Analytical Biochemistry 470 (2015) 1–6

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Time-resolved fluorescence-based assay for rapid detection of *Escherichia coli*

Janne Kulpakko *, Kari Kopra, Pekka Hänninen

Laboratory of Biophysics and Medicity Research Laboratories, University of Turku, 20520 Turku, Finland

ARTICLE INFO

Article history:
Received 5 June 2014
Received in revised form 25 August 2014
Accepted 4 September 2014
Available online 16 September 2014

Keywords:
Escherichia coli
Bacteriophage
Luminescence
Urinary tract infection

ABSTRACT

Fast and simple detection of pathogens is of utmost importance in health care and the food industry. In this article, a novel technology for the detection of pathogenic bacteria is presented. The technology uses lytic-specific bacteriophages and a nonspecific interaction of cellular components with a luminescent lanthanide chelate. As a proof of principle, *Escherichia coli*-specific T4 bacteriophage was used to infect the bacteria, and the cell lysis was detected. In the absence of *E. coli,* luminescent Eu^{3+}–chelate complex cannot be formed and low time-resolved luminescence signal is monitored. In the presence of *E. coli,* increased luminescence signal is observed as the cellular contents are leached to the surrounding medium. The luminescence signal is observed as a function of the number of bacteria in the sample. The homogeneous assay can detect living *E. coli* in bacterial cultures and simulated urine samples within 25 min with a detection limit of 1000 or 10,000 bacterial cells/ml in buffer or urine, respectively. The detection limit is at the clinically relevant level, which indicates that the method could also be applicable to clinical settings for fast detection of urine bacteria.

Pathogenic microorganisms present a global threat. In developing countries threats are linked to hygienic and environmental issues, and in industrialized settings threats are linked to growing antimicrobial resistance [1]. Among the most common causes of pathogenic infections are those caused by *Escherichia coli,* which is a common bacterium found in the human and mammalian gastrointestinal tract. There are several hundred *E. coli* types, and most of them are harmless opportunistic organisms. However, enterohemorrhagic *E. coli* (EHEC)[1] of serogroup O157:H7 is a harmful toxin-producing bacterium known to cause bloody diarrhea and hemolytic uremic syndrome. Most EHEC infections are foodborne or waterborne [2].

Of all causative agents, *E. coli* is the most common bacterium found in lower urinary tract infections. Up to 80% of these infections are caused by *E. coli* [3]. It is generally accepted that more than 100,000 bacterial cells per milliliter of urine is interpreted as a urinary tract infection [4]. In the clinics, fast urinary tract infection detection could allow immediate antibiotic treatment when needed.

Preventing epidemics requires early detection. Currently, *E. coli* is most commonly recognized by culturing methods, but these require at least 3 days for a conclusive result [5]. Apart from culturing methods there are several assays to detect *E. coli.* Immunoassays and polymerase chain reaction (PCR)-based methods can be used to detect *E. coli* O157:H7 from different sample types within a few hours. Immunoassays rely mainly on targets as somatic (O157) or flagellar (H7) antigens, and PCR relies mainly on specific genes *stx1* and *stx2* [6]. Immunoassays are limited by the use of specific antibodies that are linked to a variety of different labels and separation systems before detection. In PCR methods, the target DNA is purified and amplified to monitor the amount of the genetic material. However, PCR methods do not give information about the number of viable cells. In many cases, both of these methods require laborious enrichment from bacterial culture prior to detection. Furthermore, there is a possibility of false positives when microbes are detected using PCR. This is mainly because easily contaminating microbes and microbial DNA from previous work could still exist in the laboratory [5].

Besides traditional methods, there are several methods for the detection of *E. coli* using bacteriophages as detection elements. Methods are based on the rupture of *E. coli* membrane caused by infection, stained phages, or producing fluorescent label inside

* Corresponding author.
E-mail address: janne.kulpakko@utu.fi (J. Kulpakko).

[1] *Abbreviations used:* EHEC, enterohemorrhagic *E. coli*; PCR, polymerase chain reaction; TRL, time-resolved luminescence; NTA, 4,4,4-trifluoro-1-(2-naphthalenyl)-1,3-butanedione; TOPO, tri-*n*-octyl-phosphineoxide; DMSO, dimethyl sulfoxide; DAPI, 4′,6-diamidino-2-phenylindole; TSB, Tryptic Soy Broth; PBS, phosphate-buffered saline; UV, ultraviolet; CV, coefficient of variation; ECMDB, *E. coli* Metabolome Database; ATP, adenosine triphosphate.

