Science Based Approaches for Microbial Food Safety Management

Tom McMeekin
Australian Food Safety Centre of Excellence

Martin Cole
Centre for Food Safety and Technology, Illinois

Australian Food Safety Centre of Excellence

Food Safety: the essential ingredient

Changing Patterns of Foodborne Disease

Then	Now
Traditional pathogens	Emerging pathogens
Detected by group case	Detected by epidemiol. surveillance
Usually a local issue	Widespread, international
High dose	Low infective dose
Organism readily isolated	Risk undetected by normal culture
Normal preservation controls pathogen	May survive some processes
Low mortality, susceptible to antibiotics	High mortality, antibiotic resistant, more susceptible populations

Globalisation and its implications

- International sourcing → complex supply chains → sophisticated management required
- Potential for large and widespread outbreaks
- Cross-border translocation of infectious agents
- International regulatory harmonisation essential

Consumer dem

Consumers are dema ... foods that are totally nat ... o Calories, **NO C** ... ts and cholesterol, ... , total nutrition, low price, envi ... riendly production, 'green' ... and that guarantee perfe ... nance and immortality

(Carol Broo ... Food and Agriculture Summit, 1999)

SAFETY IS A GIVEN

SCIENCE provides KNOWLEDGE

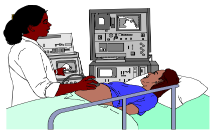
- **generation, evaluation, management and transfer of knowledge for general and specific use**

TECHNOLOGY applies KNOWLEDGE

SCIENCE	***TECHNOLOGY***
Translating knowledge	***into tools***

Knowledge demand: from Government and agencies

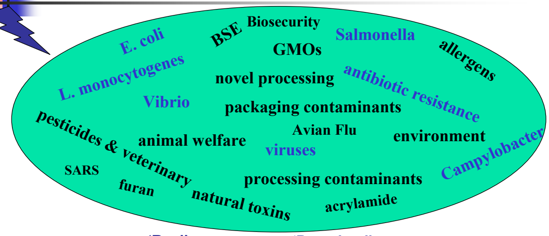
Public Health vs **Trade**



Role of governments and regulatory agencies

to protect consumer safety and to facilitate trade

Knowledge demand: from Industry/Companies



'Real' VS 'Perceived'

= Business Risk

Australian Food Safety Centre of Excellence: the fresh knowledge people

- 4 Programs
  - Core Science: pre-competitive, industry wide, strategically focussed research
  - Knowledge Transfer: collection, collation, evaluation and dissemination of knowledge. Towards an "early warning" capability
  - Education: on-line course available in food safety management in 2005; Master Classes and Workshops; Internships; Research students at multiple locations
  - Business Development: contract research and consultancy services for individual clients

The Three Paradigms of Modern Microbial Food Safety Management

HACCP

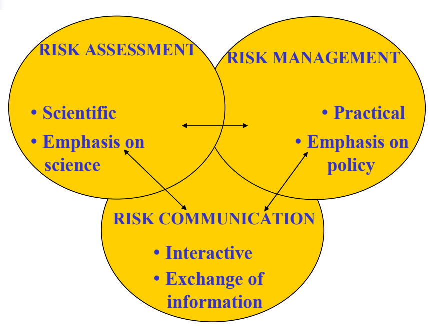
RISK ASSESSMENT

PREDICTIVE MICROBIOLOGY

Chronology of HACCP Development & Application (Hartmann 1997)

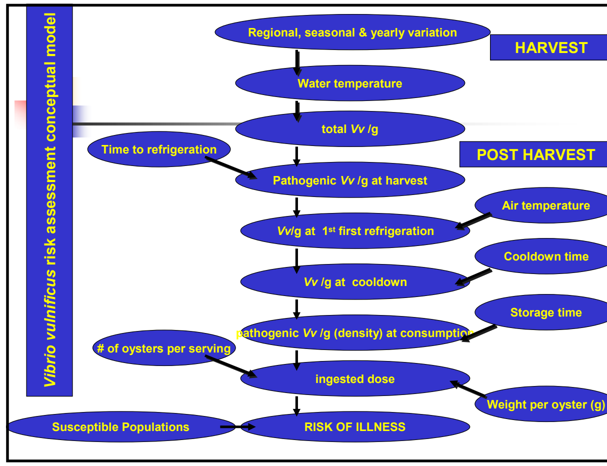
- ***1959:*** Origin of HACCP to produce safe foods for the space program
- ***1971:*** First public awareness of the HACCP
- ***1985:*** Endorsement of HACCP by US National Academy of Sciences Subcommittee on Microbiological Criteria for Foods and Food Ingredients.
- ***1989:*** NAS publication "HACCP Principles for Food Production" (revised 1992)
- ***1993:*** Approved by the Codex Alimentarius Committee of Food Hygiene
- ***1995:*** USDA, Food Safety Inspection Service regulations proposed requiring all slaughter and processing plants to develop and implement HACCP program

