Rapid, robust and low-cost detection methods for poison and microbial contamination

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1. Recent contamination incidents leading to sickness, hospitalization and death

A. Worldwide Diethylene Glycol and Ethylene Glycol Poisoning

The contamination of foods, water, medicines and ingestible household products with ethylene glycol (EG) and diethylene glycol (DEG) has resulted in many deaths, often hundreds in a single incident, even up to the present day. We summarize major incidents of lethal DEG and EG contamination in the tables below:

Substance	Location/Date	Date	Wt % EG	Fatalities	Reference
Antifreeze	Worldwide		ca 50	>2400 per year	Leikin et al (1997), Leth & Gregersen (2005)
Paracetamol Syrup	Nigeria	1990	90	196	Pharmaceutical Society of Nigeria (2007)
Wine	Netherlands	1990	1.5	0	Gomes, et al. (2002)
Drinking Water	Indiana	1990	2	6	U.S. Centers for Disease Control (1987)
Drinking Water	North Dakota	1987	7	29 ill	U.S. Environmental Protection Agency (2001)
Dialysis machine	Illinois	1985	3	4	U.S. Environmental Protection Agency (2001)
Drinking Water	New York	1985	-	1	U.S. Environmental Protection Agency (2001)

Table 1: Ethylene Glycol Poisoning

Substance	Location/Date	Date	Wt % EG	Fatalities	Reference
Acetaminophen	Nigeria	2008	17-21	84	Schier (2009)
Toothpaste	China, USA	2007	1.5-4	100s ill	Bogdanich (2007)
Antihistamine	Panama	2006	7.6-8.1	51	Schier, et al. (2008)
Cough Syrup	India	1998	17.5	36	Singh, et al. (2001)
Paracetamol Syrup	Haiti	1995	14.5-19.6	109	O'Brien, et al. (1998)
Propolis Syrup	Argentina	1992	65	15	Drut, et al. (1994), Ferrari and Giannuzzi (2005)
Paracetamol Syrup	Bangladesh	1990-92	40-48	236	Hanif, et al. (1995)
Paracetamol Syrup	Nigeria	1990		47	Okuonghae, et al. (1992)
Glycerine (medical)	India	1986	18.6	21	Pandya (1988)
Wine	Austria, Germany	1986	0.3	-	Tagliabue (1985)
Topical Cream	Spain	1985	0.65-0.72	5	Cantarell, et al. (1987)
Drinking Water	Sahara	1979	2	4	Daza (2006)
Sedative Elexiers	South Africa	1969	4.5	6	Wax (1996)
Sulfur Drug	USA	1937	50	105	Wax (1995),Osterberg (2003)

Table 2: Diethylene Glycol Poisoning

B. Salmonella outbreaks in the USA

Every year, approximately 40,000 cases of salmonellosis are reported in the United States. Because many milder cases are not diagnosed or reported, the actual number of infections may be thirty or more times greater. Children are the most likely to get salmonellosis. The rate of diagnosed infections in children less than five years old is higher than the rate in all other persons; young children, the elderly, and the immunocompromised are the most likely to have severe infections. It is estimated that in the United States approximately 400 persons die each year with acute salmonellosis [U. S. CDC website].

A large fraction of reported salmonella infections are caused by eggs or egg-related products: up to 77% of reported outbreaks with identified food vehicles have been reported to have been caused by Grade-A shell eggs or foods that contained such eggs [Louis, *et al.* (1988), Mishu, *et al.* (1994)].

Most cases of salmonella infections are isolated, local cases, which affect single or few people; however, mass outbreaks of salmonella infections also can occur, often in food served by chain restaurants, or those distributed throughout large regions through supermarkets. We summarize larger mass outbreaks of salmonella [CDC (2010)] in the United States since 2006 in the table below:

Substance	Location/Date	Date	Wt % EG	Fatalities	Reference
2010	Raw Alfalfa Sprouts		44	0	7
2010	Marie Callender's Cheesy Chicken	18 states	44	0	16
2010	Frozen rodents (for reptile feed)	17 states	34	0	1
2010	Eggs from Wright County Eggs and Hillandale Farms in Iowa	multistate	1600		
2010	Italian-style meats at Daniele International Inc.	44 states	272	0	52
2010	Restaurant Chain A	multiple states	47	0	15
2009	Raw Alfalfa Sprouts	14 states	235	0	15
2009	Pistachios				
2009	Water frogs	31 states	85	0	16
2008	Cantaloupes	16 states	52	0	16
2008	Malt-O-Meal Rice/Wheat Cereals	15 states	32	0	23
2008	Peanut Butter	15 states	28	0	
2007	Banquet Pot Pies	35 states	272	0	65
2007	Dry Pet Food	18 states	62	0	10
2007	Peanut Butter	44 states	425	0	71
2007	Veggie Booty	20 states	65	0	
2006	Tomatoes	21 states	111	0	22