http://dx.doi.org/10.1016/j.ab.2014.09.002
0003-2697/

bacterial cells. T4 bacteriophage binding to *E. coli* cell surface causes transitory ion leakage right after the phage DNA injection into the host cell [7]. This observation has led to sensor applications using ion leakage or luminescence labels. Ion leakage can be measured qualitatively by potassium-sensitive chip within 30 min [8]. Another similar method used carbon electrode printed phage microarray [9]. The detection method requires ion-free samples prior to *E. coli* infection; thus, urine samples as such are not compatible. Instead of using ions from bacterial cell breakage, there are experimental methods using the bacteriophages directly [10]. These methods combine immunomagnetic separation and fluorescently stained bacteriophages. Another approach is based on expressing green fluorescent protein on bacteriophages' phage coat protein. The fluorescent protein multiplies simultaneously with the number of phages, and the fluorescence is enhanced. This method has an assay time of several hours [11,12]. Edgar and coworkers reported a method combining the use of biotinylated bacteriophages and streptavidin-coated quantum dots. The drawback is that the bacteriophage must be engineered, and the method takes 1 h to give results [13].

Here we present homogeneous lanthanide chelate-based *E. coli* detection using time-resolved luminescence (TRL). The method combines the use of nonspecific lanthanide chelate structure and bacteriophages' specific ability to infect certain bacterial types or species. The method can be used to observe bacterial cells whether they are intact or lysed. The method enables *E. coli* detection in a simple microtiter plate format with sensitivity of 1000 *E. coli* cells/ml.

Materials and methods

Materials and reagents

$EuCl_3$, 4,4,4-trifluoro-1-(2-naphthalenyl)-1,3-butanedione (NTA), sodium citrate tribasic dihydrate, tri-*n*-octyl-phosphineoxide (TOPO), dimethyl sulfoxide (DMSO), DNA sodium salt (type XIV from herring testes), and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Tryptic Soy Broth (TSB) powder was purchased from Becton Dickinson (Sparks, MD, USA). Phosphate-buffered saline (PBS) from Lonza (Basel, Switzerland) was used for the dilution of bacteriophages. Other chemicals and analytical-grade solvents were purchased from Sigma–Aldrich. Urine samples were taken from 5 healthy volunteers. *E. coli* strain B ATCC 11303 was used as the host for the lytic phage. *Bacillus subtilis* (BGA) Spore Suspension DSM 618 (10^7 cfu/ml) was purchased from Merck (cat. no. 1.10649.0001). Wild-type lytic bacteriophage T4 from Carolina Biological Supply (Burlington, NC, USA) was used in this study.

Instrumentation

Freshly grown *E. coli* cells were sonicated with a tip sonicator (Branson Sonifier 450, Geneva, Switzerland). The TRL signal was measured with a Victor^2 multilabel counter (Wallac, PerkinElmer Life and Analytical Sciences, Turku, Finland) at 615 ± 5 nm using a 340 ± 5-nm excitation wavelength and 400-μs delay and decay times. Visualization of the bacterial cells was performed with a Leica TCS SP5 MP microscope (Leica Microsystems), LASAF software (Leica Application Suite), HyD (GaAs) detector, and 100× oil objective.

Bacteria and bacteriophages

Viability of phages was studied by phage plaque assay according to instructions in the literature [14]. *B. subtilis* was used as control bacteria that bacteriophage T4 is unable to infect. *E. coli* inoculation from stock was cultured overnight in 6 ml of TSB medium at 37 °C with shaking. From culture, bacterial cells were transferred to fresh medium in order to achieve logarithmic growth. Cell concentration was estimated using optical density at 600 nm (OD_{600}) measured with a spectrophotometer. Bacteriophages in SM buffer (10 mM $MgSO_4$, 100 mM NaCl, 0.01% gelatin, and 50 mM Tris–HCl, pH 7.5) were added to bacterial culture after OD_{600} reached 0.1. A comparison of active and inactive phages was made. Bacteriophage inactivation was performed using ultraviolet (UV) radiation treatment for 10 min or heating for 20 min at 85 °C [15]. A plaque assay was performed after the inactivation process to verify the inactivation.

Visualization of bacterial cells

Multiphoton microscopy was used to image live E. coli cells. The bacteria from the phase of logarithmic growth were centrifuged and washed before sonication or bacteriophage infection. Intact bacteria were used as a control sample that had no bacteriophage infection. Samples were incubated with DAPI staining (1 μg/ml final concentration) for 20 min. Bacteria were centrifuged and washed once with PBS and transferred to the cover slide.

Homogeneous assay for detection of E. coli

The assays using bacteriophages were performed in 96-well microtiter plates with four replicates. In a typical assay, 10 μl (10^5 phages) of bacteriophages in SM buffer and 100 μl of the bacterial sample in physiological saline were added to the plate. After 20 min of incubation at 37 °C with vigorous shaking, 7 μl of 2 mM citrate at pH 7.5 was added to the wells. After 2 min of incubation at room temperature, a 5:3:3 mixture of $EuCl_3$, NTA, and TOPO at Eu^{3+} was added to the wells in 3 μl of water. The final concentrations of $EuCl_3$, NTA, and TOPO were 6.0, 3.6, and 3.6 μM, respectively. The Eu^{3+}/NTA/TOPO chelate complex was prepared in DMSO before dilution to water in order to avoid possible aggregations. After 5 min of incubation, the TRL signals were measured.

