PACT OF ANIMAL HUSBANDRY AND SLAUGHTER TECHNOLOGIES ON MICROBIAL CONTAMINATION OF MEAT: NITORING AND CONTROL

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microbial flora transferred to carcasses during slaughter is a reflection of the care taken on the slaughter floor and of the types and bers of micro-organisms acquired by the animal on the farm or in the period between farm and slaughter. These micro-organisms may ude food-poisoning organisms able to cause illness in the consumer, or micro-organisms responsible for spoilage of the product. Vast gress has been made in reducing the contamination at slaughter and thereby extending shelflife of meat. In contrast, international stics still clearly show that meat and meatproducts are responsible for a major fraction of all foodborne infections. This latter aspect ot determined by the numbers of micro-organisms but by the bacterial composition of the animal's gut flora at slaughter. Preventive ity assurance along the whole production- and processingline is therefore the only effective means of controlling the microbiological ty and quality of meat. This includes hazard analysis techniques to identify critical control points and procedures for monitoring the robiological status of production animals and carcasses since most of the critical control points cannot be totally controlled. At early es in the production line colonisation with pathogens should be prevented. Subsequently, good slaughter practices will assure carcasses ood total microbiological quality. This paper deals with microbiological monitoring systems which can be used at different stages of duction and processing to control the microbiological quality of poultry- and pigmeat.

INTRODUCTION

rying amounts of microbial contamination may be found on surfaces of freshly slaughtered carcasses. This arises by direct or indirect ntact with the animal's hide, legs or hooves, with gut contents or faecal material or contaminated equipment. Upon storage and handling se micro-organisms will lead to either early spoilage or form a potential source of food-poisoning. Several microorganisms regularly esent on meats and meat-products (e.g. Salmonella, Campylobacter, Listeria) cause serious concern. Prevention of contamination therefore ves both aspects of economy as well as public health. Vast progress has been made in reducing the contamination at slaughter nijders,1988) and in extending the shelf-life of meat by the introduction of hygienic working procedures in combination with refrigerated rage and (gas)packaging (Farber et al 1990, McMullen and Stiles, 1990, Mulder et al 1990). On the other hand the statistics clearly show at foods of animal origin still plays a major role in the transmission of zoonotic diseases (Genigeorgis,1987). Apparently, more attention s been paid to technological improvements in relation to spoilage then to the prevention of pathogens in the foodchain. It may, however, so be argued that under the present production conditions, never an absolute guarantee can be given that the gut flora of so-called healthy imals do not harbour micro-organisms pathogenic for humans. It is therefore interesting to analyse what has been done so far to prevent ntamination of carcasses with pathogens.

raditional control of food-operations has relied on inspection to judge compliance with accepted good manufacturing practices, food laws d laboratory testing of the end product. This approach has several shortcomings since the laws or codes do not always clearly state what e requirements are, or distinguish between critical operations and those that have little effect on hygiene. The temptation is, therefore, inspect or concentrate only on those parts of the process where compliance can be evaluated easily, rather than those which are critical. ntil recently, microbiological testing was concentrated at the end of the production-chain, the slaughterline, the part of the process were so most of the technological improvements have been implemented and changes in numbers of micro-organisms relatively easily can be onitored and therefore could provide a false sense of security. Over the last few years, several new approaches have been developed for e detection and identification of micro-organisms (Huis in 't Veld et al.,1988) which possess the potential to be implemented in specific

areas of the production process (Huis in 't Veld and Hofstra, 1991).
This paper discusses the failures and successes in monitoring animal husbandry for pathogenic micro-organisms. In addition the slaughter technologies on microbial contamination of meat is reviewed.

2. PROCESS INTEGRATED MICROBIOLOGY

To evaluate whether a food operation meets commercial requirements and complies with the law, quality control personnel enforcement officials have traditionally inspected an operation for compliance with accepted good practices and taken samples for laboratory testing. But as is well known microbiological testing only identifies effects and usually neither identifies nor controls A major portion of the activities of quality control departments have therefore been directed toward observations and measurements little or no relationship to microbiological hazards. Indeed, the use of testing to control microbiological hazards in foods has limitations. These are:

- the problem of sampling and examining a sufficient number of sample units to obtain meaningful information on the microbial status of the herd, flock or batch of food (Kilsby and Pugh, 1981).
- the constraints of time and costs to obtain results.

Perishable products such as meats cannot be stored pending the results of microbiological analysis and with deep frozen or stored products costly warehousing is necessary. This approach has failed so far, also foodborne-infections could not be prevented in Another approach is needed and has been developed, a conceptual and structural approach where multidisciplinary teams work not to solve the problem but to control the process and prevent foodinfections and food poisoning by longitudinal integrated safety (Tompkin, 1990; ICSMF, 1988). There are nowadays exciting new developments in rapid microbiology, but these will only be accepted they fit in newly developed quality assurance systems. The use of process microbiology instead of food-microbiology is therefore There is an increasing understanding nowadays that the microbiological status of a food is the result of a chain of events. It is the microbiological safety of a food can only be guaranteed when the overall processing, including breeding, fattening, quality management, slaughter technologies, storage, distribution and handling by the consumers, are taken into consideration. microbiological quality assurance of food is not only a matter of control, but also of a careful design of the total process chain to obtain an endproduct that is microbiologically safe. Industry and food microbiologists have now generally adapted quality systems based on the principles of the HACCP concept (Table 1).

Implementation within certain areas of the food industry, however, has been hampered in some cases by a lack of knowledge or the bility to control the critical points in the production process. If the microbiological quality of processed foods can be assured by the process itself, microbiological control of the end product may then be omitted. Combinations of high quality raw materials of time-temperature-tolerance as well as over production-processing-packaging are of paramount importance. Both the possibility of the microbiological quality of foods and the need for quality control points, strongly depend on the nature of the foods concerned. contrast to highly processed foods, foods of animal origin possess a relatively low level of quality confidence. This is mainly complexity and the length of the meat production chain and the lack of absolute control over critical control points in the total and processing line. Generally speaking, the better the quality of a product can be assured by longitudinal integrated safety assurance less need there is for extensive microbiological monitoring. However, it has to be realised that the majority of critical control points production are classified as CCP2, critical control points which cannot be absolutely controlled (ICSMF, 1988). As a consequence microbiological monitoring at specific sites in the chain is necessary for checking that the requirements have been met or that specifications have to be tightened up.

e 1. Stages in the application of HACCP

tification of Hazards	- micro-organisms
analysis	- toxins, residues etc
tification and	- ranking risks according to severity and frequency
sification of CCP	- where must control be exercised
ption control options	- how absolute is control (CCP1* or CCP2*)
ption monitoring options	- effectiveness
	- utility
	- reliability
rcise control	- accuracy
	- implement quality assurance

P1 is a location, practice, process or procedure where control is possible in order to prevent hazardous situations, whereas at CCP2 sible hazards are minimised but not totally controlled.

re preventive systems also will include new developments such as predictive microbiology especially at CCP's (McMeekin and Olley, ; Roberts, 1989; Bratchel et al., 1989; Zwietering and Huis in 't Veld, 1990). The quality of a product will become less dependant n microbiological testing but more and more from knowledge, design and logistics of the total production-, processing- and distribution n. In addition, control will be exercised through predictions based on mathematical modelling at critical control points. Microbiological itoring can however never be omitted. Therefore, there is a need for simple and rapid microbiological tests which can be adapted to echnology and logistics of specific production processes. It is clear that traditional microbiological approaches cannot meet these high irements.

MICROBIOLOGICAL PROBLEMS ASSOCIATED WITH MEAT PRODUCTION

microbiological conditions of the live animal are of paramount importance for the microbiology, especially the safety, of the consumer- dy endproduct in relation to food-borne infections. The most important Salmonella serotypes isolated from human, poultry and pigs in Netherlands in the period 1989 - 1990 are presented in Table 2. This example clearly shows the relation between human Salmonellosis foods of animal origin.

ble 2

The most important Salmonella serotypes by man, chicken and pig
(Source: Dutch Salmonella Centre, National Institute of Public Health and Environmental Protection, Bilthoven)

	MAN %		CHICKEN %		PIG %	
	1989	1990	1989	1990	1989	1990
S. typhimurium	44.9	39.7	16.9	18.4	81.2	77.6
S. enteritidis	20.1	29.5	19.5	10.0	1.0	<1
S. virchow	6.2	6.8	8.6	13.1	<1	0
S. hadar	2.2	2.6	9.9	18.8	0	<1
S. infantis	2.4	1.9	11.3	14.2	1.9	1.5
S. panama	1.8	1.4	<1	<1	1.0	2.4
S. brandenburg	2.4	<1	<1	<1	3.4	1.3
S. london	<1	<1	<1	<1	2.2	1.1

Therefore it is important to control pathogenic micro-organisms in the live bird, not only those which negatively influence the he... of the animal but also those which may cause illness in humans by transmission of foods of animal origin. At later stages in ... production chain, the main focus should be directed towards the prevention of contamination during the slaughter process. To... forming units or the number of Enterobacteriaceae provide indications about the hygiene or good manufacture practices exer... consequently about the safety and quality of the final product. Microbiological control, therefore, must include rapid, simple ... detection or monitoring systems for pathogenic micro-organisms as well as methods for measuring hygiene during slaughter and pr...

