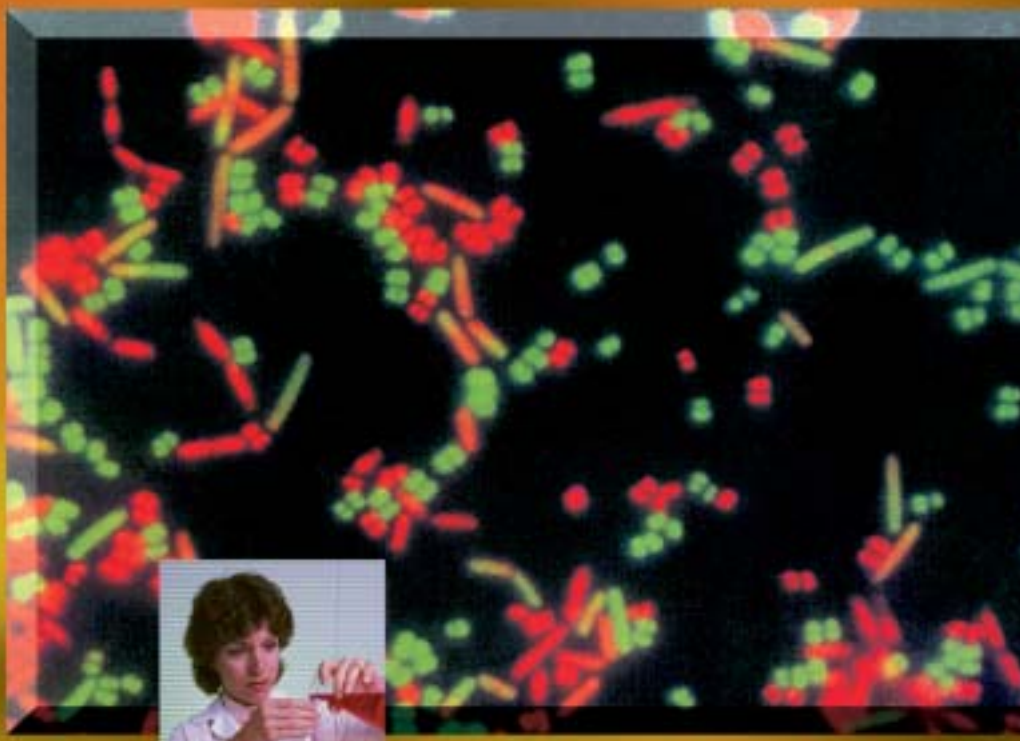
FINAL REPORT

Project Armis No. 4204

Control and Detection of Food-Borne Pathogens



The National Food Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

RESEARCH REPORT NO 3

Contents

CONTROL AND DETECTION OF FOOD-BORNE PATHOGENS

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SUMMARY

The objective of this study was to develop rapid methods for the detection of bacteria from food.

Traditional methods for the detection of bacteria from foods rely on culturing of the bacteria onto agar plates. These traditional cultural methods are very time consuming taking three days to determine a total viable count and five to seven days to detect specific pathogenic bacteria. Currently available rapid methods are largely unsuitable for use in industrial based laboratories. They lack sensitivity, are expensive and complex to perform, often requiring specialised personnel and significant capital expenditure. The absence of rapid cost effective methods for bacterial detection poses particular difficulties in the implementation of effective HACCP (hazard analysis critical control point) management systems.

During this project, rapid methods for the detection of bacteria were developed and validated.

- A rapid method was developed for the determination of total viable counts of meat samples. This method was based on a membrane filtration epifluorescent procedure and was applied to fresh and processed meat samples. The technique took 15 minutes to carry out which compares with 3 days for the standard plate count. There was an excellent correlation between the rapid and standard method (r^2 = 0.87-0.93).
- A rapid method was developed for the detection of specific pathogens of concern to the food industry including *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Salmonella*. This method involved an overnight enrichment of the food sample to be tested followed by the isolation of the bacteria from the enrichment broth using a novel surface adhesion membrane capture procedure. The captured bacterial pathogens were detected using either fluorescent labelled monoclonal antibodies or DNA hybridisation techniques (PCR). This rapid method had a detection level of 1 bacterium per 25g of food sample and took 20 - 24h to carry out which is a considerable improvement on

traditional methods which take 5-7 days. Methods for detection of the three pathogens were validated for a range of fresh and processed foods and shown to give excellent agreement with standard cultural techniques.

