Supporting Information

Rapid Detection of Urinary Tract Infection in 10 Minutes by Tracking Multiple Phenotypic Features in a 30-Second Large Volume Scattering Video of Urine Microscopy

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S1. The prototype LVSi system

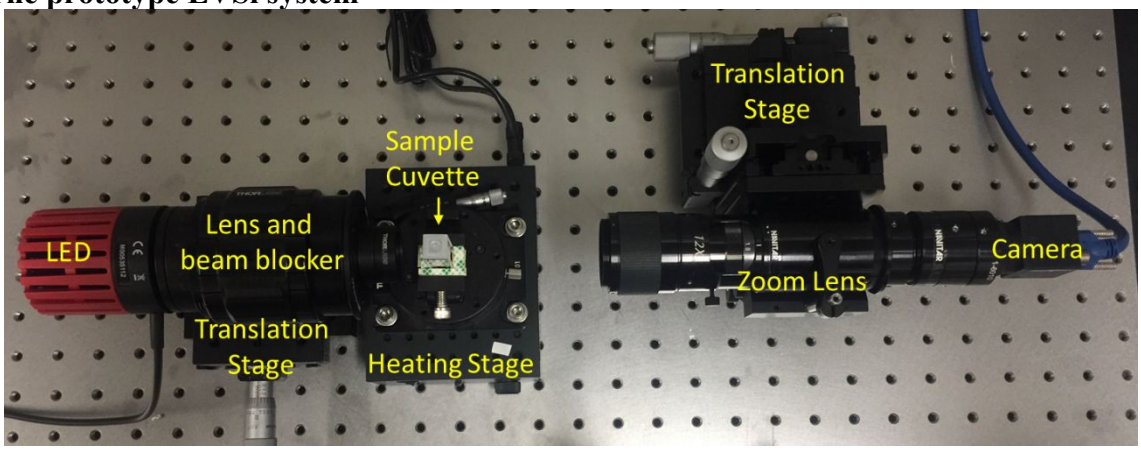

Fig S1. Photo of the prototype LVSi system. The optical system includes one LED light, one zoom lens, and one camera. An electrical heating stage with a temperature control was used to maintain the temperature of the sample in cuvette at 37 ºC.

S2. Evaluation of tracking accuracy

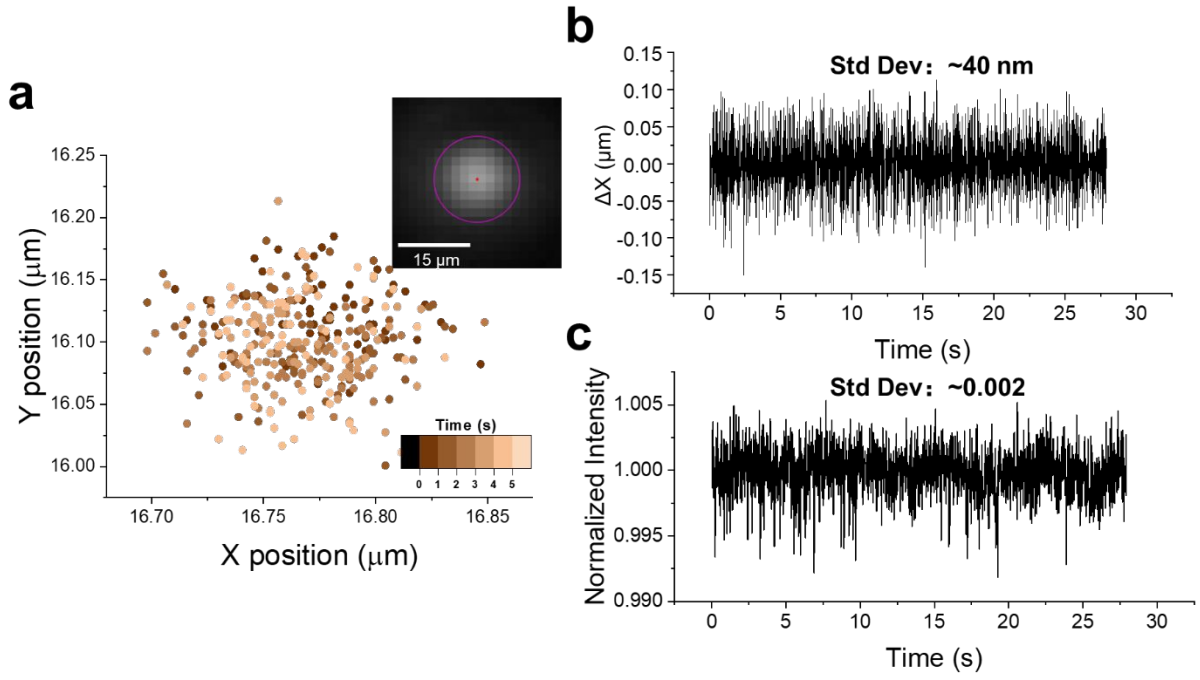

Figure S2. Evaluation of tracking accuracy of an immobilized particle. The intensity and position over time of an immobilized particle is tracked (a). The bright spot in the image is tracked with ~40 nm accuracy (standard deviation) by fitting the intensity distribution with a Gaussian function (b). The detection accuracy (standard deviation) of normalized intensity is ~0.002 for the current system (c).

