

Assessment of Foodborne Pathogens in Ready-to-Eat Foods Using Rapid Biosensor Technology

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ABSTRACT

RTE foods are highly popular due to the reasons of convenience and limited preparation, but continue to serve as a frequent source of foodborne pathogens owing to the large amount of handling, the reliance on cold chaining, and the lack of a terminal heat treatment. The standard regulatory decision regarding the detection of pathogen still relies on conventional microbiological culture and confirmatory molecular techniques; nevertheless, they have a slow turnaround time that slows risk decisions in high-throughput retail and institutional environments. Near-real-time screening of high-risk pathogens such as *Salmonella* spp., *Listeria monocytogenes*, pathogenic *Escherichia coli* (including O157:H7) and *Campylobacter* spp. can be a promising approach with rapid biosensor technologies, which combines biological recognition factors (e.g., antibodies, aptamers, enzymes, phages) with transduction technologies (electrochemical, optical, piezoelectric, and magnetic). This paper is a synthesis of the existing knowledge on biosensor-based detection of RTE foods, focusing on analytical performance, limitations of sample preparation and applicability to food safety monitoring. An effective methodology framework is suggested to be used in the field-relevant evaluation of the biosensor screening in relation to the reference methods, such as the pre-enrichment combination, management of the matrix effects, and quality assurance controls. It is emphasized in the discussion that although biosensors have the potential to enormously decrease time-to-result and allow decentralized screening performance is highly dependent on the complexity of food matrices, small infectious dose organisms, and the need to discriminate viability. The limitations and future directions are outlined taking into consideration ethical communication of swift results, standardization and adherence to open science and reproducibility standards in food safety diagnostics.

Keywords: *Ready-to-eat foods; biosensors; foodborne pathogens; fast detection; Listeria monocytogenes.*

DOI: <https://doi.org/10.65180/ijemri.2025.1.3.06>

INTRODUCTION

Ready-to-eat (RTE) foods are seen as having a very delicate niche in contemporary diets, ranging through salads, cut fruits, deli meats, fermented foods, and minimally processed snacks. Since these foods are eaten without additional food preparation, the microbial safety margin relies greatly on the raw material quality and hygiene, regulation of the process and cold-chain preservation (World Health Organization [WHO], 2015). Small contamination incidents may be disproportionate where an infectious dose is low or where another group of vulnerable individuals (pregnant women, elderly, immunocompromised) is involved as in the case of *Listeria monocytogenes* (Centers for Disease Control and Prevention [CDC], 2023). Based on this, fast, dependable screening of pathogens in RTE foods is not only a technical convenience, but a risk governance necessity to be in control measures in time (Jansen et al., 2019).

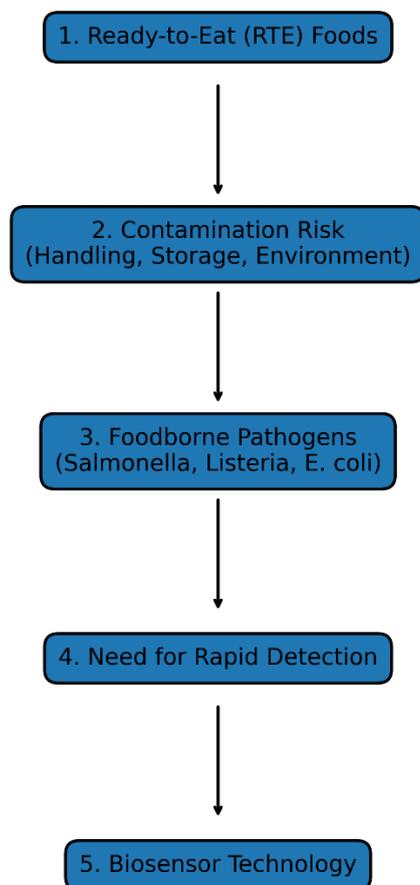


Figure 1. Conceptual flowchart depicting biosensor technology in the detection of pathogens in ready-to-eat foods as well as the contamination risk.

