A flow through assay for rapid, bedside stratification of bloodstream bacterial infection in critically ill patients: a pilot study

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Supplementary Information

A

Septiflo-N color chart: histogram			
Color code	Red	Green	Blue
	255	112	182
	255	144	195
	253	169	207
	255	197	230
	255	226	255

B

Septiflo-P color chart: histogram			
Color code	Red	Green	Blue
	255	126	182
	255	164	203
	255	204	236
	255	226	255

Fig. S1. Histogram color charts for **(A)** Septiflo-N and **(B)** Septiflo-P.

Table S1: PCR reagents and thermocycling conditions

PCR Components	**Reagent (µl)**		**Thermal cycle conditions**
	Gram (-)	**Gram (+)**	
Taq buffer	2.5	2.5	Initial denaturation: 95°C 5min Denaturation: 95°C 30s Annealing: 52.1°C 50s Primer extension: 72°C 40s Final extension: 72°C 10 min
5 mM $MgCl_2$	2.2	2.2	
2 mM dNTPs	2	2	
50 picomoles forward primers	1	2	
50 picomoles reverse primers	1	2	

Taq enzyme	0.7	0.7	
Template DNA	2	2	
Distilled water	8.6	6.6	
Total volume	20	20	

Table S2: Parametric optimization for the bioassay development

Parameters	**Reagents and conditions**	**Optimized parameters**
Membranes	SCNM-I, CX-1	CX-1
Membrane surface blocking agent	BSA (0.5, 1, 2, 3 and 4 w/v %)	1% w/v BSA
Incubation time for surface blocking agent	0.5, 1, 2, 3, and 4 min	2 min
Membrane surface wetting agent	Tween 80 (0.1, 0.2, 0.3, 0.5, 1% v/v) Tween 20 (0.1, 0.2, 0.3, 0.5, 1% v/v) Pluronic F68 (0.1, 0.2, 0.5 % w/v)	0.1% Tween 20 for Septiflo-P 1% Pluronic F68 for Septiflo-N
AuNP-conjugate passivating agent	PEG (10^2, ….10^4 M) BSA (0.1 and 0.01% w/v)	PEG (1:10^4 M) (AuNP:PEG)
AuNP-conjugate concentration	0.5 to 3 nM	1 nM AuNPs (Septiflo-P) 2 nM AuNPs (Septiflo-N)
Number of AuNP-conjugate injections	1 x 50 uL 2 x 50 µL and 3 x 50 µL	2 injections
Incubation time (sample, surfactant and blocking agent)	0.5, 1, 2, 3, and 4 min	2 min
Washing step	PF water (25, 50 and 100 µL)	50 µL PF water

Table S3: Clinical data showing patient status. Note: The samples with PCT levels < 0.25 ng/mL were considered non-infectious/non-septic. Annotation: NA: Not available, N: Negative, Y: Positive, NE: Not elevated, NG: No growth, MRSA: Methicillin Resistant Staphylococcus aureus

