FRESH PRODUCE SAFETY CENTRE
AUSTRALIA & NEW ZEALAND

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FPSC 2025 Innovation Agenda

Rapid Diagnostic Methods for Foodborne Pathogens: Outlook for the Fresh Produce Industry

July 2021

About the FPSC

The Fresh Produce Safety Centre Australia & New Zealand brokers connections and collaborations with global leaders in fresh produce to build industry capacity and capability that delivers safer fresh produce to consumers. We do this through innovation, knowledge and leadership. More **here**.

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Please read our disclaimer **here**.

Contents

List of Acronyms

AMP	Antimicrobial Peptides
ELISA	Enzyme-Linked Immunosorbent Assay
FSANZ	Food Standards Australia New Zealand
HPLC	High Performance Liquid Chromatography
IMS	Immunomagnetic Separation Assays
LAMP	Loop-Mediated Isothermal Amplification
LCR	Ligase Chain Reaction
LMS	Lateral Flow Assay
LOC	Lab-On-a-Chip
MIPs	Molecular Imprinted Polymers
NASBA	Nucleic Acid Sequence-Based Amplification
NATA	National Association of Testing Authorities
NGS	Next Generation Sequencing
PCR	Polymerase Chain Reaction
POC	Point-Of-Care
R & D	Research and Development
RDMs	Rapid Diagnostic Methods
RPA	Recombinase Polymerase Amplification
RTE	Ready To Eat
WGS	Whole Genome Sequencing

List of Tables and Figures

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1 EXECUTIVE SUMMARY

1.1 Research objectives

One of the key issues facing the fresh produce industry is the time, expertise and cost of testing for pathogenic microorganisms or indicator organisms in order to make sure that produce is safe for the consumer.

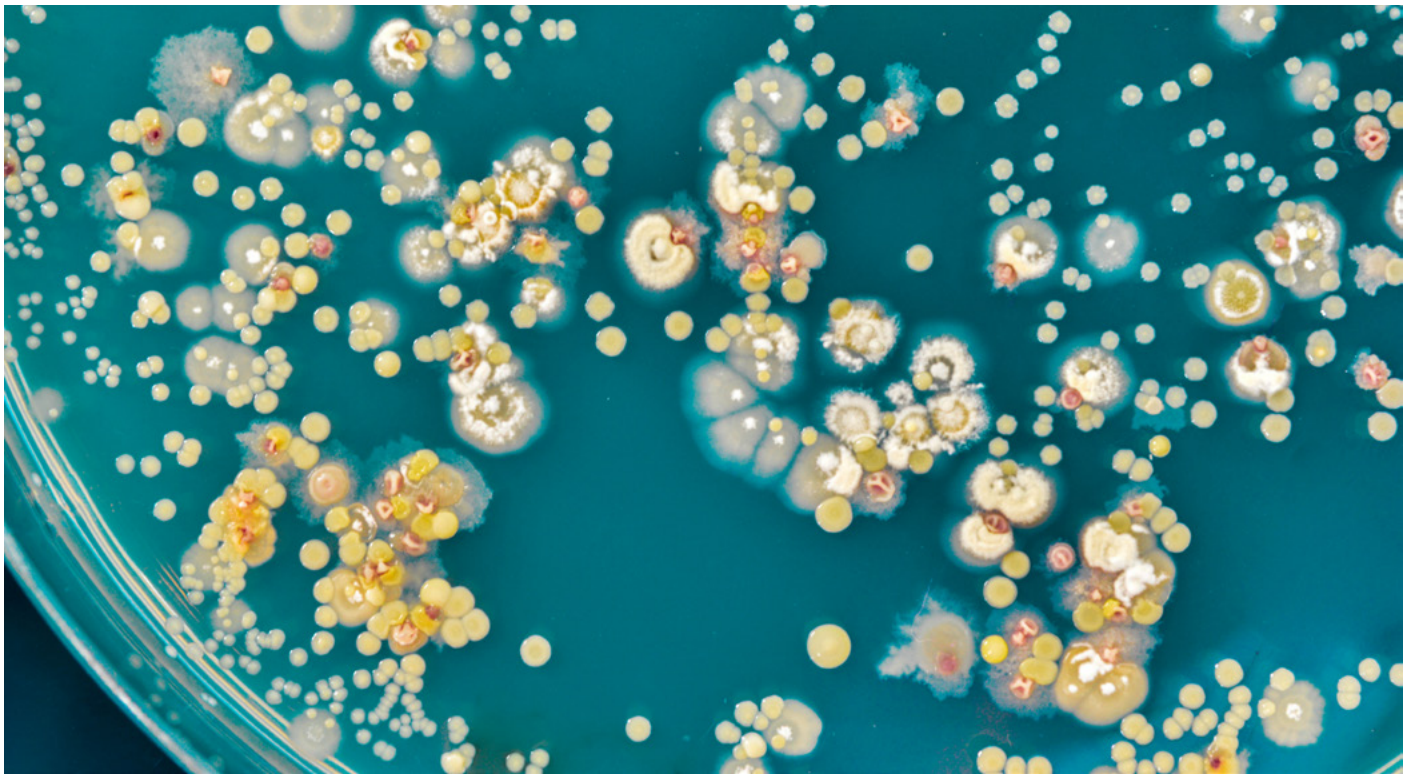
- **Time:** Timely results are a key factor for fresh produce to enable potential risks to be detected before produce departs the warehouse. Most conventional tests for microbial detection take between 1-3 days to complete, meaning produce may be on shelves before results are returned.
- **Expertise:** Another factor is the ease of use and expertise/equipment required for testing. Most samples need to be sent to a specialist lab, requiring high levels of expertise and sophisticated equipment. This prevents or restricts the ability for this testing to occur on farm.
- **Cost:** Tests are required for audits and certification by a National Association of Testing Authorities (NATA)-accredited laboratory can run into the hundreds of thousands of dollars annually.

Conventional methods of pathogen detection are typically slow, laborious, and not precise to the species or subspecies level, involving culturing of bacteria or relatively difficult and expensive culture-independent procedures such as various types of gene amplification (e.g. polymerase chain reaction [PCR]), enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC), gas or mass spectrophotometry.

1.2 Key findings

1.2.1 What are rapid diagnostic methods and why do we need them?

Given the drawbacks to conventional testing methods outlined above, there is a pressing need for rapid, accurate, sensitive, and cost-effective diagnostic testing methods and devices. Ideally, it will be possible for testing to be performed on-site by staff using inexpensive, simple and robust point-of-care (POC) devices, requiring only a minimal level of training to ensure reliable results. These types of devices and techniques are often grouped as "Rapid Diagnostic Methods" (RDMs).



Bacterial colonies growing on an agar plate

Industry interviews identified the following main reasons the fresh produce industry is interested in the development and application of RDMs.

- Current methods (generally culture-based) are time-consuming, expensive and not sufficient for rapid detection in fresh produce samples.
- Rapid, sensitive, inexpensive and reliable detection is needed to give real-time results.
- Faster results will mitigate and prevent outbreaks of foodborne illness, improving delivery times, safety and profitability.

1.2.2 What are the main types of rapid diagnostic methods?

In recent decades, a range of culture-independent methods have become well established for pathogen detection, and they can be sorted into four main categories:

i Nucleic acid-based methods (various types of PCR, ligase chain reaction [LCR], nucleic acid sequence-based amplification [NASBA] and loop-mediated isothermal amplification [LAMP])

ii Immunological-based methods (ELISA, lateral flow immunoassay and immunomagnetic separation assays [IMS]).

iii Biosensor-based methods, including nano-biosensors.

iv Lab-on-a-chip (LOC) and point-of-care methods incorporating some or a number of the above methods into small, robust, inexpensive and easy to use devices incorporating all steps from sampling preparation to detection.

1.2.3 What challenges are there to the use of rapid diagnostic methods in the fresh produce industry?

In the specific context of RDMs for foodborne pathogens, requirements for sensors include the specificity to distinguish the target pathogen in a complex product, sensitivity to detect bacteria or viruses directly, and the ability to provide results within a short time in a "real world" setting.

Detection of pathogens in a fresh product is dependent on a number of variables including:

- Type and structure of the produce;
- How produce is grown, processed and handled;
- If there is a potential for internalisation of pathogens, which requires internal sampling. Internal sampling introduces other complicating factors due to the food matrix and will generally require additional preparation steps before detection, and / or may preclude certain types of RDMs.

Hence, there may well be a need to develop a specific RDM for each fresh produce product.

Risk factors for foodborne pathogen contamination are also highly context-dependent for each type of produce. Integration of rapid diagnostic testing methods into the fresh produce industry will likely necessitate risk assessment on a case-by-case basis, and there may be implications for best practice in terms of the best RDMs to apply, frequency of testing, at what point(s) in the process and supply chain it is most effectively applied, and the stringency of tests required. ***It is likely that there will often be a trade-off between the variables of speed, accuracy, and cost, with each producer or distributor needing to determine their specific requirements of a RDM and how and when it would most effectively be integrated into their processes.***

1.2.4 What are the most promising types of RDMs for the fresh produce industry?

Despite the inherent challenges presented to RDMs by the fresh produce industry, there are still a number of promising emerging diagnostic techniques. ***Microfluidic chip (LOC devices) and paper-based devices (e.g. lateral flow test strips) appear promising in "real world" testing scenarios and offer the advantage of being affordable, sensitive, specific, easy to use and quick to provide results.*** The detection methods in such devices are generally based on different types of biosensors, sometimes with a pre-sensing step such a LAMP or IMS also incorporated. Colorimetric, fluorescent and electrical detection signals are all common, with further data interpretation sometimes provided by inputting these signals into smart phone apps or the like.

The incorporation of nanomaterials is capable of increasing the optical and magnetic properties of biosensors and paves the way for increased sensitivity and precision sensing in LOC / POC devices in field settings.

1.2.5 What will drive uptake of rapid diagnostic methods in the fresh produce industry?

It is essential to create clear understandings of the potentials and limitations of rapid diagnostic methods and their potential within the fresh produce industry. ***It is important that the realistic expectation of a suite or "toolbox" of useful RDMs that could be selected for their suitability by each producer is expected and understood by producers as an outcome of further research.*** Industry bodies have an important role to play in compiling information about the requirements and preferences of industry members and translating this information to lead to the development of useful, robust prototype RDMs. Funding for research and development (R & D) and prototypes will also need to be secured. These prototypes will then need to be extensively tested in the field to ensure that existing challenges to translating promising laboratory techniques to "real world" settings can be overcome.

Challenges inherent to the fresh produce industry, such as the inhibitory effects of the sample matrix of macerated tissue samples, the need for careful consideration of the types (internal vs external samples) and timing of sampling throughout the growing, packaging or handling process, and the wide variation in types and risk profiles of produce types, must also be communicated and understood by the industry if RDMs are to be used successfully and efficiently.

1.3 Conclusions and recommendations

The fresh produce industry has high inherent variation – between produce types, growing techniques, handing and processing methods and the eventual use of the product by the consumer. These increase the challenges to finding appropriate RDMs that can provide fast, reliable and inexpensive results in a "real world" setting. Various LOC and POC devices incorporating biosensors / nanobiosensors are promising methods that can meet all these requirements. ***The identification and detection of foodborne pathogens by LOC biosensors has many appealing properties because of their high sensitivity, robust performance, virtually real-time unenriched quantification, cost-effectiveness, multiplexing ability, and the prospect of on-site detection in an easy-to-use device.*** However, it is unrealistic to expect a single LOC / POC device to be suitable for the entire fresh produce industry. Instead, ***we suggest a suite of methods may be investigated, developed and validated, allowing each producer to choose the method(s) most suitable for their needs from this "toolbox".***

2 BACKGROUND AND CONTEXT

The aim of this project is to use technology forecasting to prioritise research and development for rapid diagnostics for three common pathogens in fresh produce. This project has three main objectives:

i Review published literature to identify promising emerging techniques for the rapid and specific detection of microorganisms or indicator organisms.

ii Identify a suite of the most promising emerging techniques for the Australian and New Zealand fresh produce industry.

iii Provide recommendations for further research and evaluation of specified rapid diagnostic methods.

2.1 Food pathogens and illness

Every year, millions of people worldwide are affected by gastrointestinal illnesses caused by bacteria, viruses and parasites, transmitted via contaminated food or water. The most frequent causes of food-borne disease include bacterial pathogens such as *Campylobacter* and *Salmonella* species and viruses such as Norovirus and Hepatitis A.

