Supplementary Information

Quantifying neutrophil phagocytes by droplet digital cell PCR for diagnosis of bacteremia

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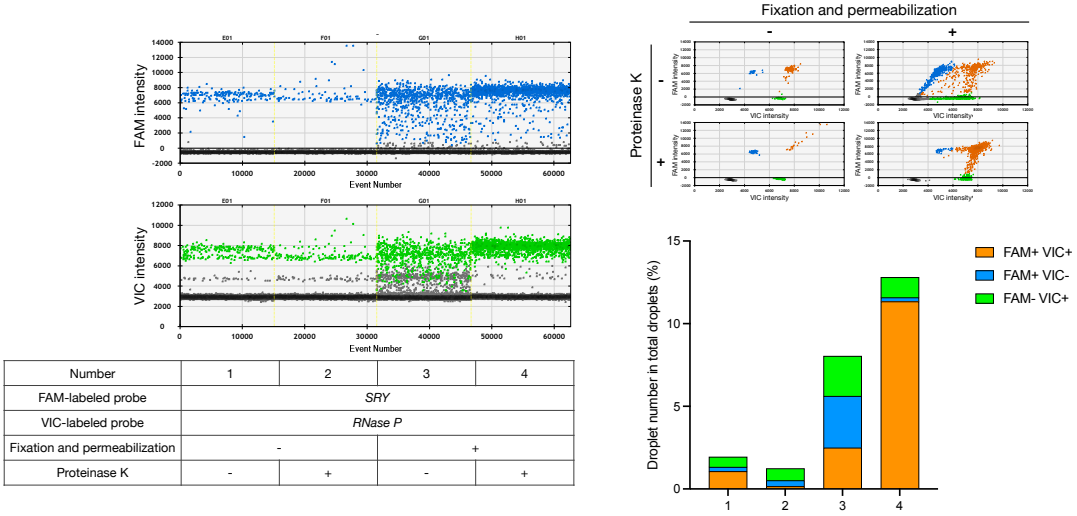
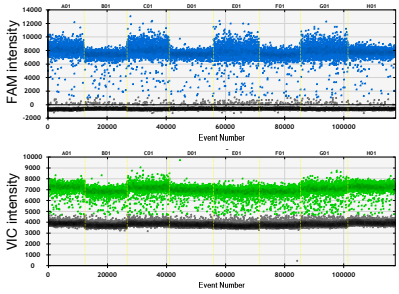
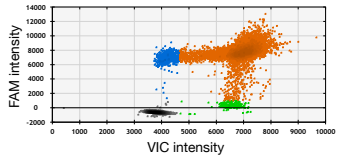
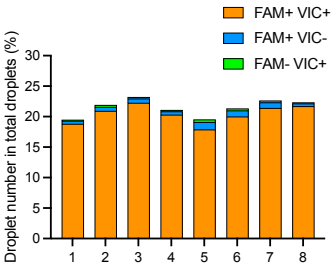


Number	1	2	3	4
FAM-labeled probe	*SRY*			
VIC-labeled probe	*RNase P*			
Fixation and permeabilization	-		+	
Proteinase K	-	+	-	+

Supplementary Fig. 1: Fixation, permeabilization, and mild proteolytic digestion are required for ddcPCR to avoid noise and maximize signal. ddcPCR was carried out to amplify both *SRY* and *RNase P* simultaneously and label each gene with FAM and VIC, respectively, in neutrophils. The graph in the lower right panel is derived from the scatter plots in the upper right panel.