S.N.	PCT (ng/ml)	Neutrophils (%)	WBC (cells/µl)	Toxic granules	Platelets (lakhs/µl)	Blood culture	CRP (mg/L)	Gram +ve PCR	Gram-ve PCR	Septiflo		Sepsis status
										P	N	
1	0.06	93%	9640	Present	2.76	NG	NE	N	Y	N	Y	N
2	0.06	77%	15720	Present	3.33	NA	NA	Y	Y	Y	Y	N
3	0.07	63%	10050	Present	3.87	NG	96	NA	NA	N	N	N
4	0.07	70%	15760	Present	0.51	NA	NA	N	N	N	N	N
5	0.08	60%	10090	Present	1.66	MRSA	6	Y	Y	Y	Y	N
6	0.09	86%	13320	Present	1.54	NG	NE	Y	Y	Y	Y	N
7	0.11	73%	4570	Absent	1.88	NA	NA	N	Y	N	Y	N
8	0.12	76	9410	Absent	2.01	NG	6 - 12	NA	NA	N	N	N
9	0.13	81%	11340	Present	3.95	NA	NA	Y	Y	Y	Y	N
10	0.13	76	5200	Absent	1.29	NG	24	NA	NA	N	N	N
11	0.13	87	6230	Present	1.59	NG	NE	NA	NA	N	Y	N
12	0.13	80%	19320	Present	3.23	NG	NE	N	Y	N	N	N
13	0.14	61	6810	Absent	2.11	NA	NE	NA	NA	N	N	N
14	0.15	58	9640	Absent	2.14	NG	NE	NA	NA	N	N	N
15	0.15	62	7940	Absent	2.05	NA	NA	NA	NA	N	Y	N
16	0.15	73	9320	Absent	3.13	NG	NA	NA	NA	Y	N	N
17	0.15	65	8550	Absent	2.81	NA	NA	NA	NA	N	N	N
18	0.17	79	7900	Absent	2.13	NA	NA	NA	NA	N	Y	N
19	0.17	70	5140	Absent	1.9	NA	NE	NA	NA	N	N	N
20	0.17	49	9270	Absent	2.25	NA	NE	NA	NA	N	Y	N
21	0.18	60	6320	Absent	2.9	NA	NE	NA	NA	N	Y	N
22	0.18	67	10550	Absent	2.58	NA	NE	NA	NA	Y	Y	N
23	0.18	NA	NA	NA	NA	NA	NA	N	Y	N	N	N
24	0.2	79%	7430	Present	0.67	NA	NA	N	Y	N	Y	N
25	0.21	76%	22980	Present	2.49	NA	NA	N	Y	N	Y	N
26	0.21	74%	18670	Absent	3.42	NG	NA	NA	NA	Y	Y	N
27	0.25	80%	7600	Present	0.74	NG	NA	N	Y	N	Y	Y
28	0.28	75 %	7640	Absent	1.5	NG	12	N	Y	N	Y	Y
29	0.3	70%	10140	Present	1.69	NG	NA	N	Y	N	Y	Y
30	0.33	88 %	12550	Present	1.14	NG	24	N	Y	N	Y	Y
31	0.35	63	4550	Absent	3.67	NA	NA	NA	NA	Y	Y	Y
32	0.5	76%	3460	NA	1.56	NG	24	N	Y	N	Y	Y
33	0.52	72%	10580	Present	1.42	NA	NA	Y	N	Y	N	Y
34	0.55	64%	7150	Present	0.49	NA	NA	NA	NA	Y	Y	Y
35	0.58	91	11270	Present	1.72	NA	NA	N	Y	N	Y	Y
36	0.6	89%	21150	Present	2.1	NG	NA	N	Y	N	Y	Y
37	0.61	89%	9670	Present	2.04	NG	NA	N	Y	N	Y	Y
38	0.67	81%	7680	Present	2.73	NA	NA	NA	NA	N	Y	Y
39	0.7	75	13700	Present	3.31	NA	NE	NA	NA	Y	Y	Y
40	0.73	95%	5730	present	1.31	NA	48	NA	NA	Y	Y	Y
41	1.19	77%	13120	Present	3.49	NA	NA	N	N	N	N	Y
42	1.4	78%	28400	Present	3.31	NG	NA	NA	NA	Y	Y	Y
43	1.5	85%	16970	Present	2.96	NG	NA	NA	NA	Y	Y	Y
44	2.7	88%	9500	Present	1.21	NG	96	NA	NA	Y	Y	Y
45	3.9	36%	10020	Present	3.42	NG	96	NA	NA	Y	Y	Y
46	4.11	92%	17460	Present	3.5	NG	NA	N	Y	N	Y	Y
47	6.14	80%	9460	Present	1.86	NA	192	N	Y	N	Y	Y
48	7.4	82%	11000	Present	6.41	NG	NA	NA	NA	Y	Y	Y
49	8.86	94%	29120	Present	1.14	NG	192	Y	Y	Y	Y	Y
50	8.9	90%	10760	Present	0.49	NG	192	Y	Y	Y	Y	Y
51	13.11	88%	17900	Present	1.59	NG	>384	Y	Y	N	Y	Y
52	14.6	93%	23220	Present	1.24	NA	384	Y	Y	N	Y	Y
53	15.12	50%	9770	Absent	2.85	NG	NA	Y	Y	Y	Y	Y
54	17.11	91%	16850	Present	1.49	NG	192	N	Y	N	Y	Y
55	38.4	77%	9920	Present	2.22	NG	96	NA	NA	N	Y	Y

