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Sirius L Tube Luminometer FB12/Sirius Software

Rapid Bacterial Detection with the Sirius L Tube Luminometer

Introduction

Over recent years there has been a growing need for methods for the rapid detection of bacteria, particularly for applications such as hygiene and environmental monitoring, infection control, the manufacture of biologicals and cell culture contamination testing. For such applications results are required quickly, preferably in less than 24 hours and more preferably within the hour, with methods offering the simplicity and ease of use that would allow them to be adapted for use in situ outside of the laboratory. Useful Biology have developed a rapid, simple and highly sensitive bioluminescence based assay that allows the detection of bacteria in as little as 15 minutes.

The assay detects the activity of an enzyme specific to bacteria which catalyses the formation of ATP from its native substrate and ADP and has a short half-life in the environment; the assay therefore will only detect live bacteria and is able to distinguish them against a background of ATP or eukaryotic cells. The enzyme is highly conserved and actively expressed in greater than 97% of bacterial species. ATP formed by the enzyme is detected using a firefly luciferase based bioluminescent reaction; a single reagent is utilised that lyses both Gram negative and positive bacteria, provides the substrates for the bacterial enzyme and includes the bioluminescent detection system.

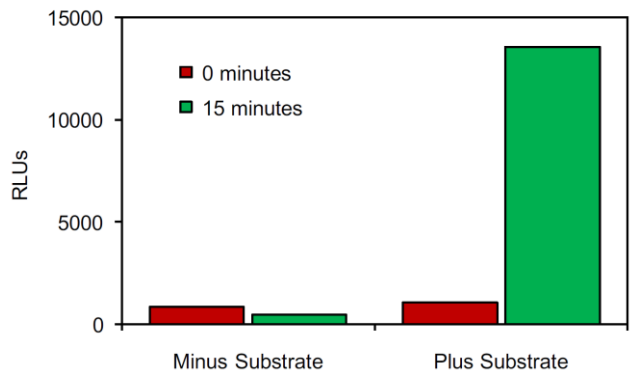

Figure 1. Two detection reagents were prepared, one lacking the bacterial enzyme's native substrate. When tested with 10000 CFU per ml of E. coli the reagent lacking the substrate showed a decrease in light output and therefore ATP over time and the reagent containing the substrate an increase.

The assay can typically detect 100 CFU or less by measuring an increase in ATP (light output) over time when bacteria are present compared to a decrease in ATP over time when they are not. Measurement may be made continuously in a kinetic mode or simply by making 2 measurements, the first after a 5 minute incubation period following addition of a reagent and the second after a further 15 minute incubation period; a ratio may then be calculated that when above 1 signifies the presence of bacteria and below 1 absence. The assay is amenable to use in both high throughput plate based and low throughput tube based formats.

Material

- Sirius L tube luminometer (Titertek-Berthold)
- Rapid Bacterial Detection Assay (Useful Biology)
- Lenticule Discs (Public Health England)

Methods

Bacterial samples at known CFUs per ml were prepared either in water, bacterial culture media or mammalian cell culture media. 1 ml of sample was added per tube along with 0.2 ml of detection reagent; after a 5 minute incubation at room temperature a measurement of light output was made, after a further incubation of 15 minutes a second reading was made. The Sirius L tube luminometer was connected to a Windows based computer running Sirius software. 1 second integrated readings of light output were made. Ratios were calculated by read 2/read 1 where a ratio >1.0 was considered to be indicative of the presence of bacteria in a sample and a ratio <1.0 indicative of absence (or presence below the limit of detection of the assay).

Results

The detection of both Gram negative and positive species of bacteria was demonstrated using the Useful Biology Rapid Bacterial Detection Assay and the Sirius L luminometer with a limit of detection of 100 CFU per ml. The presence of bacterial contamination in a mammalian cell culture was detected without any interference from the cultured cells.

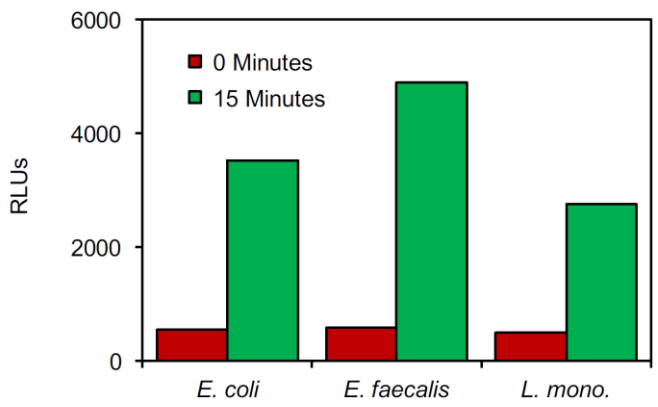

Figure 2. Both Gram negative and positive species were detected (Escherichia coli, Enterococcus faecalis, Listeria monocytogenes). Samples were diluted to 1000 CFU per ml in water.

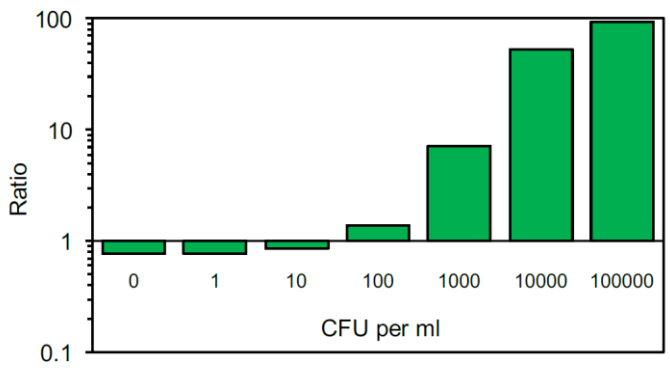

Figure 3. The assay and luminometer were able to detect 100 CFU per ml of E. faecalis. Samples were diluted at known CFUs in LB bacterial culture media

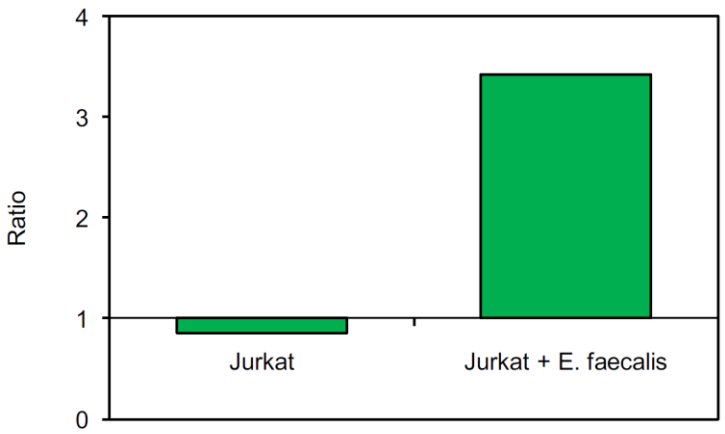

Figure 4. A culture sample of the mammalian Jurkat cell line (10000 cells per ml) produced a negative result in the assay. When E. faecalis was added to the cell culture at 1000 CFU per ml a positive result was obtained.

Summary:

When combined with a small portable luminometer such as the Sirius L tube luminometer the Useful Biology Rapid Bacterial Detection Assay proves to be a viable platform for the detection of bacterial contamination in a variety of sample sources both as a laboratory based test and an onsite test at point of sampling.

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