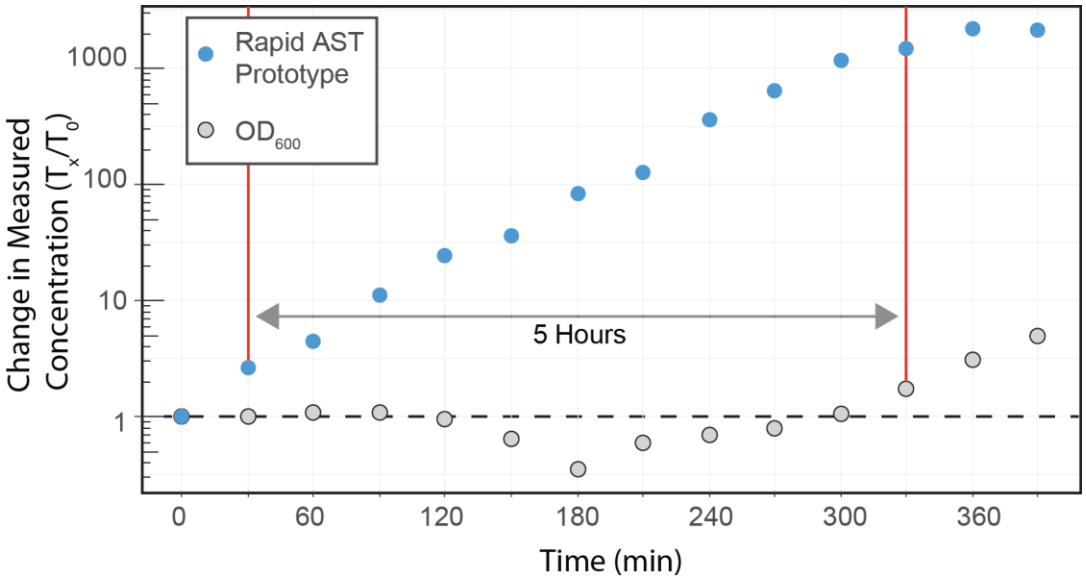
Supplementary Materials

Supplemental Figure 1. Comparison of detection sensitivity of rapid AST prototype and conventional optical density instrumentation. A culture of wild type *E. coli* (ATCC 295922) at a concentration of 1 x 10^3 CFU/ml was incubated for 6.5 hours and measured on both instruments at 30 min intervals. To display the data on the same plot, each time point was calculated as the "change in measured concentration" by dividing by the first time point measurement (T_0). Results demonstrate the superior sensitivity of the rapid AST device using discrete counting compared to traditional bulk measurements such as optical density, where a single doubling time of 30 minutes is enough to induce a detectable change, compared to at least 5 hours using OD_{600}.



Supplemental Table 1. CFU plate verification data for bacterial dilution curve.

Replicate	Expected Concentrations (CFU/mL)	Actual Concentrations (CFU/mL)
A	1×10^2	523.33
B	1×10^2	416.67
C	1×10^2	460.00
average		466.67
A	1×10^3	4190.00
B	1×10^3	2523.33
C	1×10^3	3120.00
average		3277.78
A	1×10^4	21033.33
B	1×10^4	25166.67
C	1×10^4	36300.00
average		27500.00
A	1×10^5	223000.00
B	1×10^5	212000.00
C	1×10^5	318666.67
average		251222.22
A	1×10^6	2343333.33
B	1×10^6	1930000.00
C	1×10^6	4030000.00
average		2767777.78

Supplemental Table 2: CFU Plate Verification Data for Contrived Samples (AST method validation)

Day 1 CFU Data: Back Calculations for Actual Concentration in Growth Medium								
	T0		**T1**		**T2**		**T3**	
WT *E. coli*	56800		67100		390000		5700000	
WT *E.coli* + Kanamycin	15700		0		0		0	*
***E. coli* KR**	15900		38500		630000		2600000	
***E. coli* KR + Kanamycin**	35700		18400		510000		1350000	
Day 2 CFU Data: Back Calculations for Actual Concentration in Growth Medium								
	T0		**T1**		**T2**		**T3**	
WT *E. coli*	18000		161000		1700000		6800000	
WT *E.coli* + Kanamycin	26400		0		0		0	*
***E. coli* KR**	47500		51000		259000		3320000	
***E. coli* KR + Kanamycin**	74800		57000		169000		1610000	
Day 3 CFU Data: Back Calculations for Actual Concentration in Growth Medium								
	T0		**T1**		**T2**		**T3**	
WT *E. coli*	655000		141000	**	1930000		7720000	
WT *E.coli* + Kanamycin	457000		0		0		0	*
***E. coli* KR**	319000		365000		1070000		2550000	
***E. coli* KR + Kanamycin**	175000		431000		1000000		2890000	

* for T1 to T3, no growth, assume remaining CFU/ml same as T0 concentration

**Note: T1 CFU plate showed lower colony numbers than expected/plating error (evident by next two time points)

Supplemental Table 3: Individual data comparing rapid and conventional AST results. Drug codes: AX = amoxicillin-clavulanate, CP = ciprofloxacin, CZ = ceftazidime , FF = fosfomycin, NF = nitrofurantoin. R = "resistant", S = "sensitive".

