

Supplementary Information

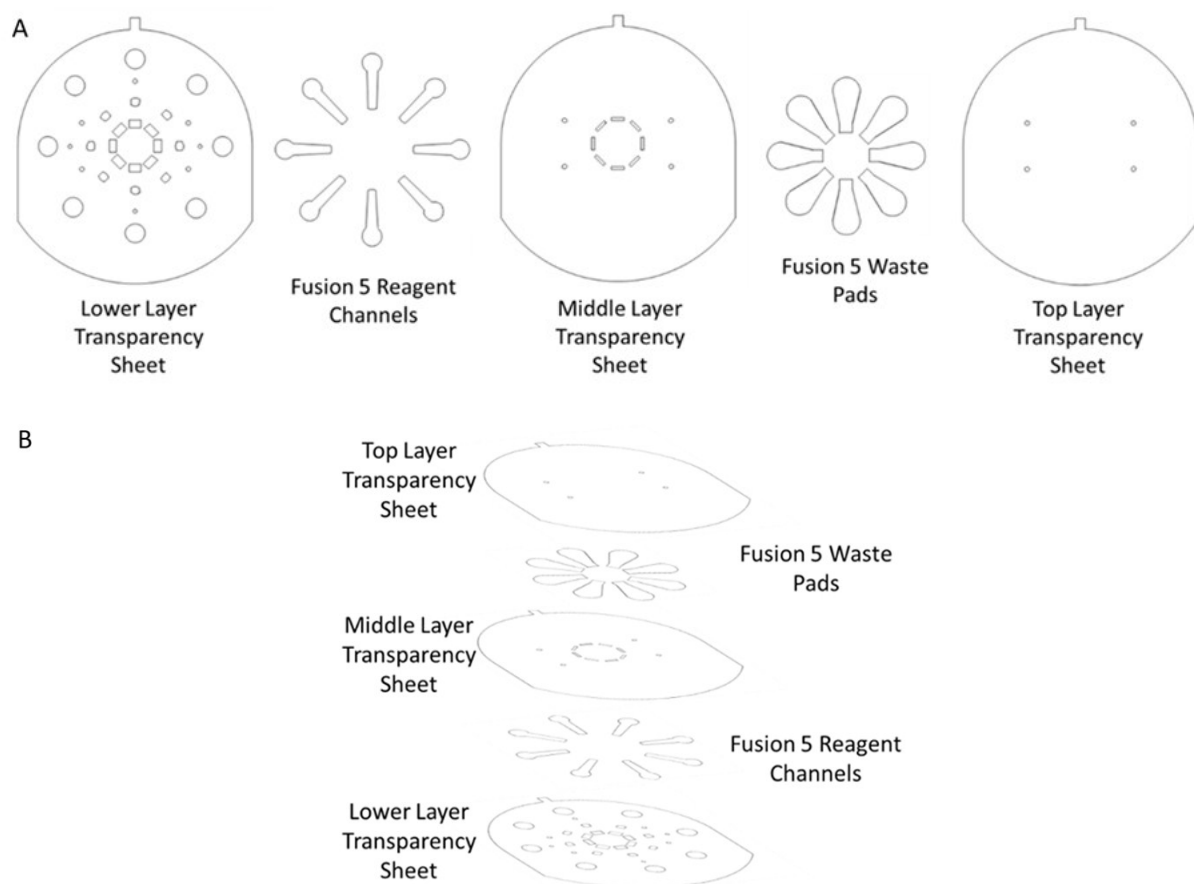


Figure S1: Designs for the different layers of transparency and paper pieces that make up the reagent card (A) and their stacking orientation before lamination (B). The lower transparency sheet is coated with a hydrophobic coating called NeverWet from Rust-Oleum.