4. MICROBIOLOGICAL PROBLEMS ASSOCIATED WITH PIG MEAT

S. typhimurium appears to be the most important serotype in pigs (Table 2). It is not easy to rear Salmonella-free animals. Only ... approaches, including herding, fattening and slaughtering, will eventually lead to pathogen free animals. Measures that must ... decrease the incidence of Salmonella include: the establishment of Salmonella free areas in which the animals are reared; the ... of the animals against environmental contamination; the use of Salmonella free feed and water; the establishment of Salm... breeding stock and the transport of animals under conditions which would not allow contamination (WHO VHP/83.42). Strict ... of hygiene in pig fattening brings immediately demonstrable benefits in elimination of salmonella infections (Oosterom and N... 1983).

The length of stay of slaughter pigs is another important factor in the manipulation of the salmonella contamination of pig ... (Morgan et al. 1987).

In contrast to poultry, where C. jejuni is the predominant specie, pigs are believed to be the most important source of E. coli. Su... as yet not much in known about the epidemiology of Campylobacter species. However, both species can be regularly isolated ... faeces. Faecal carriage rates of Campylobacter spp. among pigs may be up till 100%. However, muscle tissue of healthy ... principle, free of micro-organisms. Campylobacter spp. are not very hardy organisms and as a consequence so environmental cont... of carcasses by this organism appears to be insignificant. Our preliminary investigation shows that even as the contamination of ... pig before chilling is up to 30%, the recovery rate after overnight chilling is lower as 3%.

Nevertheless, contamination of muscle tissue during the slaughter process by e.g. faeces, skin contaminated tools and equipment, ... clothing, should be prevented. Although this contamination seems, to a certain extent, to be inevitable the level can be ... decreased substantially by bad or good slaughter procedures. It is important to know the critical control points in order ... microbiological contamination at each step or operation in breeding, slaughtering and processing process (Figure 1A). It is ess... critical control points must be monitored, not only visually but also bacteriologically, continuously or periodically to ensure that ... under control (NCR, 1985). For monitoring the critical points in the slaughter and processing line it is not necessary to search for ... pathogenic organisms in the first place. At this stage of the meat production line, it is more important and makes more sense to ... general level of contamination than to look for specific pathogenic micro-organisms such as *Salmonella.* In the ideal situation, ... of pathogenic micro-organisms in herds should have been established before and the logistics of the process should first allow sl... of (specific) pathogenic free animals in order to avoid cross-contamination of the slaughter-line. In addition it has been sh... pathogenic micro-organisms often have a highly heterogeneous distribution on carcasses, which makes detection and a consistent ... policy very difficult at this stage (Mossel,1982).

By scalding at the right temperature, the number of aerobic colony forming units per square cm on the pork skin is reduced by ... The highest reduction of the bacterial load on the skin is achieved in the singeing or flaming oven.

In the blackscraping and polishing machine pig carcasses are often severely recontaminated. If cleaning and disinfection are ... effectively, considerable quantities of dirt (hairs, parts of the epidermis, etc.) remain in the machinery. Large numbers of bacteria ... overnight on this debris and cause a more or less severe contamination of the carcasses during slaughtering. Consequently, if clean... disinfection are inadequate the blackscraping and polishing machinery acts as a continuous source of bacterial contamination. Aerob... counts of 4.2 $\log_{10}$N per cm^2 were found on pork skins after they had passed the polishing machinery, which had not been ... appropriately. When cleaning was carried out more systematically but without daily disinfection, the contamination of the car...

Sal. enteritidis p. 99

ced to 3.3 $log_{10}N$ per cm^2. Mechanizing the cleaning procedure by using rotating spraying devices in a daily disinfection programme lted in a further reduction of contamination by 0.5 $log_{10}N$ per cm^2 (Corstiaensen et al., 1986). However, the complicated construction is machinery makes cleaning difficult and elaborate. In order to make cleaning and disinfecting easier and more effective, (voluntary) elines could be set for the quality and style of the construction of slaughter equipment, and for the types of materials which can be It is not the aim to cramp the style of the designers of such equipment, but to avoid the bringing onto the market of equipment which fficult to clean. In the production of food, hygiene is of the utmost importance. Great differences exist between several slaughterlines respect to cleaning an disinfection of the equipment. At this point, much can be learned from the dairy industry.

"Clean" part

e bacterial load of the skin at the end of the so-called unclean part is already high, it will remain at that high level. In that case even l deficiencies during evisceration do not have much influence on the total contamination measured by total viable counts.
ever, it is necessary to monitor what exactly happens during the evisceration procedure in the so-called "clean" part. Enterobacteriaceae erations make sense in that view. Enterobacteriaceae-counts give a reflection of the faecal contamination in this part of the ghterline. All measures taken in this part of the slaughter process must be focused on good slaughtertechniques. Especially damage to intestine and contamination of the carcass must be avoided. An integral part of the complex of GMP is paying attention to the training instruction of personnel working on the slaughterlines. However, a warning note should be sounded here. Hygienic working practices not simply a matter of teaching to a sample of employees. Measures intended to improve and control hygiene in a company are complex require long-term investment. A strategy has to be developed to involve people in hygiene programs (Gerats, 1990). Results during last decade shows that only in a totally controlled production system a systematically improvement of hygiene has been achieved. This been confirmed by microbiological monitoring.

MICROBIOLOGICAL PROBLEMS ASSOCIATED WITH POULTRY MEAT

e primary breeding company keeps relatively small numbers of pedigreed birds, which are selected for various commercial aracteristics, such as feed conversion and body confirmation. These birds are multiplied by the breeder to form basic grand-parent stock; m there parent breeding stock will be produced, sold and further multiplied to rear large numbers of broiler chickens. As the breeding ramid is descended, there is a gradual increase in the pressure on the live stock: being kept in larger numbers are higher stocking nsities.

should be clearly understood that Salmonellosis in poultry is extremely rare. Occasionally some serotypes will cause disease in young cks. More common is the situation where Salmonella infection takes place by symptomless carrier birds. Due to the large numbers in hich broilers are kept in a common environment, the relatively small number of symptomless carrier birds may produce high percentages contaminated birds at the broiler house level where stocking densities are extremely high.

Pathogenic micro-organisms and emerging new pathogens

he presence of potentially pathogenic microorganism in poultry products make exporting countries more vulnerable to undesirable import rriers. The necessity for better hygienic procedures in the whole production chain becomes more evident. Methods for production of oultry meat have changed : an increased number of birds per m^2, reared in climate controlled houses in which microorganisms may spread sily within the flock.

ampylobacter jejuni/coli, Salmonella enteritidis and other serotypes and haemorrhagic Escherichia coli O 157 H7 are examples of icroorganisms of concern. These organisms have got the opportunity to increase in live animals, probably as a consequence of intensive imal husbandry practices. Salmonella and Campylobacter bacteria can therefore be present in healthy animals. In the intestinal tract of oultry the number of colony forming units (cfu) of Salmonella range from 100-1000 per g. With Campylobacter the situation differs :

Campylobacters can be isolated in numbers ranging from 10^5-10^7 cfu per g of intestinal material.
Problems with this high number of potentially pathogenic microorganisms present in the intestinal tract may arise during slaug
to cross-contamination the whole flock can become contaminated. It is evident that the aim of preventive measures taken during
out should be to decrease the number of Salmonella and other potentially pathogenic microorganisms in the live bird.
Figure 1B gives a typical scheme for the procedures applied in different poultry processing plants. Depending on the phase of mea
the individual processing steps are with or without human labour. From a microbiological point of view there are several steps
critically to control the microbiological contamination of products and equipment.
In this figure the processing plant starts with supply/ transport. By mentioning the critical points in the phase prior to the sla
process it is recognized that conditions of transport from the rearing farms to the slaughterhouse also contribute to the contam
skin and feathers with faecal material. Cleaning and disinfection of transport cages or containers after each transport therefore is
In terms of HACCP this also should be considered a CCP.
Modern poultry processing means a high rate of production, more than 6000 birds per hour. Developments as the introduction
stage cleaning and scalding have resulted in a decrease in microbial counts on the carcasses as well as in the scald water (Veerk
1991). Less cross-contamination can therefore be expected by using this equipment. Automatic cleaning and disinfection of e
equipment is commercially available now. This equipment is the first part in a chain of machines which are able to clean and di
place.