Table 3: Salmonella outbreaks in the USA from 2006-2010

C. Escherichia Coli Outbreaks in the USA

In 1982, an investigation by the U.S. Centers for Disease Control and Prevention (CDC) of two outbreaks of severe bloody diarrhea, associated with the same fast food restaurant chain, identified a new strain of *E. coli* that had not previously been recognized as a pathogen [Riley, *et al.* (1983), Wells, *et al.* (1983)]. In the years since the discovery of this pathogen, *E. coli* O157:H7 has become increasingly prominent, causing an estimated 20,000 illnesses and 250 deaths each year in the United States alone [Armstrong, *et al.* (1996)]. *E. coli* can be passed from person to person, but serious *E. coli* infection is more often linked to contaminated food, including:

- raw milk
- fruit juice that isn't pasteurized, such as apple cider
  drinking water, e.g. unchlorinated water gets poisoned with *E. Coli* after pipes burst [Swerdlow, *et al.* (1992), Olsen, *et al.* (2002), Carter, *et al.* (1987), Ackman, *et al.* (1997)}
- vegetables grown in cow manure or washed in contaminated water
- undercooked ground beef (used for hamburgers)

We summarize recent *E. Coli* outbreaks in the USA from 2006-2010 [U.S. Centers for Disease Control (2010)] in the table below:

Substance	Location/Date	Date	Wt % EG	Fatalities	Reference
2010	Apple Cider	1 state	7	0	3
2010	Shredded lettuce	5 states	33	0	N/A
2009	Beef from National Steak and Poultry	16 states	21	0	N/A
2009	Beef from Fairbank Farms	8 states	26		N/A
2009	Beef from JBS Swift Beef Company	9 states	17	0	12
2009	Prepackaged Cookie Dough	30 states	72	0	34
2008	Kroger/Nebraska Ltd	7 states	49	0	27
2008	Totino's/Jeno's Pizza	4 states	71	0	53
2007	Topp's Ground Beef Patties	8 states	40	0	21
2006	Taco Bell		52	0	N/A
2006	Fresh Spinach		102	0	N/A

Table 4: E.Coli outbreaks in the USA from 2006-2010

D. Cholera Outbreaks Worldwide

Cholera is an infection of the small intestine that is caused by the bacterium *Vibrio cholerae*, whose main symptoms are profuse watery diarrhea and vomiting. Transmission is primarily via fecal contamination of food and water due to poor sanitation. Cholera affects 3-5 million people and causes 100,000–130,000 deaths a year as of 2010, mostly in the developing world [Reidl and Klose (2002)]. In the early 1980s, death rates are believed to have been greater than 3 million a year [Sack, *et al.* (2004)]. It is difficult to calculate exact numbers of cases; many cases are not reported due to concerns that the report of an outbreak may have a negative impact on local tourism [Sack, *et al.* (2006)]. Cholera remains both epidemic and endemic in many areas of the world [Sack, *et al.* (2004)].

Although much is known about the mechanisms behind the spread of cholera, this has not led to a full understanding of what makes cholera outbreaks happen some places and not others. Lack of treatment of human feces and lack of treatment of drinking water greatly facilitate its spread, but bodies of water can serve as a reservoir and seafood shipped long distances can spread the disease. Cholera was not known in the Americas for most of the 20th century, but it reappeared towards the end of that century and seems likely to persist [Blake (1993)].

Typically, about 10^8 bacteria must be ingested to cause cholera in a normal healthy adult [Sack, *et al.* (2004)]. This minimum dose, however, is less in those with lower gastric acidity; furthermore, children are also more susceptible, with two- to four-year-olds having the highest rates of infection[Sack, *et al.* (2004)].

