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Supplementary Materials for

Rapid identification of health care–associated infections with an integrated fluorescence anisotropy system

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The PDF file includes:

table S1. Target sequences recognized by established detection keys.

Category	Target	DNA sequence (5' - 3')
Gram-positive	*Streptococcus*	GAAGAACGAGTGTGAGAGTGGAAAGTTCACACTGTGACGGTATCTTACCAGAAAGGGACGGCTAACTA
	Enterococcus	GAAGAACAAGGACGTTAGTAACTGAACGTCCCCTGACGGTATCTAACCAGAAAGCCACGGCTAACTAC
	Clostridium	GTCTTCAGGGACGATAATGACGGTACCTGAGGAGGAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT
	Lactobacillus	GGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAA
	Corynebacterium	CCTTTCGCAACCGACGAAGCTTTTGTGACGGTAGGTTGAGAAGAAGCACCGGCTAACT
	Bacillus	GTTGTTAGGGAAGAACAAGTGCGTTCAAATAGGGCGGCACCTTGACGGTACCTAACCAGAAAGCCACGG
Gram-negative	*Haemophilus*	TCATGGCATGCGGCCTTGCGGTCCCGCACTTTCATCTTCCGATTCTACGCGGTATTAGCGAC
	Proteus	GGAGGAAGGTGATAAGGTTAATACCCTTNTCAATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCC
	Citrobacter	ACGGAGTTAGCCGGTGCTTCTTCTGCGAGTAACGTCAATTGCTGCGGTTATTAACCACAACACCTTCCTCC
	Serratia	TCAGCGGGGAGGAAGGTGGTGAACTTAATACGTTCATCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAA
	Stenotrophomonas	AATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACATTGA
	Legionella	TTCAGTGGGGAGGAGGATTGATAGGTTAAGAGCTGATTGATTGGACGTTACCCACAGAAGAAGCACCGGCT
	Neisseria	TTTGTCAGGGAAGAAAAGGCTGTTGCTAATATCAGCGGCTGATGACGGTACCTGAAGAATAAGCACCGGCTA
	Moraxella	TTAAGTGGGGAGGAAAAGCTTGTGGTTAATACCCACAAGCCCTGACGTTACCCACAGAATAAGCA
	Bacteroides	TCATTAGACATAAAGTGCAGTCATGTCATGTCATACTGTTTTGTCATGTCATAATATGAATAAGGATCGGCT
	Mycobacterium	TCTTTCACCATCGACGAAGGTCCGGGTTCTCTGGATTGACGGTAGGTGGAGAAGAAGCACCGGCCAA

table S1 (continued). Target sequences recognized by established detection keys.

Gene	Protein name	DNA sequence (5' - 3')
ramA	Transcriptional activator	TACCGACCAGCGGGTTTATGATATCTGCCTGAAATACGGCTTTGATTCGCAGCAGAC
KPC	Carbapenem-hydrolyzing beta-lactamase	CGGCATAGTCATTTGCCGTGCCATACCCTCCGCAGGTTCCGGTTTTGTCTCCGACTG
NDM	Beta-lactamase	CGCATTGGCATAAGTCGCAATCCCCGCCGCATGCAGCGCGTCCATACCGCCCATCTTGT
OXA	Beta-lactamase	ACCCAACCTGTTAACCAACCTACTTGAGGGGTTACAGCCATTCCCCAGCCGCTTTTTG
IMP	Beta-lactamase	TGAATATTTAGCTTGTACCTTACCGGATTTTTTCAAAAGTTCATTTGTTAATTCAGAT
VIM	Beta-lactamase	TAGCCGAGGTAGAGGGGAACGAGATTCCCACGCACTCTCTAGAAGGACTCTCATCGAGCGG
SPM	Beta-lactamase	CGTTAAATGCACGGTTGGGGATGTGAGACTACAGTCTCATTTCGCCAACGGCCTTTTC
CTX-M	Beta-lactamase	CGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGC
TEM	Beta-lactamase	TTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAA
ampC	Beta-lactamase	GCGTCAAATCGACACTACCCGACATGTTGAGTTTTATT CATGCCAACCTTAACCCACAGA
vanA	Vancomycin/teicoplanin A-type resistance	TCCAATTCGTCCGCGCTATTGACTTTTTTCACACCGAAGGATGAGCCTGAAC

table S2. Summary of a set of ARV factors targeted in this study (*19*, *20*, *29*, *30*).

Gene	Protein name	Function	Positive strain
nuc	Thermonuclease	*S. aureus* specific factor important for in vivo survival	HA-MRSA (ATCC BAA-1720), CA-MRSA (ATCC BAA-1707), MSSA (ATCC 25923)
femB	Aminoacyltransferase	*S. aureus* specific factor essential for methicillin resistance, affecting level of resistance	HA-MRSA (ATCC BAA-1720), CA-MRSA (ATCC BAA-1707), MSSA (ATCC 25923)
mecA	Penicillin binding protein 2A	Methicillin resistance determinant	HA-MRSA (ATCC BAA-1720), CA-MRSA (ATCC BAA-707)
PVL	Panton-Valentine Leukocidin	A cytotoxin, one of the β-pore-forming toxins, affecting virulence of *S. aureus*	CA-MRSA (ATCC BAA-1707), MSSA (ATCC 25923)

table S3. DNA sequences used in this study.