S3. Comparison of intensity fluctuation

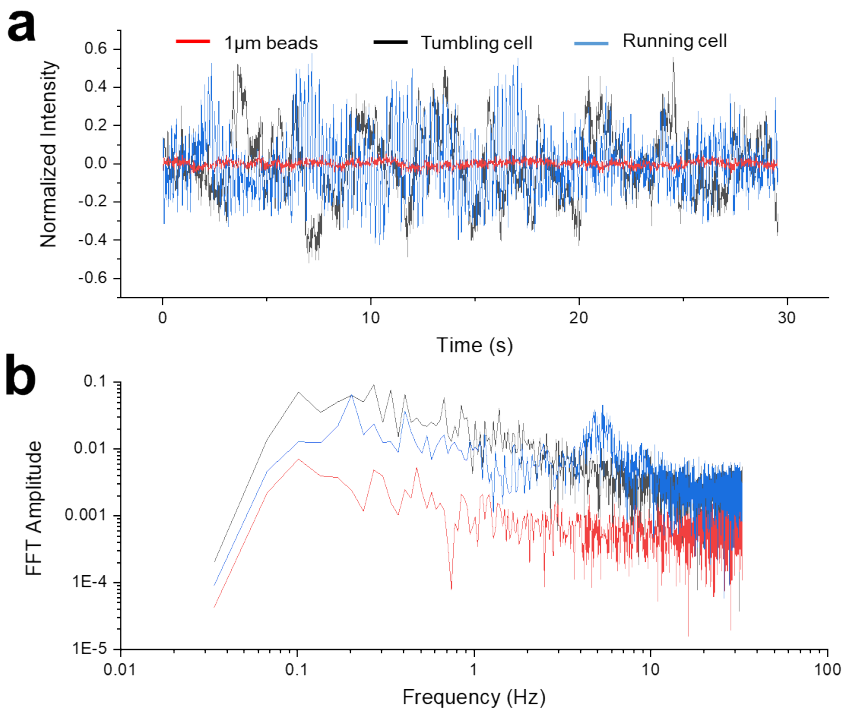

Figure S3. Normalized intensity pattern of (a) 1 μm beads and *E. coli* cells with two different swimming patterns (tumbling and running) and the (b) corresponding Fast Fourier Transform (FFT) results.

S4. Differentiation of *E. coli* and *K. pneumoniae* by phenotypic features tracking