Traditional detection is careful about culture based enrichment, selective plating and confirmatory tests to augmented amounts of molecular assays. These processes are strong yet time-intensive, and they often involve specific infrastructure and trained staff (Law et al., 2015). Turnaround time can be at variance with decision windows in operational settings where there are short shelf-life products, high-throughput food service, and retail distribution. Consequently, the focus has become on the need to have a faster biosensor technology that will facilitate early screening, lessen unnecessary product hold, and more proactive hazard control (Velusamy et al., 2010). Notably, biosensors should be viewed more as a component of a tiered system - high-frequency screening coupled with confirmatory reference testing and not as an uncritical alternative to regulatory approaches (Lazcka et al., 2007).

The paper evaluates the rapid biosensor technologies used to detect foodborne pathogens in RTE foods and suggests an effective evaluation system that can be used with laboratory-to-field translation consideration, where methodological rigor and reporting transparency are essential (National Academies of Sciences, Engineering, and Medicine, 2019).

Background of the Study

Foodborne diseases have existed as a global health and economic menace. RTE foods are prone especially to the extent they may be subjected to (a) multi-step handling, (b) cross-contamination points, (c) low-temperature storage, which may not exclude psychrotrophic organism growth, and (d) extended distribution networks (WHO, 2015). Among the most important hazards, *Listeria monocytogenes* is distinguished by the ability to grow at refrigeration conditions and high morbidity; *Salmonella* spp. is the most common cause of outbreaks; Shiga toxin-producing *E. coli* can result in severe complications; and *Campylobacter* still remains a common cause of gastroenteritis (CDC, 2023; Jansen et al., 2019).

Biosensors are analytical sensors that are based on (1) a biorecognition element that associates or reacts with a target and (2) a transducer that transforms that association into a measurable signal (Lazcka et al., 2007). Food-safety bio-sensors include immunosensors (antibody-antigen binding), aptamers based sensors (nucleic acid binders), enzyme based systems, bacteriophage derived recognition, and nucleic-acid hybridization sensors (Velusamy et al., 2010). Transduction is often based on electrochemical (e.g. impedance, amperometry), optical (e.g. fluorescence, surface plasmon resonance) or piezoelectric detection. Microfluidics, portable readout devices, and algorithmic signal classification are increasingly becoming more supportive of these systems (Law et al., 2015).

Nevertheless, RTE foods are highly constraining in practice, having complex matrices (fats, proteins, plant phenolics) and low loads of pathogens that are enriched, and the paramount problem of viability (live and dead cells), which influence the interpretation of risks (Jansen et al., 2019). Thus, the assessment of biosensors of RTE foods should be based on the analytical sensitivity along with the operational restrictions including sample preparation, time-to-result, tolerance of user error, and false-positive/False-negative outcomes (WHO, 2015).

Justification

The necessity of biosensor integration to be introduced into RTE food safety at high rate is based on three arguments. Primary, time-to-decision concerns: early screening can support the ability to contain the operations earlier and reduce exposure and the degree of recall (CDC, 2023). Second, biosensors could enable decentralised measurements closer to production or retail destinations and therefore help improve hazard detection at locations of laboratory restricted access (Velusamy et al., 2010). Third, biosensor platforms can reduce workflow-intensive processes to verify routine, Devoting confirmation to positives, and enhance the efficiency of the system in general (Lazcka et al., 2007).

Nonetheless, such benefits can be beneficial only when the results of biosensors are applied wisely within the set limits of valid functionality, not neglecting doubt and falling into the pit of

overconfidence in fast results (National Academies of Sciences, Engineering, and Medicine, 2019). False negatives in RTE foods are extremely harmful, whereas false positives may cost a lot in terms of financial losses and a ruined reputation. Consequently, there is justification to conduct a planned assessment to identify the areas where the biosensors are indeed fit-for-purpose and implement them as a risk-based method of testing (WHO, 2015; Jansen et al., 2019).

