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Evaluation of β-glucuronidase assay for the detection of *Escherichia coli* from environmental waters

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Abstract
β-Glucuronidases are hydrolytic enzymes responsible for the breakdown of carbohydrates. Specifically, β-Glucuronidases cleave the terminal β-D-glucuronic acid residue from the non-reducing terminus of a mucopolysaccharide chain. In humans, these enzymes are found in the lysosome of many tissue types. Loss of β-Glucuronidase activity results in a metabolic disease known as sly syndrome. One pharmaceutical application for these enzymes is theme tabolism of glucuroni dated prodrugs into active pharmacological compounds. As expression and activities of β-Glucuronidases vary substantially between tissue types and disease states, these enzymes have been used to achieve targeted activation of oncotherapeutic compounds, some of which may be toxic to healthy cells not associated with malignancy or disease. It is thus important to have knowledge of the β-Glucuronidase activity in the tested sample to determine whether the prodrug or active form will predominate. BioVision's β-Glucuronidase Activity Assay Kit provides a quick, reliable fluorometric method for measurements of β-Glucuronidase activities of samples and tissue lysates. The provided substrate, which is specific to β-Glucuronidases, is cleaved into a fluorescent product in the presence of β-Glucuronidase.

Keywords: β-glucuronidases, fluorometric, pharmacological compounds, malignancy or disease

Introduction
Bacteria which were β-d-galactosidase and β-d-glucuronidase positive or expressed only one of these enzymes was isolated from environmental water samples. The enzymatic activity of these bacteria was measured in 25-min assays by using the fluorogenic substrates 4-methylumbelliferyl-β-d-galactoside and 4-methylumbelliferyl-β-d-glucuronide. The enzyme activity, enzyme induction, and enzyme temperature characteristics of target and non target bacteria in assays aimed at detecting coliform bacteria and *Escherichia coli* were investigated. The potential interference of false-positive bacteria was evaluated. Several of the β-d-galactosidase-positive non target bacteria but none of the β-d-glucuronidase-positive non target bacteria contained unstable enzyme at 44.5 °C. The activity of target bacteria was highly inducible. Non target bacteria were induced much less or were not induced by the inducers used. The results revealed large variations in the enzyme levels of different β-d-galactosidase- and β-d-glucuronidase-positive bacteria. The induced and non induced β-d-glucuronidase activities of *Bacillus* spp. and *Aerococcus viridans* were approximately the same as the activities of induced *E. coli*. Except for some isolates identified as *Aeromonas* spp., all of the induced and noninduced β-d-galactosidase-positive, noncoliform isolates exhibited at least 2 log units less mean β-d-galactosidase activity than induced *E. coli*. The noncoliform bacteria must be present in correspondingly higher concentrations than those of target bacteria to interfere in the rapid assay for detection of coliform bacteria.

Escherichia coli in the Environment: Implications for Water Quality and Human Health
Escherichia coli is naturally present in the intestinal tracts of warm-blooded animals. Since *E. coli* is released into the environment through deposition of fecal material, this bacterium is widely used as an indicator of fecal contamination of waterways. Recently, research efforts have been directed towards the identification of potential sources of fecal contamination impacting waterways and beaches. This is often referred to as microbial source tracking. However, recent studies have reported that *E. coli* can become "naturalized" to soil, sand, sediments, and algae in tropical, subtropical, and temperate environments. This phenomenon raises issues concerning the continued use of this bacterium as an indicator of fecal contamination.

In this research, it is elaborate that the relationship between *E. coli* and fecal pollution and the use of this bacterium as an indicator of fecal contamination in freshwater systems. This is also elaborate recent studies showing that *E. coli* can become an active member of natural microbial communities in the environment, and how this bacterium is being used for microbial source tracking and impact of environmentally-"naturalized" *E. coli* populations on water quality.

***Escherichia coli*: the best biological drinking water indicator for public health protection**

Public health protection requires an indicator of fecal pollution. It is not necessary to analyse drinking water for all pathogens. *Escherichia coli* is found in all mammal faeces at concentrations of 10 log 9^{-1}, but it does not multiply appreciably in the environment. In the 1890s, it was chosen as the biological indicator of water treatment safety. Because of method deficiencies, *E. coli* surrogates such as the 'fecal coliform' and total coliforms tests were developed and became part of drinking water regulations. With the advent of the Defined Substrate Technology in the late 1980s, it became possible to analyse drinking water directly for *E. coli* and, simultaneously, total coliforms inexpensively and simply. Accordingly, *E. coli* was re-inserted in the drinking water regulations. *E. coli* survives in drinking water for between 4 and 12 weeks, depending on environmental conditions temperature, microflora, etc. Bacteria and viruses are approximately equally oxidant-sensitive, but parasites are less so. Under the conditions in distribution systems, *E. coli* will be much more long-lived. Therefore, under most circumstances it is possible to design a monitoring program that permits public health protection at a modest cost. Drinking water regulations currently require infrequent monitoring which may not adequately detect intermittent contamination events; however, it is cost-effective to markedly increase testing with *E. coli* to better protect the public's health. Comparison with other practical candidate fecal indicators shows that *E. coli* is far superior overall.

