

Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Carbon nanoparticles in lateral flow methods to detect genes encoding virulence factors of Shiga toxin-producing *Escherichia coli*

P. Noguera, G.A. Posthuma-Trumpie, M. van Tuil, F.J. van der Wal, A. de Boer, A.P.H.A. Moers, A. van Amerongen

NALFIA optimization

(In this section the selection of the optimal conditions for NALFIA strip manufacturing and use is described)

The intensity of the test line for Cy5 (16S) and DIG (*eae*) for strips manufactured with different amounts of antibody, and used with different amounts of PCR product, is shown in Fig. S2. As can be seen for Cy5, good line intensity (around 3×10^5 pixels) was achieved when NC was coated with at least 300 $\mu\text{g/mL}$ and using (at least) 1 μL PCR product. For DIG, a good signal was achieved with 100 $\mu\text{g/mL}$ antibody and 0.1 μL PCR product (similar procedure was done for the other antibodies). For convenience, the final amount of PCR volume selected was the same for all factors amplified. Final NALFIAs were run in microtiter wells containing a mixture of 1 μL of PCR product (sample), 1 μL carbon-neutravidin conjugate and 98 μL RB.

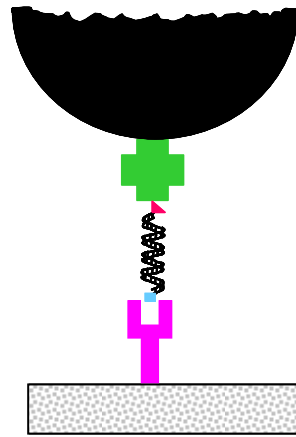


Fig. S1 Detection of a double-labelled amplicon between a carbon nanoparticle and a specific antibody

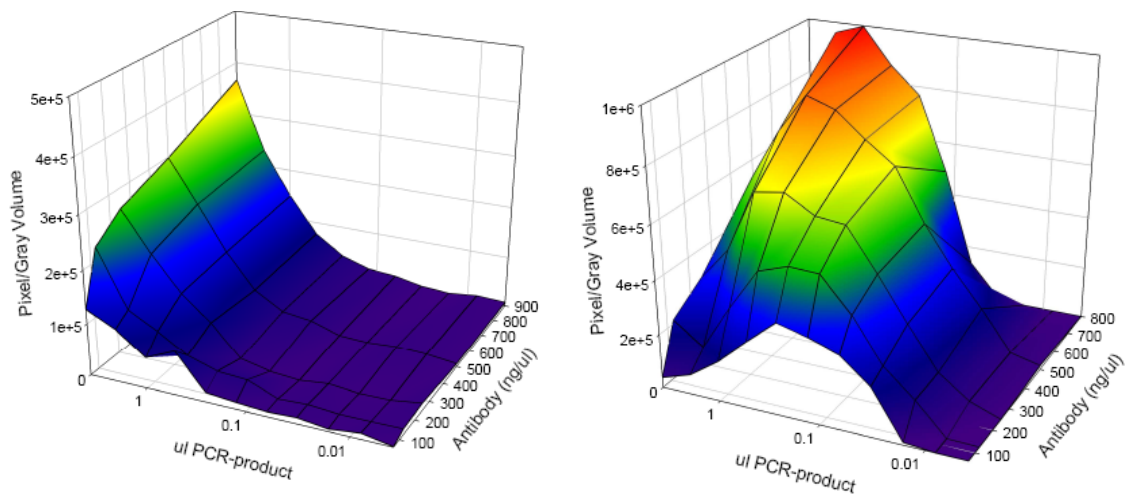


Fig. S2 Line intensity expressed as pixel grey volume as a function of the amount of antibody sprayed on the nitrocellulose membrane and the amount of PCR product used. Left: Cy5 (16 S); Right: DIG (*eae*)

Table S1 Performance of qualitative assays. Definition of terms and reliability parameters

Results achieved in the test procedure	Results achieved in the reference method			Total Q (=TP+FP) Q' (=FN+TN) S (=P+P'=Q+Q')
	Positive	Positive	Negative	
		True Positive (TP)	False Positive (FP)	
	Negative	False Negative (FN)	True Negative (TN)	
	Total	P (= TP + FN)	P' (= FP + TN)	

Parameter	Equation
Sensitivity	TP/P
Specificity	TN/P'
Positive predictive value	TP/Q
Negative predictive value	TN/Q'
Efficiency (E)	(TP+TN)/S
Agreement by chance (A)	(PQ+P'Q')/S ²
Kappa coefficient (k)	E-A/(1-A)

Table S2 Results achieved by NALFIA with selected *E.coli* strains. Identification of genes encoding the virulence factors for each of the six strains

Sample number	Results					Strain	Effectiveness*
	vt1	vt2	eae	ehxA	16S		
7	-	+	+	+	+	E32511	100 %
2	+	+	+	+	+	EDL 933	100 %
1	-	-	+	+	+	DVI 828	100 %
3	+	-	+	+	+	TB 154A	100 %
5	-	+	-	+	+	B2F1	100 %
4	-	-	+	-	+	Rdec-1	100 %
6	-	-	-	-	-	water	100 %

+/-: Presence/Absence

* see Table S1

Table S3 Reliability parameters for different *E.coli* virulence factors

	vt1	vt2	eae	ehxA	16S
Sensitivity	0.85	1.00	1.00	0.97	1.00
Std. Err.	0.08	0.00	0.00	0.03	0.00
Specificity	0.96	0.89	1.00	1.00	1.00
Std. Err.	0.04	0.07	0.00	0.00	0.00
Positive predictive value	0.94	0.94	1.00	1.00	1.00
Std. Err.	0.05	0.04	0.00	0.00	0.00
Negative predictive value	0.90	1.00	1.00	0.94	1.00
Std. Err.	0.05	0.00	0.00	0.00	0.00
Efficiency	0.92	0.96	1.00	0.98	1.00
Std. Err.	0.04	0.03	0.00	0.02	0.00
Kappa	0.83	0.91	1.00	0.95	1.00
Std. Err.	0.08	0.06	0.00	0.05	0.00