Supplemental data

Albumin Adsorption onto Surfaces of Urine Collection and Analysis Containers

Mary K. Robinson[1], Samuel P. Caudill[1], David D. Koch[2], James Ritchie[3], Glen Hortin[4], John H. Eckfeldt[5], Sverre Sandberg[6], Desmond Williams[7], Gary Myers[8], W. Greg Miller[9*]

Experimental Design.

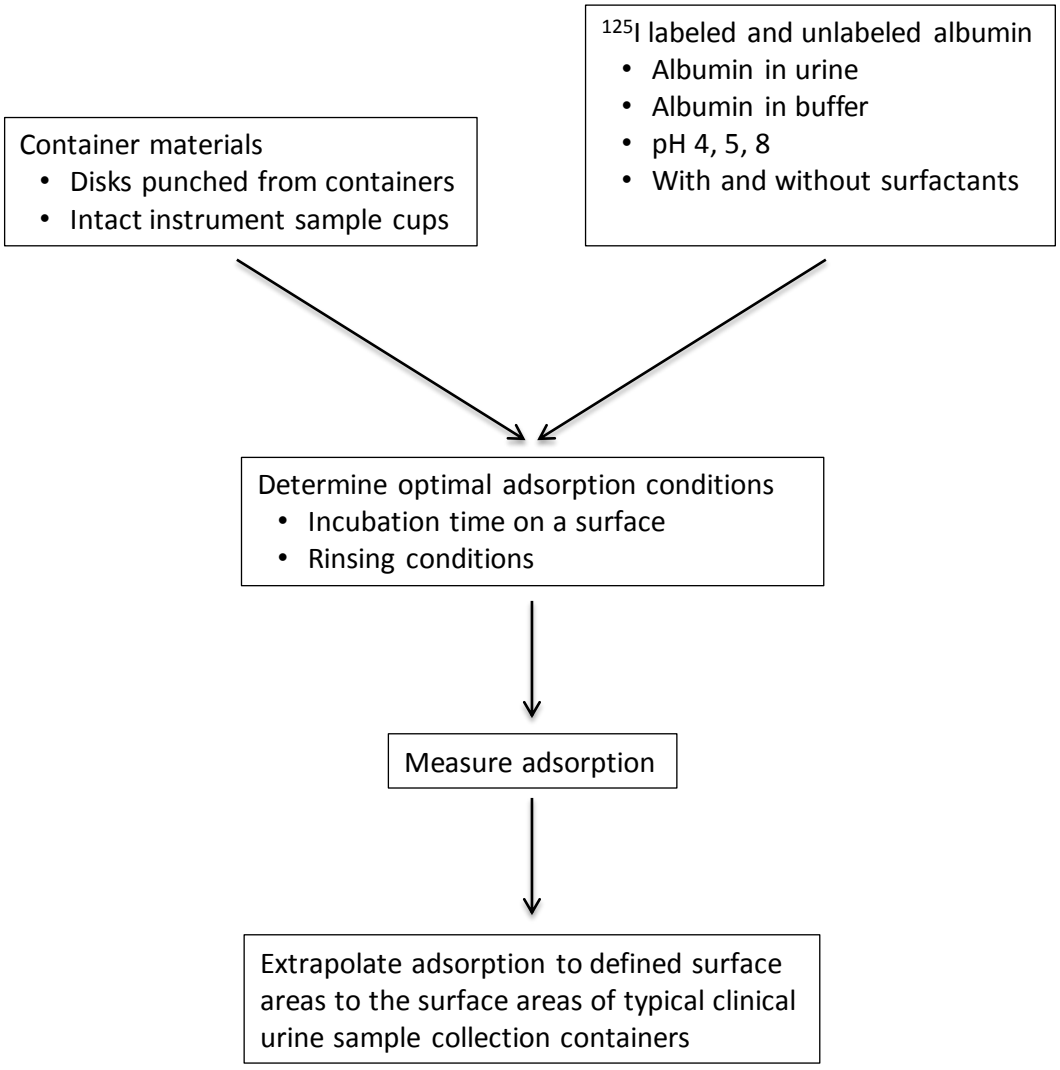

Figure S1. Diagram of the experimental design used in this investigation.

Table S1. Materials investigated for adsorption of albumin to their surfaces.

