2025, 65(7): 2785-2798
CSTR: 32112.14.j.AMS.20240796
DOI: 10.13343/j.cnki.wsxb.20240796

微生物学报
*Acta Microbiologica Sinica*
http://journals.im.ac.cn/actamicrocn

Review 综述

# ATP 生物发光法检测抗菌药敏感性影响因素的研究进展

于莹[1]，林淦[1]，王佳慧[1]，周喆麒[2]，付玉和[3]，宋厚辉[1]，程昌勇[1*]，吴芹[1*]

1 浙江农林大学 动物医学院，浙江省畜禽绿色生态健康养殖应用技术研究重点实验室，动物健康互联网检测技术浙江省工程研究中心，浙江省动物医学与健康管理国际科技合作基地，同一健康和食品安全一带一路国际联合实验室，中澳动物健康大数据分析联合实验室，浙江 杭州
2 衢州市衢江区农业农村局，浙江 衢州
3 浙江领与生物科技有限公司，浙江 杭州

于莹, 林淦, 王佳慧, 周喆麒, 付玉和, 宋厚辉, 程昌勇, 吴芹. ATP 生物发光法检测抗菌药敏感性影响因素的研究进展[J]. 微生物学报, 2025, 65(7): 2785-2798.
YU Ying, LIN Gan, WANG Jiahui, ZHOU Zheqi, FU Yuhe, SONG Houhui, CHENG Changyong, WU Qin. Influencing factors of ATP bioluminescence-based antimicrobial susceptibility testing: a review[J]. Acta Microbiologica Sinica, 2025, 65(7): 2785-2798.

**摘　要：**抗菌药敏感性试验在抗菌药残留检测、细菌性疾病的预防和治疗领域具有重要意义。传统的抗菌药敏感性试验需要经过细菌培养和菌落计数等过程，耗时且劳力。ATP 生物发光法已被广泛用于评估食品和医疗卫生中的细菌污染，似乎是一种快速、灵敏的抗菌药敏感性测试替代技术。本文就 ATP 生物发光法的性能影响因素进行综述，主要综述这些因素如何影响该方法的检测能力，为临床、环境和食品分析中的抗菌药敏感性分析提供参考。

**关键词：**抗菌药敏感性试验；ATP 生物发光；影响因素

资助项目：国家重点研发计划(2023YFD1801000)；浙江农林大学科研发展基金(2021LFR044)
This work was supported by the National Key Research and Development Program of China (2023YFD1801000) and the Zhejiang A&F University Talents Starting Program (2021LFR044).
*Corresponding authors. E-mail: CHENG Changyong, lamge@zafu.edu.cn; WU Qin, wuqin@zafu.edu.cn
Received: 2024-12-10; Accepted: 2025-02-24; Published online: 2025-03-28

# Influencing factors of ATP bioluminescence-based antimicrobial susceptibility testing: a review

**YU Ying[1], LIN Gan[1], WANG Jiahui[1], ZHOU Zheqi[2], FU Yuhe[3], SONG Houhui[1], CHENG Changyong[1*], WU Qin[1*]**

1 Key Laboratory of Applied Biotechnology on Green-eco-healthy Animal Husbandry of Zhejiang Province, Zhejiang Provincial Engineering Research Center for Animal Health Diagnostics & Advanced Technology, Zhejiang International Science and Technology Cooperation Base for Veterinary Medicine and Health Management, Belt and Road International Joint Laboratory for One Health and Food Safety, China-Australia Joint Laboratory for Animal Health Big Data Analytics, College of Veterinary Medicine, Zhejiang A&F University, Hangzhou, Zhejiang, China

2 Qujiang District Bureau of Agriculture and Rural Affairs, Quzhou, Zhejiang, China

3 Zhejiang Ling Yu Bio-Sci&Tech Co., Ltd., Hangzhou, Zhejiang, China

**Abstract:** Antimicrobial susceptibility testing (AST) is of great significance for the detection of antibiotic residues, as well as the prevention and treatment of bacterial diseases. Conventional methods of AST are time-consuming and labor-intensive due to the processes of bacterial culture and colony counting. ATP bioluminescence has been widely employed to assess bacterial contamination in food and healthcare and appears to be a rapid and sensitive alternative to conventional methods of AST. This review illustrates the influencing factors of ATP bioluminescence-based AST in detail. It mainly focuses on how these factors influence the detection performance of the method, giving insights into AST in clinical, environmental, and food analyses.

**Keywords:** antimicrobial susceptibility testing; ATP bioluminescence; influencing factors

Antimicrobial agents are common for clinically preventing and treating bacterial diseases in humans and animals[1-3]. As shown in Figure 1, it is the metabolic process of antibiotics after being consumed during the entire food chain. However, inappropriate use of antibiotics can lead to residues in the body of humans and animals, food, medical facilities, and the environment, which threatens human and animal's health. However, the presence of antibiotic resistance to pathogenic bacteria is generally considered a main risk to public health[4].

To avoid the further emergence of antibiotic resistance, it is important to monitor antibiotic usage clinically and to monitor antibiotic residual levels in the body of humans and animals[5-7], foods[8], medical facilities[9], and the environment[10]. Moreover, it is of great significance to monitor the occurrence of antibiotic resistance and screen appropriate antibiotics or antibiotic combinations against drug-resistant bacteria, as this can result in the treatment failure of bacterial infection in clinical settings[11]. However, traditional antibiotic susceptibility tests (ASTs) take 18−24 h, therefore there is a need for developing faster and more accurate ASTs. To overcome the shortcomings of traditional ASTs, researchers have developed ASTs based on ATP bioluminescence with shorter detection times and

