



Rapid detection of *Chlamydia trachomatis* in human urine using a B cell-based biosensor

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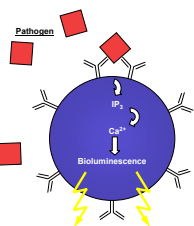
Introduction

There exists a clear need for the development of rapid, sensitive methods that enable point-of-care detection of clinically relevant viruses and bacteria. Here we describe the application of the CANARY™ technology to the detection of *Chlamydia trachomatis* elementary bodies in human urine. Results indicate that CANARY™ is capable of detecting as few as 200 *C. trachomatis* elementary bodies in urine samples using a procedure that requires 5 minutes.

Technology Description

The CANARY™ technology consists of cell lines that are genetically engineered to recognize a specific pathogen, responding to its presence by emitting a luminescent signal that can be detected using a standard luminometer. The potential of this technology has been demonstrated by its application to the detection of >20 different viral and bacterial pathogens to date (Rider et al., 2003). In both simple and complex samples CANARY™ biosensor cells demonstrate sensitivity and specificity that rivals PCR in the detection of such pathogens as *E. coli* O157:H7 and *Bacillus anthracis*. Furthermore, this technology provides results in as little as 2 minutes and can be applied by individuals with a minimum of technical expertise, making it ideal for routine application in many settings.

Construction of new biosensor cell lines is relatively simple and requires only that a monoclonal antibody be available for the target of interest. cDNAs encoding the light and heavy chains of the antibody are cloned into a vector that targets antibody to the cell surface. These vectors are transfected into a parental cell line expressing a bioluminescent protein, wherein antibodies are expressed and localized to the cell surface. Exposure of the biosensor cell line to its target pathogen triggers release of calcium from internal stores, thus activating the luminescent properties of the marker protein (see figure below).



Schematic of the CANARY™ Technology

CANARY™ Instrumentation

Equipment required for detection of the CANARY™ response is off-the-shelf technology. A high-speed benchtop microcentrifuge is used to concentrate pathogen at the bottom of a tube and the B cell reagent is brought into contact with the pathogen using a small minifuge with a horizontal rotor. A small single-tube luminometer records the response of the biosensor cells, and data is stored and analyzed on a laptop computer.



Second generation instrumentation
-combines centrifuges and luminometer
-laptop computer interprets results using custom algorithm

Off-the-shelf instrumentation
-centrifuges
-luminometer
-laptop computer



I. Experimental Methods

Measurement of target in non-complex samples.

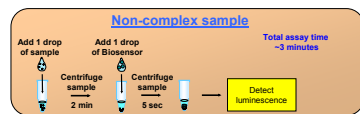
Enumerated bacteria were spiked into 250 µl assay buffer at various concentrations and samples were centrifuged for 2 minutes at 10,000 x g. Biosensor reagent was added to the tube containing the sample for testing and the sample was centrifuged for 5 seconds, then placed in a single-tube luminometer and luminescence measured for a total of 60 seconds.

Measurement of *C. trachomatis* elementary bodies in urine.

Urine samples were obtained from healthy volunteer donors who had given informed consent. 1ml aliquots of urine samples were spiked with various concentrations of enumerated *C. trachomatis* elementary bodies and processed as shown in Panel VI. Briefly, spiked donor urine was centrifuged through a 5µm filter unit for 2 minutes at 10,000 x g and urine was decanted. The pellet was washed by vortexing in assay buffer, followed by centrifugation for an additional 2 minutes. B cell reagent was added to the tube and centrifuged for 5 seconds to bring the B cells into contact with target, and tubes were placed into the luminometer to record the luminescent output.

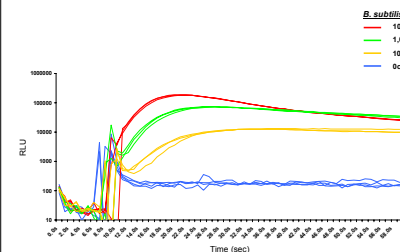
II. Procedure for detection of pathogen in liquid matrixes using CANARY™

CANARY™ provides a rapid method for detecting the presence of bacteria or viruses in liquid samples. In non-complex matrixes the sample is centrifuged at 10,000 x g to pellet the pathogen. Biosensor is added and the sample centrifuged briefly to bring the biosensor in contact with the pathogen, initiating the luminescent response.



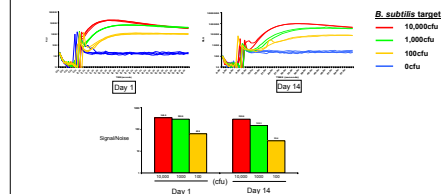
III. Sensitivity of the CANARY™ B cell reagent

Various concentrations of *Bacillus subtilis* were added to assay buffer and centrifuged at 10,000 x g to pellet the bacteria. Biosensor cells specific for *B. subtilis* were added and the sample was centrifuged for an additional 5 seconds, then placed in the luminometer to record the response.



