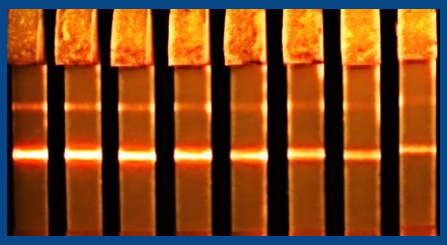
SEB QUANTUM DOT LATERAL FLOW

A 15-minute fluorescent strip test for staphylococcal enterotoxin B

How the strip works, how it compares with colloidal gold, and what it detects in chicken rinsate and whole milk, measured from the original reader images.



SEB strips under UV light: the lower (test) line glows red in proportion to SEB

10 ng/mL
SEB in chicken rinsate
clear test line, in-well concentration

78 ng/mL
SEB in whole milk
clear test line, in-well concentration

≥ 5-fold
less SEB than gold
quantum dots vs colloidal gold in buffer

15 min
time to result
read by eye under UV or on a reader

Why a faster SEB test

Staphylococcal enterotoxin B (SEB) is one of the toxins that *Staphylococcus aureus* releases into food. It survives cooking, and small amounts can cause vomiting and cramps within hours. Food-testing laboratories commonly use ELISA or latex agglutination to detect it. ELISA is sensitive but takes hours and a plate reader; agglutination is quicker but less sensitive.

Lateral flow strips give an answer in minutes without an instrument, but most use colloidal gold, whose red-purple line fades from view at low toxin levels. We built an SEB strip that reports with red-fluorescent quantum dots instead. Under ultraviolet (UV) light the quantum dot line glows against a dark membrane, so a smaller amount of SEB gives a line you can still see or measure.

This article summarizes that work, published as Ford, Sutton, Mitchell and Bruno, *Journal of Fluorescence* (2025), and re-examines the original strip images with one consistent measurement so that the numbers can be compared directly.

How the strip works

The test is a sandwich immunoassay. A mouse monoclonal antibody against SEB is biotinylated and bound to streptavidin-coated red quantum dots (CdSe/ZnS, about 650 nm emission). This detection premix is dried onto the conjugate pad. When the sample flows up the strip, SEB in the sample is bound by the detection antibody, and the complex is caught at the test line by a second anti-SEB antibody. Goat anti-mouse IgG at the control line catches the detection antibody whether or not SEB is present, so a control line shows that the strip ran (Figure 1).

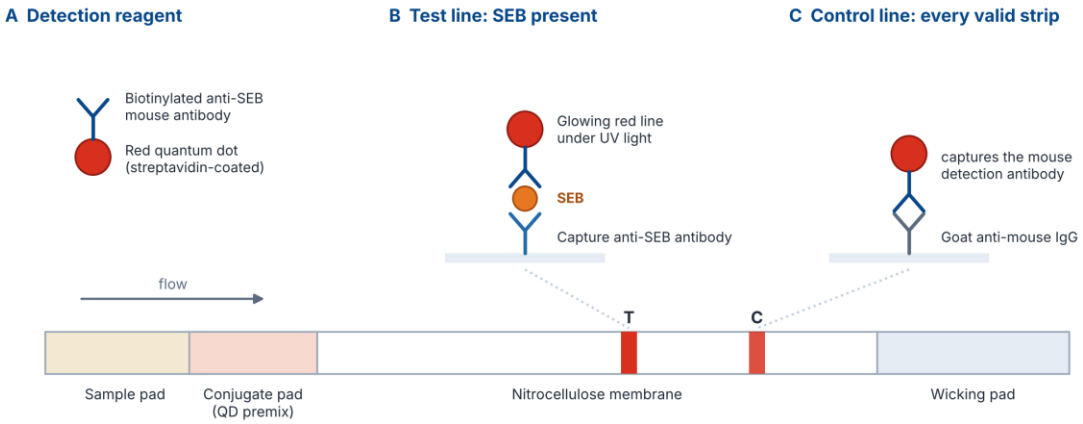


Figure 1. *The SEB quantum dot strip (diagram). (A) Detection reagent: biotinylated anti-SEB mouse antibody on a streptavidin-coated red quantum dot. (B) With SEB present, the toxin bridges the detection antibody to the capture antibody at the test line (T). (C) Goat anti-mouse IgG at the control line (C) captures the detection reagent on every strip that runs correctly.*

