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LAMP assays coupled to automated wastewater sample purification for in-field detection of foodborne pathogens

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INTRODUCTION

Programmes developed in response to the COVID-19 pandemic have demonstrated the utility of wastewater monitoring as a tool to determine the presence and/or prevalence of pathogens that pose a public health risk. In the case offoodborne disease (FBD), more research is needed for in-field diagnostics technology to improve the speed and efficiency of FBD event detection and characterisation.

Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification method that combines rapidity, high specificity, and sensitivity. Cost-efficient and easy-to-use, it represents a powerful alternative to PCR for in-field diagnostics. We have developed LAMP assays for the detection of seven foodborne pathogens: Salmonella spp, Listeria monocytogenes, Norovirus, Adenovirus, Astrovirus, Rotavirus and Sapovirus, with either fluorometric (quantitative) or colour-change (qualitative) reading outputs. Coupled to an automated wastewater sample purification system, this work could open the path to the development of a rapid on-site FBD surveillance capability.

LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

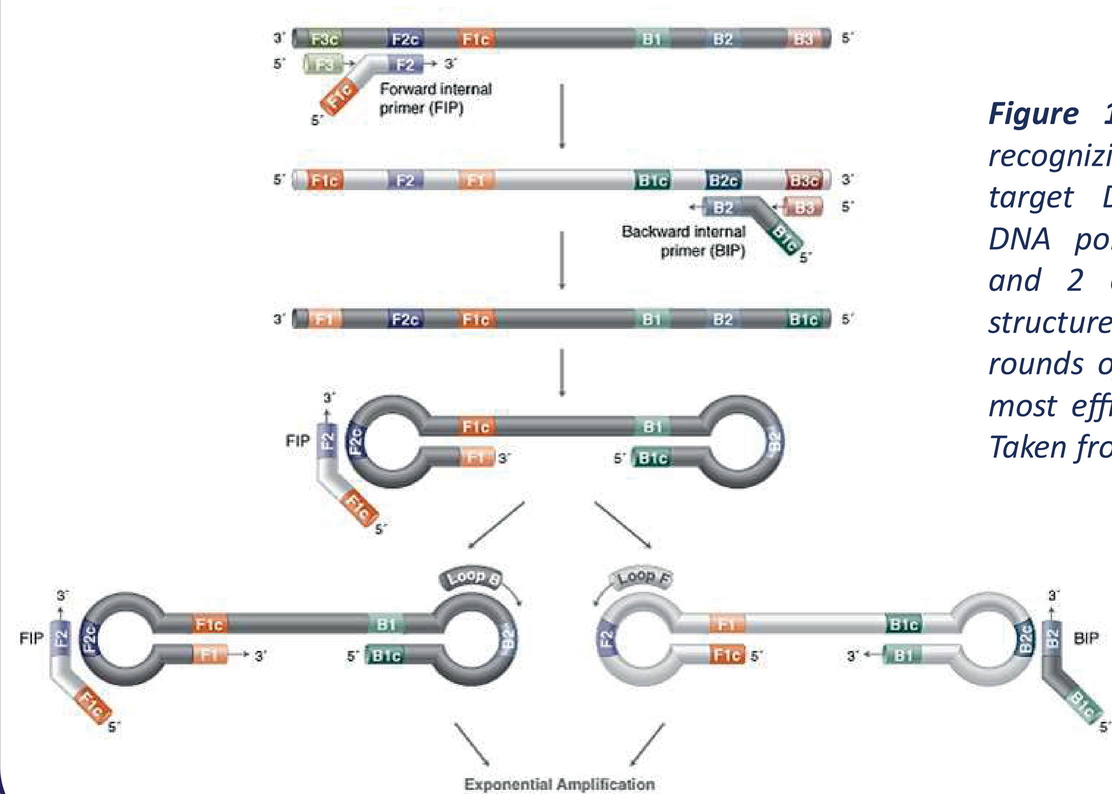

Figure 1. LAMP uses 4-6 primers recognizing 6-8 distinct regions of target DNA. The strand-displacing DNA polymerase initiates synthesis and 2 ofthe primers form loop structures to facilitate subsequent rounds of amplification. LAMP works most efficiently between 63 – 65 °C. Taken from (1).

