CHAPTER 3

MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN

The study was carried out in a class C[1] abattoir which processes on average 30-40 heads of cattle per day in the Hammanskraal region of South Africa. The study was carried out during the winter season. The experimental design that was followed is given in Figure 3.1. This involved a hygiene evaluation of the plant, a hazard analysis and a microbiological analysis. The hygiene evaluation was done using standard hygiene evaluation sheets from the Red Meat Abattoir Association of South Africa (RMAA) in conjunction with FAO hygiene assessment forms of abattoirs adapted from Church & Wood (1992) as shown in Appendix 1. The hazard analysis was done by studying the step by step practices employed in the processing and assessing them in detail to identify sources and mechanisms of contamination so as to be able to determine critical control points. All this was done concurrently with collection of microbial baseline data. The information then formed the guidelines for the training programme for personnel on the processing line. Subsequently, microbial data (HACCP data) was collected and compared with baseline data to assess whether training had any significant effect on microbial loads.

3.1.1 Hygiene evaluation

Hygiene is basic to the safety and quality of food throughout the world and is the responsibility of every one at work and every individual has a role in keeping the plant in good sanitary condition (Sprenger, 1995). A system of controlled movement of staff, product equipment and materials should be developed and organized so that contamination is not carried from one area to the next (Church & Wood, 1992). The need for the hygiene evaluation is because basic hygiene or good manufacturing practices form the foundation for a HACCP system. This ensures that the system once fully

[1] Class C abattoirs can slaughter a maximum of 50 herd of cattle a day.

implemented is focused on the product and any potential food illness or food spoilage can be controlled as emphasized in the previous sections (Lee & Hathaway, 1998).

