Development of a new LAMP assay for diagnosing the main meningitis pathogens

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Introduction

Meningitis is a serious disease with significant burden in low- and middle-income countries. Current diagnostic tests are expensive and difficult to use in secondary health centres where resources are limited. LAMP assays could be an alternative method to bring molecular detection closer to patients. This study therefore aimed to develop standardized LAMP assays that could detect in parallel reactions the four main pathogens causing meningitis using improved gene targets

Methods

- ✓ Three LAMP reaction conditions were tested to determine the best method : the method of **McKenna et *al.,* 2011** , the method of **Tanner et Evans, 2014** and the method of **Kim et *al*., 2012**;
- ✓ Optimisation tests were carried out for the LAMP condition, which did not work in the standard condition, by varying the temperature and by varying the concentration of DNA polymerase.
- ✓ Following the tests, the LAMP condition described by **Kim et *al*., 2012** was chosen as the best performing.
- ✓ The samples were subjected to the LAMP test and the results were analysed. The performance of the test was evaluated using various formulae, including sensitivity, specificity, negative predictive value and positive predictive value.

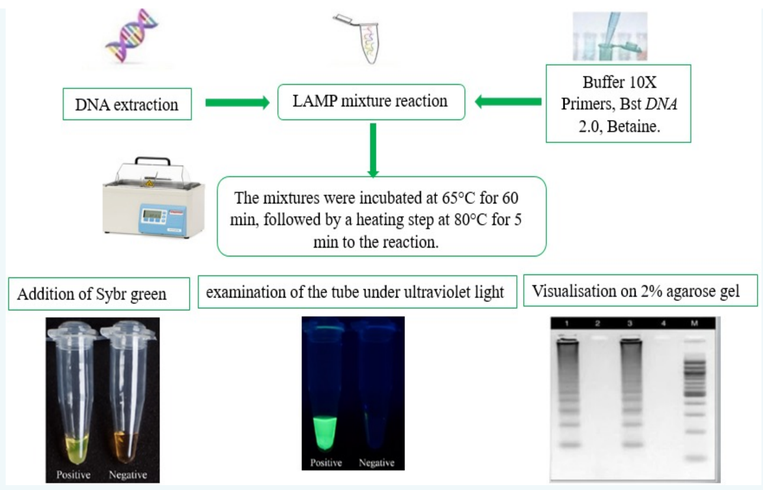

Figure 1: Flow diagram of LAMP test for detection of *Haemophilus influenzae,* Group b *Streptococcus, Neisseria meningitidis* and *Streptococcus pneumoniae*

Target	FP	FN	TP	TN	Sensitivity (%)	Specificity (%)	PPV (%)	VPN (%)
fucK	0	0	7	42	100	100	100	100
sodC	0	0	13	36	100	100	100	100
cfb	0	1	6	42	86	100	100	97
psaA	0	0	8	41	100	100	100	100

FP : False Positive, FN : False Negative, TP : True Positive, TN : True Negative, PPV : Positive predictive value, NPV : negative predictive value

Table 2: LAMP assay statistics

Target	Name	Séquence
Fuck	F3-*fucK*	GATGTTTTCCAAAAAATGGCTAA
	B3-*fucK*	TCCAACTTTTTCACCTGCA
	FIP-*fuck*	CCATTGTGTGATCTGTAGTGAATTGCGTTTTTATTTCGTCA ATGCT
	BIP-*fuck*	CGGGAACATCAATGATGACAAACATGGTTATTACTTAAAC CCAGC
	LB-*Fuck*	TTGGGATCCATCGATTTTAGCATC
Cfb	F3-*Cfb*	GGTGCATTGTTATTTTCACCA
	B3-*Cfb*	TCAACACTAGTAATAGCCTCA
	FIP-*Cfb*	GCCATTTGCTGGGCTTGATTATTACTTTTAGTACATGCTGA TCAAGTGAC
	BIP-*Cfb*	AGCTTGATCAAGATAGCATTCAGTTTTTTTAACCGGTTTT TCATAATCTGTTC
	LF-*Cfb*	ACATGATTTACCACTTGTGGAG
Sod C	F3-*Sod C*	GTAACAAAGATGTGGGTACAG
	B3-*Sod C*	CCATGGGTAACCATGTTGT
	FIP-*Sod C*	GCCTTCGCTTAATCCTTGTAAATCATTTTTGACTATTACTG AATCTAACTATGG
	BIP-*Sod C*	CCAAGCTGTGAGCCAAAAGATTTTCACCTTTAGGATCCC AGTG
	LB-*Sod C*	GACAGCTGGTTTAGGCGC
PsaA	F3- *PsaA*	CCAAGTGCCTACATCTGG
	B3-*PsaA*	AATCATAGCGATGACGACTA
	FIP-*PsaA*	GCAAGTAATGTTGCTCATTTTCTGATTTTATTGCATATTGC CAGTGC
	BIP-*PsaA*	CCTTCCAACGGAACCGGTATTTTTCGAGTACTCAAACGG AGTTC
	LB-*PsaA*	GAACAAGTGGCTTATTTGGATAACG

