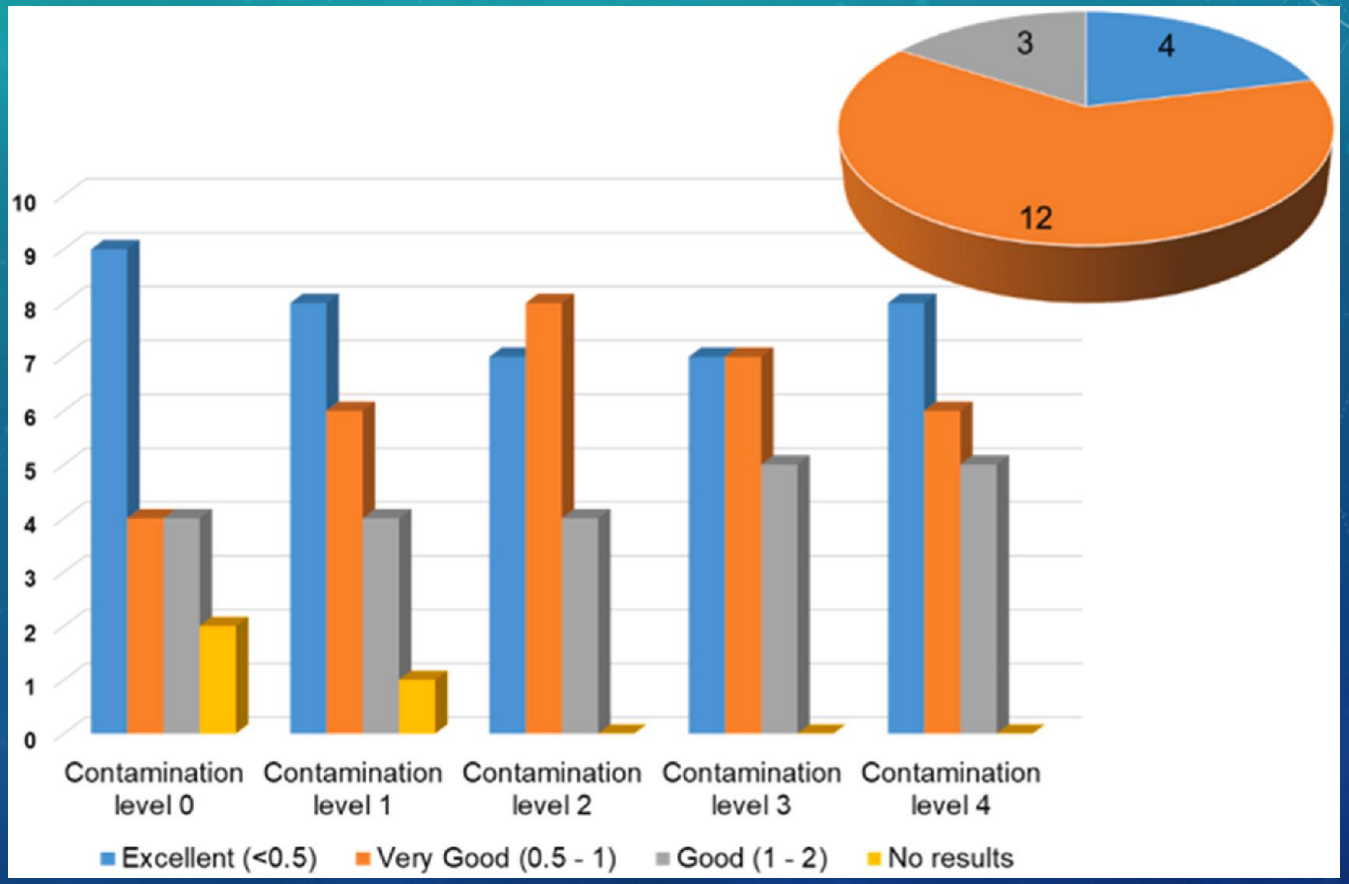
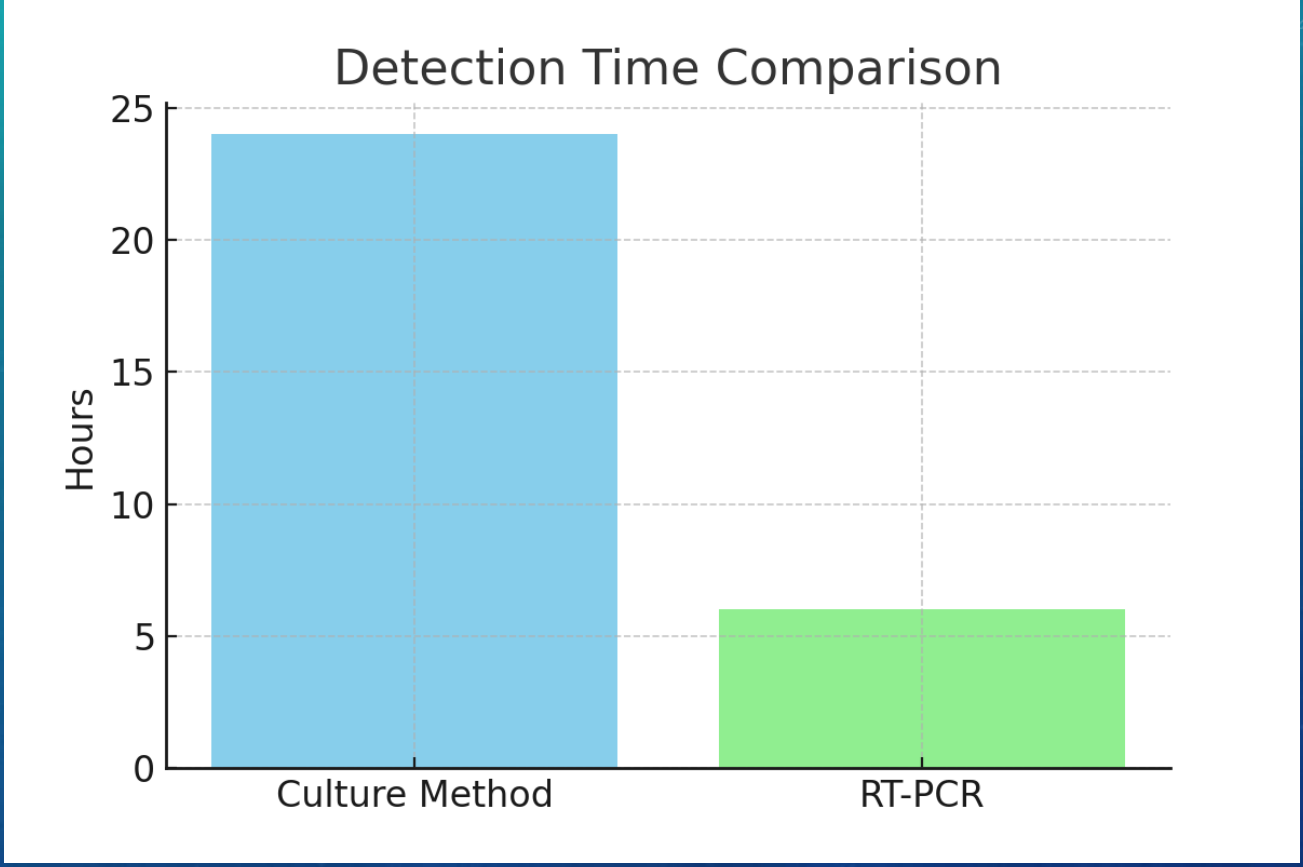
RESULTS (2ND STUDY): Z-SCORES OF THE ALTERNATIVE METHOD