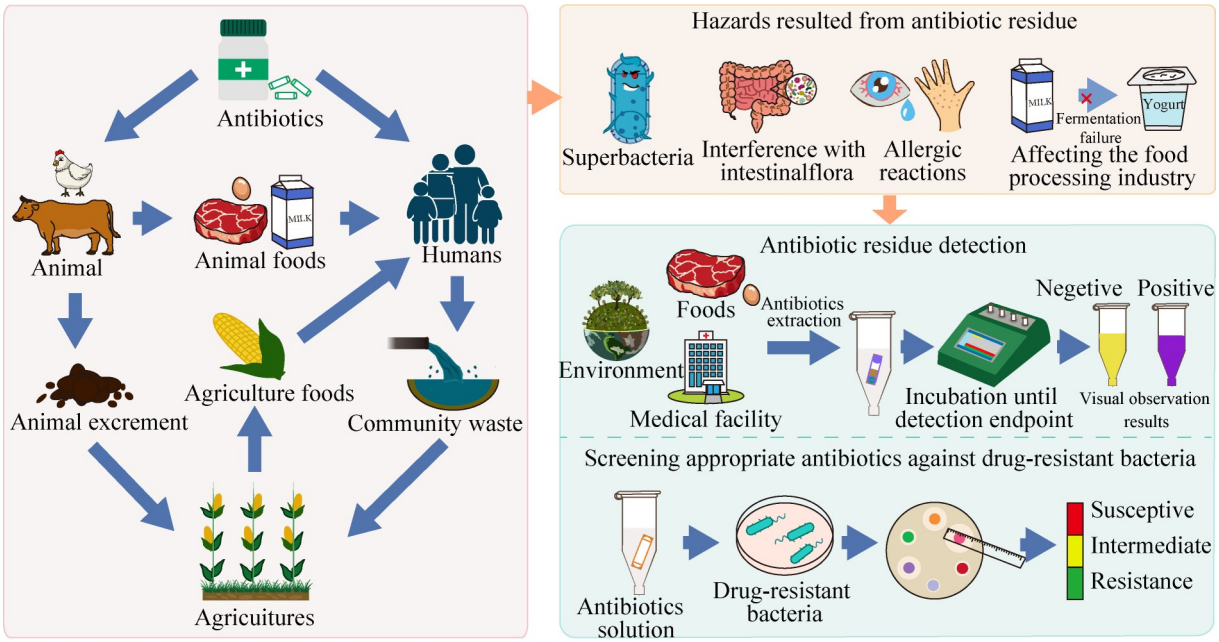


Figure 1 The consequences caused by inappropriate usage of antibiotics and its corresponding solutions.

higher sensitivity[11-13].

To promote the development of ATP bioluminescence-based ASTs, this review illustrates how these influencing factors influence the detection capability of ATP bioluminescence-based ASTs, which supports a theoretical and practical basis for the future research on ATP bioluminescence-based ASTs and has important significance for ensuring of food safety and public health.

## 1 Principle of the ATP bioluminescence-based ASTs

ATP is the primary energy source for the metabolism of all living cells. When the growth of bacteria is inhibited, the ATP content in bacterial cells decreases or disappears immediately[14]. Therefore, ATP can be used as a marker of viable bacteria. The test principle of the present detection system is based on the inhibitory effect of antibiotics on bacteria, which reduces the content of ATP in the bacteria[13]. As illustrated in Figure 2, if there are no antibiotics in the samples, the bacteria would grow normally. The luciferin would be oxidized to oxyluciferin and emit bioluminescence with the role of $O_2$, $Mg^{2+}$, and ATP (released from live bacteria by benzalkonium chloride) [15]. Moreover, the bioluminescence intensity corresponds to the content of ATP in live bacteria[12]. In contrast, if there are antibiotics in the samples (including bacteriostatic and bactericidal drugs), the antibiotics would inhibit or kill the bacteria, resulting in a decreased content of ATP in the bacteria. Furthermore, the decrease in ATP is estimated according to the formula (1).

Inhibition rate= (bioluminescence intensity of negative control − bioluminescence intensity of samples)/bioluminescence intensity of negative control×100%[13] (1)

Accordingly, the antibiotic residue levels are consistent with the inhibition rate. In past years, some ATP bioluminescence-based ASTs have been developed. For example, an ATP bioluminescence

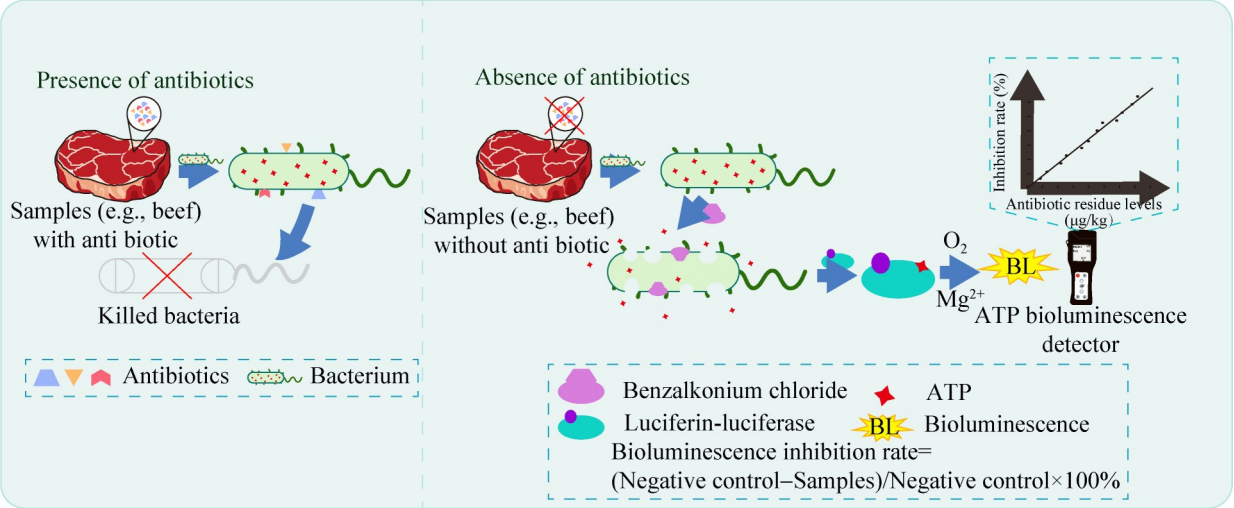


Figure 2 Schematic diagram for the detection principle of ATP bioluminescence-based ASTs.

assay with *Geobacillus stearothermophilus* ATCC12980 as an indicator bacterium was developed to detect multiclass antibiotic residues in animal derived foods[13]; an ATP bioluminescence assay was developed to evaluate antibiotic combinations against drug-resistant *Pseudomonas aeruginosa*[12]. Moreover, Puig-Collderram et al. indicated that the results of the ATP bioluminescence assay correlated with the traditional colony count method, and the ATP bioluminescence assay was less laborious and faster than the traditional colony count method[12].

