Electronic Supplementary Material (ESI) for Chemical Communications

A Prototype Point-of-Use Assay for Measuring Heavy Metal Contamination in Water Using Time as a Quantitative Readout

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Supporting Information

Materials:

All reagents were purchased commercially and were used as received unless otherwise noted. All DNA sequences were purchased from Integrated DNA technologies in IDTE buffer (pH 7.5). Deionized water was purified by filtration and irradiation with UV light. The papers used were Whatman Chromatography Paper Grade I and Boise Aspen 30 Printer Paper (92 brilliant, 30% postconsumer content), and the adhesive used was 3M™ Super 77™ Multipurpose Adhesive. The laminate was Protac™ Ultra UV (8.0 mil) with a Drytac® JetMounter™ JM26 laminator. Devices and laminate were cut using an Epilog mini 24 CO_2 laser.

Sequences of DNA Strands:

Adenosine Assay[1]

Adenosine A: 5'- biotin-AAAAAAAAAAAACCCAGGTTCTCT -3'

Adenosine B: 5'- TCACAGGTAAGTAAAAAAAAAAA-biotin -3'

Adenosine C: 5'- TTTTTTACTCATCTGTGAAGAGAACCTGGGGGAGTATTGCGGAGGAAGGT -3'

Lead Assay[2]

Lead A: 5'- biotin-
AAAAAAAAAAAAACAGACATCTCTTCTCCGAGCCGGTCGAAATAGGTGTAG -3'

Lead B: 5'- biotin-AAAAAAAAAAAATGTCCGATGCTACACTATrAGGAAGAGATGTCTGT -3'

Mercury Assay[3]

Mercury A: 5'- TCTCAACTCGTAAAAAAAAAAAA-biotin -3'

Mercury B: 5'- biotin-AAAAAAAAAAAACGCATTCAGGAT -3'

Reagent **d**: 5'- TTCGTGTTGTTCCTGTTTGCG -3'

Formation of Aptamers for Assays:

Adenosine Aptamer

The adenosine aptamers were formed as described in reference 1.

Beads: To 500 µL of Adenosine B (25 µM) was added 250 µL of Sphero™ streptavidin magnetic particles (1% w/v) (Spherotech, Lake Forest, IL) and 250 µL of buffer. The suspension was mixed for 24 h in the dark at room temperature and then collected by magnetic separation. The beads were washed three times using buffer and redissolved in 500 µL of buffer to give a 0.5% (w/v) working solution. The formed DNA sequence is referred to as Bead-Adenosine B.

Assay: To 200 µL of Adenosine A (12.5 µM) was added 25 µL of streptavidin glucose oxidase (1 mg/ml) (Rockland Immunochemicals Inc., Gilbertsville, PA) and 575 µL of 200 mM phosphate buffer (pH 7.5).

The solution was mixed at room temperature for 24 h in the dark and then collected using a Pall nanosep® 100K omega centrifugal filter. The sample was washed three times with buffer and then redissolved in 300 µL of buffer to give a working solution. The formed DNA sequence is referred to as GOX-Adenosine A. To 200 µL of Bead-Adenosine B was added 100 µL of GOX-Adenosine A and 100 µL of Adenosine C. The solution was mixed at room temperature in the dark for 90 min and then collected by magnetic separation. The suspension was washed three times with 40 mM HEPES buffer (pH 8.0) and redissolved in HEPES buffer. The final volume of buffer used was varied to change the concentration of adenosine aptamer solution added to the device.

Control: To 100 µL of Adenosine A (12.5 µM) was added 625 µL of streptavidin (2 µM). The solution was mixed at room temperature for 24 h in the dark and then collected using a Pall nanosep® 10K omega centrifugal filter. The sample was washed three times with buffer and redissolved in 300 µL of buffer to give a working solution. The formed DNA sequence is referred to as Strep-Adenosine A. To 200 µL of Bead-Adenosine B was added 100 µL of Strep-Adenosine A and 100 µL of Adenosine C. The solution was mixed at room temperature in the dark for 90 min and collected by magnetic separation. The suspension was washed three times with 40 mM HEPES buffer (pH 8.0) and redissolved in HEPES buffer. The final volume of buffer used was varied to change the concentration of adenosine aptamer solution added to the device.

Lead Aptamer

Beads: To 500 µL of Lead A (25 µM) was added 250 µL of Sphero™ streptavidin magnetic particles (1% w/v) (Spherotech, Lake Forest, IL) and 250 µL of buffer. The suspension was mixed for 24 h in the dark at room temperature and collected by magnetic separation. The beads were washed three times using buffer and redissolved in 500 µL of buffer to give a 0.5% (w/v) working solution. The formed DNA sequence is referred to as Bead-Lead A.

*Reagent **1***: To 200 µL of Lead B (12.5 µM) was added 25 µL of streptavidin glucose oxidase (1 mg/ml) (Rockland Immunochemicals Inc., Gilbertsville, PA) and 575 µL of 200 mM phosphate buffer (pH 7.5). The solution was mixed at room temperature for 24 h in the dark and collected using a Pall nanosep® 100K omega centrifugal filter. The sample was washed three times with buffer and redissolved in 300 µL of buffer to give a working solution. The formed DNA sequence is referred to as GOX-Lead B. To 300 µL of Bead-Lead A was added 300 µL of GOX-Lead B. The solution was mixed at room temperature in the dark for 90 min and collected by magnetic separation. The suspension was washed three times with 40 mM HEPES buffer (pH 8.0) and redissolved in 300 µL of HEPES buffer to give a 0.5% (w/v) working solution.

