Supplement for

Identification of bacteria in mixed infection from urinary tract of patient's samples using Raman analysis of dried droplets

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Content

Supplemental Table S1: Overview of patients in the study

Patient ID	Sex	diagnosis / visit	source of urine	Comment
1	m	long-term catheter change	catheter / urine bag	
2	m	long-term catheter change	catheter / urine bag	
3	m	UTI	catheter / urine bag	
4	m	cystofix catheter change	catheter / urine bag	
5	f	long-term catheter change	catheter / urine bag	
6	m	long-term catheter change	catheter / urine bag	
7	f	urolithiasis follow-up	midstream urine	same patient as sample 53
8	m	long-term catheter change	catheter / urine bag	
9	m	double J-stent removal	midstream urine	
10	f	stent change	midstream urine	
11	m	long-term catheter change	catheter / urine bag	same patient as sample 24
12	m	long-term catheter change	catheter / urine bag	
13	m	UTI follow-up	midstream urine	
14	m	bladder neck sklerosis	midstream urine	
15	m	long-term catheter change	catheter / urine bag	same patient as sample 17
16	m	BPH	midstream urine	
17	m	long-term catheter change	catheter / urine bag	same patient as sample 15
18	m	prostate abscess	midstream urine	
19	f	stent change	midstream urine	
20	m	long-term catheter change	catheter / urine bag	
21	m	long-term catheter change	catheter / urine bag	
22	f	urolithiasis follow-up	midstream urine	
23	f	voiding dysfunction	midstream urine	
24	m	long-term catheter change	catheter / urine bag	same patient as sample 11
25	m	long-term catheter change	catheter / urine bag	
26	f	stent change	midstream urine	
27	f	recurrent UTI	midstream urine	
28	f	pouch follow-up	navel pouch / urine bag	
29	m	stent change	midstream urine	
30	m	BPH	midstream urine	
31	f	double J-stent placement	midstream urine	
32	f	follow-up	midstream urine	
33	m	BPH	midstream urine	
34	f	stent change	midstream urine	
35	f	double J-stent change	midstream urine	
36	f	urolithiasis follow-up	midstream urine	
37	f	incontinence	midstream urine	
38	f	long-term catheter change	catheter / urine bag	
39	m	long-term catheter change	catheter / urine bag	
40	m	seminom resection	midstream urine	

41	f	double J-Stent placement, Urolithiasis	midstream urine	
42	m	cystoscopy	midstream urine	
43	m	urethral stricture	midstream urine	
44	f	recurrent UTI	midstream urine	
45	f	double J-stent change	midstream urine	
46	m	BPH	catheter / urine bag	
47	m	retrograde ureteropyelography	midstream urine	
48	m	TURB	catheter / urine bag	
49	m	phimosis	midstream urine	
50	m	ileum conduit follow-up	ileum-conduit / urine bag	
51	m	acute UTI, nephrolithiasis	midstream urine	
52	m	TURB	midstream urine	
53	f	nephrolithiasis	midstream urine	same patient as sample 7
54	f	TURB	midstream urine	
55	m	BPH follow-up	midstream urine	
56	m	recurrent UTI, TURB	midstream urine	
57	m	prostate cancer follow-up	midstream urine	
58	m	TURB	midstream urine	
59	m	TURB	midstream urine	
60	m	urethral stent change	midstream urine	spectral QC failed, excluded
61	m	long-term catheter change	catheter / urine bag	no bacterial growth, excluded
62	f	hydronephrosis, UTUC	midstream urine	no bacterial growth, excluded
63	f	incontinence	midstream urine	no bacterial growth, excluded
64	m	percutane nephrostomie change	catheter / urine bag	no bacterial growth, excluded

BPH.. Benign Prostatic Hyperplasia;

TURB.. Transurethral bladder tumour resection

Supplemental Table S2: Summary of clinical isolates included in the study

Family	**Species or Genus**	**# of spectra**	**Strain**	**Gram type**	**origin**
Enterobacteriaceae	*E. coli*	350	*E. coli* ReWhINP01 1	**negative**	patient#01
			E. coli urRP005		patient#05
			E. coli urRP007		patient#07
			E. coli urRP009		patient#09
			E. coli urRP010		patient#10
			E. coli urRP013		patient#13
			E. coli urRP014_1		patient#14
			E. coli urRP018		patient#18
			E. coli urRP020		patient#20
			E. coli urRP028		patient#28
			E. coli urRP034		patient#34
	Klebsiella spp.	430	*K. pneumoniae* urRP003		patient#03
			K. pneumoniae urRP025		patient#25
			K. pneumoniae urRP027		patient#27
			K. pneumoniae urRP028		patient#28
			K. pneumoniae urRP032		patient#32
			K. pneumoniae urRP047		patient#47
			K. pneumoniae urRP060		patient#60
			K. oxytoca urRP011		patient#11
			K. oxytoca urRP012		patient#12
			K. oxytoca urRP013		patient#13
			K. oxytoca urRP034		patient#34
			K. oxytoca urRP061		patient#61
			K. aerogenes urRP057		patient#57
			K. aerogenes bk021932		clinical
	Enterobacter cloacae	180	*E. cloacae* Va065668		clinical
			E. cloacae Va066050		clinical
			E. cloacae Va066085		clinical
			E. cloacae Va066363		clinical
			E. cloacae complex urRP037		patient#37
			E. cloacae complex urRP051		patient#51
	Citrobacter koseri	70	*C. koseri* urRP004		patient#04
			C. koseri urRP050		patient#50
Morganellaceae	*Proteus mirabilis*	250	*P. mirabilis* urRP012		patient#12
			P. mirabilis urRP021		patient#21
			P. mirabilis urRP026		patient#26
			P. mirabilis urRP029		patient#29
			P. mirabilis urRP030		patient#30
			P. mirabilis urRP039		patient#39
			P. mirabilis urRP045		patient#45
	Providencia rettgeri	120	*P. rettgeri* urRP003		patient#03
			P. rettgeri urRP051	**negative**	patient#51
	Morganella morganii	60	*M. morganii* urRP012		patient#12