International adoption of the Codex Risk Analysis Framework



Chronology of Development Application of Quantitative Microbial Risk Assessment

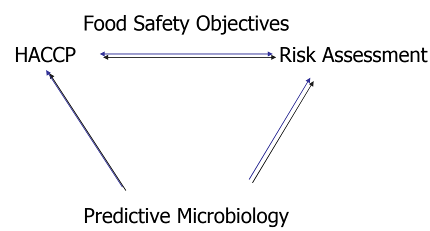
- **1978**: Mossel (Food Technology Australia). 1979 Mossel & Drion (Antonie van Leeuwenhoek).
- **1993**: WTO GATT Uruguay Round
- **1993**: Hathaway - Risk Assessment procedures used by the Codex Alimentarius Commission Joint 1978 / 79: FAO / WHO Food Standards Program 20th Session of the CAC Geneva



Chronology of Predictive Microbiology Development & Application

- **1922**: Esty & Meyer - thermal process for *C. botulinum*
- **1964**: Spencer & Baines - fish spoilage
- **1971**: Nixon - fish spoilage
- **1973**: Olley & Ratkowsky - fish spoilage (universal spoilage curve)
- **1981**: Genigeorgis - probability modes for *C. botulinum*
- **1981**: Roberts & Gibson - probability models for *C. botulinum*
- **1983**: Roberts & Jarvis - first use of term PREDICTIVE MICROBIOLOGY

Three-way Interaction



New Approaches to Risk Management

ALARA
ie 'As low as reasonably achievable'
BUT:
-Technological capabilities vary
-Idea of 'reasonable' varies

→

Public Health Based Goals
-eg yearly incidence of Listeriosis below 4 cases/million of population
BUT:
-in terms of population
-not related to specific foods

The Issue Behind the Issue:

Equivalence: Do two systems of food safety risk management (e.g. inspection, HACCP, processing) provide the same degree of public health protection?

Food Safety Objective

The maximum frequency and/or concentration of a [microbiological] hazard in a food at the time of consumption that provides the *Appropriate Level Of Protection* [ALOP].

CCFH, Bangkok, October 2001

$H_o - \Sigma R + \Sigma I \leq FSO$

- **FSO = food safety objective**
- **H_o = initial level of the hazard**
- **ΣI = total increase (growth or recontamination)**
- **ΣR = total reduction (inactivation or removal)**
- **≤ = preferably less than, but at worst equal to**
- **FSO, H_0, R, and I are expressed in $\log_{10}$ units**

H_o Control Measures

Controlling initial levels of a hazard

- Avoiding food with a history of contamination or toxicity
  - i.e. raw milk, raw molluscan shellfish harvested under certain conditions
- Selecting ingredients
  - i.e. pasteurised liquid eggs or milk
- Using microbiological testing and criteria to reject unacceptable ingredients or products

ΣI Control Measures

Preventing an increase of the hazard

- Preventing contamination
  - i.e. adopting Good Hygiene Practices (GMPs) that minimize contamination during slaughter, separating raw from cooked ready-to-eat foods, using aseptic filling techniques
- Preventing growth of pathogens
  - i.e. chilling and holding temperatures, pH, relative humidity, preservatives

ΣR Control Measures

Reducing the level of a hazard

- Destroying pathogens
  - i.e. disinfectants, pasteurisation, irradiation
- Removing pathogens
  - i.e. washing, ultrafiltration, centrifugation

Definitions

- Performance criterion
  - The required outcome of a step* or a combination of steps which can be applied to assure a food safety objective is met. (can be a level of hazard)
- Process criterion
  - The control parameters of a step* or combination of steps that can be applied to achieve the performance criterion.