*Reagent **2***: To 200 µL of Lead B (12.5 µM) was added 625 µL of streptavidin (2 µM). The solution was mixed at room temperature for 24 h in the dark and collected using a Pall nanosep® 10K omega centrifugal filter. The sample was washed three times with buffer and redissolved in 300 µL of buffer to give a working solution. The formed DNA sequence is referred to as Strep-Lead B. To 300 µL of Bead-Lead A was added 300 µL of Strep-Lead B. The solution was mixed at room temperature in the dark for 90 min and collected by magnetic separation. The suspension was washed three times with 40 mM HEPES buffer (pH 8.0) and redissolved in 300 µL of HEPES buffer to give a 0.5% (w/v) working solution.

Mercury Aptamer

*Reagent **c***: To 500 µL of Mercury A (25 µM) was added 250 µL of Sphero™ streptavidin magnetic particles (1% w/v) (Spherotech, Lake Forest, IL) and 250 µL of buffer. The suspension was mixed for 24 h in the dark at room temperature and collected by magnetic separation. The beads were washed three times using buffer and the supernatant was removed and the beads were stored for use later. The formed DNA sequence is referred to as Reagent **c**.

*Reagent **3***: To 200 µL of Mercury B (12.5 µM) was added 25 µL of streptavidin glucose oxidase (1 mg/ml) (Rockland Immunochemicals Inc., Gilbertsville, PA) and 575 µL of 200 mM phosphate buffer (pH 7.5). The solution was mixed at room temperature for 24 h in the dark and collected using a Pall nanosep® 100K omega centrifugal filter. The sample was washed three times with buffer and redissolved in 300 µL buffer to give a working solution. The formed DNA sequence is referred to as Reagent **b**. To the solid Reagent **c** was added 300 µL of Reagent **b**, and the solution was split into two 150 µL aliquots. To one aliquot was added 100 µL of Reagent **d** and 750 µL of 40 mM HEPES buffer (pH 8.0) to give a 0.1% (w/v) working solution.

*Reagent **4***: To the second aliquot of Reagent **c** and reagent **b** was added 850 µL of 40 mM HEPES buffer (pH 8.0) to give a 0.1% (w/v) working solution.

Devices for Performing Assays Using a Single Analyte:

The first step in establishing simultaneous assays for lead(II) and mercury(II) involved using a paper device that contained only two channels rather than four (i.e., one channel leading to a "start" region and the other to a "stop" region). We used this simplified device along with aptamers for adenosine (Fig. S1) to demonstrate that the configuration of the device and the use of aptamers enabled quantitative assays based on measurements of time. We also optimized the sensitivity of the assay by altering the quantity of the aptamer reagents. These preliminary studies revealed a quantitative relationship between the concentration of adenosine and the time-based measurement (Fig. S2a). These studies also revealed that the quantity of the aptamer in layer 5 had a profound impact on the limit-of-detection for the assay, where higher quantities of aptamer correlated with lower limits-of-detection (Fig. S2b). In fact, the limit-of-detection for adenosine can be varied from 16 mM to 5 nM, with dynamic ranges of ~1000-fold for each variation in the quantity of the aptamer.

Layout of the Device for Performing Assays Using a Single Analyte:

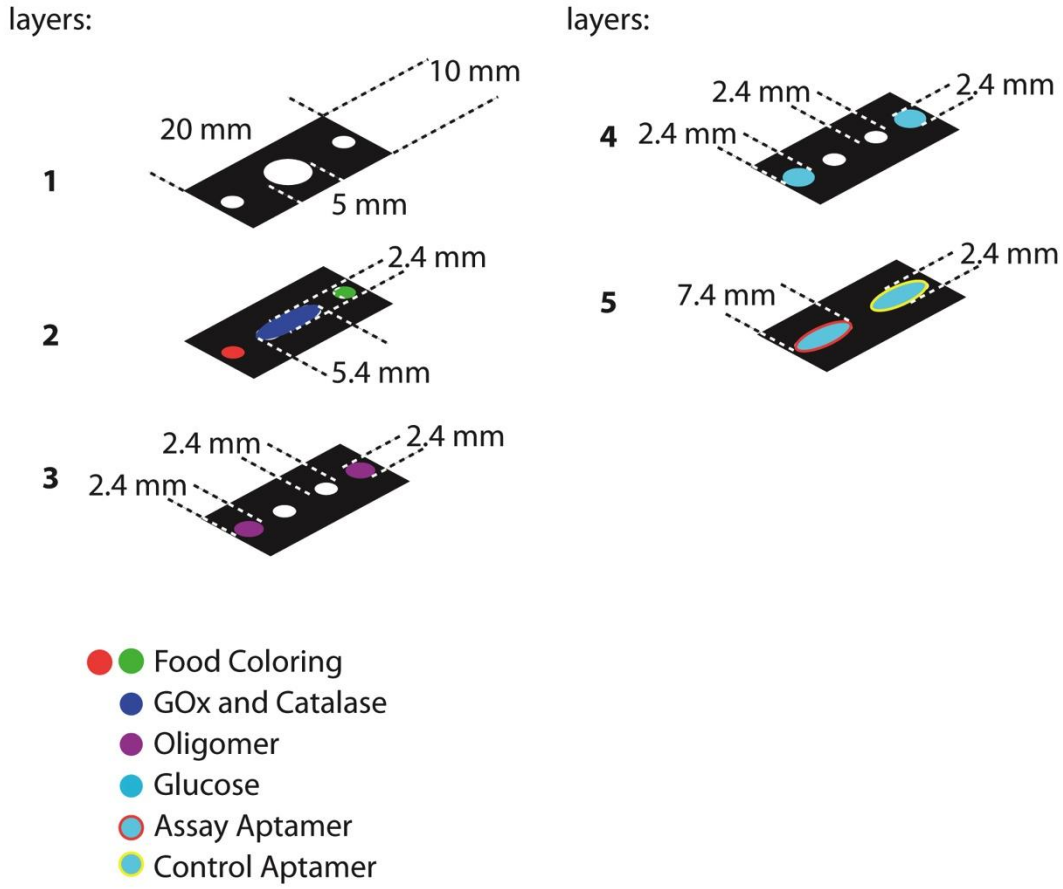

Figure S1. Expanded view of the two channel device. The device is 10 mm wide × 20 mm long × 1.8 mm thick.