## 2 Influence factors of ATP bioluminescence-based ASTs

As shown in Figure 3, these are the important components and influencing factors of ATP bioluminescence-based ASTs. Firstly, the target analytes (antibiotics or bacteria) are captured from samples during the process of sample pre-treatment. The sample pre-treatment can reduce the matrix interferences and improve specificity by using a capture probe (e. g., phage, aptamer), and thus extend the practicability of the methods. After sample pre-treatment, the captured bacteria or antibiotics are incubated with antibiotics (for captured bacteria), bacteria (for captured antibiotics), nutrients, sensitizers, etc. in a microbiological culture system. If the bacteria were sensitive to antibiotics, they will be killed by them. In contrast, the bacteria would survive normally. Afterward, the ATP in the surviving bacteria can be extracted by ATP extractants (e. g., benzalkonium chloride). Moreover, the luciferin will be oxidized to oxyluciferin and emit bioluminescence with the role of $O_2$, $Mg^{2+}$, and the extracted ATP. During the process of ATP bioluminescence-based ASTs, the influencing factors include sample pre-treatment, bacteria strains, bacteria concentration, nutrients, sensitizers, pH, non-microbial ATP, ATP extractants, and luciferase activity. However, how these factors influence the performances of ATP bioluminescence- based ASTs is shown in detail as follows.

### 2.1 Sample pre-treatment

#### 2.1.1 For evaluating antibiotic residual levels

The sample pre-treatment methods can help

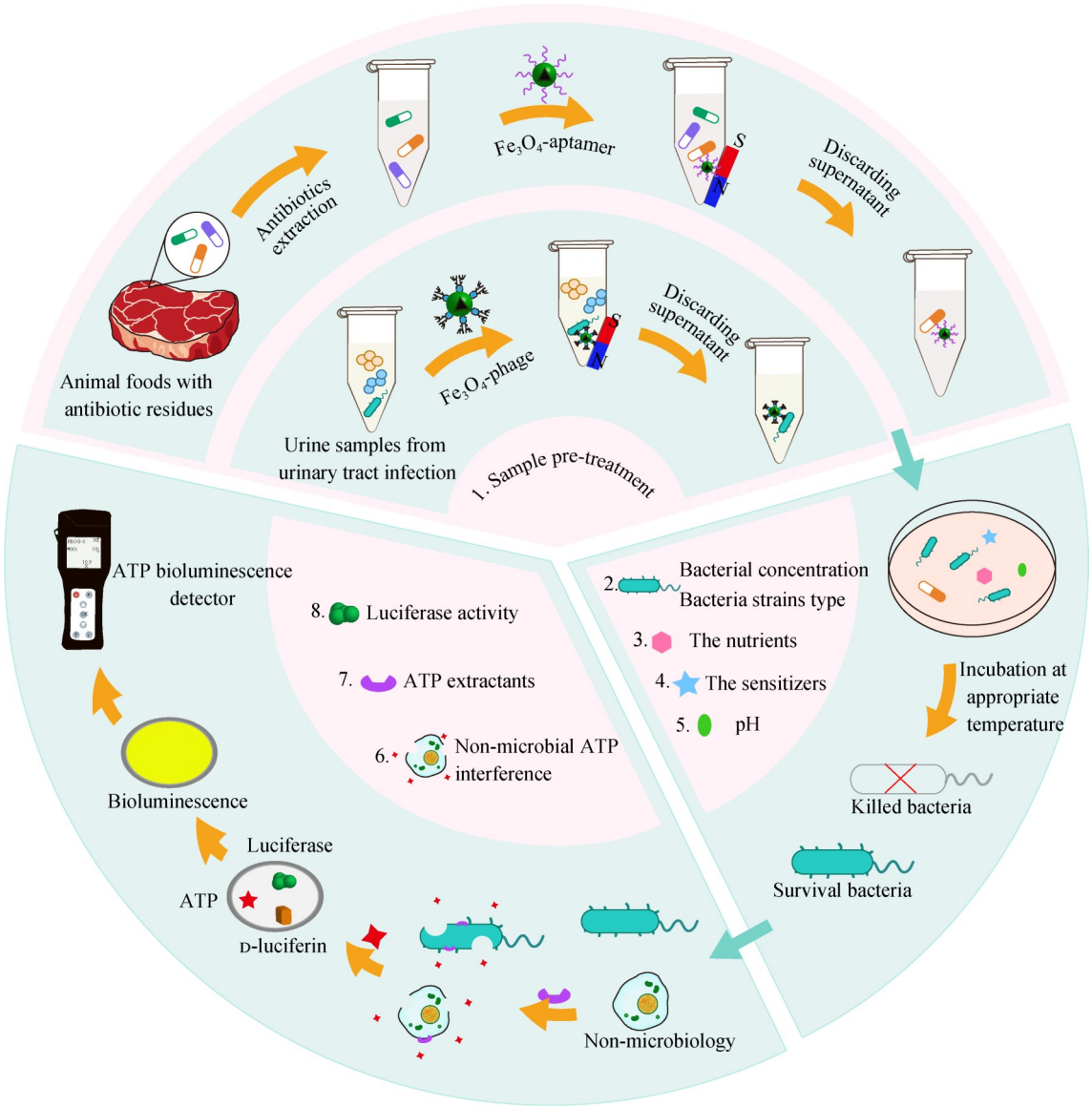


Figure 3 Schematic diagram for the influence factors of ATP bioluminescence-based ASTs.

improve the applicability of the methods by reducing matrix interferences. The interferences in animal matrices, such as impurities and natural bacteriostatic substances, are extracted during the extraction process, which can lead to false negative and false positive results. Some studies eliminated impurities by centrifugation[16]. However, these measures still cannot completely eliminate natural bacteriostatic substances in matrices. In the research of Pikkemaat et al. [16], a 0.1 mol/L phosphate buffer (pH 6.0) was added to the kidney to dilute the interferences and adjust the tissue's pH. Nonetheless, the amount of phosphate buffer added to the tissue was small, making the tissue unable to mix with the phosphate buffer adequately. The detection results might have been better if enough phosphate buffer had been added to the tissue. It was reported that incubation in a water

bath at 80 ℃ for 10 min can effectively inhibit natural antibacterial substances in animal matrices[17]. Wu et al. also used a similar method to decrease the matrix interferences from matrices[18]. If the incubation in a water bath and phosphate buffer were operated together, the detection results would be better. Thanks to the development of specific receptors (e. g., antibodies, aptamer) and magnetic nanoparticles, the target analytes are captured by specific receptors and can be quickly separated by magnetic nanoprobes and external magnets, which can reduce matrix interferences further and improve the specificity of assays. The receptors and magnetic nanoparticles have already been used in other antibiotic residue detection methods for sample pre-treatment. For example, ssDNA was used as the sensing probe integrated with a gold nanoparticle, which responds rapidly to ampicillin with a low limit of detection (0.556 pmol/L)[19]. In future studies, the receptors and magnetic nanoparticles incorporated into the existing sample pre-treatment methods of ATP bioluminescence-based antibiotic residues detection methods, the detection results would be better.