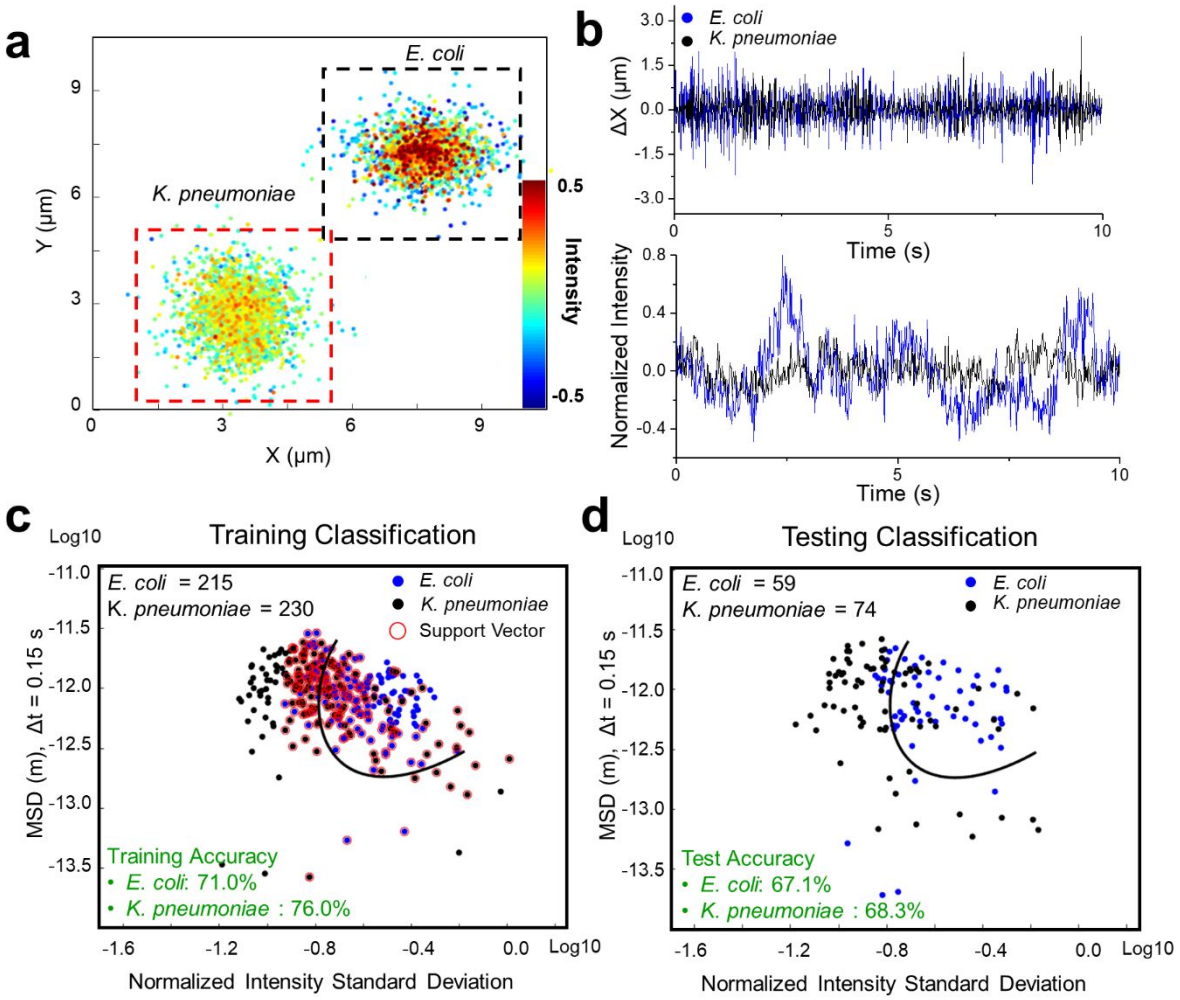

Figure S4. Differentiation of *E. coli* and *K. pneumoniae* by single cell phenotypic features tracking. (a) Single cell motion and intensity mapping for *E. coli* and *K. pneumoniae*. (b) Comparison of the corresponding intensity fluctuation and micro-motion of single *E. coli* and *K. pneumoniae* cells. (c) Training results obtained from individual pure cultures of *E. coli* (n = 215) and *K. pneumoniae* (n = 230) with machine learning classification (Support Vector Machine, SVM) based on mean squared displacement (MSD) of single cell motion and normalized intensity standard deviation (NISD) of single cell intensity. (d) Testing results obtained from individual pure cultures of both *E. coli* cells (n = 109) and *K. pneumoniae* cells (n = 74) with the trained machine learning classification (Support Vector Machine, SVM) model.

S5. The ROC curve for threshold determination with the first 20 clinical urine samples

To determine an infection threshold, the receiver operating characteristic (ROC) curve was constructed using the relative amounts of cells/all particles (N_{Cell}/N_{Total}) as a predictor, and results were evaluated to determine the infection threshold for UTIs. From the ROC curve for the first 20 samples, of which 10 were positive and 10 were negative via clinical testing, we determined the infection threshold of 0.5 with a sensitivity of 90% and a specificity of 100%.

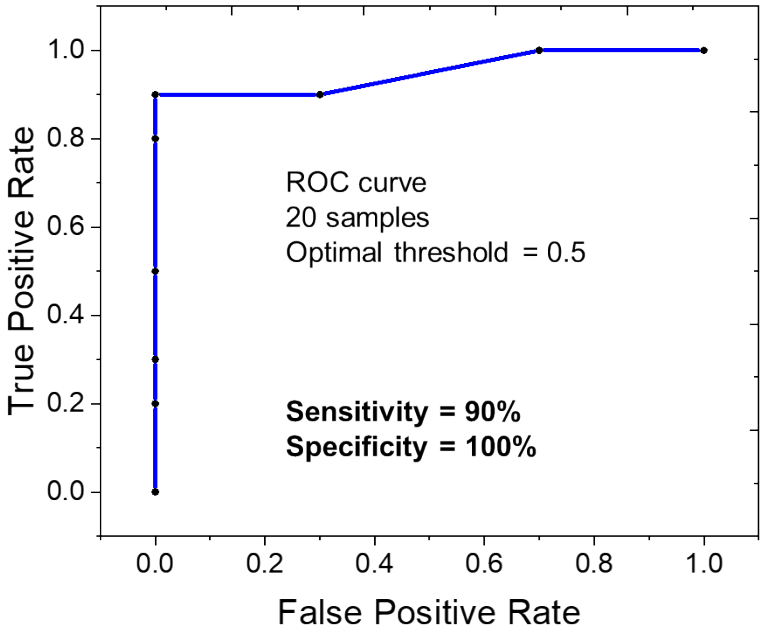

Figure S5. The ROC curve for threshold determination with the first 20 clinical samples.

S6. Classification of different samples with the trained model of distinguishing *E. coli* from urine particles