Recent Cholera outbreaks include the 2010 Cholera outbreak in Haiti following the large earthquake, which caused 1,034 fatalities and 167,000 hospitalizations [MSNBC (2010a)]. In August 2010, 12 of the 36 states in Nigeria were affected with Cholera; 6400 cases have been reported with 352 reported deaths, which the health ministry blamed on heavy seasonal rainfall and poor sanitation [MSNBC (2010b)]

E. Malaria Worldwide

Malaria is a mosquito-borne infectious disease of humans caused by eukaryotic protists of the genus *Plasmodium*. It is widespread in tropical and subtropical regions, including much of Subsaharan Africa, Asia and the Americas. The disease results from the multiplication of malaria parasites within red blood cells, causing symptoms that typically include fever and headache, in severe cases progressing to coma, and death. Malaria is commonly associated with poverty, and can indeed be a cause of poverty [Gollin and Zimmermann(2007)] and a major hindrance to economic development.

Each year, there are more than 243 million cases of malaria, killing nearly a million [World Health Organization (2009)] The majority of deaths occur in sub-Saharan Africa, and primarily affect young children [Snow, *et al.* (2005)]. We summarize the number of cases and deaths in a recent year, organized by WHO region [World Health Organization (2009)] in the table below:

WHO region	Cases	Deaths
AFR	208,000,000	767,000
AMR	1,000,000	1,000
EMR	9,000,000	52,000
EUR	0	0
SEAR	24,000,000	40,000
WPR	2,000,000	3,000
Total	243,000,000	863,000

Table 5: Estimated Malaria Cases in 2008 by region

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2. Materials and Methods

A. Sample and enzyme preparation for detection of ethylene glycol

Samples, S, containing ethylene glycol (obtained from Sigma Aldrich SAJ first grade) were mixed with household products and medicines at different mass percentages. They were prepared freshly for each experiment and were weighed out using an analytic balance (Mettler). The samples, S, were prepared in the following ratios:

EG mass fraction	EG (mg)	Substances (mg)	Buffer (g)
1	135	0	2.365
0.60	81	360	2.059
0.30	40.5	630	1.8295
0.15	20.25	765	1.71
0.06	8.1	846	1.65
0.03	4.05	873	1.62
0.015	2.025	886.5	1.61
0.006	0.81	894.6	1.6
0.003	0.405	897.3	1.6
0.0015	0.2025	898.65	1.6

Table 6: Ethylene glycol sample preparation

To prepare the enzyme stock solutions, an alcohol-dehydrogenase-NAD reagent (A) was made by adding 15 mL of Tris-HCl buffer, pH 8.8, 0.1M (Bio-Rad) to 50 mg NAD (Sigma Aldrich N8535). In mixture B, 0.1 ml of Tris-HCl buffer, pH 8.8, 0.1M (Bio-Rad) was added to 100 mg yeast alcohol dehydrogenase (USB/Affymetrix #10895). To start a sample reaction, 120 µl of the sample, S, were placed in a round 6.50 mm glass tube (Durham Culture Tubes 6.50). Next an enzyme mixture, C, containing 480 µl of solution B and 40 µl of solution A was prepared. All volumes were confirmed by weighing with a scale (Mettler Toledo). To start the reaction in our device, 240 µl of C were added to each tube containing sample, S. A 5.4 wt % EG sample in buffer was always run in parallel as a control.

B. Sample and enzyme preparation for detection of diethylene glycol and of alcohols

Samples, S, containing Diethylene Glycol (Sigma Aldrich Reagent Plus 99%) in different products were prepared freshly for each experiment and were weighed out using an analytic balance (Mettler). The samples were prepared in the following ratios:

DEG mass fraction	DEG (g)	Substances (g)	Buffer (g)
1	11.96	0	30
0.80	0.912	0.228	0.572
0.60	0.684	0.456	1.144
0.40	0.456	0.684	1.316
0.20	0.228	0.912	1.088
0.10	0.114	1.026	0.974
0.06	0.046	1.095	2.505
0.04	0.023	1.117	2.483
0.01	0.011	1.129	2.471

Table 7: Diethylene glycol sample preparation

Stock solutions A and B (see A, Ethylene Glycol) were prepared. In addition stock solutions of 0.05 wt% Amplex Ultrared in DMSO (solution D), 0.044 wt% Horseradish Peroxidase Type 1 (Sigma Aldrich P8125) in Tris-HCl buffer, pH 7.8, 0.1M (solution E), 12 wt% Peroxidase from Enterococcus faecalis (Megazyme, E.C. 1.11.1.1) in phosphate buffer, pH 6.0, 0.1M (solution F) and 0.2 mg/ml Flavin Adenin Dinucleotide (Sigma Aldrich) in deionized water (solution G) were prepared. The final enzyme mixture H contained 480 µl of solution B, 40 µl of solution A and 20 µl each of the solutions D, E, F and G. The reaction was started and read out as described for EG above. For the DEG samples, a reference sample of 5.4 wt % DEG and for alcohols a sample of 5.4×10^{-3} wt % was always run in the second chamber as a control.