In the context of the fresh produce industry, the threat posed by these illnesses varies considerably and is generally not high, but is of great concern, given the seriousness of the response required should an outbreak occur.

Table 1. Most Common Foodborne Pathogen illnesses in Australia

Foodborne pathogen illness	Reported cases per 100,000 people per year
Campylobacteriosis	114
Non-typhoidal salmonellosis	61
Rotavirus infection	17.2
Cryptosporidiosis	13.7
Shigellosis	5.4
Shiga-toxin producing E. coli infection	1.1
Hepatitis A	0.9
Typhoid fever	0.6
Paratyphoid fever	0.3
Listeriosis	0.3
Norovirus infection	*not notifiable

* data from National Notifiable Diseases Surveillance System, Department of Health, Australian Government. http://www9.health.gov.au/cda/source/cda-index.cfm

2.2 Microbiological standards, testing guidelines and regulations

The principal authority on safety standards for the fresh produce industry is Food Standards Australia New Zealand (FSANZ). Its role is to determine standards, encapsulated in the Australia New Zealand Food Standards Code: https://www.foodstandards.gov.au/code/Pages/default.aspx and to produce associated guidelines for food production and retail. New Zealand and Australia share regulations on food composition and labelling (chapters 1 and 2) of the Food Standards Code while the FSANZ food safety standards (chapters 3 and 4) only apply to Australia. New Zealand has specific standards: https://www.mpi.govt.nz/legal/compliance-requirements/food-standards/

At present, for most fresh produce types there are no legislated regulatory requirements in terms of meeting specific criteria for microbiological limits. The notable exceptions are cultured beans and sprouts and ready-to-eat (RTE) products that contain fresh produce as an ingredient. For the fresh produce industry, this mainly pertains to produce types that are minimally processed, such as leafy greens, cut fruits etc. Food safety criteria, including limits for levels of pathogenic microorganisms, are specified for these categories.

Safe pathogen limits for fresh produce types other than RTE products, beans, and sprouts are not enforced. However, there is still an obligation for fresh produce suppliers and retailers to manage food safety risks, and to ensure that produce is safe and suitable for consumption. Fresh produce businesses are currently allowed the freedom to implement pathogen control measures as they see fit to ensure the safety of their products. In this sense, the fresh produce industry is self-regulated.

Notably, FSANZ is currently considering the need for regulatory and non-regulatory food safety risk management measures for melons, berries, and leafy greens. These measures may (or may not) reinforce the need for rapid diagnostic testing of these produce types.

2.3 Focus pathogens

As a starting point for this study, the following four pathogen types were selected as the focus for investigating the progress of appropriate rapid diagnostic methods:

- Shiga-toxin producing serovars of *Escherichia coli*;
- *Campylobacter jejuni* and *C. coli*;
- Pathogenic serovars of *Salmonella enterica*; and
- *Listeria monocytogenes*

E.coli (STEC), *Salmonella spp*. and *L. monocytogenes* were chosen as they are among the most common causes of food borne diseases. *C. jejuni* and *C. coli* were included as markers for contaminated water sources, with water widely used in fresh produce processing. There may also be the potential for contamination with these bacteria from improperly composted manure used in organic produce farming. For this reason, they were included despite being generally associated with meat, and in particular, poultry.

2.4 Current methods of pathogen detection

Conventional methods of pathogen detection are typically slow, laborious, and not precise to species or subspecies level, involving culturing of bacteria or relatively difficult and expensive culture-independent procedures such as various types of gene amplification, enzyme-linked immunosorbent assay, high-performance liquid chromatography, gas or mass spectrophotometry.

2.4.1 Limitations of current methods

One of the key issues facing the fresh produce industry is the time, expertise and cost of testing for pathogenic microorganisms or indicator organisms in order to make sure that produce is safe for the consumer.

- **Time:** Timely results are a key factor for fresh produce to enable potential risks to be detected before produce departs the warehouse. Most conventional tests for microbial detection take between 1-3 days to complete, plus the time taken to get the samples to the lab. Produce is shipped to packhouses/distribution centres or directly to stores to be on shelves within 1-3 days of harvest; often before these test results are returned.

- **Expertise:** Another factor is the ease of use and expertise/equipment required for testing. Most samples need to be sent to a specialist lab for testing, requiring high levels of expertise and sophisticated equipment. This prevents or restricts the ability for this testing to occur on farm.

- **Cost:** Tests are required for audits and certification by a NATA accredited laboratory and can run into the hundreds of thousands of dollars annually.

2.5 What are rapid diagnostic methods and why do we need them?

Given the drawbacks to conventional testing methods outlined above, there is a pressing need for rapid, accurate, sensitive, and cost-effective diagnostic testing methods and devices. Ideally, it will be possible for testing to be performed on-site by staff using simple and robust point-of-care devices, requiring only a minimal level of training to ensure reliable results. These types of devices and techniques are often grouped as "Rapid Diagnostic Methods" (RDMs).

There has been an increasing focus on developing strategies that incorporate all steps from sampling preparation to detection in miniaturised POC devices – a "lab-on-a-chip" approach – as an alternative to traditional laboratory benchtop devices. Typically, these are microfluidic paper-based or chip-based devices that exploit different mechanical and biological techniques to detect very low concentrations of pathogens in food samples. Nanomaterial technology is also increasingly finding applications within these devices. However, despite advances, challenges remain for translating POC / LOC methods into practical field settings.

Table 2. Comparison of Conventional and Emerging Fresh Produce Testing Technologies

Diagnostic test		Targets	Approx. Time	Cost $	Specificity %	Sensitivity %	Expertise required	Special instruments required
Conventional methods	Bacteria culture	Pathogen	1-2 days	3-6	100	100	Yes	Yes
	ELISA	Pathogen	6 hrs	10	70-90	61-99	Yes	Yes
	qPCR	Pathogen	4 hrs	20	100	80-100	Yes	Yes
	CG	Chemical	30 mins	20-30	95	99	Yes	Yes
	HPLC	Chemical	30 mins	20-30	95	99	Yes	Yes
Emerging methods	LOC / POC devices	Pathogen and chemical	20-30 mins	2	100	80-100	No	No

Abbreviations: ELISA: enzyme-linked immunosorbent assay; qPRC: quantitative polymerase chain reaction; HPLC: high performance liquid chromatography; GC: gas chromatography; LOC: lab-on-a-chip; POC: point-of-care.

* modified from Choi et al, 2019

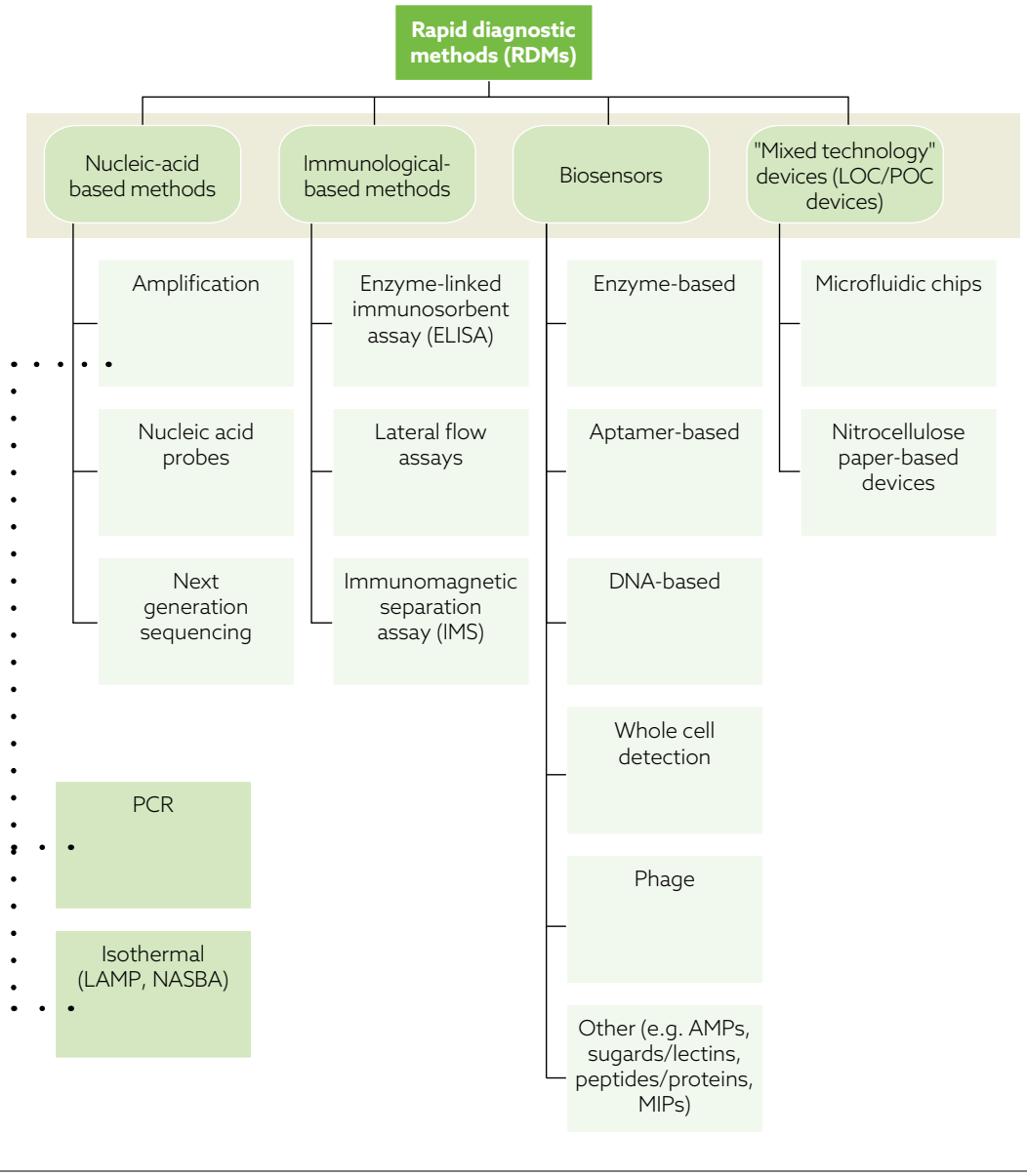


Figure 1. Types of Rapid Diagnostic Methods

Each of these types of RDM and their possible application to the fresh produce industry are discussed in detail in Section 5.1.

2.5.1 What are the main types of rapid diagnostic methods?

In recent decades, a range of culture-independent methods have become well established for pathogen detection, and they can be sorted into four main categories:

i Nucleic acid-based methods (various types of PCR, LCR, NASBA and LAMP)

ii Immunological-based methods (ELISA, lateral flow immunoassay and IMS).

iii Biosensor-based methods, including nano-biosensors.

iv LOC / POC methods incorporating the one or a number of the above methods into small, robust, inexpensive and easy to use devices incorporating all steps from sampling preparation to detection.

3 METHODOLOGY

The methodology employed was to conduct a literature review and combine this with a series of interviews with experts in rapid diagnostic methods. These two approaches, a literature review and key expert interviews, fed back into one another; reviewing of recent literature assisted in identifying the most appropriate experts for interviews, whereas information provided by interviewees helped to guide the direction and scope of the literature review.

The first step was to review relevant scientific literature to compile and refine information on current and emerging diagnostic technology and methods, and their potential for successful application in the Australian and New Zealand fresh produce industry. This literature review is not presented as exhaustive, as the literature on the topic is extensive. However, the papers reviewed (with some abstracts presented in section 9, and the full list of papers in section 10) present an overview of the key methods.

For the second part of the methodology, fifteen experts, representing research institutions, fresh produce companies, regulatory and advisory agencies for the food industry, or commercial testing companies, were interviewed to gain expert opinion and insights on the outlook for rapid diagnostic testing in the fresh produce industry.

3.1 Goals of interview process

The interviews aimed to gain additional insights about the industry perspective, and to validate findings and information emerging from the literature review. The experts were selected for their ability to provide information on:

- Typing and identification of specific foodborne microbial pathogens;
- Current and emerging rapid diagnostic technologies, including their advantages, disadvantages, and cost; and
- The specific regulatory requirements and scope for uptake of rapid diagnostic testing for pathogens in Australian and New Zealand fresh produce supply chains.

A list of interviewees and interview questions are in Appendices 1 and 2.