Pseudomonadaceae	*Pseudomonas spp.*	920	*P. aeruginosa* urRP004		patient#04
			P. aeruginosa urRP008		patient#08
			P. aeruginosa urRP018		patient#18
			P. aeruginosa urRP024		patient#24
			P. aeruginosa urRP026		patient#26
			P. aeruginosa urRP039		patient#39
			P. aeruginosa urRP046		patient#46
			P. aeruginosa urRP056		patient#56
			P. aeruginosa Va065276		clinical
			P. aeruginosa Va065845		clinical
			P. aeruginosa Va065994		clinical
			P. aeruginosa Va066070		clinical
			P. putida urRP005		patient#05
Moraxellaceae *	*Acinetobacter ursingii*	30	*A. ursingii* urRP040		patient#40
Enterococcaceae	*Enterococcus spp.*	420	*E. faecalis* ReWhINP01 3	**positive**	patient#01
			E. faecalis urRP005		patient#05
			E. faecalis urRP009		patient#09
			E. faecalis urRP012		patient#12
			E. faecalis urRP013		patient#13
			E. faecalis urRP014		patient#14
			E. faecalis urRP018		patient#18
			E. faecalis urRP020		patient#20
			E. faecalis urRP023		patient#23
			E. faecalis urRP033		patient#33
			E. faecalis urRP041		patient#41
			E. faecalis urRP043		patient#43
			E. faecium urRP060		patient#60
Corynebacteriaceae *	*Corynebacterium amycolatum*	30	*C. amycolatum* urRP038		patient#38
Streptococcaceae	*Streptococcus spp.*	370	*S. mitis/oralis* urRP015		patient#15
			S. mitis/oralis urRP038		patient#38
			S. mitis/oralis urRP040		patient#40
			S. agalactiae urRP024		patient#24
			S. anginosus urRP031		patient#31
Staphylococcaceae	*Staphylococcus spp.*	440	*S. epidermidis* urRP017		patient#17
			S. epidermidis urRP019		patient#19
			S. epidermidis urRP053		patient#53
			S. epidermidis urRP054		patient#54
			S. epidermidis urRP058		patient#58
			S. warneri urRP020		patient#20
			S. aureus urRP022		patient#22
			S. pettenkoferi urRP031		patient#31
			S. saprophyticus urRP056		patient#56

*these families (Moraxellaceae and Coreynebacteriaceae) were included in training the Gram type model, however, they were left out for training the family model.

Supplemental Table S3: Summary information on artificial mixture concentrations. Concentration in colony forming units (CFU) was inferred from colony counts on agar plates of 100 µl of bacterial suspension after mixing. In addition, number of spectra per mixture is given in the third column.

Artificial mixture	Batch	Number of spectra	Strain 1	Concentration 1 (CFU/ml)	Strain 2	Concentration 2 (CFU/ml)	Ratio conc. 1/ conc. 2
Mix_1	1	8102	*E. coli* urRP041	1.275x10^{7}	*E. faecalis* urRP041	5.75x10^{6}	2.2
	2	21543		2.295x10^{8}		1.53x10^{8}	1.5
	3	10896		1.865x10^{8}		8.8x10^{7}	2.1
	4	10990		2.28x10^{8}		1.11x10^{8}	2.1
	5	11590		1.99x10^{8}		1.43x10^{8}	1.4
Mix_2	1	10903	*E. coli* urRP059	1.03x10^{8}	*E. faecalis* urRP056	2.6x10^{7}	4.0
	2	9384		2.055x10^{8}		1.125x10^{8}	1.8
Mix_3	1	12707	*E. coli* urRP065	1.74x10^{8}	*S. warneri* urRP020	7.8x10^{7}	2.2
	2	10952		2.55x10^{8}		8.65x10^{7}	2.9
Mix_4	1	10955	*E. coli* urRP018	3.17x10^{8}	*S. aureus* urRP022	1.7x10^{7}	18.6
	2	6666		1.275x10^{8}		2.4x10^{7}	5.3
	3	10994		1.115x10^{8}		3.9x10^{7}	2.9

Supplemental Table S4: Summary of Raman bands and assignment
(based on comparison with references 1–7)

Band No.	Position (cm^{-1})	Origin	Band No	Position (cm^{-1})	Origin
1	622	C-C twisting-phenylalanine	26	1127.5	C-N (amine), C-C (lipids), and C-O str, cytochrome c
2	642	Tyrosine	27	1157	C-C str vibrations of the polyene chain of carotenoids
3	667.5	DNA, Guanine	28	1175	C-H wagging of tyrosine, guanine and cytosine, fatty acids (stearic acids)
4	681	Ring breathing modes in the DNA bases	29	1207.5	C-C_6H_5 stretch, phenylalanine, tryptophan, stretching P=O
5	698		30	1230	Amide III
6	722.5	Adenine, CH_2 rocking vibration	31	1250	Amide III
7	747.5	DNA, cytochrome c	32	1265	C-N and N-H stretching (amide III); thymine and adenine (ring breathing); CH_2 lipid def, saccharides
8	782.5	PO_2^-,Cytosine, uracil, thymine ring breathing	33	1280	Amide III
9	790	O-P-O stretch of DNA	34	1312.5	Guanine, CH def, cytochrome c, CH_2/CH_3 twisting, wagging, bending modes of lipids
10	815	Tyrosine; C-O-P-O-C-RNA binding	35	1340	Adenine, CH def, CH_2 and CH_3-fatty acids and protein def; N-H

					stretching (amide III); C-C stretching – tryptophan, guanine
11	827.5	Tyrosine	36	1362.5	Pyoverdine
12	852.5	‚Buried' tyrosine, C-O-C stretching og glycosidic linkage; teichuronic acid of Gram-positive cell wall	37	1375	COO-symetric and asymmetric stretching-peptidoglycan; CH modes of glucosamines
13	880	Tryptophan def ring	38	1395	
14	900	C-O-C str	39	1420	Adenine, guanine, -C-O vibration modes-peptidoglycan; CH modes of glocosamines
15	920	C-C str, alpha-helix, C-O-C glycos bond	40	1450	CH_2CH_3 bending-proteins
16	937.5	Stretching C-C (amide III)	41	1485	Guanine, Adenine, CH def; Pyoverdine
17	962.5	C-N str (amide lipopolysaccharides	42	1525	In-phase C=C vibrations of the polyene chain of carotenoids
18	987.5	Peptidoglycan; C-C stretching protein; =CH lipids	43	1535	
19	1005	Phenylalanine	44	1555	Tryptophan; exopolysaccharides
20	1032.5	C-H in-plane, Phe	45	1578	Exopolysaccharide-associated band, Adenine, guanine (ring stretch); -C-O vibration modes – peptidoglycan
21	1050	C-O stretching vibrations, carbohydrates, C-C, -C-OH deformations	46	1605	Phenylalanine; Pyoverdine; C-C aromatic ring chain vibrations, C-C ring stretching-phenylalanine, tyrosine and tryptophan
22	1065	C-N stretching vibrations (protein), chain C-C stretching (lipids)	47	1620	Tyrosine; amide I, lipids
23	1080	Chain C-C str (lipids), C-O, C-C str	48	1670	amide I, lipids, C=C stretching vibration
24	1097.5	PO_2^-symmetrical stretching, Carbohydrates, C-C, C-O, -C-OH def	49	2850	CH_3 and CH_2 stretching vibrations
25	1107	PO_{2-} - stretching vibrations in DNA, C-C-(CH_3) vibration	50	2935	

