EVALUATION OF A HYDROGEL BASED POINT-OF-CARE DIAGNOSTIC APPROACH FOR *NEISSERIA GONORRHOEAE* IDENTIFICATION

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BACKGROUND

In resource limited settings, appropriate laboratory infrastructure for the diagnosis of *Neisseria gonorrhoeae* (Ng) infections is often absent¹. The use of reliable and affordable point-of-care tests (POCTs) could provide accurate diagnosis of Ng. A hydrogel-based RT-PCR platform (Aquila Diagnostic Systems Inc.) was previously shown to be successful for diagnosing pathogens such as Herpes simplex virus and *Plasmodium falciparum* from clinical specimens such as blood and genital swabs^{2,3}. The platform is compact and does not require highly trained personnel. As a potential POCT to diagnose Ng infections, we evaluated this hydrogel-based platform to identify Ng DNA isolated from cultures using novel, Ng specific primers.

METHODS

REFERENCES

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RESULTS

TABLE 1. Evaluation of Eleven Primer Pairs Using *N. gonorrhoeae*, non-*N. gonorrhoeae* and non-*N. gonorrhoeae* Species

Bacterial Species	# Isolates	Amplifications with Primer Pairs										
		3	8-4	16	17-1	17-2	21-5	31-2	2	31-3	8-3	13
<i>N. gonorrhoeae</i>	130	130	130	130	130	130	130	130	129	102	130	130
<i>N. flava</i>	2	0	0	0	0	0	0	0	0	0	1	2
<i>N. subflava</i>	2	0	0	0	0	0	0	0	0	0	0	1
<i>N. elongata</i>	2	0	0	0	0	0	0	0	0	0	0	0
<i>N. mucosa</i>	3	0	0	0	0	0	0	0	0	0	0	1
<i>N. lactamica</i>	4	0	0	0	0	0	0	0	0	0	2	2
<i>N. perflava/sicca</i>	5	0	0	0	0	0	0	0	0	0	0	3
<i>N. flavescens</i>	2	0	0	0	0	0	0	0	0	0	0	1
<i>N. polysacchareae</i>	4	0	0	0	0	0	0	0	0	0	1	2
<i>Neisseria species</i>	6	0	0	0	0	0	0	0	0	0	0	0
Non- <i>Neisseria</i> species	10	0	0	0	0	0	0	0	0	0	0	0

- Seven primer pairs (3, 8-4, 16, 17-1, 17-2, 21-5, 31-2) had 100% sensitivity and specificity (Table 1)
- The hydrogel method was 100% sensitive and specific with 100 ng/μL Ng DNA (Table 2)
- While having a positive Ng diagnosis by melt curve analysis, most samples contained negative threshold cycle (Ct) values (Table 2)
- A final concentration of 4X SYBR dye resulted in favourable Ct values (Table 2)

TABLE 2. Evaluation of Hydrogel Methods Using Primer Pairs 3, 17-1 and 21-5

Primer Pair	Method	DNA Conc. (ng//μL)	# Ng Isolates		Based on Tm	
			Identified by Tm value ^a	Positive Ct values	Sensitivity (%)	Specificity (%)
3	Hydrogel	50	44	0	88	100
3	Hydrogel	70	46	33	92	100
3	Hydrogel	100	50	0	100	100
3	4X SYBR ^b	100	50	50	100	100
3	RT-PCR control	70	50	50	100	67 ^c
17-1	Hydrogel	50	50	0	100	100
17-1	Hydrogel	70	50	0	100	100
17-1	Hydrogel	100	50	0	100	100
17-1	RT-PCR control	70	50	50	100	100
21-5	Hydrogel	50	47	0	94	100
21-5	Hydrogel	70	50	0	100	100
21-5	Hydrogel	100	50	0	100	100
21-5	RT-PCR control	70	48	49	96	100

^a*N. gonorrhoeae* isolates n=50; ^bAmplification with 4X SYBR dye was only performed with primer pair 3; ^cOne *Escherichia coli* isolate was identified as *N. gonorrhoeae* positive

CONCLUSIONS

- 3 novel primer pairs successfully identify Ng DNA isolated from cultures in the hydrogel platform
- These primers represent new diagnostics
- The hydrogel platform will be tested using clinical specimens (i.e. urines, self-collected vaginal or rectal swabs)
- The hydrogel platform has potential as a POCT for Ng diagnosis