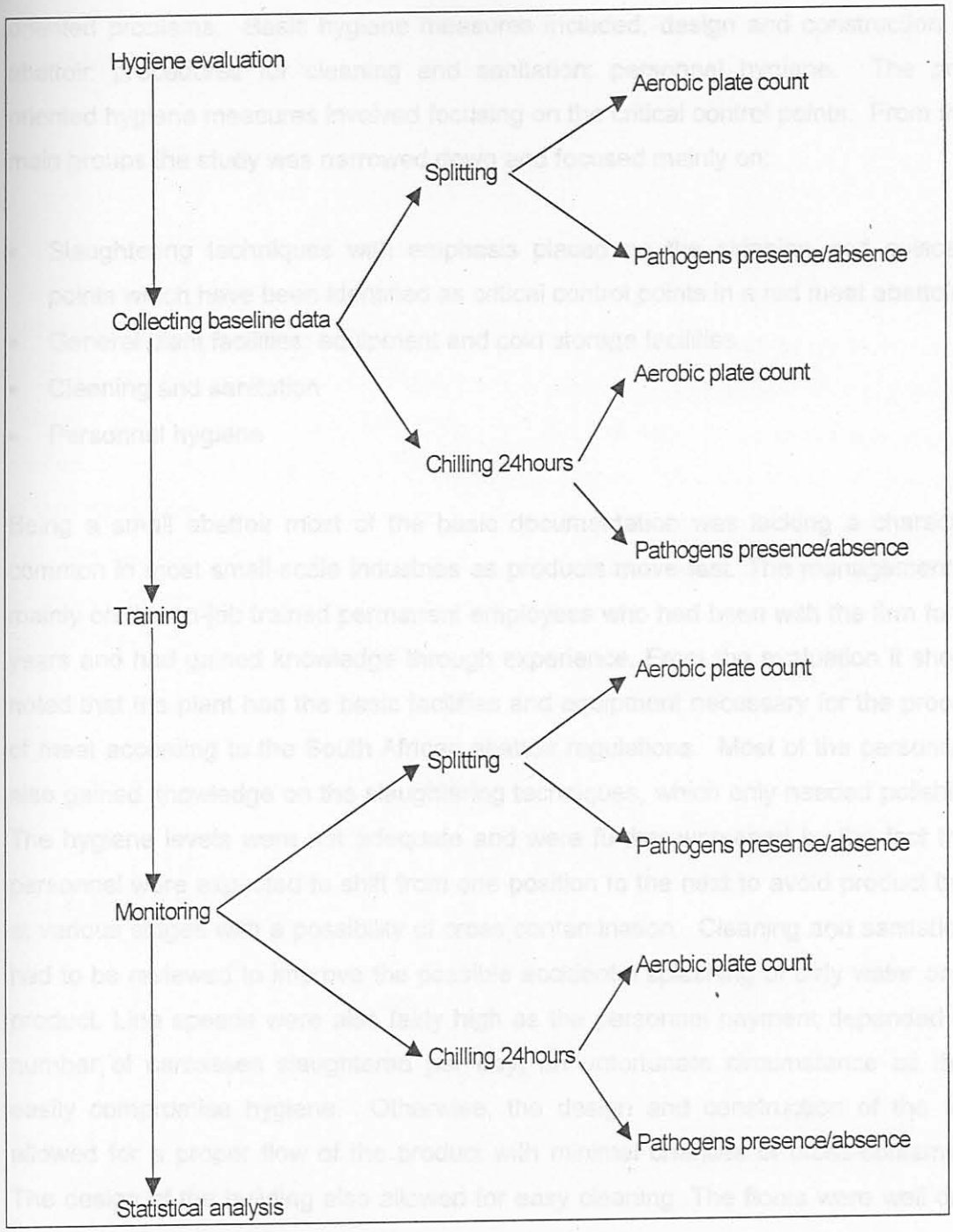

Figure 3.1: Experimental design for the study.

Note: Sampling was done after splitting and not at skinning and evisceration of the carcass to avoid reducing the chain speed of the carcasses.

Hygiene evaluation of the plant was carried out from the lairage to the dispatch over a period of 6 weeks. The evaluation was divided into basic hygiene problems and process oriented problems. Basic hygiene measures included; design and construction of the abattoir; procedures for cleaning and sanitation; personnel hygiene. The process oriented hygiene measures involved focusing on the critical control points. From the two main groups the study was narrowed down and focused mainly on:

- Slaughtering techniques with emphasis placed on the skinning and evisceration points which have been identified as critical control points in a red meat abattoir
- General plant facilities: equipment and cold storage facilities
- Cleaning and sanitation
- Personnel hygiene

Being a small abattoir most of the basic documentation was lacking a characteristic common in most small-scale industries as products move fast. The management relied mainly on the on-job trained permanent employees who had been with the firm for many years and had gained knowledge through experience. From the evaluation it should be noted that the plant had the basic facilities and equipment necessary for the processing of meat according to the South African abattoir regulations. Most of the personnel had also gained knowledge on the slaughtering techniques, which only needed polishing up. The hygiene levels were not adequate and were further worsened by the fact that the personnel were expected to shift from one position to the next to avoid product build up at various stages with a possibility of cross contamination. Cleaning and sanitation also had to be reviewed to improve the possible accidental splashing of dirty water on to the product. Line speeds were also fairly high as the personnel payment depended on the number of carcasses slaughtered per day, an unfortunate circumstance as this can easily compromise hygiene. Otherwise, the design and construction of the abattoir allowed for a proper flow of the product with minimal chances of cross-contamination. The design of the building also allowed for easy cleaning. The floors were well drained, washable and of suitable material.

While the evaluation was ongoing baseline data on the microbial levels of the plant was also being collected over a five weeks period. The baseline values were used to

develop preliminary HACCP plans for specific critical control points and to provide reference values to evaluate the impact of the HACCP program. Temperature profiles of the chilled carcasses were taken after 24 hrs using probe thermometers. The study was done according to National Advisory Committee on Microbiological Criteria for Food guidelines on HACCP for raw beef (see Table 2.9).

3.1.2 Hazard analysis

The hazard analysis consisted of inspection of the plant to identify sources and modes of contamination. Potential critical control points at which the hazards could be controlled were identified. To fully comprehend the impact of the slaughtering techniques and hygiene procedures on the microbial loads of the carcass the flow of the product was followed step-by-step as outlined in Figure 3.2. The skinning, evisceration and chilling stages are depicted Figure 3.3, 3.4 and 3.5 respectively. Single operations involved either individuals or a group of workers. The total number of people in physical contact with the carcass between slaughter and chilled storage were 12. These were divided as follows: two at stunning, four skinners, two at evisceration, one at splitting, one at inspection and two at chilling stage.