4 INTERVIEW RESPONSES: RAPID DIAGNOSTIC TESTING: WHAT DOES THE INDUSTRY NEED?

4.1 Key considerations

In order to ensure food safety and minimise the occurrence of foodborne illness, it is critical to test produce for the presence of foodborne pathogens. This highlights the need for rapid, sensitive, and selective methods to detect pathogens. There has been huge progress in both detection and separation/concentration techniques in recent years. The application of nanotechnology and other technological advances into biosensors can make RDM technology into rapid, simple, accurate, and portable devices that may fulfill the demand of food industries to have point-of-care testing. Industry experts were interviewed about:

- Priority pathogen types for the fresh produce industry;
- How they envisioned integration of RDMs into the fresh produce industry's supply chains and processes.

4.2 Priority pathogen types

As outlined in section 2.3, the following four pathogen types were selected as the focus for investigating the progress of appropriate rapid diagnostic methods:

i Shiga-toxin producing serovars of *Escherichia coli*;

ii *Campylobacter jejuni* and *C. coli*;

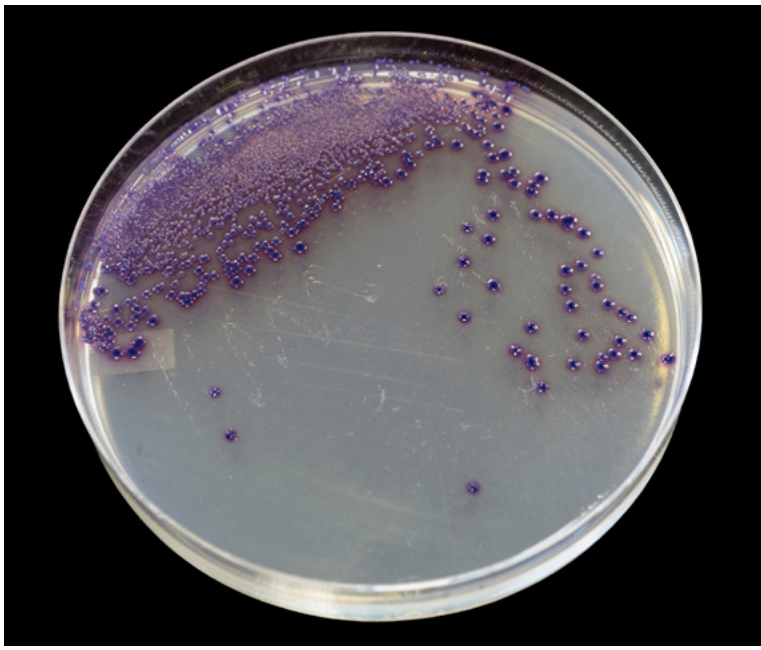
iii Pathogenic serovars of *Salmonella enterica*; and

iv *Listeria monocytogenes*.

There was unanimous support from the interviewees for the inclusion of *E.coli*, *Salmonella spp*. and *L. monocytogenes*. The inclusion of *Campylobacter sp* was questioned as outbreaks of this pathogen are typically not associated with fresh produce. Several interviewees did speculate that its potential for causing outbreaks from contaminated produce might be underestimated. However, the consensus was that Campylobacter spare unlikely to be of the same magnitude of risk as the other three pathogen types under consideration.

Several additional pathogens were suggested as potential threats to human health via outbreaks from contaminated produce. These included two viruses – Norovirus and Hepatitis A, the protozoan parasite *Cyclospora cayetanensis*, and the bacteria *Yersinia*.

After consideration, it was decided not to pursue these alternative suggestions. For the sake of simplicity, and to limit the scope of this study to a clear list of pathogens with consensus support for their importance, *E.coli*, *Salmonella spp*. and *L. monocytogenes* were used as the basis for investigating current and emerging rapid diagnostic methods.



Colonies of *E. Coli* bacteria growing on an agar plate

4.3 Integration of RDMs into fresh produce supply chains and processes

An important initial step towards the introduction and adoption of rapid diagnostic testing in the fresh produce industry will be to establish a shared, clear understanding of:

i What the intended purpose of rapid testing will be (***the question of why***); and

ii The best way to implement or integrate rapid testing in existing produce supply chains (***the question of how***).

Both matters have implications for the technical requirements of rapid diagnostic tests. There was some variation in interviewees' suggestions about why and how rapid testing could most effectively be integrated into the fresh produce industry.

Regulatory considerations surrounding adoption of rapid diagnostic testing were also discussed with the interviewees and garnered a wide range of responses.

4.3.1 Why does industry need Rapid Diagnostic Methods?

Responses as to the need for RDMs in the fresh produce industry could be summarised as:

- Current methods (generally culture-based) are time-consuming, expensive and not sufficient for rapid detection in fresh produce samples.
- Rapid, sensitive, inexpensive and reliable detection is needed to give real-time results.
- Faster results will mitigate and prevent outbreaks of foodborne illness, improving delivery times, safety and profitability.

4.3.2 What is the intended purpose of RDMs?

A commonly expressed view was that the primary purpose of rapid testing should not be to simply assure the microbiological safety of specific lots or batches of produce, but that instead, it should be viewed and applied as a tool to verify the efficacy of hygiene systems and pathogen control processes in place in a produce processing and supply chain. Returning quick information on pathogen levels by the use of RDMs could provide rapid feedback on program performance.

4.3.3 How should RDMs be used in the industry? Two-stage testing

None of the interviewees held the view that rapid methods would be likely to completely supplant standard culture-based testing in the immediate future. However, there was widespread support among interview participants for the potential introduction and use of rapid diagnostic testing as part of a two-stage testing approach, in conjunction with current reference (or 'gold standard') testing methods for bacterial pathogens. In this approach, rapid methods would be applied as a first stage screening on-site, then any sampled items returning positive results would be sent for second stage testing using standard culture methods. This approach is also entirely compatible with using rapid testing primarily as a means of verifying pathogen control processes. Over time, this might change as rapid testing becomes proven.

Multiple interviewees indicated that fresh produce companies, distributors, retailers and growers would be far more likely to support development and adoption of rapid testing methods for use as a verification tool in pathogen control programs, if:

i rapid testing remained free from regulatory and accreditation requirements;

ii companies performing rapid testing were under no obligation to act upon positive results in a specified manner.

As part of a two-stage testing approach, rapid testing would be considered as a 'pre-regulatory' test, whereas at the second stage, conventional testing method would remain subject to current regulations.

4.3.4 General technical considerations

The immediate goal of rapid diagnostic testing is to obtain an initial result on pathogen presence or concentration as quickly as possible, which ideally, will be prior to the point at which the produce leaves the farm or facility, and prior to reaching the consumer. It will also need to achieve this with an acceptable level of reliability, and at an acceptable cost. However, the relative importance of each of these variables – time, accuracy, and expense – may vary according to the specific needs of the user.

- **Time:** Two variables play into how quick a rapid diagnostic testing method needs to be – at what point(s) in the supply chain the testing is done, and the post-harvest shelf life of the produce. Exactly when (or how often) in the supply chain rapid testing will be performed will vary depending on the produce type and the nature of the post-harvest processing.
- **Accuracy (sensitivity, specificity, multiplexing):** Ideally, a rapid diagnostic test method will be highly accurate, and produce a negligible level of both false negative and false positive results. In practice, this may be difficult to achieve, and test users may need to decide which of these two types of false results is best to minimise in their circumstances.
- **Expense:** The cost of integrating rapid diagnostic testing into the production and distribution process will vary in importance for fresh produce distributors, and probably often in line with the scale of the operation. For many small produce companies or growers, the cost to the business may be the most important factor. Larger scale operations will probably be a better position to afford rapid testing, and so may be more focused on the degree to which it enhances the microbiological safety of their products, whether the end goal is improved public safety, competitive advantage, or avoidance of costs (financial and reputational) associated with any produce recalls or foodborne illness incidents.

It is likely that there will often be a trade-off between the above variables – speed, accuracy, and cost. Producers and distributors will need to determine their specific requirements for rapid testing in order to tailor the type of testing they adopt, and how and when it would most effectively be integrated into their processes.

4.3.5 Regulatory framework considerations

The question of how rapid diagnostic approaches should fit into the regulatory frameworks for foodborne microbiological safety in Australia and New Zealand was discussed at length with the interviewed experts. The issue is complicated, and there were diverse opinions. The discussion assumed that rapid testing methods for pathogens will at some point become a viable, affordable, and widely available option for the fresh produce industry.

Many interviewees suggested there will be tension between government and industry over how they will want rapid testing methods to be applied and regulated. An understandable aim of government regulators would be to promote increased use of rapid testing in the interest of consumer safety; enforceable regulations requiring increased screening of produce prior to hitting retail shelves would be one potential means of driving industry adoption of available rapid methods. Additionally, or alternatively, regulations could be brought in that require companies to act upon positive rapid test results in a specific manner.

On the other hand, produce companies are wary that additional testing by rapid methods could generate considerably more presumptive detections of pathogens than occurs presently; being forced by regulation to act on every instance, via measures such as stops on distribution, produce recalls or withdrawals, will cost them considerable time and money.

The easiest path to acceptance of new methods will be for them to meet equivalent standards of performance as current culture-based methods. Even if rapid testing is only to be used as a first stage, with the expectation that all positive results will be followed up by conventional reference testing, it would still be best practice for the industry if all rapid testing were to be done using validated methods, independently accredited by an agency such as NATA. The simplest regulatory approach of all could be to allow voluntary use of rapid diagnostic testing, requiring only that it be performed using validated methods.

4.3.6 How should RDMs be regulated?

Where regulatory mechanisms specify microbiological limits for fresh produce, they also prescribe that the reference method used to test for relevant pathogens must be the most recent Australian Standard (AS5013) or ISO method, at a minimum. Alternatively, the method can be 'any other validated method that provides equivalent sensitivity, reproducibility, and reliability'. If a rapid diagnostic method is to be used to replace the current Australian Standard reference method, then that rapid method will need to be validated as at least equivalent to the reference method.

On the other hand, if rapid testing is applied as stage one of a two-stage process, then there may be no *prima facie* need for the rapid methods to be validated (as it could be described as 'pre-regulatory' testing).

Demonstrating equivalence with existing reference tests is likely to be the easiest path to acceptance and adoption of rapid methods by the industry. However, the independent inter-laboratory testing that would be required for validation and verification of any new rapid method would be an additional and not insignificant hurdle for any research and development program.

As a final point, regulation of microbiological safety of produce is complicated by differing requirements for access to domestic and various international export markets.

5 POTENTIAL AND LIMITATIONS OF EMERGING METHODS FOR RAPID DETECTION OF PATHOGENS

5.1 Types of rapid diagnostic methods

The occurrence of foodborne pathogens in food is a primary concern of both consumers and food producers. Early screening for foodborne pathogens is essential for controlling the outbreaks of epidemics. Thus, much research is focused on developing quick, reliable, robust, sensitive, and specific diagnostic tools to identify the pathogens present. In recent decades, a range of technologies have been developed for more rapid detection of bacteria, viruses, or parasites in clinical, environmental, or food samples. Many of these technologies continue to undergo improvement and additional novel technologies are likely to emerge over time. In the case of fresh produce testing, these technologies are developed into diagnostic devices, methods, and protocols for direct detection of foodborne pathogens or for detection of 'indicator' organisms that signal the food has encountered conditions that could have led to contamination by (non-detected) pathogens.

Rapid diagnostic methods are often sorted into three categories, depending on the underpinning technology:

i Nucleic acid-based methods;

ii Immunological-based methods; and

iii Biosensors

There is sometimes overlap or combination of these categories within a testing device, particularly in miniaturised "lab-on-a-chip" devices, or point-of-care diagnostic devices, an alternative to laboratory benchtop devices. These "mixed technology" devices are discussed in section 5.1.4.

Finally, holistic integration of rapid testing and data analytics for predictive risk modelling are being increasingly investigated and are discussed in section 5.1.5.