Quantum dots against colloidal gold

To compare reporters, we titrated SEB toxoid in running buffer on strips that used the same detection antibody, once with colloidal gold and once with the quantum dot premix (Figure 2). Each strip received a smaller amount (roughly two-fold steps) of a 1 mg/mL toxoid stock in 150 µL of running buffer; concentrations below are SEB toxoid per milliliter of running buffer.

- **Colloidal gold:** the test line was faint at 0.008 µL of stock (53 ng/mL) and barely visible at 0.004 µL (27 ng/mL).
- **Quantum dots:** the test line was still clearly visible at 0.0008 µL (5.3 ng/mL), the smallest amount tested.

The quantum dot strip therefore detected at least 5-fold less SEB than the gold strip. Because the series stopped while the line was still visible, the true difference may be larger. The two titrations were run separately, several months apart, so this is a comparison of two runs rather than a side-by-side test.

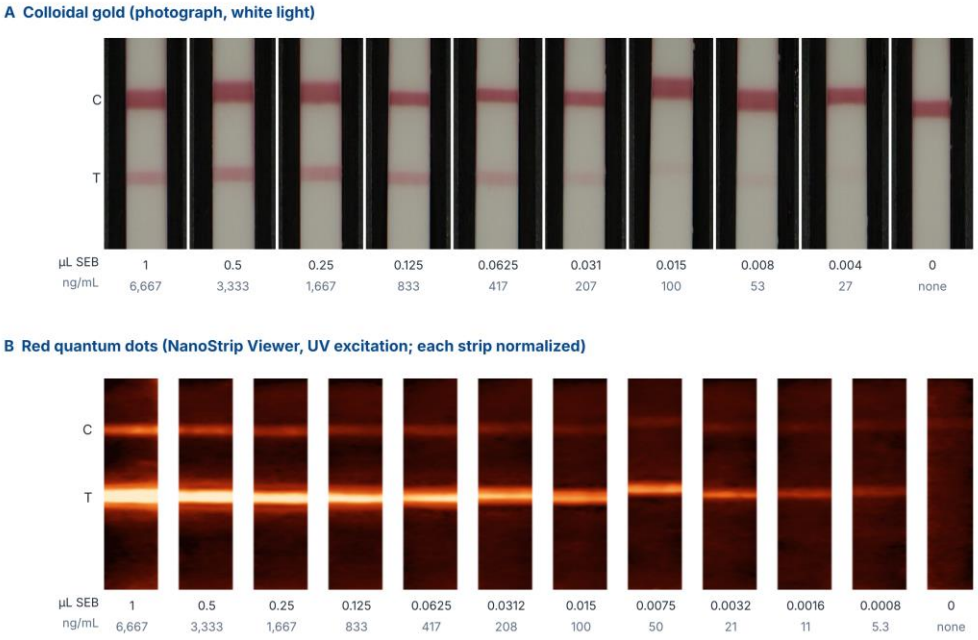


Figure 2. *SEB toxoid titrated in 150 µL of running buffer, one strip per amount, read at 15 minutes. (A) Colloidal gold conjugate of the anti-SEB detection antibody, photographed under white light. (B) Quantum dot premix dried on the conjugate pad, imaged in the NanoStrip Viewer under UV excitation. Because the reader did not illuminate the tray evenly, each strip was divided by its own membrane background before display, and all strips were then shown with one shared false-color scale. Concentrations are ng of SEB toxoid per mL of running buffer.*

Performance in chicken rinsate and whole milk

Food samples bring fat, protein and particles that can slow flow, raise background and quench fluorescence. We spiked SEB toxoid into chicken rinsate (sterile deionized water massaged over raw chicken thighs) and into whole milk. Each sample was mixed with running buffer in the test well, and the series ran in two-fold steps from 5,000 ng/mL. Concentrations are final concentrations in the test well. Strips were imaged in the NanoStrip Viewer after 15 minutes (Figure 3A and 3B).

