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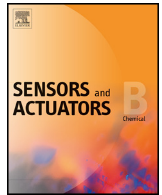
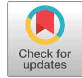


# Low cost bioluminescence imaging as an alternative to *in vivo* bioassays for quantifying biologically active staphylococcal enterotoxin type E



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ABSTRACT

*Staphylococcus aureus* is a major causative agent implicated in outbreaks of food poisoning. It acts through the production of a range of toxins including staphylococcal enterotoxin type E which has been associated with foodborne outbreaks in the USA, UK and France. While tests such as ELISA exist to detect the toxin's molecules, existing methods to distinguish active toxin from inactivated are costly and require the use of live animal testing. In this study we constructed and evaluated a low cost CCD camera device in conjunction with a cell based assay for active SEE employing a genetically engineered T-cell line with a luciferase reporter regulated by nuclear factor of activated T-cells combined with a B-cell line for toxin presentation. The emitted light intensity from the T-cells is proportional to SEE concentration over an 8-log range and can discern the active form of toxin that sickens consumers, from toxin inactivated by heat treatment. The assay was verified in sample food matrices by spiking white grape and peach mango juices as well as apple cider. The low cost imaging device compared favorably against the expensive commercial luminometer plate reader using the same cell based assay. The low cost camera device should improve the availability of active toxin testing, especially in resource poor regions.

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## 1. Introduction

*Staphylococcus aureus* is an important and frequent clinical and foodborne human pathogen [1]. It synthesizes more than two dozen staphylococcal enterotoxins (SEs) and produces emetic and immune responses in its victims by interaction with the gastrointestinal tract and through activation of the immune response. These modes of action appear to be related, though the connection between the mechanisms has not been elucidated. Loss of superantigen activity has been shown to be correlated with loss in enterotoxicity through mutation studies [2,3,4]. Activation of the immune response begins with the binding of SEs by the major histocompatibility complex (MHC) class II expressed on the surface of antigen presenting cells (APC). Once bound, the molecule is presented to T-cells [5] which also bear receptors that bind and recognize SEs via specific sequences in the TcR Vβ chain domains. Massive activation of the immune system ensues with roughly 20 percent of the naïve T-cell population being stimulated [6], resulting in T-cell proliferation [7], secretion of cytokines including interferon-γ (IFN-γ) [8] and tumor necrosis factor (TNF) [9] and expression of the receptor CD154 on the surface of CD4$^+$ T cells [10].

Staphylococcal enterotoxin E (SEE) poses a serious risk to public health and safety and was detected in the blood of patients with rheumatoid arthritis [11]. It has also been associated with foodborne outbreaks in the UK [12], France [13], and the USA [14]. One study found SEE in 18.4% of instances of animal origin food contaminated with SEE; the second most prevalent subtype [15]. Previous methods for the detection of active SEE focus on the emetic effect of the toxin and involve the administration of toxin laden sample to live monkeys or kittens either by gavage or intravenously [14,16,17,18,19]. The toxin can also be detected chemically by enzyme-linked immunosorbent assay (ELISA), typically by using microplate reader, or by mass spectrometry (MS) [19,20], but those methods are unable to discern active toxin from inactivated toxin. This is important because food that has been treated or processed to reduce or eliminate toxin by inactivation may still test positive with these non-mechanistic assays. The monkey and kitten assays have the drawback that they are expensive to perform and require the maintenance of a supply of live animals. Additionally, there are ethical and regulatory concerns against the use of animals in

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toxicity testing. The recent passage of the Lautenberg Chemical Safety Act, promotes the development and use of alternatives to animal testing for chemical toxicity methodologies.

We present here sensitive assay for quantifying biologically active staphylococcal enterotoxins. This alternate *in vitro* approach uses low cost imaging technology to take advantage of a cell based chemilumiscence bioassay for detecting SEE. This assay utilizes SEE's stimulation of genetically engineered Jurkat T cell-line, combined with a Raji B-cell line that presents the SEE to the engineered T cell line. We demonstrate the effectiveness of a cooled camera system to quantify the light emitted by the T cell expressing the luciferase reporting gene.

## 2. Materials and methods

### 2.1. Materials

The materials for fabricating analysis plates, i.e. 1/8" thick black polymethacylate (PMMA), thin polycarbonate sheet, and 3 M$^{TM}$ 9770 double sided adhesive transfer tape, were obtained from Piedmont Plastics, Inc., Beltsville, MD. SEE toxin was purchased from Toxin Technology, Sarasota, FL. Inactivated SEE was prepared by autoclaving for 30 min at 121 °C. Media reagents; RPMI 1640, fetal calf serum (FCS), MEM non essential amino acids, sodium pyruvate, hygromycin B, and penicillin/streptomycin, were purchased from Gibco/Invitrogen, Carlsbad, CA. Bio-Glo reagent was obtained from Promega Sunnyvale, CA.