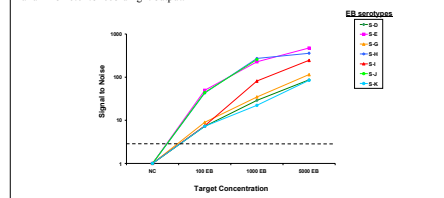
IV. Stability of the CANARY™ B cell reagent

Various concentrations of *Bacillus subtilis* were added to assay buffer and analyzed with CANARY™ biosensor as described in Experimental Methods. B cell reagent was used immediately after preparation or after storage at 4C for 14 days. Results are shown as raw luminescent units (RLU) or expressed as signal/noise (S/N), a ratio of the luminescent signal (in RLU's) in the presence of bacteria to that in the presence of assay buffer alone.



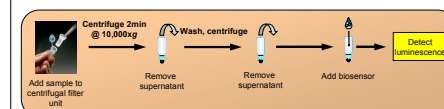
V. Sensitivity of CANARY™ for Chlamydia trachomatis elementary bodies

C. trachomatis elementary bodies were added to assay buffer at the indicated concentrations and pelleted by centrifugation for 2 minutes at 10,000 x g. CANARY™ B cells specific for *C. trachomatis* were added and samples were centrifuged for an additional 5 seconds, then placed in a luminometer to record light output.



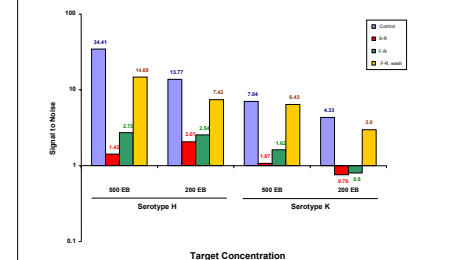
VI. Strategy for detecting Chlamydia trachomatis elementary bodies in urine

Up to 1ml of urine sample is processed by centrifugation through a 5µm filter unit at 10,000 x g. The supernatant is then discarded and the sample washed with assay buffer, then centrifuged for an additional 2 minutes. After replacing the supernatant with assay buffer, B cell reagent is added and a brief centrifugation step brings the reagent in contact with the sample, initiating the luminescent response.



VII. Detection of *C. trachomatis* in human urine

Urine samples were spiked with the indicated concentrations of *C. trachomatis* elementary bodies of serotypes K and H. The samples were then processed for detection with the CANARY™ biosensor by centrifugation alone (S-R), by passage through a centrifugal filter unit (F-R), or by passage through a centrifugal filter unit followed by washing. Each sample was tested in triplicate and compared to an unspiked negative control to determine signal to noise (S/N). Positive control samples comprise elementary bodies spiked into assay buffer.



Conclusions

The CANARY™ technology provides a valuable tool in the detection and identification of pathogenic agents. By taking advantage of the signal amplification pathways intrinsic to mammalian B cells, CANARY™ enables detection of low levels of target organism in a number of matrixes, including urine, blood, nasal swabs, and environmental samples.

Principal advantages of the CANARY™ technique include:

- detection time of 5 minutes or less
- ability to detect bacteria, viruses, toxins, and nucleic acids
- ability to detect as few as 50 organisms per sample
- low cost of instrumentation

The results presented here indicate that CANARY™ has the capacity to detect as few as 200 *C. trachomatis* elementary bodies in human urine in as little as 5 minutes with a minimum of sample processing. These results compare favorably with antibody-based kits that have a limit of detection of 2,000-20,000 pathogenic organisms in urine (Kuipers et al., 1995). Furthermore, the speed and simplicity with which CANARY™ can be performed is a significant advantage over the laborious and time-consuming steps that are required for alternative methods such as PCR and ligase chain reaction (LCR), which typically require 60-90 minutes sample preparation and approximately 4 hours assay run time. Given our experience using CANARY™ biosensors for pathogenic targets in complex matrixes we anticipate that simple improvements in sample processing will contribute to higher sensitivity of CANARY™ in clinical samples such as urine and blood, while maintaining total sample preparation and assay time to less than 10 minutes.

References

- Kuipers, J.G., K. Scharmann, J. Wollenhaupt, E. Nettelbreker, S. Hopf, and H. Zeidler. 1995. Sensitivities of PCR, MicroTrak, ChlamydiaEIA, IDEIA, and PACE 2 for purified *Chlamydia trachomatis* elementary bodies in urine, peripheral blood, peripheral blood leukocytes, and synovial fluid. *J. Clin. Microbiol.* 33:3186-3190.
- Rider, T.H., M.S. Petrovick, F.E. Nargi, J.D. Harper, E.D. Schwoebel, R.H. Mathews, D.J. Blanchard, L.T. Bortolin, A.M. Young, J. Chen, and M.A. Hollis. 2003. A B cell-based sensor for rapid identification of pathogens. *Science* 301:213-215.



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