Fabrication of the Device for Performing Assays Using a Single Analyte:

Patterning Paper

The paper was patterned according to the procedure described in reference 4.

Device for Assays of a Single Analyte

The devices were assembled according to the procedure in reference 4; the layout of the device is shown in Figure S1. The paper used for all layers was Whatman Chromatography Paper Grade I. Layer 2 contained 1 µL of food coloring (1:5 food coloring: deionized-water) pre-deposited in the outer regions. The central region contained 8 µL of immobilized glucose oxidase (0.25% w/v). The procedure for preparing the immobilized glucose oxidase is described in the section entitled "Immobilization of Enzymes on Polystyrene Beads". Layer 2 also contained 8 µL of immobilized catalase (0.25% w/v). The immobilized catalase was deposited in layer 2 and then allowed to air dry at room temperature for 45

minutes before the immobilized glucose oxidase was added and allowed to air dry. The outer two circles in layer 3 were predeposited with 0.25 µL of oligomer **5** (4 mM) in THF.[5] The outer circles in layer 4 were predeposited with 3 µL of glucose (50 mM). Both channels in layer 5 were predeposited with 3 µL of trehalose (1 M) and dried in ambient conditions for 30 minutes. The start channel in layer 5 (left-hand channel) was then pre-deposited with 3 µL of the assay aptamer, while the stop channel (right-hand channel) was pre-deposited with 3 µL of the control aptamer. All layers were air-dried in ambient conditions for 30 minutes after the reagents were deposited.

The devices were assembled as sheets (20 cm × 20 cm) by aligning the edges of each layer on top of each other and using 3M™ Super 77™ Multipurpose Adhesive to adhere individual layers together.[4] The sheets of devices were then pressed using a Drytac® JetMounter™ JM 26 laminator with medium pressure. Using a CO_2 laser (Epilog Mini 24 Laser), individual devices were cut out with tabs left between devices to allow for processing of the devices as a sheet. In a sheet of Protac™ Ultra UV (8.0 mil) (22 cm × 22 cm), holes (d = 5 mm) were cut using the CO_2 laser to align with individual devices in the sheet of devices. The devices were laminated between two sheets of 22 cm × 22 cm Protac™ Ultra UV (the cut sheet covering layer 1) using a Drytac® JetMounter™ JM 26 with medium pressure. Following lamination, individual devices were cut out using scissors.

Immobilization of Enzymes on Polystyrene Beads

Catalase: To 1 mL of catalase (20 mg/mL) in 100 mM phosphate-buffered saline (pH 7.4) was added 5 mg biotin-X-NHS (EMD Chemicals, San Diego, CA) and mixed at room temperature for 4 hours to form biotin-catalase (b-catalase). The b-catalase was purified three times using PD-10 prepacked desalting column (GE Healthcare, Buckinghamshire, UK), washing with 100 mM phosphate-buffered saline (pH 7.4) and then concentrating by lyophilization. To 4 mg of b-catalase was added 1 mL of 9 µm-diameter Sphero™ streptavidin magnetic particles (1% w/v) (Spherotech, Lake Forest, Il) and the resulting solution was mixed for 3 hours. The streptavidin beads were washed four times with HEPES buffer (40 mM, pH 8.0), concentrating by centrifugation between washes and then concentrated by lyophilization. The lyophilized powder was dissolved in 2 mL of HEPES buffer to give an immobilized catalase working solution (0.25% w/v).

Glucose Oxidase: To 4 mg of biotin-glucose oxidase (b-GOx) (Rockland Immunochemicals Inc., Gilbertsville, PA) was added 1 mL of 9 µm-diameter Sphero™ streptavidin magnetic particles (1% w/v) (Spherotech, Lake Forest, IL) and the resulting solution was mixed for 3 hours The streptavidin beads were washed four times with HEPES buffer (40 mM, pH 8.0), concentrating by centrifugation between washes and then concentrated by lyophilization. The lyophilized powder was dissolved in 2 mL of HEPES buffer to give an immobilized glucose oxidase working solution (0.25% w/v).

Procedure for Performing Assays for a Single Analyte:

The assay time was measured as follows: to layer 1 was added 60 µL of sample. When the "start" region turned green, a timer was started. The assay time was recorded when the "stop" region turned red. Six replicate tests were performed for each sample and both the highest and lowest assay times were removed from the data set to account for errors arising from failures during the device fabrication process.

Data for the Assay for Adenosine:

Table 1. Assay times for detecting adenosine in a single channel assay device containing 0.00625% (w/v) beads containing adenosine aptamer in layer 5. There were 6 replicates for each concentration of adenosine. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values.