C. Assay enzyme and pH optimization

We screened five different alcohol dehydrogenases for their specificity in reacting with DEG, compared to glycerol and cough syrup, which contains both glycerol and PEG. We measured the fluorescence product in a plate reader from our assay on 100% cough syrup and pure glycerol samples (corresponding to 0% DEG, as defined in the sample prep procedure for DEG), using 5 different ADH enzymes. For a control sample, we measured pure buffer (Tris-HCl pH 7.8 0.1M) with one enzyme (USB). We determined the "relative interference" of each enzyme by dividing the initial reaction gradient (200 sec) of each sample by the control, and found that the yeast alcohol dehydrogenase #10895 from USB/Affymetrix had the highest relative reactivity of DEG relative to both glycerol and cough syrup, as shown by the data in the table below.

Enzyme manufacturer	Product #	relative interference: glycerol	relative interference: cough syrup
Sigma	A7011	10.82	6.11
USB	10895	7.24	2.58
Worthington	LS001069	12.92	5.64
AppliChem 1	A7827	15.69	6.46
AppliChem 2	A7892	11.43	6.85

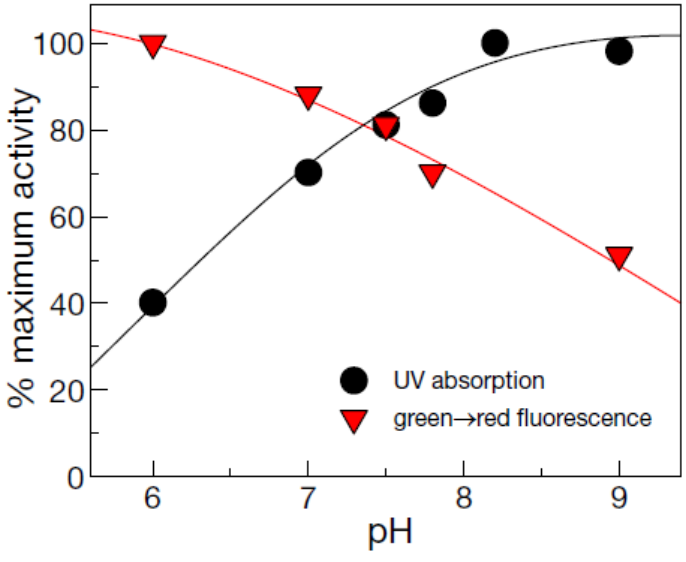
Table 8: relative activity of different alcohol dehydrogenase enzymes

We also optimized the pH of the assay solution. NAD and FAD were most stable at neutral pH; alcohol dehydrogenase was most active above pH 8; NADH Peroxidase was most active at pH 5, as summarized in the table below.

Enzyme	pH range, stability, activity notes	Source
HRP	6.0–6.5 (84% of maximum activity at pH 7.5); stability pH optimum: 5.0–9.0	Brenda Enzymes (www.brenda-enzymes.info)
NADH Peroxidase	90% of maximum activity at pH 5.0, 32% of maximal activity at pH 8.5; 50% activity at pH 7.0	communication with Megazyme Intl, Ltd.
Alcohol Dehydrogenase	pH optimum: 7.8-9	Brenda Enzymes
NAD/NADH	The coenzyme is stable for about a week at 4 °C and neutral pH, but decomposes rapidly in acids or alkalis.	Sigma Aldrich
FAD	Very stable near neutral pH	communication with Sigma Aldrich

Table 9: pH dependence of enzyme activity

By varying the buffer pH from 6 to 9, we observed the highest overall signal to-noise levels between pH 7.5-8, where both fluorescence absorption are at a high percentage of their maximum activity, as shown in the figure. The use of NADH Oxidase instead of NADH Peroxidase made the assay unstable, as NADH Oxidase solution decays within minutes at room temperature.