FOODBORNE PATHOGENS AND GENE TARGETS

Organism	Target gene
Salmonella	inva
Listeria monocytogenes	iap
Norovirus GI	ORF1/ORF2
Norovirus GII	ORF1/ORF2
Adenovirus (F41)	Penton base
Astrovirus	HAstVgp2
Rotavirus	NSP3
Sapovirus	ORF1

Table 1: List ofthe foodborne pathogens and the gene targets for this study. The specificity and cross-reactivity of each primer set were tested in silico.

ASSESSMENT OF THE LAMP PRIMER SETS

Fluorometric LAMP experiments were first run to quantitatively assess the LoD and detection time (t_d) for each target (Figure 3).

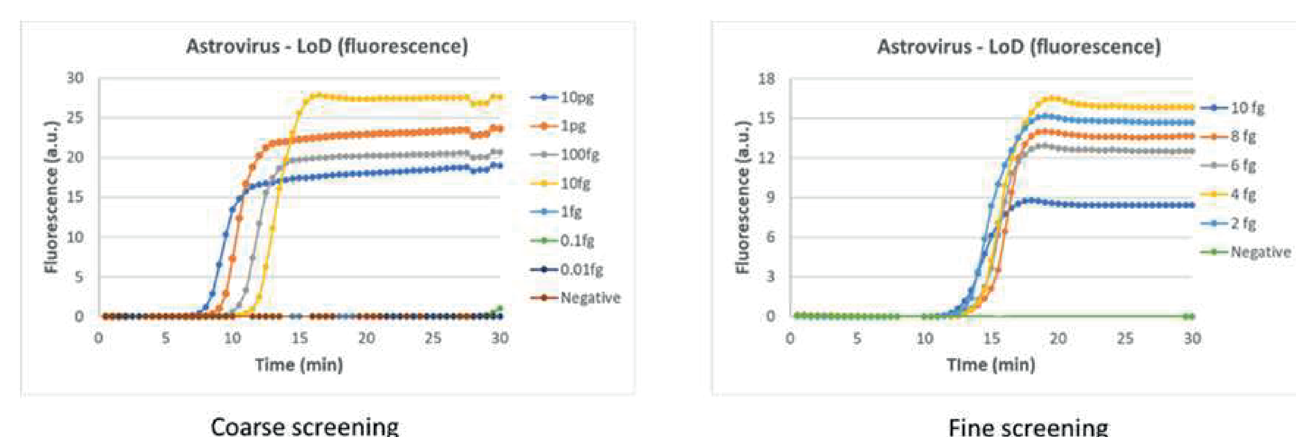

Figure 2. Left: Coarse screening ofthe LoD. Right: the LoD is determined more accurately by screening between the concentrations ofthe last positive amplification and first negative result ofthe coarse screening.

LoD and t_d experiments were run for each target using fluorescence and colour change (Figure 3) as reading outputs (Tables 2 and 3). Fluorescence output allowed for direct comparison with results by qPCR.

Target	Fluo LAMP LoD (fg)	t_d at LoD (min)	Cq eq. at LoD (qPCR)	Copy number (copies / µL)
Salmonella	0.1	15	37	3
Listeria	0.1	10	33	16
NGI	0.5	17	31	62.5
NGII	1	12	36	10
Adenovirus	1	15	37	390
Astrovirus	2	14	31	216.5
Rotavirus	0.1	11	35	6.5
Sapovirus	1	17	32	4209

Table 2: Results ofthe LoD and td experiments using fluorescence as the reading output. The equivalenttarget copy number was calculated using NEBioCalculator (2).

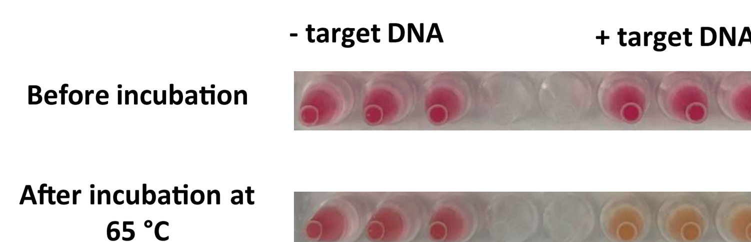

Figure 3. LAMP reactions with colour change reading output. DNA amplification leads to a decrease ofthe pH ofthe solution, leading to a colour change from magenta to yellow.