Table 3. Categories of Rapid Diagnostic Methods

Category	Methods	Includes	References
Nucleic acid-based	Amplification of DNA/ RNA Nucleic Acid Probes Next Generation Sequencing (NGS)	Polymerase chain reaction (e.g. standard and multiplex PCR) Isothermal amplification (e.g. LAMP, NASBA, RPA)	Zhao et al., 2014 Zhang et al., 2019 Wang and Salzar, 2016 Jayan et al., 2020 Notomi et al., 2000 Toldra et al., 2018 Zhong et al., 2019 Zhong and Zhao, 2018 Ma et al., 2018 Hyeon et al., 2017 Notomi et al., 2000
Immunological-based	Enzyme-linked immunosorbent assay (ELISA) Lateral Flow Assays Immunomagnetic Separation Assay (IMS)		Choi et al., 2019 Zhang et al., 2019 Koczula and Gallotta, 2016 Jayan et al., 2020 Song et al., 2016 Law et al., 2015

Table 3. Categories of Rapid Diagnostic Methods *(continued)*			
Biosensors / Nanobiosensors	Enzyme-based Antibody-based Aptamer-based DNA-based Whole cell detection Phage Other biosensing elements	 Antimicrobial Peptides (AMPs) Sugars / lectins Peptides / proteins MIPs	Templier et al., 2016 Kobun, 2020 Jayan et al., 2020 Nguyen et al., 2019 Zhang et al., 2019 Ali et al., 2017 Diaz-Amaya et al., 2019 Wang and Salazar, 2016 Wang et al., 2020 Banerjee and Bhunia, 2009 Ghasemi-Varnamkhasti et al., 2012 Mishra et al., 2012 Housaindokht et al., 2018 Bang et al, 2013 Niyomdecha et al., 2018 Chen et al., 2018 Lu and Gunasekavan, 2019 Wilson et al., 2019 Choi et al., 2019 Yang et al., 2018 Taylor et al, 2006 Law et al., 2015
"Mixed technology" LOC / POC devices	Microfluidic chips Nitrocellulose paper-based devices		Choi et al. 2019 Kobun, 2020 Jayan et al., 2020 Zhang et al., 2019 Wang et al., 2020 Yan et al., 2016 Valderrama et al., 2016 Toldra et al., 2018 Law et al., 2015 Nguyen and Wereley, 2019

5.1.1 Nucleic acid-based methods

These methods are based on the detection of specific gene sequences (signature sequences) from the genetic material (DNA or RNA) of the target organism. The sequences may be selected in such a way that they can detect a particular group, genus, species, or even the strain of micro-organism. Where foodborne pathogens produce toxins that can cause illness, as in the case of *E. coli* O157:H7, the toxin-producing genes can be also be targeted in nucleic acid assays, to distinguish between harmful and harmless strains that are closely related (Zhao et al., 2014).

Nucleic acid methods can be divided into three categories:

- Amplification of DNA or RNA, either by
  - polymerase chain reaction (PCR) or
  - isothermal amplification (LAMP, NASBA etc.);
- Nucleic acid probes; and
- Next-generation sequencing (NGS).

5.1.1.1 Amplification methods

Standard and multiplex PCR

Standard PCR is the most widely used molecular biology technique for amplifying specific DNA sequences from low to high quantities, which then enables detection or measurement by various methods. Prior to PCR, samples taken typically require pre-processing to separate pathogens from other food matrix components, followed by concentration of the pathogen cells.

Several different pathogen types can be detected simultaneously in multiplex PCR, where multiple pairs of primers are incorporated in the same amplification reaction. This has the advantage of requiring less time and effort to obtain multiple results, but it is more difficult to design and develop a robust and efficient multiplex PCR, as it requires careful optimisation of a number of reagent concentrations and reaction conditions.

A general disadvantage of PCR assays used for detection of foodborne pathogens is that the PCR amplification process will target DNA from both live and dead cells. Some studies have developed PCR assays for the detection of only live bacterial DNA.

Advances such as Quantitative reverse transcription PCR (RT-qPCR) have improved the speed and sensitivity of PCR testing, but they still generally require trained personnel, specific non-portable laboratory equipment and are expensive to perform.

PCR machine

Isothermal amplification

Various isothermal amplification methods have been developed that perform at a single constant temperature (unlike PCR which requires temperature cycling); isothermal devices and instrumentation can thus be far simpler, smaller and require considerably less energy. These characteristics are conducive to integration of isothermal amplification with microfluidic systems in simplified lab-on-a-chip devices. As a further advantage over PCR, isothermal amplification methods are less sensitive to polymerase inhibiting compounds that may be found in crude plant cell extracts.

The most promising methods for integration into field-deployable and/or microfluidic devices include LAMP, NASBA and RPA. Of these, Loop-mediated isothermal amplification is the most widely used isothermal amplification method, and it provides superior specificity and sensitivity to other methods (Zhao et al., 2014). The simple structure of LAMP chips make them amenable to miniaturization and mass production. For example, LAMP has been combined with microfluidics into chip devices that also incorporate magnetic bead-based sample preparation for detecting foodborne pathogens (Zhang et al., 2019).

NASBA has also been used to detect various foodborne pathogens, and as it detects RNA sequences it is a reliable indicator for viable cells. It has been incorporated into microfluidic-based and LOC devices, including systems with real-time fluorescence detection and devices incorporating sample purification and concentration (Zhang et al,. 2019).

Recombinase polymerase amplification (RPA) has been combined with immunoassays in lateral flow dipstick devices with colorimetric output visible to the naked eye. It has also been successfully used in multiplex applications, and incorporated into portable LOC and microfluidic-based devices for pathogen detection (Zhang et al., 2019).

5.1.1.2 Nucleic acid probes

Nucleic acid probes utilise species-specific DNA probes that hybridize with the target species genetic material. The probes are labelled with a reporter, which emits a signal of some kind that can be detected and measured, for example, chemiluminescence which can be read with a luminometer. Various types of nucleic acid probes for the identification of bacteria are available for clinical use, but at this stage appear to be focused on medical samples such a blood, rather than fresh produce.

5.1.1.3 Next generation sequencing

The advent of next generation sequencing theoretically allows for the non-targeted detection of multiple pathogens and may give information on the strains and genomes of the bacteria detected. NGS is increasingly being utilised in research in food microbiology, with most studies focusing on whole genome sequencing (WGS) of bacterial isolates (Hyeon et al., 2017) and the exploration of the microbiome associated with specific commodities. These studies highlight the diversity of microbes associated with foodstuffs, but few studies have, as yet, explored the use of NGS to screen fresh produce for the presence of human pathogens.

5.1.1.4 Nucleic acid-based methods and the fresh produce industry

In general, nucleic-acid-based methods have high sensitivity and require a shorter time than conventional culture-based techniques for detection of foodborne pathogens and toxins, but most of them require trained personnel and expensive instruments, which limit their use in a practical environment. The required preparation steps before analysis also add to the complexity and time required for any nucleic acid-based rapid detection method. Emerging isothermal amplification methods such as LAMP and NASBA may be a good prospect for detection of pathogens and toxins in resource limited settings, especially as they may be incorporated into POC devices as a gene amplification step. Their ability to also detect parasites and viruses may make them of interest to certain sectors of the fresh produce industry.

5.1.2 Immunological-based methods

These types of tests are all based on antibody – antigen reactions. In nature, the body produces specific antibodies in response to invading pathogens. These antibody molecules can be produced in the laboratory and used in tests to identify an antigenic component of a pathogen or toxin. The most important characteristic of an antibody is its ability to recognise only the target antigen in the presence of other organisms and interfering food components. The main forms of immunological-based methods are:

i Enzyme-linked immunosorbent assay (ELISA);

ii Lateral Flow Assays;

iii Immunomagnetic Separation Assay (IMS).

5.1.2.1 Enzyme-linked immunosorbent assay

The ELISA is an immunological assay commonly used to measure antibodies, antigens, proteins or glycoproteins in biological samples. Each ELISA measures a specific antigen, and kits for a variety of antigens are widely available, particularly in the field of medicine. A common form of an ELISA test is the "double antibody sandwich" assay kit. These are characterised by their fast turnaround time (around 6 hours), ease of use, and relatively small footprint in the laboratory. However, they still require a level of expertise to operate, and are expensive per sample (Choi et al., 2019).

5.1.2.2 Lateral flow assays

Lateral flow assay (LFA) is a widely used method for the qualitative analysis of target analytes and involves the use of immuno-chromatographic strip for point-of-care detection of microorganisms in the sample matrix. A paper-based strip consisting of a sample pad, conjugate pad, absorbent pad, and nitrocellulose membrane works based on the capillary flow of liquid sample containing target analytes and recognition elements such as antibodies. LFA can be employed for the simultaneous detection of multiple pathogens with high sensitivity. For example, Song et al. (2016) detected two foodborne pathogens of Shigella boydii and E. coli O157:H7 in bread, milk, and jelly. LFA can be employed for the detection of bacterial pathogens in many fields, but the lack of quantification capability limits its application in the food industry. Interfering compounds can also reduce the absorption of analyte molecule through the membrane and sample pre-treatment is normally required, which may be an issue for many fresh produce items (Choi et al., 2019).

5.1.2.3 Immunomagnetic Separation Assay

Although not a direct diagnostic technique, immunomagnetic separation, a procedure that utilises immunomagnetic beads as capturing reagents, has been developed for microbial isolation and identification. IMS both purifies and concentrates microorganisms. In the laboratory, the process works by incubating the beads and sample, allowing time for reaction to occur between the antibodies and the pathogen of interest. Subsequently, the beads are pulled to one side of the container using a magnet, isolating the pathogen of interest from the rest of the sample, which is removed and discarded. IMS-based sample preparation has potential for combining with other methods into POC or LOC devices for detecting foodborne pathogens, and has been used in this capacity in some studies already (Zhang et al., 2019).

5.1.2.4 Immunological-based methods and the fresh produce industry

The specificity and the sensitivity of immunological methods depend on the binding strength of the specific antibody to its antigen, and they work well for food matrixes without being influenced by factors such as other non-target cells, DNA, and proteins. However, ELISA requires specialised equipment, expertise and associated high costs per sample. Lateral flow immunoassay has potential, particularly in the form of "dipstick devices" but issues with sample preparation and the influence of fresh produce matrices on the test devices would need to be worked through. IMS also has potential as part of a POC or LOC device arrangement and has been utilised in this capacity in some studies already.

5.1.3 Biosensors

A biosensor acts as a detection tool that measures biological or chemical reactions by producing signals proportional to an analyte's concentration in the reaction. Clark Jr and Lyons invented the first recognised biosensor in 1962, which is used to monitor blood gas concentrations during cardiovascular surgery.

A biosensor is composed of two main parts:

i a bio-element or sensing element which undergoes biochemical reactions with the analyte; and

ii a transducer that collects information from the biological part, amplifies and produces a signal that can be read.

Recent advances in biosensor approaches to detect foodborne microorganisms in different food matrixes are spread across various device platforms utilizing different bio-elements and transducers. Advances in parallel research areas such as microfluidics, microelectromechanical systems, and nanomaterial technology have also pushed forward the development and use of biosensors.

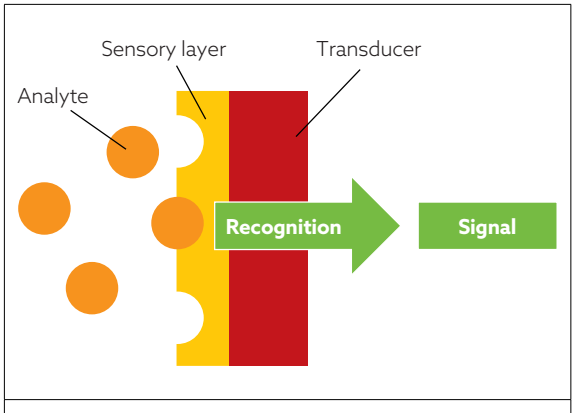


Figure 2. General Functioning of a Biosensor

Biosensors can be characterised by the biorecognition element:

i **Enzyme-based.** An enzyme-based biosensor is an analytical device that uses enzymes in the biorecognition process, the enzyme acting as a bioreceptor with a specific mode of action, usually associated with oxidation. For example, Ghasemi-Varnamkhasti et al. (2012) investigated detecting the aging of beer utilizing enzymatic biosensors based on cobalt phthalocyanine. Additionally, enzymatic biosensors have been used in the dairy industry, where screen-printed carbon electrode were modified with enzymes and incorporated into a flow cell to quantify three organophosphate pesticides in milk (Mishra et al., 2012).

ii **Antibody-based.** The mechanism of these sensors is based on the affinity of antibodies towards particular antigens. Immunosensors are widely used to identify microbial agents in food for rapid and high-sensitivity reactions. The theory is based on the immobilisation of antibodies, known as immunorecognition elements on the surface of the electrode. For example, Kozitsina et al. (2017) produced a low-cost, simple-to-use immunosensor based on antibody-modified magnetite (Fe3O4-SiO2-NH2) nanoparticles for the rapid detection of *Staphylococcus* aureus in foods.