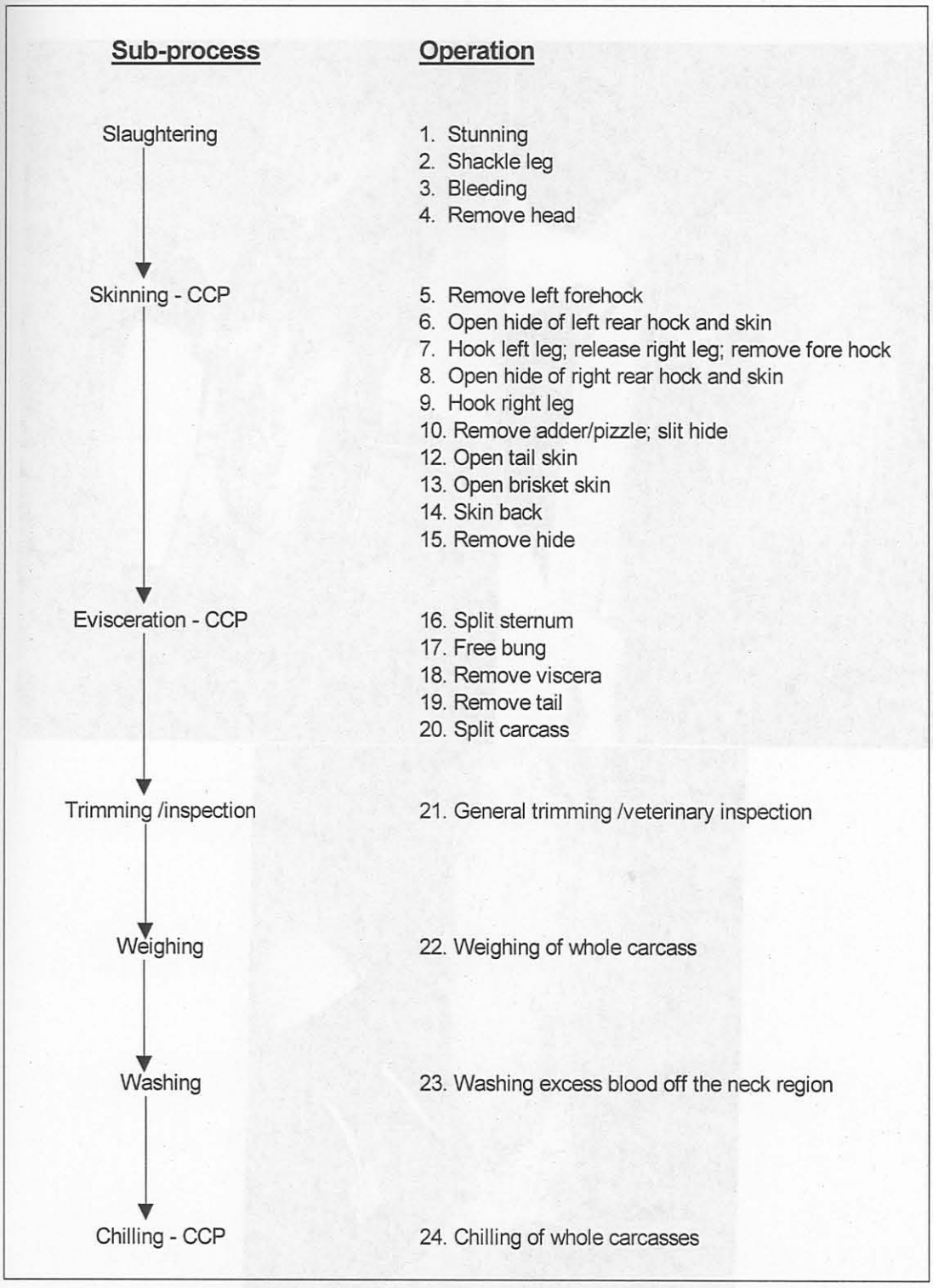

Figure 3.2: Flow chart and critical control points of beef processing steps for the abattoir under study.