Supplemental Table S5: Prediction results for patients´ samples using the PCA-LDA family model. Abbreviation of family is as introduced in Table 2, main manuscript. Prediction results are given in the left part of the table. Yellow highlighted numbers identify bacterial families that were identified by microbiological analysis. Green background color highlights correct predictions, while red background color hightlights wrong predictions. „Agg. Gram type“ summarizes the predictions of the family model with respect to Gram type, while „Gram model“ presents the results of the 2-class Gram-type model. Results of routine microbiological analysis are presented at the right part of the table. Gram type color code of the the ground truth corresponds to the prediction of the family model. Species names typed in red indicate bacterial species not included in the family model. CFU/ml gives the bacterial concentration (in colony forming units, CFU) in 1 ml of the original urine sample as determined by microbiological plating.

	prediction											**Ground truth: microbiological findings**					
	Family model								**Gram model**								
ID	**Eba**	**Mor**	**Pse**	**Eco**	**Sta**	**Str**	**# spec**	**Agg. Gram type**	**Neg.**	**Pos.**	**Gram model**	**Gram**	**CFU/ml**	**bacteria.1**	**bacteria.2**	**bacteria.3**	**bacteria 4**
1	0.51	0.17	<0.1	1	0	<0.1	389	mixture	0.32	1	mixture	negative	10^5	*P. aeruginosa*	*C. koseri*		
2	0.25	0.15	1	<0.1	<0.1	0	982	negative	1	<0.1	negative	mixture	$>10^5$	*E. coli*	*P. putida*	*E. faecalis*	
3	1	0.62	<0.1	0	0	0	1779	negative	1	<0.1	negative	negative	$>10^5$	*E. coli*			
4	<0.1	0.92	1	<0.1	<0.1	<0.1	2745	negative	1	<0.1	negative	negative	$>10^5$	*P. aeruginosa*			
5	1	0.23	0.19	<0.1	<0.1	<0.1	2099	negative	1	<0.1	negative	mixture	10^5	*E. coli*	*E. faecalis*		
6	0.39	1	0.47	<0.1	<0.1	<0.1	3188	negative	1	<0.1	negative	negative	$>10^5$	*E. coli*			
7	<0.1	1	<0.1	<0.1	<0.1	<0.1	1341	negative	1	<0.1	negative	mixture	100	*K. oxytoca*	*M. morganii/ P. mirabilis*	*E. faecalis*	
8	<0.1	1	<0.1	<0.1	<0.1	<0.1	1340	negative	1	<0.1	negative	mixture	10^5	*E. coli*	*P. aeruginosa*	*K. oxytoca*	*E. faecalis*
9	0	1	0.22	0	0	0	1557	negative	1	<0.1	negative	mixture	$>10^5$	*E. coli*	*E. coli* 2	*E. coli* 3	*E. faecalis*
10	0	1	<0.1	<0.1	<0.1	<0.1	2360	negative	1	<0.1	negative	positive	100	*S. epidermidis*			
11	0.27	0.14	0.14	<0.1	0.23	1	1680	mixture	1	0.47	mixture	mixture	$>10^5$	*E. coli*	*P. aeruginosa*	*E. faecalis*	
12	<0.1	0.19	1	<0.1	<0.1	<0.1	5420	negative	1	<0.1	negative	negative	5.10^4	*P. mirabilis*			
13	0	0	<0.1	<0.1	1	<0.1	104	positive	0.49	1	mixture	positive	100	*S. aureus*			
14	0	1	0	<0.1	<0.1	<0.1	8093	negative	1	<0.1	negative	positive	5,000	*E. faecalis*			
15	<0.1	1	0.40	<0.1	<0.1	<0.1	5593	negative	1	<0.1	negative	mixture	10^5	*P. aeruginosa*	*S. agalactiae*		
16	0.41	1	0.18	<0.1	<0.1	0.27	4199	mixture	1	<0.1	negative	negative	10^5	*K. pneumoniae*			
17	<0.1	1	0.31	<0.1	<0.1	<0.1	2705	negative	1	<0.1	negative	negative	10^5	*P. aeruginosa*	*P. mirabilis*		
18	<0.1	1	0.40	<0.1	<0.1	<0.1	2560	negative	1	<0.1	negative	negative	$>10^5$	*K. pneumoniae*			