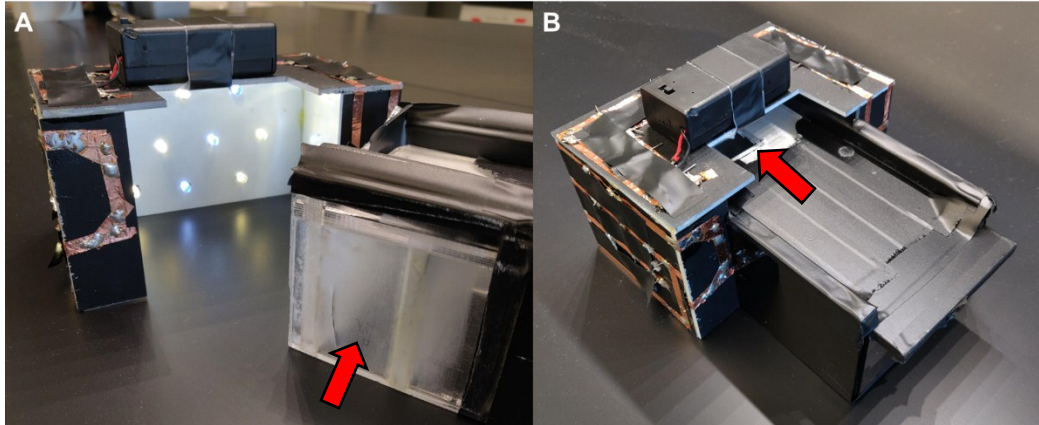


Figure S2: Light box opened to show lighting inside. Red arrow highlighting area where sample layer sits (A). Light box put together - red arrow highlighting the hole used to take a picture with a smartphone (B).

Video 1: In the included video three food coloring dyes are dried on different reagent layers. When the device is assembled, the buffer washes the dye from the reagent layer, through the sample layer and into the waste pad. The video shows the food coloring washing into the waste pad. The order of colors is Yellow, green, and purple and the video is sped up 8x. The nut that is placed on the device was used to hold the sample layer insert in place. Further iterations of the device did not require the nut, but the new devices made it more difficult to visualize the flow of food coloring into the waste pad. The video also shows how different sized waste pads could be incorporated into the device to increase or decrease the volume of washing buffer.

Image processing algorithm: The image processing employs a standard flood-fill algorithm available in the OpenCV computer vision library (see citation #45 in main paper). Flood-fill is an example of a region segmentation algorithm. As used here, flood-fill finds a single contiguous region of pixels grown out from a seed pixel known to be in our sample. This is typically the center pixel in the image. Flood-fill starts with the seed and then recursively adds adjacent pixels to the region until no similar adjacent pixels are left to include. Because there is a distinct border around the color sample, at which the addition of pixels stops, the resulting region nicely contains the pixels from the sample and does not include extraneous pixels of the border or from elsewhere in the image. The precise settings used for flood-fill are captured in the following example of our call to the OpenCV algorithm:

```
cv2.floodFill(image, mask, seed, (255, 0, 0), (4,4,4), (4,4,4), floodflags)
```

The above line of the floodFill function call takes the following inputs:

1. RGB image of the sample layer.
2. Mask is a pixel padding added to the four sides of the image.
3. Seed represents the starting point of the floodfill algorithm.
4. The fourth parameter represents the color by which the flood-filled region needs to be repainted.

- The next two parameters represent the lower and upper color difference between the current pixel and its neighboring pixel. As we are dealing with RGB space, so we have 3 values representing threshold for each of the color space.
- The final parameter defines the connectivity value that is the number of pixels to be considered around the current pixel.

The blue masked region in Figure S3 shows the region of a sample layer that was analyzed with the flood-fill algorithm. The automated flood-fill algorithm was compared to the analysis performed with NIH ImageJ (Fig. S3). When plotted against each other, the manual and automated analysis are nearly identical with a slope of 0.986 ± 0.014 and an r^2 of 0.99996. In addition to reducing manual labor, the automated analysis can discard pixels that are inconsistent with neighboring pixels due to glare, increasing analysis accuracy.

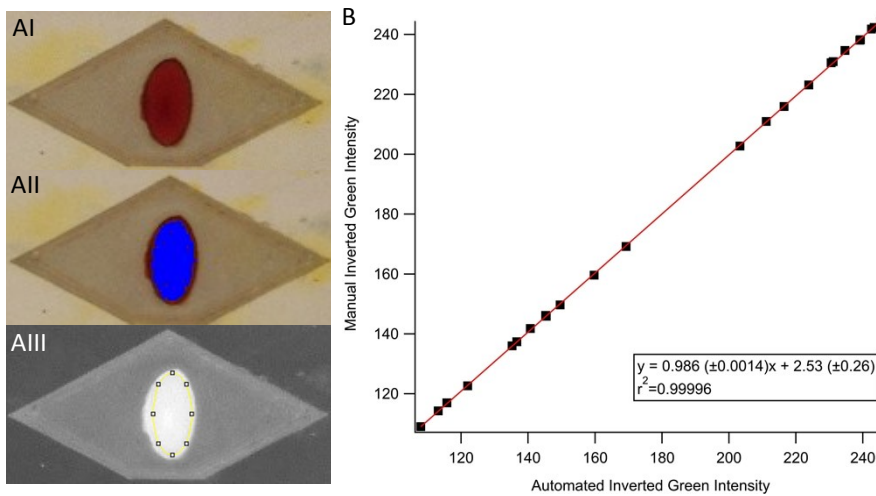


Figure S3: Original image (AI), area of original image analyzed by flood fill algorithm (AII), manual analysis of one-color channel. Inverted green intensity found using a manual analysis vs the inverted green intensity found with the automated algorithm.

