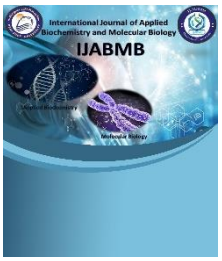
International Journal of Applied Biochemistry and Molecular Biology (IJABMB)



Biological and Biochemical Assay in the identification of clinical samples

Israa Abdel Munem Yaseen[1], Ameerah Abdulmunem Yaseen Al-doori[2], Shelan Weli Hasan[3], Hayfaa Hakeem Lateef Abbas AL-Bayati[4], Aqeel Majid Alabbasi[1], Batol Imran Dheeb[3*]

1University of Samarra/College of Education /Department of Biology, Samara, Iraq

2University of Samarra/College of Applied Sciences/Department of Applied Chemistry, Samara, Iraq

3Samara University, Faculty of Applied Sciences, Department of Pathological Analysis, Samara, Iraq

4 Environmental Research Center, University of Technology, Baghdad, Iraq

***Corresponding author:**
Batol Imran Dheeb
University of Samarra/College of Applied Sciences/Department of Pathological Analysis. batoolomran@yahoo.com

Running Title: ***Biological and Biochemical Assay***

Abstract

Bioassays, or biological assays, are a crucial component of contemporary pharmaceutical development and scientific study. Because they enable researchers to assess the biological activity of substances, whether they are medications, hormones, or environmental pollutants, these adaptable methods are essential in the domains of biology, pharmacology, and medicine. Examine the importance, varieties, and uses of biological assays in this article, delving into their intriguing realm. One difficult and important aspect of industry and medicine is the quick detection and identification of microbes. Conventional procedures, including culture media and biochemical testing, are known for being labor-intensive and time-consuming. Screening methods, on the other hand, entail the rapid and affordable classification of bacterial and fungal isolates, but modern analysis necessitates thorough reporting of microorganisms using molecular techniques. This review's objective is to conduct a Biological and Biochemical Assay in the identification of clinical samples .

Keywords: Bioassay, techniques, cytotoxicity, detection, ELISA

1 .Introduction

A biological assay is a type of scientific procedure used to measure a substance's impact on a living creature or living biological system to determine its potency or concentration (1). These tests are essential for determining the biological activity of a wide range of substances, including hormones, growth factors, toxins, and medications. Researchers can gain a better understanding of a substance's pharmacological characteristics, toxicity, and therapeutic potential by assessing its biological activity (1) . Finding the dose at which a specific proportion of people or creatures display the response is the aim of tests, in which the response is either present or missing. To determine the Lethal Dose (LD50) of a chemical for a population of test participants, for instance, researchers in toxicology employ quantal tests (1). Graded assays. The biological assays can also be classed based on whether they are performed in isolated cells or tissues, or in living animals. Drug discovery and development are two significant areas where biological assays are critical. Biological assays are used extensively by pharmaceutical companies to find prospective drug candidates, test efficacy, and define safety profiles. Throughput Screening (HTS) assays are commonly used to screen thousands of chemicals for possible medicinal activity (2). Toxicology and biological tests. Toxicology and biological assays are key tools for determining the safety of chemicals, insecticides, and environmental contaminants. Toxicity assays are used to identify the negative effects of chemicals on living organisms, which aid in risk assessment and regulatory decisions (3). Microorganisms critical to human survival, yeasts, molds, and bacteria, have been studied for both productivity and their bad effects. Biotechnology is linked inextricably to biotechnology, and sciences, food medicine, genetic engineering, and other areas of life remain unchanged (4). Microorganisms pose a risk to industry