19	0.76	1	0.47	<0.1	0.21	0.96	6418	mixture	1	<0.1	negative	negative	10^5	*E. coli*	*K. pneumoniae*	
20	<0.1	0.91	1	<0.1	<0.1	<0.1	7247	negative	1	<0.1	negative	negative	10^5	*P. mirabilis*		
21	<0.1	1	<0.1	<0.1	<0.1	<0.1	5073	negative	1	<0.1	negative	negative	$>10^5$	*S. marcescens*	*P. mirabilis*	
22	0.20	1	<0.1	0.31	0.37	0.12	1928	mixture	1	0.22	mixture	positive	100	*S. pettenkoferi*	*S. anginosus*	
23	0.18	0.43	1	<0.1	<0.1	<0.1	2779	negative	1	<0.1	negative	negative	10^5	*K. pneumoniae*		
24	<0.1	<0.1	1	<0.1	0.15	<0.1	5543	mixture	1	0.24	mixture	mixture	10^5	*E. coli*	*E. faecalis*	
25	0.33	0.29	1	<0.1	<0.1	<0.1	6740	negative	1	<0.1	negative	negative	$>10^5$	*E. coli*	*K. oxytoca*	
26	<0.1	1	<0.1	<0.1	<0.1	<0.1	2001	negative	1	<0.1	negative	negative	$>10^5$	*E. cloacae* complex		
27	<0.1	1	0.13	<0.1	<0.1	<0.1	9702	negative	1	<0.1	negative	positive	1,000	*S. mitis/oralis*	*C. albicans*	*C. amycolatum*
28	<0.1	0.47	1	<0.1	<0.1	<0.1	5311	negative	1	<0.1	negative	negative	$>10^5$	*P. aeruginosa*	*P. mirabilis*	*E. coli*
29	<0.1	1	<0.1	<0.1	<0.1	<0.1	6089	negative	1	<0.1	negative	mixture	100	*A. ursingii*	*S. mitis/oralis*	
30	<0.1	0.19	<0.1	<0.1	1	0.82	890	mixture	1	<0.1	negative	mixture	100	*E. coli*	*E. faecalis*	
31	<0.1	1	0.40	<0.1	<0.1	<0.1	8835	negative	1	<0.1	negative	negative	10^5	*E. coli*		
32	<0.1	0.62	0.26	0.36	0.32	1	5096	mixture	0.92	1	mixture	positive	10^5	*E. faecalis*		
33	0.4	0.28	0.21	<0.1	<0.1	1	1031	mixture	1	0.24	mixture	negative	10^5	*E. coli*		
34	<0.1	0.49	0.12	<0.1	0.14	1	582	mixture	0.87	1	mixture	negative	1,000	*P. mirabilis*	*E. coli*	
35	<0.1	1	0.10	<0.1	<0.1	<0.1	7904	negative	1	<0.1	negative	negative	$>10^5$	*P. aeruginosa*		
36	<0.1	1	0.11	<0.1	<0.1	0.23	5296	mixture	1	<0.1	negative	negative	10^5	*E. coli*	*K. pneumoniae*	
37	<0.1	1	<0.1	<0.1	<0.1	<0.1	2646	negative	1	<0.1	negative	negative	50,000	*E. coli*		
38	<0.1	1	<0.1	<0.1	<0.1	<0.1	9691	negative	1	<0.1	negative	negative	10^5	*C. koseri*	*E. coli*	
39	0.69	0.49	0.28	0.17	1	0.75	2253	mixture	1	0.25	mixture	negative	10^5	*P. rettgeri*	*E. cloacae*	
40	<0.1	1	<0.1	<0.1	<0.1	<0.1	9378	negative	1	<0.1	negative	negative	100	*E. coli*		
41	<0.1	1	<0.1	<0.1	<0.1	<0.1	2705	negative	1	<0.1	negative	positive	10^5	*S. epidermidis*		
42	<0.1	1	<0.1	<0.1	<0.1	<0.1	9154	negative	1	<0.1	negative	positive	100	*S. epidermidis*		
43	<0.1	1	0.5	<0.1	<0.1	<0.1	7718	negative	1	<0.1	negative	mixture	10^5	*P. aeruginosa*	*S. saprophyticus*	*E. faecalis*
44	1	0.42	0.39	<0.1	0.13	0.18	6849	mixture	1	<0.1	negative	negative	10^5	*K. aerogenes*		
45	<0.1	1	<0.1	<0.1	0.56	0.40	3278	mixture	1	0.23	mixture	positive	50,000	*S. epidermidis*		
46	<0.1	0.11	1	<0.1	<0.1	<0.1	2199	negative	1	<0.1	negative	negative	10^5	*E. coli*		
47	<0.1	0.20	1	<0.1	<0.1	<0.1	6811	negative	1	<0.1	negative	mixture	1,000	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*E. faecium*
48	0.32	1	0.47	<0.1	<0.1	0.27	4951	mixture	1	<0.1	negative	negative	10^5	*K. oxytoca*		

49	<0.1	0.73	<0.1	<0.1	0.21	1	2029	mixture	1	0.46	mixture	positive	1,000	*S. warneri*	*S. dysgalactiae spp. Equisimilis*		
50	0.21	1	<0.1	<0.1	<0.1	<0.1	8732	negative	1	0.11	mixture	negative	>10^5	*E. coli*			
51	<0.1	1	<0.1	<0.1	<0.1	<0.1	10629	negative	1	<0.1	negative	positive	10^5	*S. aureus*			
52	<0.1	1	<0.1	<0.1	0.24	<0.1	5841	mixture	1	<0.1	negative	positive	>10^5	*S. epidermidis*			
53	<0.1	1	<0.1	<0.1	0.10	0.46	816	mixture	1	0.19	mixture	mixture	100	*K. oxytoca*	*S. epidermidis*	*C. amycolatum*	*E. faecalis*
54	<0.1	0.38	0.29	<0.1	1	<0.1	1308	mixture	1	<0.1	negative	negative	10^4	*E. coli*	*K. pneumoniae*		
55	<0.1	1	<0.1	<0.1	<0.1	<0.1	6652	negative	1	<0.1	negative	mixture	>10^5	*Lactobacillus jensenii*	*S. marcescens*	*E. faecalis*	
56	1	0.72	0.32	<0.1	<0.1	<0.1	7879	negative	1	<0.1	negative	negative	10^5	*K. pneumoniae*			
57	1	0.43	0.67	<0.1	0.314	<0.1	1023	mixture	1	<0.1	negative	positive	500	*E. faecalis*			
58	<0.1	1	<0.1	<0.1	<0.1	<0.1	11019	negative	1	<0.1	negative	negative	10^5	*C. koseri*			
59	<0.1	1	0.22	<0.1	<0.1	<0.1	2563	negative	1	<0.1	negative	mixture	10^4	*E. faecalis*	*P. aeruginosa*		

Supplemental Table S6: For a few patient's samples two different dried droplets were prepared and measured. In Supplemental Table S4 only results for the scan with more spectra after Raman quality assessment were kept. Here, results from both batches are compared. Table outlines is as explained for Supplemental Table S4.

		prediction											**Ground truth: microbiological findings**				
		Family model								**Gram model**							
ID	**batch**	**Eba**	**Mor**	**Pse**	**Eco**	**Sta**	**Str**	**# spec**	**Agg. Gram type**	**Neg.**	**Pos.**	**Gram model**	**Gram**	**CFU/ml**	**bacteria.1**	**bacteria.2**	**bacteria.3**
4	1	<0.1	0.92	1	<0.1	<0.1	<0.1	2745	negative	1	<0.1	negative	negative	$>10^5$	*P. aeruginosa*		
4	2	0	0.34	1	0	0	0	1599	negative	1	0	negative	negative	$>10^5$	*P. aeruginosa*		
17	1	0	1	<0.1	0	0	0	1160	negative	1	<0.1	negative	negative	10^5	*P. aeruginosa*	*P. mirabilis*	
17	2	0	1	0.31	0	0	0	2705	negative	1	<0.1	negative	negative	10^5	*P. aeruginosa*	*P. mirabilis*	
22	1	<0.1	1	<0.1	<0.1	<0.1	<0.1	847	negative	1	<0.1	negative	positive	100	*S. pettenkoferi*	*S. anginosus*	
22	2	0.20	1	<0.1	0.31	0.37	0.12	1928	mixture	1	0.22	mixture	positive	100	*S. pettenkoferi*	*S. anginosus*	
24	1	0	<0.1	1	0	0.15	0	5543	mixture	1	0.24	mixture	mixture	10^5	*E. coli*	*E. faecalis*	
24	2	0.22	0.11	0.72	1	0.83	0.44	60	mixture	0.67	1	mixture	mixture	10^5	*E. coli*	*E. faecalis*	
26	1	<0.1	1	<0.1	<0.1	<0.1	<0.1	2001	negative	1	<0.1	negative	negative	$>10^5$	*E. cloacae complex*		
26	2	0	1	<0.1	0	0	0	1601	negative	1	0	negative	negative	$>10^5$	*E. cloacae complex*		
50	1	0.21	1	<0.1	<0.1	<0.1	<0.1	8732	negative	1	0.11	mixture	negative	$>10^5$	*E. coli*		
50	2	0.36	1	0.21	<0.1	0.21	<0.1	2255	mixture	1	<0.1	negative	negative	$>10^5$	*E. coli*		