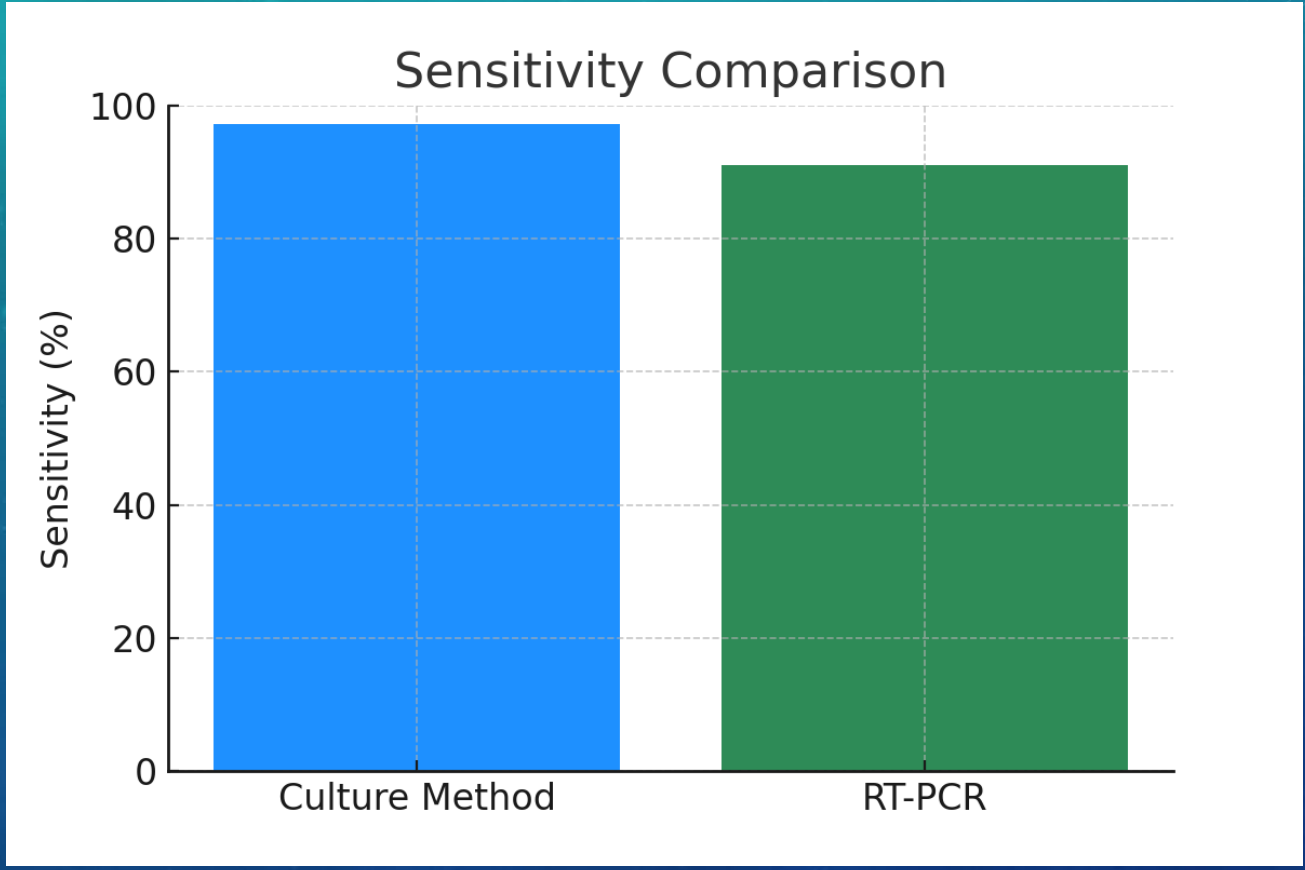
KEY RESULTS (2ND STUDY)