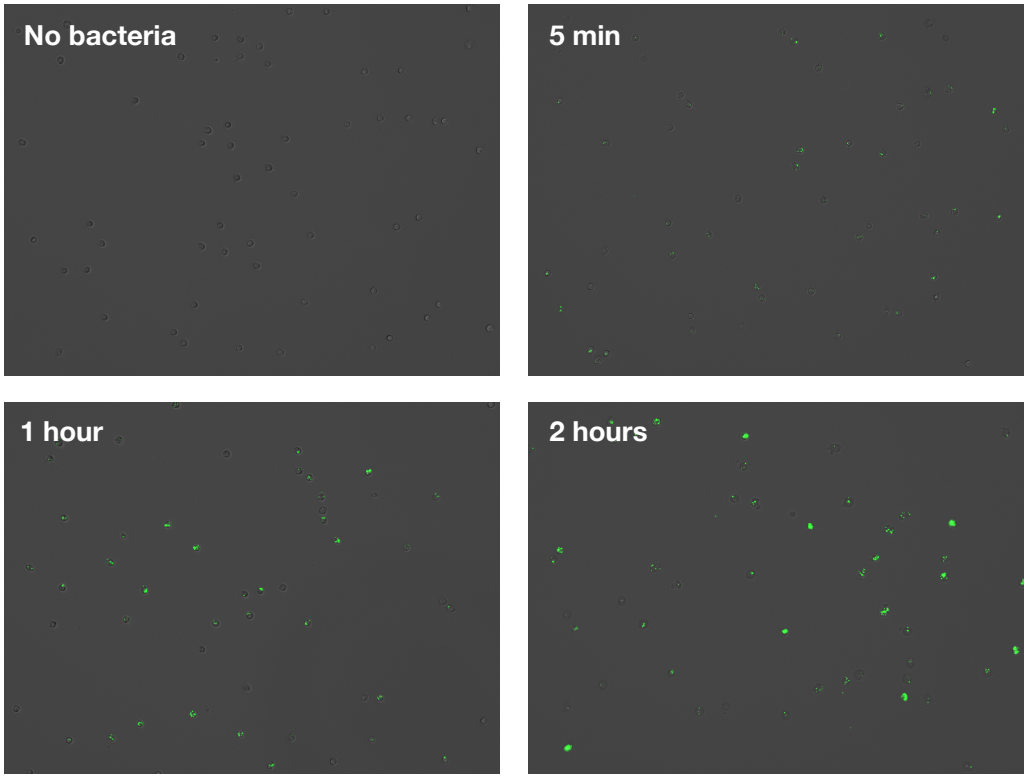




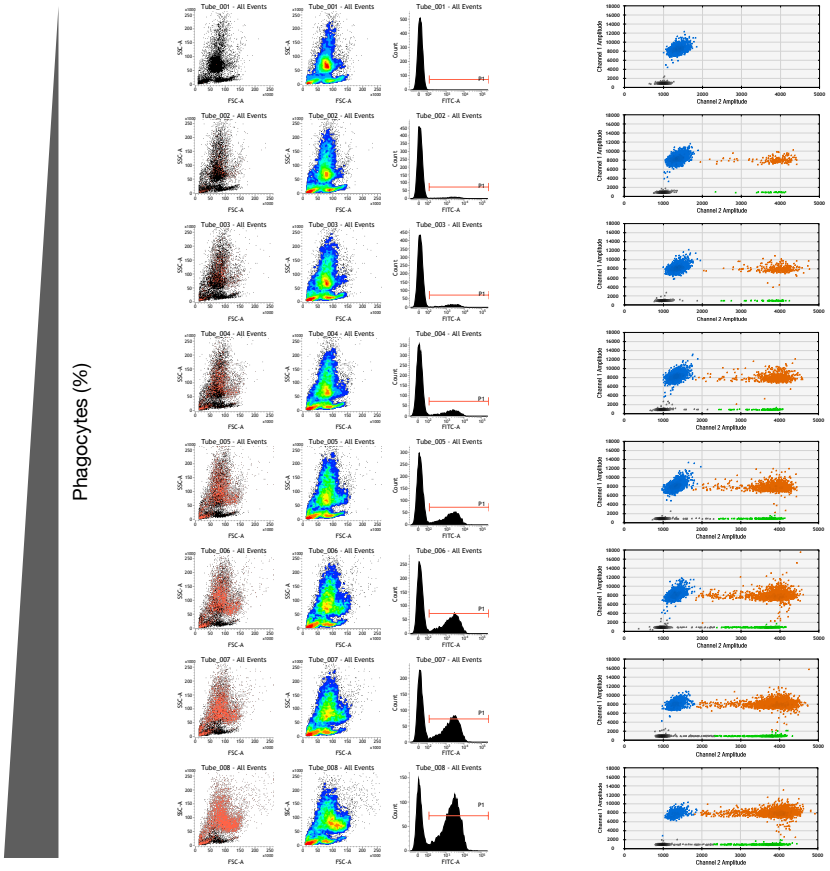


Number	1	2	3	4	5	6	7	8
FAM-labeled probe	*HBB*							
VIC-labeled probe	*RNase P*							
Fixation and permeabilization	5 min at room temp.				10 min at room temp.			
Proteinase K	50 ug/mL		100 ug/mL		50 ug/mL		100 ug/mL	
	40°C	55°C	40°C	55°C	40°C	55°C	40°C	55°C