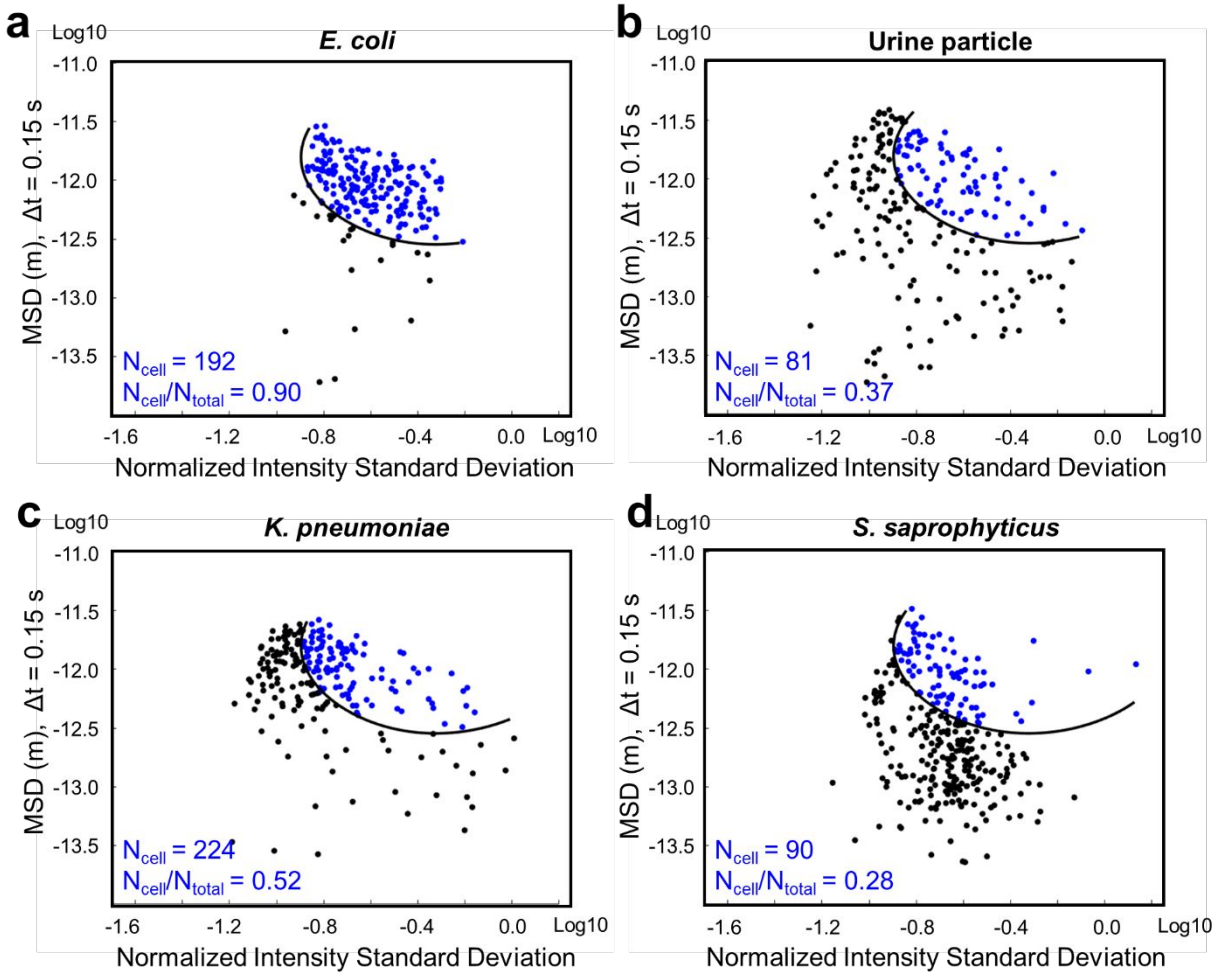

Figure S6. The testing classification results with the trained model (distinguishing *E. coli* from urine particles) for different samples. (a) Pure culture of *E. coli* cells. (b) Pooled, healthy urine sample with no bacteria present. (c) Pure culture of *K. pneumoniae* cells. (d) Pure culture of *S. saprophyticus* cells. (a-d) The *E. coli* – urine particle trained SVM model classifies bacterial cells as blue dots and non-bacterial particles as black dots.

S7. Clinical urine sample rapid detection results

Table S1. LVSi-RD detection (N_{cell}/N_{total}) results of UTI for 104 human patient samples compared with clinical results and on-site plating validation results.