MATERIALS AND METHODS

Total viable count

Bac*light *direct count

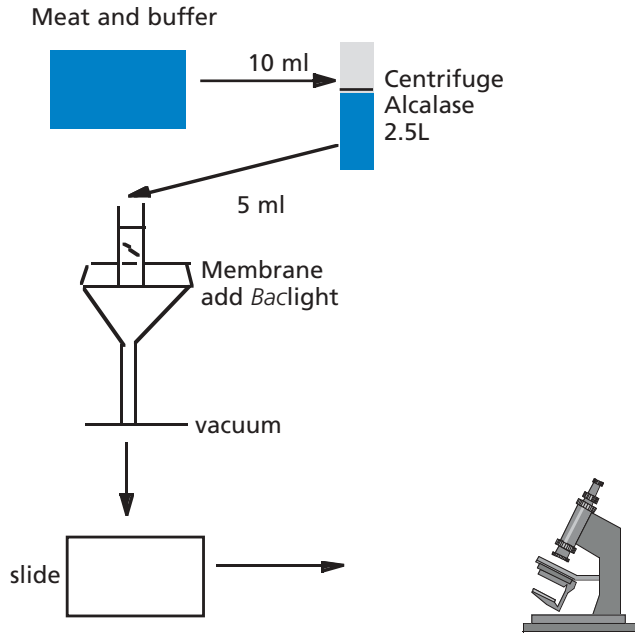
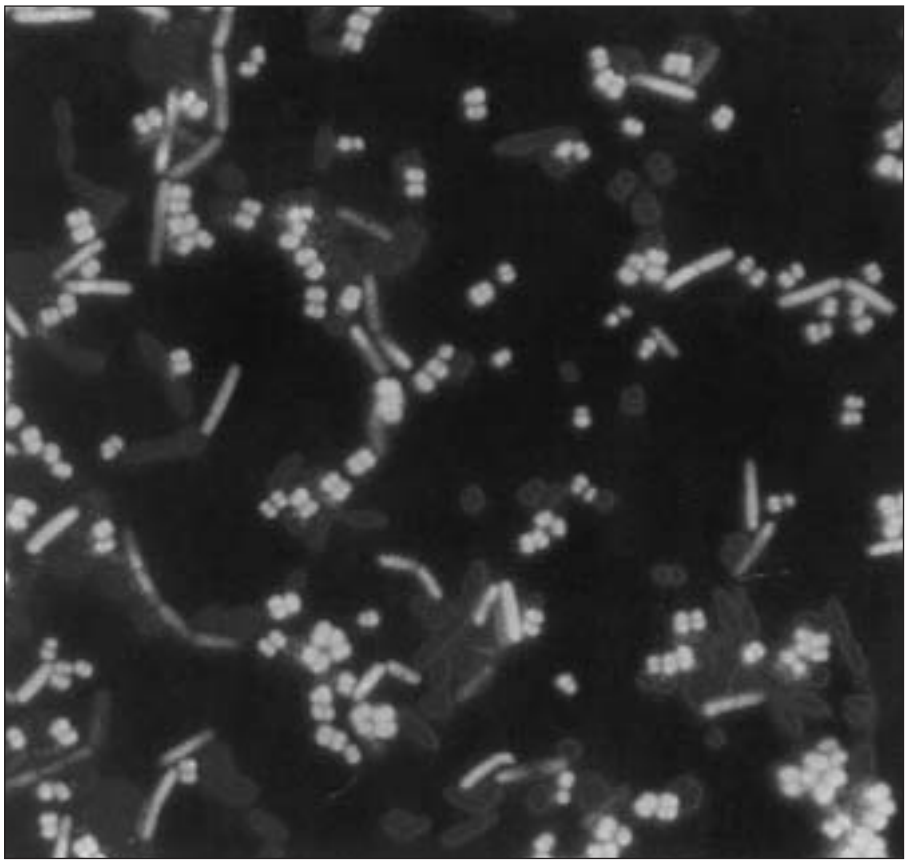