due to their genetic traits and metabolic activity .Bioassay contains specific qualities that allow the production of hormones and antibiotics .On the one hand, their single features enable the production of other therapeutic compounds and antibiotics, hormones, and amino acids (5). Furthermore, food and food-related products are produced, and components such as lignocellulose biomass are degraded for second-generation ethanol or biogas (6). At the same time, some bacteria's genetic characteristics and metabolic capacities make them harmful to both industry (food spoilage) and human health (7). Indeed, it is thought that approximately 1400 microbes can cause human diseases. Pathogenic bacteria alone are responsible for 350 million cases of foodborne illness (8). Every year the foodborne infections occur, resulting in around 128,000 hospitalizations and 3000 deaths. Enterotoxigenic Escherichia coli, Vibrio cholerae, Aeromonas spp., enterotoxigenic Bacteroides fragilis, Clostridium difficile, and Cryptosporidium parvum are among the microorganisms responsible for serious infections and mortality in children, accounting for nearly all deaths (4). Microorganisms' pathogenicity and potential application in biotechnological processes. Two factors determine the potential application of microorganisms in biotechnology. Other strains' pathogenicity is governed by their genetic traits and biochemical abilities (9). The biological material taxonomic classification of the following characterization, identification, and subsequent industrial applications and infection therapy will be possible following the characterization, identification, and subsequent taxonomic classification of biological material. Industrial and infection therapeutic applications will be feasible in the near future. Though systematics is concerned with species variety, relationships, and potential interactions, taxonomy is the hierarchical classification of organisms based on descendants of the nearest common ancestor .

Taxonomy and systematics

Taxonomy and systematics are sometimes used interchangeably, yet they are two distinct concepts. Hierarchical categorization words, like kingdom and division, class, family, genus, species, and strain, are well-known and widely used (9). Systematics and microorganism identification research are inextricably linked and have a mutual influence. Hence, categorization and systematics. Accurate identification has ramifications for taxonomy classification and microbiological systematics. As a result, the more precise its identification, the more thorough research on a particular microorganism (11). As a result, the "polyphasic" methodology is based on morphological and biochemical data that is supplemented by molecular technique data. The common strategy is regarded as an essential foundation for microbial identification and categorization when combined with fingerprinting techniques and 16S rRNA genes, molecular other molecular markers (12). Accurate microbe identification is critical for scientists working in a wide range of applied research and industrial fields, including clinical microbiology and food production. There are numerous criteria for identifying the number of methods employed in microbial identification; nonetheless, they can generally be classified as direct or indirect approaches (13).

Clinical diagnostics: In the field of medicine, biological tests

Clinical Diagnostics: Biological tests are used in medicine to diagnose and monitor a variety of diseases (13). Immunoassays, for example, are used to discover specific biomarkers or antibodies that are indicative of infections or disorders such as the human immune system. HIV/AIDS, and COVID-19. Biological assays are used in biotechnology research to assess the performance of genetically modified organisms, validate recombinant protein production, and follow bioprocess progression. Environmental monitoring: Bioassays

are used by scientists to assess the effects of pollutants on aquatic and terrestrial ecosystems (14). These assays provide useful information on ecosystem health as well as potential hazards to animal and human populations (15).

Challenges and upcoming directions

Despite their apparent importance, biological assays present distinct challenges. Some of these challenges include biological response variability, the need for uniformity, and the ethical issues of animal research in in vivo studies. In recent years, there has been a growing interest in exploring alternative methods to animal testing, such as in vitro research using cell cultures and organoids (16). Advances in automation, robotics, and data processing have improved the efficiency and accessibility of biological assays for researchers (17). Bioassays and biochemical procedures are essential in a variety of scientific and industrial domains, including medication discovery, environmental monitoring, and quality assurance. Bioassays evaluate the effects of chemicals on living systems, whereas biochemical approaches investigate the chemical and biological processes that occur within those systems (18).

Bioassay applications

Bioassays are used to identify new pharmaceutical candidates, assess efficacy, and define safety profiles (19). Toxicology: Bioassays investigate the toxicity of chemicals, pesticides, and environmental pollutants to living beings. Bioassays are utilized for environmental monitoring, including analyzing water quality, wastewater discharges, and the impact of new technologies and infrastructure (20). Bioassays examine the biological activity and potency of pharmaceutical products, including herbal therapies, to guarantee consistency and quality. Bioassays are used in biopharmaceutical manufacturing to monitor and regulate production processes, ensuring product quality and consistency (21).