Sample #	Sample ID	Color	N_{Cell}/N_{Total}	LVSi-RD Call	Clinical Lab Call*	Plating Validation Call#	Comments#
3	ATU120319_03	Milky	0.90	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
6	ATU120319_06	Milky	0.77	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
7	ATU120319_07	Clear	0.69	Positive	Positive	Positive	10,000-100,000 cfu/mL ESCHERICHIA COLI
9	ATU120319_09	Milky	0.68	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
10	ATU120319_10	Yellow	0.93	Positive	Positive	Positive	>100,000 cfu/mL KLEBSIELLA PNEUMONIAE
12	ATU121019_02	Clear	0.83	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
14	ATU121019_04	Clear	0.22	Negative†	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
16	ATU121019_06	Cloudy	0.61	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
18	ATU121019_08	Cloudy	0.76	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
20	ATU121019_10	Milky	0.57	Positive	Positive	Positive	>100,000 cfu/mL KLEBSIELLA PNEUMONIAE
23	ATU010720_03	Milky	0.82	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
24	ATU010720_04	Milky	0.66	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
26	ATU010720_06	Milky	0.64	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
27	ATU010720_07	Cloudy	0.80	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
28	ATU010720_08	Cloudy	0.72	Positive	Positive	Positive	>100,000 cfu/mL KLEBSIELLA PNEUMONIAE
34	ATU011420_04	Milky	0.93	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
35	ATU011420_05	Milky	0.58	Positive	Positive	Positive	10,000-100,000 cfu/mL ESCHERICHIA COLI
38	ATU011420_08	Clear	0.56	Positive	Positive	Positive	10,000-100,000 cfu/mL ESCHERICHIA COLI
39	ATU011420_09	Cloudy	0.77	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
40	ATU011420_10	Milky	0.65	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
41	ATU012120_01	Cloudy	0.55	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
42	ATU012120_02	Milky	0.88	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
47	ATU012120_07	Clear	0.86	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
48	ATU012120_08	Milky	0.78	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
49	ATU012120_09	Milk	0.30	Negative†	Positive	Positive	>100,000 cfu/mL GRAM NEGATIVE BACILLUS
51	ATU012820_01	Cloudy	0.70	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
52	ATU012820_02	Milky	0.40	Negative†	Positive	Positive	10,000-100,000 cfu/mL ESCHERICHIA COLI
56	ATU012820_06	Milky	0.71	Positive	Positive	Positive	>100,000 cfu/mL GRAM NEGATIVE BACILLUS
57	ATU012820_07	Clear	0.77	Positive	Positive	Positive	10,000-100,000 cfu/mL GRAM NEGATIVE BACILLUS
60	ATU012820_10	Cloudy	0.82	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
61	ATU020420_01	Milky	0.94	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
62	ATU020420_02	Clear	0.70	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
63	ATU020420_03	Cloudy	0.88	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
68	ATU020420_08	Milky	0.83	Positive	Positive	Positive	>100,000 cfu/mL GRAM NEGATIVE BACILLUS
69	ATU020420_09	Clear	0.42	Negative†	Positive	Positive	>100,000 cfu/mL GRAM NEGATIVE BACILLUS
71	ATU021120_01	Cloudy	0.86	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
72	ATU021120_02	Milky	0.73	Positive	Positive	Positive	10,000-100,000 cfu/mL ESCHERICHIA COLI
79	ATU021120_09	Cloudy	0.47	Negative†	Positive	Positive	10,000-100,000 cfu/mL ESCHERICHIA COLI
80	ATU021120_10	Yellow	0.21	Negative†	Positive	Positive	10,000-100,000 cfu/mL ESCHERICHIA COLI
84	ATU021820_04	Clear	0.82	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
85	ATU021820_05	Cloudy	0.62	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
86	ATU021820_06	Milky	0.74	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
87	ATU021820_07	Yellow	0.45	Negative†	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
90	ATU021820_10	Clear	0.59	Positive	Positive	Positive	10,000-100,000 cfu/mL KLEBSIELLA PNEUMONIAE
93	ATU022520_03	Cloudy	0.90	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
94	ATU022520_04	Clear	0.41	Negative†	Positive	Negative†	10,000-100,000 cfu/mL ESCHERICHIA COLI
95	ATU022520_05	Milky	0.71	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
96	ATU022520_06	Milky	0.81	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
100	ATU022520_10	Clear	0.94	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
101	ATU031020_01	Cloudy	0.88	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI
102	ATU031020_02	Cloudy	0.83	Positive	Positive	Positive	>100,000 cfu/mL ESCHERICHIA COLI