The demonstration of cell breakage with sonication was performed with washed *E. coli* cells kept 15 min on ice before sonication for 2 min in an ice bath. Sonication was repeated until the cloudy suspension became translucent.

Results and discussion

Here we described a new method to detect bacteria. The assay uses the rupture of the *E. coli* cells with bacteriophages, in which a vast number of different molecules from the cell contents are released to the surroundings (Fig. 1). The cell disruption method has an effect on the appearance of the treated bacteria but possibly also on the contents released from the cell as well (see Fig. S1 in online supplementary material). A lanthanide chelate complex Eu^{3+}/NTA/TOPO (5:3:3) and citrate ions were used to detect these leaked molecules. Low luminescence signal is observed when bacterial cells are intact, which occurs from the ability of citrate ion to chelate free Eu^{3+}. In the presence of ruptured cells, the released intracellular content provides protection from the citrate ion and enables the formation of the luminescent Eu^{3+}/NTA/TOPO chelate complex.

In the first state, the bacteriophage infection was simulated by breaking cells with ultrasound waves. The ultrasound method was chosen because it can be applied easily without adding chemicals that could interfere with the chelate complex formation. A more than 20-fold increase in the TRL signal was monitored with ruptured cells compared with unbroken cells. The method of how cells

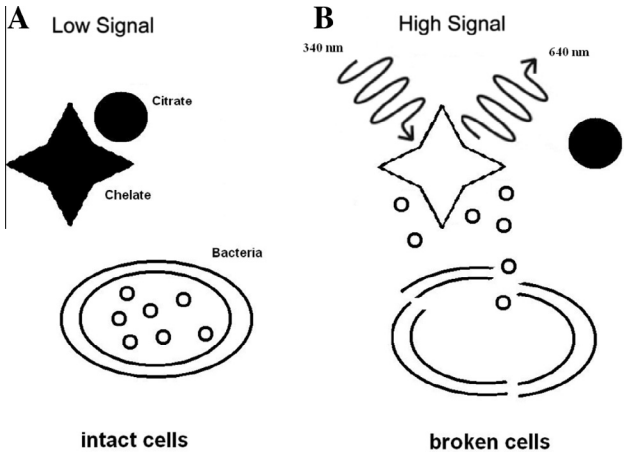

Fig.1. Principle of TRL detection-based assay for *E. coli.* (A) Low TRL signal is monitored in the presence of intact *E. coli* bacteria and chelate. (B) Ruptured bacterial cells release cellular contents to the surroundings, causing Eu^{3+}–chelate complex formation and increased TRL signal.

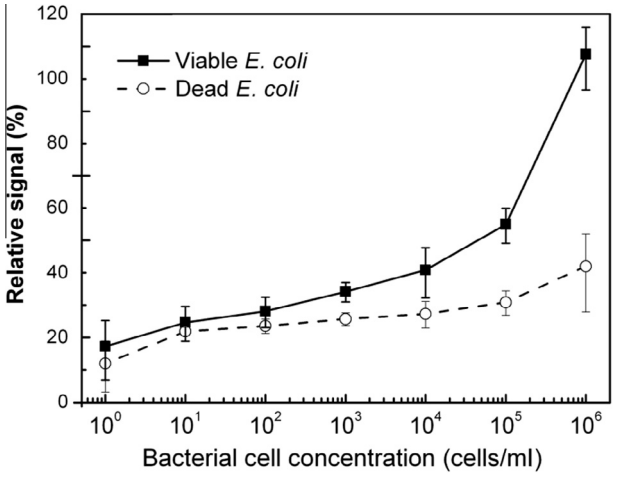

Fig.3. Calibration curves measured with *E. coli* rapid detection assay. *E. coli* was detected using a specific T4 bacteriophage for living *E. coli* cells from the early logarithmic growth phase and dead metabolically inactive cells. The bacteriophage concentration was 1×10^8/ml.

are ruptured has an effect on the TRL signal (Fig. 2). Ultrasound treatment clearly caused a different TRL signal than intact cells had. Ultrasound rupture of cells caused a more than 20-fold increase in the signal.

Calibration curves were measured for living and dead *E. coli* to test assay sensitivity with the method based on lytic bacteriophages and Eu^{3+} chelate (Fig. 3). Dead cells were taken from late stationary phase, and these cells also were UV-radiated to ensure the absence of living cells. The curves indicate that assay measures the leaked content of the cell instead of the whole cell.