**step = point, procedure, operation or stage in food chain, including raw materials, from primary production to final consumption

(ICMSF, 1998)

Example: RTE short shelf-life chilled meal Hazard = *Listeria monocytogenes*

Food Safety Objective	*Performance Criteria*	*Process Criteria*
To achieve a level of < 100/g at the point of consumption	**A 6D reduction**	**Heat** **2 min @ 70° C** **z=7.5° C**
		UHP **7.5 min @ 500MPa** [1]
		Ionizing irradiation **2.7 kGy @ 0° C** **4.6 kGy @ -5° C** [2]

[1] (Smelt et al, 1997)

[2] (Thayer and Boyd, 1995)

Control measures for *Enterobacter sakazakii*

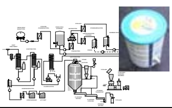
Reducing the level of contamination through a heating step of the reconstituted powdered infant formula prior to use

$$H_0 - \Sigma R + \Sigma I < FSO$$

Reducing the concentration/prevalence of intrinsic contamination

Minimise the chance of contamination of reconstituted formula during preparation

Minimize the growth of E. sakazakii following reconstitution prior to consumption



Food Production Chain

Raw Materials

$H_0 - \Sigma R + \Sigma I \leq PO_1$

Last level from previous step becomes initial level at next step

Processor

$H_0 - \Sigma R + \Sigma I \leq PO_2$

Distribution & Retail

$H_0 - \Sigma R + \Sigma I \leq PO_3$

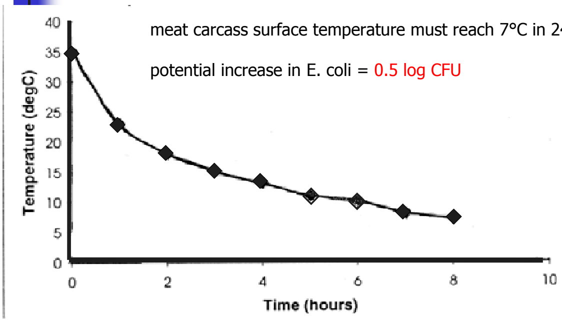
Consumption by consumer

$H_0 - \Sigma R + \Sigma I \leq FSO$

OUTCOMES/EQUIVALENCE

meat carcass surface temperature must reach 7°C in 24h:

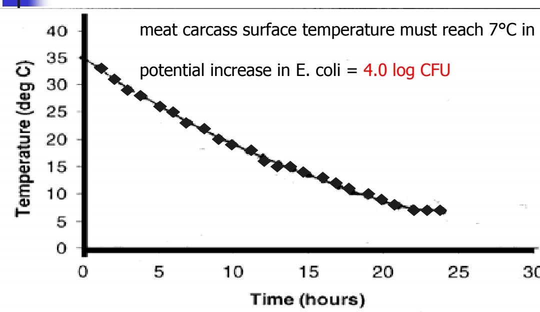
potential increase in E. coli = 0.5 log CFU



OUTCOMES/EQUIVALENCE

meat carcass surface temperature must reach 7°C in 24h:

potential increase in E. coli = 4.0 log CFU



PREDICTIVE MICROBIOLOGY:
the quantitative microbial ecology of foods

- When you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot…. your knowledge is of a meagre and unsatisfactory kind.
  - Lord Kelvin.

From retrospective analysis using traditional microbiological techniques towards real time estimates of microbial population behaviour

Predictive software

Biomathematics (mathematical models)

Bioinformatics (database)

Predictive microbiology application software: science meets technology

- Pathogen Modeling Programme (USA) - www.arserrc.gov/mfs/pathogen.htm
  - 37 models of growth, survival and inactivation
  - Frequently updated (version 7.0)
  - Available free of charge during the last 15 years
  - ~ 5000 downloads per year
- GrowthPredictor (UK) - www.ifr.ac.uk/Safety/GrowthPredictor/
  - Based on data previously used in the FoodMicromodel software
  - 18 models for growth of pathogenic bacteria
  - Available free of charge since 2003
- Sym'Previus - www.symprevius.net
  - French predictive microbiology application software under development

A new initiative to pool food microbiology data and expertise

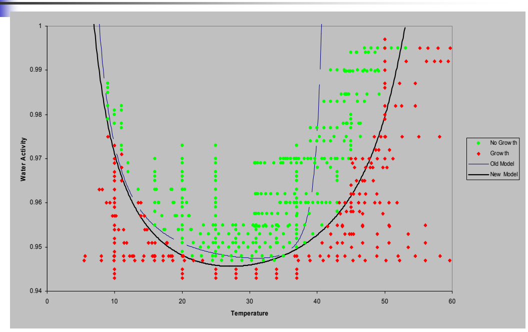
www.combase.cc

A Combined, common database of microbial responses to food environments

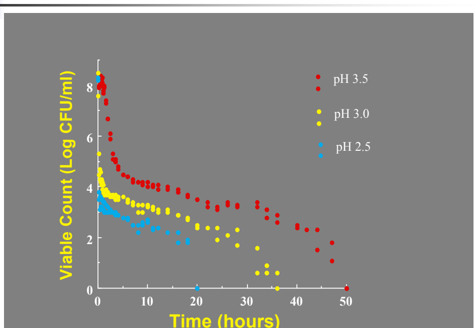
Practical applications may be developed from a hierarchy of knowledge