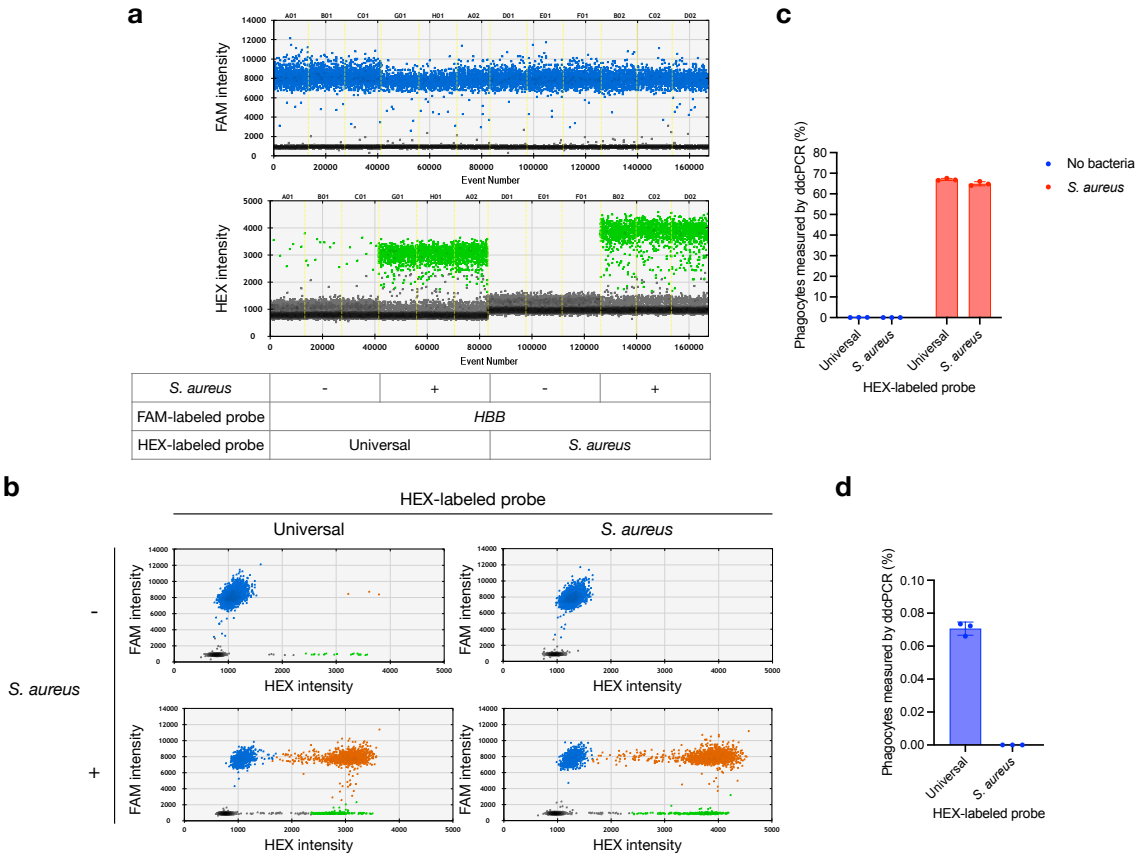
Supplementary Fig. 2: Optimal conditions of fixation, permeabilization, and Proteinase K were determined. ddcPCR was performed to amplify both *HBB* and *RNase P* simultaneously and label each gene with FAM and VIC, respectively, in neutrophils that were fixed, permeabilized, and treated with Proteinase K in the indicated conditions.