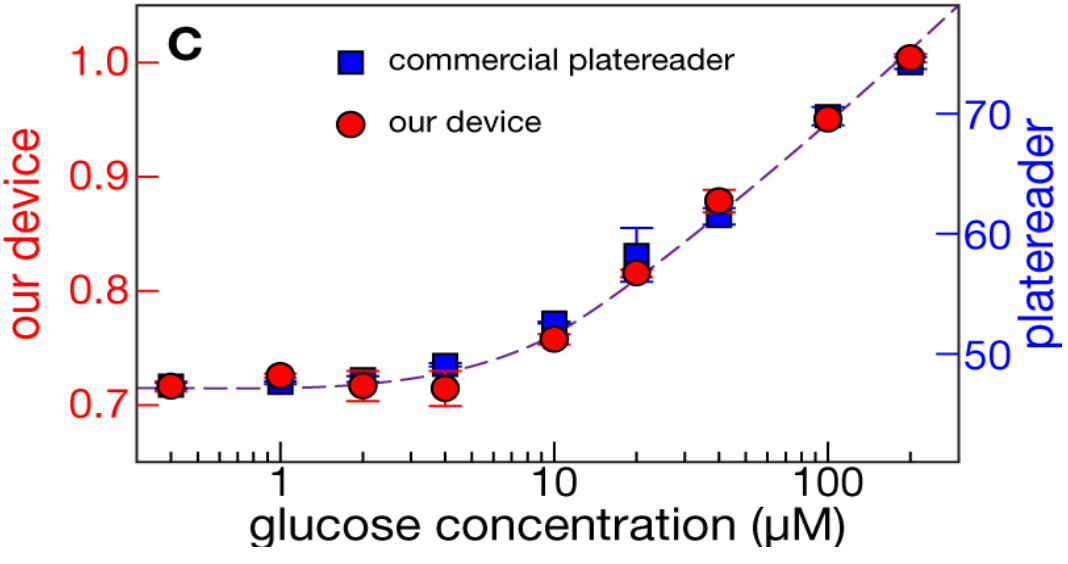
D. Fluorescence detection of glucose and comparison with commercial plate reader

The device has a sensitivity comparable to a commercial plate reader, as was tested by comparing the fluorescence emission from a standard glucose assay in both the device and a commercial plate reader. The chemistry for our glucose assay was the Glucose Oxidase Assay Kit from Invitrogen (Amplex Red Glucose/Glucose Oxidase Assay Kit - Cat. No. A22189). In this assay, glucose oxidase reacts with d-glucose to form d-gluconolactone and H_2O_2. In the presence of horseradish peroxidase (HRP), the H_2O_2 then reacts with the Amplex Red reagent in a 1:1 stoichiometry to generate the red fluorescent oxidation product, resorufin. Initially the contents of the kit were warmed up to room temperature. 60 µl DMSO were then added to an Amplex Red vial. A 1× reaction buffer was prepared by dilution with water. 10 U/ml HRP and 100 U/ml Glucose Oxidase solutions were prepared using the reaction buffer according to the protocol provided by Invitrogen. Using the 1× reaction buffer, a 400 mM (72 mg/ ml) d-glucose stock solution was weighed out, and then serially diluted down to desired concentrations between 400 and 1 µM d-glucose. The reagent mixture was prepared and contained 4.75 ml reaction buffer, 100 µl HRP, 100 µl glucose oxidase, and 50 µl Amplex Red.

To start a reaction for measurement in our device, 175 mg of glucose samples were added to 175 mg of the reaction mixture in a round 6×50 mm glass tube (Durham Culture Tubes 6×50). A positive control (200 µM) was always run in parallel, in the second sample chamber. The absorption and fluorescence values were monitored in the detectors for 5 minutes (i.e. initial kinetics). After 30 minutes, the stationary fluorescence values were re-measured.

To start a reaction in the fluorescent plate reader (Molecular Devices, SPECTRAmax™ GEMINI XS) 50 µl of glucose samples/controls were added to 50 µl of reaction mixture in a 96 well-plate. The reactions were monitored for 30 minutes in 15 s intervals (ex: 530nm; em: 590nm).