	Target	Strand name	DNA sequence (5' - 3')
	Universal	Amplicon	GGTAAGGTTCTTCGCGTTGC**N***TCGAATTAAACCACATGCTCCA
		Limiting primer	GGAGCATGTGGTTTAATTCGA
		Excess primer	GGTAAGGTTCTTCGCGTT
	Reporter	Template	CATGAATCTACTCGACGATATTGCTCAACTGTCATAAACTTCTGAGGA
		Primer	TCCTCAGAAGTTT
		FAM-DNA	(FAM)-TATCGTCGAGTAGATTCATG
Species differentiation	*Escherichia*	Amplicon	TGCGGGTAACGTCAATGAGCAAAGGTATTAACTTTACTCCCTTCCTCCA
		Limiting primer	GGAGGAAGGGAGTAAAGTTAATACCTTTG
		Excess primer	TGCGGGTAACGTCAATGAG
	Klebsiella	Amplicon	ACGGCAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCCAATCGCCAAGGTTATT AACCTTATCGCCTTGCCTCCA
		Limiting primer	GGAGGCAAGGCGATAAGGT
		Excess primer	ACGGCAGTTAGCCGGTGCTTCT
	Acinetobacter	Amplicon	GAGTTAGCCGGTGCTTATTCTGCGAGTAACGTCCACTATCCCTAGGTATTAACTA GAA
		Limiting primer	TCTAGTTAATACCTAGGGATAGTGGACGTT
		Excess primer	GAGTTAGCCGGTGCTTATTCTGCGAG
	Pseudomonas	Amplicon	TATTCTGTTGGTAACGTCAAAACAGCAAGGTATTAACTTACTGCCCTTCCTCCA
		Limiting primer	GGAGGAAGGGCAGTAAGTTAATACCTTG
		Excess primer	GCTTATTCTGTTGGTAACGTCAAAACAG
	Staphylococcus	Amplicon	GTAGTTAGCCGTGGCTTTCTGATTAGGTACCGTCAAGATGTGCACAGTTACTTA CACATATGTTCTTCCCA
		Limiting primer	GGGAAGAACATATGTGTAAGTAAC
		Excess primer	GTAGTTAGCCGTGGCTTTCT
Resistance and virulence	*nuc*	Amplicon	CACCTGAAACAAAGCATCCTAAAAAAGGTGTAGAGAAATATGGTCCTGAAGCAA
		Limiting primer	TGCTTCAGGACCATATTTCTCTAC
		Excess primer	CACCTGAAACAAAGCATCCTAAA
	femB	Amplicon	TGAATTGAGCAAAACGGACGGCCCAATTCTAAACCTTGCTTCTGGA
		Limiting primer	CCAGAAGCAAGGTTTAGAATTG
		Excess primer	TGAATTGAGCAAAACGGACGGC
	mecA	Amplicon	ATGAAGGTGTGCTTACAAGTGCTAATAATTCACCTGTTTGAGGGTGGATAGCAGA
		Limiting primer	CTGCTATCCACCCTCAAACAGG
		Excess primer	ATGAAGGTGTGCTTACAAGTGC
	PVL	Amplicon	CACCTGATAAGCCGTTAGAGATATTAATATCTCCACCATAAGAATAACCTACCGA
		Limiting primer	CGGTAGGTTATTCTTATGGTGGAGAT
		Excess primer	CACCTGATAAGCCGTTAGAGATATT

* The nucleobase in bold letter (N) indicates Adenine (A) for *Escherichia, Klebsiella, Acinetobacter* or Thymine (T) for *Pseudomonas, Staphylococcus*.

Supplementary Figures

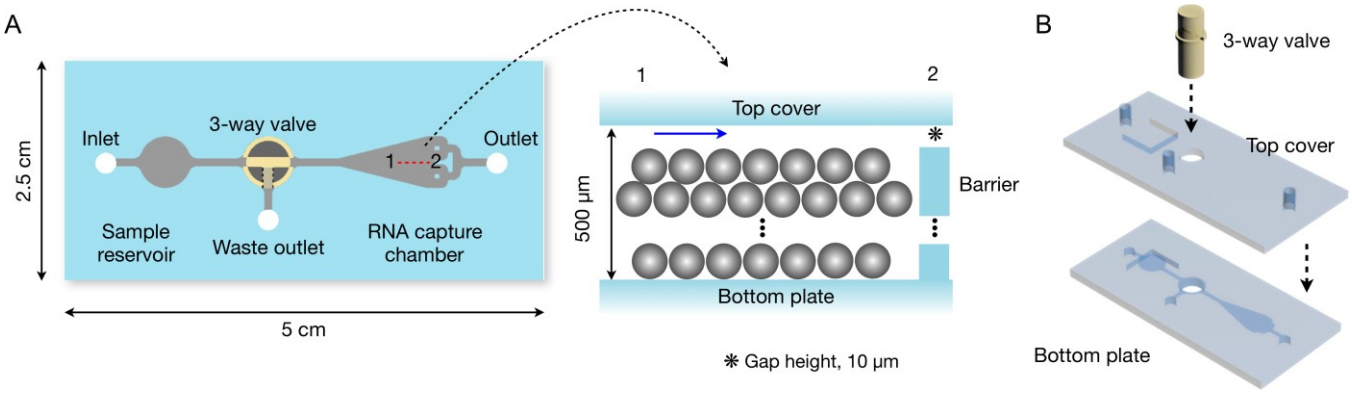

fig. S1. Schematic of the plastic cartridge for RNA extraction. (A) Top view. The device has a RNA capture chamber that is filled with glass beads. The 3-way valve (push-button style) is used to direct waste product to a separate outlet. The RNA chamber has weir style barrier (right) to retain glass beads while allowing for fluidic flow. Gap height (❊), 10 µm. **(B)** Device assembly. The bottom plate and the top cover were separately injection-molded, and glued together.