Sample #	Sample ID	Color	IC90/IC0	LVSi-RD Call	Clinical Lab Call*	Plating Validation Call#	Comments#
1	ATU120319_01	Milky	0.25	Negative	Negative	Negative	No growth after 1 day of incubation.
2	ATU120319_02	Yellow	0.32	Negative	Negative	Negative	No growth after 1 day of incubation.
4	ATU120319_04	Yellow	0.22	Negative	Negative	Negative	No growth after 1 day of incubation.
5	ATU120319_05	Yellow	0.23	Negative	Negative	Negative	No growth after 1 day of incubation.
8	ATU120319_08	Clear	0.26	Negative	Negative	Negative	No growth after 1 day of incubation.
11	ATU121019_01	Milky	0.14	Negative	Negative	Negative	No growth after 1 day of incubation.
13	ATU121019_03	Yellow	0.37	Negative	Negative	Negative	No growth after 1 day of incubation.
15	ATU121019_05	Clear	0.32	Negative	Negative	Negative	No growth after 1 day of incubation.
17	ATU121019_07	Clear	0.19	Negative	Negative	Negative	No growth after 1 day of incubation.
19	ATU121019_09	Yellow	0.17	Negative	Negative	Negative	No growth after 1 day of incubation.
21	ATU010720_01	Clear	0.35	Negative	Negative	Negative	No growth after 1 day of incubation.
22	ATU010720_02	Clear	0.13	Negative	Negative	Negative	No growth after 1 day of incubation.
25	ATU010720_05	Clear	0.28	Negative	Negative	Negative	No growth after 1 day of incubation.
29	ATU010720_09	Clear	0.39	Negative	Negative	Negative	No growth after 1 day of incubation.
30	ATU010720_10	Yellow	0.22	Negative	Negative	Negative	No growth after 1 day of incubation.
31	ATU011420_01	Yellow	0.20	Negative	Negative	Negative	No growth after 1 day of incubation.
32	ATU011420_02	Clear	0.44	Negative	Negative	Negative	No growth after 1 day of incubation.
33	ATU011420_03	Cloudy	0.13	Negative	Negative	Negative	No growth after 1 day of incubation.
36	ATU011420_06	Clear	0.22	Negative	Negative	Negative	No growth after 1 day of incubation.
37	ATU011420_07	Yellow	0.25	Negative	Negative	Negative	No growth after 1 day of incubation.
43	ATU012120_03	Clear	0.36	Negative	Negative	Negative	No growth after 1 day of incubation.
44	ATU012120_04	Yellow	0.15	Negative	Negative	Negative	No growth after 1 day of incubation.
45	ATU012120_05	Yellow	0.13	Negative	Negative	Negative	No growth after 1 day of incubation.
46	ATU012120_06	Cloudy	0.31	Negative	Negative	Negative	No growth after 1 day of incubation.
50	ATU012120_10	Yellow	0.20	Negative	Negative	Negative	No growth after 1 day of incubation.
53	ATU012820_03	Yellow	0.30	Negative	Negative	Negative	No growth after 1 day of incubation.
54	ATU012820_04	Yellow	0.43	Negative	Negative	Negative	No growth after 1 day of incubation.
55	ATU012820_05	Cloudy	0.33	Negative	Negative	Negative	No growth after 1 day of incubation.
58	ATU012820_08	Cloudy	0.13	Negative	Negative	Negative	No growth after 1 day of incubation.
59	ATU012820_09	Clear	0.30	Negative	Negative	Negative	No growth after 1 day of incubation.
64	ATU020420_04	Cloudy	0.25	Negative	Negative	Negative	No growth after 1 day of incubation.
65	ATU020420_05	Yellow	0.21	Negative	Negative	Negative	No growth after 1 day of incubation.
66	ATU020420_06	Yellow	0.19	Negative	Negative	Negative	No growth after 1 day of incubation.
67	ATU020420_07	Clear	0.47	Negative	Negative	Negative	No growth after 1 day of incubation.
70	ATU020420_10	Clear	0.40	Negative	Negative	Negative	No Growth after 1 day of incubation
74	ATU021120_04	Yellow	0.14	Negative	Negative	Negative	No growth after 1 day of incubation.
75	ATU021120_05	Yellow	0.09	Negative	Negative	Negative	No growth after 1 day of incubation.
76	ATU021120_06	Yellow	0.09	Negative	Negative	Negative	No growth after 1 day of incubation.
77	ATU021120_07	Yellow	0.19	Negative	Negative	Negative	No growth after 1 day of incubation.
78	ATU021120_08	Cloudy	0.31	Negative	Negative	Negative	No growth after 1 day of incubation.
81	ATU021820_01	Milky	0.31	Negative	Negative	Negative	No growth after 1 day of incubation.
82	ATU021820_02	Milky	0.39	Negative	Negative	Negative	No growth after 1 day of incubation.
83	ATU021820_03	Yellow	0.29	Negative	Negative	Negative	No growth after 1 day of incubation.
88	ATU021820_08	Yellow	0.47	Negative	Negative	Negative	No growth after 1 day of incubation.
89	ATU021820_09	Yellow	0.42	Negative	Negative	Negative	No growth after 1 day of incubation.
91	ATU022520_01	Milky	0.05	Negative	Negative	Negative	No growth after 1 day of incubation.
92	ATU022520_02	Yellow	0.13	Negative	Negative	Negative	No growth after 1 day of incubation.
97	ATU022520_07	Cloudy	0.29	Negative	Negative	Negative	No growth after 1 day of incubation.
98	ATU022520_08	Clear	0.44	Negative	Negative	Negative	No growth after 1 day of incubation.
99	ATU022520_09	Yellow	0.14	Negative	Negative	Negative	No growth after 1 day of incubation.
103	ATU031020_03	Cloudy	0.44	Negative	Negative	Negative	No growth after 1 day of incubation.
104	ATU031020_04	Clear	0.24	Negative	Negative	Negative	No growth after 1 day of incubation.

***** Reference method; standard microbiological plating results generated by the Mayo Clinic microbiology lab.
[#] On-site validation results generated by standard microbiology plating upon sample receiving.
[†] Disagreement between LVSi-RD and reference method results.

S8. Initial sample validation results

On-site initial bacterial load validation is performed with sample microbiology plating and colony counting. Upon receipt, urine samples were subjected to serial dilutions and plated on LB agar for colony enumeration. This plating validation provides initial bacterial concentration reference and reveals any viability changes during sample storage and transportation. After LVSi-RD, we obtained an estimation of the bacterial cell number with SVM clustering, which provides the calculated cell concentration per mL for comparison.