Supplemental Table S7: Prediction results for patients´ samples that had to be excluded from detailed analysis as only one Raman spectrum was kept after Raman quality assessment (patient 60) or no bacterial growth was observed (patients 61-64). *Candida albicans* was detected as pathogen in two patients (Pat. 61 and Pat. 64). However, due to sample preparation (filtration through 5 µm pores) it is very unlikely that fungal cells were within the dried droplet. Table outlines is as explained for Supplemental Table S4.

	prediction											**Ground truth: microbiological findings**				
	Family model								**Gram model**							
ID	**Eba**	**Mor**	**Pse**	**Eco**	**Sta**	**Str**	**# spec**	**Agg. Gram type**	**Neg.**	**Pos.**	**Gram model**	**Gram**	**CFU/ml**	**Pathogen 1**	**Pathogen 2**	**Exclusion criterion**
60	<0.1	1	<0.1	<0.1	<0.1	<0.1	1	negative	1	0	negative	mixture	10^5	*K. aerogenes*	*S. haemolyticus*	only 1 Raman spectrum
61	0.68	1	0.82	<0.1	0.13	<0.1	749	mixture	1	<0.1	negative	Fungi	$>10^5$	*C. albicans*		no bacterial growth
62	<0.1	1	0.68	0.08	0.45	<0.1	16685	mixture	1	<0.1	negative	n.a.		None		no bacterial growth
63	<0.1	1	0.21	0.32	0.51	0.27	193	mixture	1	0.16	mixture	n.a.		None		no bacterial growth
64	<0.1	1	<0.1	<0.1	<0.1	<0.1	9054	negative	1	<0.1	negative	Fungi	$<10^5$	*C. albicans*		no bacterial growth

Notices:

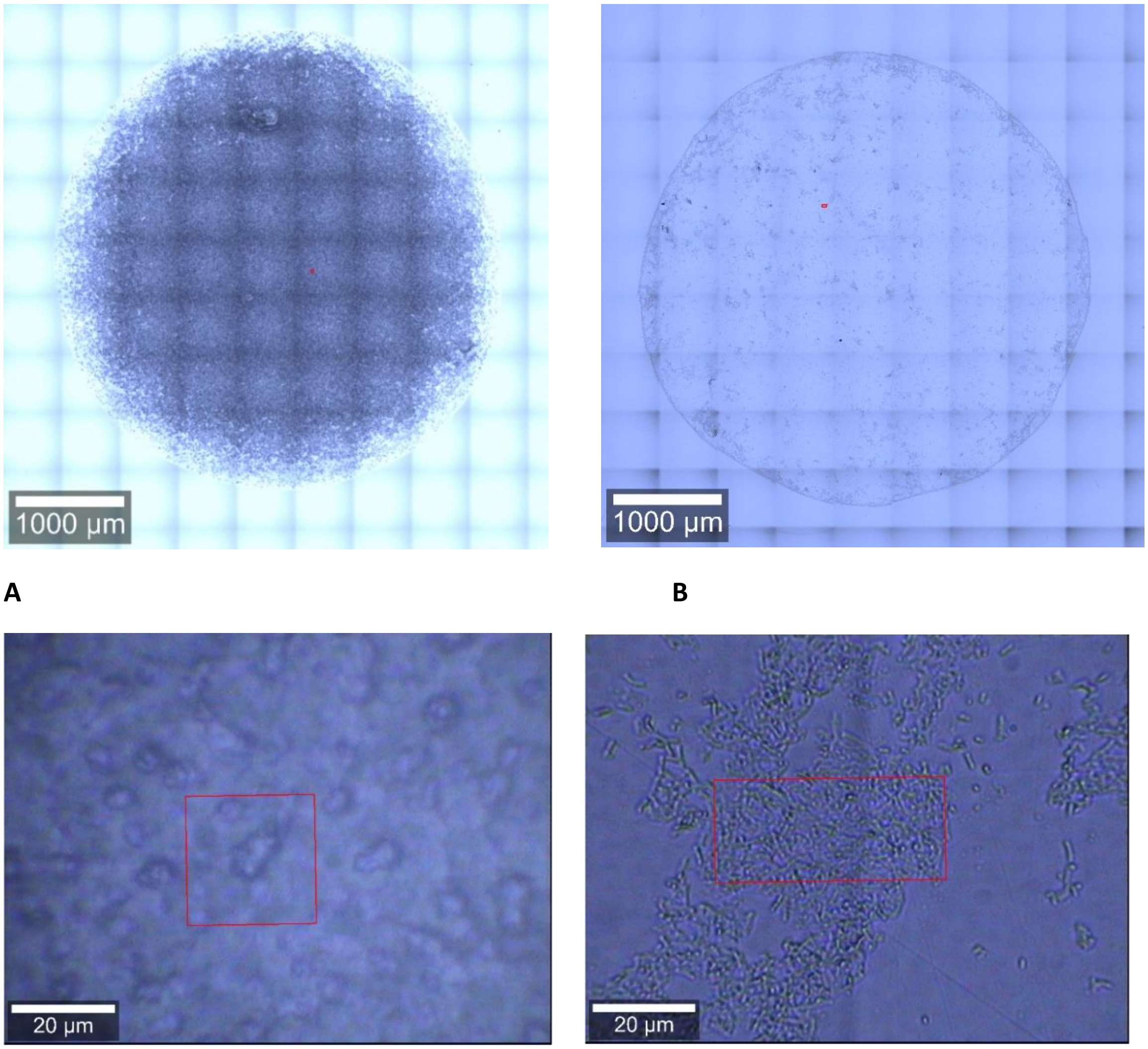
Patient sample 60 contained very low concentration of bacteria cells (250 CFU/ml after filtration, Microbiological labs declared 1000 CFU/ml), after washing steps, the pellet wasn´t seen by eye, and distinguish of dried droplet was possible, but very hard. For this case we focus on small area of several individual cells, resulting in very low S/N ratio for proposed Raman experimental routine. For this sample, we observed 10-times more colonies on our MH2 agar plates before filtering than after filtering

For sample 61, we observed colonies on MH2 agar plates only before filtering. It corresponds to Microbiological findings. Urine was turbid with "particles" (stones, debris), it was possible to observe a droplet.

Sample 62 originated from midstream shadowed-pyuria urine collected after 14-days unacid + sultamicillin treatment. It was hard to filter it, no pellet was observed by eye after washing steps, as for sample 60, it was hard but possible to see the droplet. No growth was observed on MH2 agar plates after 24 hours.

