



Real-time testing of foods: the Holy Grail?

*There may be a bad bug in food
Whose presence is simply no good
For the public's protection
We want fast detection
Immediate, if only we could.*

Introduction

The approach to quality assurance and control in the food industry has changed, especially with the widespread implementation of preventative, process-oriented food safety plans grounded in Hazard Analysis Critical Control Point (HACCP) and risk assessment principles. However, microbiological analysis of foods remains critical to the management of quality and safety of food products, particularly with respect to the detection of pathogens.

The time to complete tests has decreased significantly but, the required sensitivity of the test, the physiological state of the target analyte, the food matrix and associated non-target microflora, all constrain further acceleration of testing and limit the potential for achieving real-time testing of foods, particularly when testing for pathogens such as *Salmonella*. While real time testing may be the ultimate goal, is it food microbiology's Holy Grail?

Evolution of tests and times

Traditional pathogen testing typically involves the use of multiple stages of culture, with various tests taking between 2-7 days. For example, testing for *Salmonella* involves two periods of enrichment followed by plating, taking 3



Figure 1. Real-time testing – is it food microbiology's Holy Grail?

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days to a negative result, and a further 1-2 days to confirm a positive. In real-world terms, a short shelf-life product may have been distributed, sold or even consumed before the most lengthy tests are complete. Under such circumstances, testing is retrospective.

The development of an array of detection methods, including those based on immunoassay or amplification of nucleic acids¹, along with improvements in enrichment, have led to significant decreases in the time to complete testing, from 5 days, to 12-24 hours, with the real possibility of testing in one shift (6-8 hours).

Constraints

The major constraints to the further acceleration of testing and to, ultimately, achieving real-time testing, are the sensitivity of the test, the initial population and physiological state of both the target and non-target microorganisms in foods, and the matrix itself. This is particularly true when testing for pathogens such as *Salmonella*.

In this case, in which the target is capable of causing disease when ingested even at a low population, a qualitative test is performed, typically aiming to detect as little as one colony forming unit (cfu) in as much as 25g of food for a single sample unit test. This equates to finding something the size of a milk carton in a swimming pool, filled not with water, but with a complex matrix, such as peanut butter or minced beef.

If the target analyte is present in a food, it typically occurs in very low numbers.

While a pathogen such as *Salmonella* may be found in high numbers in a raw flesh food, in many foods that undergo substantial processing, the population of the organism will be reduced, potentially to zero. Also, non-replicating pathogens, especially viruses, may be present in very low numbers, but still represent a significant hazard. Nucleic-acid based amplification techniques^{1,3} offer the potential for very rapid detection of a single cell or particle of an infectious agent, yet even these methods are often less sensitive in practice than in theory.

Even if a small proportion of the population survives food processing, cells of the target are then frequently injured. This issue of injury becomes increasingly crucial as the time of testing decreases. If injury occurs, recovery and initiation of vegetative growth can be delayed significantly^{4,7}. Studies have shown that even minor variations in the resuscitation procedure, such as the composition of enrichment media^{7,8} can have a profound effect on the time to recovery and rate of outgrowth.

Low initial numbers and the potential impact of injury limit the time in which a target organism recovers and grows, constraining the minimum time taken for a population to reach the threshold required for some rapid methods, such as ELISA¹.

The ambiguity of the physiological state of the target calls into serious question the ability to directly detect a pathogen. As many foods are subject to one or more processes deleterious to microorganisms, the target of detection may be alive (fully vegetative or injured) or dead. If the method of detection does not assess viability, a positive result may not reflect a true health hazard associated with the food under test. On the other hand, acceptance of a false-positive result leads, at the least, to product rework or disposal.

It has been proposed that reverse-transcriptase PCR answers the question of



viability. RNA species are turned over rapidly in viable cells and are considered to degrade rapidly upon cell death. However, there is evidence that RNA may survive in cells rendered inactive by typical food processes⁹.

The background microflora present in the food may interfere with testing, sometimes to a significant degree¹⁰. With regard to rapid testing involving some form of enrichment, overgrowth of target by background may occur. In the case of direct, nucleic acid-based detection, assay of target nucleic acid may be overwhelmed by non-target nucleic acid, should a non-specific chemical extraction method be used. Potentially, target cells may be extracted specifically using, for example, antibody-coated immuno-magnetic beads¹¹, though the viability of such cells may still be in doubt.

Benefits

Very rapid or, potentially, real-time testing benefits both the producer and

consumer. For example, faster turnaround in testing translates into less inventory being held, improving the economics of production, including a reduction in storage space. Fast or immediate availability of test results allows for positive release; product is held while testing is in progress, and is only released when testing is completed.

Real-time testing would not only benefit the producer, lessening the impact of process failure, thus reducing rework, and potentially eliminating the need for disposal or recall, but also the consumer, by reducing the risk of spoilage or disease associated with the distribution of contaminated product.

Conclusion

In conclusion, while the time taken to complete the microbiological testing of foods has decreased significantly, factors including complexity of the food matrix, the composition of the background flora and the population and physiological

state of the target microorganism constrain the development of true real-time testing. Like King Arthur's knights, food microbiologists may never find their Holy Grail.