Identification Number[a]	Material[b]	Source
1	Hydrophilic-coated polyethylene terephthalate centrifuge tube	Sumitomo-Bakelite, Japan
2	Polystyrene centrifuge tube	Becton Dickinson
3	Polypropylene urine specimen container	Ben Meadows
4	Polypropylene urine specimen container	Fisher Scientific
5	Polyethylene urine specimen container	Fisher Scientific
6	Polypropylene urine specimen container	Fisher Scientific
7	Polyethylene terephthalate centrifuge tube	Corning
8	Polypropylene centrifuge tube	Van Waters and Rogers
9	Polypropylene centrifuge tube	Corning
10	Polypropylene centrifuge tube	Globe
11	Polypropylene urine specimen container	Globe
12	Polystyrene centrifuge tube	Becton Dickinson
13	Polypropylene urine specimen container	Fisher Scientific
14	Teflon centrifuge tube	Van Waters and Rogers
15	Polypropylene centrifuge tube	Fisher Scientific
16	Polyethylene 24-hour amber urine collection jug	Fisher Scientific
17	Polysulfone centrifuge tube	Unidentified
18	Polyethylene urine specimen container	Unidentified
19	Polypropylene screw cap for container #3	Ben Meadows
20	Polystyrene sample cup	Roche
21	Hydrophilic coated polyethylene terephthalate vial	Sumitomo-Bakelite, Japan
22	Polyethylene sample cup	Roche

[a] Note that not all materials were included in all experiments. However, the same identification numbers were used consistently in all figures and tables.

[b] Disks were cut from materials 1-19 and used to measure adsorption from albumin containing solutions. Materials 20-22 were used intact.

Kinetics of Adsorption. To assess the time course of adsorption, we incubated solutions for times from 0.5 to 16 hours in two instrument analysis cups (PE and PS) and on disks from one container (500 mL PP urine collection cups, material 3). Solutions on the disks began to dry out after about 6 hours. Data for the PE and PS sample analysis cups and data for the PP disks are shown in Figures S1A and S1B below. We chose to use 5 hours for our incubations because adsorption appeared to be complete and further time duration could compromise the disk experiments due to potential influence from evaporation.

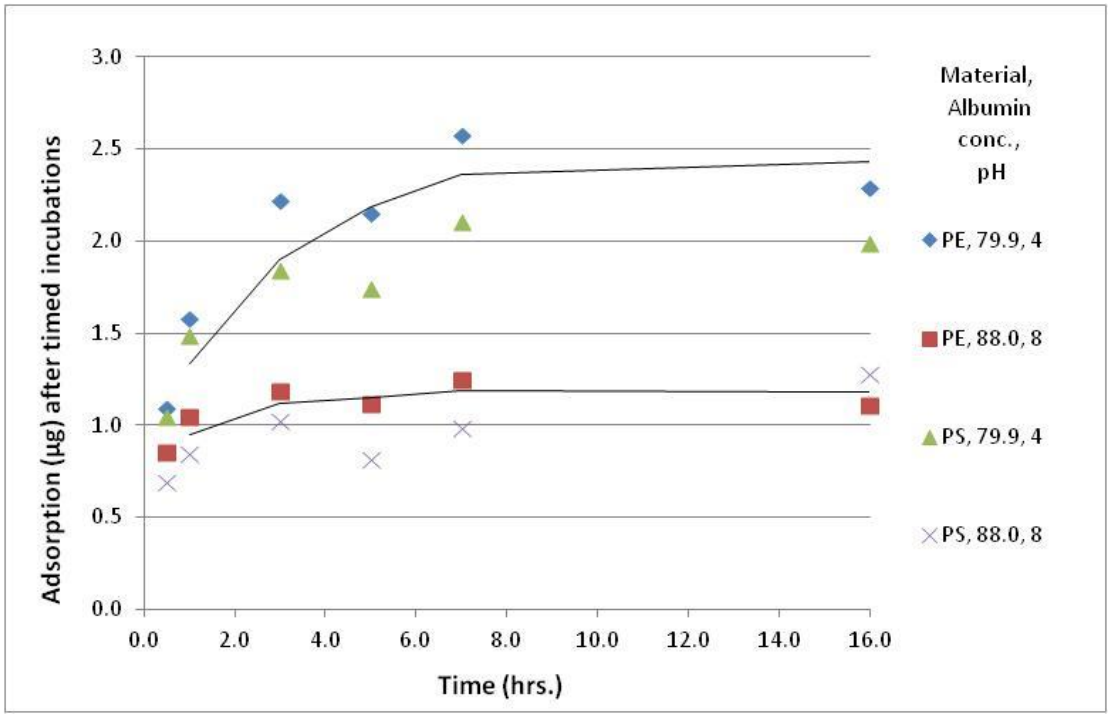

Figure S2. Kinetics of albumin adsorption to polyethylene (PE) and polystyrene (PS) sample cups after incubation of albumin solutions (80 and 88 mg/L) at pH 4 and pH 8 for 5 hours. Results are the means of duplicate measurements.

Rinsing Efficiency. To assess rinsing efficiency we incubated six container materials with 4 urine samples containing albumin at concentrations of 0.4 and 47 mg/L (pH 4) and 4.5 and 90 mg/L (pH 8), with added ^{125}I-labeled HSA. We counted separately: 1) the solutions removed from the container materials, 2) 6 separate water rinses of equal volume (100 or 200 µL), and 3) the container material itself after all rinses were completed. We determined that five rinses were adequate for our studies. Figure S2 below shows results for one of the solutions (90 mg/L, pH 8) with all six materials. Graphs for all four concentrations with all six materials, were similar.