### 2.1.2 For evaluating the sensitivity of pathogenic microorganisms to antibiotics

Unlike antibiotic residue detection, the sample pre-treatment of incubation at high temperature is not suitable for evaluating the sensitivity of pathogenic microorganisms to antibiotics. Nonetheless, after bacterial phenotypic identification, the samples can be diluted with a detection buffer to reduce matrix interference. For example, the samples were centrifuged at 2 000×*g* for 10 min to remove the blood corpuscles, and the resulting supernatant and bacterial pellet were homogenized and then diluted 30 000 times with the broth[20]. Urine specimens with pathogenic bacteria were diluted with trypticase soy broth and were introduced into the detection system by Ivančić et al. [21]. Above all, these evaluation experiments of pathogenic microorganisms' sensitivity to antibiotics need bacterial phenotypic identification, which is more complex to operate and time-consuming. The introduction of receptors and magnetic nanoparticles promotes the sample pre-treatment efficiency. The target bacteria can be captured by specific receptors, and quickly separated by magnetic nanoprobes with external magnets, which can not only reduce matrix interferents futher, but also perform bacterial phenotypic identification. For example, the urinary tract infections pathogen species in an artificial urine samples with capture antibodies immobilized on the carrier, which was then quantified *via* an ATP bioluminescence assay and given personalized antibiotic therapy[22]. However, antibodies are complicated to prepare, high-cost, and susceptible to harsh environments (e. g., pH, temperature, organic reagents). Nonetheless, aptamers are easy to synthesize, stable to preserve for a long time, and have a wider range of potential targets. For example, Wang et al. developed a hand-held agitator array modified with hyperbranched aptamer probes for the specific adsorption extraction of *Salmonella typhimurium* in complex water samples[23]. However, the aptamers require long incubation times with analytical targets. Moreover, the conformation of aptamers is inconstant, and thus the target capture rate is variable. Compared to antibodies and aptamers, phages can be produced on a large scale easily and inexpensively in most microbiology laboratories. Furthermore, phages can distinguish between live and dead bacteria. An ATP bioluminescence assay was developed with phage-functionalized magnetic

beads for rapid and sensitive detection of *Vibrio parahaemolyticus* in aquatic products by Du et al.[24]. The *Vibrio parahaemolyticus* were captured and enriched by phage VPHZ6 coupled to a magnetic bead, which shortened detection time and improved method sensitivity[24]. Furthermore, Li et al. developed a similar ATP bioluminescence assay for *Staphylococcus aureus* detection[25]. The phage-functionalized magnetic beads brought a tenfold increase in sensitivity during detection in skim milk and chicken samples, with high recoveries of *Staphylococcus aureus* in samples[25]. However, the phages are susceptible to harsh environments (pH, temperature, organic reagents). Above all, these specific receptors (antibody, aptamer, phages) have great potential but some shortcomings. Therefore, in future studies, the sensitivity and specificity of these specific receptors should be further improved.

## 2.2 Test bacteria

### 2.2.1 Bacterial strains type

The sensitivity of different kinds of bacteria to antibiotics is different. Moreover, the sensitivity of a single bacterial strain to various antibiotics is different. Therefore, many research studies were conducted to screening antibiotics or antibiotic combinations to overcome bacterial infections clinically. In general, many researchers commit themselves to screen antibiotic or antibiotic combinations to overcome urethritis[26] and bacteremia[27] clinically. Even with the presence of antibiotic resistance, there are still no appropriate antibiotics to prevent infections. Therefore, there is a need to develop new drugs or new antibiotic combinations to fight against bacterial infections[28]. Moreover, in the antibiotic detection field, researchers focus on screening test bacteria that are sensitive to as many antibacterials as possible. The commonly used test bacteria are *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus stearothermophilus*, *Micrococcus luteus*, *Escherichia coli*, etc. Among them, *Bacillus stearothermophilus* is the most used test bacterium for its sensitivity to more antibiotics. Moreover, it is environmentally friendly and shortens the detection time of assays by growing quickly[18,29]. However, *Bacillus stearothermophilus* is insensitive to certain antibiotics (especially quinolones). Therefore, it is necessary to improve the sensitivity of test bacteria to antibiotics by other methods or develop engineered bacteria with higher sensitivity to antibiotics.

### 2.2.2 Bacterial concentration

As we know, bacterial concentration influences the therapeutic effect of antibiotics and therapeutic time clinically. Furthermore, in the antibiotic detection field, the bacterial concentration in the detection system is inversely proportional to the sensitivity and detection time of the method. The higher bacterial concentration results in lower sensitivity and a shorter detection time, while the lower bacterial concentration results in higher sensitivity and longer detection time. Moreover, some natural bacteriostatic substances in samples can inhibit the growth of test bacteria in the detection system and thus produce false positive results and longer detection time[5,29-30]. For that, some studies increased the concentration of indicator bacteria in the kit's medium to reduce the interference of natural bacteriostatic substances in samples. For example, the STOP with *Bacillus subtilis* ($10^7$ CFU/mL) as an indicator bacterium was developed for screening antibiotics in the kidney[5]. Based on the STOP, the LAST was developed to detect antimicrobial agents in urine by increasing the concentration of *Bacillus subtilis* spores ($10^9$ CFU/mL) in the kit's medium to reduce

the interference of natural bacteriostatic substances in the urine[5]. However, if the study increases the concentration of test bacteria in the kit's medium, it would prolong the detection time. Therefore, it is crucial to screen the appropriate test bacterial concentration in ASTs.

## 2.3 The nutrients in the medium

The nutrients in medium components are related to the recovery of bacteria strains and the growth of bacteria, which affects the detection time in ATP bioluminescence-based ASTs. A lack of nutrients would slow the growth rate of bacteria, thus resulting in a longer detection time. Wu et al. [18] found that the bacteria growth inhibition based-ASTs were sensitive to kinds of antibiotics in antimicrobial agent diluents, milk, egg, and honey, and the detection time of the method in milk, honey, chicken egg, and antimicrobial agents diluents were 3, 3.25, 3.5, and 3.75 h, respectively, because the milk, chicken egg, and honey are richer in nutrients than antimicrobial agents diluents, which can promote the growth of bacteria in the medium and shorten detection time. Moreover, some bacillus strains were used as test bacteria and the bacillus spores need germination factors to germinate and then grow. Therefore, the $Ca^{2+}$ used in the medium promoted the germination of *Bacillus stearothermophilus* spores[6,30]. Furthermore, the medium broth was used to prepare samples and thus improve the nutrients in the medium. For example, Matsui et al. [20] developed a rapid ATP bioluminescence method to detect levofloxacin-resistant bacteria in blood specimens. The blood specimen was centrifuged and then diluted 30 000 times with broth to obtain bacterial suspension for ATP measurement[20]. Some ATP-Bioluminescence assays were used to rapidly identify uropathogenic microbes. And the trypticase soy broth was used to dilute urine specimens to support enough nutrients for bacterial growth[21-22]. However, the existing ASTs still have a long detection time. Therefore, it is necessary to shorten detection time by improving the nutrient conditions in the medium further.

