

**Supplementary Table S1.** Detailed clinical information of BC-/ddPCR<sup>+</sup> samples.

Number	ddPCR results	Anti-bacterial agents	Culture results of other sites	Clinical diagnosis	Laboratory examination
<b>Probable BSI</b>					
17	<i>Klebsiella</i>	Ceftazidime Itraconazole Sulfamethoxazole (SMZ) Caspofungin Isopamicin Sulperazon Vancomycin Tigecycline Voriconazole	<i>Klebsiella pneumoniae</i> (endotracheal tube) <i>CRKP</i> (faeces)	Acute hemorrhagic necrotizing pancreatitis Severe pneumonia Acute lymphocytic leukemia (ALL)	WBC (10 <sup>9</sup> /L):0.39 CRP (mg/L):139 PCT (µg/L): <0.05
20	<i>Streptococcus</i> <i>EBV</i>	Meropenem Vancomycin	<i>Methicillin-resistant</i> <i>Staphylococcus aureus</i> (MRSA, throat swab) <i>C. albicans</i> (sputum) <i>S. aureus</i> (sputum) <i>S. anginosus</i> (hydrothorax) <i>E. coli</i> (faeces)	Sepsis Septic shock Pyopneumothorax Severe pneumonia	WBC (10 <sup>9</sup> /L):10.15 CRP (mg/L): >170 PCT (µg/L):27.52
53	<i>E. coli</i>	Sulperazon Vancomycin	<i>Enterobacteriaceae</i> (faeces) <i>Enterococcus</i> (faeces)	Sepsis Bacterial pneumonia Hepatoblastoma	WBC (10 <sup>9</sup> /L):1.08 CRP (mg/L): 88 PCT (µg/L): 0.16
59	<i>Klebsiella</i>	Sulperazon	<i>E. asburiae</i> (pus)	Sepsis	WBC (10 <sup>9</sup> /L):0.24

62	<i>Streptococcus</i>	Vancomycin	<i>S. viridans</i> (throat swab)	Bronchopneumonia	CRP (mg/L): 57
	CoNS			Retinoblastoma	PCT (µg/L): 0.37
	<i>Klebsiella</i>	Sulperazon	<i>Klebsiella pneumoniae</i> (oral fluid)	Sepsis	WBC (10 <sup>9</sup> /L):0.43
<b>Possible BSI</b>		Vancomycin	<i>Enterobacteriaceae</i> (faeces)	Intestinal infection	CRP (mg/L): 98
		Fluconazole	<i>Enterococcus</i> (faeces)	ALL	PCT (µg/L): 2.91
1	<i>Bacteroides</i>	Meropenem	<i>Proteobacteria</i> (faeces)	Neuroblastoma	WBC (10 <sup>9</sup> /L):0.46
	<i>fragilis</i>	Vancomycin	<i>C.albicans</i> (faeces)	Respiratory tract infection (RTI)	CRP (mg/L):123
	VZV	Itraconazole		Autoimmuneencephalitis (AE)	PCT (µg/L):3.85
2	CoNS	Isopamicin	<i>P. Aeruginosa</i> (sputum, endotracheal intubation)	Medulloblastoma	WBC (10 <sup>9</sup> /L):3.27
		Levofloxacin		Pneumonia	CRP (mg/L):6
		Itraconazole		Central respiratory failure	PCT (µg/L): <0.02
8	<i>Klebsiella</i>	Meropenem	<i>P. Aeruginosa</i> (hydrocele)	Epididymitis	WBC (10 <sup>9</sup> /L):39.79
	<i>E. coli</i>	Vancomycin			CRP (mg/L):60
					PCT (µg/L):2.26
16	<i>A. Baumanii</i>	Isopamicin	<i>Carbapenem-resistant Klebsiella pneumoniae</i> (CRKP, faeces)	Sepsis	WBC (10 <sup>9</sup> /L):0.27
	CoNS	Imipenem and Cilastatin Sodium	<i>P. Aeruginosa</i> (faeces)	Pneumonia	CRP (mg/L):81
		Linezolid		Acute monocytic leukemia (AML)	PCT (µg/L):0.06
		Voriconazole			
		Tigecycline			
21	<i>Streptococcus</i>	Isopamicin	<i>Enterobacteriaceae</i> (faeces)	Sepsis	WBC (10 <sup>9</sup> /L):16.9
		Sulperazon	<i>Enterococcus</i> (faeces)	Septic shock	CRP (mg/L): 104