5.2 Visual monitoring

Although often forgotten, visual observation is an important means of monitoring the critical points in slaughtering and processing
If dirt can still be seen after cleaning and disinfection has been completed, bacteriological examination is wasted work. Checklis
be used regularly to objectively monitor the effectiveness of the cleaning process at the critical points in the slaughterline. The
should be given as: good, sufficient, insufficient or bad (Gerats et al. 1981). A mean score for each machine can then be calcul

5.3 Microbiological monitoring

The frequency of the microbiological evaluation should be specified and the results must be recorded systematically and a
employees is necessary. This should also include monitoring for pathogenic micro-organisms at farms. Unfortunately not much
been given to this part of the product line.

In the Netherlands two microbiological tests are used for monitoring the slaughtering process. One is based on checking the
cleaning and disinfection of equipment before slaughtering starts. The other method assesses the bacterial population per square
surface of carcasses or cuts during processing. (Snijders, 1988) or per gram for poultry (Mulder, 1978).
The disinfection of machinery surfaces which come into close contact with carcasses can be monitored regularly with agar cont
Many years of experiences in The Netherlands have shown that small plates (3.2 cm in diameter) filled with plate count agar
for this purpose (Snijders 1988). After sampling and incubation at 37°C for 24 hours the number of colonies on the plates is
classified. A general judgement about the hygienic state of the equipment can be obtained by calculating the mean score for
machines/objects.
The bacterial condition of the surface of the pig carcasses is a useful parameter in appraising the overall hygiene in a slaughterho
an indication of the effectiveness of hygiene processing and support the visual inspection during processing. For this purpose, the
method gives the most reliable and reproducible results (Nortje et al. 1982, Snijders, 1977). In this method three discs of tiss
surface area of 5 cm^2 each are punched out of the back, abdomen and cheek of the carcass. After plating and incubation, the
colony count and the Enterobacteriaceae count on violet red bile glucose agar can be calculated. With this kind of monitoring it
to assess the effect of changes in the processing of pigs (Snijders, 1988), and poultry. (Mulder et al., 1978; Bolder and Mul

the next years the EC zoonosis order will come into force. In this order a mandatory monitoring system for herds or flocks is foreseen. veral microorganisms to be monitored are named, however looking at the poultry production chain monitoring with respect to Salmonella tamination is the most important issue.

tical control points with reference to Salmonella contamination can basically be divided in two categories: the first is critical because the possibility of massive multiplication due to infections or suboptimal storage, the second because of the role in the transmission routes the organism in the whole production chain (cross-contamination).

nitoring of the live animal should be aimed to predict the contamination rate of herds and flocks at slaughter. Of course informations the (Salmonella) status of breeding flocks, hatcheries etc is of importance. The actuality of the contamination status of a flock to be livered to the slaughterhouse, however exceeds the importance of findings in earlier phases of the production.

nure sampling and examination at three to four weeks of age at the rearing farms predicts the current contamination situation. Dependent the result of the examination specific herds or flocks can be slaughtered in a different plant or in a special slaughter order : Salmonella- e animals before Salmonella contaminated animals. The latter approach is applied in those poultry integrations where sampling and mination for Salmonella is done routinely. By monitoring the situation of contamination of the live flocks, sampling of processed ducts for pathogens can be minimal. The only motive for doing so is to control whether products become contaminated through the vironment, men or equipment in the slaughterhouse. On the other hand L.monocytogenes on freshly slaughtered hogs can hardly be ected, while the majority of primals are often contaminated with this micro-organism. Thus, environmental contamination of primals Listeria is significant and growth may occur in drip water, wet surfaces and water left standing in drains. This appears an important tical control point for Listeria.

NOVEL MICROBIOLOGICAL MONITORING APPROACHES

mber of alternative rapid analytical techniques in food microbiology are available now. (Huis in 't Veld et al., 1988). A summary is sented in Table 3.

certain areas of the food industry, automated instrumental microbiological analyses such as impedimetry and turbidimetry have had a nificant impact as a hygiene parameter (total counts and Enterobacteriaceae) in routine food analysis. Bioluminescence, the ATP assay, also found a wide application as a hygiene parameter, as it provides almost instantaneous information about total bacterial contamination a product or a surface, provided that at least 10,000 micro-organisms/ml are present in the suspension to be measured. In contrast to se tests, immunological assays or nucleic acid hybridisation tests can provide information on specific bacterial species or virulence types d also about microbial metabolites (toxins etc.).

e of the major concerns among microbiologists with respect to the implementation of new techniques is their sensitivity, particularly en applied to complex samples like foods. Indeed, the sensitivity of these techniques is by no means high enough to allow direct detection specific bacteria.

Table 3: Potential novel microbiological techniques for use in food operations and their characteristics

	characteristics	detection pathogens	user friendly	costs	speed	sensitivity(*)	automa
Impedimetry	Bacterial metabolism/growth	+/-	+	high	4-24h	10^5	+
Turbidimetry	Bacterial growth	-	+	high	6-24h	10^5	+
Bioluminescence	ATP	-	+/-	variable	< 1h	10^4-10^5	+
Fluorescence/DEFT	Morphology/viability	-	-	variable	< 1h	10^2-10^3	+/-
Immuno-assays	Specific antigens and metabolites	+	+	moderate	2-6h	10^4-10^5	+/-
DNA technology	Specific DNA sequences(genes)	+	+/-	moderate /high	2-6h	10^2	+
Flowcytometry	Morphology/metabolism	+	+/-	high	0.5-2h	10-10^2	+

(*) sensitivity in expressed as the number of cells/ml to yield a detection signal

In principle, modern methods, based on Bio-analytical reagents (monoclonal antibodies, DNA-probes and primers) in combination with automated instruments may meet the requirements for implementation in food operations. In contrast to the present situation, where methods are being "prescribed" to food manufacturers , future microbiological quality control should preferentially be exercised by combinations of certified bio-analytical reagents and techniques such as immuno-assays, various DNA hybridizations techniques, automated culture systems and flow cytometry (Shapiro, 1990; Patchet et al 1991.). These approaches will provide information on the level of specificity desired: from total bacterial counts through family and genus levels down to subspecies or virulence types within a species. Generally speaking two types of tests are required: 1) very sensitive and reliable tests at critical control points and 2) less sensitive, rapid and used-friendly tests for screening or monitoring purposes.

6.1 Impedimetry and Turbidimetry

When microbial growth and metabolism take place in a culture medium, changes in electric impedance (Z) will occur. The time for these changes to reach a threshold value (i.e. the detection time, DT) is inversely proportional to the initial inoculum (Firstenberg-Eden, 1984).

When small numbers of microorganisms are introduced into a broth the initial impedance changes are too small to be registered above the initial circuit noise of the instrument. Not until there is quite a large microbial population (10^5-10^6 CFU/ml) of actively metabolizing microorganisms can the instrument recognize these changes. This directly uncovers one of the drawbacks of the technique, the impossibility of quickly detecting low levels of microbial contamination.

Since their introduction these methods have only incidently been used for the determination of microbial contamination (total colony forming units or total numbers of Enterobacteriaceae). Recently methods for the detection of specific pathogenic micro-organisms have been introduced (Smith et al 1989; Ogden, 1988).

Another instrumental technique which is based on bacterial metabolism is turbidimetry (Manninen et al 1990; Mattilla 1987). During growth of microorganisms in liquid media changes in turbidity will occur which can be measured spectroscopically. The turbidimetry method is also relatively slow for the estimation of products with a low initial contamination. Most applications, so far, have been aimed at estimating the initial contamination level (Schulz et al 1988).