Bioassays are used to research the function of biological molecules

Bioassays are used to explore the function of biological molecules, detect biological hazards, and evaluate the effects of Enzyme kinetics, mechanism, and substrate selectivity are studied using biochemical techniques (14). Protein identification and quantification are accomplished by procedures such as purification, electrophoresis, and mass spectrometry. Metabolomics is the study of small-molecule metabolites in biological systems to better understand cellular processes (15). Genomics and transcriptomics examine gene expression and control through biochemical and molecular biology approaches (16). Drug Metabolism Studies: Biochemical techniques are applied to investigate drug processing and metabolism by the body. Environmental monitoring involves using biochemical methods to assess pollutants and toxins in environmental samples (17). Biochemical methods concentrate on the chemical and molecular interactions of a biological system. A cell-based assay is used to determine the action of a putative medicine by examining its influence on cell growth or viability (18).

Biochemical Method

An enzyme assay for determining an enzyme's Michaelis-Menten constant (Km) and maximum reaction velocity (Vmax). In essence, bioassays and biochemical procedures are complementary tools for comprehending biological systems and assessing the impact of diverse substances on living organisms and their surroundings (15).

Validation of bioassay techniques

Validation of bioassay is considered useful application in the field of herbal drug research. The definition, premise, and goal of the Bioassay (15) is defined as "a set of reagents that produces a detectable signal, allowing a biological process to be quantified." (16) USP 42 1030 defines bioassay as "Analysis (as of a drug) to quantify the biological activity or activities of one or more

analysis by determining its capacity for producing an expected biological activity on a culture of living cells (in-vitro) or on test organisms (in-vivo), expressed in terms of units". As per European Pharmacopeia 10 (17).

Biological procedures

Biological methodologies are proposed for assessing certain substances and preparations whose potencies cannot be satisfactorily guaranteed by chemical or physical examination. Throughout these assays, the principle of comparison with a standard preparation is used to determine how much of the biochemical to be tested lead to generating the same biological effect as a certain amount (19).

A biological assay, often known as a bioassay, is a method for determining the effect of a drug on a specific type of living matter or biological test system (20). Bioassays can be performed using either direct or indirect methods. The term "direct bioassay" refers to the identification of the concentration of a chemical required to elicit a specific response (21). For example, the efficacy of powdered digitalis leaves is evaluated by the concentration required to stop a cat's heart. Indirect bioassays may be determined by comparing the reactions of equal concentrations of authentic standard and sample to produce a preset response (22). A bioassay can be conducted: in vivo and ex vivo, also called in vitro. In an in-vivo bioassay, animals are given different dilutions related to standards and test compounds to measure their potency using concentration-response curves. These investigations utilized both colony and naive mice, although colony animals were preferred (23). Before being employed in a bioassay, animals must be healthy and have had time to acclimate to their surroundings (24). Ex vivo bioassays assess the activity of test compounds using laboratory-cultured cell lines or their same tissue from biopsies of humans and animals or their organs; bioassays that use living tissues or cells from animals necessitate a management strategy like that of an in-vivo assay. In-vitro bioassays are used to determine

the ability of one or more components to elicit predicted biological activity in grown cell lines derived from malignancies, non-transformed cells, and engineered cell lines. In vitro refers to "the technique performed for a given procedure outside of the living organism and artificial environment (25). Bioassays are further grouped into techniques connected to living species that are utilized, such as isolated organ vertebrates and entire animals, lower creatures (bacteria, fungi, insects), cultured cells, organs / cultured tissue, and isolated subcellular systems (26). Bioassays can be used to analyze any herbal drug or (isolated) chemical molecules, as well as for pharmaceutical quality control (QC) in the biotechnology industry. The bioassay investigation can be performed using four ways to investigate the bioactivities of a specific sample. To explore the bioactivities of a specific sample(s), bioassay research can be conducted using four main methods (1). Using a single bioassay technique to demonstrate a single pharmacological activity (e.g., antidiabetic), (2) Using a specific bioassay with different procedures to discover different types of bioactivities, (3) Using a nonspecific particles to detect multiple activities; cytotoxicity can be used to identify antibacterial, antibacterial, anticancer, and insecticidal activity, and (4) Using a combination of bioassays to identify a specific or multiple activities (27). The bioactivity of a sample can be measured at several stages. Stage 1 entails improving and confirming the dynamic signal range of sample responses as well as their variability in each biosystem. The second stage is to characterize and assess the relative potency of the sample was determined by comparing the reference material (28). Anti-diabetic, anti-bacterial, anti-fertility, anti-cancer, anti-inflammatory, anti-viral, diuretic activity, and anti-emetic assays are some of the primary screens which General bioactivity can be tested. Hepatotoxicity bioassays can be carried out with high-throughput screening (HTS) on a 96- or 384-well microtiter plate. There are two types of bioassays: cell-free or biochemical, and cell-based.