Table 1: Target primers designed for the detection of *Haemophilus influenzae,* Group b *Streptococcus, Neisseria meningitidis* and *Streptococcus pneumoniae* by lamp assay

Results

- ✓ Of all the targets tested, only SodC, psaA, cfb and fucK showed signs of amplification using all three reaction conditions. However, the LAMP reactions using Thermopol buffer and the Isotherm were not reproducible. These reaction conditions were therefore abandoned. The final reaction using 1X housemade buffer worked well, was reproducible and therefore was selected to perform the validation assays.
- ✓ The LAMP test targeting the *fucK* gene showed a sensitivity, specificity, positive predictive value and negative predictive value of 100%. All *Haemophilus influenzae* samples tested positive, while the other bacteria in the panel tested negative (Table 2; figure 2).
- ✓ The LAMP test targeting the cfb gene revealed that 6 out of 7 Streptococcus agalactiae were detected by the test, while the other bacteria in the panel were negative. Sensitivity was therefore 86%, specificity and positive predictive value 100% and negative predictive value 97% (Table2; figure 3).
- ✓ The LAMP test targeting the *sodC* gene showed a sensitivity, specificity, positive predictive value and negative predictive value of 100%. All *Neisseria meningitis* samples tested positive, while the other bacteria in the panel tested negative (Table 2;figure 4).
- ✓ The LAMP test targeting the *psaA* gene showed a sensitivity, specificity, positive predictive value and negative predictive value of 100%. All *Streptococcus pneumoniae* samples tested positive, while the other bacteria in the panel tested negative (Table 2;figure 5).

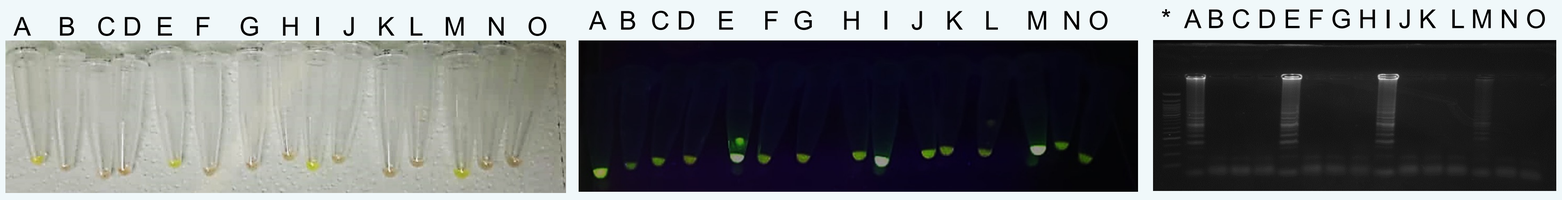

Figure 2: Visualisation of products resulting from the LAMP assay targeting the *H. influenzae fucK* gene