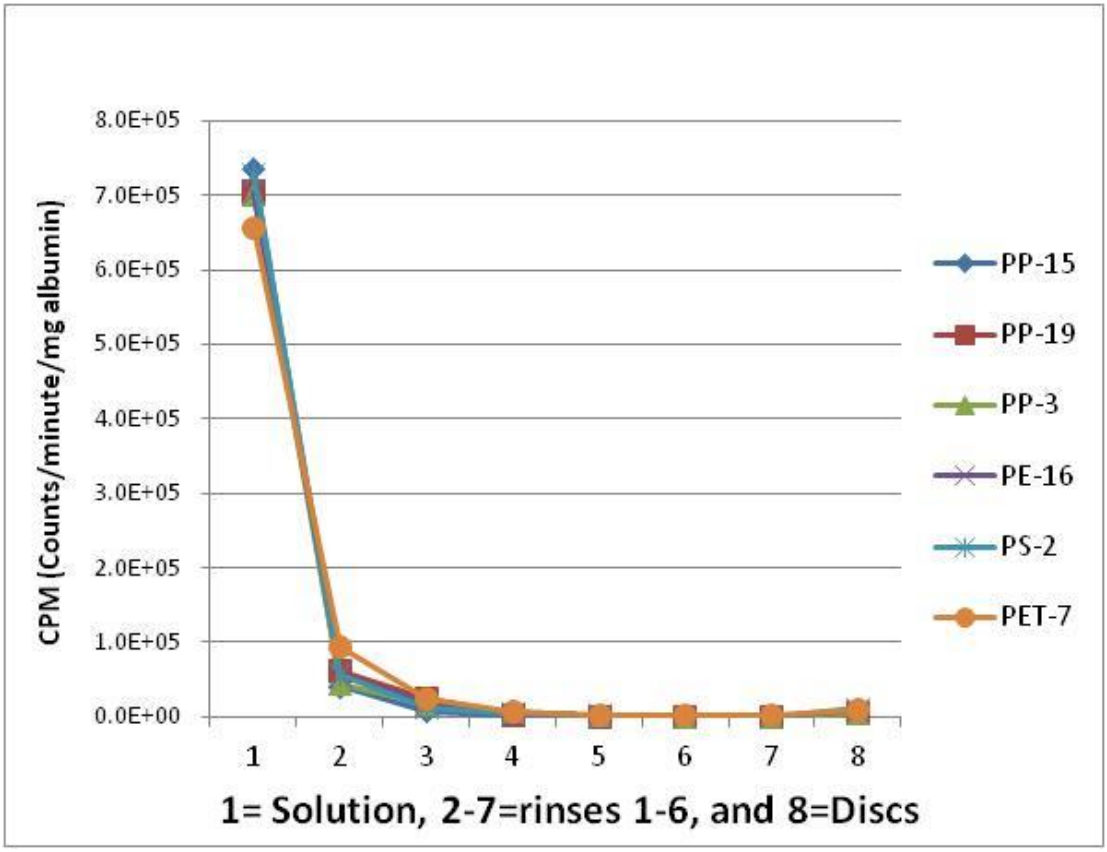

Figure S3. Efficiency of rinsing for ^{125}I labeled HSA spiked into urine with concentration of 90 mg/L and pH 8. The horizontal axis is the initial urine (1), the number of rinses (2-7) and the disk after all rinses (8). The 6 materials are shown in the figure legend; the number refers to the material number used throughout the report.

Effects of Specific Activity on Adsorption. Literature reference (6) implies that adsorption measurements using ^{125}I-labeled albumin solutions require specific activities >10^7. To assess the effects of specific activity on adsorption we incubated solutions containing variable ratios of labeled to unlabeled HSA and confirmed this requirement. Adsorption decreased with increasing specific activities up to 10^7, but remained approximately the same at higher specific activities.