2

Luminescent ATP assay

very rapid and sensitive ATP assay, based on the firefly ATP luminescent reaction, has long been used in analytical and clinical research oratories. Recently, modifications of this method were developed as an alternative to the traditional plate count techniques in routine robiological analysis (Stanley 1989; Stewart 1990). The firefly luminescent reaction can be summarized in the following reaction:

$+ LH_2 + ATP \dashrightarrow E.LH_2\text{-}AMP + PP$

$LH\text{-}AMP \xrightarrow{O_2} \text{oxyluciferin} + CO_2 + AMP + \text{light}$

= luciferase; LH = luciferin; E.LH -AMP = enzyme-bound luciferyl adenylate; PP = pyrophospate

e total light output of a sample is directly proportional to the amount of ATP present and can be quantified in so called Luminometers. inimum of at least 10^4 cells is required to give a signal. ATP measurements can be used for measuring efficiency of cleaning surfaces d utensils

DNA-HYBRIDISATION TECHNOLOGY

NA technology can be used for detection of specific micro-organisms as well as groups of bacteria.

o types of DNA hybridisation-based methods can be distinguished. Firstly the hybridisation with a preferably non-radioactively labelled NA probe. In the DNA probe technique (Fig 2.), bacteria are applied to a solid phase, usually a nitrocellulose or nylon filter. After lysis he bacteria the DNA is fixed to the filter. Subsequently, labelled DNA probes are added and allowed to react with their counterstrand, present. In this type of reaction, the DNA present in the sample is not multiplied. Its sensitivity is therefore dependant on the initial ount of DNA. This is also the case in the newer application of DNA probes, i.e. the hybridisation in solution, followed by capture of e target DNA or RNA by a second probe as developed by gene-trak systems, Framingham, MA, USA (King et al., 1990)

all cases described so far (selective) pre-enrichment procedures are needed to obtain the minimum number of cells required for the DNA bridization test (approximately 10^5 cells/ml). This can be done along the lines of the classical microbiological methodology. The method, g. for Salmonella, allows the identification of the bacteria within 2 to 3 days, including sample pretreatment (Rose et al 1991). In this se, Salmonella probes derived from single copy DNA or from genes with a low copy number can be used. Yet bacteria generally possess tween 10^4 and 10^5 ribosomes and consequently as many copies of 16S and 23S RNAs. Ribosomal RNAs may therefore be a naturally plified target for hybridisation probes (Haun and Göbel,1987).

he other approach is the probe or primer induced DNA amplification e.g. the polymerase chain reaction (PCR). In the PCR type reactions g.3) small, but specific DNA probes (primers) are added to the DNA sample. If they meet their complementary strand, they will bridise. A subsequent polymerase activity doubles the initial amount of specific DNA. This cycle can be repeated, in principle, for an limited number of times (Saiki et al 1988).

veral specific DNA probes, suitable for use in food microbiological analyses, are now available for implementation. Universal Salmonella obes and PCR primers, Campylobacter species specific probes and PCR primers and several probes for E. coli virulence types and higella species have been constructed (Hofstra and Huis in 't Veld, 1990). However, the enzymatic reaction is strongly influenced by the atrix in which the determination have to be carried out. Sample pretreatment for these methods may be limited by a cleaning up step or NA extractions.

ree different areas relevant for application of DNA probe technology are:

- identification of micro-organisms, e.g S. enteritides or S. typhymurium
- detection of micro-organisms, absence of e.g. Salmonella
- typing of micro-organisms, e.g. for epidemiological studies

7.1 Identification of microorganisms

Identification can be performed on pure cultures by the classical DNA-hybridisation technique. A particular set of labelled DNA may replace the, biochemical and serological identification systems, generally used today. We have selected random DNA-fragm the development of probes specific for the different Campylobacter species. Probes specific for C. jejuni, C. coli and the ther Campylobacters have been developed. These probes were used for the identification of previously isolated and characterised campy species (Huis in 't Veld et al 1991). In all cases, a very good correlation with the few classical biochemical tests at hand was o DNA hybridisation technology can therefore be used for identification, especially for those microorganisms which are difficult to with classical methodology, as in the case of Campylobacters.

7.2 Detection of microorganisms

In the above mentioned hybridisation methods, the DNA present is not multiplied. Its sensitivity is therefore dependant on the initia of DNA. When high numbers of cells are available as in pure cultures, this is no problem. In many practical situations, howe numbers of pathogenic microorganisms present in foods or other specimens must be detected. In these cases tedious and time co pre-enrichment procedures have to be performed. This can be overcome by using in vitro DNA amplification procedures like the po chain reaction (PCR).

Sequencing of the C. jejuni probe led to a set of primers which could be used to detect as low as one single C. jejuni cell. Howev these promising results one should not conclude that DNA amplification methods like PCR will detect a single cell in natural samp have conducted experiments in which chicken meat was seeded with different numbers of C. jejuni cells, after which the ce recovered by rinsing with peptone-physiological salt solution. Samples were analyzed by classical plating on selective blood agar the PCR reaction. The percentage recovery by plating was generally between 50-75%. In parallel experiments, specific C. jejuni P performed in the rinsing fluid after a short washing procedure. Numbers as low as 10 cells per 10 ml of rinsing fluid were detec with varying efficiency. One of the major problems with DNA technology is the sample pretreatment. How can cells be separa the foodmatrix? A very promising technique is immunoseparation (Skjerve et al 1990), the isolation of cells by binding onto magne coated with a specific antibody. The cells bound to the beads can subsequently be detected by cultivating on agars (Vermunt et al ATP (Torensma et al, 1992), PCR or an immuno-assay.

7.3 Typing of microorganisms

In epidemiology, it is very important to elucidate the type and origin of microbial infections. Generally, biochemical and serolo in combination with phage typing are being used. A major drawback of these techniques is that they are genus/species specific and be performed at specialised institutes. Furthermore, these techniques rely mainly on the availability of highly specific (rabbit) antis are difficult to standardise with the consequence that international standardisation is almost impossible. DNA technology opportunity to overcome most of these problems by a generally applicable combination of Restriction Enzyme Analysis (REA) w more DNA probes. The basic principle of this Restriction Fragment Length Polymorphism technique (RFLP, DNA-fingerp presented in Figure 4. Several variation on this basic principle are possible.

On the basis of these DNA patterns, typical fingerprints of each isolate can be defined. C. coli isolates can be easily distingu the other species, but also within the species C. coli variations in DNA patterns between the different serotypes were observed. possible to discriminate between Campylobacter coli isolates from different pig farms. RFLP typing holds therefore great p studying the epidemiology of zoonoses.

IMMUNO-ASSAYS

Immunological assays based on polyclonal or monoclonal antibodies are performed as Enzyme-linked immunosorbant assays (ELISAs), radio-immuno-assays (RIAs), fluorescent techniques or agglutination tests. ELISAs can be designed in a number of ways as shown in Fig. Over the last few years, several modifications on the classical ELISA procedure have been introduced. They claim either a higher sensitivity, specificity or rapidity and above all simplicity. The choice of the format very much depends on the type of molecules to be detected and the required specificity or sensitivity. For example small molecules such as hormones can only be detected in so called competitive ELISAs. ELISAs are usually carried out in a microtitre tray. Alternatively, polystyrene or ferrous metal beads can be used as solid phases. The latter have the advantage that a magnetic device can be used to collect the beads for the washing steps (immunoseparation). Immuno-assays allow the detection micro-organisms directly via the detection of specific antigens (Mattingly et al 1985; van Poucke, 1990) or indirectly via changes in the antibody titre in serum of infected animals. In addition, residues, antibiotics and microbial metabolites, such as toxins, can be detected in the picogram to nanogram/gram range. Fig.5 shows the double antibody blocking ELISA assay which has currently been introduced in the Netherlands for monitoring S. enteritidis infections in poultry.

BIOSENSORS

Another powerful tool for monitoring infections are biosensors (Robinson, 1991), in particularly immunosensors based on surface plasmon resonance (SPR, Severs et al 1992). The technique offers a number of potential advantages over conventional immuno-assays. The detection is quick and in real time, while the immunochemical reaction takes place. Moreover, the system is easy to use, does not need highly sophisticated environments and can easily be automated for decentralised testing. The SPR immunosensor is based on changes in the reflection of laser-light at a metal-liquid surface, due to a change in refractive index. Local refractive index changes, produced by binding (of serum)antibodies to immobilised antigens, disturb this field and the plasmons arise at a different angle of incidence. This shift can be detected by measuring a calibrated change in intensity of the reflected beam at a fixed angle of incidence with a pindiode The sensitivity of the SPR immunosensor normally depends on the molecular weight of the ligand to be detected. Antibodytiters in serum can be detected directly after binding to the antigen coated slide. Small molecules (Mw < 5000 D), such as residues and hormones, cannot be detected directly, because binding to the antibody coated surface hardly changes the refractive index. This can be overcome by the introduction of 0.3-micron latex particles coated with the small molecule to be detected which will lead to a dramatic change in refractive index and consequently to an increased sensitivity. Biosensors based on SPR have just been introduced in clinical chemistry, they certainly, as will be shown later, hold promises for future monitoring residues, hormones or microbial infections in animal husbandry.