Many chemicals are used in HTS forms (28). When developing an HTS bioassay, some practical problems should be considered. Plate format, plate type, final test assay homogeneity, reagent combination/addition, stability, volume, and compound addition have all been extensively studied (29). Many parameters, including reagent solubility, reagent stability, reagent aggregation, order of reagent addition and instrumentation, cell culture plastic, culture media, culture conditions, serum, cell cycle, and pH, temperature, ion concentration, and passage numbers, may cause changes in the bioassay (30). The bioassay's different variables demand the introduction of appropriate authentic standard(s) (31). Without such standard(s), the bioassay's validity cannot be determined. Furthermore, the performance of each bio-system in use should be verified (32). A biological test (bioassay) is the technique of measuring the activity of an identifiable or unidentified agent on living material, such as bronchial, uterine, or vascular muscle contractions. It is employed only when chemical or physical approaches are impractical, such as in the case of a mixture of active compounds, an incompletely purified product, or when no chemical method has been devised (33).

Biological standardization

One sort of bioassay is biological standardization. It comprises comparing an unknown potency material to an international or national standard in order to develop pharmaceutical and research preparations. Insulin and vaccines are examples of findings that are expressed as units of a material rather than weight (33). Biological standardization is a subset of bioassays. It entails matching unknown potency material to an international or national standard to create preparation for use in treatments and research (34).

Biochemical assays

Biochemical assays are intended to assess a drug's affinity for its target. They also apply to both enzyme targets and receptors (20). They do not reveal

the pharmacological mechanism of the drug action, such as but their usefulness is predicated on whether it is inhibitory or stimulatory (agonists or antagonists), on the assumption that human binding activity is equal at the native human target. When functional studies are carried out in vitro or in vivo, the significance becomes clear (35). Other studies have questioned the effectiveness of cell-based and biochemical testing (36). Bioassays are used by pharmacologists to test the biological characteristics of pharmaceutical substances during early development over massive data generated by As the discovery program develops from early exploratory research to clinical evaluation, the tests grow more thorough as the molecules become more complicated, and the drug development cycle approaches clinical stage (32). This is the stage at which the biopharmaceutical properties, which necessitate intact physiological systems and testing of absorption, distribution, elimination, and toxicity metabolism (ADMET) properties over massive data generated by combinatorial (37).

Cytokines: From Technology to Therapeutics

The physicochemical assays panel may provide a wealth of information about impurities, and so on. The biological assay, the structure of protein components, is the only one that can quantify biological activity (2). A bioassay is classified as a 'functional' assay, and no physicochemical test can quantify function. However, for some peptide hormones with less complex structures than the bulk of long-established physicochemical cytokines, tests can now be utilized as surrogate correlates for biological activity(9). High-throughput screening for biological activity creates a large amount of data but does not distinguish between known and novel chemicals. As a result, a dereplication step is necessary (32). The NCI's dereplication technique for antitumor and HIV inhibitory tests included first HPLC fractionation with diode array detection. Fractions were plated into 96

microtiters well plates, daughter plates were created for biological testing, and molecular weights were determined using MS-ES (36).

Common hormone assay: Direct application to the surface of the target organ .