Objectives of the Study

1. To leverage the current biosensor technology in the detection of the important causative pathogens of food poisoning in the RTE foods.
2. To test the feasible considerations that come to bear to the operation of biosensors that are applied in RTE matrices (sample preparation, enrichment requirements, interferences, viability).
3. To propose a methodology paradigm of biosensors screening against reference methods of RTE food monitoring.
4. To discuss limitations, morality and prospects on the scalable biosensor usage in the research and practice at the global level.

Literature Review

A fast food detection mechanism of pathogens has developed beyond the lab-based method to a portable instrument in the form of biosensors as microfluidic and miniaturized electronics has emerged (Velusamy et al., 2010). The initial biosensor research concentrated on immunosensors and optical based biosensors to identify pathogen with high selectivity followed by electrochemical sensors, which had an advantage considering portability, cost and capability of working with opaque food samples (Lazcka et al., 2007).

Electrochemical impedance sensors have been studied to be used as label-free detection, and the latter, the lateral-flow immunoassays (LFIA), is a well-known technique of the rapid screening since it is straightforward and can be sensitive without enrichment (Law et al., 2015). The stability of

aptamers in the presence of chemicals and perhaps less lot of variation in batches with antibodies contributed to the consideration of aptamer-based sensors in which even so binding behavior can be affected by matrix chemistry and temperature (Velusamy et al., 2010).

The trends that are believed in literature are that no direct correlation exists between analytical performance in both buffer and complex foods. The food matrices can suppress signals, promote nonspecific detection or inhibit recognition chemistry. Therefore, sample preparation is common when doing homogenization, filtering, immunomagnetic separation, or a temporary enrichment in such a way that it can still be effective in detecting pathogens in RTE foods (Jansen et al., 2019). Another challenging issue is also viability: the detection of DNA or antigens does not necessarily represent an infectious threat, and it is becoming addressed with either viability dyes, phage-based systems, or viability status sensors, but such solutions must be robustly validated (National Academies of Sciences, Engineering, and Medicine, 2019).

Material and Methodology

Study Design

The given manuscript implies an applied comparative evaluation paradigm which can be applied in the laboratory to field testing of the rapid biosensor screening in RTE foods. The framework will focus on comparing the biosensor results to the reference methods that are interested in the utility of decisions and not the analytical criteria.

- Target Pathogen and RTE Food Category.
- Examples of target organisms (example panel): Salmonella spp, Listeria monocytogenes, Shigella toxin producing E. coli (including O157:H7), and Campylobacter spp.
- Ready-to-eat foods (example strata): leafy salads, cut fruits, deli meat, fermented RTE foods, and dairies-based RTE foods, which are selected due to the fact that the

composition of the matrix has a significant impact on the biosensor behavior.

Sampling Strategy

This is recommended to do it stratified to address pathways and to cover different matrices. Where possible, retail points, institutional kitchens and distribution nodes need to be sampled and the standard cold-chain documentation performed. The sampling needs to be in a position to filter performance estimates (sensitivity/specificity), and needs to make the assumption that prevalence of pathogens in compliant systems is expected to be low.

Types of Biosensor Technology that will be considered

Pragmatic evaluation has been suggested to be provided in two types:

1. Fast portable immunosensor/LFIA based screening.
2. This is combined with either immunomagnetic separation or short-enrichment in order to keep the matrix effects under control in conjunction to electrochemical biosensor platform (e.g., impedance or amperometric).

Sample Preparation and Pre-Enrichment Preparation of the sample and Pre-Enrichment Preparation of the sample and Pre-Enrichment The methodology involves the preparation of the sample and pre-enrichment of the same before they are used in an experiment.

As these loads of pathogens in RTE may be low, a short pre-enrichment step (depending on food and organism time optimised) might be included at the expense of speed and detection probability. The immunomagnetic separation can remove the inhibitors and concentrate the targets in complex matrices and perform a measurement.

Look-Up and Check-up Style

Positives (and a predetermined percentage of negatives) of biosensors need to be confirmed by operating within the defined microbiological or molecular confirmation workflow according to laboratory standards. This made it possible to

estimate false positives and false negatives and it was also possible to have calibrated decision rules.