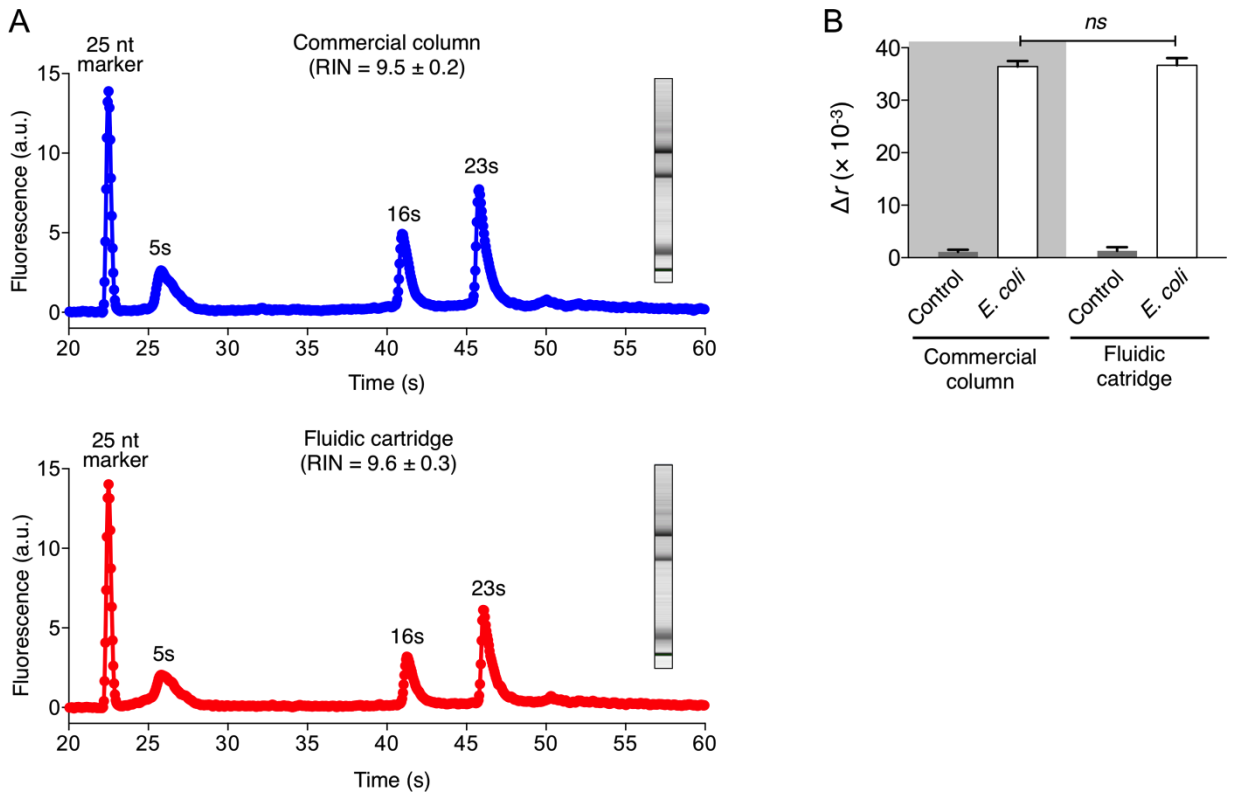

fig. S2. Comparison between the fluidic cartridge and a commercial column. Equal volumes of *E. coli* lysates were flown through a commercial column (Zymo Research) and the fluidic cartridge for total RNA extraction. **(A)** The RNA integrity was assessed by Bioanalyzer (Inset: 'virtual gel' pattern from the instrument). The RNA integrity number (RIN) indicated that high quality RNA was collected by the fluidic cartridge (RIN = 9.6 ± 0.3). The number was comparable to that of a commercial column (RIN = 9.5 ± 0.2). **(B)** The RNA extracted by a commercial column and the fluidic cartridge was amplified by asymmetric RT-PCR and detected by the PAD. The observed Δr values were statistically identical (two-tailed *t*-test, $P > 0.64$).