Fig 1. *Bac*light direct count method for total viable counts

The method developed for the determination of total viable counts is outlined in Fig 1. This method was based on a membrane filtration epifluorescent technique and involved pretreatment of the meat sample by

centrifugation, surfactants and a proteolytic enzyme, alcalase 2.5L. The treated sample was filtered through a polycarbonate membrane (0.6mm), stained with a fluorescent viability stain (*Bac*light) and viewed using an epifluorescent microscope with a 100W mercury vapour light source and a 100X oil immersion plan objective. Viable (live) bacteria were stained green and non viable (dead) bacteria stained orange (Photo 1).The number of viable bacteria per gram of meat was determined by counting the green fluorescing cells in twenty random fields of vision.



▲ *Bacterial cells isolated from a meat sample onto a membrane, stained with Bac*light *viability stain and viewed under an epifluorescent microscope (100X).*
Note: live cells are stained green and dead cells are red/orange as on the front cover illustration.

Pathogen detection

Isolation of pathogens

A novel rapid method based on surface adhesion was developed for the isolation of pathogens (*Listeria monocytogenes*, *Yersinia enterocolitica*, *Salmonella enteritidis*) from meat (Fig 2). Meat samples (25g) were incubated at 30°C for 18h in Buffered Peptone Water (225ml). Pathogens were isolated by surface adhesion onto a polycarbonate membrane which was attached to a glass microscope slide and immersed for 15 min in the enriched sample. Pathogens including *Listeria*, *Salmonella* or *Yersinia* adhering to the membrane were visualised by either immunofluorescence or by DNA hybridization (PCR)