The assay consists of adding bacteriophage, citrate, and Eu^{3+}–chelate to the sample and measuring the TRL signal. The cell lysis assay was performed within 25 min total assay time. The limit of detection, calculated as +3 standard deviations above the signal from intact cells, was 1000 cells/ml (Fig. 3). Bacterial cell concentrations exceeding 1×10^7 cells/ml result in a signal that starts to slow down, and a slight decrease of signal is seen with even higher concentrations (data not shown). It is possible that cell disruption is more difficult to achieve with higher bacteria concentrations, or all of the citrate molecules are already used by released ions.

A set of control experiments was arranged to exclude possible artifacts that could cause signal differences (Fig. 4). A research

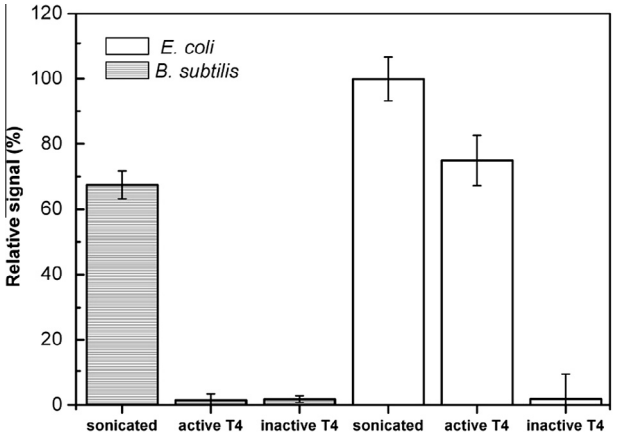

Fig.4. *B. subtilis* and *E. coli* bacterial cells in three different experimental conditions (sonicated, active, and inactive *E. coli*-specific bacteriophages added). The bacterial cell concentration was 1.0×10^5/ml, and the bacteriophage concentration used was 1.0×10^8/ml.

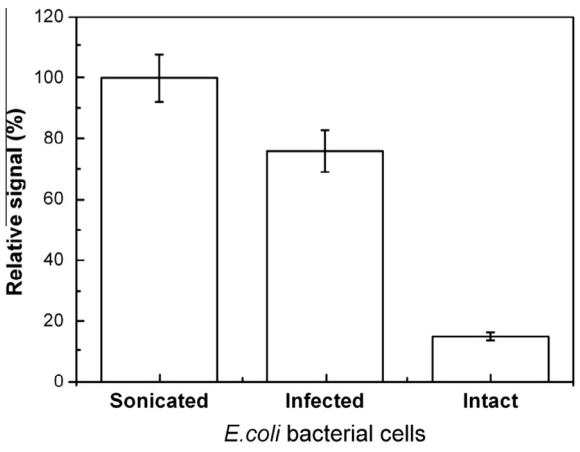

Fig.2. Response of *E. coli* rapid detection assay for sonicated, infected, and intact control *E. coli* cells. The freshly grown *E. coli* bacterial cell concentration in all three conditions was 1×10^7 cells/ml. The results are shown as means ± standard deviations of four replicates.

question was whether the TRL signal is dependent on cell disruption and whether the signal is species specific when phages are used. Active normal phages were used to infect *E. coli* cells. This was compared with inactive phages that were UV-irradiated and heated. Then different bacterial species were exposed to phages or sonication. The method of sonication affects all bacterial cells, but phages lyse only a narrow host range of cells. Phage-mediated cell disruption was expected to cause a similar TRL signal increase as the disruption caused by sonication. However, phage is unable to infect nonhost cells and release cell contents. Therefore, the TRL signal was not expected to increase. Observed results confirmed this expectation that different bacterial species cause a similar TRL signal when their cell contents are released. Fig. 4 shows that sonication affects *B. subtilis* cells in a very similar way as it affects *E. coli* cells. The specific cell lysis with *E. coli* occurs only with active phages and host bacterial cells. The assay was performed in accordance with the protocol described earlier except that sonicated cells were measured 5 min after sonication.

The method applicability for urinary tract infection screening was performed directly on the urine sample (Fig. 5). Bacteriophages were added to urine to infect any living *E. coli* cells, and