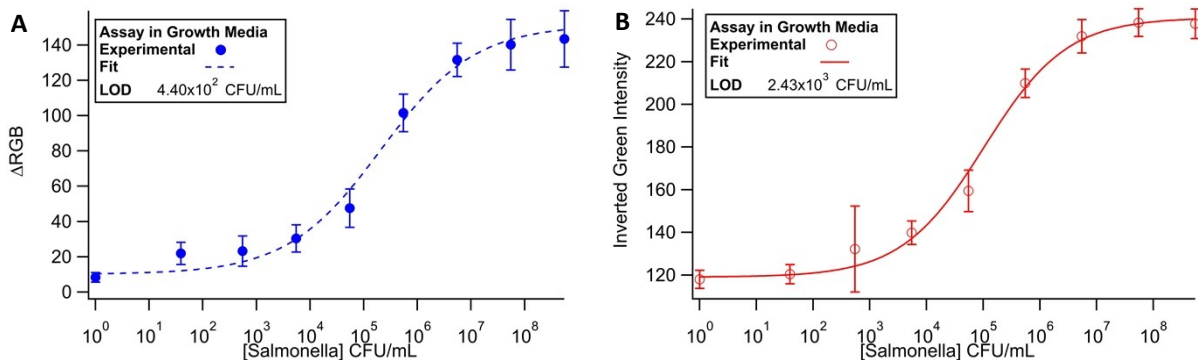


Figure S4: Dose response curves created using ΔRGB (A) and inverted green (B).

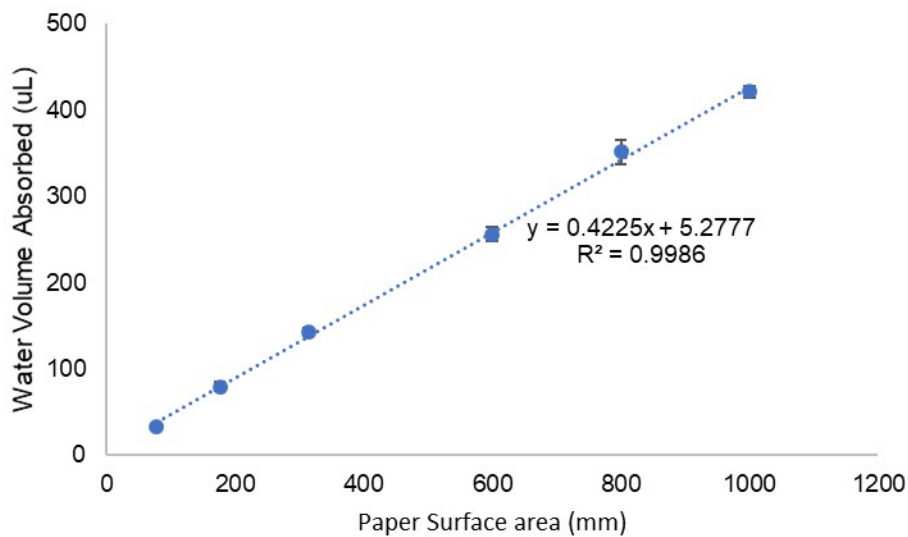


Figure S5: Buffer volume/mm² absorbed by Fusion 5

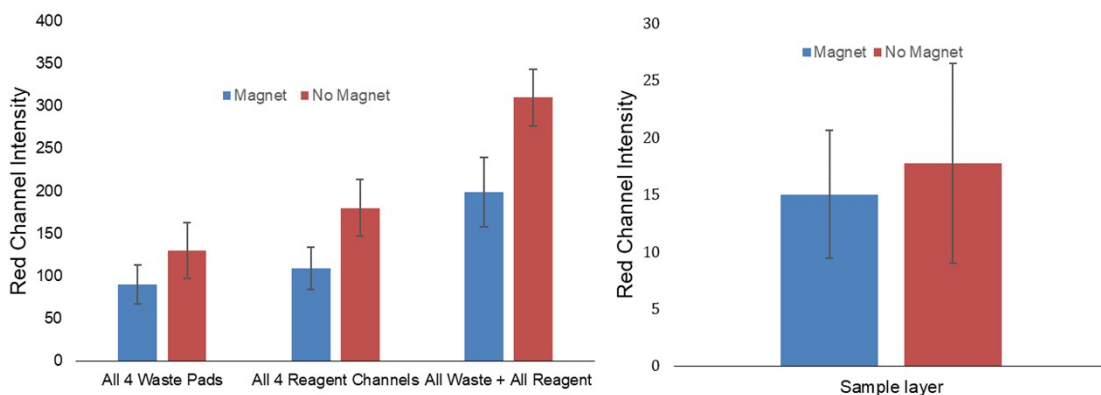


Figure S6: Magnetic bead retention with and without a magnet. A) Fluorescent intensity of sample layer before and after 4 washes in the device. B) Summed fluorescent intensities of the reagent layer and waste pads used for washing.