Patient 63 was under 12-days prophylactical treatment after surgery, the pellet was nearly invisible and lost after 1st washing step. It was hard but possible to observe the droplet. We detected concentration of 10 CFU/ml on MH2 agar plates, which is below LOD for Microbiological findings.

For sample 64, small pellet was seen after washing steps, there wasn´t problem to find position with enough material for Raman spectra experiment. We observed on MH2 agar plates before filtration the number of colonies corresponding to concentration of 1.5 x 10^4 CFU/ml, and no growth after filtration step. It corresponds to microbiological findings of Candida albicans presence.

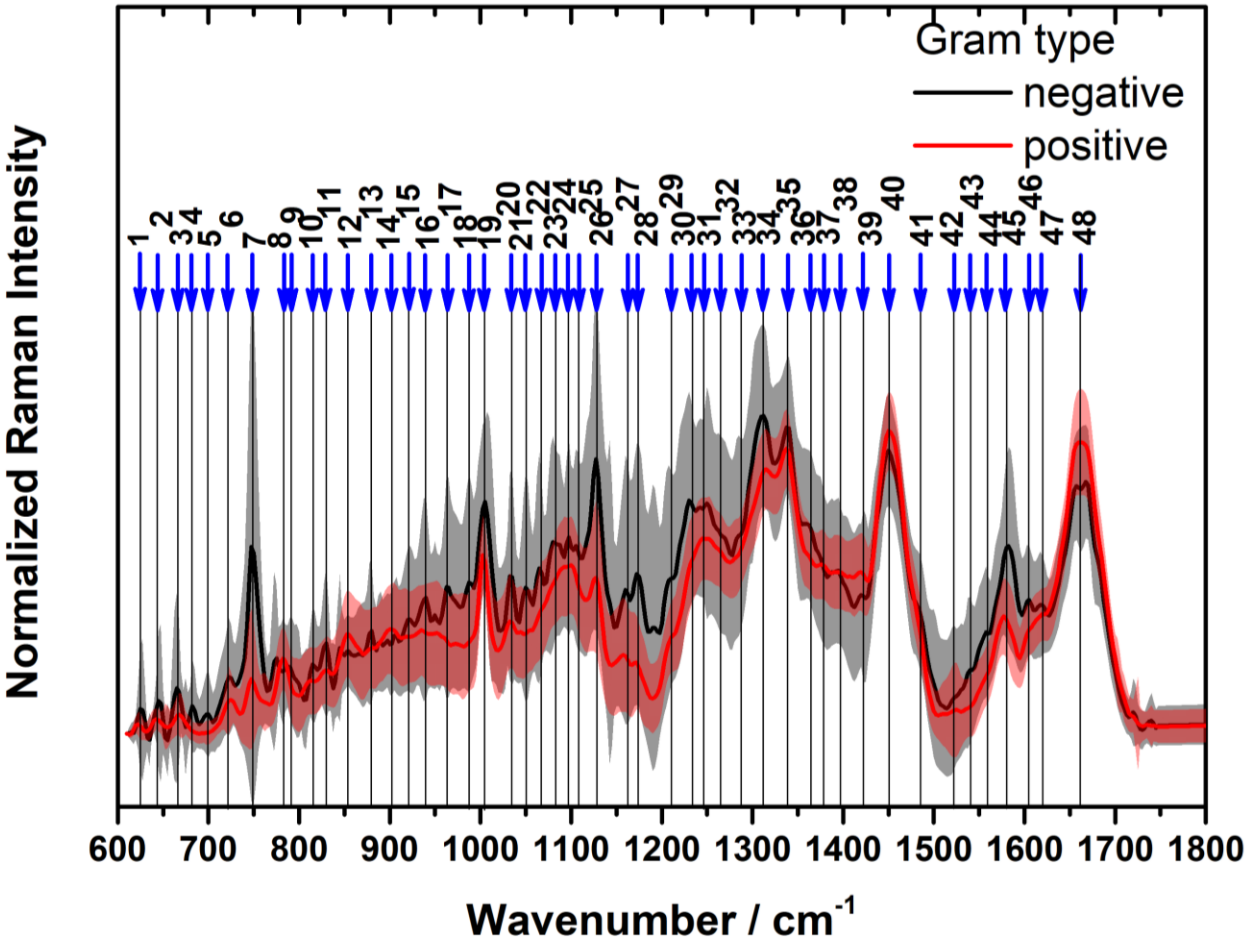


A **B**

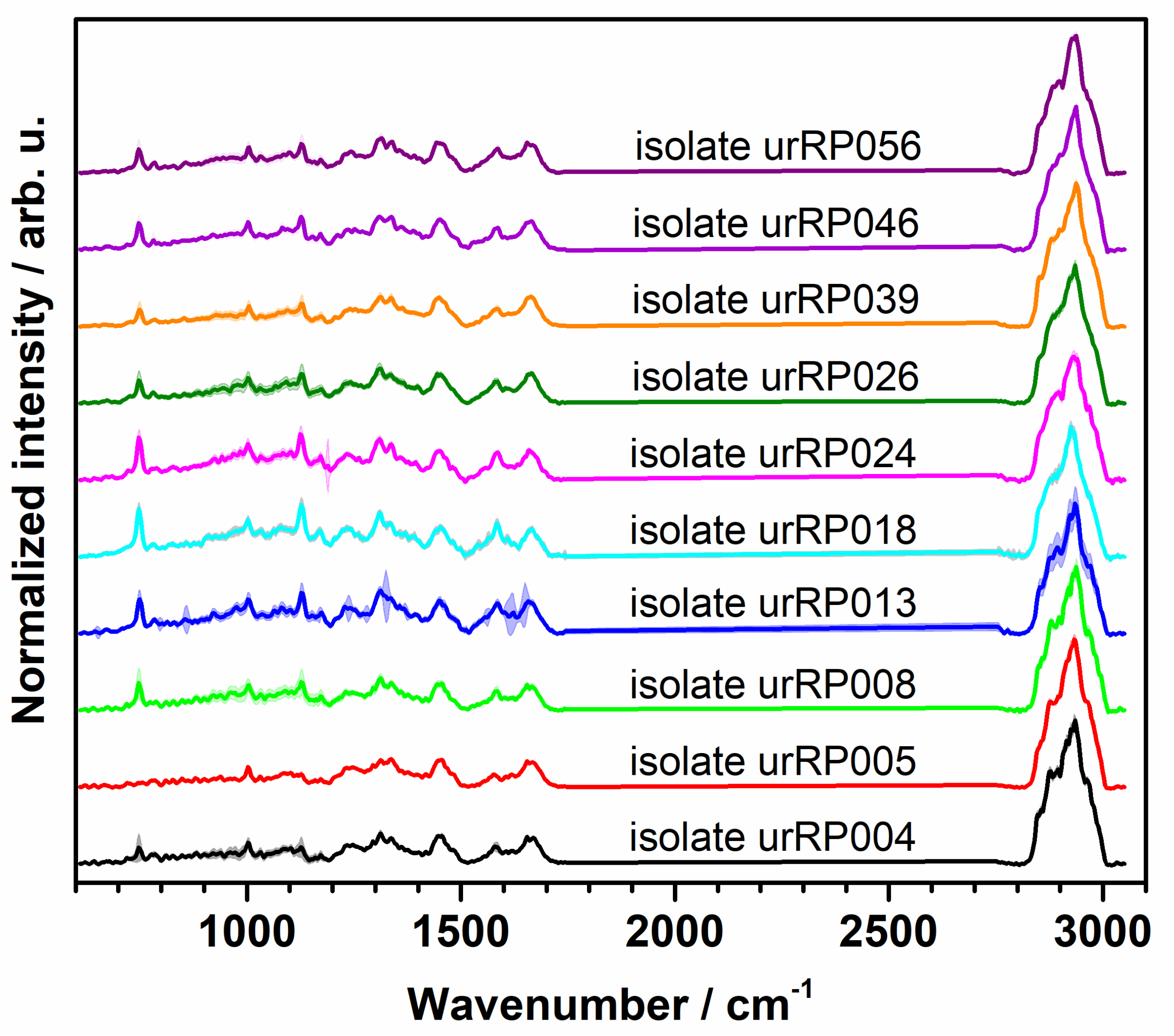
C D

Supplemental Figure S1: Bright field images of typical a typical dried droplet. **A**: Dense bacterial lawn is visible when the bacterial suspension had higher concentration of cells. **B:** Bacteria are mainly found at the outer rim (coffee ring effect) if only low bacterial