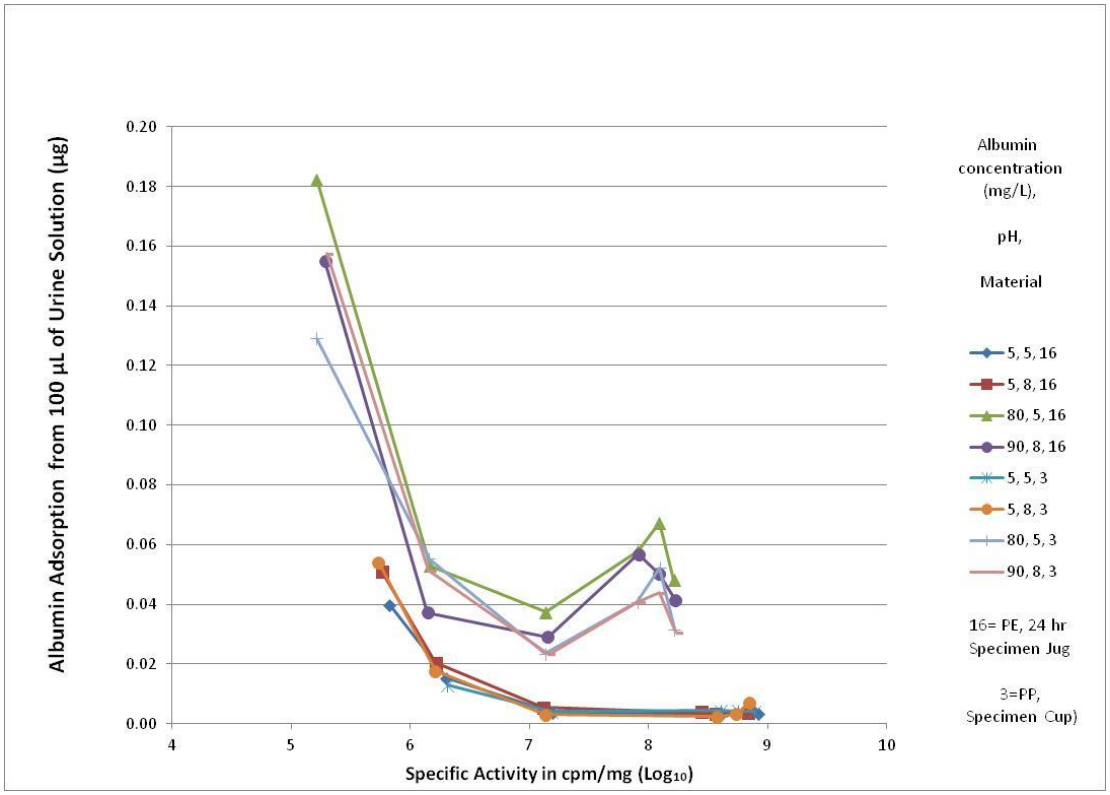

Figure S4. Effects of Specific Activity on Adsorption. Urine was supplemented with HSA and contained ^{125}I-labeled HSA adjusted to specific activities of 105-1010 cpm/mg of albumin. We measured adsorption at approximately 5 mg/L and 80-90 mg/L albumin concentrations and at pHs of 5 and 8 for two materials, polypropylene and polyethylene. Results are the average of duplicates. Materials: Polyethylene 24-hr collection jug (16); Polypropylene specimen cup (3).