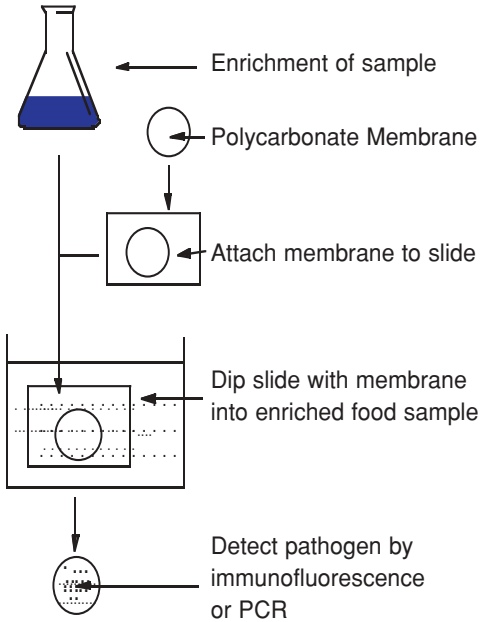


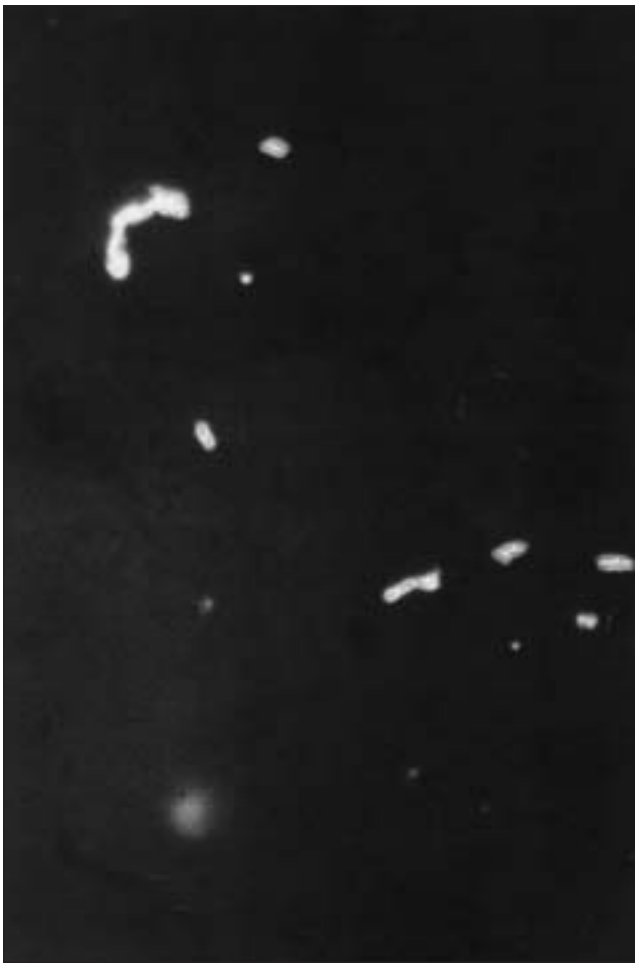
Fig 2. A rapid surface adhesion based technique for the detection of pathogens

Immunofluorescence

The membrane was coated with 0.5 ml of a monoclonal antibody specific for the target pathogen. The antibody had a fluorescent label (FITC) which allowed stained bacteria to be viewed under an epifluorescent microscope with a 60 X oil immersion plan objective. Twenty fields of vision were examined for the presence of bright green fluorescing cells with a distinctive halo appearance (Photo 2).

DNA hybridization

The membrane was placed in an eppendorf tube and the DNA was chemically extracted from the target bacteria. This process also dissolved the membrane. The extracted DNA was amplified in a PCR assay using DNA primers for a specific genetic region of the target bacterial pathogen. The PCR product was detected by gel electrophoresis.



Listeria monocytogenes *cells isolated from a meat sample onto a membrane, stained with FITC and viewed under an epifluorescent microscope (60X)*

RESULTS AND DISCUSSION

Total viable counts

Bac*light *direct count

The developed method using the viability staining procedure (*Bac*light direct count method) was successfully applied to fresh and processed meats. There was an excellent correlation between the *Bac*light direct method and the standard plate count for mince beef (r^2=0.92), cooked ham (r^2= 0.90), bacon rashers (r^2= 0.91) and frozen burgers (r^2 = 0.93). The *Bac*light technique measures viable cells only. This is an advantage particularly for processed meats which contain a significant proportion of injured and dead cells. Previous methods such as the acridine orange direct count (AODC) yielded a total count (viable and non viable cells) and gave a very poor correlation with the standard plate count. This is illustrated in Fig 3 which compares the results obtained using an acridine orange and a *Bac*light method with the standard plate count method for cooked ham samples. The technique takes approximately 15 min to carry out and is suitable for routine use in a factory laboratory.

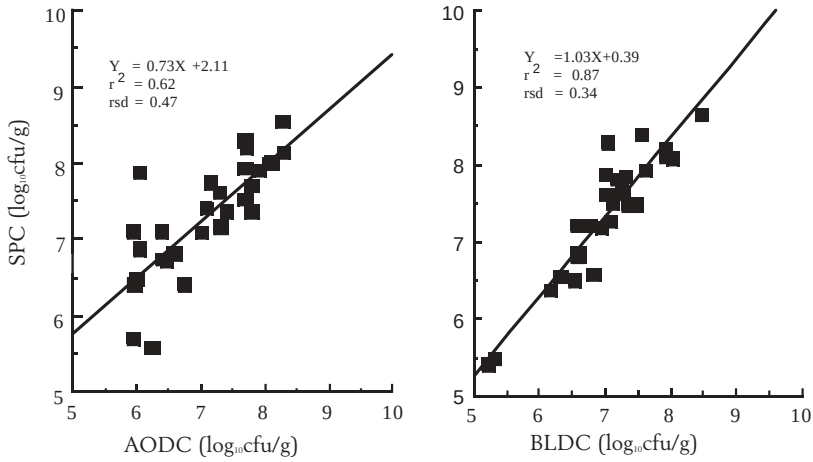


Fig 3. Relationship between the acridine orange direct count (AODC) and *Bac*light direct count (BLDC) methods and the standard plate count (SPC) for cooked ham (n=28).