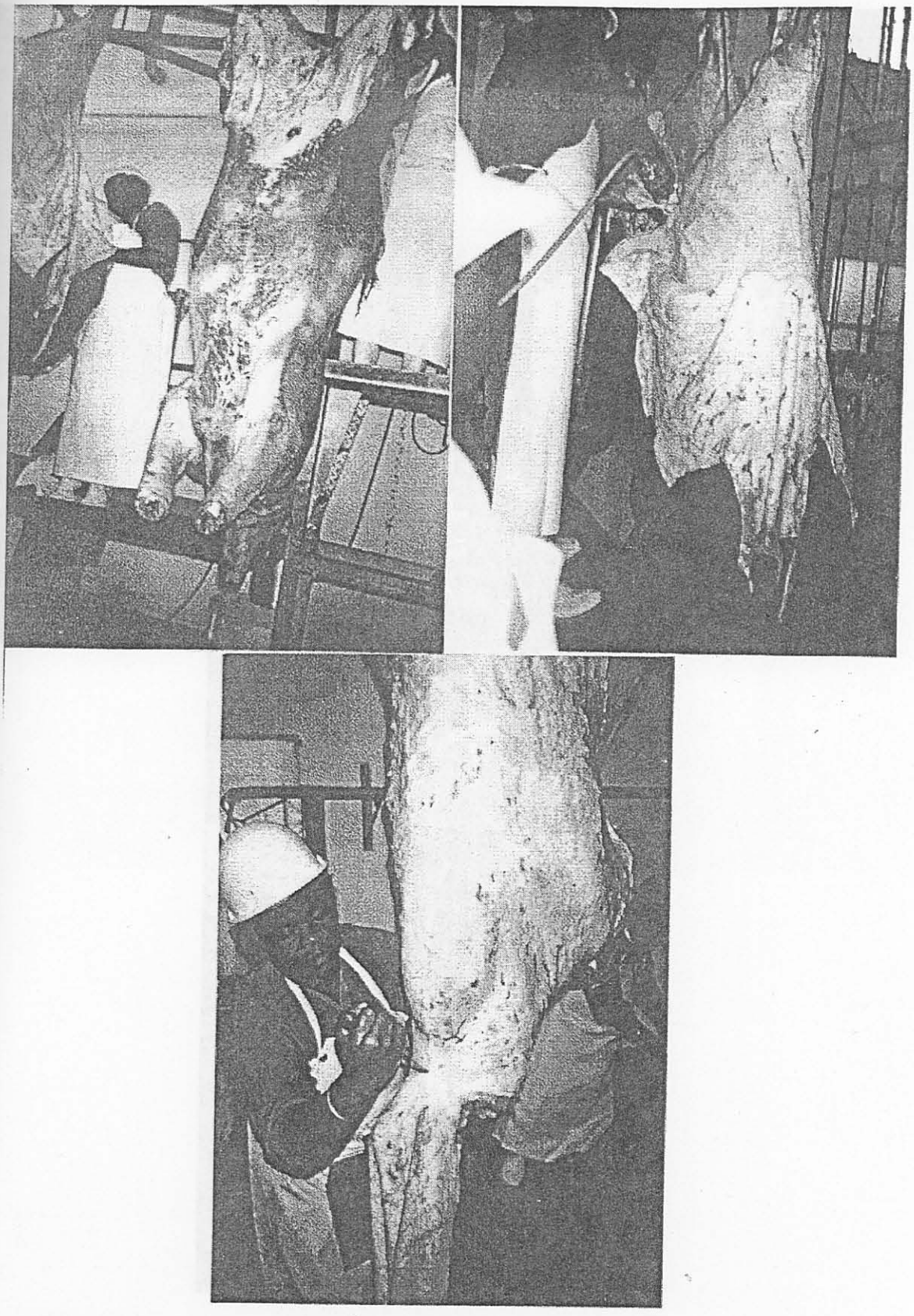

Figure 3.3: Pictorial presentation of the skinning steps.