In vitro: A dose is administered to tissue segments. The method of hormone entrance into cells, as well as cell activation in the tissue's core, remains unknown (9). Cells that are separated but still intact, tissue cells, and hormones are subject to artifacts and damage caused by reagents such as enzymes and the technique used in their synthesis. Cell fragments that have separated are also vulnerable to the effects of liberated intracellular products, such as enzymes, which are only a fraction of the normal intracellular reaction involved (33). Hormone receptor solution. Reflects only the hormone's ability to bind to receptors, not its ability to begin intracellular activities (35). Antiserum has specificity due to a combination of antibodies with multiple specificities; antibodies seldom interact with and activate receptor sites on hormone target cells; exceptions include some thyroid autoantibodies. Monoclonal antibody. Each clone's antibody has a distinct specificity, indicating that it binds to the same binding site on the antigen. Natural receptors in animals, such as tissues and hormone receptors, have more repeatable specificity, although each antiserum and monoclonal antibody has its own distinct range of specificity (4).

Conclusion

Bioassays for determining the identity of microbiological samples have evolved in recent decades. Despite their limitations, culture and microscopy are two of the most extensively used PCR and microbial threat detection techniques. MS has been proven to be successful, rapid, and simple for identifying microbial samples, and other genetic approaches are especially important for non-culture bacteria. It only applies to pure isolates and cannot be used with complicated samples since they may interfere with

the conclusion. Chromatography-based technologies (such as HPLC and LC-MS) can help to speed up the procedure .Bacterial detection limits development will remain a critical task in clinical microbiology in the future. Combining these (and potentially additional) techniques and equipment will undoubtedly increase pathogen detection skills.

References

1. Bisen P.S., Debnath M., Prasad G.B. In: Microbes in Applied Research: Current Advances and Challenges. Bisen P.S., Debnath M., Prasad G.B., editors. Wiley-Blackwell; Oxford, UK: 2012. p. 699
2. Salih II, Seddiq SH, Hashim SS, Dheeb BI.(2022). Application of Omics and Proteomics in Fungi. AIP Conference Proceedings;4(2):34-35
3. Manafi M. Fluorogenic and chromogenic substrates in culture media and identification tests. Int. J. Food Microbiol. 1996;31:45–58. doi: 10.1016/0168-1605(96)00963-4. [DOI] [PubMed] [Google Scholar]
4. Ramamurthy T., Ghosh A., Pazhani G.P., Shinoda S. Current Perspectives on Viable but Non-Culturable (VBNC) Pathogenic Bacteria. Front. Public Heal. 2014;2:103. doi: 10.3389/fpubh.2014.00103. [DOI] [PMC free article] [PubMed] [Google Scholar]
5. Bochner B.R. Global phenotypic characterization of bacteria. FEMS Microbiol. Rev. 2009;33:191–205. doi: 10.1111/j.1574-6976.2008.00149.x. [DOI] [PMC free article] [PubMed] [Google Scholar]
6. Dheeb, B. I. (2015). Antifungal Activity of Alkaloids and Phenols Compounds extracted from black pepper Piper nigrum against some pathogenic fungi. Journal of Biotechnology Research Centre, 9(2), 46-54.
7. Lincoln R.J., Boxshall G.A., Clark P.F. A Dictionary of Ecology,

Evolution, and Systematics. Cambridge University Press; Cambridge, UK: 1998. p. 298. [Google Scholar]