56	65.5	90%	13860	Present	0.47	NG	NA	N	Y	N	Y	Y
57	97.9	90%	12310	Present	0.21	NG	96	NA	NA	N	Y	Y
58	109	76%	9630	Present	1.1	E. coli	48	NA	NA	Y	Y	Y
59	150	79%	6600	Present	2.91	NG	96	NA	NA	N	Y	Y
60	663.8	76%	2750	NA	1.14	NA	96	Y	Y	Y	Y	Y

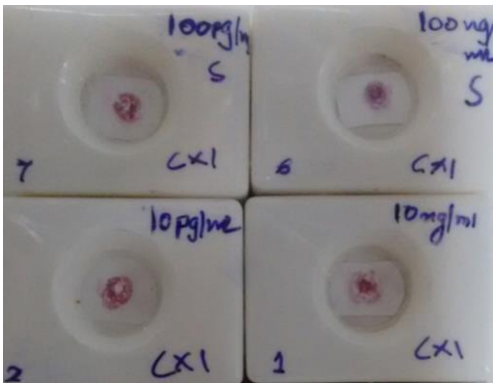


Fig. S2. Representative images for Septiflo assay performed without surfactant.

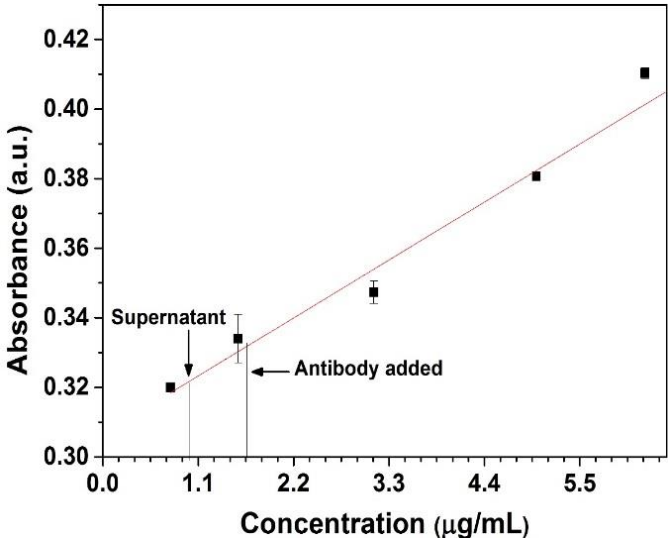
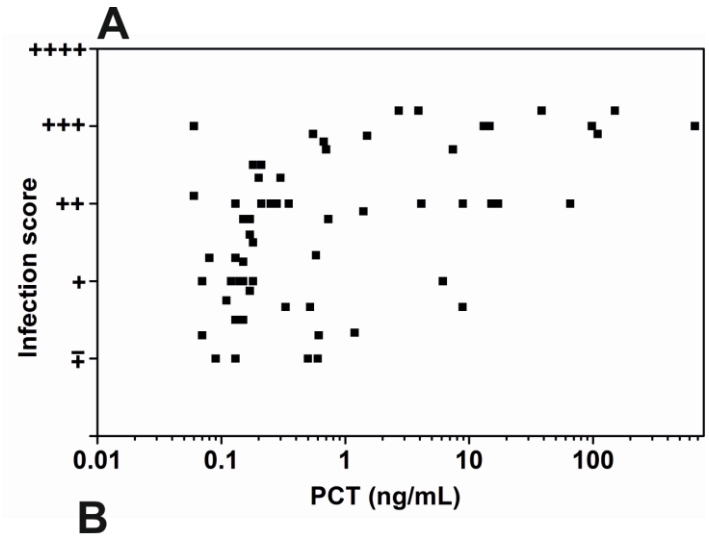


Fig. S3. Calibration graph obtained using Bradford's assay for the quantification of number of antibodies conjugated per AuNP. The results indicated AuNP:mAb molar ratio of ≈1:6.