## 2.4 The sensitizers in medium

No single bacterial strain is sensitive to all kinds of antibiotics. Nevertheless, the sensitizers can help improve the sensitivity of test bacteria to antibiotics. The most common sensitizers are trimethoprim (TMP) and chloramphenicol (CAP). TMP and CAP are used to improve the bacteriostatic effect of sulfonamides and tetracyclines through different bacteriostatic mechanisms compared to the target antibiotics group[18,30]. Moreover, some ASTs use sensitizers with the same bacteriostatic mechanism as the target antibiotics group. For example, tylosin was added to the NK-B&M plate agar medium to increase the sensitivity of this test plate to macrolides, furthermore, the supplement cloxacillin was added into the detection hole to improve the bactericidal activity of β-lactams[16]. Similarly, gentamicin was used to improve the sensitivity of *Bacillus stearothermophilus* to aminoglycosides[7]. Above all, these sensitizers work with antibiotics against non-resistant bacteria. When drug-resistant or multi-drug-resistant bacteria emerge, such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, it prompted efforts to develop new antibiotics to overcome this problem. For that, researchers devoted themselves to developing new therapeutic strategies against resistant bacteria by combining antibiotics with sensitizers, nanoparticles, natural products, bacteriophages, antibodies, photodynamic therapy, etc.[31-32]. All of these sensitizing means have potential but

shortcomings. Moreover, the development of sensitizers is an ongoing research area, and new types of sensitizers may emerge in the future.

## 2.5 The pH value in the medium

The commonly used antibiotics include β-lactams, aminoglycosides, tetracyclines, macrolides, sulfonamides, lincosamides, and quinolones. Among these antibiotics, the aminoglycosides, macrolides, sulfonamides, lincosamides, and quinolones have more robust bacteriostasis activity in alkaline conditions, while the β-lactams and tetracyclines work better in acid conditions. Therefore, researchers used different antibiotics to overcome urethritis[33] and bacteremia[20] clinically, depending on their bacteriostatic activity at the optimal pH. Furthermore, in the detection field, the detection medium's pH is responsible for the growth of test bacteria and the bacteriostasis effect of antibiotics. Therefore, some ASTs use multiple Petri dishes at different pH levels for corresponding antibiotics. For example, Dang et al. developed a two-plate method with the test bacteria *Bacillus subtilis* to screen tetracyclines, sulfonamides, and quinolones in shrimp on two Petri plates (pH 6.0 and 7.5) [34]. The EC four-plate method was developed to detect β-lactams, aminoglycosides, and quinolones in muscle and kidney with three Petri dishes in pH 6.0, 7.2, and 8.0, respectively, using *Bacillus subtilis* as the test bacterium. Above all, these ASTs are conducted *in vitro*. Therefore, the cases are different when the ASTs are present *in vivo*. Therefore, the sensitivity of bacteria to antibiotics *in vivo* can be improved not only by adjusting pH value.

## 2.6 The non-microbial ATP interferences

The main purpose of the ATP bioluminescence method is to measure the amount of ATP in test bacteria. However, non-microbial ATP in the sample, such as free ATP and ATP in animal somatic cells, is the major factor that affects the accuracy and precision of the ATP bioluminescence method[35]. Therefore, it is essential to eliminate the interference of non-bacterial ATP. To reduce the interference of free ATP, some studies used apyrase or enzyme combinations to hydrolyze free ATP[36]. Furthermore, to improving the activity of apyrase, some researchers developed a recombinant *Shigella flexneri* apyrase, which revealed superior activity by eliminating the extracellular ATP[37]. Nonetheless, Wu et al. [13] showed that the amount of ATP in tissue was negligible compared to the amount of ATP in indicator bacteria. The reasons for this are that ATP is extremely unstable and easy to degrade at high temperatures, and the sample pre-treatment method of water bath (80 ℃, 10 min) seriously degrades the content of ATP in tissues[13]. Moreover, the content of ATP in tissues continually degrades during incubation at 65 ℃ in the incubator. Accordingly, there is no need to eliminate non-bacterial ATP in tissues for considering the simplification of test operation. However, when the ASTs are present in other temperatures (e.g., 37 ℃) and without incubation at 80 ℃ for 10 min, it should reevaluate whether there is needed to eliminate non-bacterial ATP in samples.

## 2.7 ATP extractants

The extraction efficiency of bacterial ATP is vital for the accuracy of the ATP bioluminescence method detection results. Therefore, some extraction methods should be able to break the cell membrane entirely and release ATP quickly. Secondly, ATPase can be denatured and inactivated to protect the extracted ATP from being hydrolyzed by ATPase. Thirdly, they are no effect on luciferase

activity, or the effect is little[38]. ATP can be extracted from living organisms by various methods, and different ATP extractants are applied to specific living organisms. For example, trichloroacetic acid (TCA) is suitable for extracting ATP from microorganisms (such as *Escherichia coli* and *Bacillus subtilis*) and somatic cells. However, TritonX-100 is only suitable for extracting ATP from somatic cells such as whole blood cells, erythrocytes, and granulocytes. It was reported that cationic surfactant benzalkonium chloride (BAC) could break the structure of bacterial cell walls and fully release ATP with the same extraction effect as TCA[13]. Moreover, it can keep ATP stable and not be hydrolyzed by ATPase to a certain extent. In addition, it has less effect on luciferase activity than TCA does.

