PATH-SAFE
WS3a

Rapid diagnostic technologies for foodborne pathogens

PathSafe webinar series

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Pathsafe - Workstream 3a Technology Readiness Level (TRL) study

Workpackage 1

Literature review of technologies

Technology Readiness Level framework

Clarivate Web of Science™

Scopus®

Database of technologies & levels

Workpackage 2

Stakeholder map and database

Stakeholder requirements & feedback

Workpackage 3

Selection of 2(?) technologies for pilot study

Plan and conduct pilot study

WS3a - Scope

Horizon scanning and technology readiness level study, with in-field testing of rapid diagnostic technology

Target pathogens: Norovirus, Campylobacter, Salmonella, Listeria, Clostridium, Indicator organisms

Sample matrixes: Water, Meat, Shellfish, Dairy, Swabs, Animal feed, Fish, Fresh produce, RTE (Ready to Eat products)

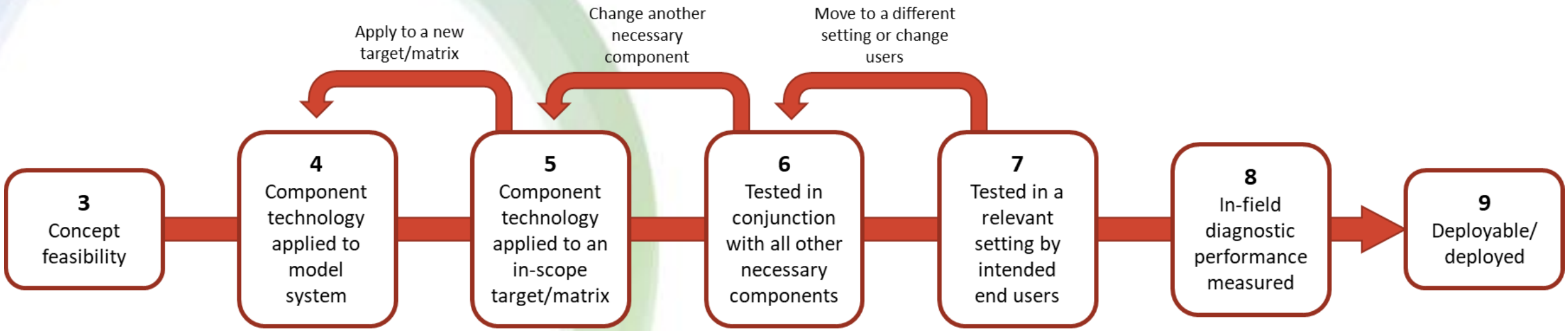
Overview of Literature Review

- Broad scope which returned 28142 Papers .
- 8489 eliminated due to duplication.
- 16485 eliminated due to lack of relevance or no additional content.
- 3168 papers collated into categories of technologies for full review.

Results Technology Review

Technology	References per pathogen						
	Norovirus	Campylo-bacter	Clostridium	Listeria	Salmonella	E. coli	TOTAL
Polymerase chain reaction (PCR)	1	1	3	1	11	7	**190**
LAMP/Loop mediated Isothermal amplification	1	5	1	9	33	31	**314**
Other isothermal amplification methods	5	0	1	12	23	17	**455**
Lateral flow tests	0	1	1	6	11	11	**185**
Biosensor	11	5	2	14	27	45	**677**
Crude extraction method	2	5	5	3	21	22	**129**
Aptamers	5	2	1	8	24	24	**168**
CRISPR (Clustered regularly interspaced short palindromic repeats)	2	2	0	4	11	10	**163**
ATP bioluminescence	0	0	0	1	1	6	**14**
Nanopore sequencing	0	1	0	0	0	1	**26**