To put numbers on what the eye sees, we measured the test line on every strip from the saved reader images in the same way: the brightness at the test line above a straight baseline fitted to the membrane on either side, expressed as a percentage of that baseline (Figure 3C). We count a line as **clear** when it stands at least 5% above background; strips without SEB read −1.8% (rinsate) and −0.3% (milk).

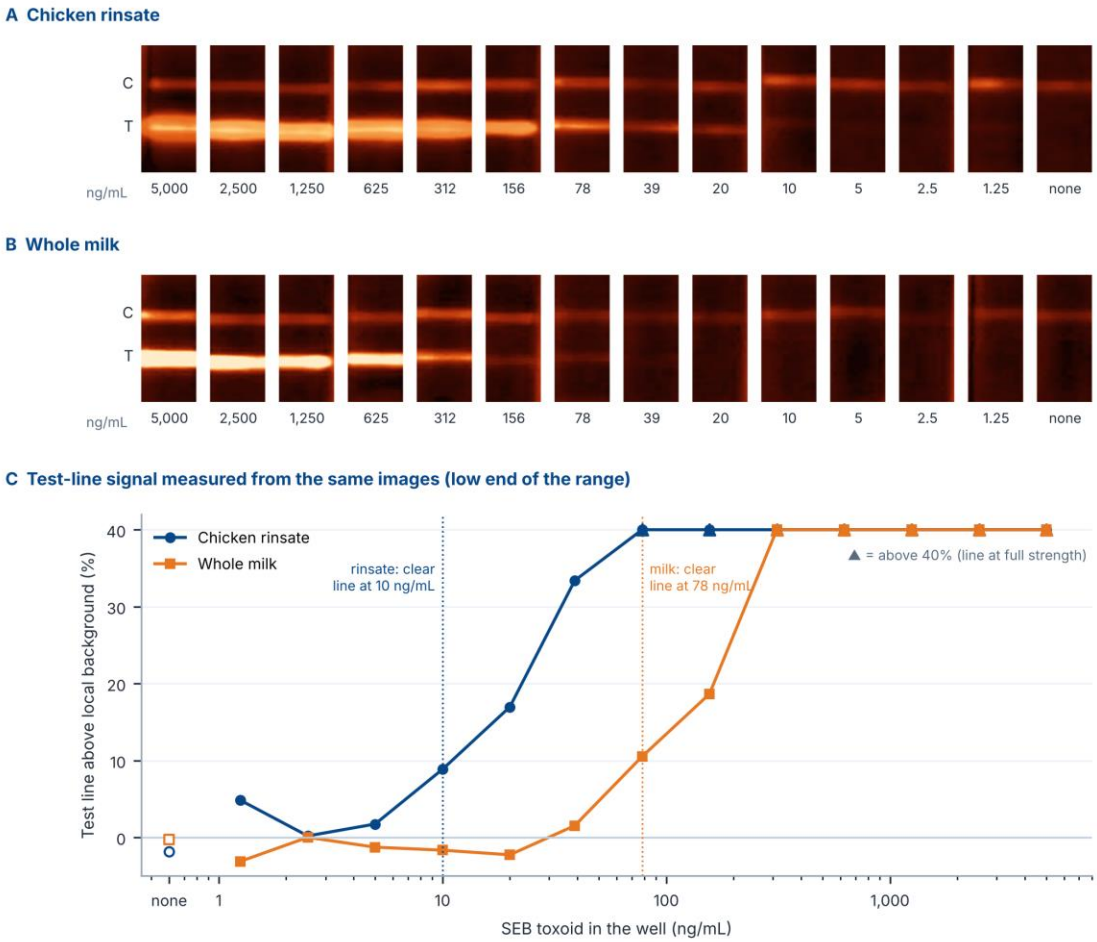


Figure 3. *SEB toxoid spiked into (A) chicken rinsate and (B) whole milk, one cassette per concentration, imaged in the NanoStrip Viewer at 15 minutes. Each strip was divided by its own membrane background to correct uneven illumination across the reader tray, then all strips were shown with one shared false-color scale. (C) Test-line signal measured from the same images as percentage above local background, plotted for the low end of the range; triangles mark strips above 40%. Open symbols: no-SEB strips. Dotted lines: lowest concentration with a clear line.*