### 2.2. Cell lines and culture

Raji B cell line (Burkitt's lymphoma ATCC #CCL-86) was obtained from the American Type Culture Collection, Rockville, MD. A Jurkat cell line (T cell leukemia) genetically modified for stable expression of the luciferase reporter gene under control of NFAT response element was obtained from Promega, Sunnyvale, CA. RPMI 1640 containing 10% FCS, 1% MEM non essential amino acids and 100 nM sodium pyruvate was used as basic cell culture medium further supplemented with 200 μg/mL hygromycin B for Jurkat cells or with 100 units/mL penicillin and 100 μg/mL streptomycin for Raji cells. Cells were cultured in a 37 °C incubator under a humidified 5% $CO_2$ atmosphere.

### 2.3. Photo detector apparatus

The low cost photodetection device was comprised of a 16-bit greyscale cooled CCD camera and a custom fabricated assay plate. The camera was an astronomical model SXVF-M7 (Adirondack Video Astronomy, Hudson Falls, NY), to which was mounted a 12 mm f1.2 lens (Spytown, Utopia, NY). Assay plates were laser machined from black PMMA sheet to which 3 M$^{TM}$ 9770 adhesive transfer tape was attached to one surface. Wells were laser machined as straight through 7 mm round holes. Attachment of a thin polycarbonate sheet to the side of the plate with adhesive completed the formation of the wells with final dimensions 7 mm in diameter and 3.2 (1/8") deep. The use of black material reduces the transfer of light between wells.

### 2.4. Image processing

The camera in Fig. 1b used to capture images incorporates a semiconductor charge couple device (CCD) sensor, which converts incident photon energy into an electric signal. An advantage of the astronomical cooled CCD SXVF-M7 camera is it's high quantum efficiency and response linearity, as well as its ability to generate high-quality, low noise images. The images were analyzed and the photon signal intensity quantified with ImageJ [21]. The average sensor background signal was subtracted from sample images. This background signal was recorded by capturing images with exposure and gain settings identical to those used to image samples. Each data point was calculated as the mean pixel intensity value represented by a digital number in three samples.

### 2.5. SEE assay

To detect and quantify SEE, samples were incubated with Raji cells and Jurkat reporter cells in black 96 well microplates with clear bottoms. Each well contained $1 \times 10^5$ Jurkat cells in a 50 μL volume without hygromycin B and $5 \times 10^4$ Raji cells in a 25 μL volume plus a 25 μL sample containing 4 x SEE of final concentration. The incubation period was 5 h at 37 °C followed by a 10–15 min equilibration to room temperature. Detection of luciferase expressed by the Jurkat reporter cells in response to SEE was by the addition of 100 μL Bio-Glo reagent per well and a 5–10 min incubation period at room temperature. The luciferase enzyme activity was detected according to the Bio-Glo manufacturer's instructions. Luminescence was quantified per well using either a commercial plate reader or the photo detector apparatus. In the latter case, 80 μL of the reaction mixture was transferred from each well of the 96 well plate to wells in a custom analysis plate constructed as described above, and analyzed accordingly.

## 3. Results

### 3.1. Quantitative bioluminescence assay for measuring SEE

Experiments were performed to test the suitability of CCD camera imaging for quantification of the bioluminescence response of Jurkat cell genetically engineered to express firefly luciferase as a reporter gene for SEE detection. Concentrations of SEE from 100 ng/mL to 100 fg/mL were applied to the bioassay mixture of Jurkat and Raji cells and after 5 h incubation the assay sample mixture was transferred to 9-well sample chips (Fig. 2 C1, 100 ng/mL – Fig. 2 A3, 100 fg/mL). The Point Grey Research astronomy CCD camera was first used to quantify the light intensity from the luciferase-catalyzed luciferin reaction. However, with this camera, the signal levels of the cellular luminescence were too low to be discerned against the background. Leaving the shutter open for longer light exposure permits an increase in signal but also increases the thermal noise generated within the silicon structure of the CCD. The 16-bit grayscale, astronomical cooled CCD SXVF-M7 camera equipped with Pentax 12 mm f1.2 lens was selected to address this problem. This camera is computer controlled for image acquisition and analysis. As illustrated by Fig. 1c APC were plated with the reporter T cells line in a plate and incubated with SEE. The photon intensity is detected by CCD camera converting photons carrying image information into electronic signal after striking a silicon wafer CCD chip to generate an image. The CCD chip is cooled to reduce the background thermal noise. Fig. 2 shows an example image of the assay plate in which the cooling reduced the noise and improved the sensitivity of the assay. Increased light intensity level corresponded to increased SEE concentration with dose dependency.

### 3.2. Detection limit of SEE using CCD measurements or luminometer plate reader

The average light signal intensity of each pixel from three image was quantified by free imaging software ImageJ [21]. The average background signal was subtracted from the sample images and the optical brightness intensity reported in analog-digital units (ADU) and plotted against SEE concentrations. Fig. 3A shows that the light intensity is proportional to SEE concentration over an 8-log range

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