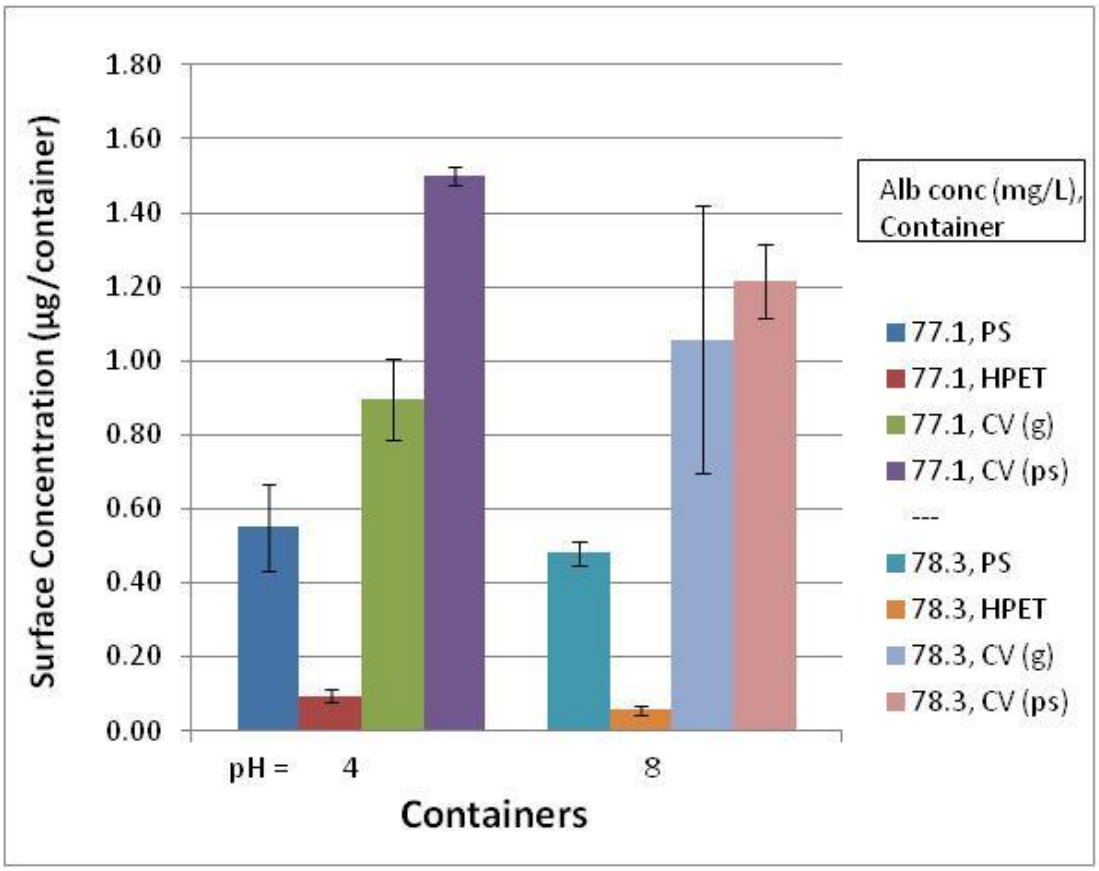

Figure S5 Albumin adsorption onto PS and HPET centrifuge tubes and glass and polystyrene counting vials after incubation for 5 hours at pH 4 and 8 for 77-78 mg/L HSA in PBS spiked with ^{125}I-labeled HSA. Results are the average of triplicates. Error bars are 2 standard deviations. Materials: PS=polystyrene and HPET=hydrophilic-coated centrifuge tubes. CV(g) and CVps= glass and polystyrene counting vials.

Table S2. Albumin adsorbed onto surfaces from urine spiked with ^{125}I -labeled HSA after incubation for five hours at room temperature.

Materials[a]	Albumin adsorbed from 5.3 mg/L in urine at pH 5, ng/cm^2 (%)	Albumin adsorbed from 5.8 mg/L in urine at pH 8, ng/cm^2 (%)	n	p-values comparing adsorption from 5.3 and 5.9 mg/L albumin in urine at pH 5 vs. 8	Albumin adsorbed from 87 mg/L in urine at pH 5, ng/cm^2 (%)	Albumin adsorbed from 91 mg/L in urine at pH 5, ng/cm^2 (%)	n	p-values comparing adsorption from 88 and 91 mg/L albumin in urine at pH 5 vs. 8
2	2.97 (0.47)	2.62 (0.38)	4	0.74	17.13 (0.16)	12.38 (0.11)	4	0.56
3	2.25 (0.35)	4.56 (0.66)	7	0.27	14.95 (0.14)	17.17 (0.16)	7	0.72
4	1.90 (0.30)	1.88 (0.27)	5	0.96	11.37 (0.11)	8.14 (0.07)	5	0.33
5	3.42 (0.54)	4.85 (0.70)	4	0.03	32.13 (0.31)	27.19 (0.25)	4	0.45
6	1.88 (0.30)	2.29 (0.33)	4	0.12	14.90 (0.14)	14.23 (0.13)	4	0.91
7	2.21 (0.35)	2.45 (0.35)	4	0.67	22.96 (0.22)	20.47 0.19)	4	0.86
8	2.30 (0.36)	3.22 (0.46)	4	0.22	17.19 (0.17)	15.31 (0.14)	4	0.77
9	2.40 (0.38)	2.48 (0.36)	4	0.91	13.78 (0.13)	15.93 (0.15)	4	0.81
10	2.53 (0.40)	2.59 (0.37)	4	0.92	17.29 (0.17)	11.59 (0.11)	4	0.23
11	1.80 (0.28)	2.12 (0.31)	5	0.55	12.00 (0.12)	9.60 (0.09)	5	0.52
12	4.61 (0.73)	6.47 (0.93)	4	0.30	37.32 (0.36)	22.38 (0.21)	4	0.07
13	1.72 (0.27)	2.01 (0.29)	4	0.41	15.26 (0.15)	12.21 (0.11)	4	0.48
14	1.57 (0.25)	1.80 (0.26)	4	0.34	12.03 (0.12)	7.16 (0.07)	4	0.28
15	2.18 (0.34)	2.45 (0.35)	4	0.61	11.39 (0.11)	13.89 0.13)	4	0.54
16	3.24 (0.51)	2.93 (0.42)	7	0.74	24.52 (0.24)	12.40 (0.11)	7	0.10
19	1.98 (0.31)	2.25 (0.32)	4	0.46	19.98 (0.19)	9.95 (0.09)	4	0.05
20	1.25 (0.17)	2.06 (0.25)	8	0.11	24.97 (0.21)	8.71 (0.07)	8	0.01
21	1.54 (0.21)	1.62 (0.20)	8	0.69	25.65 (0.25)	13.41 (0.11)	8	0.03

[a] Materials 1-19 were disks cut from: hydrophilic-coated centrifuge tube (1), polystyrene centrifuge tubes (2 and 12), polypropylene urine specimen containers (3, 4, 6, 11 and 13), polyethylene urine specimen containers (5 and 18), polyethylene terephthalate centrifuge tube (7), polypropylene centrifuge tubes (8, 9, 10, and 15), teflon centrifuge tube (14), polyethylene 24-hour urine collection jug (16), polysulfone centrifuge tube (17), and polypropylene screw cap for container #3 (19). Materials 20-22 were: polystyrene sample cups (20), hydrophilic coated vials (21), and polyethylene sample cups (22).