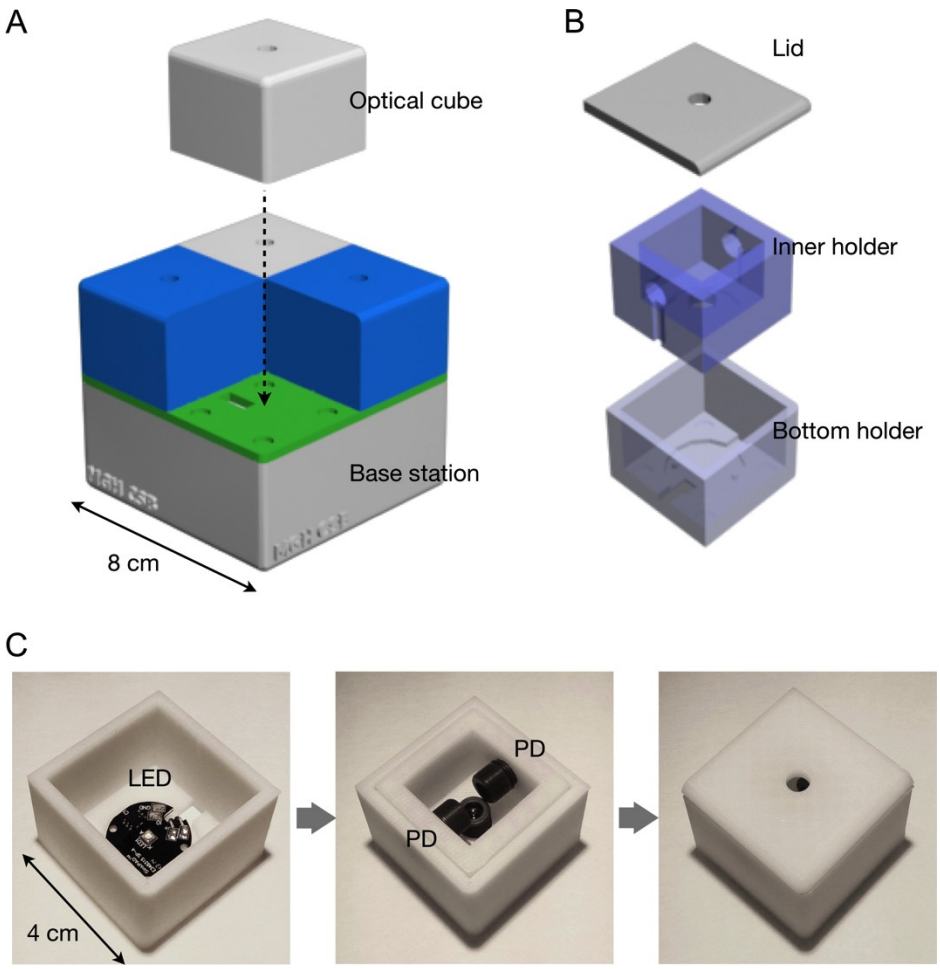

fig. S3. The optical detection system in the PAD. (A) The system has a modular structure. The base station contains control electronics, including a microcontroller and signal processing units. Four optical cubes are plugged into the base station. **(B)** Blow-up schematic of an optical cube. **(C)** Optical cube assembly. A LED circuit was positioned on the bottom holder. The inner holder, mounted with photodiodes (PDs), focusing lenses, and optical filters (polarizers), was inserted into the bottom holder and capped with a lid.

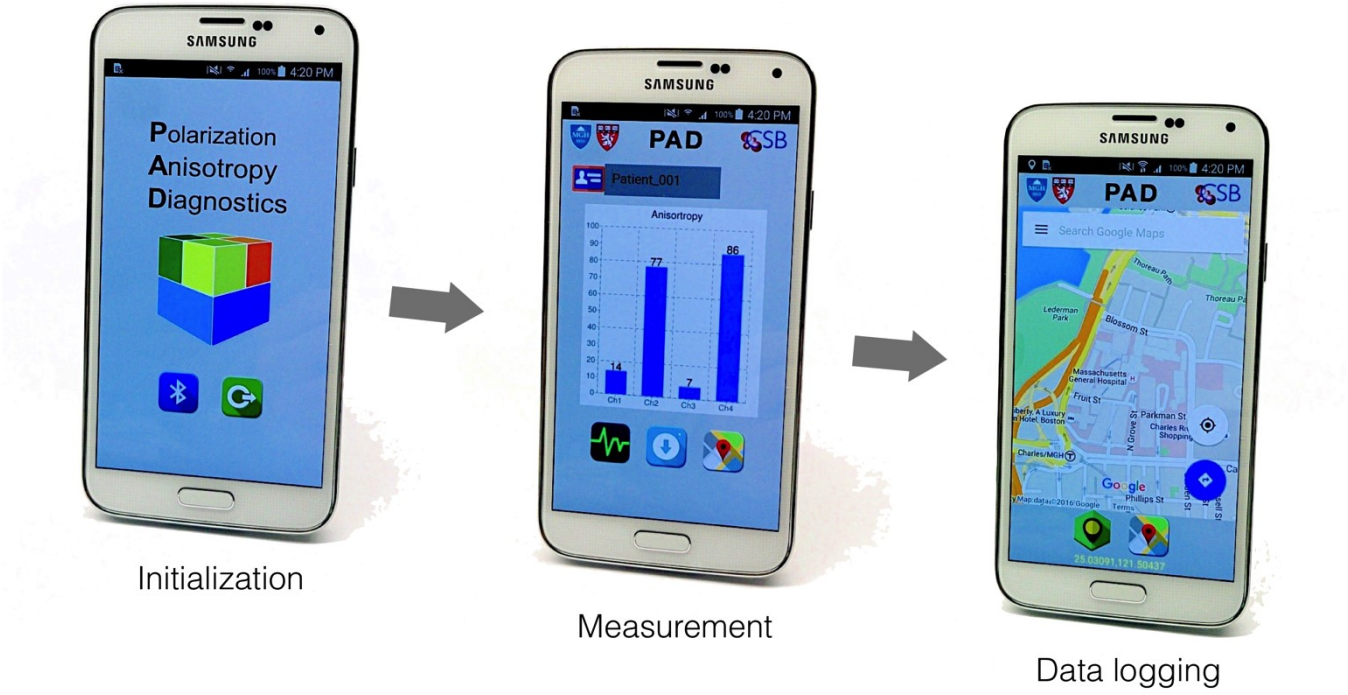

fig. S4. Snapshots of a PAD application. The PAD App has three main pages, namely, system initialization, measurement, and data storage with the geographic information.

