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Are point of management assays relevant for food safety in the poultry industries?

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ABSTRACT

The current pandemic has ensured considerable attention has been paid to the role of the approach termed 'Point of Care' diagnostics. Indeed, the term 'RAT' (Rapid Antigen Test) and RAT hunting now have totally different meaning to that widely understood before 2020. In the veterinary field, including food safety, the term used for these types of rapid *in situ* assays is 'Point of Management' (POM) assays. In this article, we describe our recent research on low cost, low technology, in-house style POM assays in the field of food safety as applied to the poultry industries. We then discuss what are the advantages and disadvantages of these low cost, low technology POM assays.

Keywords: *Campylobacter*, chicken, food safety, isothermal, LAMP, point of management, poultry, *Salmonella*.

Key food safety pathogens for the poultry industries

As with all food production systems, the potential for food safety pathogens to enter the system and cause human illness is a key issue for the Australian poultry industries. Two genera dominate the food safety issues linked to the poultry industries – *Campylobacter* and *Salmonella*.

Over 96 million cases of food-borne illness linked to *Campylobacter jejuni* and *C. coli* are estimated to occur globally each year.[1] The Australian infection rate of 146.9 cases per 100 000 population[2] is one of the highest among the industrialised countries.[3] Importantly, while not the only source of *Campylobacter*, it is recognised that in Australia, undercooked poultry are a major source.[4]

Salmonella is second only to *Campylobacter* as the most notified enteric pathogen in Australia.[2] As with *Campylobacter*, while there are multiple sources of human infections, raw and undercooked foods, eggs, and to a lesser degree poultry meat, are often associated with *Salmonella* infections.[5]

Food-borne pathogens impose costs onto both broad society as well as the production system. While figures are not available for Australia, a recent report estimated that campylobacteriosis costs the US (population >10 times that of Australia but with 10 times lower *Campylobacter* infection rate[3]) in the range of US$1.5–US$6.9 billion per year depending on the calculation method.[6] The reduced income and increased expenses associated with *Salmonella* in the Australian egg industry were estimated to cost $7 million annually in 2015–2016.[7] The scale of these economic impacts to both broad society as well as the producers emphasises the need for improved control of food-borne pathogens in the poultry industries.

POM assays

Our work in the development of POM assays arose from the finding that dipsticks made from untreated cellulose-based paper can bind nucleic acids in seconds, retain them during a rinse step that removes the contaminants and then release the nucleic acids when placed in a reaction buffer.[8,9] We have combined the low-cost DNA extraction technology with isothermal amplification performed in another existing innovation – the 'Diagnostic Droid'.[10] The workflow involves a centrifugation of a 2 mL aliquot of carcass rinse, a treatment of the pellet with proteinase K and heat denaturation to release the DNA. Two dipsticks are added to bind the DNA and purify it away from the contaminants. Each dipstick is then given a single wash and the DNA eluted into a loop mediated

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Fig. 1. Work flow for the *Campylobacter* Point-of-Management assay. A 2 mL aliquot of the carcass rinse is centrifuged (5 min) and the pellet resuspended, treated with proteinase K and heated to lyse cells and release DNA. The two cellulose dipsticks are added to the crude lysate to bind the released DNA. The dipsticks are then placed in a wash buffer and then in the LAMP reaction mix (one for *C. jejuni* and one for *C. coli*). The LAMP reactions proceed for 100 min at 65°C in the 'Diagnostic Droid'. The Droid monitors the reaction and interprets the result.