		Tigecycline	<i>A. Baumanii</i> (sputum)	Osteosarcoma	PCT (µg/L):0.74
		Fluconazole			
26	<i>Klebsiella</i>	Vancomycin	<i>S. viridans</i> (throat swab)	Sepsis	WBC (10 <sup>9</sup> /L):2.38
	<i>E. Coli</i>	Sulperazon	<i>A. Baumanii</i> (wound fluid)	T lymphocyte lymphoma	CRP (mg/L):68
	<i>Enterococcus</i>	Isopamicin	<i>Enterobacteriaceae</i> (faeces)	Stomatitis	PCT (µg/L):0.87
		Fluconazole	<i>Enterococcus</i> (faeces)		
29	<i>E. Coli</i>	Meropenem	<i>Enterococcus</i> (faeces)	Sepsis	WBC (10 <sup>9</sup> /L):8.78
		Vancomycin	<i>E. coli</i> (faeces)	Purulent meningitis	CRP (mg/L):153
				Pneumonia	PCT (µg/L):55.97
30	<i>E. coli</i>	Amoxicillin and clavulanate potassiun	-	Bronchopnumonia	WBC (10 <sup>9</sup> /L):17.95
					CRP (mg/L):6
					PCT (µg/L): <0.02
34	<i>E. coli</i>	Sulperazon	-	Systemic inflammatory response syndrome (SIRS)	WBC (10 <sup>9</sup> /L):5.43
		Cefurxim		Acute lymphadenitis	CRP (mg/L):27
					PCT (µg/L): 0.22
35	<i>P. Aeruginosa</i>	Sulperazon	<i>Haemophilus parainfluenzae</i> (throat swab)	ALL	WBC (10 <sup>9</sup> /L):0.18
	<i>Enterococcus</i>	Ornidazole	<i>S. viridans</i> (oral fluid)		CRP (mg/L): <5
		Caspofungin	<i>Neisseria</i> (oral fluid)		PCT (µg/L): 0.03
		SMZ			
		Meropenem			
		Voriconazole			
37	<i>P. Aeruginosa</i>	Sulperazon	<i>S. epidermidis</i> (throat swab)	Pneumonia	WBC (10 <sup>9</sup> /L):2.58
	<i>E. coli</i>	Fluconazole		ALL	CRP (mg/L):25
					PCT (µg/L): 0.14
41	<i>A. Baumanii</i>	Isopamicin	-	Intestinal infection	WBC (10 <sup>9</sup> /L):0.46

