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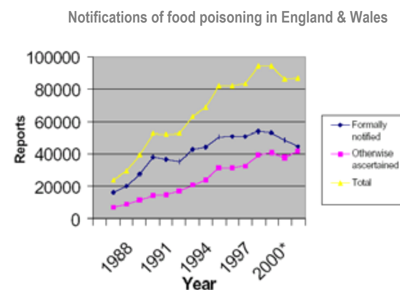
# ABL Pathogen Detection

**Advanced Biomedical (ABL) have a range of technologies* particularly suited to "one-pot" or homogeneous diagnostic tests. The proprietary technology is based around novel cytolytic peptides, which have been engineered to be sensitive to a range of environmental changes. These changes may include pH, redox, light or the presence of small molecules such as hormones, drugs-of-abuse, explosives, pesticides, antibiotics and toxins.**
**The technology has been applied to the detection of low numbers of pathogenic bacteria in food samples. In this application the peptides are made to respond to pH changes - as the immuno-targeted bacteria metabolise, the peptide senses this and brings about a release of targeted signal thus producing an eye-visible colour signal. The ABL pathogen detection technique has the following key advantages:**

- **Detects presence AND viability of pathogens rapidly (< 2 hours)**
- **Extremely sensitive - eye visible colour from 5x$10^{1}$ salmonella spiked into raw minced beef within 2 hours**
- **No false positives from unspiked controls**
- **No signal interference from 5x$10^{7}$ competitors (6 different problem organisms) - no "bulk pH" effect**
- **Requires no specialised instrumentation**
- **Reagents independently shown to be compatible with a range of foods such as minced beef, whole egg, chicken and milk**
- **Non-destructive assay allows subsequent optional confirmatory tests**
- **Generic technology which can be applied to a range of pathogens**

## Background

In recent years, the number of food poisoning cases notified to the public health laboratory service has been increasing steadily. In 1988 there were approximately 20,000 reported incidents, but by 2000 this had risen to 86,616, with the number of incidents still rising. Food poisoning accounts for more notifications than all other notifiable diseases, such as measles and meningitis, put together. Of the cases in 2000, 16,987 were directly attributed to *Salmonella* species, 62,867 to Campylobacter species, 113 to Listeria and 1,147 to verotoxin-producing *Escherichia coli* O157. It should be noted that although Listeria and *E. coli* O157 account for a small proportion of these cases, the seriousness of the illnesses caused by these infections make them important organisms in food safety




Pathogenic contamination of food presents a complex challenge that can, currently, only be addressed by heat or radiation sterilisation, which is not suitable for most foods. Conventional detection methodology, which involves successive cultures of the contaminating bacteria, is extremely labour-intensive and time-consuming (72 hours to presumptive identification), and unsuited to advising pathogen risk during food processing and manufacture. Despite many developments over the last 3 decades, no new rapid method technology has achieved the required sensitivity (1 bacterium in 25 g food) within a practical timescale (a working day).

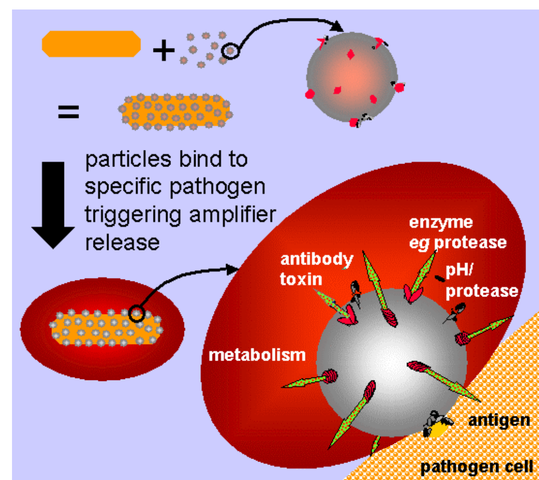
*patents apply WO 98/41535, WO 99/20252, WO99/38009

Rapid detection of pathogens would offer a great commercial advantage to companies within the food industry. Such a test would allow rapid identification of contaminated foodstuffs, allowing the decision to hold a batch of product to be reached within a matter of hours, rather than several days, allowing foods to be positively released and obviating the need for refrigerated warehousing. Further, the ability to determine viability may eliminate the need for lengthy conventional methods altogether.