- All laboratories showed performance Z-scores for the considered five contamination levels which are below the critical threshold of 2.
- Acceptability Limits (AL) of the relative level of detection (RLOD) is set at 2.5 according to ISO 16140 part 2 and in this study, the RLOD = 1.79
- Sensitivity – Reference: 97.2%, RT-PCR: 91.1%
- Specificity – Reference: 100%, RT-PCR: 97.7%

DETECTION TIME COMPARISON



SENSITIVITY COMPARISON



CHALLENGES & LEARNINGS

- Lab experience impacts performance
- Critical steps: RNA extraction & filter choice
- Polycarbonate filters perform better than cellulose for nucleic acid extraction

DISCUSSION – TECHNICAL LESSONS

- Filter type crucial – polycarbonate better for RNA recovery
- Proper lysis (vortex/bead beating) improves sensitivity
- PCR master mix must match equipment (ROX concentration)
- Requires operator training and good lab practice

DISCUSSION – OPERATIONAL INSIGHTS

- RT-PCR requires training & practice
- Risk of contamination during RNA work
- Standardisation & SOP refinements are key to success

CONCLUSIONS

- RT-PCR enables rapid detection of *E. coli* in drinking water
- Valid alternative when quick response is needed
- Could complement culture methods, not replace them
- EU-wide study supports adoption with proper validation

FOR OPEN DISCUSSION

In what context does this molecular method should have an application?

1. as a routine method? (cost/specialised staff)
2. in case of emergency (outbreak/major disaster)?
3. in need of speed?
4. In case of massive social gatherings?
5. some or all of the above?

THANK YOU