	<i>Streptococcus</i>	Sulperazon Fluconazole Vancomycin Imipenem SMZ Imipenem and Cilastatin Sodium		Skin soft-tissue infection	CRP (mg/L):41 PCT (µg/L): 0.23
44	<i>Klebsiella</i> <i>EBV</i>	Vancomycin Fluconazole Imipenem	<i>Neisseria</i> (throat swab)	Sepsis Hodgkin lymphoma Stomatitis	WBC (10 <sup>9</sup> /L):0.3 CRP (mg/L):90 PCT (µg/L): <0.02
47	<i>Klebsiella</i>	Sulperazon Fluconazole Ornidazole	<i>Neisseria</i> (throat swab) <i>S. viridans</i> (throat swab)	Pneumonia Stomatitis ALL	WBC (10 <sup>9</sup> /L):2.13 CRP (mg/L): <5 PCT (µg/L): 0.1
48	<i>Streptococcus</i>	Sulperazon	<i>Enterobacteriaceae</i> (faeces)	Sepsis Intestinal infection ALL	WBC (10 <sup>9</sup> /L): - CRP (mg/L): - PCT (µg/L): -
50	<i>A. Baumanii</i> <i>Streptococcus</i>	Meropenem Vancomycin Fluconazole Azithromycin	<i>Serratia marcescens</i> (gastric juice) <i>Enterobacteriaceae</i> (faeces)	Sepsis Septic shock Severe pneumonia B-cell lymphoma	WBC (10 <sup>9</sup> /L):5.97 CRP (mg/L):165 PCT (µg/L): 0.48
52	<i>S. aureus</i>	Meropenem Vancomycin	-	Sepsis Intestinal infection Neuroblastoma	WBC (10 <sup>9</sup> /L):0.14 CRP (mg/L): >160 PCT (µg/L): 11.71
56	<i>Klebsiella</i>	Imipenem and Cilastatin Sodium	-	Sepsis Pneumonia	WBC (10 <sup>9</sup> /L):14.57 CRP (mg/L): 23

		Isopamicin Meropenem Vancomycin Azithromycin Linezolid Ceftriaxone Fluconazole		B-cell lymphoma	PCT (µg/L): <0.05
58	<i>Klebsiella</i> <i>EBV</i> <i>CMV</i>	Sulperazon Fluconazole SMZ Voriconazole Ornidazole Imipenem and Cilastatin Sodium Isopamicin Tigecycline	-	Pneumonia Acute cholecystitis ALL	WBC (10 <sup>9</sup> /L):3.68 CRP (mg/L): 15 PCT (µg/L): 0.1
60	<i>Klebsiella</i>	Sulperazon Vancomycin	<i>Enterococcus</i> (faeces)	Sepsis RTI Embryonal rhabdomyosarcoma	WBC (10 <sup>9</sup> /L):1.38 CRP (mg/L): 102 PCT (µg/L): 1.63
64	<i>E. Coli</i>	Meropenem Vancomycin	<i>S. viridans</i> (sputum)	Sepsis Pneumonia Rhabdomyoid tumor	WBC (10 <sup>9</sup> /L):27.71 CRP (mg/L):87 PCT (µg/L): 3.61
73	<i>Enterococcus</i> <i>EBV</i>	Sulperazon Meropenem Voriconazole	<i>S. viridans</i> (throat swab) <i>Enterococcus</i> (faeces)	Pneumonia Intestinal infection ALL	WBC (10 <sup>9</sup> /L):2.2 CRP (mg/L): <5 PCT (µg/L): 0.14

75	<i>Enterococcus</i>	SMZ Sulperazon Meropenem	<i>Enterobacteriaceae</i> (faeces)	Chronic superficial gastritis (CSG) ALL RTI	WBC (10 <sup>9</sup> /L):2.15 CRP (mg/L): 133 PCT (µg/L): 0.54
<b>Presumptive false-positive</b>					
25	<i>P. Aeruginosa</i> <i>E. Coli</i>	Cefuroxim	<i>E. faecalis</i> (urine) <i>E. coli</i> (urine) <i>Enterobacteriaceae</i> (faeces) <i>Enterococcus</i> (faeces)	Systemic lupus erythematosus (SLE) Lupus nephritis	WBC (10 <sup>9</sup> /L):6.06 CRP (mg/L): <5 PCT (µg/L): -

## Interference Test

### Validation Protocol

(1) The substances and concentrations of interferences.

Interfering substance	Final concentration of sample (mg/ml)
Human hemoglobin	20
Human ferroheme	0.2

Positive templates of the pathogen DNA mix according to P1, P2, P3, P4 and P5 were diluted to 50 copies/µl using distilled water, respectively.