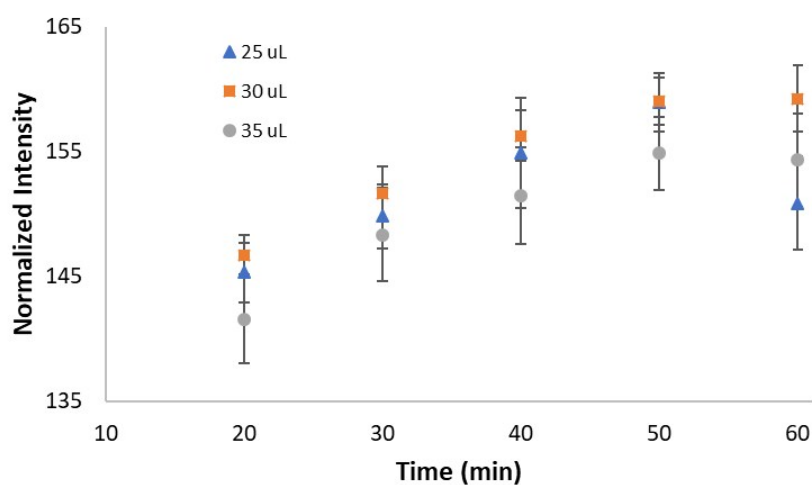
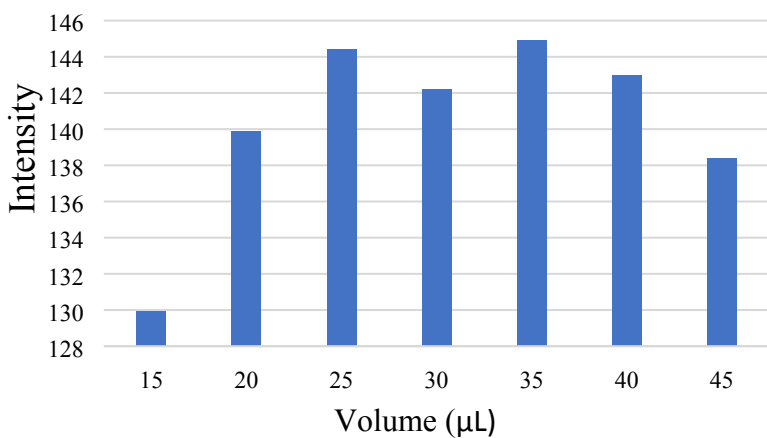


Figure S7: CPRG volume (a) and time(b) optimization. CPRG concentration was 2.5 mM and 0.2 µg of Streptavidin β-galactosidase was reacted on each sample layer. The volume of CPRG and reaction time were varied.

Table S1

	Ambient Light	Light Box
Image 1: Fluorescent light	111.1	126.2
Image 2: Traditional bulb	163.1	126.1
Image 3: Direct sunlight	158.2	133.5
Average	144.1	128.5
SD	28.7	4.2
%RSD	19.9%	3.3%

Inverted green intensity of the same sample layer in multiple ambient light environments inside and outside the light box

$$Signal = d + \frac{a - d}{1 + \left(\frac{T}{c}\right)^b}$$

Equation S1: Signal is the intensity of the colored sample layer, T is the target (Salmonella) concentration, and a – d are the 4 parameters defining the shape of the curve. The parameter a is the expected signal at T=0, b is the slope of the line at the center/steepest part of the curve, c is the target concentration at the center of the curve, and d is the maximum signal, i.e. when T is infinitely high. The coefficients assigned to the curve in Figure 5 are:

$$a=10.12 \pm 2.7; b=0.496 \pm 0.116; c=2.10 \pm 1.45 \times 10^5 \pm 0.001; d=151.42 \pm 14.1$$

The coefficients from the curve in figure 6 are:

$$a=10.413 \pm 8.46; b=0.32644 \pm 0.138; c=3.4086e+005 \pm 5.4e+005 d=173.04 \pm 37.2$$