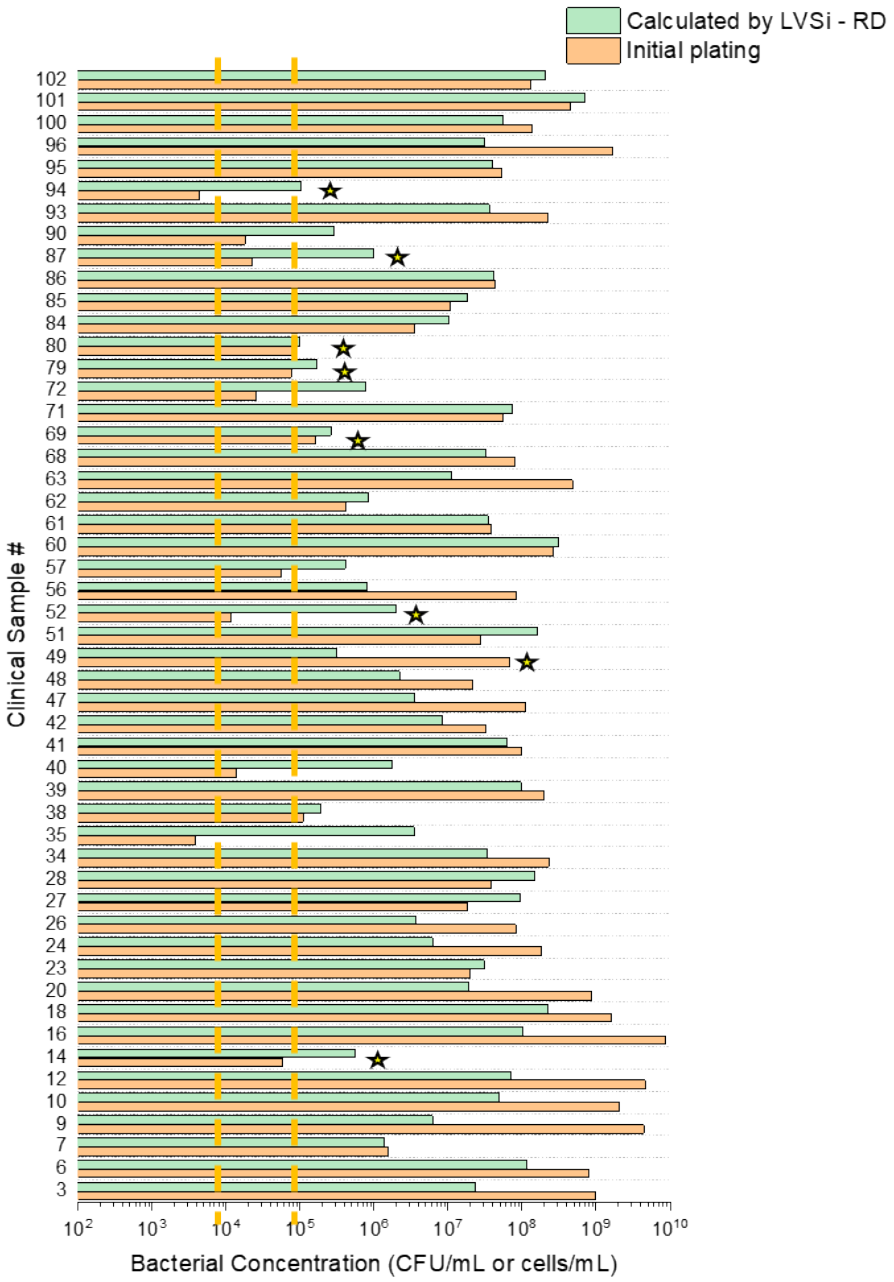

Figure S7. Initial plating validation (yellow bar) of 51 clinical positive samples and the calculated cell concentration by LVSi-RD (green bar). The yellow dashed lines indicate the clinical infection threshold (10^4-10^5 CFU/mL). The stars mark the 8 false negative samples determined by LVSi-RD.

S9. Initial and parallel plating validation result of 8 false negative samples

Parallel plating validation was performed along with LVSi-RD to test the samples post-preparation. Initial plating CFU/mL determinations (described above), calculated CFU/mL based on sample dilution, and parallel plating of sample post-preparation of eight false negative samples are presented here. Of the eight false negative samples, initial on-site plating validation found one sample (#94) to have a bacterial concentration below the clinical threshold of 10^4 CFU/mL. After all sample handling, including prewarming, filtration and dilution, the parallel microbiological plating validation results show low counts of bacterial cells (below 1,000 cells/mL). Therefore, false negative results are likely due to a combination of low initial bacterial concentration, storage, transport, and the sample handling process, most of which can be avoided with an optimized dilution scheme and quicker handling process at the point of care settings.