B

Parameter	Threshold limit	
	1 pg/mL	100 pg/mL
Accuracy (%)	67	70
Sensitivity (%)	100	74
Specificity (%)	9	64
Precision (%)	66	74
PPV (%)	65	18
NPV (%)	100	48
AUC	0.695	

Fig. S4. (A) Correlation of total infection score against PCT values and **(B)** their performance characterization taking different LPS/LTA threshold limits (see Figs. S5 and S6 and Fig. S12 for ROC analysis). The total infection score was calculated by taking the higher of the Septiflo-N/P values.

Definitions:

Accuracy = (TP + TN)/Total
Sensitivity = TP/Actual Yes
Specificity = TN/Actual No
Precision = TP/Predicted Yes
Positive predictive value (PPV) = TP / Predicted Yes
Negative predictive value (NPV) = TN / Predicted No

where, TP = True Positive, TN = True Negative

n = 60	Predicted NO	Predicted YES	
Actual NO	TN = 2	FP = 20	22
Actual YES	FN = 0	TP = 38	38
	2	58	60

Fig. S5. Performance classification of Septiflo assay against PCT based on the highest score of Septiflo-P/N and LPS/LTA <1 pg/mL threshold limit.

n = 60	Predicted NO	Predicted YES	
Actual NO	TN = 11	FP = 30	41
Actual YES	FN = 12	TP = 7	19
	23	36	60

Fig. S6. Performance classification of Septiflo assay against PCT based on the highest score of Septiflo-P/N and LPS/LTA <100 pg/mL threshold limit.

n = 31	Predicted NO	Predicted YES	
Actual NO	TN = 3	FP = 0	3
Actual YES	FN = 2	TP = 26	28
	5	26	31

Fig. S7. Performance classification of Septiflo-N against PCR assuming LPS < 1 pg/mL as threshold.

n = 31	Predicted NO	Predicted YES	
Actual NO	TN = 3	FP = 0	3
Actual YES	FN = 7	TP = 21	28
	10	21	31

Fig. S8. Performance classification of Septiflo-N against PCR assuming LPS < 100 pg/mL as threshold.

n = 31	Predicted NO	Predicted YES	
Actual NO	TN = 20	FP = 0	20
Actual YES	FN = 2	TP = 9	11
	22	9	31

Fig. S9. Performance classification of Septiflo-P against PCR assuming LTA <1 pg/mL as threshold.

n = 31	Predicted NO	Predicted YES	
Actual NO	TN = 21	FP = 0	21
Actual YES	FN = 5	TP = 5	10
	26	5	31

Fig. S10. Performance classification of Septiflo-P against PCR assuming LTA <100 pg/mL as threshold.

n = 21	No. of LTA negative cases	No. of LPS positive cases	Sensitivity
Septiflo-N (100 pg/mL threshold)	**21**	**13**	**62%**
Septiflo-N (1 pg/mL threshold)	**21**	**17**	**81%**

Fig. S11. Out of a total of 21 LTA negative samples with high PCT levels, 62% tested positive for LPS (assuming 100 pg/mL LPS cut-off in blood) and 81% assuming 1 pg/mL cut-off. Of all the 31 patient samples tested with PCR, 96% cases (n=28) showed Gram-negative bacterial presence out of a total n=29 infection cases. This corroborated well with our Septifo-N results which tested positive in 90% (n=26) of the total infection cases.