Summary of TRL framework



Primarily lab-based activity

On-site activity

Individual component technologies

Sample-to-result solutions

Any target or sample type/model system

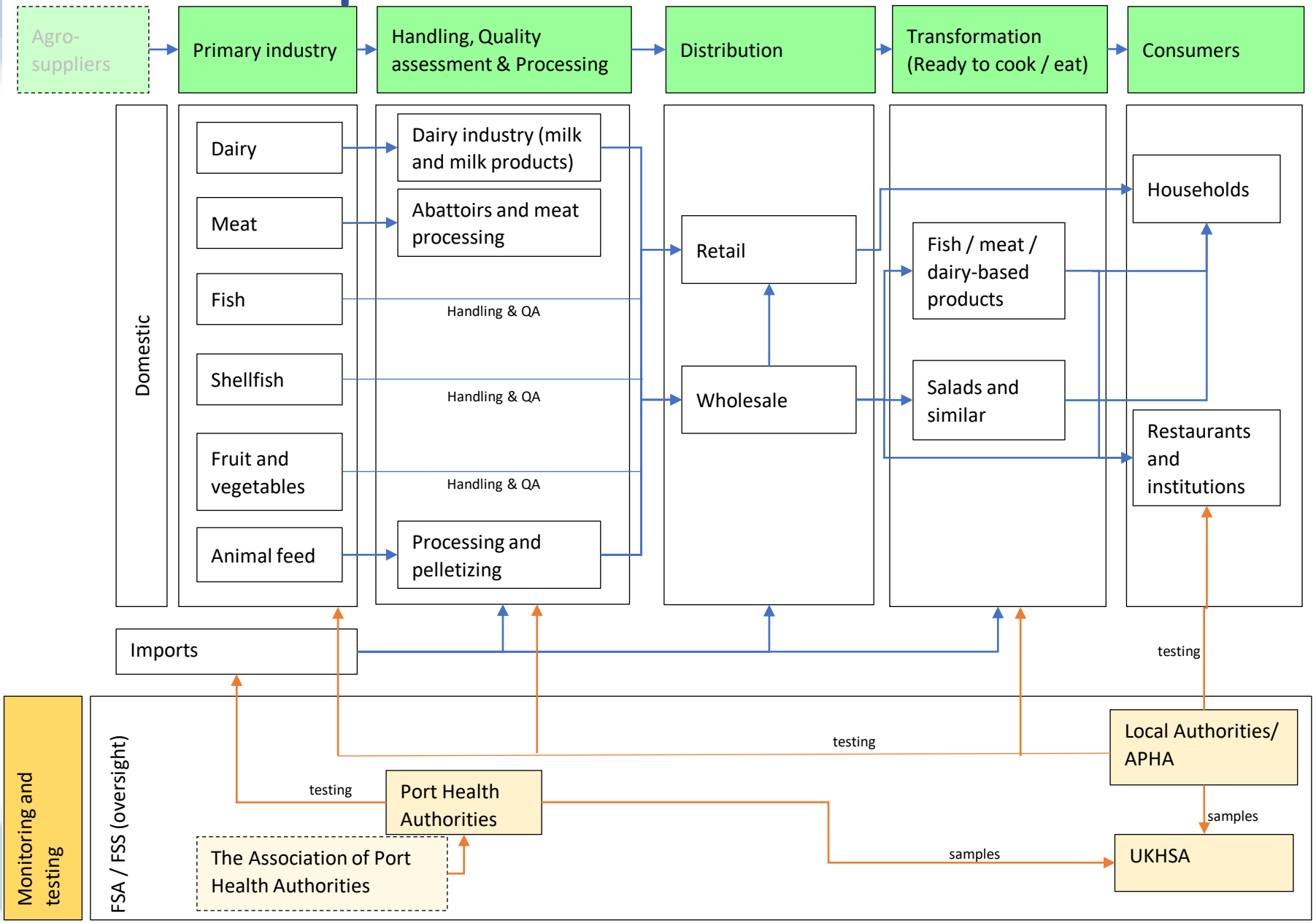
Specific, in-scope target and/or sample type

Database with TRLs assigned

- TRL assessment tool was used to answer questions about each technology we were assessing to guide the TRL assignment.