Adenosine (nM)	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 6 (s)	Average (s)	Standard Deviation (s)
0	1	7	10	20	21	28	14.5	7.1
10	6	7	12	27	27	142	18.3	10.3
25	8	10	26	26	33	42	23.8	9.7
50	48	52	68	73	113	256	76.5	25.9
100	27	141	203	204	215	-	190.8	33.6
250	306	315	315	322	324	333	319.0	4.7
500	521	545	583	596	631	656	588.8	35.5
1000	1225	1345	1375	1382	1391	1404	1373.3	19.9

Table 2. Assay times for detecting adenosine in a single channel assay device containing 0.05% (w/v) beads containing adenosine aptamer in layer 5. There were 6 replicates for each concentration of adenosine. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values.

Adenosine (nM)	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 6 (s)	Average (s)	Standard Deviation (s)
0	1	1	5	22	39	2700	16.8	17.4
10	4	7	8	16	33	82	16.0	12.0
50	114	132	152	152	172	180	152.0	16.3
100	68	274	287	294	298	301	288.3	10.5
500	55	442	454	485	488	521	467.3	22.8
1000	11	665	881	889	925	1067	842.5	119.7

Table 3. Assay times for detecting adenosine in a single channel assay device containing 0.083% (w/v) beads containing adenosine aptamer in layer 5. There were 6 replicates for each concentration of adenosine. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values.

Adenosine (nM)	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 6 (s)	Average (s)	Standard Deviation (s)
0	6	11	12	12	21	33	14.0	4.7
1	1	2	2	10	16	20	7.5	6.8
5	18	22	25	32	41	52	30.0	8.4
10	8	32	42	47	67	74	47.0	14.7
50	61	67	88	109	110	138	93.5	20.4
100	265	289	296	304	344	381	308.3	24.6
1000	892	906	911	914	915	-	911.5	4.0

Table 4. Assay times for detecting adenosine in a single channel assay device containing 0.25% (w/v) beads containing adenosine aptamer in layer 5. There were 6 replicates for each concentration of adenosine. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values.

Adenosine (nM)	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 6 (s)	Average (s)	Standard Deviation (s)
0	1	2	4	7	14	21	6.8	5.3
50	1	1	10	13	15	23	9.8	6.2
100	13	15	28	28	33	40	26.0	7.7
500	10	68	79	84	90	236	80.3	9.3
1000	126	132	142	143	149	158	141.5	7.0
10000	113	170	181	197	202	405	187.5	14.7
100000	28	326	431	452	524	-	433.3	81.8

Table 5. Assay times for detecting adenosine in a single channel assay device containing 0.5% (w/v) beads containing adenosine aptamer in layer 5. There were 6 replicates for each concentration of adenosine. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values.

Adenosine (nM)	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 6 (s)	Average (s)	Standard Deviation (s)
0	2	2	3	10	25	630	10.0	10.6
5	2	5	12	13	15	39	11.3	4.3
10	4	10	20	50	54	58	33.5	21.8
50	12	24	55	55	59	-	48.3	16.3
100	62	90	101	109	123	132	105.8	13.9
500	100	136	151	163	177	-	156.8	17.4
1000	275	370	433	437	438	670	419.5	33.1
5000	22	450	462	463	464	-	459.8	6.6
10000	788	789	794	814	822	824	804.8	15.8

Table 6. Assay times for detecting adenosine in a single channel assay device containing 1.0% (w/v) beads containing adenosine aptamer in layer 5. There were 6 replicates for each concentration of adenosine. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values.

Adenosine (nM)	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 6 (s)	Average (s)	Standard Deviation (s)
0	9	11	11	13	14	21	12.3	1.5
1	1	2	3	21	22	26	12.0	11.0
5	11	12	45	48	52	89	39.3	18.4
10	77	86	87	116	138	138	106.8	25.1
50	127	150	151	160	172	173	158.3	10.2
100	118	182	218	244	292	306	234.0	46.3
500	539	564	575	602	603	608	586.0	19.6
1000	854	874	889	908	942	993	903.3	29.3

Effect of the Quantity of the Aptamer on the Limit-of-Detection of the Assay:

Table 7. Change in limit of detection with mass of aptamer–beads added to devices for detecting adenosine.

Aptamer–Beads (µg)	LOD (nM)
0.1875	15892.5
1.5	11977.0
2.49	5071.9
7.5	116.0
15	14.7
30	5.1

We used 30 µg of bead-bound aptamer reagent in layer 5 to achieve the 5 nM limit of detection, but quantities beyond 15 µg slow the distribution of sample within the device.