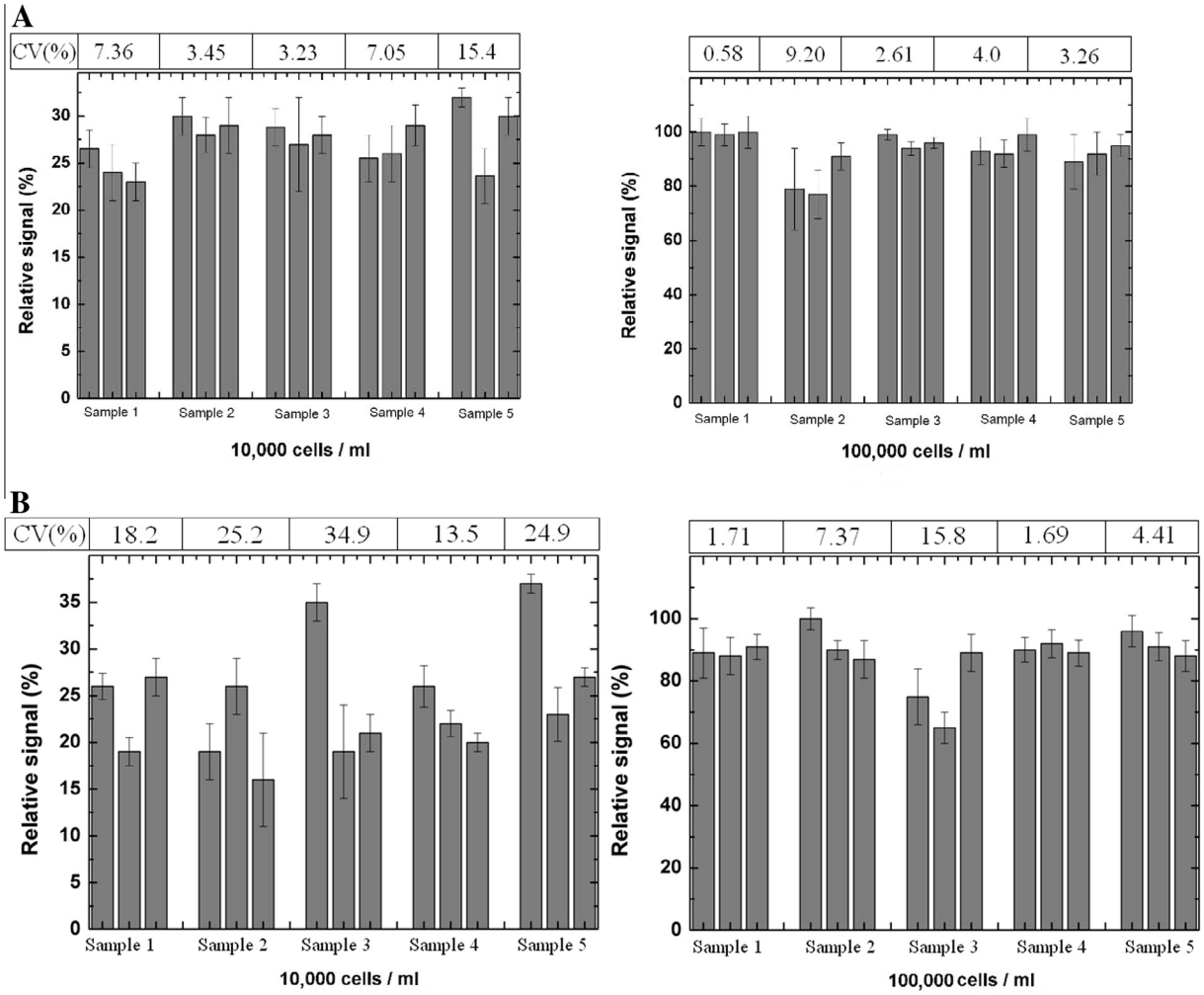

Fig.5. Intra-assay repeatability (A) and inter-assay reproducibility (B) of *E. coli* detection in 5 spiked urine samples. The relative signal was calculated as active phage signal minus inactive control phage signal. The error bars indicate 1 standard deviation from the mean. The intra-assay repeatability represented the variability in the values from three repeated measures of the same prepared sample. The inter-assay reproducibility was determined by analyzing the same samples in three separate runs carried out on different days. CVs were calculated from the triplicates.

inactive bacteriophages were used as a control. The monitored signal/background (S/B) ratio is decreased for the sample containing 100,000 cells compared with the sample containing 10,000 cells.

The intra-assay repeatability represented the variability in the values from three repeated measures of the same prepared sample. The inter-assay reproducibility was determined by analyzing the same samples in three separate runs carried out on different days. Intra-assay repeatability (Fig. 5A) shows that coefficients of variation (CVs) are in an acceptable range in both 10,000 and 100,000 cells/ml (Fig. 5B). The spiked bacteria for this experiment were grown on different days; therefore, the growth stage might have been different for each experiment.

Observing the physiological status of bacterial cells is an important feature, for instance, when antimicrobial susceptibilities are studied [16]. There are several fluorescent probes existing for studying the cell condition. These are mainly for flow cytometric monitoring for bacterial cell status, for example, dead/alive assays. The probe enters specifically dead or compromised cells through damaged membrane areas, whereas intact cells prevent probe entrance. This class of fluorescent probes binds to nucleic acids in the cell nucleus [17]. Furthermore, separating between living and dead cells can be made by measuring the fluorescent product of glucose-6-phosphate dehydrogenase that is leaking from damaged cells to the surrounding medium [18].