However, results showed that *Listeria monocytogenes* was tolerant to BAC[39]. Therefore, there is a need to develop new ATP extraction methods, which cannot produce resistance to ATP extractants easily. It was reported that Au and Ag nanoparticles were also used to extract ATP[38]. Moreover, results showed that gold nanoparticles stabilized with BAC not only extracted ATP effectively but also enhanced ATP bioluminescent signal intensity depending on metal-enhanced bioluminescence[38]. Nevertheless, these methods cannot selectively extract ATP from targeted bacteria, because they can hardly distinguish different sources of bacteria. To that end, Kim et al. effectively extracted ATP from targeted bacteria following antibody-conjugated gold nanorods and photothermal lysis[40]. The introduction of antibody-conjugated gold nanorods achieved the specific recognition of targeted bacteria. The photothermal lysis phenomenon is associated with electromagnetic radiation of gold nanorods by near-infrared (NIR) irradiation. The introduction of photothermal lysis avoids the risk of bacterial resistance during ATP extraction and processing. Nonetheless, in future studies, the electromagnetic radiation ability of gold nanorods can be improved further by preparing nanohybrids with other photothermal nanomaterials, such as $Fe_3O_4$ and GO.

## 2.8 Luciferase activity

It is known that practical applications of luciferases are hindered by their delicate nature against harsh conditions (e. g., chemical agents, temperature)[41]. For instance, ATP extractants, such as TCA, TritonX-100, and BAC, have some inhibitory effects on luciferase. Moreover, the luciferase-luciferin system is required to be stored and shipped either between 4 °C and 8 °C or −20 °C, which is limited application for point-of-care and field analysis, especially in remote areas or low-resource settings. To solve these problems, Hattori et al.[42] isolated a luciferase-LL-2LLL7L490K with low sensitivity to BAC. It is a commercially available enzyme mutant that maintains high enzymatic activity in the presence of higher concentrations of BAC, ensuring that ATP can be extracted effectively. Branchini et al. developed a novel luciferase PLG2 to study the enhanced catalytic performance of the embedded enzyme PpyLit and found that the embedded enzyme had two times more catalytic efficiency and 1.4 times more bioluminescence quantum yield than the original *Photinus pyralis* enzyme[43]. Above all, several attempts were performed to improve luciferase activity by genetic mutation or site-directed mutagenesis for modifying the luciferase enzymes.

Nevertheless, there is still needed to protect luciferase activity during the application process of luciferase. As we all know, gold nanoparticles

provide excellent biocompatibility for enzyme immobilization. Therefore, some researchers investigated the results of luciferase proximity with gold nanoparticles and hybrid gold nanoparticles. Results showed that the gold nanoparticles improved the activity of luciferases 3−5 times higher than that of free enzymes[44]. Moreover, the signal stability improved in the presence of nanoparticles. To improve the long-term stability of enzymes without special storage conditions, some studies immobilized reagents (both enzyme and substrate) on solid support (such as paper). However, a portion of luciferase loses enzyme activity after immobilization on a solid support, which inevitably increases total assay cost[45]. Some researchers developed an innovative freeze-drying procedure, which enabled luciferase and reagents immobilized on paper to show higher reproducibility and enzyme stability than previously reported methods[45]. To improve the luciferase activity on a solid support further, the luciferase can be immobilized onto nanosized supports. As we all know, metal-organic frameworks (MOFs) have been shown as a versatile host platform for the immobilization of proteins, with the potential to protect proteins in harsh conditions. Therefore, some studies combined a new luciferase mutant with zeolitic imidazolate framework-8 (ZIF-8) to stabilize the activity of the luciferase-luciferin system[46]). Results showed that the ZIF-8 not only provided a five-fold increase in the bioluminescence signal intensity but also significantly improved the stability of the sensor[41]. However, the performances of nanosized supports for luciferase should be improved in future study by expanding surface area of nanosized supports and improving their resistance to harsh conditions.

# 3 Perspective

Above all, this review illustrates the influencing factors of ATP bioluminescence-based ASTs in detail. These ASTs can expand the detection spectrum, improve sensitivity, and shorten detection time by optimizing the bacterial types, bacterial concentrations, and medium components. Moreover, their sensitivity can be further improved by optimizing the ATP bioluminescence system (non-microbial ATP interferences, ATP extractants, and activity of luciferase). Additionally, it can expand their applicability by optimizing the sample pre-treatment method.

The future ATP bioluminescence-based ASTs will be developed with higher rapidity, higher sensitivity, and easier of operation so that anyone can operate them by following the instructions. Moreover, the miniaturized test instrument is easy to carry around and suitable for on-site monitoring. These ASTs can timely monitor the antimicrobial residue levels in food, medical care, and the environment, and thus timely prevent and manage it. Moreover, it can determine whether a particular strain of bacteria is developing drug resistance and determine the most effective antibiotic treatment for a bacterial infection, which promotes the development of new drugs and drug combinations. It is believed that the ATP bioluminescence-based method will be widely used in all fields of people's daily lives, and promote the improvement of people's living standards under the continuous advancement and perfection of ATP bioluminescence detection technology.

# Author Contributions

YU Ying: Literature review and writing of the review text; LIN Gan: Literature review and

writing of the review text; WANG Jiahui: Literature review; ZHOU Zheqi: Literature review; FU Yuhe: English editing; SONG Houhui: English editing; CHENG Changyong: Determination of the review theme and English editing; WU Qin: Determination of the review theme, revision of the review text, and English editing.

## Declaration for conflict of interests

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## REFERENCES


[1] CAIRNS KA, UDY AA, PEEL TN, ABBOTT IJ, DOOLEY MJ, PELEG AY. Therapeutics for vancomycin-resistant enterococcal bloodstream infections[J]. Clinical Microbiology Reviews, 2023, 36(2): e0005922.

[2] BROUWER S, RIVERA-HERNANDEZ T, CURREN BF, HARBISON-PRICE N, de OLIVEIRA DMP, JESPERSEN MG, DAVIES MR, WALKER MJ. Pathogenesis, epidemiology and control of group A *Streptococcus* infection[J]. Nature Reviews Microbiology, 2023, 21: 431-447.

[3] ZENG M, XIA J, ZONG ZY, SHI Y, NI YX, HU FP, CHEN YJ, ZHUO C, HU BJ, LV XJ, LI JB, LIU ZY, ZHANG J, YANG WJ, YANG F, YANG QW, ZHOU H, LI X, WANG JH, LI YM, et al. Guidelines for the diagnosis, treatment, prevention and control of infections caused by carbapenem-resistant Gram-negative bacilli[J]. Journal of Microbiology, Immunology and Infection, 2023, 56(4): 653-671.

[4] RAGASSA S, BERHANU G. Antibiotic use, awareness of antimicrobial resistance and residue in veterinary professionals and farmers in selected districts of Kellem Wollega zone, Ethiopia[J]. Veterinary Medicine, 2023, 14: 159-175.