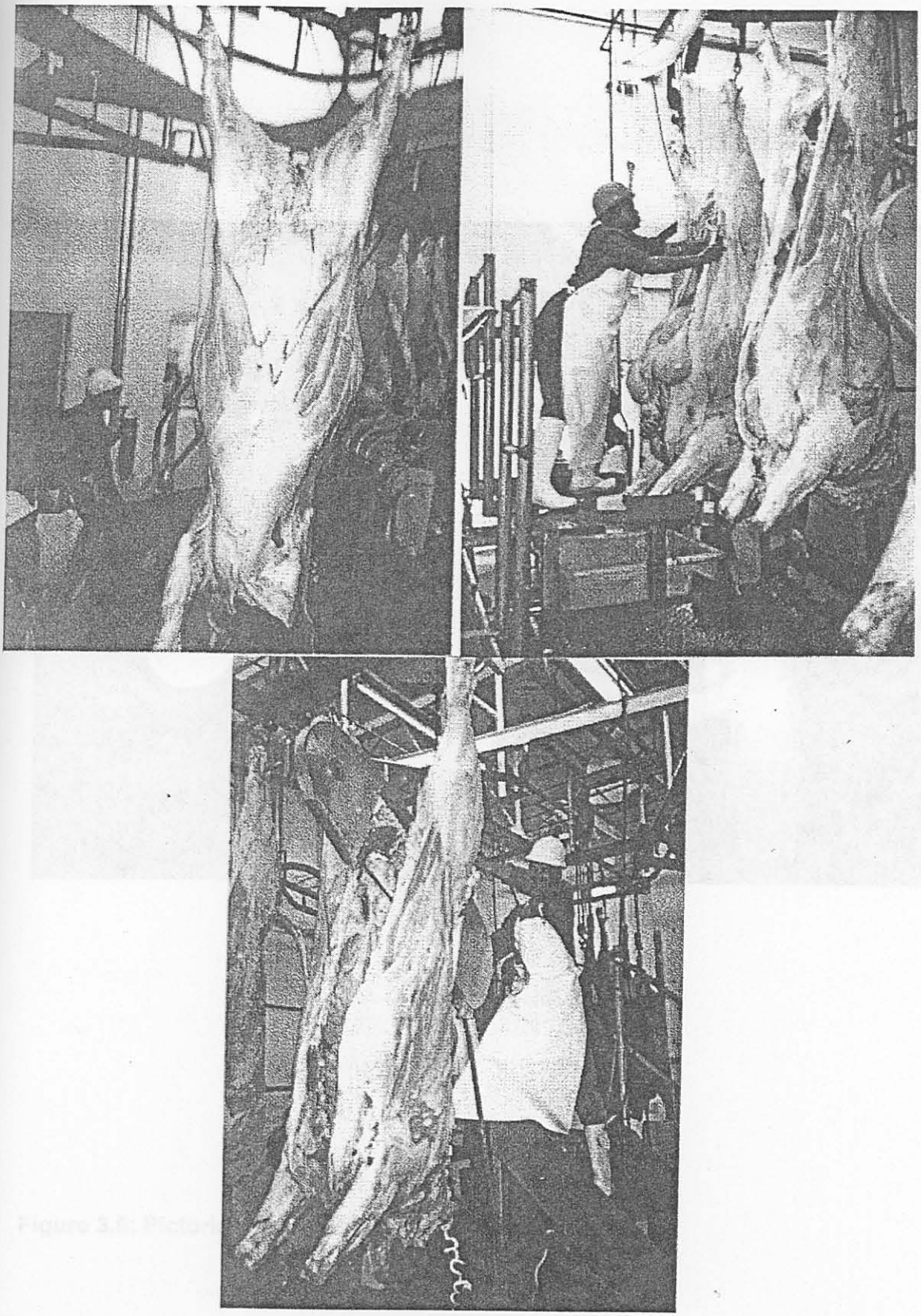

Figure 3.4: Pictorial presentation of the evisceration steps.