In this study, we demonstrated a novel method to observe the cell physiological state using TRL detection. Using environment-sensitive europium–NTA–chelate reacting with cell interior molecules, we were able to estimate not only the physiological state of the bacterial cells but also the number of specific bacterial species. With inactivated bacteriophages, the TRL signal alteration is not detectable because cells remain intact and cell content remains inside of the cell. For this reason, it can definitely be stated that specific bacteriophages capable of lysing the cells are truly the factor behind the assay. Broken bacterial cells release myriad molecules capable of binding both the citric acid and chelate. Furthermore, it is possible that proteins can protect europium–NTA–chelate, and this might be the major reason for the phenomenon observed.

Many dead/alive assays for bacterial and eukaryotic cells are based on intracellular DNA because of its abundant presence inside the cell. The DNA concentration inside the *E. coli* cell ranges from 11 to 18 mg/ml [19]. For this reason, the role of DNA in our assay was studied. DNA concentrations from 5.0×10^{-10} to 5.0×10^{-5} g were spiked to bacterial sample, which had 100,000 bacterial cells/ml (Fig. 6). From this experiment, it can be concluded that DNA has very little role in causing the signal difference in our assay because the DNA concentration is strongly diluted in the sample medium. In addition, increasing DNA concentrations cause the opposite effect of broken cells.

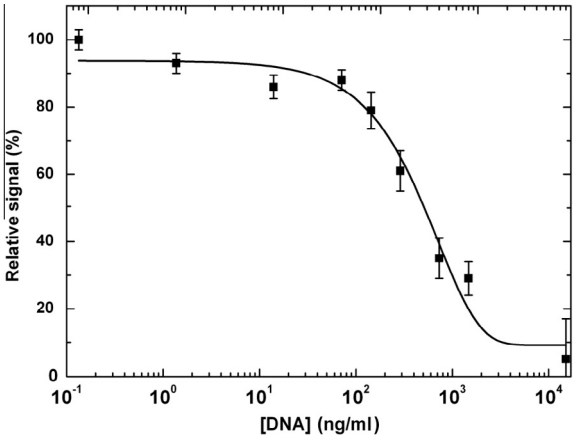

Fig.6. DNA concentration effect with *E. coli* rapid detection assay. DNA concentrations 1.5 ng to 1.5×10^4 ng/ml are shown. The concentrations were spiked to bacterial sample, which had 100,000 bacterial cells/ml. The results are shown as means ± standard deviations of three replicates.

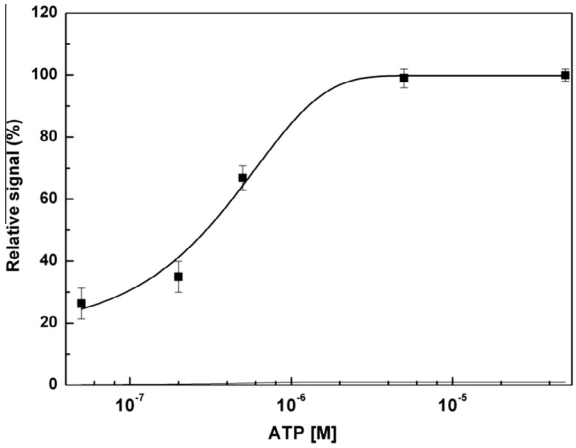

Fig.7. ATP concentration effect with *E. coli* rapid detection assay. Concentrations from 0.5 to 500 nM were spiked to bacterial sample, which had 100,000 bacterial cells/ml. The results are shown as means ± standard deviations of three replicates.

According to the *E. coli* Metabolome Database (ECMDB), living *E. coli* has calcium (Ca^{2+}, 0.1 mM), chlorine (Cl^-, 6.0 mM), sodium (Na^+, 5 mM), and potassium (K^+, 200 mM) ion concentrations in cell cytosol. Considering the average volume of a single *E. coli* bacterial cell (1.0×10^{-9} µl) and the sample volume of 112 µl, ion concentration changes are inconsequential to cause disturbances for europium–chelate complex. Therefore, a plausible explanation for the increase of luminescence could involve protective cell cytosolic proteins guarding europium ion from chelating citrate molecules. Instead, many *E. coli* metabolites leaking from the cells might have a role in causing the luminescence increase observed with broken cells. These are compounds such as phosphate, pyrophosphate, NADP (*nicotinamide adenine dinucleotide phosphate),* and especially ATP (adenosine triphosphate) involved in energy metabolism. These compounds are present in the cells mainly in the active phase of growth when cells are dividing. These molecules are not abundant in dormant or dead cells.