concentration could be achieved in the sample. Dimensions of scanning area were represented by the zoomed area **C:** corresponding to droplet. **A** (20 um per 20 um), and **D**: corresponding to droplet **B**. Variations are introduced by varying bacterial load of the patitents' urine samples. In future analysis, this could be included in the interpretation of the results as being a diagnostic indicator, i.e. revealing the bacterial concentration present in the urine sample (which is currently also reported during routine diagnostics).

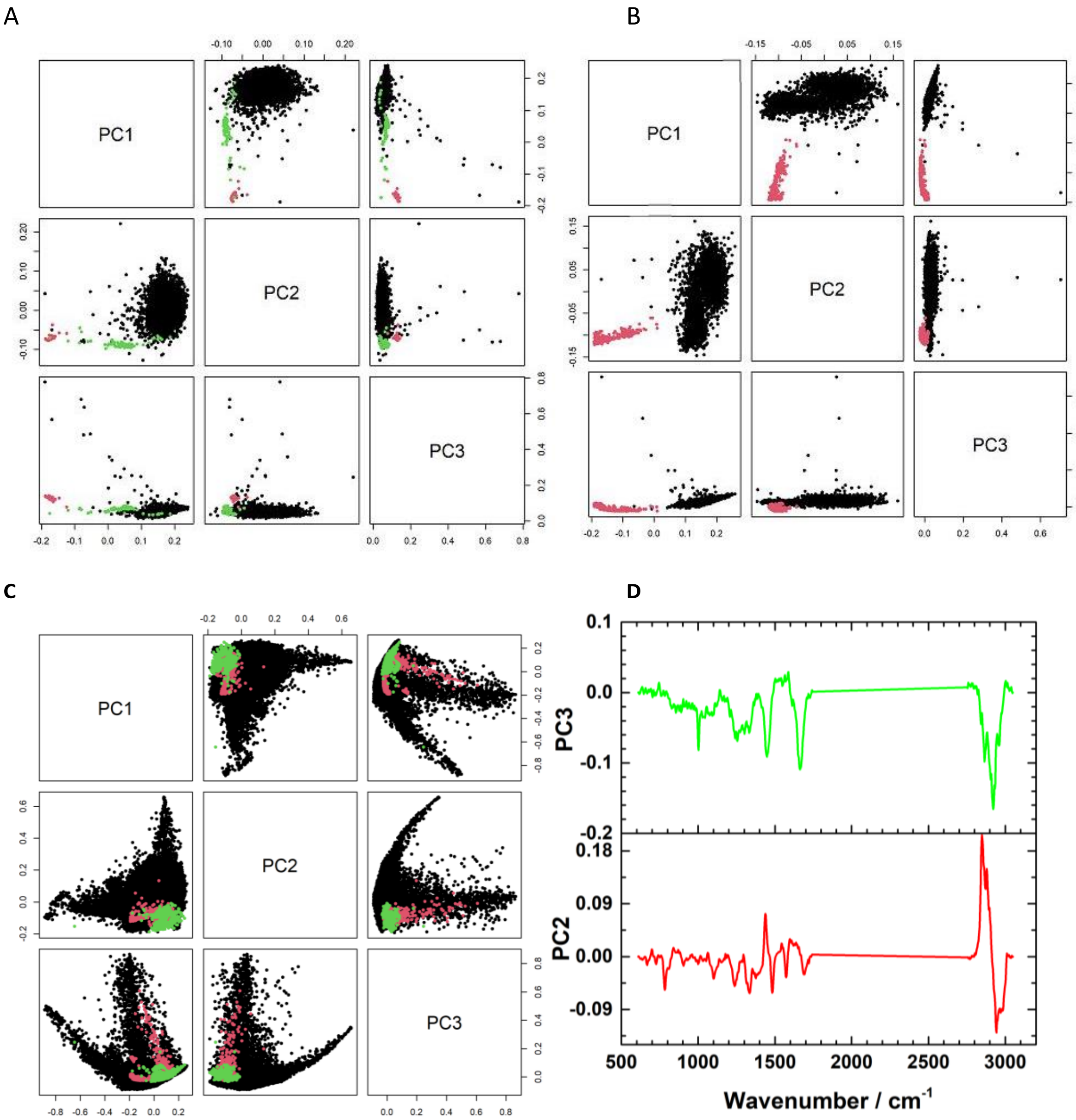
The zoomed-in bright field images clearly show that only bacteria were present in the dried droplet and no crystals from the urine, thus, indicating that the washing procedure successfully removed background material.



Supplemental Figure S2: Detailed view on normalized mean Raman spectra of Gram negative (black) and Gram positive bacteria (red) together with standard deviation (shown as shadow). Raman band assignment is given in Supplemental Table S2.