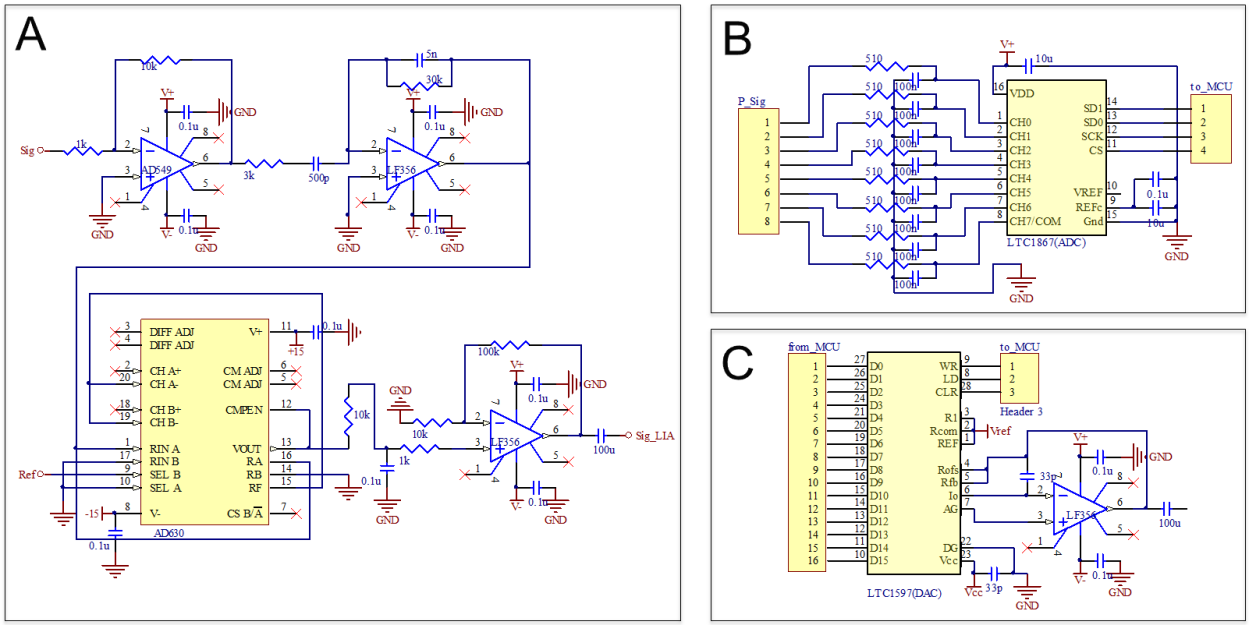

fig. S5. Circuit diagrams of the detection system. (A) Schematic of a custom-designed current amplifier circuit (AD549; Analog Devices), an active band pass filter, an analog lock-in circuit (AD630; Analog Devices), and a low pass filter. **(B)** A 16-bit analog-to-digital converter (LTC1867; Linear Technology) with a configurable 8-channel analog input multiplexer (MUX) was used. **(C)** A 16-bit digital-to-analog convertor (LTC1597; Linear Technology) was used to deliver the modulated control signal to the LED driver.

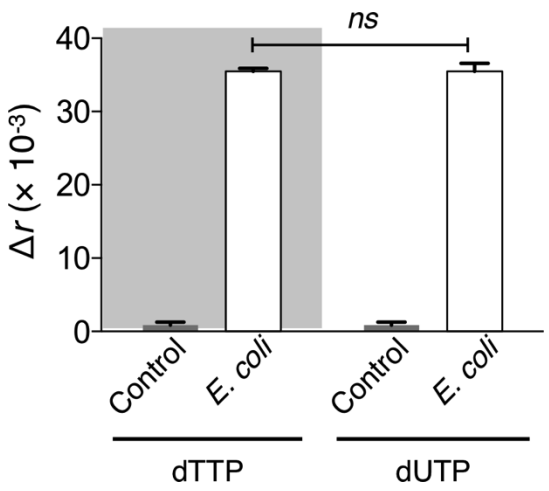

fig. S6. The effect of dUTP on the PAD assay. As compared to the original assay condition in which dTTP was used (left), no difference was observed when dTTP was replaced with dUTP (right). The observed Δr values in both cases were statistically identical (two-tailed *t*-test, $P > 0.99$).

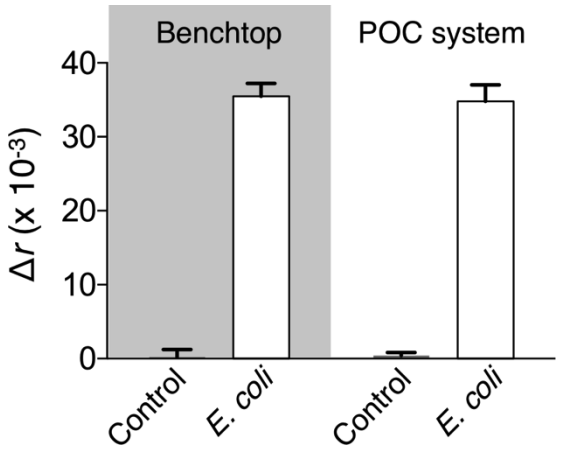

fig. S7. Portable PAD system. For on-site, point-of-care (POC) operation, the PAD detection was paired up with a miniaturized themocycler (MiniPCR; Ampylus). The performance of a benchtop equipment and the POC-PAD was statistically identical (two-tailed *t*-test, $P > 0.69$).

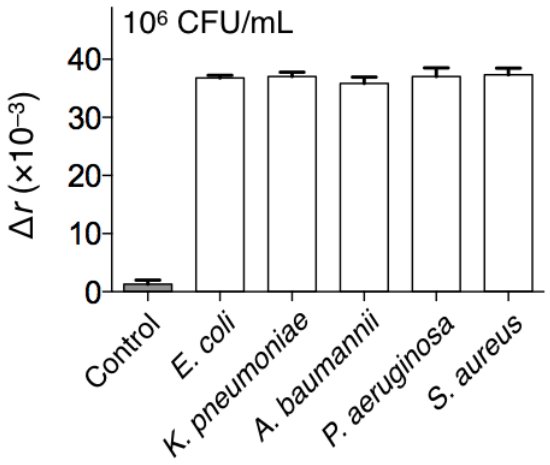

fig. S8. Lyophilized probes. The PAD reagents were lyophilized, stored for 2 weeks in ambient condition, and then used for bacterial detection. Five different HAI pathogens (10^6 CFU/mL) were detected with lyophilized reagents. The signal levels were comparable to those obtained by fresh reagents (**Fig. 3B**). All experiments were performed in triplicate, and the data are displayed as mean ± s.d.