The fluorometer channel of our device performs quite similarly to a commercial plate reader, as shown in the figure below:



E. Sample preparation of E. Coli, Salmonella and Cholera bacteria in foods and water

We grew cultures of E. coli (strain: DH5alpha), E. Salmonella (strain: LT2 Delta PhoP/Q S typhi) and Vibrio Cholera (strain: VC O395NT). Bacteria were stained with 2.5 µM Syto 85 (Invitrogen Cat. No. S11366) in deionized water for 3-30 minutes at 250 rpm and 30 °C in the dark; the resulting solutions of stained bacteria are referred to as samples I. The concentration of bacteria in each solution I was measured using the absorption value at 600 nm (Nanodrop 2000). In addition, we stained samples of water (J), milk (K) and egg whites (L) with 2.5 µM Syto 85. Water (J) and milk (K) samples were stained directly as described above. Egg whites (L) were first diluted at a volume ratio 1:1 with deionized water, then vortexed and filtered with a 100 µm filter (BD). The filtrate was centrifuged at 4300 rpm for five minutes and the pellet was reconstituted with water at the same volume of the original egg white sample (L). We now prepared mixtures (M) of stained bacteria (I) with the respective stained products (J, K, L) at different mass fractions. Mass fractions were determined using a scale (Mettler Toledo). To optically measure M using our detectors, 360 µl of a stained sample mixture M were placed in a round 6.50 mm glass tube (Durham Culture Tubes 6.50). All volumes were confirmed by weighing the samples (Mettler Toledo). A negative, buffer-only control was run in parallel and measured in the detectors.

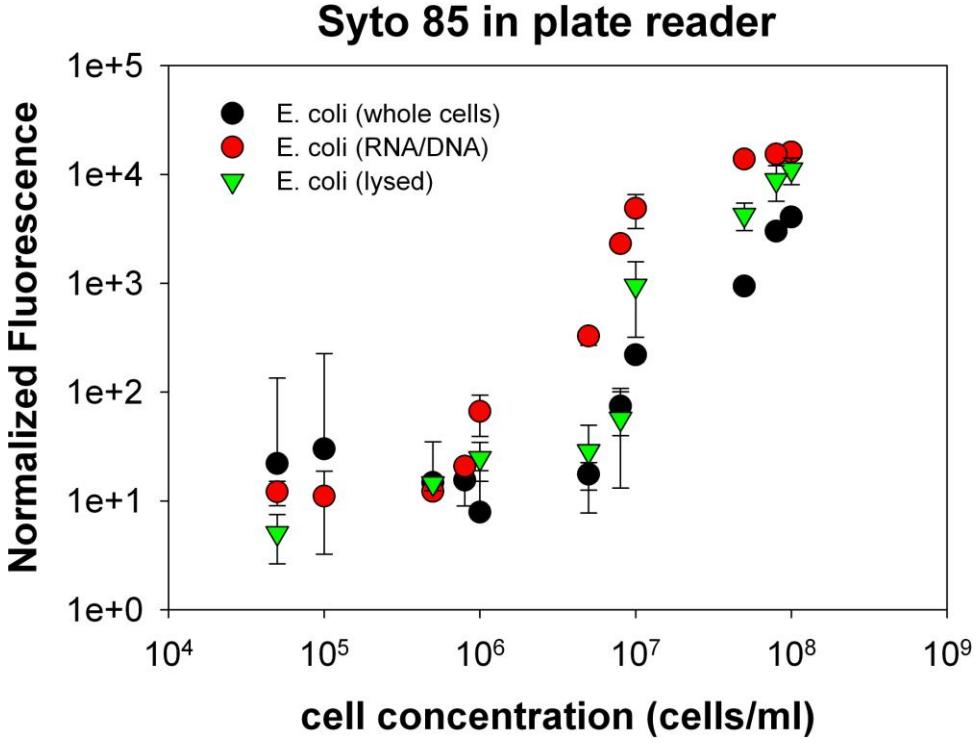
F. Sample preparation for the malaria model of yeast in red blood cells

Baker's yeast (2.86 Mio yeast cells/ml in distilled water) was stained with 5 µM Syto 85 (Invitrogen Cat. No. S11366) in deionized water for 5-60 minutes protected from light. After centrifugation, the bacteria were reconstituted with an equi-volume amount of water in 0.5 g/ml sucrose (yielding solution N). The concentration of bacteria of the resulting solution, N, was measured using the absorption value at 600 nm (Nanodrop 2000). The same procedure was used to stain 2.86 Mio. cells/ml bovine red blood cells (Lampire Biologicals #7240807) in sucrose-water, yielding stained solution O. After cell staining, mixtures P containing the components N and O at different mass fractions were prepared utilizing a scale (Mettler Toledo). For the measurement in our device, 360 µl of a stained sample mixture P (prepared above) was placed in a round 6.50 mm glass tube (Durham Culture Tubes 6.50). The volumes were confirmed by weighing the samples (Mettler Toledo). A negative, buffer-only control was run in parallel.