(2) Verification method:

a). Verification of human hemoglobin: 2 ml negative plasma was taken, and added into 3  $\mu$ l (150 copies) positive template P1 or P2, P3, P4, P5 and 20 mg/ml human hemoglobin, respectively. The detection was carried out for 5 times following the manufacturer's instruction.

b). Verification of human ferroheme: 2 ml negative plasma was obtained and added to 150 copies (3  $\mu$ l) positive template P1 or P2, P3, P4, P5, and 0.2 mg/ml of human ferroheme. The detection was performed for 5 times following the manufacturer's instruction.

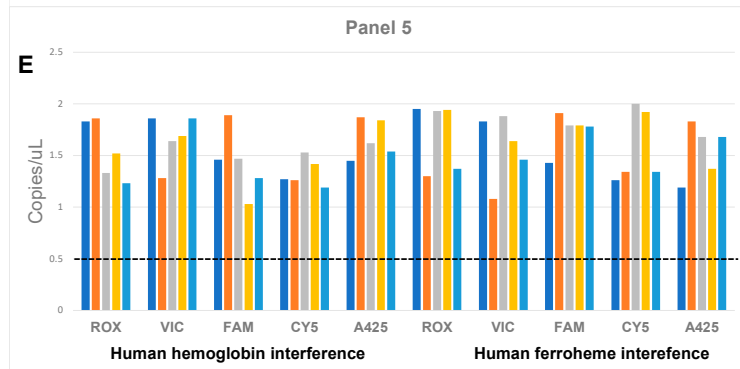
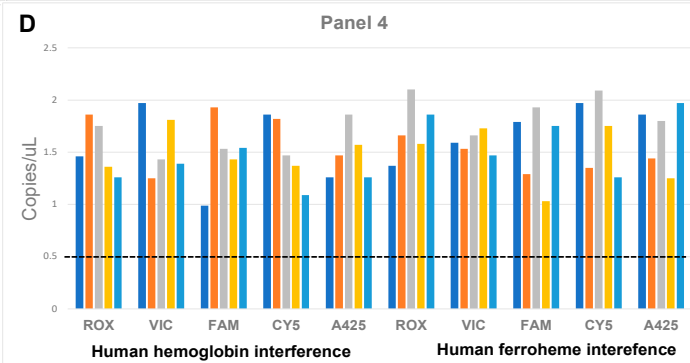
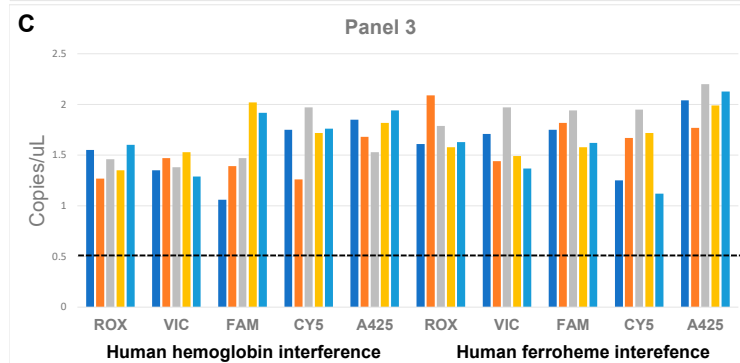
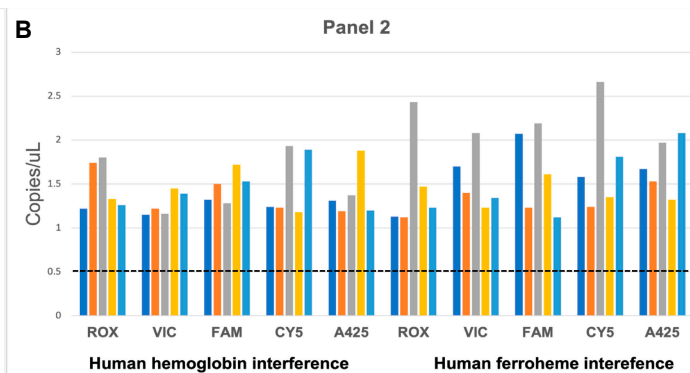
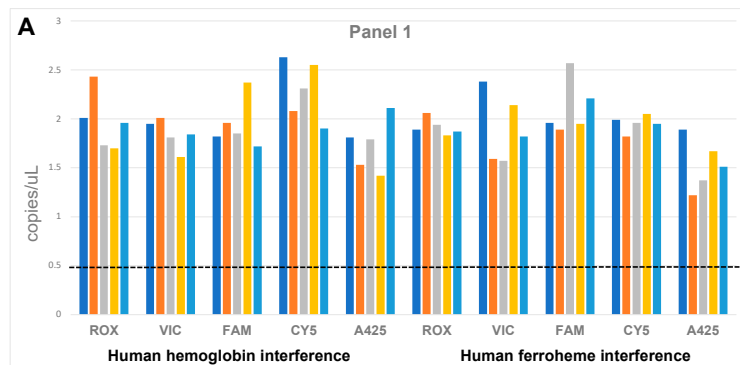
### Diagnostic Criteria