- Models developed and validated in specific products - Sea Food Spoilage Predictor
- Models developed in laboratory media and validated in specific foods - Food Spoilage Predictor
- Models developed in laboratory media and evaluated against traditional criteria - Process Hygiene Index
- Patterns of microbial behaviour without explicit mathematical description
- Rigorous analysis of published data indicating likely patterns of behaviour as a precursor to model development

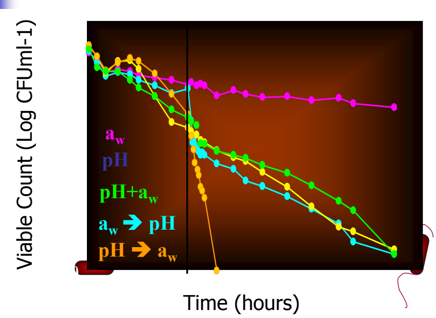
Growth/No Growth Model for *E. coli*



Response of *E. coli* M23 to lethal pH (35°C)

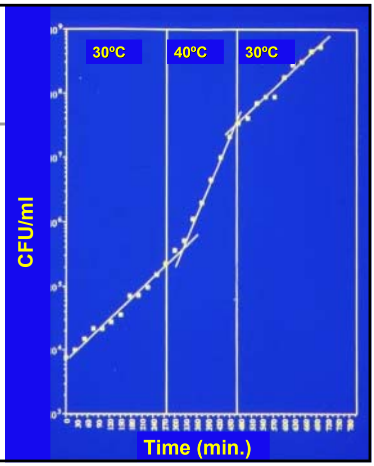


Constraint Sequence

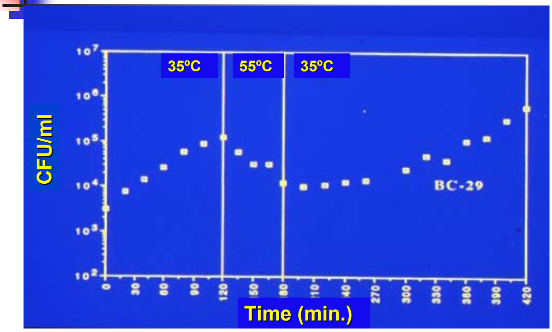


S.thermophilus

Temperature cycled between 30 and 40°C

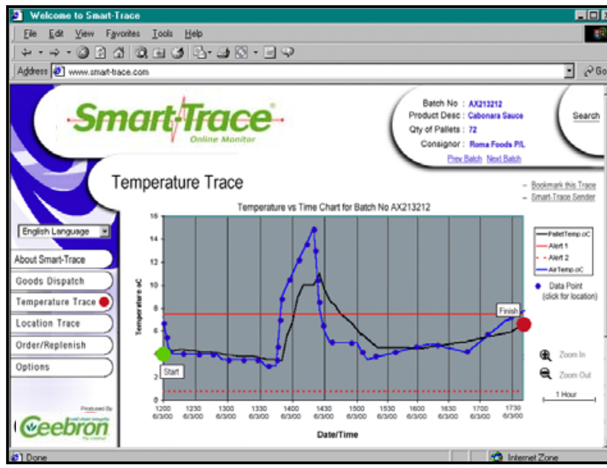


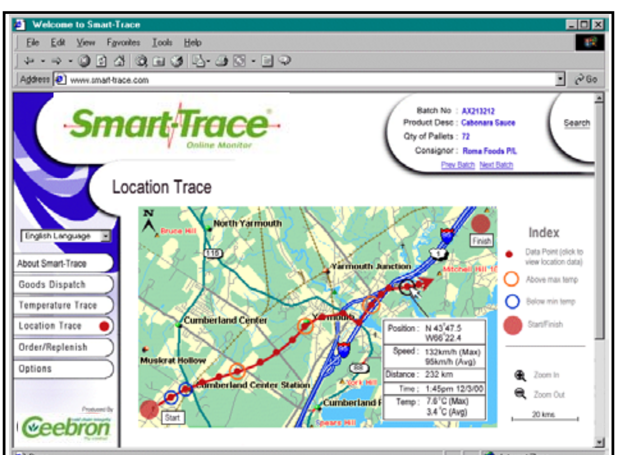
S.thermophilus



Traceability and supply chain integrity

- Traceability is demanded from 1 January 2005 by the European Food Law (EEC/178/2002). Simple traceability demanded – the one up, one down model
- At the same time there is increasing interest in electronic chain traceability systems that communicate with finance software, business systems and work as an integrated part of production management
- Specifications for electronic chain traceability have been suggested (www.tracefish.org; www.eufoodtrace.rog) and several traceability software packages are available
- Radio Frequency Identification (RFID)/wireless technology





Combining predictive models and RFID technology into a food safety management tool

- *E.coli* "megamodel" for temperature, water activity, pH and lactate concentration
- Development: Ross et al. 2003 Int. J. Food Microbiol. 82: 33-44.
- Evaluation: Mellefont etal. 2003 Int. J. Food Microbiol.82: 45-58.
- Application: Development, by AQIS, of a Refrigeration Index for carcass chilling in revised Export Meat Orders