Sample	Clear test line	Trace only	No line
Chicken rinsate	10 ng/mL and above (9% above background at 10 ng/mL)	5 ng/mL (2%)	2.5 ng/mL (0%); no-SEB strip
Whole milk	78 ng/mL and above (11% above background at 78 ng/mL)	39 ng/mL (2%)	20 ng/mL and below; no-SEB strip

Table 1. *Detection in food samples, from the measured test-line signal in Figure 3C. Concentrations are final ng of SEB toxoid per mL in the test well, after mixing with running buffer. One strip per concentration.*

Whole milk needed about eight times more SEB than chicken rinsate for a clear line, consistent with milk fat and casein interfering with flow or fluorescence. Above about 80 ng/mL in rinsate and 300 ng/mL in milk the test line was at full strength, so the strip reads presence and rough amount rather than an exact concentration at high levels.

A note on the lowest rinsate strip. The single 1.25 ng/mL rinsate strip read 4.8% above background, but the 2.5 ng/mL and 5 ng/mL strips beside it read 0.2% and 1.7%. Because the signal does not follow the dilution series, we do not count it as detection here. The visual limit of 1.25 ng/mL in the published paper rests on this strip; replicate strips at 1 to 10 ng/mL would settle where the limit lies.

Specificity against other biological toxins

A test intended for food and biodefense samples must not light up for other toxins. We ran strips with 1 µL of toxoid stock of SEB, botulinum neurotoxin A, botulinum neurotoxin B, abrin and ricin, and with tetrodotoxin and saxitoxin as BSA conjugates at 75 ng/mL in the well. Only SEB produced a test line; the control line formed on every strip (Figure 4, Table 2).

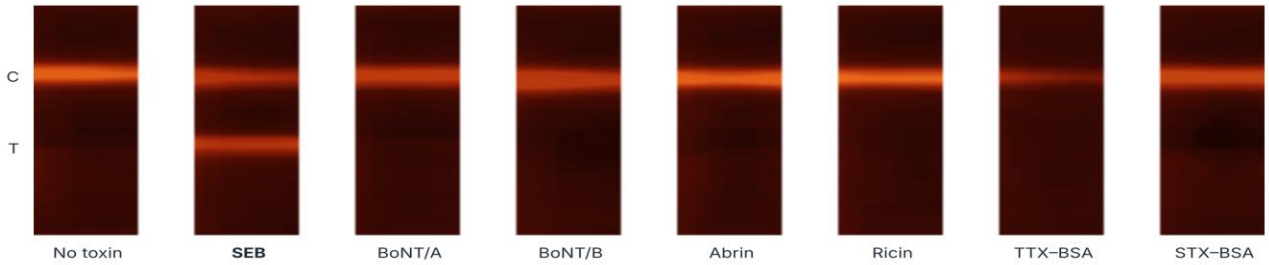


Figure 4. *Specificity. One cassette per toxin, imaged in the NanoStrip Viewer at 15 minutes. Toxoids were added as 1 µL of stock; tetrodotoxin (TTX) and saxitoxin (STX) were added as BSA conjugates at 75 ng/mL. Each strip was normalized to its own membrane background and shown on the same false-color scale as Figures 2B and 3. BoNT, botulinum neurotoxin.*

Sample	Test line (reader units)	Control line (reader units)
No toxin	0	13,614
SEB toxoid	10,840	11,494
BoNT/A toxoid	0	15,451
BoNT/B toxoid	0	17,425
Abrin toxoid	0	16,481
Ricin toxoid	0	13,030
Tetrodotoxin–BSA	0	3,813
Saxitoxin–BSA	0	18,292

Table 2. *NanoStrip Viewer readings for the strips in Figure 4, recorded 15 minutes after the cassettes were placed in the reader.*

Reading the strips

The quantum dot line can be seen by eye under a handheld UV lamp. For numbers, the strips were read on the NanoStrip Viewer, a camera-based fluorescence reader built with our partner Nanohmics. It images 2 to 12 strips or up to 5 cassettes at once, which makes it practical for titrations and side-by-side comparisons.