iii **Aptamer-based (biomimetic).** Aptasensors (a biosensor utilizing aptamers, single stranded DNA or RNA binding elements) is one of the biomimetic biosensors commonly used in the food industry. The function of aptamers is to bind to a non-nucleic acid target with high specificity. For example, Housaindokht et al. (2018) developed a low-cost aptamer-based biosensor to specifically detect *E. coli* O157: H7 by using a single-wall carbon nanotube modified screen-printed electrode. The biosensor was able to detect the pathogen in low concentration ranges such as would be found in fresh produce samples and was also proven to be effective on a real sample such as tap water.

iv **DNA-based.** Based on the binding characteristics of single-strand nucleic acid to its corresponding strand in a given sample. The interaction occurs when the stable hydrogen bond is formed between the two strands of nucleic acids. A DNA-based biosensor consists of pathogen-specific detector sequences that are immobilised to build a solid support

microarray. For example, a study conducted by Bang et al. (2013) demonstrated the use of DNA microarray to detect *L. monocytogenes* in milk.

v **Whole cell detection.** The identification of a foodborne pathogen by cellular structures or cell-based bioreceptors may depend on the whole-cell or portion of the cell capable of performing a specific bond with the target organism. In cell-based biosensors, two transduction phases and an entire cell are required to act as a molecular recognition element. For example, Banerjee and Bhunia (2010) successfully tested three prototypes of a B-lymphocyte (Ped-2E9) cell-based biosensor for the simultaneous identification of foodborne pathogens found in food and beverages, including *L. monocytogenes*, enterotoxigenic *Bacillus*, and *Serratia*.

vi **Phage.** The use of bacteriophages (a virus that may bind to specific receptors on the bacterial surface to inject genetic material inside the bacteria) as elements of biorecognition to detect the presence of a foodborne pathogen in the sample has become an emerging trend. The identification of bacterial receptors is made through the bacteriophage protein tail spike, and is a promising technique for pathogen detection in a food sample as it is highly specific. For example, Niyomdecha et al. (2018) demonstrated the successful use of M13 bacteriophage in a capacitive flow injection system to detect *Salmonella spp*.

vii **Other elements.** Other types of biorecognition elements used in biosensors include antimicrobial peptides (AMPs), sugars/lectins, other peptides/proteins and molecular imprinted polymers (MIPs). All of these have been investigated as of potential use in the recognition of food-borne bacterial pathogens in recent years.

Or by the nature of the signal detected by the transducer:

i **Optical.** Involves light sources and optical materials to generate a light beam with specific features. This light is then transmitted to a modulating agent (the type of sensing head), which is connected to a photodetector. Optical biosensors are widely applied to detect the presence of foodborne pathogens in food samples due to sensitivity and selectivity. For example, Chen et al. (2018) developed an optical biosensor in combination with immunomagnetic separation, urease catalysis, and pH indication to rapidly identify the presence of L. monocytogenes in lettuce samples.

ii **Electrochemical.** An electrochemical biosensor can assess biological / chemical reactions involving immobilised biomolecules and target analytes, using measurable electrical properties such as current or potential which change via the creation or consumption of ions. Theoretically, the electrical signal can correlate to the concentration of the target analyte. In recent years, the electrochemical biosensor has attracted attention for the detection of foodborne pathogens because they are sensitive, low cost, can be used in turbid media and can be miniaturised (Kobun, 2020). For example, Lu and Gunasekaran (2019) developed and manufactured an electrochemical immunosensor for simultaneous identification of two mycotoxins in food samples.

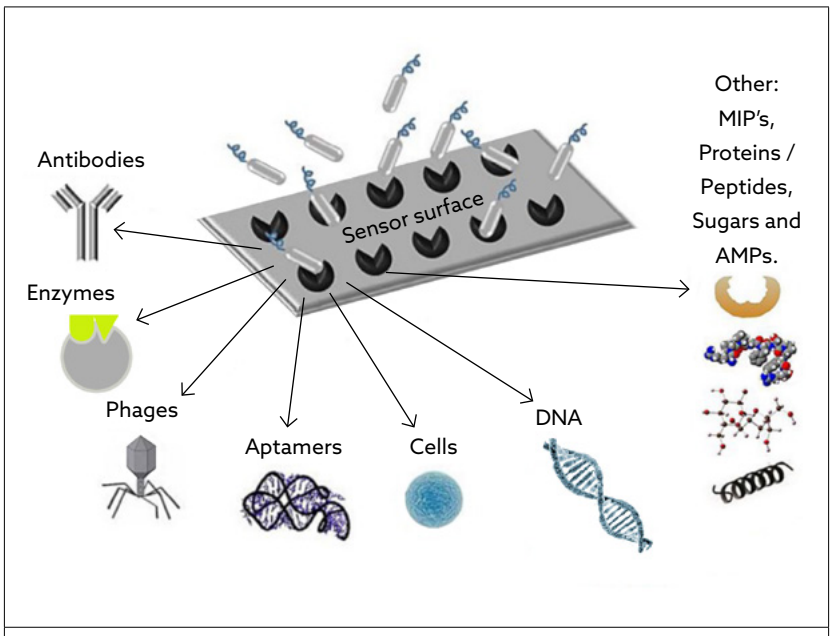


Figure 3. Overview of the Different Ligands Integrated in Biosensing Platforms

Modified from Templier et al 2016.

5.1.3.1 Nanotechnology and biosensors

Nanomaterials (nanoparticles, nanofibers) are incorporated into biosensors to improve their characteristics and are also driving miniaturization.

Nanomaterials may increase surface area and provide a strong bond to the bioreceptor to improve electrode response characteristics. Nanomaterials also provide higher catalytic capacity, improved bio-compatibility, and lower mass transfer resistance by increasing the volume to surface area ratio. In the same way, the larger surface area of the transducer can provide improved conductivity and sensitivity, as well as lower detection limits. Nanomaterials such as carbon nanotubes can also be incorporated into the transducer to achieve higher electron transfer rates and improved transducer operation, reducing analysis times. The sensing capability of sensor systems can be enhanced by the use of nanomaterials such as magnetic nanoparticles, carbon nanotubes, nanotubes, quantum dots, nanowires or nanochannels. Nanobiosensors have many functions and are highly versatile for food analysis due to their unique characteristics, which can produce fast, low cost, user-friendly, and high sensitivity biosensors. For example, Wilson et al. (2019) used iron oxide (Fe3O4) magnetic nanoparticles functionalised with a sensing bio-element to detect *E. coli*, *Staphylococcus aureus*, and *Salmonella typhimurium* in apple juice.

5.1.3.2 Biosensors and the fresh produce industry

It is important to recognise that ***the "perfect" bioreceptor does not exist***, each one having its own advantages and disadvantages. In the fresh produce industry, it is unlikely that a single biosensor will be suitable for all types of produce or pathogens. ***By necessity, the industry will need to be prepared to adopt a "toolbox" of various biosensors most suited to their needs.*** Nevertheless, by combining several types of bioreceptors and sensing approaches, it is nowadays possible to quickly detect a wide range of pathogenic microbes in a variety of samples (Templier et al., 2016, Yang et al., 2018, Choi et al., 2019, Kobun, 2020). The selection and discovery of new synthetic sensing molecules, i.e. aptamers, peptides, or small molecules, and the use of nanotechnology could lead to the development of more stable and efficient sensors. The final challenge is the integration of these different biosensors into small and portable devices providing rapid, accurate and inexpensive results.

5.1.4 Mixed technology methods: LOC / POC devices

Recently, there has been an increasing focus on developing strategies that incorporate all steps from sampling preparation to detection in miniaturised "lab-on-a-chip" (LOC) devices, or point-of-care (POC) diagnostic devices, as an alternative to laboratory benchtop devices. These devices incorporate multiple laboratory processes in a semi-automated and miniaturised format, replacing traditional expensive, time-consuming and laborious methods. The possibilities of combining various rapid methods, including nucleic-acid-based methods, immunological-based methods, and biosensor-based methods into a device with excellent reliability, ease-of-use, and rugged construction will increase the success rate of systems during on-site testing. Often, these devices can be microfluidic paper-based devices, such as lateral flow assays, that exploit different mechanical, chemical, and biological techniques to detect very low concentrations of pathogens in samples. LOC devices can also merge biosensing and microfluidic technologies to integrate one or more laboratory functions on a single electronic microchip, often providing reduced cost, increased speed of diagnostics, and ease of use for a range of industrial, environmental, and clinical applications. Nanomaterial technology is also increasingly finding applications within such devices.

5.1.4.1 Microfluidic chip devices

Microfluidics is the science and technology of devices that use tens-to-hundred micrometre channels that can move or control small volumes of fluids. It is a new multidisciplinary field that includes chemistry, fluidic mechanics, biology, nanomaterials, nanotechnology, and engineering known as the lab-on-a-chip due to its miniaturization and integration characteristics (Jayan et al.,

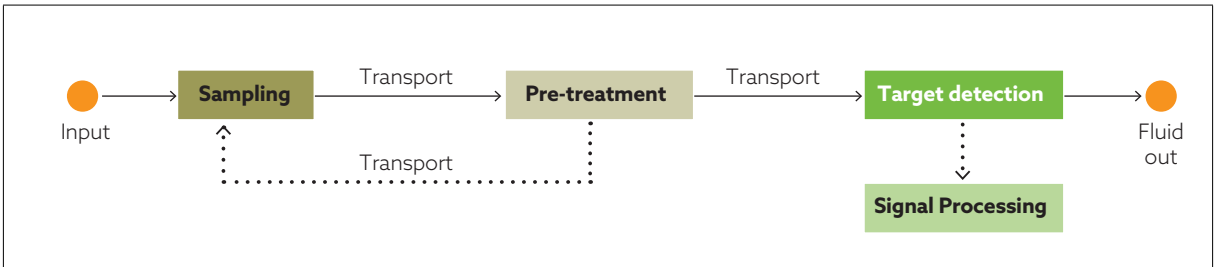


Figure 4. Generalised Flow Chart for a Lab-On-a-Chip System

2020, Kant et al., 2018). Microfluidics can be incorporated into multiple functions such as mixing, pre-treatment, reaction, separation, and detection on a chip of only a few square centimetres. Even tinier paper-based devices, particularly lateral flow test strips, are also broadly-used devices for food safety analysis. In response to demands for lower price per test, the field is rapidly switching from highly sophisticated fabrication technologies to polymer or paper-based devices that can fulfil the requirements of end users. Nitrocellulose paper-based devices offer many advantages, such as compatibility with most biological samples, ease of surface functionalization with various sensing elements, low cost, disposability, and capability to let all types of aqueous solutions moving through the devices. Additionally, the papers contain well-defined and distributed pores within their structure, which allow size dependent separation of sample molecules (Zhang et al., 2019). Both paper and chip-based microfluidic systems fulfill the values of the "green approach" in chemical analysis, using less solvents, reagents and energy than conventional lab-based systems. They also offer the advantage of being able to retain the sample for further testing if required.

5.1.1.2 LOC / POC devices and the fresh produce industry

Most existing chip-based or paper-based devices serve as a platform for the detection of pathogens based on various rapid methods, including nucleic-acid-based methods, immunological-based methods and biosensor-based methods. In the near future, all these methods may be integrated within microfluidic LOC systems and connect to smart phone technologies to generate the next generation of LOC / POC systems (Zhang and Liu, 2016). With the correct application of a number of these technologies simultaneously, broader ranging and more accurate technologies could be developed. It is these integrated devices that are most commonly commercialised, and have the most promise for rapid integration as part of testing regimes within the fresh produce industry. ***Devices utilizing isothermal nucleic acid amplification (e.g. LAMP), IMS or lateral flow immunoassays coupled with various forms of biosensors / nanobiosensors would be those of most interest to the fresh produce industry.***

As with biosensors themselves, there are advantages and disadvantages to each type of LOC device, and each specific type of producer will need to adopt devices that suit their own particular needs from within a "toolbox" possibly accredited by or developed by industry bodies in conjunction with researchers.

5.1.5 Holistic data analytics

A future direction is the holistic integration of rapid testing and data analytics for predictive risk modelling. Such data analytics may eventually be integrated with artificial intelligence/machine learning for predictive risk modelling of pathogen contamination – with sampling and data integration all the way from harvest to consumption. These systems are still in their infancy, but may well be of interest to the fresh produce industry in the future.