Pathogen detection

Isolation of pathogens

The rapid surface adhesion method was applied to the detection of three pathogens (*Listeria monocytogenes*, *Salmonella enteritidis* and *Yersinia enterocolitica*).

The sensitivity of the surface adhesion technique can be increased by manipulation of the environmental conditions in the enrichment broth including pH and temperature. Gram positive bacteria (*L. monocytogenes*) were preferentially isolated from the enrichment broth when the pH was low (4.75) and gram negative bacteria (*Y.enterocolitica*) at a high pH value (9.00). Culture type was also important with significantly higher numbers of bacteria adhering to membranes immersed in meat cultures than in either pure or mixed cultures.

The isolated bacteria were detected by immunofluorescence or by DNA hybridization.

Immunofluorescence

The surface adhesion immunoflorescent (SAIF) method for *Listeria* and *Salmonella* and *Yersinia* had a detection level of approximately $\log_{10}3.50$cfu ml^{-1} of enrichment broth. It was established that a single enrichment step could be used for the detection of all three pathogens (Fig 4). The method was validated against traditional detection methods for all three pathogens using a varied range of retail food samples (meat, fish, poultry, dairy product, vegetable, salads) and excellent agreement was reported between rapid and standard techniques.

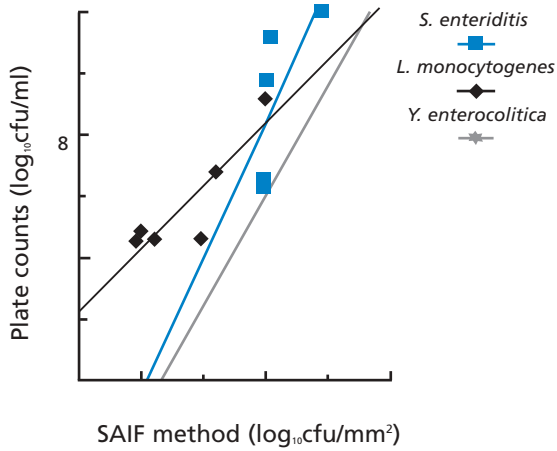


Fig 4. Comparison of a standard plate count and a surface adhesion immunoflurescent (SAIF) count for the enumeration of pathogens from a single enriched meat sample

DNA hybridization

The DNA hybridization method detects a specific genetic sequence in the pathogen and is a very specific test that can distinguish between pathogenic and non pathogenic strains. The technique has been applied and validated for the detection of *Listeria monocytogenes*, an important pathogen in the food industry. It takes approximately 22 h to carry out, has a detection level of $\log_{10}3.5$ cfu ml^{-1} enrichment broth and has been validated with 100 retail food samples.

CONCLUSIONS

- A rapid *Bac*light direct count method was developed for the determination of total bacterial counts on fresh and processed meats. The method takes 15 min to carry out and gives excellent correlation with standard plate count. This method is suitable for routine use in industry.

- A rapid surface adhesion based method was developed for the isolation of food borne pathogens (*Listeria monocytogenes*, *Salmonella* and *Yersinia enterocolitica*) from food samples. The isolated pathogens were detected by immunofluorescence or DNA hybrization. The methods had a detection level of 1 cell per 25g of food sample and were validated with a range of fresh and processed meats. These methods will be available as part of the analytical services of The National Food Centre by the end of 1998.

ACKNOWLEDGEMENTS

Teagasc acknowledges with gratitude grant aid under the Food Sub-Programme of the Operational Programme for Industrial Development. The programme is administered by the Department of Agriculture and Food supported by national and EU funds.

PUBLICATIONS

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