Repeated detection for five times, and the positive rate of each target is 100%.

### Results

The positive rate of the target was 100% for each ddPCR panel (**Figure S1**).

**Figure S1.** The results of interference test using human hemoglobin and human ferroheme. a). The verification test of interference factors on Panel 1; b). The verification test of interference factors on Panel 2; c). The verification test of interference factors on Panel 3; d). The verification test of interference factors on Panel 4; e). The verification test of interference factors on Panel 5. ROX, ROX channel; VIC, VIC channel; FAM, FAM channel; CY5, CY5 channel; A425, A425 channel.





## Conclusion

The detection performance of ddPCR panels were not interfered by with the mixing of human hemoglobin and ferroheme.

## Cross-validation Test

### Validation Protocol

(1) sample:

- a). Positive template P1: DNAs from the standard strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* were extracted and diluted, respectively. Then, the DNAs were mixed and labeled as P1.
- b). Positive template P2: DNAs from the standard strains of *Staphylococcus aureus*, *Candida albicans*, *Enterococcus faecium*, *Streptococcus pneumoniae* and *Staphylococcus epidermidis* were extracted and diluted, respectively. Then, the DNAs were mixed and labeled as P2.
- c). Positive template P3: The synthetic positive plasmids containing *KPC*, *mecA*, *OXA-48*, *NDM*, *IMP*, *vanA*, *vanM* genes were diluted, mixed together, and labeled as P3.
- d). Positive template P4: DNAs from the standard strains of *Salmonella*, *Bacteroides fancilis*, *Listeria monocytogenes*, *Haemophilus influenzae* and

*Morganella mosani* were extracted and diluted, respectively. Then, the DNAs were mixed and labeled as P4.

e). Positive template P5: DNAs from the standard products of HSV-1, VZV, EBV, CMV, HSV-2 were extracted and diluted. All nucleic acids were mixed together and labeled as P5.

(2) Verification method:

Panel 1-5 was used to detect all five positive templates P1, P2, P3, P4 and P5, and the no template control (NTC), respectively.