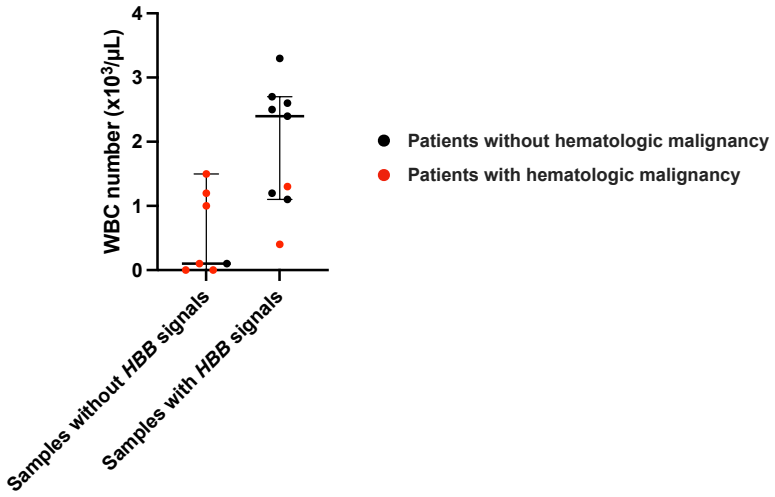
Supplementary Fig. 3: Representative fluorescence imaging of neutrophils incubated with or without fluorescence-labeled *S. aureus* for the indicated times.



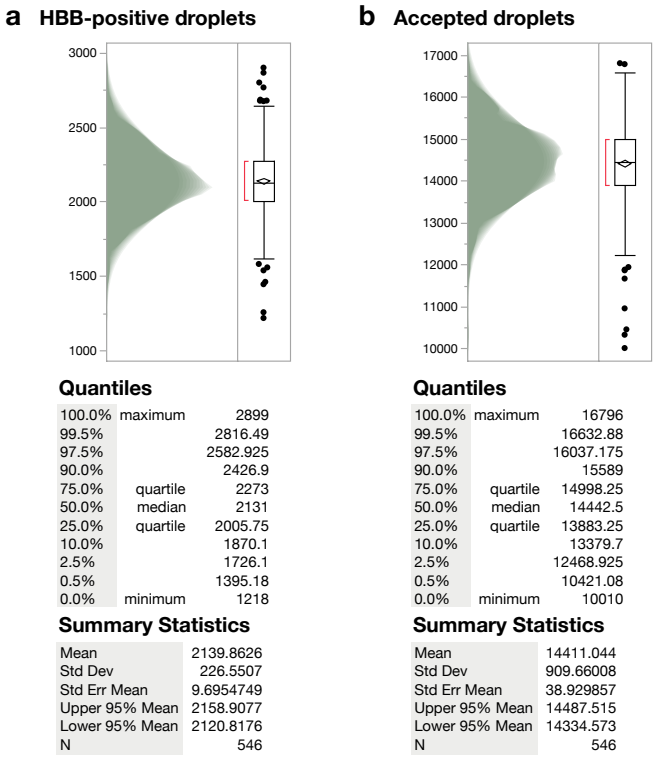
Supplementary Fig. 4: Flow cytometry and ddcPCR results acquired from neutrophils containing different ratios of phagocytes. Samples were prepared by mixing neutrophils serially with neutrophils incubated with fluorescence-labeled *S. aureus* at 37ºC for 2 hours. Each sample was analyzed by flow cytometry and ddcPCR.



Supplementary Fig. 5: Neutrophils containing *S. aureus* can be detected by a universal probe as well as *S. aureus*-specific probe. However, background noise is generated by the universal probe. **a**, ddcPCR was carried out to amplify *HBB* and 16S rRNA simultaneously and label each gene with FAM and HEX, respectively, in neutrophils incubated with or without fluorescence-labeled *S. aureus*. The 16S rRNA gene was labeled with either an universal probe or *S. aureus*-specific probe. **b**, Scatterplots of (**a**) are shown. **c**, The percentage of phagocytes measured using the universal probe is as high as that measured using the *S. aureus*-specific probe. **d**, Background noise is caused by the universal probe but not by the *S. aureus*-specific probe. The graphs of (**c**) and (**d**) represent the mean ± s.d. of three replicates.