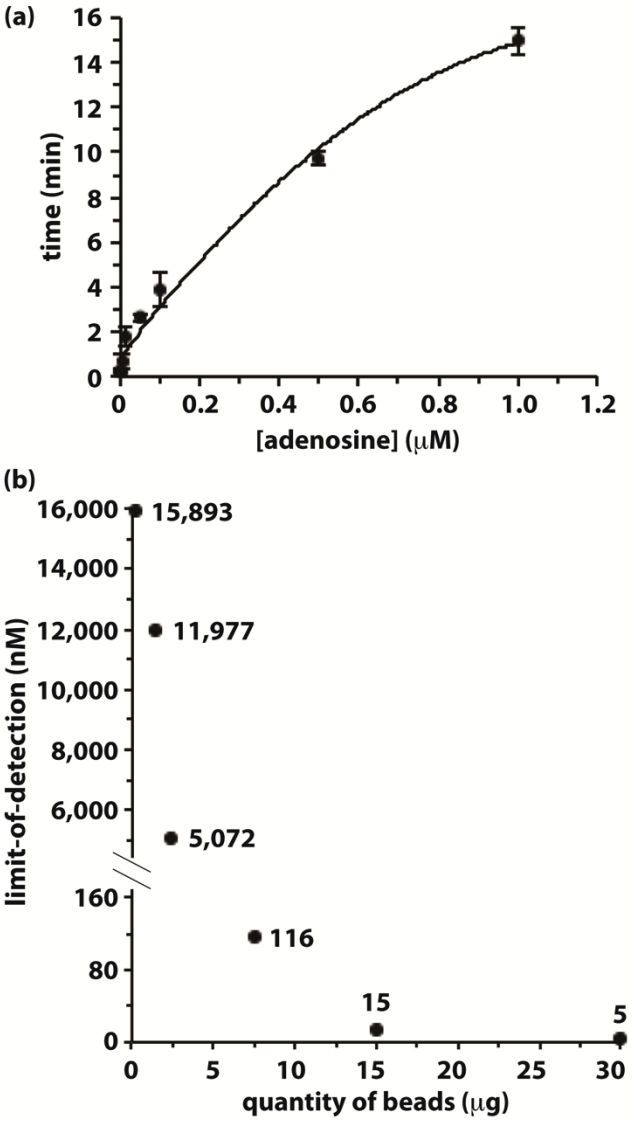

Figure S2. Detection of adenosine as a model target to show quantitative detection of an analyte using the aptamer-based assay. (a) Calibration curve showing the relationship between the measured time and the concentration of adenosine. (b) Tuning the sensitivity of the adenosine assay by varying the quantity of the adenosine aptamer (immobilized on microbeads) in layer 5 of the device for performing assays of a single analyte (Fig. S1).

Data on the Effects of Preprocessing:

Samples containing 100 nM adenosine that were spiked with either glucose or hydrogen peroxide were added to the device for performing assays of a single analyte (Figure S1). Devices that did not contain immobilized glucose oxidase and immobilized catalase were used to show the effects of glucose and hydrogen peroxide on assays lacking preprocessing.

No Preprocessing

Table 8. Assay times for detecting 100 nM adenosine spiked with glucose in a single channel assay device containing 0.5% (w/v) beads containing adenosine aptamer in layer 5. No immobilized glucose oxidase or catalase was present in layer 2. There were 6 replicates for each concentration of glucose. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values

Glucose (mM)	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 6 (s)	Average (s)	Standard Deviation (s)
0	109	234	247	256	257	290	248.5	10.7
10	8	141	164	192	192	-	172.3	24.7
25	8	62	91	96	97	-	86.5	16.5
50	3	19	23	34	71	220	36.8	23.7

Table 9. Assay times for detecting 100 nM adenosine spiked with hydrogen peroxide in a single channel assay device containing 0.5% (w/v) beads containing adenosine aptamer in layer 5. No immobilized glucose oxidase or catalase was present in layer 2. There were 6 replicates for each concentration of hydrogen peroxide. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values.

Hydrogen Peroxide (mM)	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 6 (s)	Average (s)	Standard Deviation (s)
0	109	234	247	256	257	290	248.5	10.7
0.5	1	2	7	8	11	16	7.0	3.7
10	2	13	36	38	43	64	32.5	13.3
25	1	1	2	3	10	24	4.0	4.1
50	5	6	26	32	32	44	24.0	12.3

With Preprocessing

Table 10. Assay times for detecting 100 nM adenosine spiked with glucose in a single channel assay device containing 0.5% (w/v) beads containing adenosine aptamer in layer 5. Immobilized glucose oxidase or catalase was present in layer 2. There were 6 replicates for each concentration of glucose. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values.

Glucose (mM)	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 6 (s)	Average (s)	Standard Deviation (s)
0	203	209	247	249	260	275	241.3	22.2
10	12	251	265	268	282	-	266.5	12.7
25	112	198	205	212	238	258	213.3	17.5
50	68	285	223	242	270	-	230	35.7

Table 11. Assay times for detecting 100 nM adenosine spiked with hydrogen peroxide in a single channel assay device containing 0.5% (w/v) beads containing adenosine aptamer in layer 5. Immobilized glucose oxidase or catalase was present in layer 2. There were 6 replicates for each concentration of hydrogen peroxide. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values.

Hydrogen Peroxide (mM)	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 6 (s)	Average (s)	Standard Deviation (s)
0	203	209	247	249	260	275	241.3	22.2
0.5	172	243	260	268	292	297	265.8	20.4
10	205	211	230	237	250	308	232.0	16.3
25	204	222	232	235	285	293	243.5	28.2
50	231	235	242	242	270	288	247.3	15.5