### Diagnostic Criteria

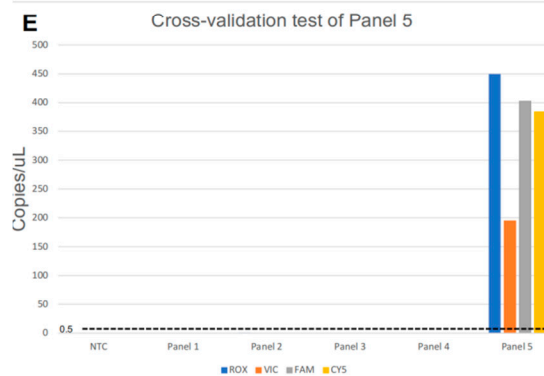
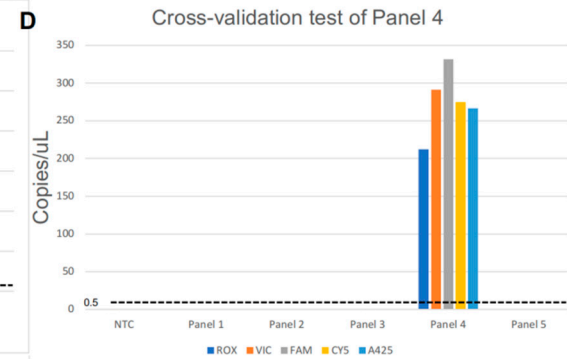
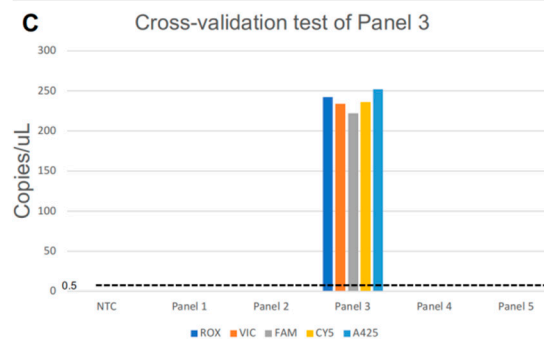
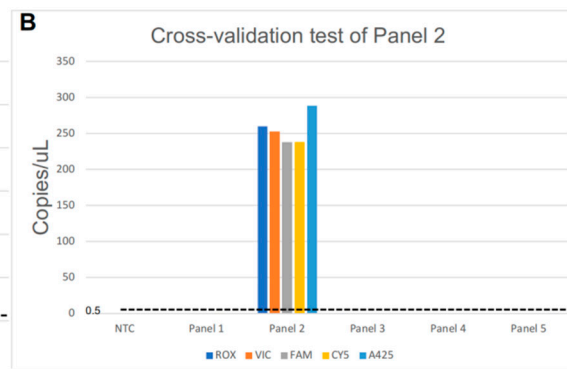
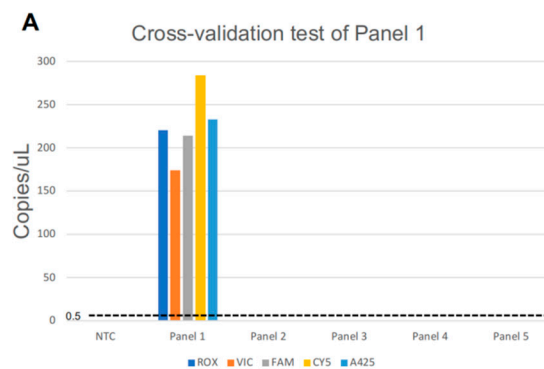
P1/P2/P3/P4/P5: FAM channel/VIC channel/ROX channel/CY5 channel/A425 channel < 0.5 copies/μl, CY5.5 channel ≥ 50 copies/μl;

Negative quality control products: FAM channel/VIC channel/ROX channel/CY5 channel/A425 channel < 0.5 copies/μl, CY5.5 channel ≥ 50 copies/μl;

Positive quality control products: FAM channel/VIC channel/ROX channel/CY5 channel/A425 channel ≥ 0.5 copies/μl, CY5.5 channel ≥ 50 copies/μl;

### Results

The ddPCR panels detected their corresponding target template, while no signal was observed from the other template DNAs (**Figure S2**).



**Figure S2.** The results of cross-validation test. a). Positive template P1 verified other four panels; b). Positive template P2 verified other four panels; c). Positive template P3 verified other four panels; d). Positive template P4 verified other four panels; e). Positive template P5 verified other four panels. ROX, ROX channel; VIC, VIC channel; FAM, FAM channel; CY5, CY5 channel; A425, A425 channel.

## Conclusion

The ddPCR panels can specifically detect their target without cross-reaction.