Methods and Materials

Isolation of β-d-galactosidase- and β-d-glucuronidase-positive environmental bacteria. Cultivable bacteria were recovered from sewage effluent (after primary treatment), polluted river water, or coastal water after membrane filtration (type GSWP Millipore filter; diameter, 47 mm; pore size, 0.22 μm) by growing them on tryptic soy agar (TSA) (Difco Laboratories) supplemented with 50 mg of 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside (X-Gal) (Sigma Chemical Co.) per liter and 50 mg of 4-methylumbelliferyl-β-d-glucuronide (MUGlu) (Diagnostic Chemicals Ltd.) per liter for 48 h at 35°C or for 72 h at 20°C. β-d-Galactosidase- and/or β-d-glucuronidase-positive bacteria were isolated by randomly picking green colonies (β-d-galactosidase positive) and/or fluorescent colonies (β-d-glucuronidase positive) from the agar plates. The colonies were purified by streaking them onto new agar and were identified on the basis of the results of Gram staining, the oxidase test, and the catalase test, as well as the API 20E and API 20NE tests (gram-negative rods) and the ID 32 Staph or ID 32 Strep test (gram-positive cocci) (bioMerieux). The isolates identified by the API systems as *Aeromonas* spp. and gram-positive rods were not further differentiated. β-d-Glucuronidase assay. The β-d-glucuronidase assay was performed like the β-d-galactosidase assay, except that the filters were placed in flasks containing 17 ml of PB (pH 6.4), 3 ml of a MUGlu solution (50 mg of MUGlu in 50 ml of PB supplemented with 1 drop of Triton X-100), and 0.1% nutrient broth (Difco Laboratories). Measurement of induced and noninduced enzyme levels the levels of enzymatic activity per cell in cell suspensions, cultivated in the presence and absence of inducer, were determined by performing the 25-min enzyme assays described above at 44.5 °C., temperature dependence. The levels of enzymatic activity per cell of induced cell suspensions were determined by performing the 25-min enzyme assays described above at 25, 35, and 44.5 °C.

Bacterial strains and culture

In total, different strains of *E. coli* were used in this study including the *E. coli* Collection of Reference Strains as ECOR; a collection of 72E. coli isolates from a range of geographic and animal backgrounds obtained from the research Centre,10 Environmental isolates were collected as part of the routine bivalve shellfish hygiene monitoring programme operated by the Centre for Environment, Fisheries and Aquaculture Sciences, *E. colistrains* were used to determine the inclusivity of the qNASBA and qPCR methods for *E. coli*. Additionally, 23non- *E. colispecies* were used to test assay exclusivity. These included species closely related to E. coli and more distantly related bacterial species. All cultures were grown overnight on either nutrient agar, LB agar, or TCBS agar at 37 Cor 30 C according to the growth requirements of individual species.

Field sampling

Water samples were collected monthly from four different locations in the South West of research area, all situated in the county of Dorset. With the exception of the tertiary treated sewage works, which was sampled on four occasions, samples were taken monthly from each site on five separate occasions between 21st January and 9th June 2016. Two litres of water were collected from each site using a sterile polypropylene bottle. The water samples were transported on ice back to the laboratory where they were stored at 4 C and used within 24 h of collection.

Result

The results of this study indicate that rapid enzyme assays without a growth-selective phase should not generally be rejected when high numbers of non target bacteria are present in environmental water samples. The obvious advantage of the 25-min assays is the short assay time. The assays can be used for early warning of accidental pollution or for monitoring water quality for recreation or aquaculture. Several of the enzyme-positive non target bacteria isolated from environmental water samples had low enzyme levels, and the influence of these organisms can be neglected except at very high bacterial concentrations. However, some non target bacteria with high enzyme levels, especially high levels of β-d-glucuronidase, could interfere seriously if they are present at high enough concentrations.

Conclusion

In conclusion, the current continuous spectrophotometric assay is significantly simpler, faster, more economical, and more sensitive than the existing discontinuous assays. The experimentalist can easily enjoy the beauty of this assay method. Such a method should be highly desired for both routine GUS as-says and for accurate kinetic analysis of the

enzyme. The development of new techniques and more sophisticated assays for the detection of genotoxicity in fresh water and in sea water has been the purpose of various projects concerning environmental biomonitoring. To achieve active management of bathing areas and to reduce risk associated with the presence of fecal pollution, tests capable of rapid on-site assessment of microbiological water quality are required. A protocol for the recovery and detection of fecal pollution indicator bacteria, *E. coli*, using β-glucuronidase (GUS) activity was developed. The developed protocol involves two main steps: sample preparation and GUS activity measurement. In the sample preparation step, syringe filters were used with a dual purpose, for the recovery and pre-concentration of *E. coli* from the water matrix and as μL reactors for bacteria lysis and GUS extraction.

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