Supplementary Fig. 6: ddcPCR can be performed for patients with neutropenia if they have greater than 1000/μL leukocytes in whole blood and do not have hematologic malignancy. *HBB* signals were detected when patients with neutropenia had greater than 1000/μL leukocytes and did not have hematologic malignancy. However, when neutropenia was associated with hematologic malignancy, *HBB* signals were often undetected even when leukocytes were greater than 1000/μL.



Supplementary Fig. 7: Distributions of the number of droplets per reaction. a, Distribution of the number of *HBB*-positive droplets per reaction. **b,** Distribution of the number of droplets that were able to be analyzed per reaction. Data were obtained from clinical validation.

Supplementary Table 1. ddcPCR results for coagulase-negative staphylococci identified by blood culture

		Phagocyte containing *S. aureus* detected by ddcPCR	
		+	-
Coagulase-negative staphylococci identified by blood culture	*S. hominis*	0	4
	S. epidermidis	0	2
	S. capitis	0	1

Supplementary Table 2. Comparison between blood culture and ddcPCR

	Blood culture	ddcPCR
Turnaround time from the reception of specimens to the report of identified bacteria	2-7 days	1 day
Target bacteria	Unlimited	Limited
Necessary blood volume	20 mL x 2 sets	1 mL
Strictly aseptic venipuncture	Necessary	Unnecessary
Blood containing antibiotics causes false negative results	Yes	Unlikely
Phagocytosis	Unknown	Known
Identified bacteria are causative	Uncertain	Certain

Supplementary Table 3. Primers and probes used in this study

Assay ID	Location of amplicon (Sequence accession number or official gene symbol)	Oligo ID	Oligonucleotide sequence and modifications (5' to 3')	Amplicon length (bp)	Design method (software)	Supplier (Catalog number)	Published Reference
RNase P	RNaseP (RPPH1) gene; single exon (GenBank: GRCh38, Chr.14:20343370)	TaqMan Copy Number Reference Assay, human, RNase P	VIC reporter, TAMRA quencher (Oligonucleotide sequence not supplied)	87	Not supplied	ThermoFisher Scientific (4403326)	This study
HBB	Hemoglobin subunit beta (HBB) gene (GenBank: GRCh38, Chr.11: 5225466-5227071)	TaqMan Gene Expression Assay	FAM reporter, MGB quencher (Oligonucleotide sequence not supplied)	95	Not supplied	ThermoFisher Scientific (4331182; assay Hs00758889_s1)	This study
SRY	Sex determining region Y (SRY) gene (GenBank GRCh38, Chr.Y: 2786855-2787741)	TaqMan Gene Expression Assay	FAM reporter, MGB quencher (Oligonucleotide sequence not supplied)	84	Not supplied	ThermoFisher Scientific (4331182; assay Hs00976796_s1)	This study
Bacterial 16S rRNA	*Staphylococcus aureus* 16S ribosomal RNA gene (GenBank: AF015929: 891-1051)	Forward primer (P891F)	TGGAGCATGTGGTTTAATTCGA	161	Described in (34)	Integrated DNA technologies	(34)
		Reverse primer (P1033R)	TGCGGGACTTAACCCAACA				
		S. aureus-specific probe	/5HEX/CCTTTGACA/ZEN/ACTCTAGAGATAGAGCCTTCCC/3IABkFQ/				
		Universal probe	/5HEX/CACGAGCTG/ZEN/ACGACARCCATGCA/3IABkFQ/				