G. Dye Optimization

In order to lower the detection limit for bacterial detection we optimized dye staining. We expected the DNA to be more easily accessible to the dye in lysed compared with whole cells. We therefore lysed the cells and compared detection limits of different dyes with each other.

Cells were *lysed* and stained with *Syto85*. In the below figure whole E.coli cells, lysed E.coli cells, as well as E.coli RNA/DNA mixtures were tested on a plate reader (which was shown to have a similar sensitivity to the introduced device, see above). It was shown that lysing E.coli cells improved the detection limit by approximately half an order of magnitude to about $7 \cdot 10^5$ CFU/ml (further testing required to confirm precise numbers). There was no significant difference between lysed E.coli cells and purified DNA/RNA mixtures, which suggests that optimizing lysis conditions would not lower the detection limit significantly further.

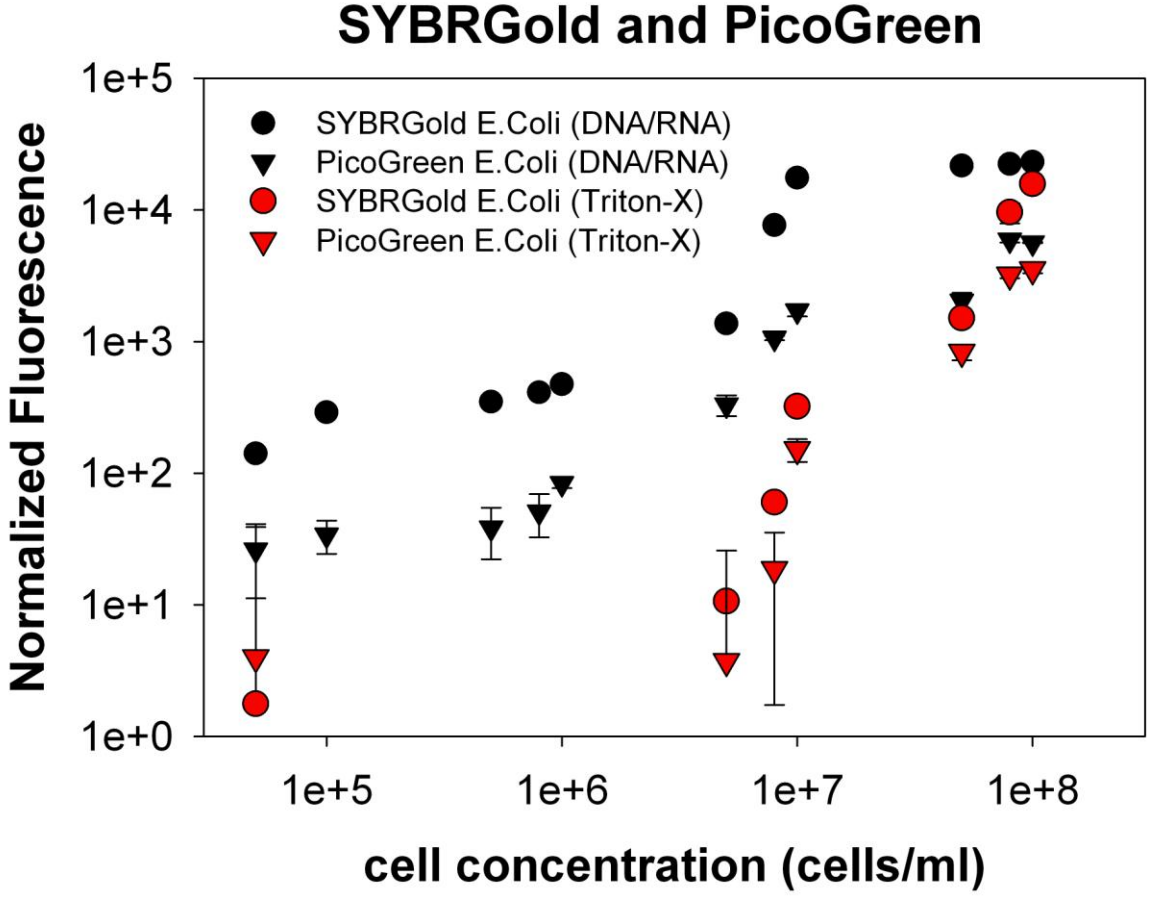


As a next step, we tested *Sytox Orange*, a dye having similar optical spectra as Syto 85 and therefore allowed the use of the current optical device setup.