The above mentioned techniques, alone or in combination, will soon find application in Longitudinal Integrated Safety Assurance systems within the production of food of animal origin. When manufacturers would develop simple and cheap instruments which can be used for measuring hygiene at CCPs, implementation of process integrated microbiology (control of hygiene) would be within reach. As the need for detection and identification of specific pathogenic micro-organisms (subspecies of Campylobacter, Salmonella, Yersinia, Listeria etc) becomes one of the most important features microbiological quality assurance, in the long run, DNA probes and immuno-assays in combination with other technologies will form the core of those automated monitoring and control systems.

10. PROBLEMS WITH THE APPLICATION OF NEW MICROBIOLOGICAL METHODS

From the above it is clear that different microbiological questions have to be answered at different stages in the production line. Also the need for quick results and the required sensitivity or specificity may vary. Consequently, different microbiological approaches should be developed and applied. As was shown before, the potential microbiological methodology is available but, unfortunately, it must be admitted that not many of these newly developed techniques have been used within the production and processing of foods. Apparently, there is a

gap between what is offered and what is suitable for implementation. The reason for this is likely to be due to the following obs

- Some of the methods require highly skilled personnel, who generally are not available in the food industry.
- The separation of the target microorganisms from the food- or faecal matrix is the most difficult step. This is usually not acco in the instructions delivered with the kit or the instrument.
- There are no objective results in some techniques. Subjective judgements have to be made by highly trained personnel.
- Operational costs per tests may be extremely high.
- Prototype versions are launched too early and industry is then stuck with expensive equipment which is outdated in 1 or 2
- The penetration into the market is limited and as a result they cannot be used for developing commercial specifications.
- Some of the methods were taken from clinical microbiology where costs is considered in a different order of magnitude.
- Most rapid methods are developed by fast moving companies based on venture capital which need a quick return on invest

Especially research should be initiated into selection of appropriate methodology, the problems of sample preparation and separa specificity of the results, all working from food matrix or critical control point towards analytical endpoints, rather then the c around.

11. APPLICATIONS OF RAPID METHODS IN MONITORING ANIMAL HUSBANDRY, SLAUGHTER AND PROCESS

Rapid tests with two different characteristics are needed: 1. highly sensitive tests for the accurate and reliable determination of the or presence of specific pathogens. 2. cheap, rapid, simple and consumer-friendly screens which need not be as sensitive and re the first category.

One very important aim is to confirm Salmonella free pigs and poultry just before slaughter. In this way it will be possible to sele and flocks to avoid cross-contamination during slaughtering. Monitoring faecal samples of pigs and poultry from farms who hav to be able to conduct good quality assurance systems, might be an approach. At the breeding farms it is of crucial importa management secures that the animals are free of pathogenic micro-organisms because spreading from these central points will enormous impact at later stages of the chain (vertical transmission). Although differences between pig- and poultry-breeding c ignored, the main task of the management is to pay attention to environmental conditions such as housing, external contacts, quality Sensitivity and simplicity instead of time are the most important factors, consequently monitoring by classical microbiology can at this stage. The disadvantage of regular analysing environmental- and faecal samples in this way is that it is relatively expensive of labour, materials and time, requires skilled personnel and a microbiological infrastructure and is therefore difficult to carry breeding farm. It is therefore useful to develop a "rapid" screen to detect Salmonella-carrier pigs which can be used for large-scale purposes on farms. Such a screen would need to involve few materials, require low labour input, be able to handle multiple sa produce presumptive results less than 2 working days after sample collection. Furthermore, the procedure would have to be able potentially low numbers of Salmonella to at least 10^5 cells/ml, without allowing competing flora to overwhelm the salmonellas or the material to interfere with the presumptive test. Among several selective enrichment broths tested, Muller-Kauffmann tetrathionate incubated overnight at 42°C, maintained the lowest ratio of Salmonella to competing flora and identified all inoculated samples postenrichment in M broth plus novobiocin (MbN) reduced the number of false positive results in subsequent ELISAs.

The ability of the screen to detect the presence of Salmonella cells in 100 naturally contaminated faeces was compared with con isolation procedures (Table 4). Of the 34 positive samples identified by conventional isolation, a commercial ELISA (BacTrace, K and Perry Laboratories) identified 19 (56%), another Salmonella ELISA screen (Organon-Teknika) identified 22 (65%), a m different samples (76%). Using the modified protocols there were 9 false positives from the Salmonella-tek ELISA and 1 from the The best of the classical isolation methods studied was 48h enrichment in Muller-Kauffmann cultured on XLD and brilliant g which identified 28 (82%) of the positive samples.

ble 4.

Isolation and detection of Salmonella from naturally contaminated pig faeces using classical procedures and the screen for faecal salmonellas. One hundred samples were tested and a total of 34 were Salmonella-positive according to classical isolation procedures.

dia and methods	No. positive	% total positives	false positives
sical isolation(*)			
, 24h			
, 48h	25	74	+
	28	82	+
en postenrichment(*)			
, 24h; MbN,4h			
ken	24	71	+
Trace			
monella-tek	19	56	1
bined BacTrace	22	65	9
Salmonella-tek	26	76	10

as identified by positive colonies on XLD- and Brilliantgreen (BG) agar.
suspect colonies on XLD and BG agar negative by polyvalent O-serum agglutination test.

screen protocol has been developed specifically for pig faeces although minor adjustments may allow for its application to other faecal
ples as well as a range of other samples requiring monitoring for Salmonella, e.g. animal feeds or environmental samples. Results using
protocol are comparable to conventional isolation methods but the screen has the advantages that it requires low labour input, is already
ly automatable, is able to test multiple sample and give presumptive positive and negative results within 24h of sample suspension in
chment media. Although further studies are necessary in order to evaluate its efficiency as a method of detecting Salmonella-carrier
ghter-pigs in field trials, the screen is a valuable alternative to more time-consuming Salmonella isolation procedures.The total time
he test is 1,5 days and has the advantage of being both simple and the potential for automation.
e above described method detects Salmonella in general and since no pure cultures are involved, cannot identify at the subspecies level.
many cases this is not a drawback because, detection of Salmonella is the primary and most important goal. However, there are situations
en further identifications are required.

was shown before, the number of cases of human Salmonellosis caused by S. enteritidis has increased dramatically throughout the world
a connection was made with the consumption of poultry. In order to eradicate this particular micro-organism from the poultry
duction chain and poultry industry, it is essential to monitor the presence or absence of this particular serotype after which essential
sures can be taken. The S. enteritidis monitoring and eradication program for poultry in the Netherlands became effective in september
9. This program is based on periodic bacteriological cultural examination of all primary and secondary breeding flocks of both the
ler and layer sectors, hatcheries and imported poultry. Breeding flocks positive for S. enteritidis are slaughtered.
e classical Salmonella monitoring system i.e bacteriological sampling of faeces, is known to be fallible as none of the methods of
chment and selection is able to detect all positive samples (Tate et al 1990). Furthermore excretion of the organism is intermittent
Williams, 1972), thereby causing false negative results. As a means to overcome these problems, serological monitoring systems, i.e.
ing of blood for the presence of antibodies to S. enteritidis, have been proposed. From the results obtained with ELISAs based on

Lipopoysaccharide antigens (LPS), it was apparent that cross-reactions with other Salmonella species occur (Nicolas and Culle... This cross reaction was probably due to O antigen 12, which is shared by both Salmonella serotypes B and D1. Further studies (... Huis in 't Veld,1992, Zijderveld et al 1992) revealed that LPS-antigens could not be used for the detection of specific antibodies ... S. enteritidis in serum. Nevertheless, LPS antigens could be used for screening purposes i.e. measuring the antibody titers ... Salmonellas of the B and D group in order to trace down flocks suspected to be positive for S. enteritidis. In those flocks bacter... testing was intensified to confirm the presence of S. enteritidis after which slaughtering took place. Although this approach ... Netherlands has been successfully applied to reduce S. enteritidis in poultry (Table 5), it would be of great benefit to have a immu... screen which is specific for S. enteritidis and, after validation, make bacteriological analyses redundant.

Table 5. Incidence (%) of S. enteritidis in reproduction flocks and the reduction in contamination after introduction of the ... program (Edel et al 1992).