5.2 Specific challenges for rapid diagnostic methods in testing fresh produce

Despite the rapid growth RDM technologies, and the enormous effort spent on the development of biosensors and POC / LOC devices, very few devices have approached the point of commercialisation (Ali et al. 2020); ***one estimate suggests that over 80% of biosensor platforms developed at laboratory scale will never make it through to market*** (Diaz-Amaya et al. 2019). One issue is the substantial challenges involved in moving from laboratory bench technologies to device manufacture; many new technologies do not progress because the early stages of development ignore issues that arise later relating to scaling-up, economic viability, and inherent technology limitations under "real world" conditions.

In the specific context of RDMs for foodborne pathogens, requirements for sensors include the specificity to distinguish the target bacteria in a complex product, sensitivity to detect bacteria directly, and the ability to provide real-time results within a reasonable time. Detection of pathogens in a fresh product matrix is not simple and generally requires additional preparation steps before detection. The development of any rapid biosensors for detection of pathogens also relies on the type of produce and the nutrients present in these items, such as fat, proteins, and fibres. ***Hence, there may well be a need to develop a specific RDM for each fresh produce product.***

5.2.1 Variation among produce types

The term 'fresh produce' encompasses a very wide range of different horticultural product types, and there is associated wide variation in the potential for contamination by pathogenic micro-organisms. Produce items vary widely in in number of ways, including:

- Basic physical characteristics like size, shape, density, and structural complexity;

- Heterogeneity in tissue structure and density within a single unit of fresh produce – this can often include external crevices that may shelter micro-organisms from wash steps during processing;

- Whether the structure of a produce item tends to be entirely or only partially consumed (e.g. is the skin on a fruit eaten or discarded).

Further, there is considerable variation in how produce types are grown, irrigated, fertilised, harvested, processed, distributed for retail, stored, and treated by consumers prior to consumption:

- Some produce types are grown in the ground; others are grown above ground but may contact soil, and other types are unlikely to ever have direct contact with soil.

- The cleanliness of irrigation and wash process water may vary, as well as methods of water application.

- Application of fertiliser varies, including the important variable of whether treated or non-treated composted material is used.

- The extent to which produce is handled by human workers post-harvest differs between produce types, as do factors such as storage conditions and useful shelf life.

- Intrinsic differences within the same commodity line; the same product grown on different farms even within the same local area may be exposed to quite different soil conditions, fertiliser, harvesting, and processing methods.

- The degree of manual and automated processing may have an impact on the potential for pathogen colonisation of produce; processing can cause superficial damage that exposes internal tissue and provides a point of entry and source of nutrients for pathogens; manual processing may introduce human-borne pathogens.

- The way in which produce is treated prior to consumption also varies; it may or may not be washed or peeled, it may be refrigerated or stored at ambient temperatures, and most importantly, it may be eaten raw or cooked.

Risk factors for foodborne pathogen contamination and causation of illness are thus highly context-dependent for each type of product. Integration of rapid diagnostic testing methods into the fresh produce industry will often necessitate risk assessment on a case-by-case basis, and there may be implications for best practice in terms of the best RDMs to apply, frequency of testing, at what point(s) in the process and supply chain it is most effectively applied, and the stringency of tests.

In many instances, more research is required across the industry and for different produce types. Better data are needed on issues such as adhesion of pathogens to external surfaces, extent of internalisation of pathogens, rates of post-harvest growth of pathogens, the extent of damage caused by handling and processing and the effect this has on colonisation, and the potential for cross-contamination of produce types in processing and distribution centres, to name but a few.

5.2.2 Sampling and preparation

A critical component of any rapid testing protocol will be the steps taken to sample a raw produce item, and to further prepare that sample for application in a diagnostic testing device. Sampling of produce for pathogen testing typically involves either:

i Internal sampling, in which a piece of tissue from a selected part of the produce item is removed then homogenised (through grinding or maceration); or

ii External sampling, where part of the exterior surface of the produce item is either physically swabbed or is washed/rinsed with a solution.

Environmental sampling of produce processing facilities and equipment is also typically performed by swabbing of surfaces. Sampling swabs are usually subsequently rinsed or added to a solution to be used for further preparation steps or for direct testing in a device.

Most rapid diagnostic methods also require some degree of initial pre-treatment of samples before undergoing detection, to purify samples, isolate the microorganisms from interfering materials, and to concentrate the microorganisms to the point where detection is possible. This adds time and complexity to any complete protocol for rapid detection of pathogens from food samples.

5.2.3 Food matrix effects

5.2.3.1 Inhibitory compounds

The biggest challenge in preparing a food sample for rapid diagnostic testing is efficient separation of pathogenic micro-organisms from the food matrix – the heterogenous mixture of cellular components, inorganic molecules, biochemical compounds, and fluid constituting the structure and tissue contents of the food item. This is obviously a lesser concern for than for external sampling techniques such as swabbing or rinsing than for internal sampling (where the tissue sample is homogenised). Homogenization of the plant tissue creates a mix of cellular debris and releases internal molecules and compounds, many of which may potentially interfere with the ability of diagnostic methods to detect pathogens, particularly where those methods involve enzymatic reactions, as in PCR and some biosensor detection methods (Schrader et al., 2012, Wang & Salazar, 2016).

Homogenised extracts from fruits and vegetables often contain a diverse array of compounds that can inhibit nucleic acid-based detection methods (Suther and Moore, 2019). Examples of inhibitory compounds commonly present in fruits and vegetables are chlorophylls, tannins, polyphenols, polysaccharides, and pectin; all of which can have relatively high concentrations in some produce (Nero et al., 2009).

A range of methods that have been developed to remove inhibitors from sample extracts, prevent their co-extraction, or to reduce their interfering effects and may need to be utilised before detection.

Because of the broad range of fresh produce types, and their diverse range of internal substances that may interfere with detection methods ***it may be necessary to develop matrix-specific protocols for rapid diagnostic testing on a case-by-case basis.***

5.2.3.2 Acidity

There may also be simple chemical effects of the homogenised food matrix that can interfere with rapid testing methods, such as pH of the plant tissue. Many produce types, such as fruits, can be quite acidic (low pH). Usually, this can be remedied through adjustment of sample pH with appropriate buffers, but it is another issue that needs to be considered when developing matrix-specific protocols for rapid tests, particularly when transferring methods between different produce types.

5.2.3.3 Indigenous microflora

The surfaces and matrix of raw produce items are also home to indigenous microflora – diverse microbial communities including bacteria, fungi, viruses, and parasites, most of which are non-pathogenic. Any diagnostic test must be able to efficiently detect the presence of the pathogen species of interest, which is often in relatively low numbers, amongst the background of these naturally occurring microflora species.

5.2.3.4 Implications

The issue of matrix effects for fresh produce samples presents two distinct problems for development of rapid diagnostic tests for pathogens. First, for a test to be considered rapid, it generally needs to have a relatively quick and simple pre-treatment of the sample prior to sensing/detection of pathogens. A quick extract from homogenised tissue will often be crude and likely to contain interfering compounds. Adding further complexity to the preparation process to negate the effect of inhibitors will generally add both time and expense, and it may require more technical skill and/or equipment. The challenge will be to create a matrix-specific protocol that is simple enough for it be useable on site, fast enough so that the total time for turnaround of results is still useful, while remaining affordable. ***Optimizing a method and protocol may become a trade-off between sensitivity, time, and cost.*** A better sample preparation may give a more reliable result, but it could cost more and take longer.

Second, because of food matrix effects, it is very difficult to evaluate published research results that claim advances or impressive technical specifications for rapid methods. In many cases, published research papers report analytical results, based on testing of relatively simple or purified starting material (ranging from pathogen-spiked solutions, to simple fruit juice or highly purified extracts from tissue). Often, the turnaround time claimed for a method appears to exclude or overlook any steps that might have been taken to purify the sample sufficiently prior to detection. ***Applying published laboratory methods to food samples in a field setting may not be a straightforward exercise,*** and the diagnostic performance (i.e. sensitivity, specificity, limits of detection) may fall well short of the published analytical performance.

5.2.4 Internalisation of pathogens

Another important consideration for pathogen contamination of fresh produce is the extent to which pathogenic bacteria may colonise or be absorbed into the internal tissues of the produce (as opposed to adhering to external surfaces only). Although the extent of internalisation is not well understood in most cases, it is expected that it will vary significantly among different types of fresh produce. Internalisation is known to be possible in peaches, lettuce, and presumably other leafy greens.

In the context of diagnostic testing, if there is pathogen internalisation then internal sampling of produce items by homogenization of tissue samples may be necessary for successful detection of contamination. External sampling alone might suffice in many other instances. Thus, the challenge for rapid diagnostic testing protocols is deciding for specific circumstances whether it is necessary to macerate tissue samples or proceed with an external rinse or swab sample. More research data are required to establish a clearer picture of this aspect of pathogen contamination in the industry.

5.2.5 Specific protocols for each produce type: a "toolbox" approach

Given all the variables outlined above, it is likely that there will need to be different protocols and possibly different RDMs developed for each type of fresh produce. ***It is envisioned that industry bodies could investigate and validate a range of RDMs to create a "toolbox" of techniques that could then be selected and / or modified by each producer to suit their own circumstances and needs.***

Optimisation of the sampling and preparation steps required prior to biosensing or molecular testing for pathogens is also an important area, which requires further investigation. The steps necessary are also likely to vary between fresh produce types, and perhaps even between producers, should their growing, processing and handling procedures be different.

The relative importance of rapidity, accuracy and cost may also vary between producers and produce types and will be further complicated by how RDMs are treated by regulators.

In general, there is much work to be done to validate and further develop "real world" diagnostic methods, with many techniques that seem promising in the laboratory settling so far failing the challenges presented by sampling fresh food products in an industry settling. This is complicated by the inherent differences and inhibitory compounds often found in fresh produce samples, particularly in those products where potential internalisation of pathogens requires macerated tissue samples to be analysed.

5.3 Conclusion: most promising types of RDMs for the fresh food industry

Despite the inherent challenges presented to RDMs by the fresh produce industry, there are still a number of promising emerging diagnostic techniques that may well prove to be effective, rapid and cost-effective methods of identifying pathogens in fresh produce items.

In general, well-established laboratory techniques such as HPLC, gas chromatography, various forms of PCR and ELISA are unsuitable for on-site rapid testing by the fresh food industry, as they are expensive, often require specialised laboratory equipment and trained personnel, and are often adversely affected by food matrix compounds that may well be present in fresh produce samples (this is a particular problem for PCR techniques and some types of biosensor).

Microfluidic chip (LOC devices) and paper-based devices (e.g. lateral flow test strips) provide promising advances in "real world" testing scenarios and offer the advantage of being affordable, sensitive, specific, easy to use and quick to provide results. The detection methods in such devices are generally based on different types of biosensors, sometimes with a pre-sensing step such a LAMP or IMS also incorporated. Colorimetric, fluorescent and electrical detection signals are all common, with further data interpretation sometimes provided by inputting these signals into smart phone apps or the like (Zhang and Liu, 2016).

The incorporation of nanomaterials is capable of increasing the optical and magnetic properties of biosensors and paves the way for increased sensitivity and precision sensing in LOC / POC devices in field settings. In general, these ***types of LOC / POC devices incorporating biosensors or nanobiosensors are the most promising for the fresh produce industry.***

6 OUTLOOK: WHAT CAN BE DONE TO FACILITATE THE DEVELOPMENT OF RAPID DIAGNOSTIC METHODS SUITABLE FOR THE FRESH PRODUCE INDUSTRY?

6.1 Bridging the gap between research and industry: challenges

Biosensors have been under development for about 50 years and have contributed to significant contributions in the academic field for the past 10 years. Nonetheless, other than pregnancy kit test and electrochemical glucose biosensors, very few biosensors have gained global retail-level commercial success. Bhalla et al. (2016) reported that this is due to industries having a hard time in translating academic research into commercially viable prototypes. One important message that emerged from discussions with industry experts (and through reviewing research literature) is that reliance on published claims for research advances may be of limited value for technology forecasting in industry settings. It is not a simple exercise to identify the frontrunning methods based on research claims alone; when laboratory methods are subjected to independent replication or evaluation on complex food matrices under "real world" conditions, they may perform poorly. The most appropriate methods from among the available LOC / POC devices will need to be identified by evaluating in situ performance using fresh produce samples. In addition, a common limiting factor for all rapid methods is the necessary preparation of the product sample prior to the detection step; that is, purification (clean up) and concentration of the sample to a point where it is ready to be run on a rapid detection device or assay. This is of particular concern where internal tissue samples must be tested to ensure safety of particular products (e.g. leafy greens).