Target	Colorimetric LAMP LoD (fg)	t_d at LoD (min)	Copy number (copies / µL)
Salmonella	1	25	3
Listeria	0.1	15	16
NGI	0.5	20	62.5
NGII	1	15	10
Adenovirus	1	20	390
Astrovirus	1	30	216.5
Rotavirus	0.1	15	6.5
Sapovirus	0.5	17	4209

Table 3: Results ofthe LoD and t_d experiments using colour change as the reading output.

LAMP ASSAYS USING WASTEWATER SAMPLE ELUENTS

Near-source wastewater (WW) samples were purified using the extraction/purification protocol developed by 20/30 Labs and spiked eluents analysed using colorimetric LAMP. The results are summarised in Table 3.

Target	Colorimetric LAMP LoD (fg)	Copy number (copies / µL)
Salmonella	1	3
Listeria	0.1	16
NGI	0.5	62.5
NGII	1	10
Adenovirus	1	390
Astrovirus	1	216.5
Rotavirus	0.1	6.5
Sapovirus	0.5	4209

***Table 3.** Summary ofthe LoD and td experiments using WW sample eluents.*

AUTOMATED WASTEWATER EXTRACTION SYSTEM

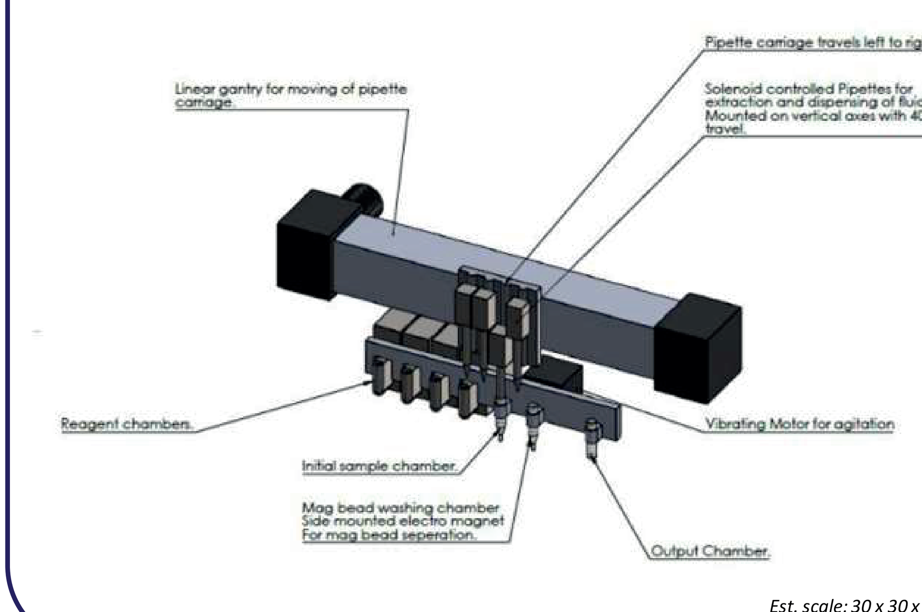

Figure 4. Proposed fully automated system for in-field sample preparation. The system will be designed to ultimately integrate autosampling and proceed with sample analysis (e.g. LAMP).

CONCLUSIONS

- **Quantitative (fluorescence) and qualitative (colour change) LAMP assays were successfully developed to detect 8 foodborne pathogens.**
- **These LAMP assays worked successfully with wastewater eluent (robustto potential carried-over inhibitors).**
- **The LAMP assays are sensitive, highly specific and rapid (t_d at LoD < 20 min).**
- **The results obtained using LAMP are comparable to those using qPCR.**
- **An automated extraction/purification system is currently being developed to provide a hands-free, rapid diagnostics solution for wastewater sample analysis.**

References

(1) https://international.neb.com/applications/dna-amplification-pcr-and-qpcr/isothermal-amplification/loop-mediated-isothermal-amplification-lamp
(2) *From the NEB website (https://nebiocalculator.neb.com/#!/dsdnaamt).*