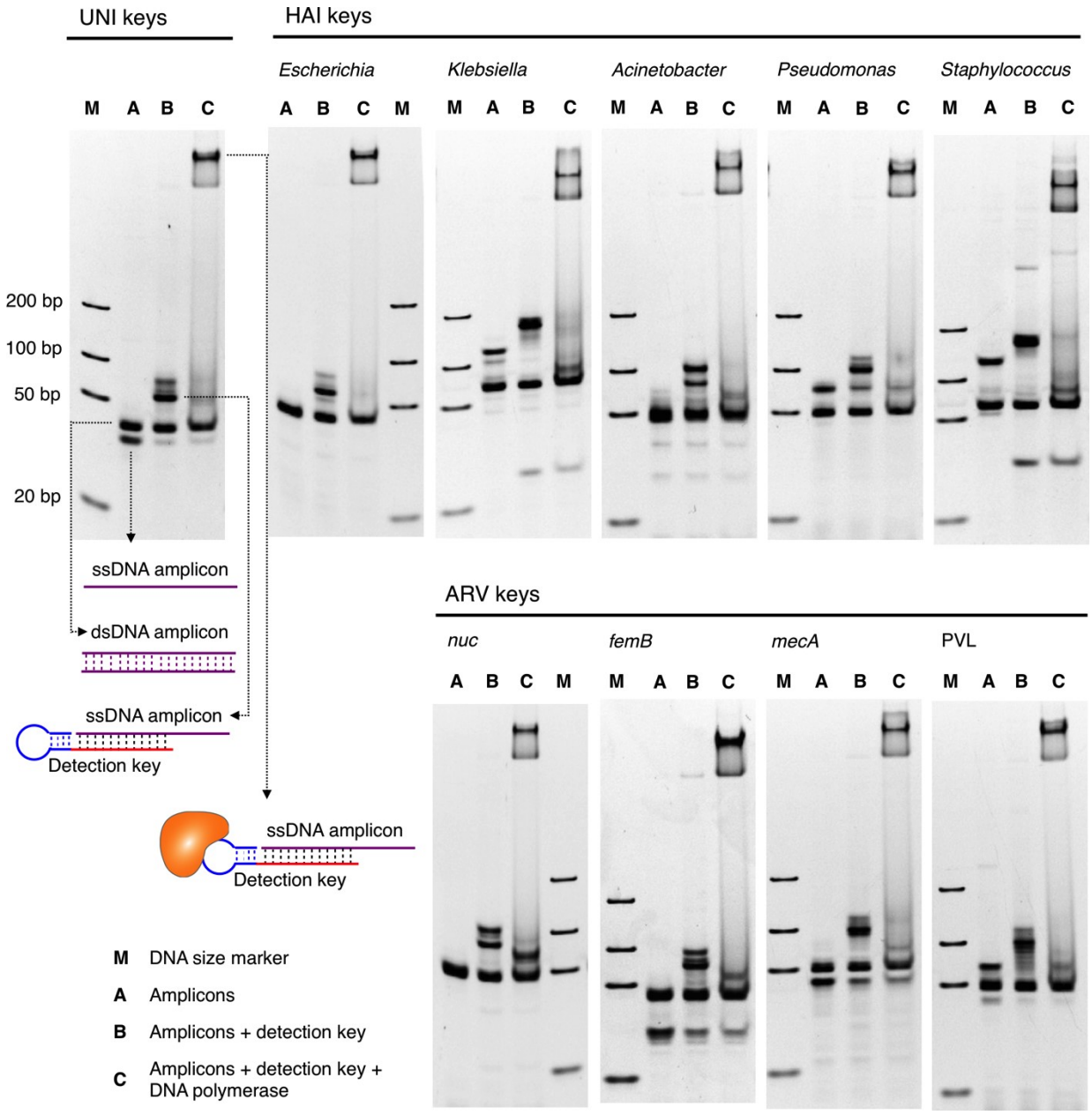

fig. S9. Electrophoretic band-shift assay. Lane A. Amplicons only. **Lane B**. Binding of amplicons with their detection keys produced new bands. **Lane C**. Adding DNA polymerase to B shifted the band for the amplicon and detection-key hybrid. This confirmed the binding between detection keys and DNA polymerase.