- **Excitation:** 370 nm UV LEDs for quantum dots and europium chelates; 505 nm cyan LEDs for phycoerythrin.
- **Detection:** a single 550 nm long-pass filter and a 1920 × 1200 pixel camera with a low-distortion lens.
- **Analysis:** software crops each strip, builds a line profile, and reports test and control line intensities; the raw image is saved so results can be re-analyzed later, as we did for Figure 3.

What these results do and do not show

- **One strip per concentration.** The detection limits above come from single titration series. Replicates are needed for a formal limit of detection.
- **Toxoid, not active toxin.** All work used recombinant SEB toxoid (BEI Resources NR-10049), which anti-SEB antibodies recognize but which is non-toxic.
- **Spiked samples.** Rinsate and milk were spiked in the laboratory; naturally contaminated food was not tested.
- **Related enterotoxins not yet tested.** Cross-reactivity with staphylococcal enterotoxins A, C, D and E has not been measured.
- **Research use.** The strips are for research use only and are not validated for food-safety or diagnostic decisions.

Methods at a glance

Component	Details
Detection reagent	Biotinylated anti-SEB mouse monoclonal antibody (Meridian Bioscience C86400M) on streptavidin-coated red CdSe/ZnS quantum dots; biotinylated with an Attogene antibody biotinylation kit and desalted on Attogene desalting columns
Test line	Anti-SEB capture monoclonal antibody (Meridian Bioscience C86220M)
Control line	Goat anti-mouse IgG (titrations)
Strip format	4.5 mm strips in Attogene lateral flow cassettes (matrix and specificity tests) or as dipsticks (buffer titrations)
SEB	Recombinant SEB toxoid, BEI Resources NR-10049, 1 mg/mL stock
Buffer titrations	150 µL of Attogene running buffer per test
Food samples	Chicken rinsate or whole milk mixed with Attogene running buffer in the test well
Read	15 minutes; by eye under UV light and on the NanoStrip Viewer
Image display	Reader images segmented into individual strips; each strip divided by a smooth fit to its own membrane background (line regions excluded), then shown on one shared false-color scale. Line measurements in Figure 3C use the same per-strip background.

Table 3. *Assay components and conditions. Full methods are in the published paper.*

Full study

Ford LP, Sutton SB, Mitchell DR, Bruno JG. Ultrasensitive staphylococcal enterotoxin B quantum dot-based lateral flow immunoassays for rapid testing of poultry rinsates and milk. *Journal of Fluorescence* (2025). https://doi.org/10.1007/s10895-025-04526-1

This article re-analyzes the study's original strip images. Where its numbers differ from the paper (the chicken rinsate limit and the gold comparison), the text above explains why.

Acknowledgments. This work was funded by the U.S. Department of Homeland Security under SBIR Phase II contract 70RWMD23C00000005. The following reagent was obtained through BEI Resources, NIAID, NIH: Staphylococcal Enterotoxin B Toxoid, Recombinant from Escherichia coli, NR-10049. The NanoStrip Viewer was designed and built by Nanohmics, Inc. (Austin, TX).

Related products

Catalog no.	Product
AU2048, AU2048-01	SEB Lateral Flow Test Kits
CO2028	Protein/Antibody Biotinylation Kit
CO2027	Desalting Columns
CO2022	Lateral Flow Cassettes
AU2054	Blank 4.5 mm Lateral Flow Strips

Table 4. *Attogene products used in or related to this work. Quantum dot, europium and phycoerythrin versions of SEB and other toxin strips are available through custom development.*

Custom lateral flow development

Need a toxin, pathogen or residue test with a fluorescent readout? The team behind this strip offers feasibility studies, antibody screening and conjugation, reporter selection (gold, quantum dots, europium, phycoerythrin), multiplex design, reader integration and manufacturing scale-up.

Attogene Corporation
3913 Todd Lane, Suite 310
Austin, TX 78744
512-333-1330
sales@attogene.com
www.attogene.com