isothermal amplification (LAMP) reaction mix for either *C. jejuni* or *C. coli*. The 'Diagnostic Droid' performs the amplification step and monitors the increase in turbidity associated with a positive LAMP reaction by illuminating the reaction tubes with an LED and measuring the amount of scattered light via a light sensor perpendicular to the LED light source. The results are automatically interpreted with no need for human involvement and are available within 2.5 h[10] (see Fig. 1 for an illustrative workflow of our POM for *Campylobacter*). The Australian chicken meat industry has a self-set target of ensuring that all chicken carcasses have less than 6000 colony forming units (cfu) which is equivalent to 12 cfu/mL in the 500 mL volume used to wash the carcass. The performance of the POM assay was evaluated using 29 rinse samples that were examined by the relevant Australian Standard method[11] (see Fig. 2 for an illustration of the work required by this Standard) as well as the POM assay.[10] A total of 26 samples were in agreement – 16 were high in the POM assay and contained more than 12 cfu/mL and 10 were low in the POM assay and had <12 cfu/mL in the culture method. While three samples gave different results in the two assays (i.e. an 11% disagreement), only two samples (i.e. 7% of samples) were a major disagreement where the POM assay result was low and the culture result was above 12 cfu/mL. It is worth noting that these two disagreements occurred on the edge of the 12 cfu/mL cut-off – being 23 and 37 cfu/mL. All samples above 45 cfu/mL in the culture method were positive in the POM assay. Overall, this work has shown the potential for a very low-cost POM assay that uses little in the way of technology to be used to semi-quantify the level of *Campylobacter* in chicken carcass rinses.

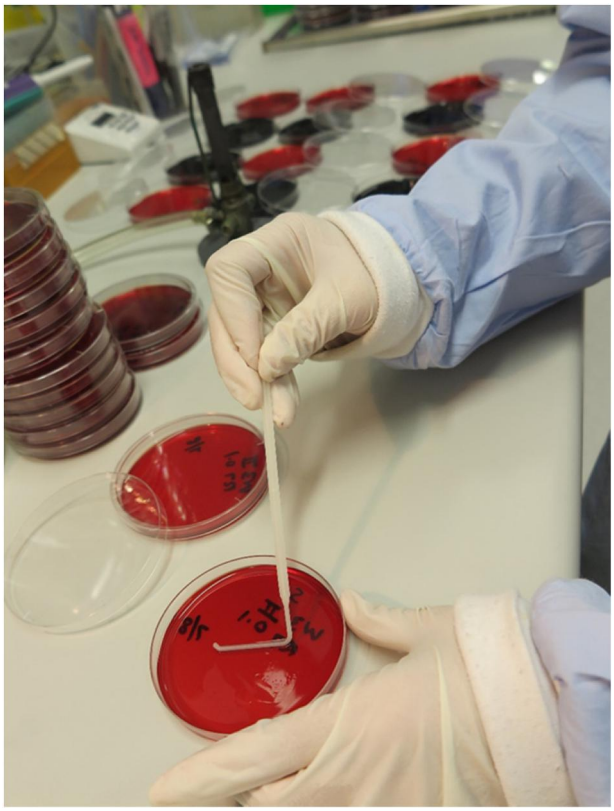

Fig. 2. Part of the work flow for conventional Australian standard methods for *Campylobacter*.

Who pays?

In evaluating the advantages/disadvantages of a POM assay there is a critical primary issue that first needs to be addressed – who pays? In a situation where a producer has a disease problem on farm, the solution of a vaccine that controls the disease results in an extra cost but returns an increased profit via less mortalities or an improved growth rate. In these circumstances, the producer sees a direct financial benefit from a new intervention or a new management tool. In contrast, producers or processing companies that introduce an intervention to reduce the level of *Campylobacter* have the cost of that intervention but no direct financial return. Society would benefit from the intervention with a lower level of campylobacteriosis in the population but the producer/processor bears the costs and gains no financial benefit. Hence, any interventions to increase food safety (such as POM assays to provide near to real time information on *Campylobacter* levels on carcasses) must be implemented with an understanding that society will benefit but that there will be no immediate financial benefit to the individual producer or processor. Clearly, an industry providing food to the population benefits from a public perception that they provide a high-quality product that is safe and healthy. The public expects safe and healthy food but often does not

understand the associated financial burdens. Hence, adoption of POM assays for food safety pathogens has to occur in a situation where no immediate benefit flows from the cost of adopting the technology.