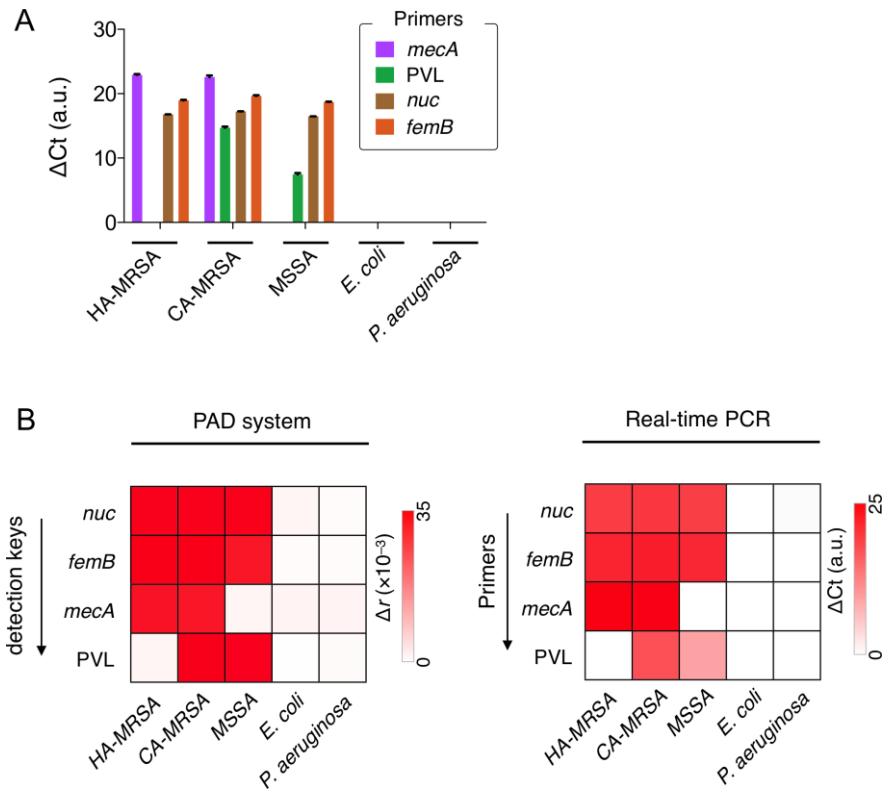

fig. S10. Detection of ARV factors. (A) Real-time PCR detection of *mecA*, PVL, *nuc*, and *femB* in bacterial species. SYBR Green assay was used. **(B)** Comparison between the PAD and the qPCR assays.

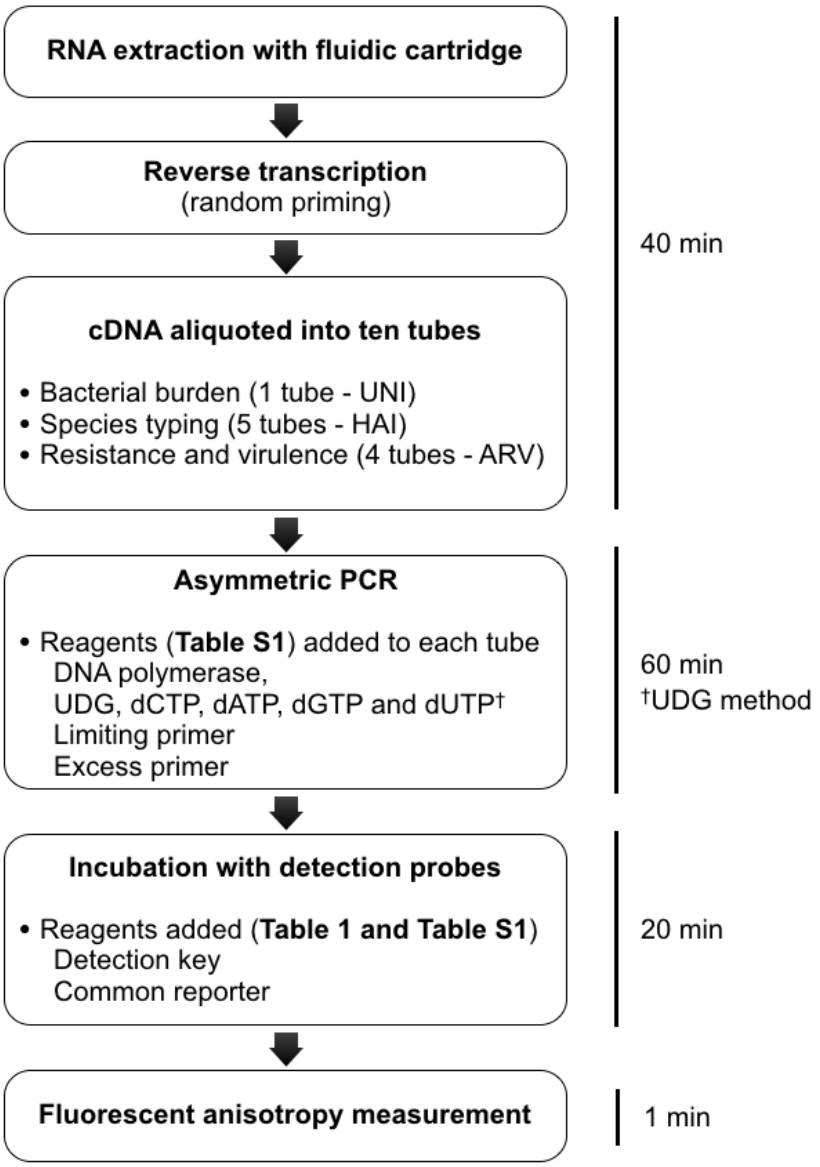

fig. S11. Overall assay procedure for clinical samples. Once the sample is aliquoted into individual tubes, the rest of the procedures are performed in the same tube.