It is known that ATP has a tendency to chelate lanthanide ions and that hydrolysis of ATP produces phosphates that can easily quench lanthanide complexes. This property has led to the development of several microtiter plate assays for studying ATPase enzymes and for detecting phosphate from environmental samples [20,21]. As mentioned, the luminescence of lanthanide complexes can be quenched, but in some cases enhancement of luminescence is observed when additional unknown ligands are involved with the complex [22]. Because of the strong presence of ATP in the living cells, the role of ATP in our assay was investigated. ATP concentrations from 5.0×10^{-8} to 5.0×10^{-4} M were spiked to bacterial sample, which had 100,000 bacterial cells/ml (Fig. 7). According to the measurements, high ATP concentrations are shown to increase TRL signal. However, according to the ECMDB, the concentration of ATP inside the *E. coli* cell ranges from 1.3 to 7.0 mM. When the small volume of the cell is considered, the released and strongly diluted ATP concentration is negligible to cause signal differences.

Urine as a sample matrix is applicable to the method described here (Fig. 7). The data indicate that bacteriophages effectively lyse the bacteria. Assay suitability to large-scale urinary tract infection scanning requires further studies with a large test panel of patient samples. For example, urine pH, ion concentrations, and blood cells can affect results. As the developed method work with urine that is full of different ions with different oxidation states, the reason for signal difference between broken and intact cells might not be due to ion leakage. The method described in this article is obviously much faster than culturing methods. PCR and immunoassay methods are laborious and require trained persons. Furthermore, those methods can measure parts of bacterial remnants or dead bacterial cells as well, leaving the living amount of cells unclear. Our method is efficient when compared with other phage-based methods [7–13]. The detection limit reached the same range as other sensitive methods, but our method required less time and flow cytometric analysis was not required [10–12]. In addition, our method is not based on ions leaking from the cells. This allows analyzing the samples containing different ionic species.

TRL signal is measured from microtiter plates that are suitable for urine samples. Urine and other biological fluids contain naturally fluorescent compounds. Hence, the conventional fluorescent assays have certain limitations. A long-lived fluorophore such as europium used with TRL signal monitoring is able to overcome these problems by having a delay between excitation and emission. This minimizes the fluorescence interference. Biological samples usually have nonspecific background fluorescence, which has a decay time of only approximately 10 ns. The TRL measurement is made after 400 µs, thereby excluding short-lived background fluorescence.

Performing the method properly requires that the bacterial cells be in the active state, enabling phage infection. Bacterial cells used in these experiments were fresh and vigorous cells from the logarithmic growth phase. These cells have active metabolism in progress. The method does not function with dormant cells with low metabolic activity. However, *E. coli* cells from urinary tract infection are expected to be in the active phase of growth. When different bacterial species were exposed to bacteriophages, the resulting fluorescence difference is obvious only with *E. coli* bacterial cells. However, it must be added that due to the different physiological states of bacteria and the unstable nature of the chelate, assay results may vary. Experiments were made by a debilitated laboratory strain of *E. coli* that has many defects on its cell wall. There are several different infectious wild-type *E. coli* bacteria, and these have naturally much stronger cell wall structures [23]. This must be considered when the assay is performed. By adjusting the chelate stability, it might be possible to further improve the assay performance. Even though the method using TRL provides an advantage for measuring biological fluids, it must be remembered that urine content differs not only between different individuals but also between different time points with one individual. When a practical assay for urinary tract infection is considered, bacteriophages must be chosen according to *E. coli* types present in urine sample. It is known that a special group of *E. coli* bacteria is known

to cause most of the urinary tract infections. These uropathogenic strains of *E. coli* are susceptible to many lytic bacteriophages such as SP6likevirus, T4likevirus, and T1likevirus [24].

Conclusions

Here, we have reported the use of luminescent chelate to distinguish between broken and intact cells. The straightforward method has the potential to recognize pathogenic *E. coli* strains from urine samples within 25 min. The method combines specific bacteriophage–bacteria interaction with unspecific interactions of chelate and its surrounding molecules. The detection limit in urine samples was in the range of urinary tract infection. The assay potentially can be used to detect several other bacterial species having a lytic phage that can be applied with the method described here. These results indicate that there is a possibility of using this method to investigate the physiological state of the cell. For instance, the leaking of eukaryotic cell membrane or viral infection could be studied. Further efforts are needed to improve stability and sensitivity of the system, the reason for signal increase should be studied in more detail, and the applicability of the assay for other bacterial species and bacteriophages should be studied.

Acknowledgments

This study was supported by the University of Turku, the University of Turku Foundation, and the Foundation for Finnish Innovators.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ab.2014.09.002.