Year	Layers			Broilers		
1989	2.8		2.8	1.7		1.7
	|	50% red.	|	|	35% red.	|
1990	1.4		| 100% red.	1.1		| 56% red.
	|	100% red.	|	|	32% red.	|
1991	0.1		0.0	0.75		0.75

Such an immunoassay was developed by Zijderveld et al (1992) in a double antibody sandwich blocking ELISA based on ... antibodies against a S. enteritidis flagella antigen (GM-DAS blocking ELISA, Fig. 5).
Compared to other ELISA formats and other S. enteritidis antigens, only the GM-DAS blocking ELISA was able to discrimina... S. enteritidis infections and infections with other serotypes. The ELISA had an sensitivity and a specificity of both 100% when ... serum samples of experimentally infected chickens. Also a field study showed promising results.
This investigation, carried out in the poultry production chain, clearly show the potential of alternative techniques for moni... presence of Salmonella in animal husbandry and consequently could also contribute to decision making which flock should be sl... first. In this way cross contamination of poultry via the slaugtherprocess can be reduced. Although most of the research so far ... focused on poultry, there are arguments for application of the same approaches in pig production and slaughtering.
As was discussed before, an interesting new development is the Surface Plasmon Resonance (SPR) immunosensor (Severs et al 19... deposited slides were coated with LPS isolated from S. enteritidis to which sera were added. After 30 min. incubation follo... washing procedure, a second sandwich antibody was added. Within a few minutes the biosensor could discriminate between sera ... or low titers against S. enteritidis (Fig.6). As has been discussed before, the LPS isolated from S. enteritidis is not specific since ... cross-reactions with Salmonella of the B and D groups have been observed. Therefore, at present research is focused in isolating ... parts of the S.enteritidis flagellum. Work is started to locate specific parts of the H antigenic part of the flagellum at the DNA ... to transfer and express these in E. coli. In this way, it is hoped to obtain an antigen with the required specificity for a user-... very rapid serotype-level screen for S. enteritidis or S. typhymurium in combination with the biosensor.
Until now monitoring pathogenic micro-organisms was focused on primary production. This is an aspect of major importance ... pathogens have been eradicated in early phases of the production line, attention in slaughter and cutting can be concentrated ... procedures of hygiene, cleaning and disinfection. At the moment monitoring systems in slaughterlines mainly rely ... microbiological methods. Only very rarely, newly developed monitoring techniques are being implemented.

sical approaches will include checking the effect of cleaning and disinfection of equipment before slaughtering starts as well as the surment of the bacterial population per cm^2 of the surface of carcasses or cuts during processing as has been discussed before.
sical microbiological methods have, however, the disadvantage that they are time consuming, relatively labour intensive and cannot utomated. Hygiene can also be monitored in an alternative way.
measurement of ATP by bioluminescence for example will provide instantaneous information about the efficiency at which a certain ace, tool or piece of equipment has been properly cleaned. Although the method is simple and can be automated, at least 10^5 cells/ml eeded to give a positive signal. The technique can therefore be used in those situations where cleaning of heavily contaminated surfaces eas has to be controlled. In addition, the increase of micro-organisms at certain surfaces can be followed.
edance can be used for the determination of microbial contamination of carcasses. The same excision method may be used except that ation and quantization takes place in the impedimeter. Fig 7. presents a typical calibration curve where the initial colony counts of meat samples are plotted against the impedimetric detection times. The same kind of plot can be generated for total counts of obacteriaceae. Poor slaughter practices will be detected within one working day.
edance technology may also be used for trend analysis of raw materials, half-products and end-products. Data obtained by impedance surements can be transferred directly to a central computer that can store data over certain periods of time. Software programs are now ilable for complex trend analysis after which data can be displayed in graphics which are readily interpreted. This method facilitates parison of trends in quality between raw material suppliers, production lines or factories and enables monitoring of line hygiene dards.

n specific pathogens have to be detected or identified at this stage of the production line, classical microbiology still is also in many ratories the method of choice. However, several test based on DNA-hybridisation technology (Gene-trak systems, Framingham, USA) or immuno-assays (Organon-Teknika, Belgium; Bactrace ELISA, Kirkegaard and Perry Laboratories Ltd; Curiale et al 1990; on et al 1990) are available. Generally, non-selective and selective pre-enrichment steps are required. Therefore, these techniques can onsidered rather as a final identification than as rapid detections. As has been discussed before, for that purpose DNA amplification ems are the method of choice. Rapid methods for the detection of Salmonella and Campylobacter on carcasses have been developed ur laboratory (Hofstra, ten Bosch and van der Plas, manuscripts in preparation). DNA-primers to be used in in vitro amplification ems were based on DNA-probes, specific for these micro-organisms. The experimental approach was first to seed swabsamples from ltry- or pigcarcasses with as low as 1-10 numbers of Campylobacters or Salmonella's. Samples were successively analysed by classical thodology and a PCR method, preceded by an alternative pre-enrichment procedure, specifically adapted to the PCR technique. This ludes two short pre-enrichments directly followed by the amplification step. The Amplified DNA was detected after separation by electrophoresis. The total time needed to detect 1-10 Salmonellas or Campylobacters amongst thousands of other micro-organisms was ut 24 hours. Of course, this technique needs further refining before it can form the basis for future microbiological quality assurance. e of the first things is to shorten the pre-treatment by different concentration steps. Immuno-separation appeared a very promising proach. Magnetic beads coated with a specific Salmonella antibody can be added to bacterial suspensions (swab samples of carcasses), ken for a short time after which the beads can be separated from the suspension by a magnetic device. The DNA is released and bsequently used as a matrix for PCR. This approach will shortly allow detection of specific pathogens in slaughterlines within one king day.

e creative combinations of immunoassays, DNA technology and automated instrumental techniques holds great promises and surely form the basis of future microbiological monitoringsystems in the production of food of animal origin.

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Fig. 1 Flowdiagram showing the different steps in the production of pig meat (A) and poultry (B).

Fig. 2 Principles of DNA-probe technique. Microorganisms are applied to a filter, lysed and fixed.
Subsequently a labelled DNA-probe is added and allowed to react with their counter strand if present.
A positive reaction can be visualised.

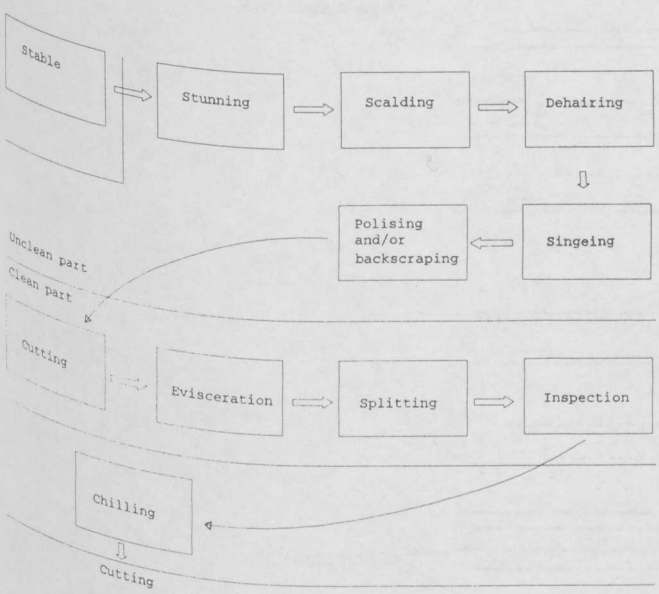
Fig. 3 Principles of Polymerase Chain Reaction (PCR).
Small specific DNA sequences ("primers") are allowed to bind to heat denaturated (separate) DNA strands. A heat stable enzyme (DNA-polymerase) extends the primers to new complementary strands after which the cycle is repeated.
An amount of DNA, equivalent to an "overnight culture", can be obtained within 2 hours.

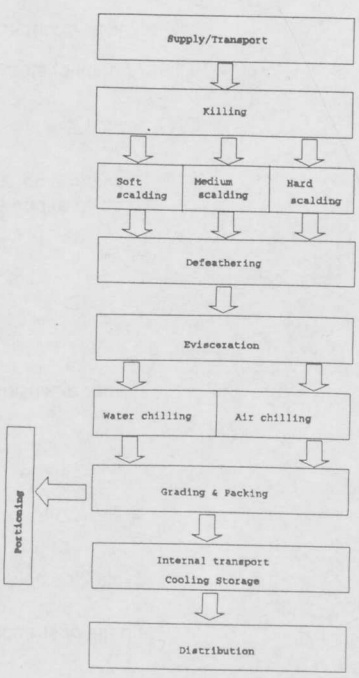
Fig. 4 When DNA strands with minor differences in DNA sequences (I) are being treated with a restriction enzyme, different products may be obtained (a, b, c and d, b, c, e resp.). These products can be separated by gelelectrophoresis (II) and visualised with a DNA-probe.