- More sophisticated information systems (e.g. **RFID** technology) combined with increasingly extensive microbial databases (e.g. ***ComBase***), will enable real time monitoring taking food safety management to new levels of precision and flexibility.

Information systems in the identification and epidemiological characterisation of strains

- Combines wide range of data:
  - Microscopy –morphology
  - Phenotypic – biochemical and nutritional tests, phenotypic
  - Chemical – chemotaxonomy (fatty acids, lipoquinones, cell wall constituents)
  - Genotypic – DNA base composition, DNA:DNA hybridisation
  - Molecular – DNA fingerprinting, DNA sequence analysis, genomics, proteomics

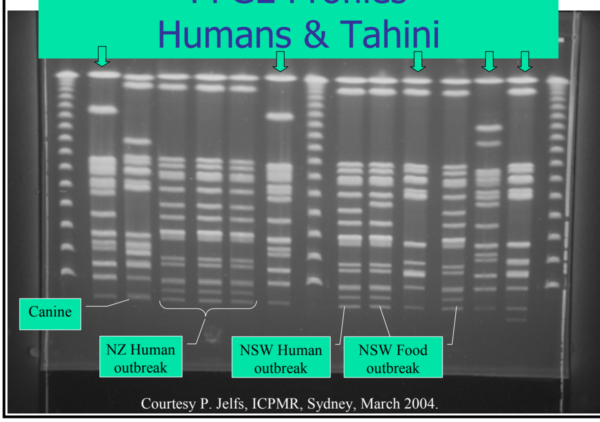
Online databases for foodborne pathogen identification

- APIweb
  - phenotypic-based identification
- PulseNet
  - accumulates pulse field electrophoretograms of E. coli and L. monocytogenes
- Pathogen Tracker 2.0 (Cornell University)
  - accumulates phenotypic and genetic data for bacterial pathogens
- National Collection of Biotechnology Information (NCBI)
  - accumulates nucleotide and protein data including genomic data

Information systems in foodborne disease surveillance

- Effective surveillance systems are critical for the detection of accidental or intentional contamination of the food supply
- Towards a solution:
  - changes to our ability to manage information in the electronic age and development of molecular methods for strain characterisation has raised our preparedness significantly. This is set to continue with increases in our capacity and ability to share data

PFGE Profiles
Humans & Tahini



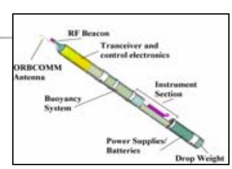
Courtesy P. Jelfs, ICPMR, Sydney, March 2004.

Combining science and engineering

SCIENCE ⟷ ***ENGINEERING and related technologies***

Providing tools for rapid and sensitive detection of food contaminants

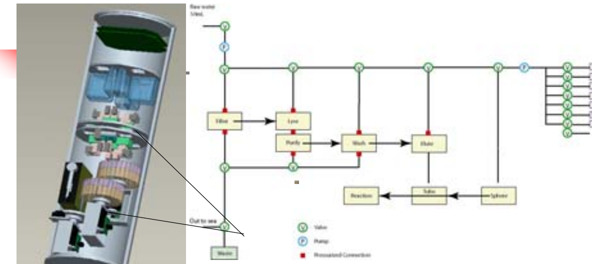
Center for Ocean Technology, University of Southern Florida
Field Sensors and Field Robotics/Automation