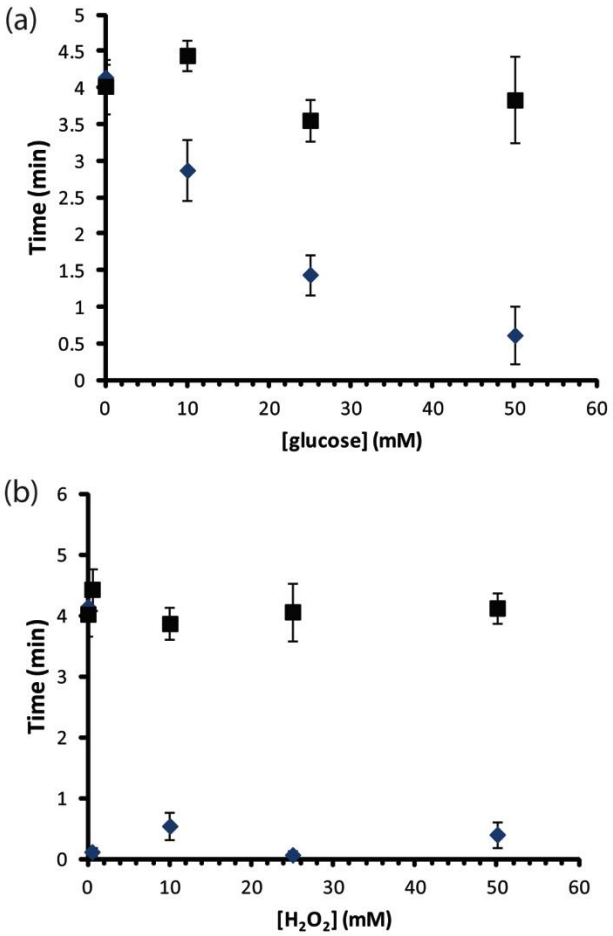

Figure S3. Effects of preproccesing on an assay for adenosine using samples spiked with either glucose or hydrogen peroxide. The samples used were 100 nM adenosine in deionized water. (a) Effects of preprocessing on samples containing glucose. The blue diamonds are devices that do not contain preprocessing, the black squares are devices containing preprocessing. (b) Effects of preprocessing on samples containing hydrogen peroxide. The blue diamonds are devices that do not contain preprocessing, the black squares are devices containing preprocessing.

Data for the Assay for Pb^{2+}:

Table 12. Assay times for detecting lead(II) in a single channel assay device containing 0.5% (w/v) reagents **1** and **2** in layer 5. There were 6 replicates for each concentration of lead. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values.

Lead(II) (nM)	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 6 (s)	Average (s)	Standard Deviation (s)
0	18	20	24	32	63	102	34.8	19.5
1	15	22	28	29	30	37	27.3	3.6
5	1	43	47	54	60	60	51.0	7.5
10	24	69	75	88	94	187	81.5	27.6
25	84	100	126	134	167	248	131.8	27.6
50	29	120	128	140	142	145	132.5	10.4
100	189	203	210	211	217	331	210.3	5.7
500	300	336	341	343	345	390	341.3	3.9
1000	40	300	357	377	395	439	357.3	41.2
100000	668	675	679	757	772	781	720.75	50.9

Data for the Assay for Hg^{2+}:

Table 13. Assay times for detecting mercury(II) in a single channel assay device containing 0.1% (w/v) reagents **3** and **4** in layer 5. There were 6 replicates for each concentration of mercury. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values.

Mercury(II) (nM)	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 6 (s)	Average (s)	Standard Deviation (s)
0	1	12	18	23	50	70	25.8	16.8
1	13	16	50	72	96	519	58.5	34.0
5	89	109	132	159	163	184	140.8	25.3
10	226	232	245	262	270	-	252.3	17.1
25	114	297	328	332	335	345	323.0	17.6
50	114	393	417	432	434	610	419.0	18.9
100	442	524	585	593	618	619	580.0	39.9
10000	90	747	799	824	859	930	807.3	47.1

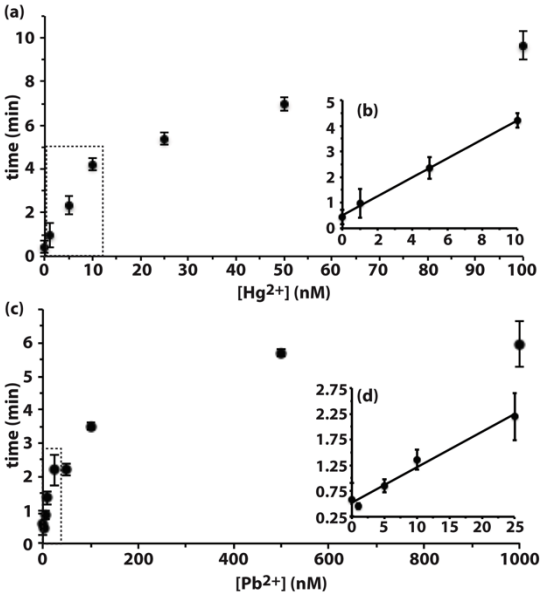

Fig. S4 Calibration curves for (a) Hg^{2+} and (c) Pb^{2+} using two-channel paper-based devices. The calibration curves were obtained at 20 ºC and 37% relative humidity. The data points are the average of four measurements and the error bars reflect the standard deviations of these averages. The insets (b) and (d) show the linear regions that are defined by the dotted rectangles. These linear regions were used to calculate the limits-of-detection values for the assays.

Layout of the Device for Performing Assays on Multiple Analytes Simultaneously:

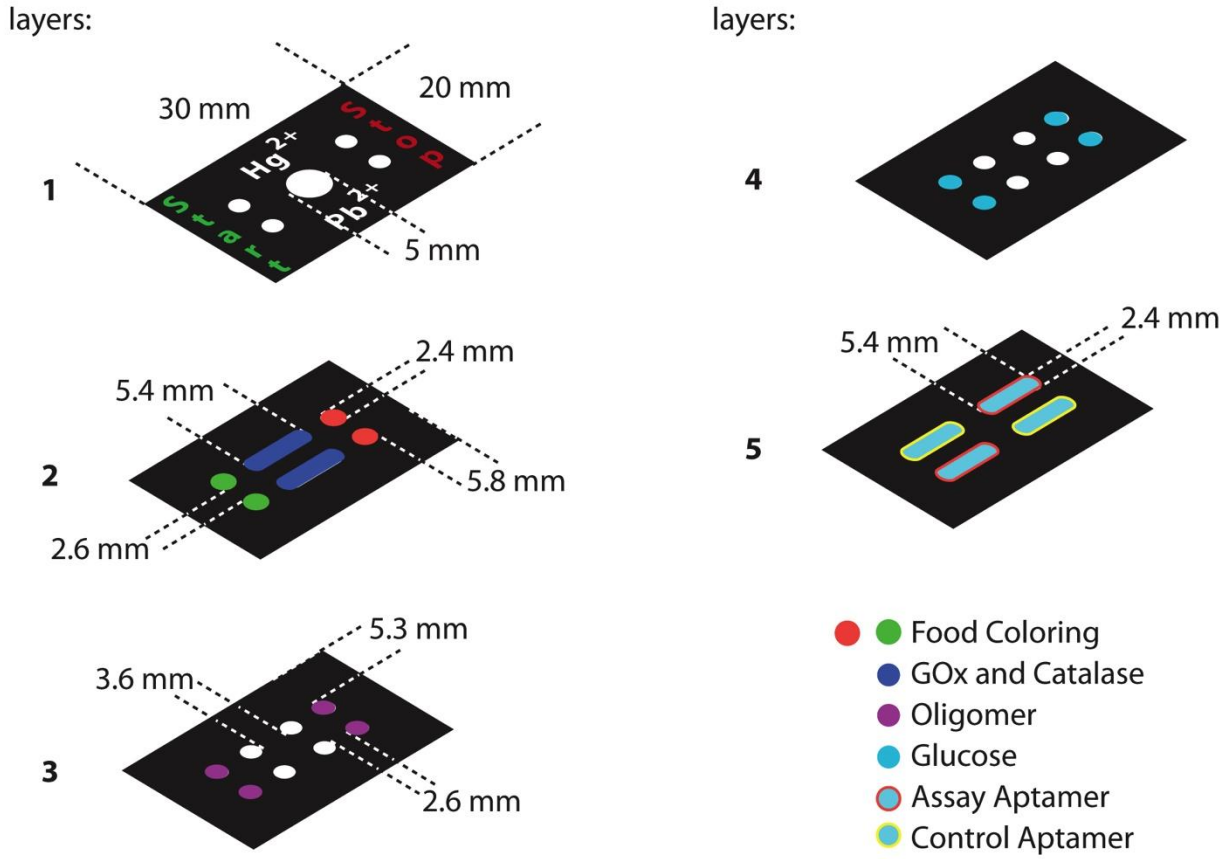

Figure S5. Expanded view of the device shown in Figure 1. The device is 20 mm wide × 30 mm long × 1.8 mm thick.

Fabrication of the Device for Performing Assays on Multiple Analytes Simultaneously:

The devices were assembled according to the procedure in reference 4; the layout of the device is shown in Figure S5. The paper used for all layers was Whatman Chromatography Paper Grade I. Layer 2 contained 1 µL of food coloring (1:5 food coloring: deionized-water) pre-deposited in the outer regions. The central region contained 8 µL of immobilized glucose oxidase (0.25% w/v). Layer 2 also contained 8 µL of immobilized catalase (0.25% w/v). The immobilized catalase was deposited in layer 2 and then allowed to air dry at room temperature for 45 minutes before the immobilized glucose oxidase was added and allowed to air dry. The outer circles in layer 3 were predeposited with 0.25 µL of oligomer **5** (4 mM) in THF.[5] The outer circles in layer 4 were predeposited with 3 µL of glucose (50 mM). All channels in layer 5 were predeposited with 3 µL of trehalose (1 M) and dried in ambient conditions for 30 minutes. For the mercury portion of the device (top row), the start channel in layer 5 (left-hand channel) was then pre-deposited with 3 µL of reagent **3**, while the stop channel (right-hand channel) was pre-deposited with 3µL of reagent **4**. For the lead portion of the device (top row), the start channel in layer 5 (left-hand channel) was then pre-deposited with 3 µL of reagent **1**, while the stop channel (right-hand channel) was

pre-deposited with 3µL of reagent **2**. All layers were air-dried in ambient conditions for 30 minutes after reagents were deposited.

The devices were assembled as sheets (20 cm × 20 cm) by aligning the edges of each layer on top of each other and using 3M™ Super 77™ Multipurpose Adhesive to adhere individual layers together.[4] The sheets of devices were then pressed using a Drytac® JetMounter™ JM 26 laminator with medium pressure. Using a CO_2 laser (Epilog Mini 24 Laser), individual devices were cut out with tabs left between devices to allow for processing of the devices as a sheet. In a sheet of Protac™ Ultra UV (8.0 mil) (22 cm × 22 cm), holes (d = 5 mm) were cut using the CO_2 laser to align with individual devices in the sheet of devices. The devices were laminated between two sheets of 22 cm × 22 cm Protac™ Ultra UV (the cut sheet covering layer 1) using a Drytac® JetMounter™ JM 26 with medium pressure. Following lamination, individual devices were cut out using scissors.

Procedure for Performing Assays on Multiple Analytes Simultaneously:

The assay time was measured as follows: to layer 1 was added 80 µL of sample. When the "start" region turned green, a timer was started. The assay time was recorded when the "stop" region turned red. Each assay (mercury and lead) was timed separately so that two times are measured, one for each analyte quantified. Six replicate tests were performed for each sample and both the highest and lowest assay times were removed from the data set to account for errors arising from failures during the device fabrication process.

Data Using De-Ionized Water:

Table 14. Assay times for detecting mercury(II) and lead(II) in a multiple channel assay device containing 0.1% (w/v) reagents **3** and **4**, and 0.5% (w/v) reagents **1** and **2** in layer 5. Samples were made using deionized water. There were 5 replicates for each sample. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values.