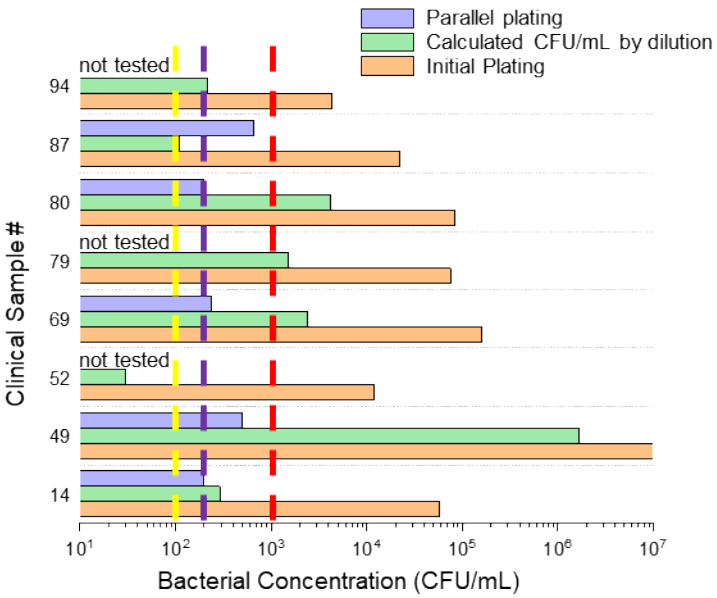

Figure S8. The comparison of initial plating, calculated CFU/mL by dilution, and the parallel microbiological plating results of eight false negatives samples. Most of the false negative samples had bacterial concentrations below 1,000 CFU/mL (red dashed line) after sample preparation. The parallel microbiological plating results are the mean value of three technical replicates. The limit of detection for initial microbiological plating (yellow bar) is 100 CFU/mL (yellow dashed line), and the limit for parallel microbiological plating (purple bar) is 200 CFU/mL (purple dashed line).

S10. Flow chart for clinical urine sample preparation

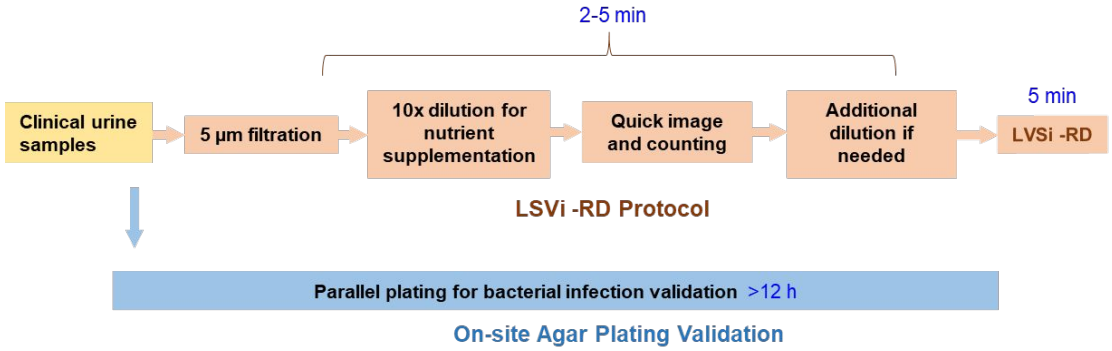

Figure S9. Workflow of sample preparation, LVSi-RD test, and plating-based validations for clinical urine samples.

S11. Flow chart for LVSi video processing and machine learning

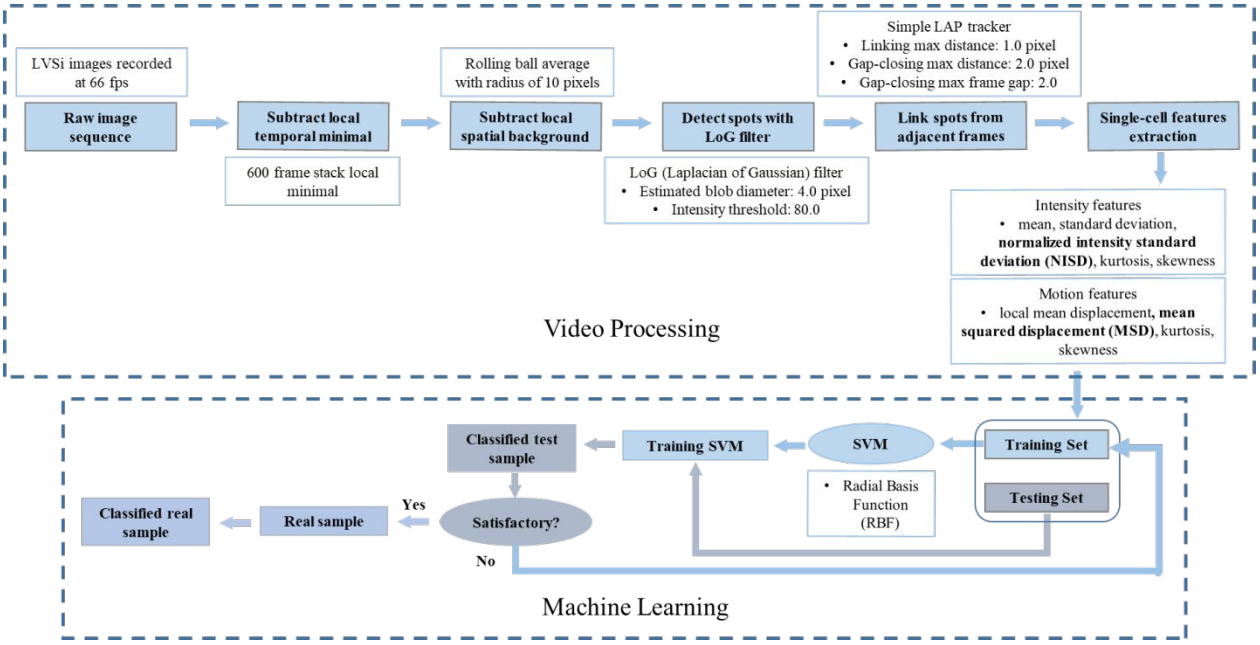

Figure S10. Flow chart for LVSi video processing and machine learning.