Technology	Highest TRL for any target/matrix (including out of scope)	TRL for in-scope target *Norovirus*	TRL for in-scope target *Campylobacter*	TRL for in-scope target *Clostridium*	TRL for in-scope target *Listeria*	TRL for in-scope target *Salmonella*	TRL for in-scope target E. *coli*
Portable real-time PCR 1	6	4	5	5	6	6	5
Portable real-time PCR 2	9 (FDA approved for SARS-CoV-2 testing)	4	4	4	5	5	5
Real-time LAMP (fluorescence)	6 (instruments are commercially available but testing by relevant end users generally has not been demonstrated)	5	5	4	5	5	5
LAMP with LFD read out	6	4	5	5	5	5	5
LFDs with antibodies	9	9	9	4	9	9	9
Chemiluminescent probes	8 or 9	4	4	4	8 or 9	8 or 9	8 or 9

Stakeholder map



Engagement approach

Strategic steer

2 focus groups & 3 interviews (FSA, DEFRA, APHA, CEFAS, UKHSA)

Operational feasibility & need

7 end-user interviews

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Operational feasibility & need

7 end-user interviews (primary industries, processing, retail, import, testing)

Needs and opportunities

Statutory testing

- Pen-side testing
- Product testing (production & port health)

vs

Non-statutory / additional testing

- Customer assurance (e.g. norovirus in shellfish)
- Hygiene testing (e.g. counter-tops)
- Production decisions (e.g. irrigation water)

Test requirements (scenario specific)

- Test performance:
  - Pathogen viability
  - Presence / absence (specific strain) vs count
  - Presumptive results & false positives
- Cost(!) vs speed
- Ease of use: training possible (issue: live cultures)

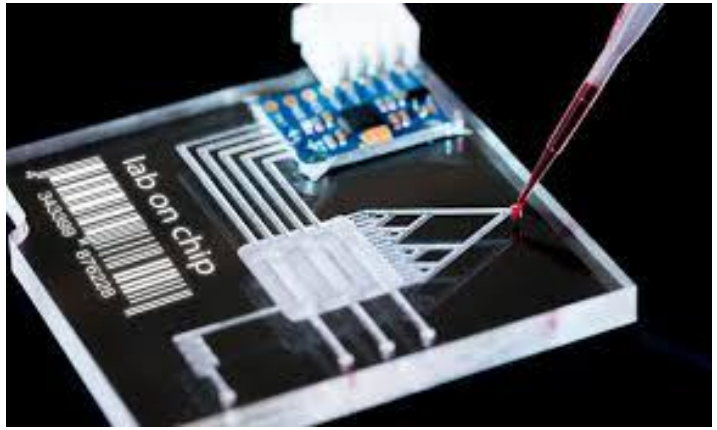
Technologies for pilot study

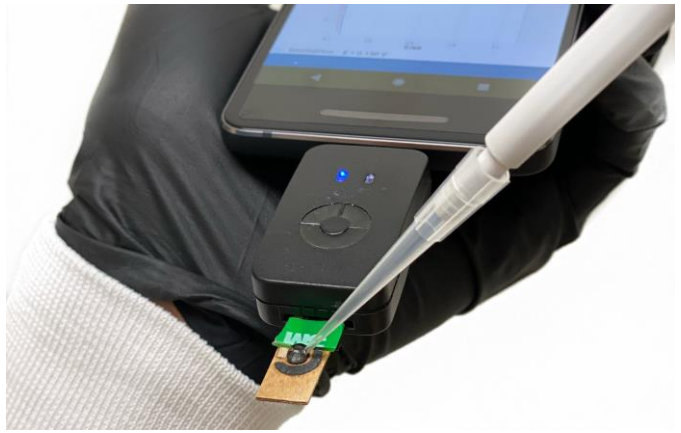
Lab-on-a-chip and biosensor devices may be promising future technologies.

- Currently only in the prototype stage for in scope pathogens.

Nanopore sequencing

- TRL 4 or 5 for in-scope pathogens
- High complexity for end users
- Long time to result





Portable real-time PCR

- Many portable real-time PCR devices are now commercially available.

- **Advantages:** can give rapid results ~ 30-60 minutes, could be used with other PCR assays in the literature, can include test controls for more confidence in results. Equivalent analytical sensitivity and specificity to laboratory tests. Can be quantitative. Commercial kits available.
- **Disadvantages:** Relatively complex protocol including pipetting steps. High costs, including buying the instrument.
- **Next steps:** TRL 6 Assessing the available kits in the lab with all necessary components, and optimise performance (with/without enrichment). TRL 7 demonstrate the technology in an in-scope setting with relevant end users.