References

[1] R. Laxminarayan, D. Heymann, Challenges of drug resistance in the developing world, Br. Med. J. 344 (2012) e1567.
[2] H. Karch, P. Tarr, M. Bielaszewska, Enterohaemorrhagic *Escherichia coli* in human medicine, Int. J. Med. Microbiol. 295 (2005) 405–418.
[3] L. Nicolle, Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis, Urol. Clin. North Am. 35 (2008) 1–12.
[4] J. Kwon, M. Fausone, H. Du, A. Robicsek, L. Peterson, Impact of laboratory-reported urine culture colony counts on the diagnosis and treatment of urinary tract infection for hospitalized patients, Am. J. Clin. Pathol. 137 (2012) 778–784.
[5] A. Deisingh, M. Thompson, Strategies for the detection of *Escherichia coli* O157:H7 in foods, Appl. Microbiol. 96 (2004) 419–429.
[6] S. Radu, G. Rusul, O. Ling, E. Purwati, M. Mustakim, S. Lihan, Rapid isolation and detection of *Escherichia coli* O157:H7 by use of rainbow agar O157 and PCR assay, Southeast Asian, J. Trop. Med. Public Health 31 (2000) 77–79.
[7] P. Boulanger, L. Letellier, Characterization of ion channels involved in the penetration of phage T4 DNA into *Escherichia coli* cells, Biol. Chem. 263 (1988) 9767–9775.
[8] N. Nikkhoo, P. Gulak, K. Maxwell, Rapid detection of *E. coli* bacteria using potassium-sensitive FETs in CMOS, IEEE Trans. Biomed. Circuits Syst. 7 (2013) 621–630.
[9] A. Shabani, M. Zourob, B. Allain, C. Marquette, M. Lawrence, R. Mandeville, Bacteriophage-modified microarrays for the direct impedimetric detection of bacteria, Anal. Chem. 80 (2008) 9475–9482.
[10] L. Goodridge, J. Chen, M. Griffiths, Development and characterization of a fluorescent bacteriophage assay for detection of *Escherichia coli* O157:H7, Appl. Environ. Microbiol. 65 (1999) 1397–1404.
[11] M. Namura, T. Hijikata, K. Miyanaga, Y. Tanji, Detection of *Escherichia coli* with fluorescent labeled phages that have a broad host range to *E. coli* in sewage water, Biotechnol. Prog. 24 (2008) 481–486.
[12] M. Oda, M. Morita, H. Unno, Y. Tanji, Rapid detection of *Escherichia coli* O157:H7 by using green fluorescent protein-labeled PPO1 bacteriophage, Appl. Environ. Microbiol. 70 (2004) 527–534.
[13] R. Edgar, M. McKinstry, J. Hwang, A. Oppenheim, R. Fekete, G. Giulian, C. Merril, K. Nagashima, S. Adhya, High-sensitivity bacterial detection using biotin-tagged phage and quantum-dot nanocomplexes, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 4841–4845.
[14] A. Brown, Benson's Microbiological Applications, , Complete Version, 9th ed., McGraw-Hill, New York, 2005.
[15] E. Pollard, W. Solosko, The thermal inactivation of T4 and lambda bacteriophage, Biophys. J. 11 (1971) 66–74.
[16] F. Mortimer, D. Mason, V. Gant, Flow cytometric monitoring of antibiotic-induced injury in *Escherichia coli* using cell-impermeant fluorescent probes, Antimicrob. Agents Chemother. 44 (2000) 676–681.
[17] J. Porter, C. Edwards, R. Pickup, Rapid assessment of physiological status in *Escherichia coli* using fluorescent probes, J. Appl. Bacteriol. 79 (1995) 399–408.
[18] R. Batchelor, M. Zhou, Use of cellular glucose-6-phosphate dehydrogenase for cell quantitation: applications in cytotoxicity and apoptosis assays, Anal. Biochem. 329 (2004) 35–42.
[19] S. Zimmerman, S. Trach, Estimation of macromolecular concentrations and excluded volume effects for the cytoplasm of *E. coli*, J. Mol. Biol. 222 (1991) 599–620.
[20] M. Turel, A. Duerkop, A. Yegorova, A. Karasyov, Y. Scripinets, A. Lobnik, Microtiterplate phosphate assay based on luminescence quenching of a terbium complex amenable to decay time detection, Anal. Chim. Acta 675 (2010) 42–48.
[21] M. Jagoda, R. Krämer, Screening of ATP hydrolysis by Zr(IV) and Eu(III) complexes, Inorg. Chem. Commun. 8 (2005) 697–699.
[22] C. Spangler, C. Spangler, M. Schäerling, Luminescent lanthanide complexes as probes for the determination of enzyme activities, Ann. N. Y. Acad. Sci. 1130 (2008) 138–148.
[23] R. Curtis, Biological containment and cloning vector transmissibility, J. Infect. Dis. 5 (1978) 668–675.
[24] A. Chibeu, E.J. Lingohr, L. Masson, A. Manges, J. Harel, H.W. Ackermann, A.M. Kropinski, P. Boerlin, Bacteriophages with the ability to degrade uropathogenic *Escherichia coli* biofilms, Viruses 4 (2012) 471–487.