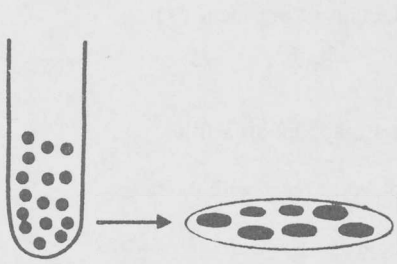
Fig. 5 Principle of the double antibody blocking Elisa.
A monoclonal antibody directed against a S. enteritidis antigen is bound to a microfiber well (1). S. enteritidis antigen is added (2), allowed to bind after which serum is added (3). After a short incubation and washing procedure, a second, enzyme-labelled, monoclonal antibody directed towards another S. enteritidis epitope is added (4). This antibody cannot bind to the epitope if the serum contained antibodies against S. enteritidis (positive serum).
A positive serum, therefore yields a negative Elisa signal.

Fig. 6 Result of a screening for antibodies against S. enteritidis in chicken sera. For experimental detail see 9. Biosensors.

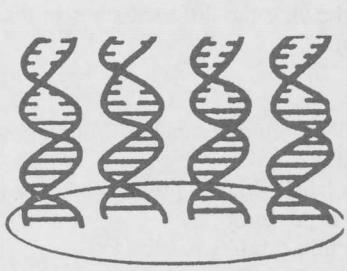
Fig. 7 Relation between aerobic colony counts and the impedance detection time for 75 samples of meat. Poor hygiene can be detected within less than a working day.



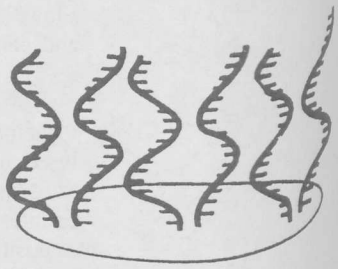




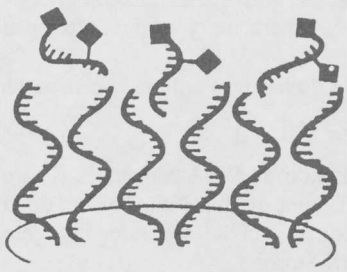
Pre enrichment and collection of micro-organisms on filter matrix.



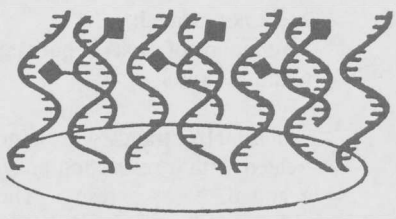
Disruption of the cells and separation of the DNA strands



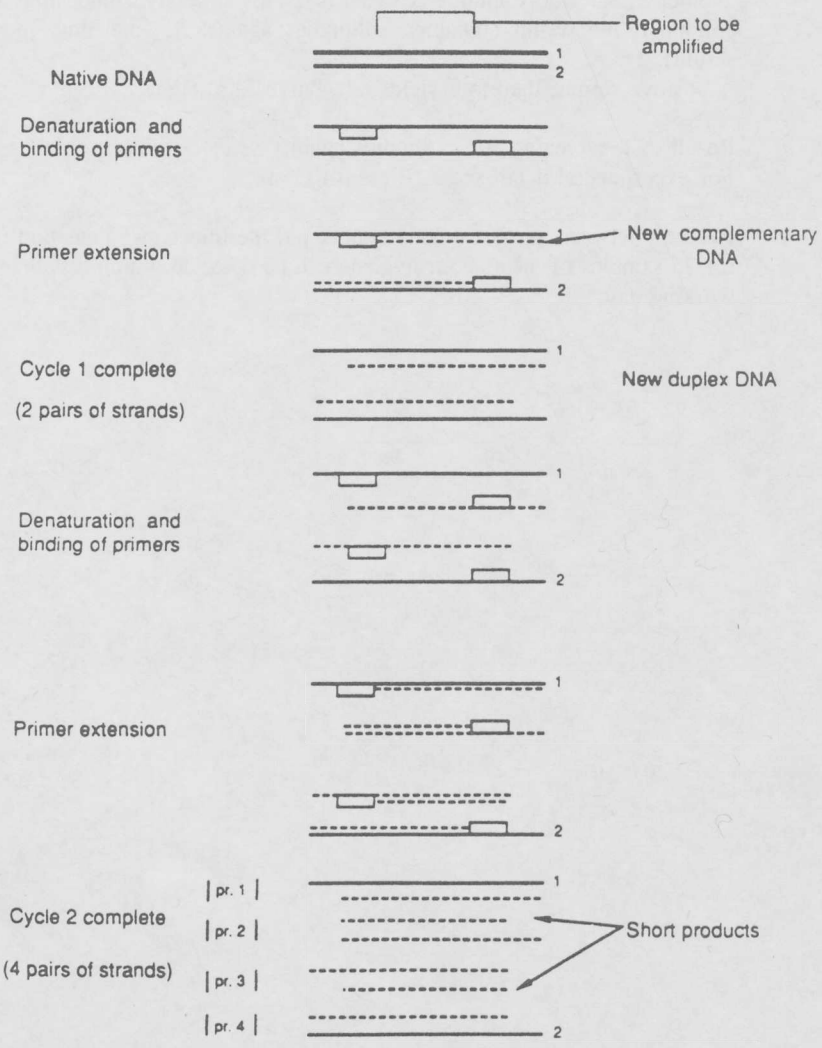
Binding DNA to matrix

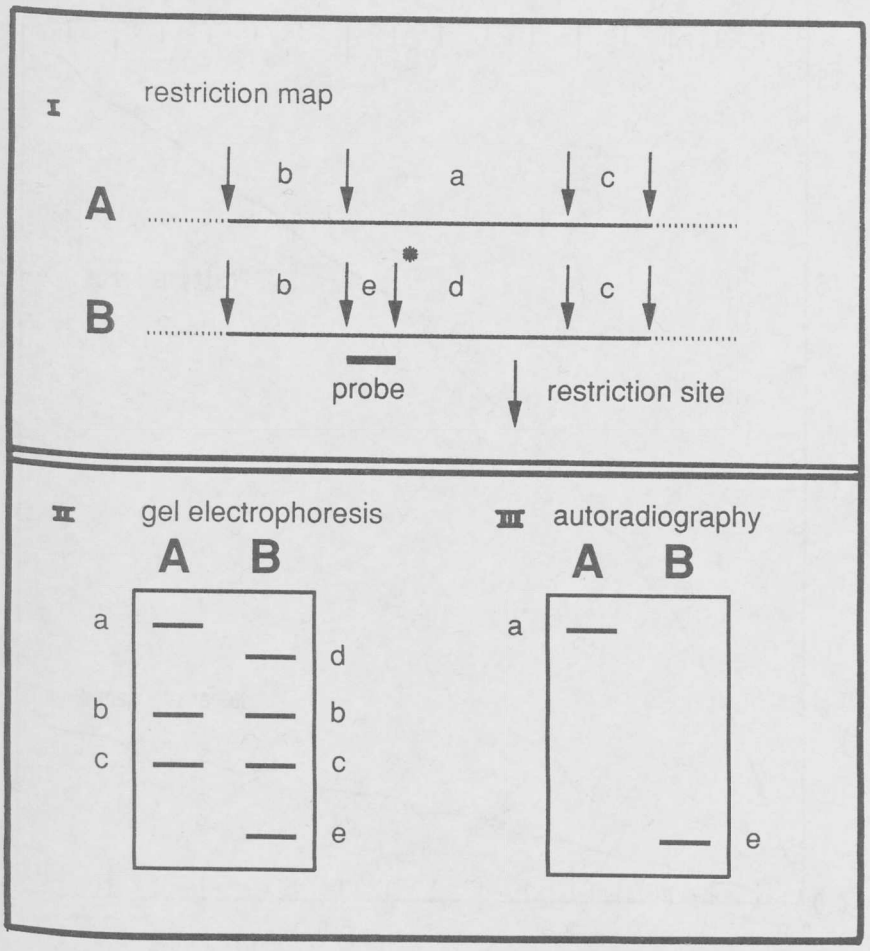


Addition of labeled DNA probes



Hybridization and detection




I
restriction map
A
b
a
c
B
b
e
d
c
probe
restriction site
II
gel electrophoresis
A B
a
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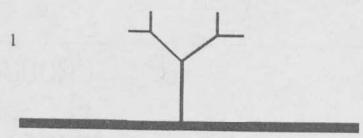
d
b
c
e
III
autoradiography
A B
a
e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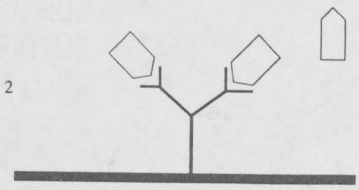
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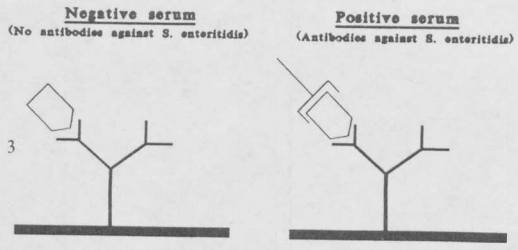
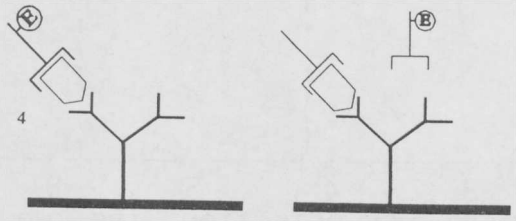