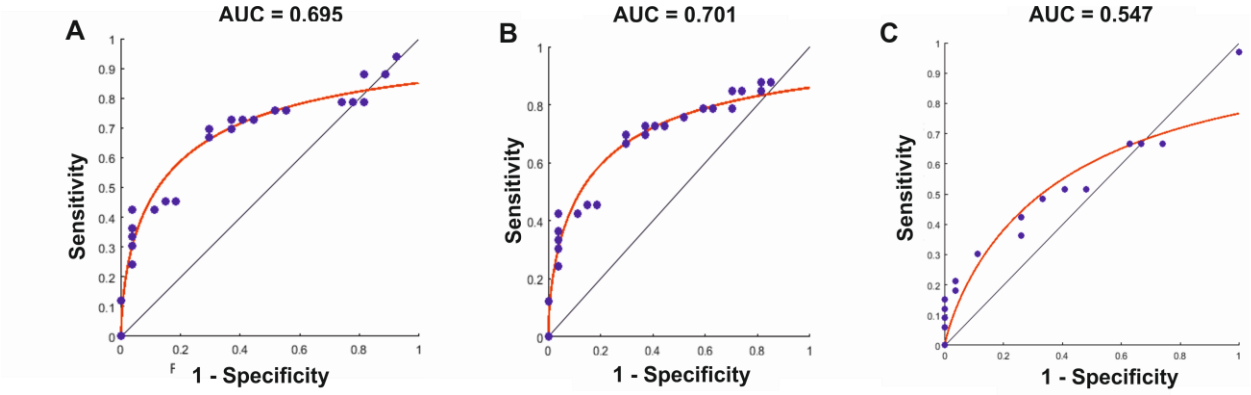


Fig. S12. Receiver operating characteristic (ROC) curves for (A) mixed infections, (B) Septiflo-N and (C) Septiflo-P, compared with PCT biomarker.

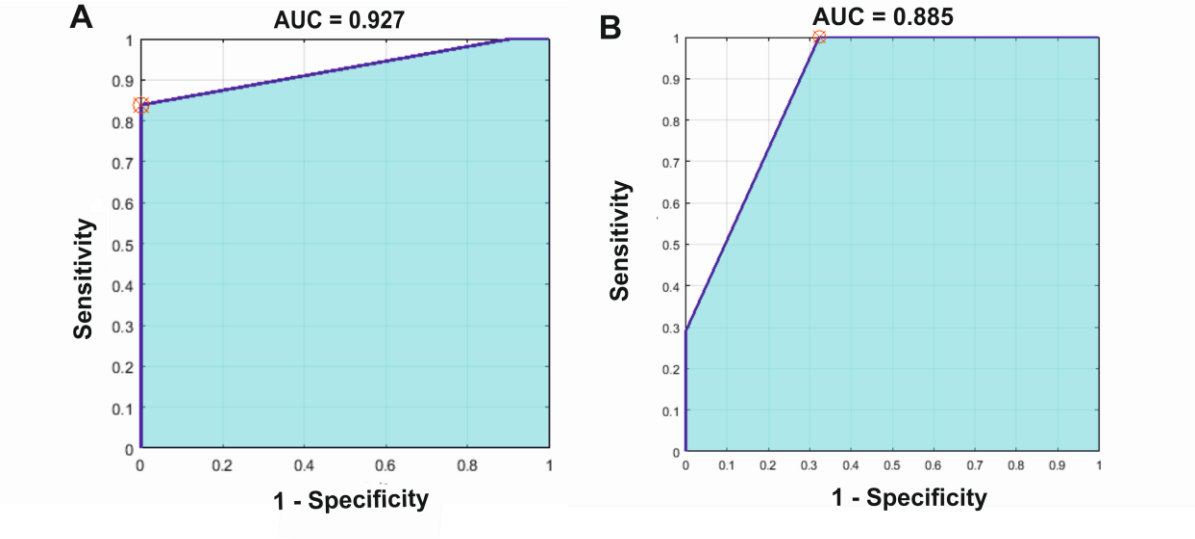


Fig. S13. ROC curves obtained for **(A)** Septiflo-N and **(B)** Septiflo-P using PCR as reference.