Sample Number	Patient ID	Drug	Rapid AST	Conventional AST	Species
1	0001	AX	R	R	*E. coli*
2	0001	CP	S	S	*E. coli*
3	0001	CZ	S	S	*E. coli*
4	0001	FF	S	S	*E. coli*
5	0001	NF	R	R	*E. coli*
6	0002	CP	R	R	*S. haemolyticus*
7	0002	FF	R	R	*S. haemolyticus*
8	0003	AX	R	R	*E. coli*
9	0003	CP	R	R	*E. coli*
10	0003	CZ	R	R	*E. coli*
11	0003	FF	S	S	*E. coli*
12	0003	NF	S	S	*E. coli*
13	0004	AX	R	R	*K. pneumoniae*
14	0004	CP	S	R	*K. pneumoniae*
15	0004	CZ	R	R	*K. pneumoniae*
16	0004	FF	R	R	*K. pneumoniae*
17	0005	CP	R	R	*P. aeruginosa*
18	0005	CZ	R	R	*P .aeruginosa*
19	0005	FF	R	R	*P. aeruginosa*
20	0006	AX	S	S	*E. faecalis/E. coli*
21	0006	CZ	S	S	*E. faecalis/E. coli*
22	0006	FF	S	S	*E. faecalis/E. coli*
23	0007	AX	R	R	*E. coli*
24	0007	CP	R	R	*E. coli*
25	0007	CZ	S	S	*E. coli*
26	0007	FF	S	S	*E. coli*
27	0007	NF	S	S	*E. coli*
28	0008	AX	R	S	*K. pneumoniae/E. faecalis*
29	0008	CP	S	S	*K. pneumoniae/E .faecalis*
30	0008	CZ	R	S	*K. pneumoniae/E .faecalis*
31	0008	FF	S	S	*K. pneumoniae/E. faecalis*
32	0009	AX	S	S	*E. coli*
33	0009	CP	S	S	*E. coli*
34	0009	CZ	S	S	*E. coli*
35	0009	FF	S	S	*E. coli*
36	0009	NF	S	S	*E. coli*
37	0010	AX	S	S	*E. coli*
38	0010	CP	S	S	*E. coli*
39	0010	CZ	R	S	*E. coli*
40	0010	FF	S	S	*E. coli*
41	0010	NF	S	S	*E. coli*
42	0011	AX	R	R	*E. coli*
43	0011	CP	R	R	*E. coli*
44	0011	CZ	R	R	*E. coli*

45	0011	FF	S	S	*E. coli*
46	0011	NF	S	S	*E. coli*
47	0012	AX	R	S	*E. coli/E .faecalis*
48	0012	CP	S	S	*E. coli/E. faecalis*
49	0012	CZ	S	S	*E. coli/E. faecalis*
50	0012	FF	S	S	*E. coli/E. faecalis*
51	0012	NF	S	S	*E. coli/E. faecalis*
52	0013	NF	S	S	*E. faecalis*
53	0014	AX	S	S	*E. coli*
54	0014	CP	S	S	*E. coli*
55	0014	CZ	S	S	*E. coli*
56	0014	FF	S	S	*E. coli*
57	0014	NF	S	S	*E. coli*
58	0015	CP	S	S	*S. haemolyticus*
59	0015	FF	S	R	*S. haemolyticus*
60	0016	AX	R	R	*K. pneumoniae*
61	0016	CP	R	R	*K. pneumoniae*
62	0016	CZ	R	R	*K. pneumoniae*
63	0016	FF	S	S	*K. pneumoniae*
64	0017	AX	S	S	*E. coli*
65	0017	CP	S	S	*E. coli*
66	0017	CZ	S	S	*E. coli*
67	0017	FF	S	S	*E. coli*
68	0017	NF	S	S	*E. coli*
69	0018	AX	S	S	*E. coli*
70	0018	CP	S	S	*E. coli*
71	0018	CZ	S	S	*E. coli*
72	0018	FF	S	S	*E. coli*
73	0018	NF	S	S	*E. coli*
74	0019	AX	S	S	*K. pneumoniae*
75	0019	CP	S	S	*K. pneumoniae*
76	0019	CZ	S	S	*K. pneumoniae*
77	0019	FF	S	R	*K. pneumoniae*
78	0020	AX	R	R	*E. coli*
79	0020	CP	R	R	*E. coli*
80	0020	CZ	R	R	*E. coli*
81	0020	NF	S	S	*E. coli*
82	0021	AX	S	S	*E. coli*
83	0021	CP	S	S	*E. coli*
84	0021	CZ	R	S	*E. coli*
85	0021	FF	S	S	*E. coli*
86	0021	NF	S	S	*E. coli*
87	0022	AX	S	S	*E.coli*
88	0022	CP	S	S	*E. coli*
89	0022	CZ	S	S	*E. coli*
90	0022	FF	S	S	*E. coli*
91	0022	NF	S	S	*E. coli*
92	0023	AX	S	S	*E. coli/E. faecalis*
93	0023	CZ	S	S	*E. coli/E. faecalis*
94	0023	FF	S	S	*E. coli/E. faecalis*

95	0024	CP	S	S	*E. faecalis*
96	0024	NF	S	S	*E. faecalis*
97	0025	CP	R	S	*E. faecium*