References

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ABRIDGED PRODUCT INFORMATION

Fluconazole is a triazole antifungal agent.

INDICATIONS: 1. Cryptococcal meningitis in patients unable to tolerate amphotericin B. 2. Maintenance therapy to prevent relapse of cryptococcal meningitis in patients with AIDS. 3. Oropharyngeal and oesophageal candidiasis in AIDS and other immunosuppressed patients. 4. Secondary prophylaxis of oropharyngeal candidiasis in patients with HIV infection. 5. Serious life-threatening *Candida* infections in patients unable to tolerate amphotericin B. 6. Vaginal candidiasis, when topical therapy has failed.

CONTRAINDICATIONS: Sensitivity to fluconazole, to related azole compounds or excipients. Concomitant use with cisapride or terfenadine.

PRECAUTIONS: PREGNANCY (Category D): lactation (has been found in breast milk at concentrations similar to plasma, hence its use in nursing mothers is not recommended); immunocompromised patients who develop rashes: allow for salt content and volume of the infusion solution: patients who develop abnormal liver function tests should be monitored for the development of more severe hepatic injury and Diflucan should be discontinued if clinical signs and symptoms consistent with liver disease develop that may be attributable to fluconazole.

****Some azoles, including fluconazole, have been associated with prolongation of the QT interval on the electrocardiogram. During post-marketing surveillance, there have been very rare cases of QT prolongation and torsade de pointes in patients taking fluconazole. These reports included seriously ill patients with multiple confounding risk factors, such as structural heart disease, electrolyte abnormalities and concomitant medications that may have been contributory. Fluconazole should be administered with caution to patients with these potentially proarrhythmic conditions.**

Drug Interactions: Oral contraceptives; warfarin; sulphonylureas; hydrochlorothiazide; phenytoin; theophylline; astemizole; cyclosporin; rifabutin; rifampicin; tacrolimus; zidovudine; short acting benzodiazepines.

ADVERSE REACTIONS: Headache; nausea; vomiting; abdominal pain; diarrhoea; skin rash; acne; mild transient elevations in hepatic transaminases; clinical hepatitis; cholestasis; fulminant hepatic failure; anaphylaxis; rare cases of leukopenia and thrombocytopenia (causal relationship not established); ****QT prolongation, torsade de pointes.**

DOSAGE & ADMINISTRATION: Normally administered orally; if not possible, by intravenous infusion (not exceeding 200 mg/hour). Base daily dose on the infecting organism and the patient's response to therapy. Continue until clinical evidence or laboratory tests indicate that active fungal infection has subsided. Patients with AIDS and cryptococcal meningitis or recurrent oropharyngeal candidiasis often require maintenance therapy to prevent relapse. Diflucan IV has been used safely for up to 14 days. Diflucan intravenous infusion is compatible with Ringer's solution: Normal saline. Avoid mixing with any other drug prior to infusion. **Adults:** *Cryptococcal meningitis:* 400 mg on day 1, then 200-400 mg daily. Continue 10-12 weeks after CSF becomes culture negative. Patients not responding to treatment for up to 60 days are unlikely to respond to Diflucan. *Prevention of relapse of cryptococcal meningitis:* 100-200 mg daily. *Oropharyngeal candidiasis:* 100 mg on day 1, then 50 mg daily for 2-3 weeks. *Oesophageal candidiasis:* 200 mg on day 1, then 100 mg daily for 2-3 weeks and in severe cases for 2 weeks following resolution of symptoms. *Secondary prophylaxis against oropharyngeal candidiasis:* 150 mg as a single dose once weekly. *Serious and life-threatening candidal infections:* 400 mg on day 1, then 200-400 mg daily for at least 4 weeks and for at least 2 weeks following resolution of symptoms. *Vaginal candidiasis when topical therapy has failed:* 150 mg as a single oral dose. **Children:** *Mucosal candidiasis:* 3 mg/kg daily. A loading dose of 6 mg/kg may be used on day 1. *Systemic candidiasis and cryptococcal infection:* 6-12 mg/kg daily. *Impaired renal function in adults and children:* reduce dose in accordance with the guidelines given for adults. **Children below 4 weeks of age:** Neonates excrete fluconazole slowly. *Weeks 0-2:* same mg/kg dosing as in older children at 72-hour intervals. *Weeks 2-4:* same dose every 48 hours.

PRESENTATION: **Hard Gelatin Capsules:** 50 mg, 100 mg, 200 mg - packs of 28; 150 mg - packs of 1. **Powder for Oral Suspension:** 35 mL bottle containing 50 mg/5 mL of orange flavoured suspension when reconstituted. **Solution for Injection:** 2 mg/mL in sodium chloride solution: 50 mL and 100 mL vials.

Pfizer Pty Ltd (ABN 500 8422 348) 38-42 Wharf Road, West Ryde, NSW 2114. Full Product Information: TGA approved 30 October 1997, Date of last amendment 18 August 2003.

(**Please note changes in Product Information at the last amendment). Abridged PI prepared 18 September 2003. *Trademark: Pfizer Inc. www.pfizer.com.au 02/04 PFXDI5378 AP35057

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