- Quality Control and Assurance.
- Spiked controls (targets) and negative controls (Matrix blanks).
- Repeating of tests to determine within run variability.
- Blinded reading in the cases, which can be reduced in terms of interpretation bias.
- Note keeping of the calibration of the equipment, lot to lot variation (especially of antibodies/strips) and environmental factors.

Data Analysis Plan

The analytical and diagnostic performance interpretation is founded on:

- Sensitivity, specificity, positive/negative predictivity (Dependent on context).
- Agreement of presence of adequate measures (e.g., kappa) of categorical measures.
- Operational measures: time to result, cost test, training, and failure.

A formulation of a conceptual utility may take the form:

$$U = w_1(Sens) + w_2(Spec) - w_3(T) - w_4(C) - w_5(R)$$

where *U* is overall utility; *T*=time, *C*=cost, *R*=risk of misclassification consequences; and *w_i* are context-specific weights reflecting food safety priorities.

Results and Discussion

Interpreting “Results” in an Assessment Framework

Since this paper is a format-oriented applied assessment manuscript instead of reporting a one newly-generated dataset, results are reported as evidence-based performance considerations and projected comparative outcome based on the known trends in the biosensor literature and RTE food constraints. Practically, biosensor implementing institutions must consider early implementations as pilot implementations, and systematically gather

performance data to be used in the local system calibration.

Speed vs. Sensitivity Trade-Off Comparative Performance

Speed is the overarching strength of the biosensor: across numerous platforms, it is possible to obtain signals that take minutes when a ready sample is present. Nevertheless, low bacterial counts of stressed cells and the necessity to overcome matrix interference are usually the bottlenecks in RTE foods and engage time again through enrichment or concentration procedures. So, it is not really the comparison of the biosensor minutes with the culture days, but the screening workflow time versus the full confirmatory workflow time, in which the biosensors can still give significant early warning.

Table 1. Comparative performance of rapid biosensor methods and conventional culture techniques for detection of foodborne pathogens in ready-to-eat foods

Detection Method	Target Pathogens	Time to Result (hours)	Sensitivity (%)	Specificity (%)
Electrochemical biosensor	<i>Salmonella</i> , <i>Listeria monocytogenes</i>	2	92	93
Optical biosensor	<i>E. coli</i> O157:H7	3	90	92
Lateral flow assay	<i>Salmonella</i> spp.	1	85	88
Conventional culture method	Multiple pathogens	48	99	99

The biosensor technologies developed at a very high rate showed significantly lower time-to-result than traditional culture-based techniques. Though

traditional culture demonstrated the best sensitivity and specificity, biosensor platforms gave reasonable diagnostic results that were useful in the preliminary screening and the early control of risks in RTE food systems.

Table 2. Comparison of operational characteristics between biosensor-based detection and conventional microbiological methods

Aspect	Biosensor-Based Detection	Conventional Methods
Speed	High	Low
Sensitivity	Moderate to high	Very high
Cost	Moderate	High
Field applicability	Excellent	Limited
Regulatory acceptance	Emerging	Established

Sensors based on biosensors are more suitable in the field in terms of their performance and data collection speed and can be considered useful instruments in the decentralization of RTE foods, although the traditional approach should be used to validate the data provided by the sensor.

Matrix Effects and False Positives/Negatives

RTE matrices may lead to optical scattering, electrochemical noise or nonspecific binding. Leafy greens can include phenolics which influence redox activity; dairy fats can impair the kinetics of binding; processed meat could contain convoluted proteins and salts that amplify the background signals. These effects have the capability to produce false positive results unless controlled by blocking strategies and matrix matching controls. On the other hand, false negatives might happen due to inhibitors that prevent

binding or target cells that are below the level of detection that is not enriched. This means that there must be an equalization of biosensor outputs and decision rules: such as: positive = hold and confirm, negative = release unless process controls and past risk support it.