Advantages of POM assays

In evaluating the advantages of the type of POM assay we have developed, our vision is that these assays are not seeking to replace the Australian Standard method[11] but rather add to the quality and relevance of the formal testing provided by the standard methods. The POM assay we have developed is as sensitive as the gold standard of culture but gives results in a little over 2 h vs 2 days required by the standard culture method. Hence, quality assurance/quality control staff can implement routine rapid, low-cost regular monitoring of the effectiveness of the processing chain in achieving the industry set target of <6000 cfu of *Campylobacter* per carcass. Given that there are around 50 processing plants for chickens in Australia and that over 663 million birds were processed in 2020, the need for cost-effective, low technology, rapid assays suitable for both the large multi-state processors as well as the much smaller state and regionally based processors is clear in our view. A POM assay would allow refinements and alterations in production parameters to be rapidly monitored for their impact on *Campylobacter* levels. POM assays should not be seen as replacements for the nationally certified standards but rather supplementary, additional tests that support the same goal – the production of a high quality, safe food product.

Disadvantages of POM assays

POM assays suffer the same generic problem that all food safety interventions suffer as outlined above (i.e. they are an additional cost). However, it should be noted that the cost of our POM approach is far cheaper than the currently available commercial instruments and assays.

There is a general acceptance that POM assays are not as sensitive as laboratory-based methods – as clearly shown in comparisons of RATs and RT-qPCR for COVID-19.[12] Our work is, at this stage, still too early to provide firm evidence about the relative sensitivity of culture and our POM assay. However, it should be noted that the three samples in disagreement all involved viable counts of less than 40 colonies on the two counting plates. As the reliable counting range for plate counts is 25–250 colonies,[13,14] it is clear that the disagreements occurred in a range where both technologies (plate counts and POM assay) were struggling to detect very low numbers of *Campylobacter*.

Conclusion

The importance of reducing the levels of *Campylobacter* on chicken carcasses for public health is well established. A European study has shown that a 2-log reduction in the number of *Campylobacter* on carcasses would result in a 30 times reduction in the incidence of campylobacterosis.[15] In our view, access to low cost, low technology, rapid POM assays is an essential requirement to ensure the level of monitoring required to achieve either the 2-log reduction suggested by the European study[15] or the industry-assigned target of 6000 cfu per carcass.

While this article has focussed on *Campylobacter* and chicken carcasses, there are other areas where POM assays for food safety pathogens could be effective tools e.g. in detecting the presence of *Salmonella* on layer farms. Few layer farms currently engage in *Salmonella* tests due to the costs of the assay, the delay in obtaining results and the distance from the farm to the laboratory. POM assays for on-farm detection of *Salmonella* would remove many of those barriers and could encourage a far more proactive quality assurance program.

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Data availability. Data sharing is not applicable as no new data were generated or analysed during this study.

Conflicts of interest. The authors declare no conflicts of interest.

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Biographies

Jillian Templeton is a microbiologist working for the Queensland Department of Agriculture and Fisheries. Jillian is a mid-career researcher who has led projects over the past 20 years to investigate the epidemiology of *Campylobacter* on Australian meat chicken farms; the development, validation and application of genotyping methods for *Campylobacter*; and the development of rapid diagnostic techniques for *Campylobacter*.

Jimmy Botella is professor of Biotechnology at the University of Queensland. Jimmy has eleven international patents in the field of Biotechnology, has founded two biotechnology companies and is a member of the Expert Scientific Panel for the Agricultural Biotechnology Council of Australia. Jimmy's research has been awarded with the Chinese Academy of Sciences Visiting Professorship for Senior International Scientists and the title of 'Pathologist of Distinction' by the International Society of Plant Pathology.

Pat Blackall is a bacteriologist working at the Queensland Alliance for Agriculture and Food Innovation at the University of Queensland. Pat has research interests in the bacterial diseases of intensively raised animals and in the food safety pathogens that are present in those industries.