Observed Analyte Detection Limits (4-5 meter pathlengths)

- Iron 0.2 nanomolar
- Copper 0.4 nanomolar
- Nitrite 0.5 nanomolar
- Nitrate 1.5 nanomolar
- Chromate 0.2 nanomolar
- Molybdate 0.4 nanomolar
- Hydrogen Sulfide 2.5 nanomolar
- Quinine Sulfate (fluorescence) 0.06 nanomolar
- Chlorophyll-a (fluorescence) 0.03 nanomolar

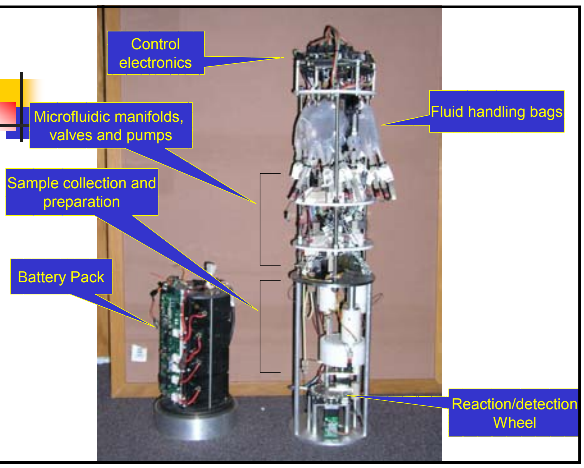
AMG (Autonomous Microbial Genosensor)



Cell Capture/Lysis
Nucleic acid purification

Amplification/detection

Demo: *K. brevis* the Florida red tide dinoflagellate



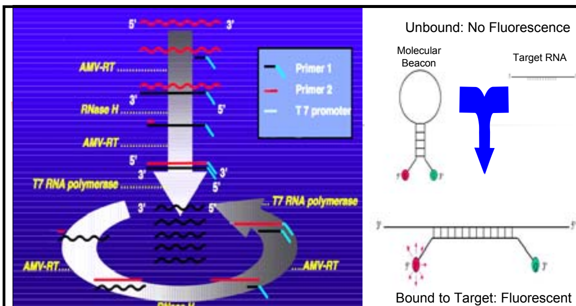
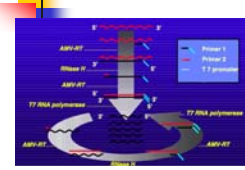


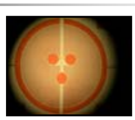
Fig. 1. How Real Time NASBA Works

NASBA is an isothermal amplification method that selectively amplifies RNA in a DNA background. NASBA uses an enzyme cocktail of T7 RNA polymerase,RNase H and reverse transcriptase (RT) and two target specific primers (P1 and P2). Primer P1 is modified at the 5' end to contain a T7 RNA polymerase promoter. The enzymes work in concert to amplify a sense RNA molecule to form a antisense RNA amplicon. Real-time detection of amplification is achieved using a molecular beacon - A single stranded fluorescently labeled oligonucleotide that has a stem-loop structure. The loop structure is complementary to an internal region of the antisense RNA, the stem is composed of a short complementary nucleic acid sequence. When the molecular beacon hybridizes to the target sequence the fluorophore and quencher are separated and the bound probe fluoresces.

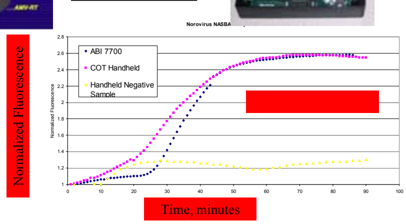
Handheld Microbial Genosensor

Eliminate complex electronics and complex detection by using Nucleic Acid Chemical Amplification Approach to Detection





Planar integrated heater/optical cell, fluidic interconnect for nucleic acid base amplification assay



Australian Food Safety Centre of Excellence

Food Safety: the essential ingredient

Consortium Partners

University of Tasmania
National Food Industry Strategy
Australian Government Department of Agriculture, Fisheries and Forestry
Food Science Australia — A joint venture of CSIRO and the Victorian Government

Core Members

Dairy Australia
GRDC Grains Research & Development Corporation
mla Meat & Livestock Australia
ozfoodnet
RIRDC Rural Industries Research & Development Corporation
Seafood Services Australia
Tasmania Department of Primary Industries, Water and Environment
Victoria Department of Primary Industries