Interviewees for this report spoke at length about the myriad practical, technical, and economic challenges that impede research on rapid diagnostic technologies, and in particular, the obstacles for progressing R & D work in this area towards prototypes and eventually commercially available devices and methods for the fresh produce industry. Understanding these challenges is important for bridging the divide between laboratory research advances and commercial solutions.

6.1.1 What does industry actually want?

One of the key outcomes of the expert interviews was the strong need to involve industry at an early point in the development process – ***only by understanding their needs, challenges and preferences can a RDM be developed that will be assured of rapid and widespread uptake.*** For example, 'dipstick'-type lateral flow devices did not appeal widely to the interviewed experts – the single-use disposable nature of these was considered unsatisfactory and discussions with industry had indicated a strong preference for electronic diagnostic devices that can be washed and reused, as a more environmentally sustainable option.

To maximise the chance of successful industry adoption, designers and developers of testing devices should first define exactly what it is that industry wants and needs, before undertaking prototype development.

It is worth noting that the current level of development in LOC / POC devices cannot be solely described by reference to published research or interviews with industry experts. Commercial and Intellectual Property (IP) constraints may inhibit the open exchange of information where a RDM is under development into a commercially available test. Further research as to currently available and developing commercial RDMs is needed in order to fully understand opportunities to use or adapt commercial RDMs for the fresh produce industry, but was beyond the scope of this report.

6.1.2 Funding

Even from the starting point of a working prototype device, the commercialisation pathway can be lengthy and expensive. If industry players do not see a fast, tangible benefit to their business, they will not be willing to take a risk on the investment, given their other financial priorities.

The scale of the fresh produce industries in New Zealand and Australia may be a barrier to effective levels of investment in rapid diagnostic technologies; there may be insufficient critical mass to drive R & D and adoption of new methods or platforms. A potential solution may be to bring together different food industries in the two countries (e.g. dairy, meat, seafood, fresh produce), in an effort to create a large enough market to attract external investment. An alternative approach to funding development and commercialisation of promising technologies

may be to try to combine smaller funding amounts from multiple sources. However, this can be difficult since many different groups may be competing against each other for those funds. It would clearly be beneficial for the produce industry to pursue a collaborative strategy for technology development that minimises competition for limited R & D investment and maximises the benefit of companies banding together to pool smaller investment amounts. However, it will first be necessary to determine the level of enthusiasm (or reluctance) of industry players to work together in this way to develop rapid testing capabilities. Any industry-wide survey of this nature should also aim to establish how much individual companies or specific produce industries are prepared to pay to adopt rapid testing methods when they become available; this information will be essential for R & D investors and biotechnology companies to understand the potential market.

6.1.3 Cost / benefit to industry

Once any new rapid testing method has been developed and validated and becomes commercially available, there is still a question of how extensively it will be adopted by industry. ***The degree of industry uptake is likely to depend on the cost of implementation, more than any other factor, especially for an industry like fresh produce where there are already small profit margins.*** New rapid methods will need to be the same price (if not cheaper) than current approaches, or if more expensive, able to deliver tangible benefits to the business by reducing time and/or effort. A parallel benefit would be in reducing the likelihood of potential food safety incidents that could be harmful to the business; the cost of rapid testing will need to be balanced against the financial and reputational costs of produce recalls or withdrawals. Again, identifying these drivers before development is a key factor in the commercial success of a new RDM.

7 WHAT CAN BE DONE TO DRIVE UPTAKE OF RAPID DIAGNOSTIC METHODS IN THE INDUSTRY?

The experts interviewed for this study offered diverse opinions when asked to discuss the principal factors that could drive the industry to adopt and integrate rapid testing methods into existing production systems.

Some held the view that R & D and integration of new rapid methods will have to be driven by industry itself, given their impression that industry players could be resistant to demands on how they operate imposed by government regulators. Rapid diagnostics could bring important benefits for consumer safety and the minimization of the reputational harm and financial losses associated with food safety incidents that demand produce withdrawals and recalls.

Other interviewees suggested that the top-down pressure of legislation and regulatory requirements may be necessary to drive uptake of rapid testing. This was based on the belief that many produce growers and companies are unlikely to adopt and pay for rapid testing methods voluntarily, since they have more immediate financial priorities like the costs of agrochemicals, water, and labour. For this reason, adoption of rapid testing by produce growers may need to be pushed by government, via legislated regulations or guidelines, or alternatively, through the insistence of someone down the line, such as produce retailers, insurance companies or end user consumers. Another driving force for some areas of the produce industry could be the specific regulatory demands of various international export markets.

7.1 Create clear understandings of the potentials and limitations of rapid diagnostic methods and their potential

The ***expectation that a single RDM can be developed to meet the needs of the entire fresh produce industry is unrealistic,*** given the wide diversity within the industry, including the types of produce, methods of growing, processing and handling techniques and consumer use of the final produce. It is important that this is clearly communicated to industry from the outset, and ***a more realistic expectation of a suite or "toolbox" of useful RDMs that could be selected for their suitability*** by each producer is expected. Industry bodies have an important role to play in the compiling of information about the requirements and preferences of industry members and translating this information to lead to the development of useful, robust prototype RDMs. Funding for R & D and prototypes will also need to be secured. These prototypes will then need to be extensively tested in the field to ensure that ***existing challenges to translating promising laboratory techniques to "real world" settings can be overcome.***

Challenges inherent to the fresh produce industry, such as the inhibitory effects of the sample matrix of macerated tissue samples, the need for careful consideration of the types (internal vs external samples) and timing of sampling throughout the growing, packaging or handling process and the wide variation in types and risk profiles of produce types must also be communicated and understood by the industry if RDMs are to be used successfully and efficiently.

The rapidity, simplicity, cost-effectiveness and portability of POC / LOC devices incorporating a range of diagnostic techniques into a single, robust and inexpensive kit makes them a key contender for RDMs applicable to the fresh produce industry. Their advantages and potential must be clearly communicated to industry if they are to achieve eventual widespread adoption.

7.2 Define clearly what the industry wants and needs from rapid diagnostic testing

Given industry variation in terms of produce types, supply chain organisation, and processing systems, it would be useful exercise to survey the industry to establish a clear understanding of the variation in requirements (and desire) for rapid testing, with a focus on the following types of questions:

- How will rapid testing be applied and integrated into existing testing programs?
- How frequently and at which point(s) during the supply chain will testing occur?
- What are the specific requirements for testing your particular produce type (internal vs external sampling, sampling points in time / handing procedure etc.)?
- What are the requirements for turnaround time, accuracy, and tolerable cost?

- What actions will be taken in response to positive results?

Understanding the diversity in requirements and expectations for rapid testing for the industry will help to determine which technologies are best pursued in terms of further funding, research, development, and evaluation through field testing of prototypes.

7.3 Potential partnerships for commercialisation

There are a number of potential partners for the commercialisation of RDMs that would be useful to the fresh produce industry. These include:

- Producers / growers / food companies;
- universities;
- biotechnology companies / kit manufacturers
- CSIRO / government agencies;
- industry partners; and
- regulators.

Each of these groups have their own agenda and priorities, but by engaging each as soon as possible in the funding, R & D, prototype, field testing and commercialisation process, their input can be incorporated into any final product, making it more robust and widely applicable.

8 CONCLUSIONS AND RECOMMENDATIONS

8.1 Key points arising from the study

Conventional methods that are commonly used to detect the presence of pathogens in fresh produce samples are highly dependent on identified microbiological, biochemical, or genetic identification. Those techniques can take significant amounts of time, as well as being expensive and necessitating the transport of samples to an off-site laboratory. An enrichment step is also often needed to identify target microorganisms that often occur in small numbers in the samples. The limitations of available conventional methods necessitates the development of a fast, sensitive, inexpensive and on-site detection method.

The fresh produce industry has high inherent variation – between produce types, growing techniques, handing and processing methods and the eventual use of the product by the consumer. These increase the challenges to finding appropriate RDMs that can provide fast, reliable and inexpensive results in a "real world" setting.

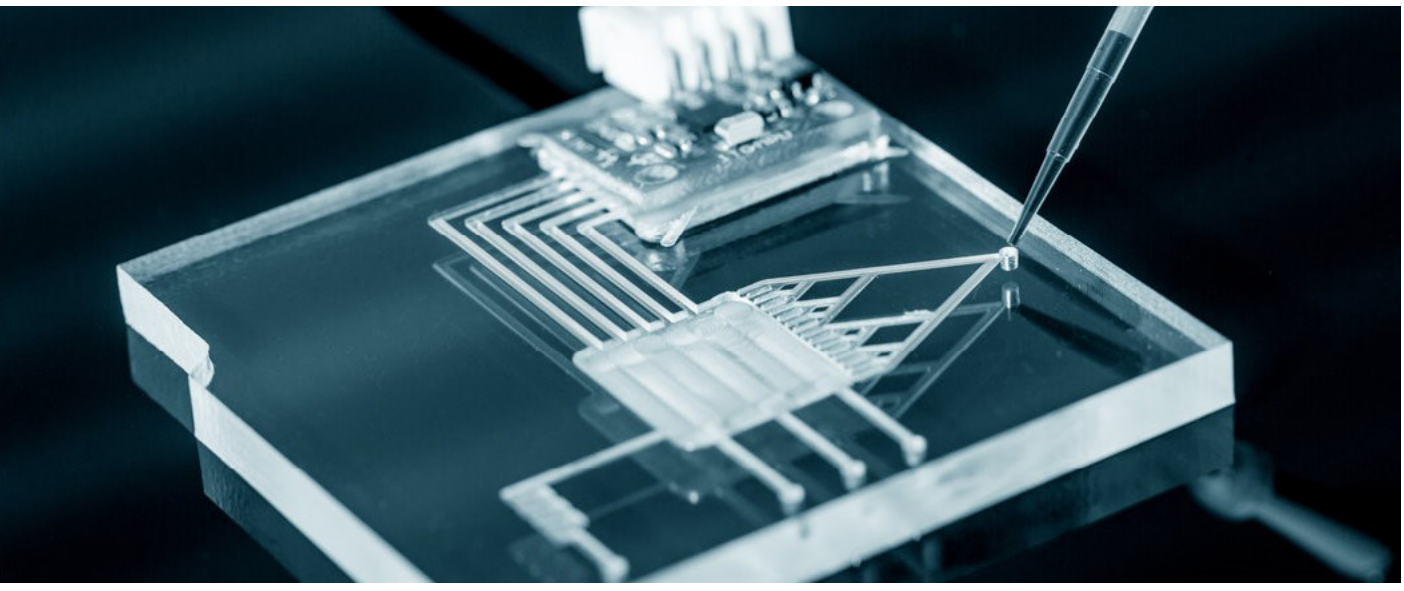
Various LOC and POC devices incorporating biosensors / nanobiosensors are a promising method that can meet all these requirements. The identification and detection of foodborne pathogens by LOC biosensors has many appealing properties because of their high sensitivity, robust performance, virtually real-time unenriched quantification, cost-effectiveness, multiplexing ability, and the prospect of on-site detection in an easy-to-use device. However, it is unrealistic to expect a single LOC / POC device to be suitable for the entire fresh produce industry. Instead, we suggest a suite of methods may be investigated, developed and validated, allowing each producer to choose the method(s) most suitable for their needs from this "toolbox".

8.2 Recommendations: Desirable directions for further research and development

The development, commercialisation and adoption of suitable RDMs in the fresh produce industry is a large and complex project. For this reason, we suggest a focus on "getting things started" at this point. For example, it may be worth focusing on:

- Two-three high priority produce groups to begin with – e.g. melons, berries, leafy greens.
- External pathogen contamination over internalised pathogens (this greatly decreases inhibitory compounds and pre-treatment of samples required).
- Engaging several industry "big players" first, and including other industry members later in the development pathway.

Given industry variation in terms of produce types, supply chain organisation, and processing systems,



Lab-on-a-chip

it would be a useful exercise to broadly survey the industry to establish a clear understanding of the variation in requirements (and desire) for rapid testing (as outlined in Section 7.2).