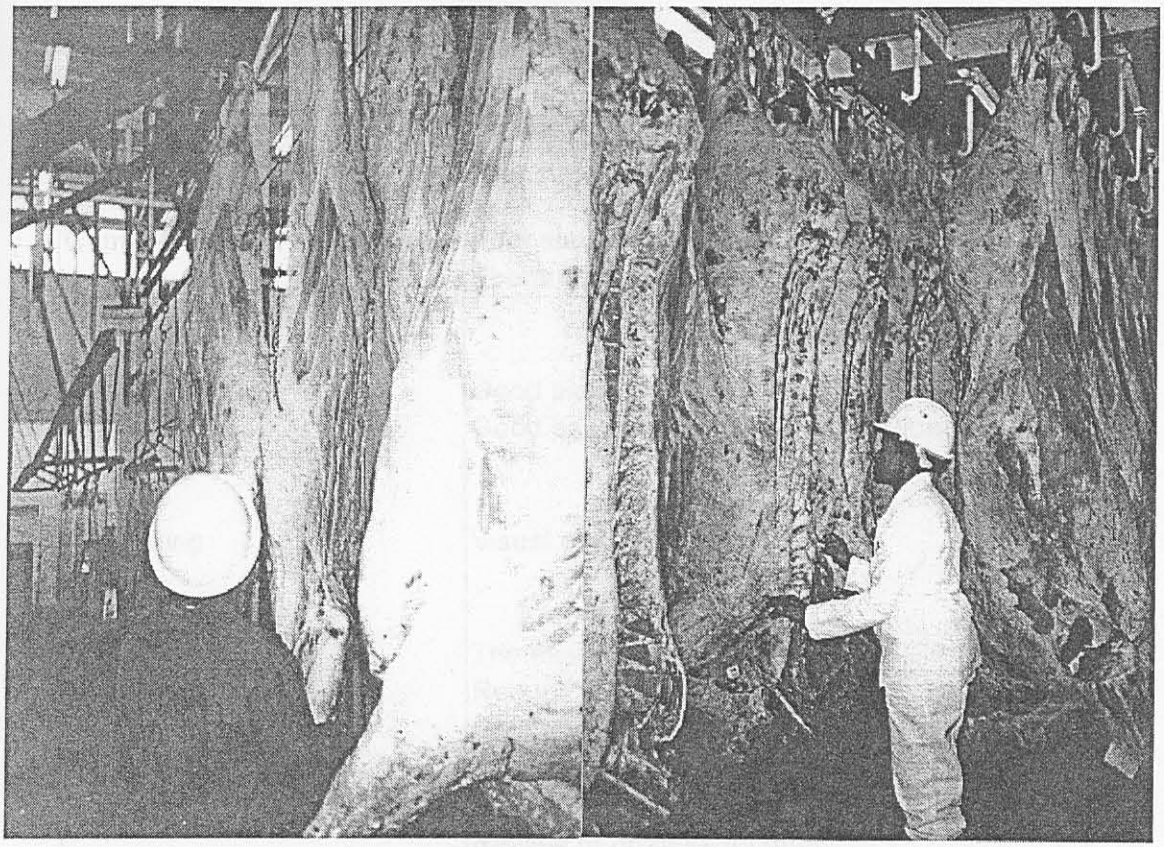

Figure 3.5: Pictorial presentation of the chilling step.

The critical control point work sheets for skinning, evisceration and chilling were prepared as indicated in Tables 3.1,3.2 and 3.3 respectively.

Table 3.1: Critical control point work sheet for the skinning step.

Process step	Skinning
Hazard	Contamination and growth of pathogens
Cause of hazard	Poor slaughtering techniques Poor sanitation of personnel and equipment
Control measure	Good slaughtering techniques Good sanitation of plant and equipment
Monitoring	Visual observation
Corrective action	Trimming Reduce chain speed GMP
verification	Microbiological analysis Review of process records
Records	Deviation/corrective action

Table 3.2: Critical control point worksheet for evisceration.

Process step	Evisceration
Hazard	Contamination and growth of pathogens
Cause of hazard	Poor slaughtering techniques-spilling of gut Poor sanitation of personnel and equipment
Control measure	Good slaughtering techniques Good sanitation of plant and equipment
Monitoring	Visual observation
Corrective action	Trimming Reduce chain speed GMP
Verification	Microbiological analysis Review of process control
Records	Deviation/corrective action

Table 3.3: Critical control point work sheet for the chilling step.

Process step	Chilling
Hazard	Growth of pathogens
Causes of hazards	Insufficient Time/Temperature maintenance
Control measures	Maintain temperature/time < 10oC within the first 24 hours
Monitoring	Periodic monitoring of carcasses and chill room temperature
Corrective action	Evaluate cause and prevent reoccurence Chill further
Verification	Periodic calibration of thermometer Review of temperature records Microbiological analysis
Records	Thermometer calibration log Deviation or corrective action

To be able to attain the targets for the CCPs, the RMAA trained the personnel on slaughtering techniques and the proper hygienic handling of the carcasses. The overall objective was to ensure that personnel were able to fully comprehend the "why" of hygiene to be able to fully respond to the "how" of hygiene and understand that hygiene and good housekeeping are a prerequisite for low microbial loads on the carcass.