Heavy Metal(s)	Assay	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Average (s)	Standard Deviation (s)
0 nM Pb^{2+} and 0 nM Hg^{2+}	**Hg(II)**	1	12	13	17	33	14.0	2.6
	Pb(II)	2	6	9	34	45	16.3	15.4
100 nM Pb^{2+} and 0 nM Hg^{2+}	**Hg(II)**	1	2	4	12	22	6.0	5.3
	Pb(II)	189	203	220	243	244	222.0	20.1
0 nM Pb^{2+} and 100 nM Hg^{2+}	**Hg(II)**	574	534	582	488	566	534.7	47.0
	Pb(II)	1	2	21	23	30	15.3	11.6
100 nM Pb^{2+} and 100 nM Hg^{2+}	**Hg(II)**	504	506	541	557	580	534.7	26.1
	Pb(II)	234	238	236	236	309	236.7	1.2
100 nM Pb^{2+}, 100 nM Hg^{2+}, 100 nM Cd^{2+} and 100 nM Zn^{2+}	**Hg(II)**	510	519	520	564	572	534.3	25.7
	Pb(II)	219	229	240	253	284	240.7	12.0

Data using Lake Water:

Sample were prepared with lake water from Tussey Lake (40° 46' 11.28" N, 77° 45' 29.88" W). The lake water was spiked with lead(II) to simulate contaminated drinking water.

Table 15. Assay times for detecting mercury(II) and lead(II) in a multiple channel assay device containing 0.1% (w/v) reagents **3** and **4**, and 0.5% (w/v) reagents **1** and **2** in layer 5. Samples were made using lake water. There were 5 replicates for each sample. To account for errors in fabricating the devices, the fastest and slowest assay times were not used in determining the average or standard deviation values.

Heavy Metal(s)	Assay	Trial 1 (s)	Trial 2 (s)	Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Average (s)	Standard Deviation (s)
0 nM Pb^{2+} and 0 nM Hg^{2+}	**Hg(II)**	1	8	9	10	221	9.0	1.0
	Pb(II)	1	1	4	13	14	6.0	6.2
100 nM Pb^{2+} and 0 nM Hg^{2+}	**Hg(II)**	1	9	15	16	62	13.3	3.8
	Pb(II)	233	241	245	246	248	244.0	2.6
10 nM Pb^{2+} and 0 nM Hg^{2+}	**Hg(II)**	1	1	1	17	69	6.3	9.2
	Pb(II)	70	80	81	83	83	81.3	1.5
1 nM Pb^{2+} and 0 nM Hg^{2+}	**Hg(II)**	2	2	5	25	27	10.7	12.5
	Pb(II)	11	30	32	52	58	38.0	12.2

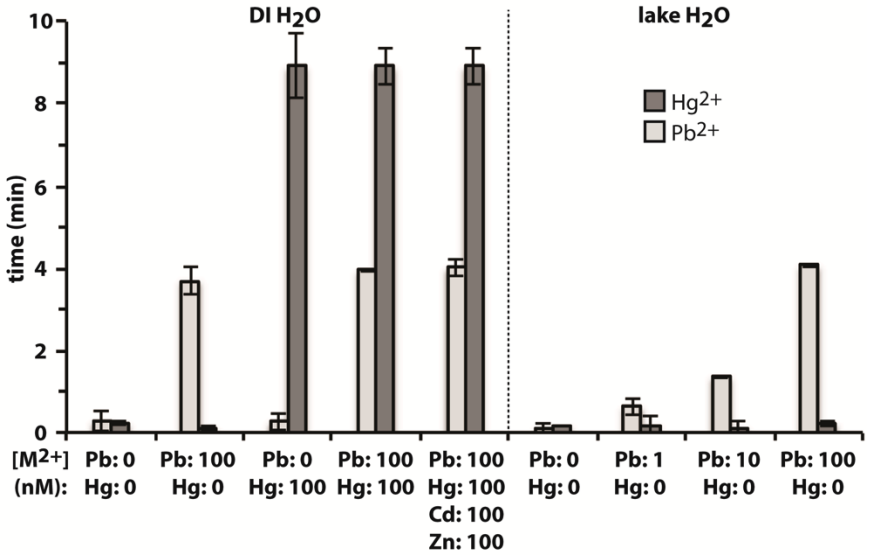

Figure S6. Select assay results using the four-channel device shown in Fig. 1. The values are the average of three measurements and the error bars reflect the standard deviations of these averages.

Stabilization of DNA Oligonucleotides

DNA in the solid state has been shown to exhibit long term stability compared to when in solution.[6] This can be further improved through the use of additives, such as trehalose, during the drying process.[7] Further stabilization can be achieved through the immobilization of the DNA onto a surface or particle.[8] Using lyophilization, the DNA can be dried while preventing aggregation of the DNA oligomers, improving the stability of the dried DNA.[9]

Breakdown of Materials Costs

Table 16. Breakdown of the materials costs for each of the prototype devices used to quantify heavy metal contaminants. All values are in $USD. Values are calculated using retail prices for materials used within each device. Quantities of aptamers and preprocessing reagents could be further optimized using a cost-benefit analysis to provide the most cost-effective devices.

Materials	Lead Device	Mercury Device	Multiplexed Device
patterned paper	$0.043	$0.043	$0.043
adhesive spray	$0.004	$0.004	$0.004
laminate sealing	$0.007	$0.007	$0.007
aptamer reagents	$0.533	$0.112	$0.645
preprocessing reagents	$0.421	$0.421	$0.425
hydrophobic oligomer	$0.001	$0.001	$0.003
Total	**$1.009**	**$0.588**	**$1.125**

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