8. Wägele J.W. Foundations of Phylogenetic Systematics. Verlag Dr. Friedrich Pfeil; Munich, Germany: 2005. p. 365. [Google Scholar]
9. Guerra-García J.M., Espinosa F., García-Gómez J.C. Trends in Taxonomy today: An overview about the main topics in Taxonomy. Zool. Baetica. 2008;19:15–19. [Google Scholar]
10. Godfray H.C.J. Challenges for taxonomy. Nature. 2002;417:17–19. doi: 10.1038/417017a. [DOI] [PubMed] [Google Scholar]
11. Dheeb,B.I, Al-Halbosiy,M.M.F, Al Lihabi,R.K, Khashman,B.M. The effects of Rubus idaeus extract on normal human lymphocytes and cancer cell line (2016). BMC genomics. 17 (6), 18.
12. Donelli G., Vuotto C., Mastromarino P. Phenotyping and genotyping are both essential to identify and classify a probiotic microorganism. Microb. Ecol. Health Dis. 2013;24:1–8. doi: 10.3402/mehd.v24i0.20105. [DOI] [PMC free article] [PubMed] [Google Scholar]
13. Yeung P.S.M., Sanders M.E., Kitts C.L., Cano R., Tong P.S. Species-Specific Identification of Commercial Probiotic Strains. J. Dairy Sci. 2002;85:1039–1051. doi: 10.3168/jds.S0022-0302(02)74164-7. [DOI] [PubMed] [Google Scholar]
14. Lagier J.-C., Hugon P., Khelaifia S., Fournier P.-E., La Scola B., Raoult D. The Rebirth of Culture in Microbiology through the Example of Culturomics to Study Human Gut Microbiota. Clin. Microbiol. Rev. 2015;28:237–264. doi: 10.1128/CMR.00014-14. [DOI] [PMC free article] [PubMed] [Google Scholar]
15. Skerman V. A Guide to the Identification of the Genera of Bacteria, with Methods and Digests of Generic Characteristics. 2nd ed. Williams & Wilkins Co.;

Philadelphia, PA, USA: 1959. [Google Scholar]

16. Abdullah HI, Hammadi SY, Hussein AS , Dheeb BI.(2019). Investigation of genetic diversity and relationships among the clinical Candida species using random amplified polymorphic DNA (RAPD) analysis., Research Journal of Biotechnology, 14(1):6–13.
17. Madigan M.T., Martinko J.M., Bender K.S., Buckley D.H., Stahl D.A., editors. Brock Biology of Microorganisms. 13th ed. Pearson; London, UK: 2012. p. 1032 .
18. Hashim S S, Mahmood Z F, Abdulateef SM, Dheeb BI.(2023). Evaluation cytotoxicity effects of Centaurea cineraria extracts against some of cancer cell lines. Biomed Pharmacol Journal .16(1): 243–249. DOI : https://dx.doi.org/10.13005/bpj/2606
19. Klenk H.-P. Culturomics as Tool in Research and Service in Culture Collections. [(accessed on 12 February 2019)]; Available online: https://www.eccosite.org/wp-content/uploads/2015/07/HP-Klenk_ECCO-XXXIV.pdf.
20. Colwell R.R. Polyphasic taxonomy of the genus vibrio: Numerical taxonomy of Vibrio cholerae, Vibrio parahaemolyticus, and related Vibrio species. J. Bacteriol. 1970;104:410–433. doi: 10.1128/jb.104.1.410-433.1970. [DOI] [PMC free article] [PubMed] [Google Scholar]
21. Yang F., Chen L., Liu Y., Li J., Wang L., Chen J. Identification of microorganisms producing lactic acid during solid-state fermentation of Maotai flavour liquor. J. Inst. Brew. 2019;125:171–177. doi: 10.1002/jib.537. [DOI] [Google Scholar]
22. Dige I., Nilsson H., Kilian M., Nyvad B. In situ identification of streptococci and other bacteria in initial dental biofilm by confocal laser scanning microscopy and fluorescence in situ hybridization. Eur. J. Oral Sci. 2007;115:459–467. doi: 10.1111/j.1600-

0722.2007.00494.x. [DOI] [PubMed] [Google Scholar]