Table S3. Albumin adsorbed onto surfaces from HSA solutions spiked with ^{125}I -labeled HSA after incubation for five hours at room temperature.

Materials[a]	Albumin adsorbed from 0.4 mg/L HSA solution at pH 4, ng/cm^2 (%)	Albumin adsorbed from 1.3 mg/L HSA solution at pH 8, ng/cm^2 (%)	n	p-values[c]	Albumin adsorbed from 76 mg/L HSA solution at pH 4, ng/cm^2 (%)	Albumin adsorbed from 83 mg/L HSA solution at pH 8, ng/cm^2 (%)	n	p-values comparing adsorption from 76 and 83 mg/L albumin in HSA solutions at pH 4 vs. 8
1	0.98 (2.05)	1.83 (1.18)	6		64.4 (0.71)	54.0 (0.54)	6	0.73
2	2.85 (5.96)	6.86 (4.41)	5		258.4 (2.84)	177.9 (1.79)	5	0.21
3	2.22 (4.64)	2.78 (1.79)	5		114.1 (1.26)	51.3 (0.52)	5	0.01
4	3.03 (6.33)	5.20 (3.34)	4		318.4 (3.5)	165.9 (1.67)	4	0.02
5	2.60 (5.43)	4.66 (3.00)	5		194.8 (2.14)	130.7 (1.32)	5	0.08
6	2.45 (5.12)	5.98 (3.85(	4		262.9 (2.89)	175.0 (1.76)	4	0.09
7	2.45 (5.12)	5.92 (3.81)	4		219.5 2.41)	244.8 (2.47)	4	0.65
8	2.87 (6.00)	6.52 (4.19)	4		233.9 (.57)	200.8 (2.10)	4	0.52
9	3.29 (6.27)	6.54 (4.21)	4		294.1 3.14)	187.4 (1.89)	4	0.08
10	3.00 (3.57)	6.96 (4.48)	4		314.8 (.46)	207.8 (2.09)	4	0.07
11	1.71 (3.57)	3.66 (2.35)	4		254.8 (2.80)	149.1 (1.50)	4	0.03
12	3.32 (6.94)	7.57 (4.87)	4		357.9 (3.94)	205.8 (2.07)	4	0.03
13	3.46 7.23)	6.39 (4.11)	4		311.4 (3.43)	194.8 (1.96)	4	0.08
14	1.99 (4.16)	3.47 (2.23)	4		189.4 (2.08)	145.9 1.47)	4	0.35
15	3.26 (6.81)	6.48 (4.17)	4		288.8 (.18)	196.3 (1.98)	4	0.13
16	2.40 (5.02)	5.78 (3.72)	4		241.2 (2.65)	261.1 (2.63)	4	0.85
17	2.56 (5.35)	6.25 (4.02)	4		246.8 (2.71)	221.8 (2.23)	4	0.58
18	2.10 (4.39)	5.88 (3.78)	4		318.9 (3.51)	152.0 (1.53)	4	0.11
20	13.71 (24.5)	29.86 (16.4)	4		1248 (11.7)	815.0 (7.02)	4	0.00
22	16.00 (28.6)	34.11 (18.8)	4		1394 (13.1)	1006 (8.67)	4	0.00

[a] Materials were as described in Table S1.
[c] P values were not determined for comparing pHs because the change was influenced by both the concentration and pH differences.

Table S4. Albumin, spiked with ^{125}I -labeled HAS, retained in urine applied to surfaces after incubation for five hours at room temperature; and mass balance for adsorption (see Table S1) and retention.