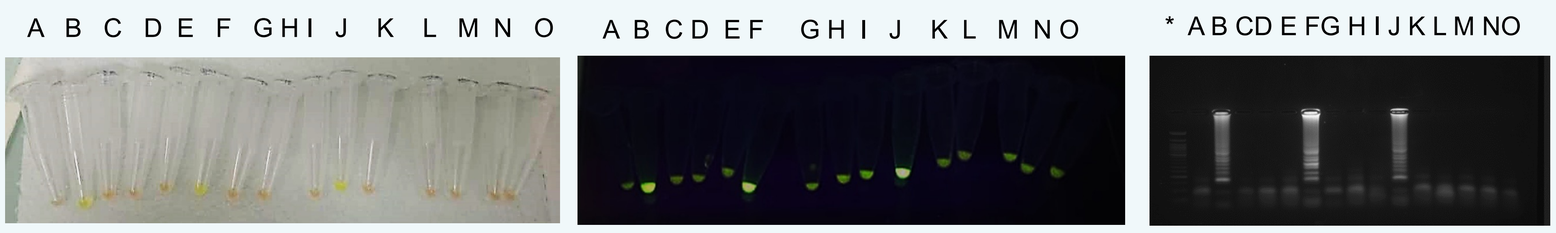

Figure 3: Visualisation of products resulting from the LAMP assay targeting the *S. agalactiae cfb* gene

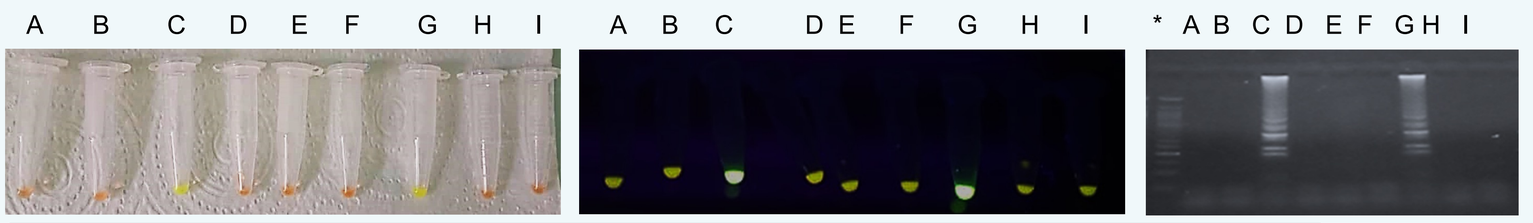

Figure 4: Visualisation of products resulting from the LAMP assay targeting the *N. meningitidis sodC* gene

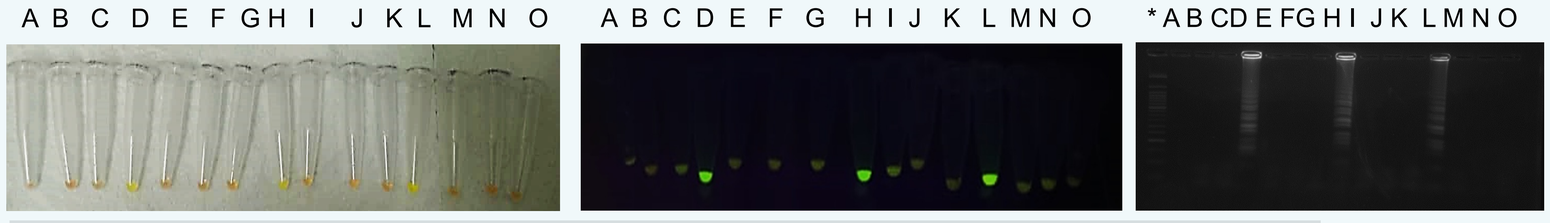

Figure 5: Visualisation of products resulting from the LAMP assay targeting the *S. pneumoniae psaA* gene

Conclusion

we have successfully developed rapid and sensitive LAMP assays for the detection of *H. influenzae, S. agalactiae, N. meningitidis*, and *S. pneumoniae.* They have been validated on DNA extracted from bacterial strains. Further validation with clinical samples is needed alongside further development to decrease the amount of sample handling required, making it more user friendly, and ensuring and easier uptake in peripheral settings. Assays using methodologies like LAMP holds great promise for improving meningitis diagnosis and could contribute to more effective management and treatment of the disease in resource limited settings.

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