23. Dheeb B I, Abdulla S N, AL-Qaysi SAS, Al-Sarraj BM & Farhan MS. 2023. Extraction of Klebsiella pneumoniae and Candida albicans biofilm and studying their cytotoxic effects on human lymphocytes. Jordan J Biol Sci., 16 (4):.726–717https://doi.org/10.54319/jjbs/160418
24. Rajwa B., Dundar M.M., Akova F., Bettasso A., Patsekin V., Hirleman E.D., Bhunia A.K., Robinson J.P. Discovering the unknown: Detection of emerging pathogens using a label-free light-scattering system. Cytometry. A. 2010;77:1103–1112. doi: 10.1002/cyto.a.20978. [DOI] [PMC free article] [PubMed] [Google Scholar]
25. Obara B., Roberts M.A.J., Armitage J.P., Grau V. Bacterial cell identification in differential interference contrast microscopy images. BMC Bioinformatics. 2013;14:134. doi: 10.1186/1471-2105-14-134. [DOI] [PMC free article] [PubMed] [Google Scholar]
26. Ahmed W.M., Bayraktar B., Bhunia A.K., Hirleman E.D., Robinson J.P., Rajwa B. Classification of Bacterial Contamination Using Image Processing and Distributed Computing. IEEE J. Biomed. Heal. Informa. 2013;17:232–239. doi: 10.1109/TITB.2012.2222654. [DOI] [PubMed] [Google Scholar]
27. Cardinale M., Kaiser D., Lueders T., Schnell S., Egert M. Microbiome analysis and confocal microscopy of used kitchen sponges reveal massive colonization by Acinetobacter, Moraxella and Chryseobacterium species. Sci. Rep. 2017;7:5791. doi: 10.1038/s41598-017-06055-9. [DOI] [PMC free article] [PubMed] [Google Scholar]
28. Schuler B., Meyer G., Peña D., Mullins O.C., Gross L. Unraveling the Molecular Structures of Asphaltenes by Atomic Force Microscopy. J. Am. Chem. Soc. 2015;137:9870–9876. doi:

10.1021/jacs.5b04056. [DOI] [PubMed] [Google Scholar]

29. Beier B.D., Quivey R.G., Berger A.J. Raman microspectroscopy for species identification and mapping within bacterial biofilms. AMB Express. 2012;2:35. doi: 10.1186/2191-0855-2-35. [DOI] [PMC free article] [PubMed] [Google Scholar]
30. Amann R., Fuchs B.M. Single-cell identification in microbial communities by improved fluorescence in situ hybridization techniques. Nat. Rev. Microbiol. 2008;6:339–348. doi: 10.1038/nrmicro1888. [DOI] [PubMed] [Google Scholar]
31. Sabnis R.W. Handbook of Fluorescent Dyes and Probes. John Wiley & Sons, Inc.; Hoboken, NJ, USA: 2015. p. 464. [Google Scholar]
32. Nam H.-M., Srinivasan V., Gillespie B.E., Murinda S.E., Oliver S.P. Application of SYBR green real-time PCR assay for specific detection of Salmonella spp. in dairy farm environmental samples. Int. J. Food Microbiol. 2005;102:161–171. doi: 10.1016/j.ijfoodmicro.2004.12.020. [DOI] [PubMed] [Google Scholar]
33. Noble R., Fuhrman J. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat. Microb. Ecol. 1998;14:113–118. doi: 10.3354/ame014113. [DOI] [Google Scholar]
34. Pierini F., Zembrzycki K., Nakielski P., Pawłowska S., Kowalewski T.A. Atomic force microscopy combined with optical tweezers (AFM/OT) Meas. Sci. Technol. 2016;27:025904. doi: 10.1088/0957-0233/27/2/025904. [DOI] [Google Scholar]
35. Xie C., Mace J., Dinno M.A., Li Y.Q., Tang W., Newton R.J., Gemperline P.J. Identification of Single Bacterial Cells in Aqueous Solution Using Confocal Laser Tweezers Raman Spectroscopy. Anal. Chem. 2005;77:4390–4397. doi: 10.1021/ac0504971. [DOI] [PubMed] [Google Scholar]

36. Props R., Monsieurs P., Mysara M., Clement L., Boon N. easuring the biodiversity of microbial communities by flow cytometry. Methods Ecol. Evolut. 2016;7:1376–1385. doi: 10.1111/2041-210X.12607. [DOI] [Google Scholar]

37. Buszewski B., Rogowska A., Pomastowski P., Złoch M., Railean-Plugaru V. Identification of Microorganisms by Modern Analytical Techniques. J. AOAC Int. 2017;100:1607–1623. doi: 10.5740/jaoacint.17-0207. [DOI] [PubMed] [Google Scholar]