+ cheaper ($5 a test, $10,000 instrument)
+ faster

+ simpler workflow
+ remote control though mobile phone app.
- Expensive ($10 a test, £20,000 instrument)

LAMP

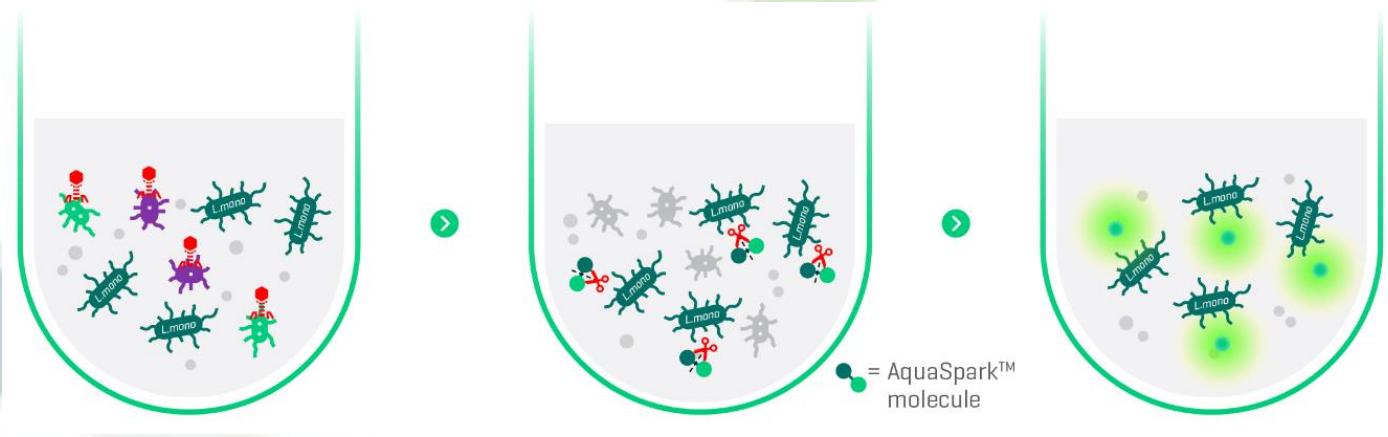
- LAMP has multiple formats with different advantages. Real-time LAMP with fluorescence (or turbidity); LAMP with simple readout (LFD or colorimetric).
- **Advantages:** analytical sensitivity and specificity equivalent to laboratory PCR. Assays available in the literature for all target pathogens.
- **Disadvantages:** Relatively complex protocol including pipetting steps. High costs, including buying the instrument. Sample to answer commercial kits are less readily available.
- **Next steps:** real-time kits: TRL 6/7/8 lab validation/verification and then field validation with end users. Other formats: TRL 5/6/7: further optimising performance in the lab, testing both colorimetric and LFD readout methods. Test the performance in a relevant setting with end users.

+ Higher sensitivity
+ real-time results and no post amplification steps
+ Some commercial kits available.

+ Lower initial costs (no instrument required)
+ Simple result interpretation

Chemiluminescence kits

- Chemiluminescent probe technology for the detection of live bacteria – currently used for surface swab samples.
- **Advantages** – simple protocol with closed system so low chance of cross contamination. Relatively cheap test ($7 & 2500 capital), high sensitivity. Detects only live bacteria. Commercial kits available.
- **Disadvantages:** requires enrichment so not a rapid test.
- **Next steps :** TRL 7/8/9: measure diagnostic performance of the tests in an in-scope scenario and get end user feedback, identify any specific logistical barriers to deployment. OR TRL 5/6: adapt tests in the lab for different matrices such as food, optimise and test performance.



Lateral flow tests

- **Advantages:** Simple protocol and interpretation of results. No instruments needed and relatively low test cost (£8). Commercial kits available for most in-scope pathogens.
- **Disadvantages:** low sensitivity unless combined with an enrichment step. Qualitative results.
- **Next steps:** TRL 7/8/9: generating performance data for an in-scope setting where LFDs are not currently used but could be useful. Assessing the generated data to see whether they are a useful test.

Technologies combined with scenarios

- *Listeria* swabs for environmental monitoring - LFDs or Chemiluminescent tests.
- *E. coli* as a faecal indicator in shellfish, meat, irrigation water with LAMP or real-time PCR
- *Norovirus* in shellfish and water – with real-time PCR or LAMP

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