Retention in urine solutions									Mass balance[b]			
Material[a]	Albumin retained from 5.3 mg/L in urine at pH 5, µg	Albumin retained from 5.8 mg/L in urine at pH 8, µg	n	p-values comparing retention from 5.3 and 5.9 mg/L albumin in urine at pH 5 vs. 8	Albumin retained from 87 mg/L in urine at pH 5, µg	Albumin retained from 91 mg/L in urine at pH 8, µg	n	p-values comparing retention from 88 and 91 mg/L albumin in urine at pH 5 vs. 8	For 5.3 mg/L albumin in urine at pH 5, %	For 5.9 mg/L albumin in urine at pH 8, %	For 87 mg/L albumin in urine at pH 5, %	For 91 mg/L albumin in urine at pH 8, %
2	0.524	0.583	4	<0.001	8.764	9.128	4	<0.001	99.4	100.8	100.0	100.0
3	0.525	0.581	7	<0.001	8.766	9.124	7	<0.001	99.5	100.8	100.0	100.0
4	0.525	0.583	5	<0.001	8.769	9.131	5	<0.001	99.3	100.8	100.0	100.0
5	0.524	0.581	4	<0.001	8.751	9.115	4	<0.001	99.4	100.8	100.0	100.0
6	0.525	0.583	4	<0.001	8.766	9.126	4	<0.001	99.4	100.8	100.0	100.0
7	0.525	0.583	4	<0.001	8.759	9.121	4	<0.001	99.4	100.8	100.0	100.0
8	0.525	0.582	4	<0.001	8.764	9.125	4	<0.001	99.4	100.8	100.0	100.0
9	0.525	0.583	4	<0.001	8.767	9.125	4	<0.001	99.4	100.8	100.0	100.0
10	0.524	0.583	4	<0.001	8.764	9.128	4	<0.001	99.4	100.8	100.0	100.0
11	0.525	0.583	5	<0.001	8.768	9.130	5	<0.001	99.3	100.8	100.0	100.0
12	0.523	0.579	4	<0.001	8.747	9.119	4	<0.001	99.4	100.8	100.0	100.0
13	0.525	0.583	4	<0.001	8.765	9.128	4	<0.001	99.4	100.8	100.0	100.0
14	0.525	0.583	4	<0.001	8.768	9.132	4	<0.001	99.4	100.8	100.0	100.0
15	0.525	0.583	4	<0.001	8.769	9.126	4	<0.001	99.4	100.8	100.0	100.0
16	0.524	0.582	7	<0.001	8.758	9.128	7	<0.001	99.4	100.8	100.0	100.0
19	0.525	0.583	4	<0.001	8.761	9.130	4	<0.001	99.4	100.8	100.0	100.0
20	1.052	1.166	8	<0.001	17.513	18.256	8	<0.001	99.5	100.8	99.9	99.9
21	1.052	1.167	8	<0.001	17.514	18.250	8	<0.001	99.5	100.8	99.9	99.9

[a] Materials as described in Table S1.
[b] Mass balance = (retained + adsorbed) / (mass in original solution) *100

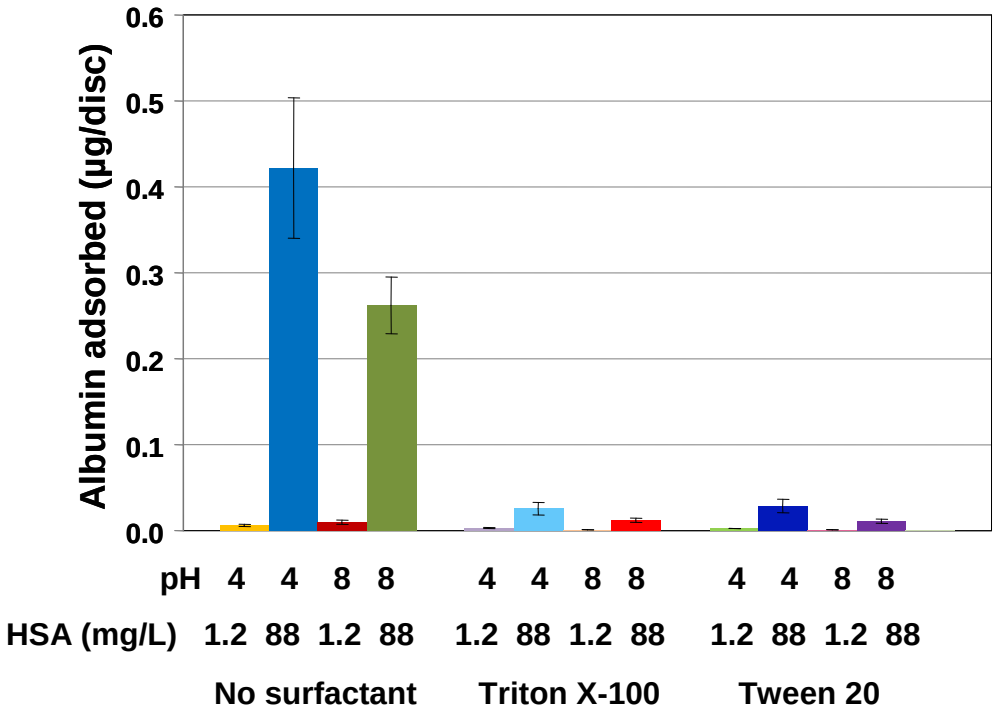

Figure S6. Albumin adsorbed to polypropylene discs from HSA in PBS spiked with ^{125}I-labeled HSA after incubation for 5 hours in the absence and presence of the surfactants Triton X-100 and Tween 20. Results are the mean of four measurements, and bars represent 95% confidence intervals.