Figure 8 in the paper shows that the detection sensitivity could be improved to 10^4 CFU/ml from ~ 10^6 CFU/ml, using Sytox Orange compared with Syto 85 in whole cells. Sytox Orange hence yields a lower detection limit than Syto 85. The fact that the purified RNA/DNA mixtures in Fig. 8 overlapped with the lysed cells further suggests that optimizing lysis conditions for Sytox Orange will not significantly improve the detection limit.

Sytox Orange was chosen as a dye, because it has similar excitation and emission spectra as Syto 85 and therefore did not require changes to the current device design. Changing the device design may allow us to further improve dye staining and therefore further lower the detection limit. As an additional test, we therefore tested the dyes Pico Green and SYBRGold, which would require different excitation LEDs than the current device. As a quick test to estimate detection limits we used a plate reader.

The below graph shows the results of lysed cells and DNA/RNA mixtures stained with SYBR Gold and Pico Green in the plate reader. The results suggest that the detection limit may be lowered to about 10^5 – 10^6 cells/ml using Pico Green or SYBR Gold. Because this limit is not lower than the achieved detection limit of Sytox Orange (10^4 CFU/ml), changing the device characteristics to match either of the two dyes is not necessary.



Dye Optimization Protocols:

Cell Lysis:
Initially we tested lysis conditions for E.coli and compared lysis protocols with lysozyme, Triton-X 100 and CelLytic B with each other. For all tested organisms, CelLytic B (bacteria) and CelLytic Y (yeast) led to higher lysis efficiencies compared with most other methods. They were therefore used, without further optimization, unless otherwise noted.

For the Triton-X lysis, E.coli cells were lysed by incubation in a Triton-X 100 (Sigma, X100-5ML) 1x buffer solution in TE (Sigma, 93283-100ML) for 20 minutes at 37 °C in a shaking incubator. They were then put through one freeze-thaw cycle in a -80 °C freezer and were allowed to lyse for another 20 minutes in a shaking incubator afterwards. For lysozyme lysis, the protocol for lysozyme from chicken egg white (L7651) from Sigma Aldrich was followed. For CellLytic B and Y we used the protocol provided by Sigma Aldrich.

For E.coli cells, we achieved higher lysis efficiencies using CelLytic B and Triton-X than with lysozyme lysis. For E.coli lysis we achieved comparable yields with Triton-X and CelLytic B. Salmonella, E.Coli and Cholera were therefore lysed using CelLytic B (Sigma, B7435-50ML) and yeast cells were lysed using CelLytic Y Plus (Sigma, C4482-50ML).

Syto-85 (with lysed cells)
Cells were lysed using CelLytic reagents and above protocols. They were then stained with 2.5 µM Syto 85 (Life Technologies) in TE buffer for 5 minutes (see earlier).

Sytox Orange
Cells were lysed and stained with 0.1 µM Sytox Orange (Life Technologies) in TE-buffer. As a control, we used purified E.Coli DNA (Affymetrix, 14380) and RNA (Affymetrix, 21185, 100 gm). After vortexing, they were incubated for 5 minutes and results were read out with a plate reader or the device introduced in this paper.

Pico Green
On the day of the experiment, an aqueous working solution of the Quant-iT™ Pico Green reagent (Life Technologies) was prepared by making a 200-fold dilution of the concentrated DMSO solution in TE. 50 µl samples of DNA at different concentrations, as well as a buffer control were put in a 96 well-plate. 50 µl Pico Green reagent were added to it and read out in a plate reader after 2 minutes of incubation (ex. 495/537 nm).

SYBRGold
On the day of the experiment, an aqueous working solution of the SYBRGold reagent (Life Technologies) was prepared by making a 1000-fold dilution of the concentrated DMSO solution in TE buffer. 50 µl samples of DNA at different concentrations, as well as buffer control were put in a 96 well-plate. 50 µl SYBRGold reagents were added to it and read out in a plate reader after 4 minutes of incubation (ex. 502/532 nm).

H. Detection Limit Analysis

The detection limit (LOD) was calculated based on Kaiser's criterion, which defines the LOD as the average of the blank plus three times the standard deviation of the blank.

$$y_{LOD} = y_{blank} + 3\delta_{blank}$$

I. Device Design

The detailed device design is documented in Lu, P., Hoehl, M., Macarthur J.B., Sims P.A., Slocum A.H., Rugged low-cost multisample, multi-wavelength UV/vis absorption and fluorescence detector, http://arxiv.org/abs/1205.6571.