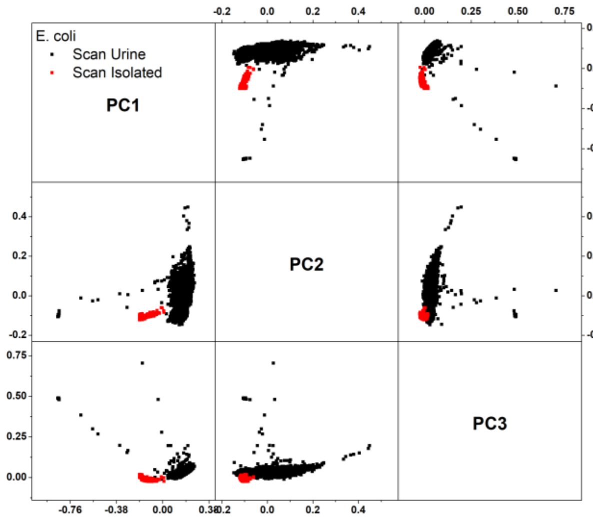


Supplemental Figure S3: Mean spectra per isolate of *Pseudomonadaceae*.

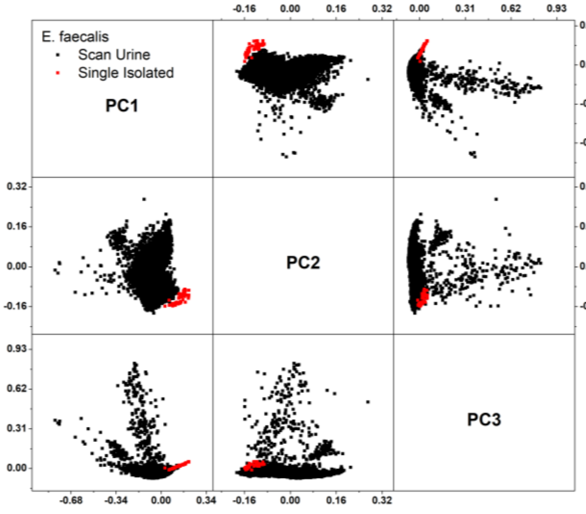


Supplemental Figure S4: PCA score plot showing the differentiation of bacteria measured directly from urine and after cultivation. **A:** Patient no. 4 *(P. aeruginosa)* , **B:** Patient no. 6 (*E. coli*); and **C:** all 14 pairs of pathogens measured directly from urine and after isolation. Samples from patients no. 3, 4, 6, 12 – 14, 16, 18, 20, 26 32, 35, 45, and 48 are included. Please note, scale is different for panels A, B and C.

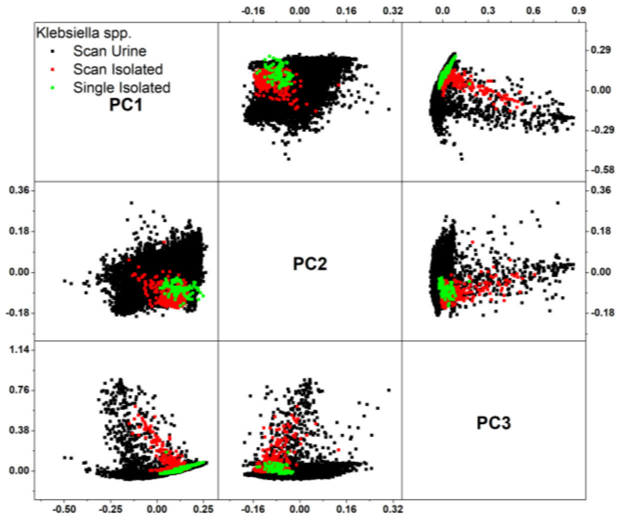
Spectra of bacteria directly from urine are shown in black, Raman spectra of the same strain after isolation and microbiological identification are depicted in red. Both were measured under same measurement conditions (scans). Raman spectra of identical strain, reflecting different measurement mode (green, individual spectrum at 10 different position), is also added to discuss effect of different measurement conditions. **D**: Loading plots of PC2 and PC3. PC1 is shown in Figure 5b, main manuscript. Microbiological findings for the samples are listed in **Supplemental Tables S5** and **S6.**



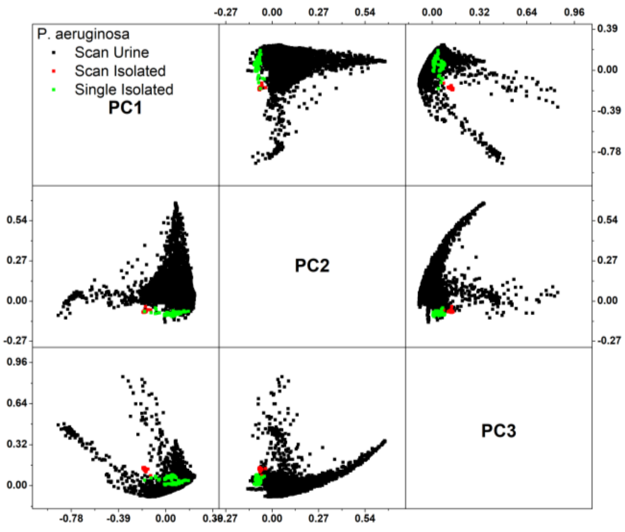
A



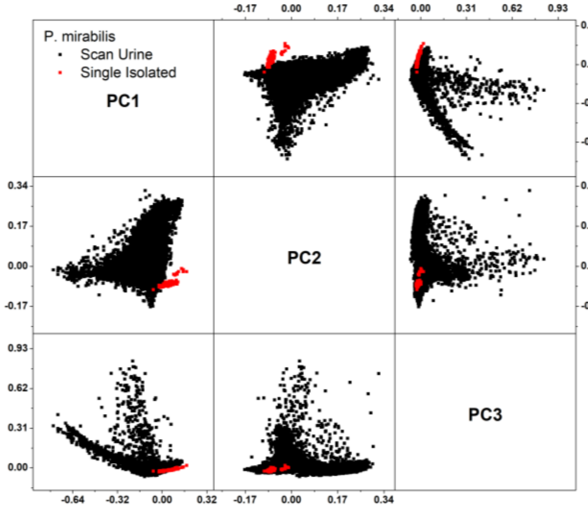
B



C



D



E

Supplemental Figure S5: PCA score plot showing the differentiation of monoinfections containing same species/genuses measured directly from urine and after cultivation. **A:** *E. coli* for Patients no. 3 and 6, **B:** *E. faecalis* for Patients no. 14 and 32, **C:** *Klebsiella* spp. for Patients no. 16, 18 and 48, **D:** *P. aeruginosa* for Patients no. 4, and 35, and **E:** *P. mirabilis* for Patients no. 12, and 20. Microbiological findings for the samples are listed in **Supplemental Tables S5** and **S6.**

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