## The Technology

The ABL pathogen detection technology utilises antibody-labelled liposomes, containing a marker, which are targeted to a specific pathogen type. These liposomes are stable preparations which are formulated to retain their payload for long periods of time, preparations retain their contents after many months. These very stable liposomes are then made sensitive to pH changes by the incorporation of specially engineered peptides with these liposomes. The peptides have been designed to be sensitive to changes in pH, so that they undergo conformational changes as the pH varies which modulates their lytic activity. At inactive pH values the peptides are not lytic and have no effect upon liposomes, however at active pH values the peptides lytic activity is triggered releasing the payload from the liposomes and resulting in a detectable signal.

Upon growth of targeted pathogens the pH in their immediate vicinity (microenvironment) is reduced by the excretion of waste metabolites. This causes the peptide to release the contents of the liposome highlighting the presence AND viability of the pathogen by detection of the eye-visible colour marker. The marker may also be fluorescence, luminescence or conductivity. The current assay is performed on agar plates, which are familiar to microbiologists and also allow the colonies to be isolated and taken for further confirmatory testing.




## Sensitivity, Selectivity & Specificity

The ABL technology displays high sensitivity and can detect extremely low numbers (<10) of pathogenic bacteria. Detection is achieved simply by the naked eye - when a coloured signal is visible, viable pathogenic bacteria are present in the sample tested. Often, novel diagnostic tests fail to deliver their promised potential once they are challenged with “real” samples. However using the ABL assay detection of 5x10$^1$ organisms (right) spiked into raw minced beef is rapidly (within 2 hours) achieved. As shown, no signal was observed with unspiked samples illustrating that the high numbers of “weed” organisms present in this matrix do not produce false

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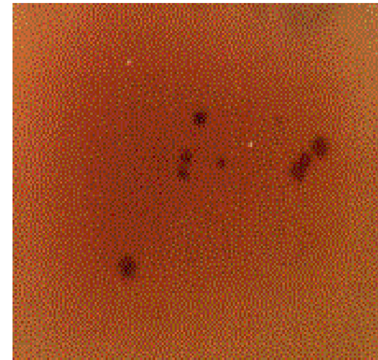
positive results. In addition, independent tests using the ABL technology have shown that the reagents are compatible with a wide range of other foods including whole egg, chicken, milk, cabbage and milk chocolate.

The ABL assay has been developed and configured specifically to avoid the effects of high numbers of competing organisms which are always present in "real" samples. The test also demonstrates excellent specificity; and no signal is observed when the assay is exposed to $5 \times 10^7$ competitor organisms. The test has been challenged with organisms which often cause problems with such tests but caused no such problems with the ABL test. A large amount of resource has been devoted to ensure this was the case, resulting in the ABL assay being specifically configured to eliminate any effect of competitors and in particular the bulk pH changes which they bring about. By using single antibody targeted liposomes, reagents are ONLY present in samples contaminated with the pathogen of interest, therefore, the signal is ONLY produced in samples which contain the pathogen of interest.

As the figures show, the ABL pathogen assay is currently presented on an agar plate format, a platform, with which, all microbiologists are familiar. Once the test has been performed and results obtained (positive or negative for viable pathogenic bacteria) overnight incubation of the plates results in colony growth of the organisms tested (right) in the area of the developed colour. If required, these colonies can then be isolated and taken for further confirmatory testing and/or typing allowing added confidence in rapidly obtained results.



**Alternative formats and further benefits**

Due to the generic nature of the ABL technology it can simply be applied to a number of applications, for example, utilising a different antibody allows detection of other bacteria such as Campylobacter, E. coli or Lysteria. In addition, the assay is available in the following alternative formats:

- Immuno-magnetic separation (IMS) compatible assay
- With a fluorescent payload which is more suitable for high-throughput applications
- Antibiotic susceptibility test for use in hospitals

**ABL, Rapid Pathogen Detection Solutions, Licences* available**

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