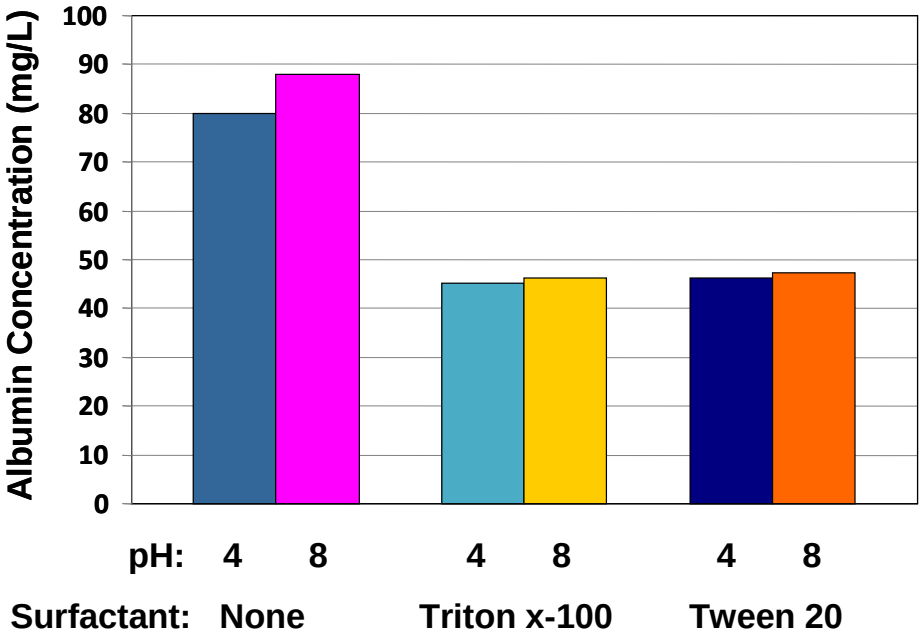

Figure S7. Albumin concentrations measured by IA for HSA in PBS in the absence and presence of surfactants Triton X-100 and Tween 20. Results are the average of duplicate measurements.

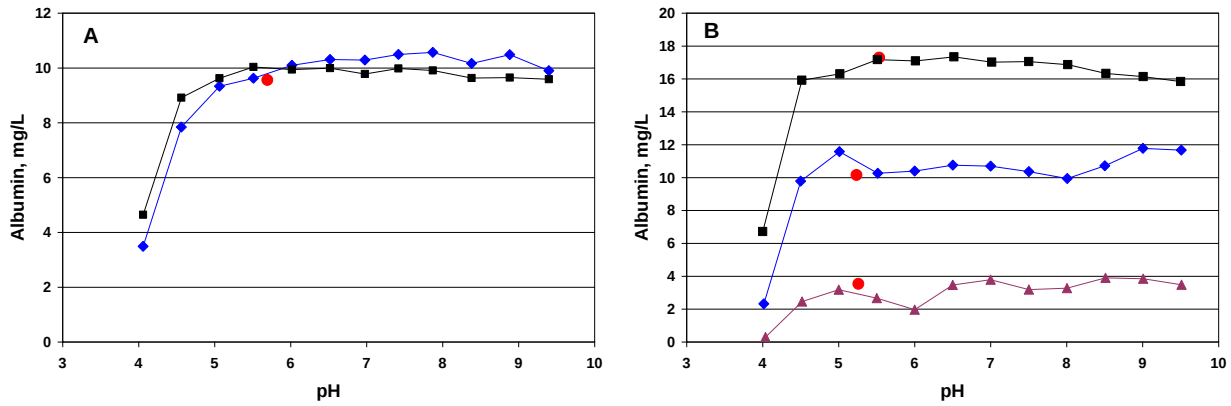

Figure S8. Albumin concentrations measured by immunoassay for urine solutions adjusted to various pH values. Results are the average of quadruplicate measurements. The red circle shows the original albumin concentration and pH for each sample. Panel A shows results for one sample after adjusting the pH to the values indicated and then readjusting the pH back to the original value. Panel B shows results for 3 additional urine samples after adjusting the pH to the values indicated.

Table S5. Influence of adsorption of albumin from urine onto containers based on worst case observations[a].

Urine albumin concentration, mg/L	Albumin adsorbed to 24 hour collection container (assume 2L fill), μg	Albumin adsorbed to 100 mL collection container, μg	Albumin adsorbed to analyzer cup (assume 0.2 mL fill), μg	Change in concentration due to adsorption to 24 hour collection container, %	Change in concentration due to adsorption to 100 mL container, %	Change in concentration due to adsorption to analyzer cup, %	Total change in albumin concentration if all 3 containers were used, %
5	3.234	0.654	0.00643	0.032	0.131	0.643	0.806
10	4.401	0.890	0.00875	0.022	0.089	0.438	0.549
20	6.735	1.362	0.0134	0.017	0.068	0.335	0.420
50	13.74	2.778	0.0273	0.014	0.056	0.273	0.343
100	25.41	5.138	0.0505	0.013	0.051	0.253	0.317
200	48.75	9.857	0.0970	0.012	0.049	0.242	0.304
300	72.09	14.58	0.1434	0.012	0.049	0.239	0.300

[a] The amount adsorbed was calculated from the linear regression for the worst case for 2 urine samples, with approximate albumin concentrations 5 and 90 mg/L, at pHs 5 and 8 to 18 plastic materials (Fig. 1A).