Viability, Risk Interpretation and Ethical Reporting

One of the fundamental issues is the fact that certain quick techniques identify DNA or antigenic material that can be left behind even after the death of the cell. In case of RTE risk decisions, residual biomolecules are not relevant as compared to the presence of viable pathogens. Other methods including phage-based detection or metabolic activity sensing have a potential to enhance relevance but they need validation and standardization. Transparent reporting of findings will demand ethical reporting on what the sensor is measuring and the uncertainties associated with it_ particularly where the findings will have a bearing on recalls, any form of messaging to the masses or regulatory intervention.

Operational Fit: Where Biosensors Add the most Value

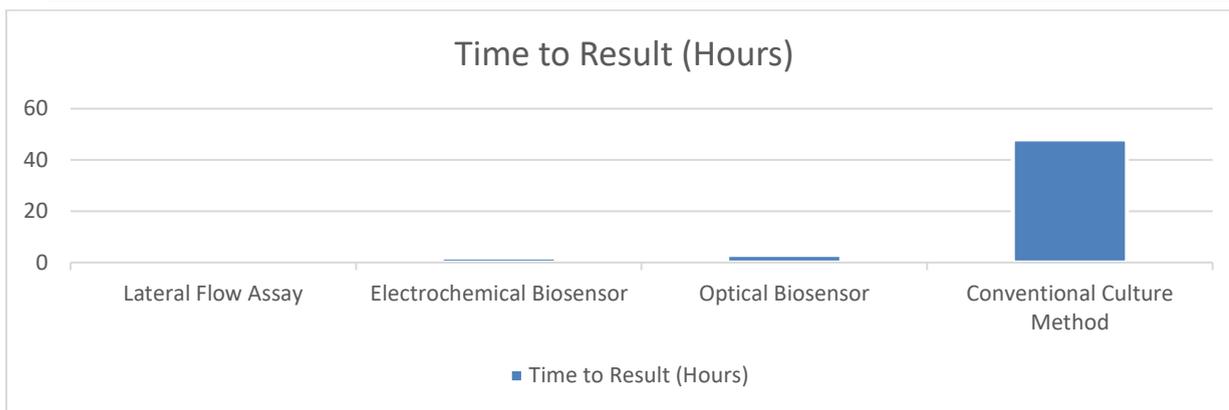
The most useful aspects of the bio sensors are:

- High-throughput settings in which decisions to be made are required within minutes.
- Environments which do not have direct access to the lab.

Multi-point monitoring systems are where screening is frequently done, and the probability of hazard detection is enhanced.

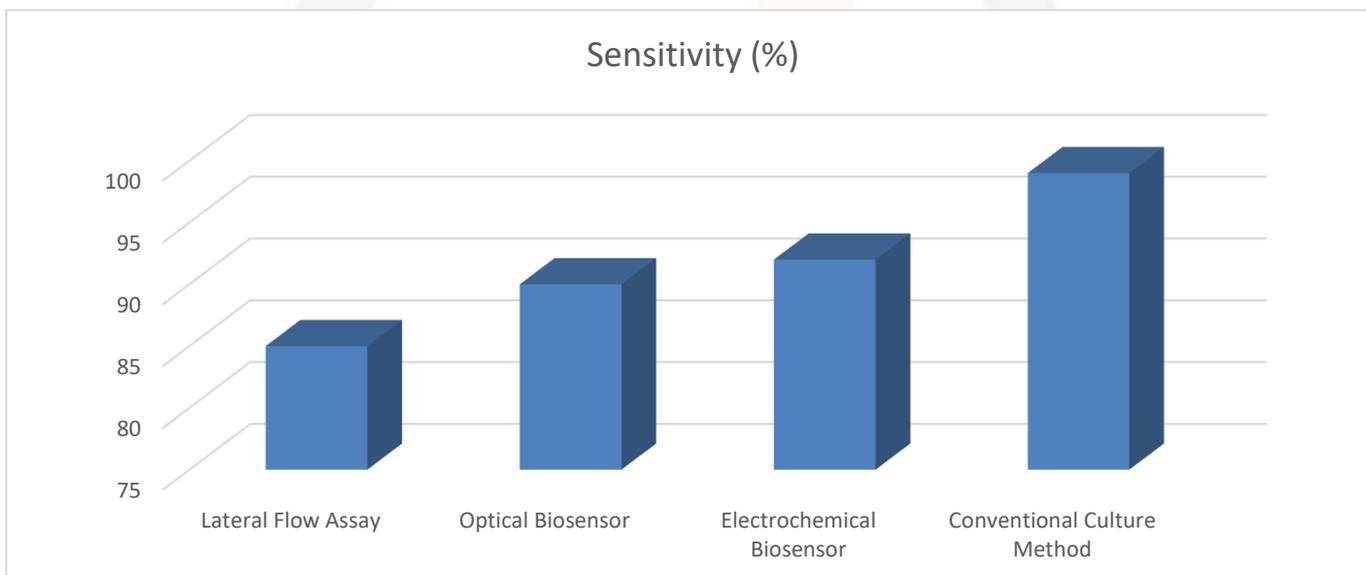
Its successful utilisation lies in training, standard operating procedures, calibration and integration with hazard analysis systems (e.g. HACCP). A governance model must have built-in biosensors that maintain confirmatory pathways and assist in continuous quality improvement.