Supplementary Table 4. dMIQE2020 checklist

ITEM TO CHECK	PROVIDED	COMMENT
1. SPECIMEN		
Detailed description of specimen type and numbers	**Y**	Methods section
Sampling procedure (including time to storage)	**Y**	Methods section
Sample aliquotation, storage conditions and duration	**N**	Samples were used on the day of blood collection without storage.
2. NUCLEIC ACID EXTRACTION		
Description of extraction method including amount of sample processed	**N**	N/A nucleic acids were not extracted.
Volume of solvent used to elute/resuspend extract	**N**	N/A nucleic acids were not extracted.
Number of extraction replicates	**N**	N/A nucleic acids were not extracted.
Extraction blanks included?	**N**	N/A nucleic acids were not extracted.
3. NUCLEIC ACID ASSESSMENT AND STORAGE		
Method to evaluate quality of nucleic acids	**N**	N/A
Method to evaluate quantity of nucleic acids (including molecular weight and calculations when using mass)	**N**	N/A
Storage conditions: temperature, concentration, duration, buffer, aliquots	**N**	N/A
Clear description of dilution steps used to prepare working DNA solution	**N**	N/A
4. NUCLEIC ACID MODIFICATION	**NA**	N/A
Template modification (digestion, sonication, pre-amplification, bisulphite etc.)	**N**	N/A
Details of repurification following modification if performed	**N**	N/A
5. REVERSE TRANSCRIPTION	**NA**	All templates measured with dPCR were DNA templates.
cDNA priming method and concentration	**N**	N/A
One or two step protocol (include reaction details for two step)	**N**	N/A
Amount of RNA added per reaction	**N**	N/A
Detailed reaction components and conditions	**N**	N/A
Estimated copies measured with and without addition of RT	**N**	N/A
Manufacturer of reagents used with catalogue and lot numbers	**N**	N/A
Storage of cDNA: temperature, concentration, duration, buffer and aliquots	**N**	N/A
6. dPCR OLIGONUCLEOTIDES DESIGN AND TARGET INFORMATION		
Sequence accession number or official gene symbol	**Y**	Supplementary Table 3

Method (software) used for design and *in silico* verification	**Y**	Supplementary Table 3
Location of amplicon	**Y**	Supplementary Table 3
Amplicon length	**Y**	Supplementary Table 3
Primer and probe sequences (or amplicon context sequence)	**Y**	Supplementary Table 3
Location and identity of any modifications	**Y**	Supplementary Table 3
Manufacturer of oligonucleotides	**Y**	Supplementary Table 3
7. dPCR PROTOCOL		
Manufacturer of dPCR instrument and instrument model	**Y**	Methods section
Buffer/kit manufacturer with catalogue and lot number	**Y**	Methods section
Primer and probe concentration	**Y**	Methods section
Pre-reaction volume and composition (incl. amount of template and if restriction enzyme added)	**N**	N/A
Template treatment (initial heating or chemical denaturation)	**Y**	Methods section
Polymerase identity and concentration, Mg++ and dNTP concentrations	**Y**	Methods section
Complete thermocycling parameters	**Y**	Methods section
8. ASSAY VALIDATION		
Details of optimisation performed	**Y**	Fig. 1c and d, Supplementary Fig. 1, and Supplementary Fig. 2
Analytical specificity (vs. related sequences) and limit of blank (LOB)	**Y**	Supplementary Fig. 5
Analytical sensitivity/LoD and how this was evaluated	**N**	LoD was not formally established.
Testing for inhibitors (from biological matrix/extraction)	**N**	Not performed
9. DATA ANALYSIS		
Description of dPCR experimental design	**Y**	Fig. 1a, Fig. 3a, and Fig. 4a
Comprehensive details negative and positive of controls (whether applied for QC or for estimation of error)	**Y**	Results section
Partition classification method (thresholding)	**Y**	Thresholds were set using the 'Autoanalyze - combined wells' function or manually set up to classify positive and negative partition. Manual thresholds are indicated by pink lines in scatterplots.
Examples of positive and negative experimental results (including fluorescence plots in supplemental material)	**Y**	Fig. 1b and Fig. 3d
Description of technical replication	**Y**	Figure legends
Repeatability (intra-experiment variation)	**N**	Not formally assessed
Reproducibility (inter-experiment/user/lab etc. variation)	**N**	Not assessed between labs/users

Number of partitions measured (average and standard deviation)	**Y**	Supplementary Fig. 7b
Partition volume	**Y**	0.85 nL (QuantaSoft v.1.7.4)
Copies per partition (λ or equivalent) (average and standard deviation)	**N**	Copy number concentrations were not used in this study.
dPCR analysis program (source, version)	**Y**	Methods section
Description of normalisation method	**N**	N/A
Statistical methods used for analysis	**N**	Copy number concentrations were not used in this study.
Data transparency	raw data available on request:	