[5] WU Q, ZHU Q, ABU BAKR SHABBIR M, SATTAR A, PENG DP, TAO YF, CHEN DM, YUAN ZH, WANG YL. The search for a microbiological inhibition method for the rapid, broad-spectrum and high-throughput screening of six kinds of antibiotic residues in swine urine[J]. Food Chemistry, 2021, 339: 127580.

[6] WU Q, ZHU Q, LIU YN, ABU BAKR SHABBIR M, SATTAR A, PENG DP, TAO YF, CHEN DM, WANG YL, YUAN ZH. A microbiological inhibition method for the rapid, broad-spectrum, and high-throughput screening of 34 antibiotic residues in milk[J]. Journal of Dairy Science, 2019, 102(12): 10825-10837.

[7] WU Q, GAO XY, ABU BAKR SHABBIR M, PENG DP, TAO YF, CHEN DM, HAO HH, CHENG GY, LIU ZL, YUAN ZH, WANG YL. Rapid multi-residue screening of antibiotics in muscle from different animal species by microbiological inhibition method[J]. Microchemical Journal, 2020, 152: 104417.

[8] NIU CY, YAN ML, YAO ZL, DOU JH. Antibiotic residues in milk and dairy products in China: occurrence and human health concerns[J]. Environmental Science and Pollution Research International, 2023, 30(53): 113138-113150.

[9] LAMBERT H, SHEN X, CHAI J, CHENG J, FENG R, CHEN M, CABRAL C, OLIVER I, SHEN J, MacGOWAN A, BOWKER K, HICKMAN M, KADETZ P, ZHAO L, PAN Y, KWIATKOWSKA R, HU X, WANG D. Prevalence, drivers and surveillance of antibiotic resistance and antibiotic use in rural China: interdisciplinary study[J]. PLoS Global Public Health, 2023, 3(8): e0001232.

[10] YU T, RAJASEKAR A, ZHANG SH. A decennial study of the trend of antibiotic studies in China[J]. Environmental Science and Pollution Research, 2023, 30(58): 121338-121353.

[11] SEVER EA, AYBAKAN E, BEŞLI Y, KARATUNA O, KOCAGOZ T. A novel rapid bioluminescence-based antimicrobial susceptibility testing method based on adenosine triphosphate consumption[J]. Frontiers in Microbiology, 2024, 15: 1357680.

[12] PUIG-COLLDERRAM B, DOMENE-OCHOA S, SALVÀ-COMAS M, MONTERO MM, DURAN X, GONZÁLEZ JR, GRAU S, OLIVER A, HORCAJADA JP, PADILLA E, SEGURA C, PRIM N. ATP bioluminescence assay to evaluate antibiotic combinations against extensively drug-resistant (XDR) *Pseudomonas aeruginosa*[J]. Microbiology Spectrum, 2022, 10(4): e0065122.

[13] WU Q, WEI HB, LEI CX, WU RL, ABU BAKR SHABBIR M, PENG DP, TAO YF, CHEN DM, LIU ZL, WANG YL. ATP bioluminescence-based method for screening of multiclass antibiotic residues in animal derived foods[J]. Microchemical Journal, 2023, 193: 108981.

[14] LIU ST, ZHAO JB, GUO YL, MA XE, SUN CM, CAI M, CHI YY, XU K. Application of ATP-based bioluminescence technology in bacterial detection: a review[J]. Analyst, 2023, 148(15): 3452-3459.

[15] CAI YY, SEAH CL, LECK H, LIM TP, TEO JQ, LEE W, TAN TT, KOH TH, EE PLR, KWA AL. Rapid antibiotic combination testing for carbapenem-resistant Gram-negative bacteria within six hours using ATP bioluminescence[J]. Antimicrobial Agents and Chemotherapy, 2018, 62(9): e00183-18.

[16] PIKKEMAAT MG, DIJK SO, SCHOUTEN J, RAPALLINI M, KORTENHOEVEN L, van EGMOND HJ. Nouws antibiotic test: validation of a post-screening