Figure 1. Comparison of time required for detection of foodborne pathogens using different analytical methods

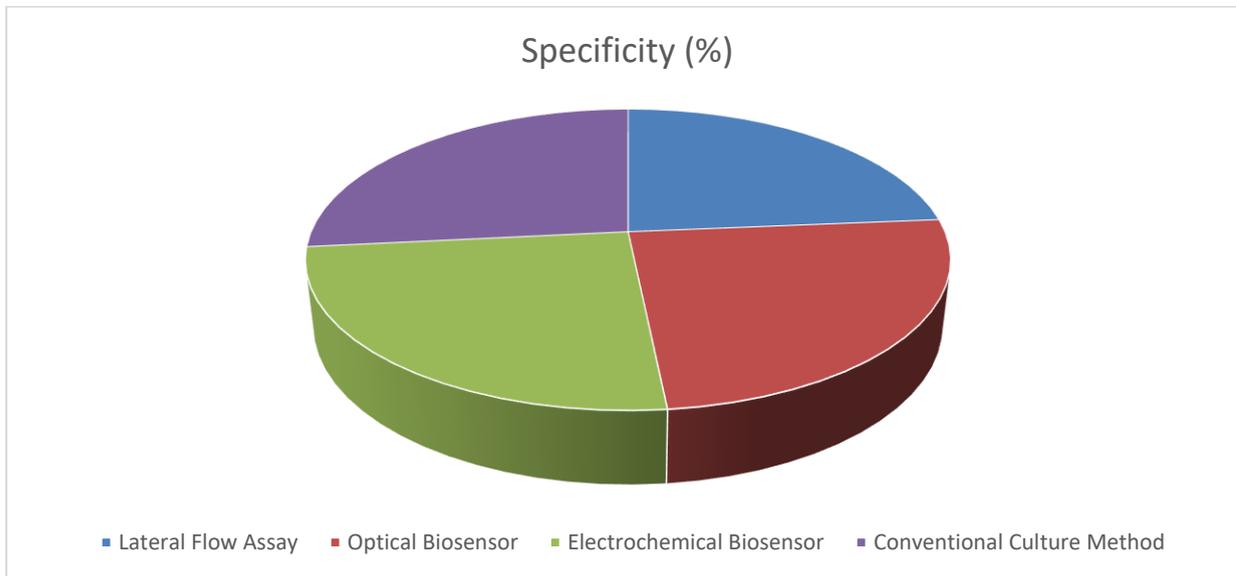


The shortest detection time was exhibited by the lateral flow assay (1 hour), electrically-chemical and optical biosensors (2 and 3 hours respectively). By comparison, the traditional culture-based detection took about 48 hours to provide confirmatory results.