Negative serum
(No antibodies against S. enteritidis)
Positive serum
(Antibodies against S. enteritidis)
3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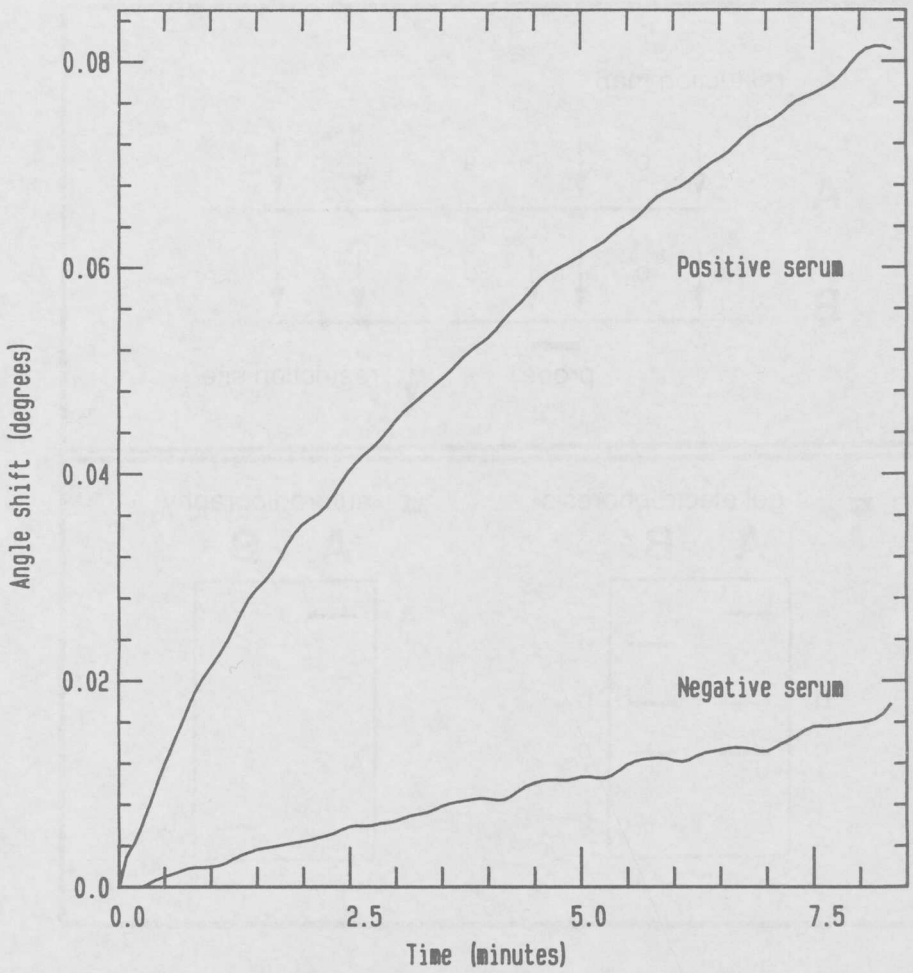
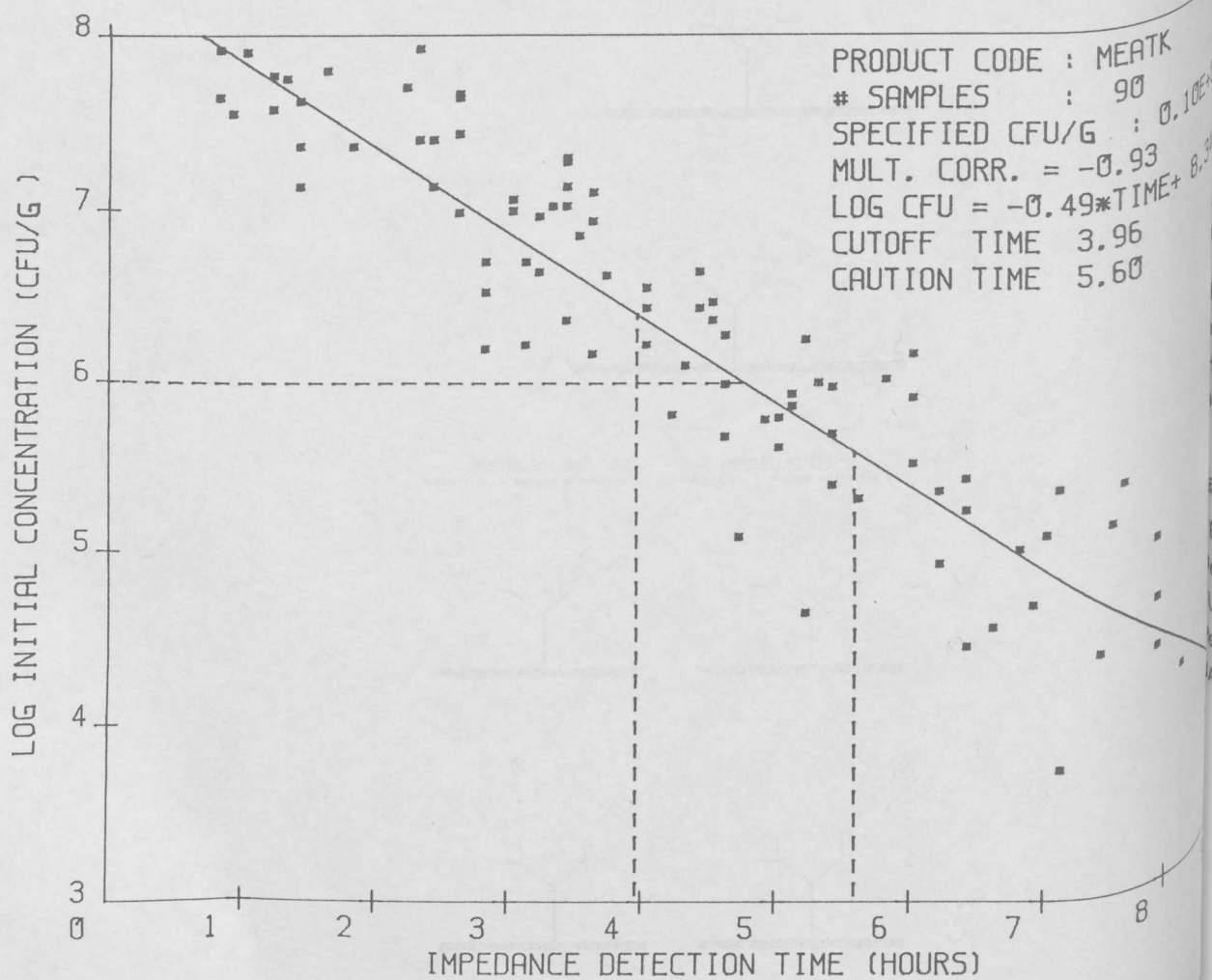

0.08
0.06
0.04
0.02
0.0
Angle shift (degrees)
Positive serum
Negative serum
0.0
2.5
5.0
7.5
Time (minutes)


PRODUCT CODE : MEATK
SAMPLES : 90
SPECIFIED CFU/G : 0.10E+
MULT. CORR. = -0.93
LOG CFU = -0.49*TIME+ 8.
CUTOFF TIME 3.96
CAUTION TIME 5.60
LOG INITIAL CONCENTRATION (CFU/G)
8
7
6
5
4
3
0
1
2
3
4
5
6
7
8
IMPEDANCE DETECTION TIME (HOURS)