method for antibiotic residues in kidney[J]. Food Control, 2009, 20(9): 771-777.
[17] STEAD S, SHARMAN M, TARBIN JA, GIBSON E, RICHMOND S, STARK J, GEIJP E. Meeting maximum residue limits: an improved screening technique for the rapid detection of antimicrobial residues in animal food products[J]. Food Additives and Contaminants, 2004, 21(3): 216-221.
[18] WU Q, PENG DP, LIU QY, SHABBIR MAB, SAJID A, LIU ZL, WANG YL, YUAN ZH. A novel microbiological method in microtiter plates for screening seven kinds of widely used antibiotics residues in milk, chicken egg and honey[J]. Frontiers in Microbiology, 2019, 10: 436.
[19] WEI XJ, LIU CB, QIN HH, YE ZW, LIU XR, ZONG BY, LI Z, MAO S. Fast, specific, and ultrasensitive antibiotic residue detection by monolayer $WS_2$-based field-effect transistor sensor[J]. Journal of Hazardous Materials, 2023, 443(Pt B): 130299.
[20] MATSUI A, NIIMI H, UCHIHO Y, KAWABE S, NODA H, KITAJIMA I. A rapid ATP bioluminescence-based test for detecting levofloxacin resistance starting from positive blood culture bottles[J]. Scientific Reports, 2019, 9(1): 13565.
[21] IVANČIĆ V, MASTALI M, PERCY N, GORNBEIN J, BABBITT JT, LI Y, LANDAW EM, BRUCKNER DA, CHURCHILL BM, HAAKE DA. Rapid antimicrobial susceptibility determination of uropathogens in clinical urine specimens by use of ATP bioluminescence[J]. Journal of Clinical Microbiology, 2008, 46(4): 1213-1219.
[22] DONG T, ZHAO XY. Rapid identification and susceptibility testing of uropathogenic microbes *via* immunosorbent ATP-bioluminescence assay on a microfluidic simulator for antibiotic therapy[J]. Analytical Chemistry, 2015, 87(4): 2410-2418.
[23] WANG M, CAO C, BI WC, LIN JY, TAN L, GAN N. On-site enrichment and detection of live *Salmonella typhimurium* using a bioluminescence sensor coupled with a hyperbranched aptamer probe-labelled stir-bars array[J]. Sensors and Actuators B: Chemical, 2022, 364: 131862.
[24] DU QL, DING YF, WANG YS, WANG J, SHAO YC, WANG XH. A novel phagomagnetic separation-ATP bioluminescence (PhMS-BL) for rapid and sensitive detection of viable *Vibrio parahaemolyticus* in aquatic product[J]. Food Chemistry, 2024, 439: 138113.
[25] LI RN, LI ZW, HUANG CX, DING YF, WANG J, WANG XH. Phage-based biosensing for rapid and specific detection of *Staphylococcus aureus*[J]. Microorganisms, 2023, 11(8): 2098.
[26] HSU YT, CHUANG TY, HSIAO JC, CHENG WM. Diagnosis and treatment outcomes of urethritis-like symptoms in young males: a retrospective cohort study[J]. Scientific Reports, 2023, 13(1): 17469.
[27] MINTER DJ, APPA A, CHAMBERS HF, DOERNBERG SB. Contemporary management of *Staphylococcus aureus* bacteremia-controversies in clinical practice[J]. Clinical Infectious Diseases, 2023, 77(11): e57-e68.
[28] XIAO G, LI JY, SUN ZL. The combination of antibiotic and non-antibiotic compounds improves antibiotic efficacy against multidrug-resistant bacteria[J]. International Journal of Molecular Sciences, 2023, 24(20): 15493.
[29] WU Q, LIU XS, DUN XL, ABU BAKR SHABBIR M, PENG DP, YUAN ZH, WANG YL. The screening and identification of six commonly used antibiotics in swine kidney by a microbiological inhibition method[J]. Microchemical Journal, 2021, 161: 105796.
[30] WU Q, ABU BAKR SHABBIR M, PENG DP, YUAN ZH, WANG YL. Microbiological inhibition-based method for screening and identifying of antibiotic residues in milk, chicken egg and honey[J]. Food Chemistry, 2021, 363: 130074.
[31] PIERI C, BORSELLI D, Di GIORGIO C, de MÉO M, BOLLA JM, VIDAL N, COMBES S, BRUNEL JM. New ianthelliformisamine derivatives as antibiotic enhancers against resistant Gram-negative bacteria[J]. Journal of Medicinal Chemistry, 2014, 57(10): 4263-4272.
[32] SHARIATI A, ARSHADI M, KHOSROJERDI MA, ABEDINZADEH M, GANJALISHAHI M, MALEKI A, HEIDARY M, KHOSHNOOD S. The resistance mechanisms of bacteria against ciprofloxacin and new approaches for enhancing the efficacy of this antibiotic[J]. Frontiers in Public Health, 2022, 10: 1025633.
[33] REYES S, LE N, FUENTES MD, UPEGUI J, DIKICI E, BROYLES D, QUINTO E, DAUNERT S, DEO SK. An intact cell bioluminescence-based assay for the simple and rapid diagnosis of urinary tract infection[J]. International Journal of Molecular Sciences, 2020, 21(14): 5015.
[34] DANG PK, DEGAND G, DANYI S, PIERRET G, DELAHAUT P, TON VD, MAGHUIN-ROGISTER G, SCIPPO ML. Validation of a two-plate microbiological method for screening antibiotic residues in shrimp tissue[J]. Analytica Chimica Acta, 2010, 672(1/2): 30-39.
[35] FRUNDZHYAN VG, PARKHOMENKO IM, BROVKO LY, UGAROVA NN. Improved bioluminescent assay of somatic cell counts in raw milk[J]. The Journal of Dairy Research, 2008, 75(3): 279-283.
[36] JIMENEZ L. Rapid methods for the microbiological surveillance of pharmaceuticals[J]. PDA Journal of Pharmaceutical Science and Technology, 2001, 55(5): 278-285.
[37] PAVANKUMAR AR, ZELENIN S, LUNDIN A, SCHULTE T, RAJARATHINAM K, REBELLATO P, ARDABILI S, SALAS J, ACHOUR A, RUSSOM A. Bioanalytical advantages of a novel recombinant apyrase enzyme in ATP-based bioluminescence methods[J]. Analytica Chimica Acta, 2018, 1025: 118-123.
[38] MOROZOVA EP, SMOLIAROVA TE, LUKYANENKO KA, KIRILLOVA MA, VOLOCHAEV MN, KICHKAILO AS, RANJAN R, KRATASYUK VA.

Metal-enhanced bioluminescence by detergent stabilized Ag and Au nanoparticles[J]. Talanta, 2023, 254: 124157.
[39] NAKAMURA H, TAKAKURA KI, SONE Y, ITANO Y, NISHIKAWA Y. Biofilm formation and resistance to benzalkonium chloride in *Listeria monocytogenes* isolated from a fish processing plant[J]. Journal of Food Protection, 2013, 76(7): 1179-1186.
[40] KIM SU, JO EJ, NOH Y, MUN H, AHN YD, KIM MG. Adenosine triphosphate bioluminescence-based bacteria detection using targeted photothermal *Lysis* by gold nanorods[J]. Analytical Chemistry, 2018, 90(17): 10171-10178.
[41] MARTÍNEZ-PÉREZ-CEJUELA H, CALABRETTA MM, BOCCI V, D'ELIA M, MICHELINI E. Super-stable metal-organic framework (MOF)/luciferase paper-sensing platform for rapid ATP detection[J]. Biosensors, 2023, 13(4): 451.
[42] HATTORI N, KAJIYAMA N, MAEDA M, MURAKAMI S. Mutant luciferase enzymes from fireflies with increased resistance to benzalkonium chloride[J]. Bioscience, Biotechnology, and Biochemistry, 2002, 66(12): 2587-2593.
[43] BRANCHINI BR, SOUTHWORTH TL, FONTAINE DM, KOHRT D, TALUKDER M, MICHELINI E, CEVENINI L, RODA A, GROSSEL MJ. An enhanced chimeric firefly luciferase-inspired enzyme for ATP detection and bioluminescence reporter and imaging applications[J]. Analytical Biochemistry, 2015, 484: 148-153.
[44] KARIMI E, NIKKHAH M, HOSSEINKHANI S. Label-free and bioluminescence-based nano-biosensor for ATP detection[J]. Biosensors, 2022, 12(11): 918.
[45] CALABRETTA MM, ÁLVAREZ-DIDUK R, MICHELINI E, RODA A, MERKOÇI A. Nano-lantern on paper for smartphone-based ATP detection[J]. Biosensors & Bioelectronics, 2020, 150: 111902.
[46] MARTÍNEZ-PÉREZ-CEJUELA H, GREGUCCI D, CALABRETTA MM, SIMÓ-ALFONSO EF, HERRERO-MARTÍNEZ JM, MICHELINI E. Novel nanozeolitic imidazolate framework (ZIF-8) -luciferase biocomposite for nanosensing applications[J]. Analytical Chemistry, 2023, 95(4): 2540-2547.