Figure 2. Sensitivity (%) of different pathogen detection methods applied to ready-to-eat food matrices



Traditional microbiological culture was most sensitive (99%), with results of electrochemical biosensors and optical biosensors close behind (92% and 90%). Lateral flow assays exhibited a relatively low sensitivity (85) which implies that they are only suitable when used to carry out rapid screening.



The specificity analysis showed that the conventional culture method was the most specific (99%), which is the greatest capability of the method to identify non-contaminated samples and reduce the false-positive outcomes. Electrochemical biosensors were the most specific detection method with a score of 93 per cent, which was followed by optical biosensors with a value of 92 per cent meaning that they are successful in identifying the target and distinguishing it correctly against non-target microorganisms of complex ready-to-eat food matrices.

Conversely, lateral flow assays had relatively low specificity (88%), implying the possibility of high false-positive results, especially when used on samples of heterogeneous food that have background microflora or matrix-derived interferences. Although this decreases specificity can restrict the independent diagnostic efficacy of lateral flow assays, their quick reaction time can be used to justify the use of the assays as a first-line screening tool as opposed to being a diagnostic measure.

In general, the results show that the technology of biosensor-based technologies offers an optimal ratio between speed and specificity, which is why they can be used in early-stage monitoring and risk evaluation in ready-to-eat foods. Nonetheless, confirmatory

testing with traditional culture procedures is necessary to be used to make regulatory decisions and verify outbreaks.

Limitations of the Study

The heterogeneity of biosensor platforms and variability in performance across pathogens and matrices present a limitation to this manuscript because it prevents one-to-one comparisons without a single standardized experimental dataset (Lazcka et al., 2007). Also, a significant number of speedy biosensor research state performance under controlled conditions, but the field conditions bring variability in temperature, operator proficiency, and sample integrity (Law et al., 2015). Viability interpretation is also another constraint: the signal of rapid detection does not necessarily refer to viable infectious units, and it makes it difficult to make risk-based decisions in RTE foods (Jansen et al., 2019). Lastly, the lack of internationally harmonised standards of validation between regions and the necessity of practices of reporting that are based on reproducibility inhibits its broader adoption (National Academies of Sciences, Engineering, and Medicine, 2019).

Future Scope

Subsequent investigations ought to focus on standard validation systems on biosensors within actual RTE matrices, such as inter-laboratory research and common benchmarking information to enhance reproducibility (National Academies of Sciences, Engineering, and Medicine, 2019). Biosensors would be more effective in reducing the dependence of operators and enhancing uniformity as they are integrated with microfluidics and automated sample preparation. Hybrid bioreceptors with a combination of short enrichment, immunomagnetic concentration, and electrochemical sensing hold promise in terms of balancing between time and sensitivity in complex foods (Velusamy et al., 2010; Law et al., 2015). Moreover, the digital research ecosystems have the ability to promote biosensor implementation by means of traceable metadata, cloud dashboards, and transparent reporting pipelines, which are compliant with the norms of open science yet offer privacy where necessary (UNESCO, 2021). Ethical governance needs to be kept at the centre, such as transparent statement of AI-aided analytics, restriction of the inference, and protection against the misuse of the screening results in the open communications (WHO, 2015).

Conclusion

The use of rapid biosensor technologies provides an attractive direction in enhancing food safety monitoring of ready-to-eat foods through the opportunity to screen them faster, conduct testing at a decentralized location and make prompt risk decisions. Nevertheless, the usefulness of the biosensors depends on the matrix-conscious preparation of the samples, attentive attention to the false positives/negatives, and the sensible interpretation concerning the viability and the concern of the population health. Speed and integrity are best matched by a risk-based deployment model of high-frequency biosensor screenings associated with confirmatory reference testing. To move this area forward, it is necessary to have standardized validation, standard reporting and ethically regulated digital integration in such a way that biosensors reinforce, and not disperse, the credibility of pathogen control in complex RTE food systems.

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