Concurrently, communicating the current state of RDMs research, their potential benefits and the challenges to their use in the fresh produce industry to the industry at large would be valuable. ***The key message, that a single RDM is unlikely, and instead a "toolbox" of validated methodologies (most likely in the form of LOC / POC devices) is to be expected as an outcome, is vital for industry understanding and eventual uptake.***

Further research into the most promising / appropriate currently commercially available LOC / POC biosensing devices also needs to be undertaken, with data from industry as to their wants / needs then applied to determine a suitable candidate for field testing, initially with a small cohort of producers.

In the longer term, investment in basic research, as well as continued engagement with industry stake-holders to ensure that promising ideas in the lab are developed into prototypes, field tested in "real world" settings and eventually commercialised in a way that is useful and wanted by industry is crucial. It is important to understand that the challenges of the fresh produce industry and the difficulties in translating laboratory success into robust, inexpensive and reliable devices for use in the field means this work will occur over a timescale of numerous years.

Sources of potential funding for this type of research also needs investigation, and it is crucial that funding includes resources for industry engagement throughout the process. This can be a challenge in the commercial setting, where intellectual property concerns and potential commercialisation and profit drivers come into play.

Currently, there are multiple groups around New Zealand and Australia working on RDMs – there would be value in bringing together people from those groups or organisations in a workshop setting to share information. It would also be highly relevant to include industry players and regulators to speak about their requirements and expectations from RDMs.

9 ABSTRACTS/EXTRACTS FROM SELECTED PAPERS

Law, J. W., Ab Mutalib, N. S., Chan, K. G., Lee, L. H. (2015) Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations *Frontiers in Microbiology* 5, 770.

Conventional methods for the detection of foodborne pathogens which based on culturing the microorganisms are selective, but they can be time-consuming and laborious. Hence, various rapid detection methods have been developed in order to overcome the limitations of conventional detection methods. Rapid methods are important for the rapid detection of foodborne pathogens in food products to prevent outbreaks of foodborne diseases and the spread of foodborne pathogens. Rapid detection methods are generally more sensitive, specific, time-efficient, labour-saving, and reliable than conventional methods.

Nucleic acid-based methods such as PCR, mPCR, qPCR, and DNA microarray have high sensitivity and they are widely used for the detection of foodborne pathogens, but these methods require trained personnel and specialized instruments. Alternative nucleic acid-based methods such as NASBA and LAMP are available for the detection of foodborne pathogens and their toxins. NASBA and LAMP are relatively sensitive, specific and cost efficient. They do not require thermocycling system therefore they are useful especially in low resource settings. Furthermore, numerous biosensors-based methods have recently emerged and employed in the field of foodborne pathogen detection due to their rapidness and cost effectiveness. Biosensors-based methods are easy to operate, and they do not require trained personnel, furthermore the techniques can be used for the detection of foodborne pathogens without sample pre-enrichment. However, improvement in food matrixes detection is still needed for these methods for on-site detection. Immunological-based methods such as ELISA and lateral flow immunoassay are also used for the detection of foodborne bacterial pathogens and their toxins. Immunological methods work best in the absence of interfering molecules in the samples such as non-targeted cells, DNA or proteins. Combination of several rapid methods for the detection of a particular foodborne pathogen is also possible as the use of only one detection method may not be sufficient to confirm the detected pathogen. Further studies on the effect of different combinations of rapid methods for foodborne pathogen detection are required in order to develop the most effective and accurate detection method.

Yang, Q., Domesle, K. J., Ge, B. (2018) Loop-mediated isothermal amplification for *Salmonella* detection in food and feed: current applications and future directions *Foodborne Pathogens and Disease* 15(6), 309–331.

Regarding new platform developments, closed-tube, "one-pot" platforms that allow rapid, sensitive, specific, and real-time amplification and detection in small, portable, robust, and user-friendly instruments will be the mainstream. The development and refinement of microfluidic devices (heat control, fluid manipulation, and monitoring method) will continue at a rather fast speed, focusing on full integration of sample preparation, amplification, and detection on one simple, small, user-friendly microdevice. Improvements in sample throughput and field amenability are also desired.

Special considerations should be given when adopting these new advancements in food and feed testing. In terms of assay development, there is currently a paucity of LAMP primers developed for specific Salmonella serovars other than Salmonella Enteritidis and Salmonella Typhimurium. LAMP assays for Salmonella serovars that are major animal pathogens are also scarce. Progresses in the areas of viable detection (Lu et al., 2009; Chen et al., 2011; Techathuvanan and D'Souza, 2012) and contamination prevention (Hsieh et al., 2014) have been made and further research is still needed. Simple and effective sample preparation methods, including DNA extraction and storage for field detection are in great demand. Further developments in non-instrumented nucleic acid amplification such as running the assays in a thermos (Kubota et al., 2013) or a pocket warmer (Zhang et al., 2018) will enable field-based food and agricultural diagnostics. Finally, there is an increasing need for matrix-specific validation of newly developed methods. Such validations should follow international guidelines before the methods can be adopted for routine use in food and feed testing.

Zhao, X., Lin, C.W., Wang, J., Oh, D.H. (2014) Advances in rapid detection methods for foodborne pathogens *J. Microbiol. Biotechnol* 24(3), 297–312.

Food safety is increasingly becoming an important public health issue, as foodborne diseases present a widespread and growing public health problem in both developed and developing countries.

The rapid and precise monitoring and detection of foodborne pathogens are some of the most effective ways to control and prevent human foodborne infections. Traditional microbiological detection and identification methods for foodborne pathogens are well known to be time consuming and laborious as they are increasingly being perceived as insufficient to meet the demands of rapid food testing. Recently, various kinds of rapid detection, identification, and monitoring methods have been developed for foodborne pathogens, including nucleic-acid-based methods, immunological methods, and biosensor-based methods, etc. This article reviews the principles, characteristics, and applications of recent rapid detection methods for foodborne pathogens.

Choi, J., Yong, K.W., Choi, J., Cowie, A. (2019) Emerging point-of-care technologies for food safety analysis *Sensors* 19, 817.

Food safety issues have recently attracted public concern. The deleterious effects of compromised food safety on health have rendered food safety analysis an approach of paramount importance. While conventional techniques such as high-performance liquid chromatography and mass spectrometry have traditionally been utilised for the detection of food contaminants, they are relatively expensive, time-consuming and labour intensive, impeding their use for point-of-care applications. In addition, accessibility of these tests is limited in developing countries where food-related illnesses are prevalent. There is, therefore, an urgent need to develop simple and robust diagnostic POC devices. POC devices, including paper- and chip-based devices, are typically rapid, cost-effective and user-friendly, offering a tremendous potential for rapid food safety analysis at POC settings. Herein, we discuss the most recent advances in the development of emerging POC devices for food safety analysis. We first provide an overview of common food safety issues and the existing techniques for detecting food contaminants such as foodborne pathogens, chemicals, allergens, and toxins. The importance of rapid food safety analysis along with the beneficial use of miniaturised POC devices are subsequently reviewed. Finally, the existing challenges and future perspectives of developing the miniaturised POC devices for food safety monitoring are briefly discussed.

Templier, V., Roux, A., Roupioz, Y., Livache, T. (2016) Ligands for label-free detection of whole bacteria on biosensors: A review *Trends in Analytical Chemistry* 79(2016), 71-79.

With the aim of getting earlier, sensitive and specific information on the presence –or absence – of bacterial pathogens, biosensors are getting an increasing interest for more than two decades. This is partly due to their reduced format, to the possibility to address several questions with a single device and also to the increasing panel of physical approaches that can be exploited for signal transducing. When designing a biosensor, the choice of the ligand motif remains a key element as it drives the efficiency and sensitivity of the assay. In this review, we propose to gather and comment different ligands used for the detection of whole cell bacteria. Because time is a crucial issue when looking for a pathogen, our attention was focused on whole cell assays and label-free methods, which enable the user to skip sampling processing steps and decrease the overall test cost.

Li, Y., Fan, P., Zhou, S., Zhang, L. (2017) Loop-mediated isothermal amplification (LAMP): A novel rapid detection platform for pathogens *Microbial Pathogenesis* 107 (2017), 54-61.

Foodborne bacterial infections and diseases have been considered to be a major threat for public health in the worldwide. Increased incidence of human diseases caused by foodborne pathogens have been correlated with growing world population and mobility. Loop-mediated isothermal amplification (LAMP) has been regarded as an innovative gene amplification technology and emerged as an alternative to PCR-based methodologies in both clinical laboratory and food safety testing. Nowadays, LAMP has been applied to detection and identification on pathogens from microbial diseases, as it showed significant advantage in high sensitivity, specificity and rapidity. The high sensitivity of LAMP enables detection of the pathogens in sample materials even without time consuming sample preparation. An overview of LAMP mainly containing the development history, reaction principle and its application to four kind of foodborne pathogens detection are presented in this paper. As concluded, with the advantages of rapidity, simplicity, sensitivity, specificity and robustness, LAMP is capable of applications for clinical diagnosis as well as surveillance of infection diseases. Moreover, the main purpose of this paper is to provide theoretical basis for the clinical application of LAMP technology.

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11 APPENDICES

Appendix 1: List of interviewees
The following is a list of the participants, in the order in which they were interviewed:

1. Prof Robert Buchanan – Director, Center for Food Safety and Security Systems, University of Maryland, USA

2. Graham Fletcher – Team Leader, Seafood Technologies, Plant & Food Research, NZ

3. Dr Rob Lake – Science Leader, Environmental Science & Research, NZ

4. Dr Craig Shadbolt – Principal Food Safety Scientist, NSW Food Authority, Australia

5. Dr Jocelyn Eason – General Manager Science, Food Innovation, Plant & Food Research, NZ

6. Dr Sharon Jones – General Manger Technical, One Harvest Ltd, Australia

7. Nicola King – Senior Scientist, Environmental Science & Research, NZ

8. Prof Nigel French – Chief Scientist – NZ Food Safety Science & Research Centre, NZ

9. Ben Daughtry – Senior Food Scientist, Food Standards Australia New Zealand, Australia

10. Dr Craig Billington – Science Leader, Environmental Science & Research, NZ

11. Dr Harry van Enckevort – Science & Technology Advisor, AsureQuality, NZ

12. Dr Ramin Khaksar – Chief Scientific Officer, Clear Labs, USA

13. Dr Andrew Kralicek – Team Leader, Plant & Food Research, NZ

14. Dr Angela Cornelius – Senior Scientist, Environmental Science & Research, NZ

Appendix 2: Interview questions
A set of ten questions, sent to the interviewees prior to the interview, was used as the basic structure for the conversation. Because of the diversity in roles and expertise among the interviewees, it was not expected that individuals would be familiar or knowledgeable on all of the topics raised by the interview questions, and this proved to be the

case. Hence, the interview questions were applied as a general framework for each conversation, with additional questions inserted into each conversation to focus on the specific expertise of the interviewee relevant to rapid testing methods or the fresh produce industry. The interviewees were also encouraged to offer any additional observations or opinions that they believed were relevant to the subject but had not been covered in the conversation.

Interview questions:

1. For this study, we have chosen to focus on the foodborne pathogens Escherichia coli (STEC), Campylobacter spp., Salmonella enterica serovars, and Listeria monocytogenes. Would you agree/disagree that these should be the priority organisms for targeting with rapid diagnostic testing of fresh produce?

2. For testing of fresh produce to be effective and commercially viable, what are the requirements in terms of speed (turnaround time), limits of detection, sensitivity and specificity?

3. Based on these criteria, how do different emerging diagnostic technologies for rapid testing compare? What are the most promising front-runners?

4. Research and development of rapid diagnostic technology often involves testing of meat or dairy samples. Are there specific technical challenges or requirements involved in rapid testing of fresh produce (e.g. in terms of sample preparation) and how might this differ between produce types?

5. How necessary and/or feasible will it be to develop multiplex tests for simultaneous detection of several different pathogen types?

6. What are the most significant technical barriers or challenges impeding commercialisation of emerging rapid diagnostic methods? What is the best pathway for commercialisation and who might make effective partners?

7. How close (or far away) do you think we are from simple and rapid benchtop or lab-on-chip diagnostic technology becoming widely available and successfully established in the fresh produce industry?

8. What steps should be taken next to further develop promising methods for the industry (e.g. in terms of testing and evaluation)? Where should investment be directed?

9. What are the critical regulatory considerations for implementing rapid diagnostic testing in the fresh produce industry? What changes to the regulatory framework or recalibration of microbiological standards will be required to enable adoption of new rapid testing methods?

10. Can you recommend any other experts we should contact for this study?

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