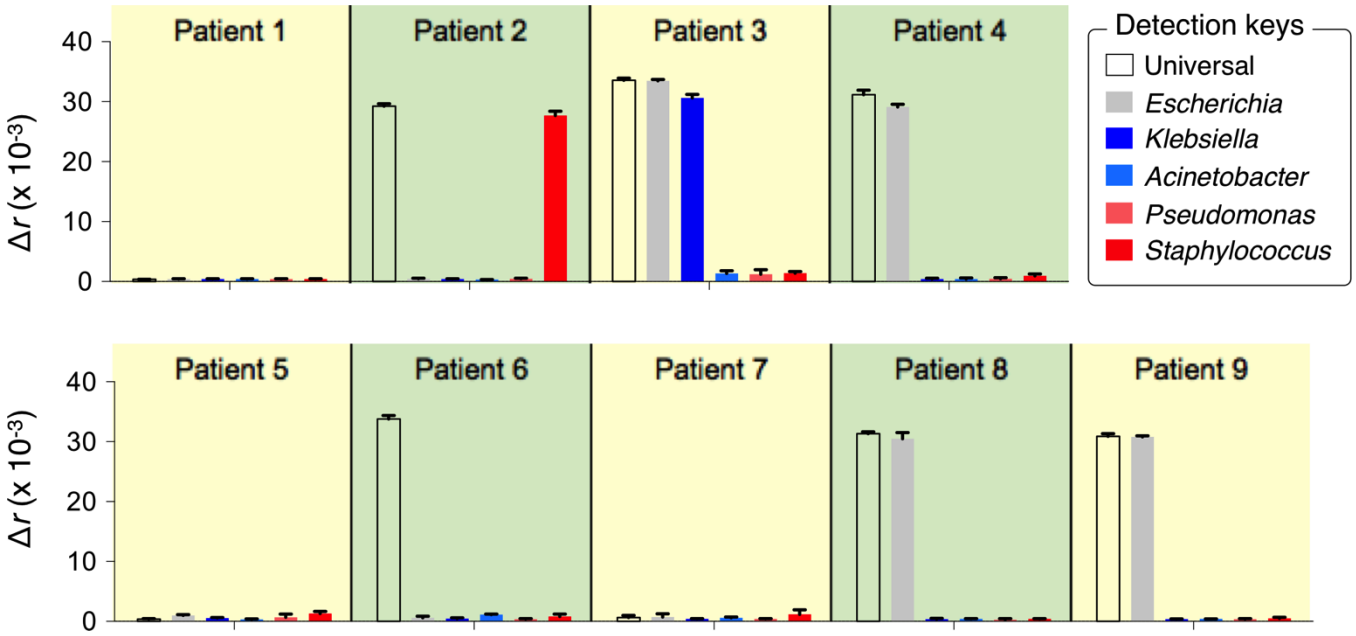

fig. S12. Universal and species-specific detection of HAI pathogens in clinical samples by the PAD system.

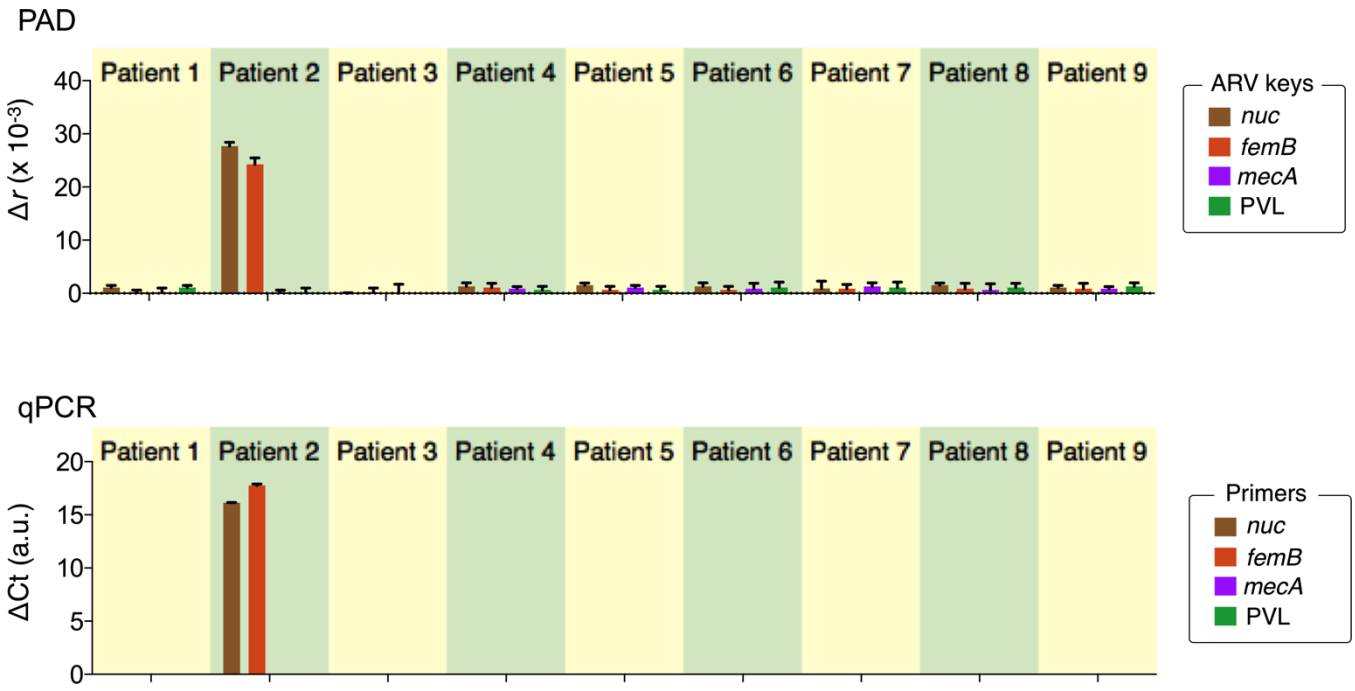

fig. S13. Detection of ARV factors in clinical samples with PAD (top) and qPCR (bottom).