3.2 MICROBIOLOGICAL ANALYSIS

Non destructive sampling (swabbing) was used as the study was carried out in a commercial abattoir where carcass excision is not practical as this affects the physical appearance of the carcass (Dorsa, Cutter & Siragusa, 1996). The nature of the swabbing method is such that the swab is rubbed on to the surface to collect the sample. The disadvantage with this method is that it does not always give precise results, as it cannot remove bacteria firmly attached to the surface (Benito, Pin, Marin, Garcia, Selgas & Casas, 1997). However, comparative studies done by Dorsa *et al.* (1996); Korsak *et al.* (1998) and Gill & Jones, (1998) on excision versus swab sampling showed that despite this disadvantage, swab sampling has more advantages than excision sampling. These includes not only maintaining the carcass surface intact but also saving on time and a considerably larger number of swabs can be examined with the same expenditure on cost.

Studies done by Untermann *et al.* (1997) also showed that there is a considerable variance of colony counts that occur within a carcass on the same day of slaughtering (section 3.3). This therefore makes this technique a practical method to examine a much more representative area of a single carcass than by excision of the carcass for hygiene monitoring of carcasses along the line. Untermann *et al.* (1997) further indicated that to get a useful trend on hygiene at least five to six swab samples from at least ten to fifteen carcasses would be necessary.

Jay (1992) indicated that for raw meat products potential safety and quality could be estimated with the use of indicator microorganisms including aerobic plate count, coliform count and *Escherichia coli* count. Coliform count and *Escherichia coli* count provide estimates of faecal contamination and poor sanitation during processing and has been recommended as appropriate for assessing the carcass dressing process for HACCP system purposes (Gill, Mcginnis, & Badoni, 1996).

3.2.1 Collection and treatment of samples

A total of 100 carcass sides were sampled from the plant throughout the study period. Of these, 50 carcasses formed the baseline data and the other 50 was sampled in the third phase of the study after training of the personnel. A total of 10 half carcasses were sampled per day (5 after splitting and 5 after chilling for 24 hours). The sampling was done on the carcasses within one hour of stunning after splitting and after 24 hours of chilling. It is important to sample the carcass soon after slaughtering, as bacteria tend to adhere to the carcass surface firmly with time. The ease and firmness with which the bacteria attach to the carcass play an important role in the sanitary quality of the meat. It should be noted that attachment is the first step in contamination and may lead to subsequent surface colonisation if conditions permit. The degree of attachment is important as it affects the swab sampling results (Benito *et al.,* 1997; Selgas *et al.*, 1993).

Samples were collected from four sites on the carcasses: the right or left brisket, right or left neck, the right or left fore leg and the right or left flank. These problem areas had the advantage of accessibility while the carcasses are on the line. The sampled points are illustrated in Figure 2.4 (Section 2.3.2.3). The choice of these sites as potential problem sites of carcasses during skinning and evisceration was based on observation during the first phase of the study (hygiene evaluation) in this particular abattoir. These sites compared well with studies by Bell (1997), Gill, Mcginnis & Bryant (1998); Gill *et al.* (1996); Korsak *et al.* (1998) and Untermann *et al.* (1997).

Sampling was done by swabbing a 10 cm^2 area of each of the four sites. A sterile gauze swab (10 x 10 cm^2 eight ply, curity gauze sponge: Johnson & Johnson, South Africa) which had been pre-moistened with 2% w/v peptone water was used (Dorsa *et al.*, 1996; Gill & Jones, 1998). The swab was rubbed horizontally and then vertically over the carcass surface (sterile gloves were worn over hands and changed between carcasses). A 100cm^2 sterile template was used to define the specific areas making a total of 400cm^2 per 1/2 carcass. The swab was then placed in a stomacher bag containing 10 ml of chilled 2% w/v peptone water. The samples were then placed in insulated containers and stored on ice while being transported

to the Council for Scientific and Industrial Research (CSIR) laboratories for microbial analysis within four hours.

3.2.2 Reagents and Materials

In the laboratory the swabs used for each carcass were pooled to form 40ml of the 2% w/v peptone water and then homogenised in a stomacher laboratory blender (Model 400 type BA 7021) for 2 minutes. A 1-ml portion of each homogenate was used to prepare 10-fold dilutions, to 10^3, in 2% w/v peptone water (Merck C134). The dilutions were spread on duplicate plates of various media except for *Salmonella* for which rapid method analysis was done for confirmation of either presence/absence. Conventional plate count analysis was carried out for the following bacteria: Total aerobic count, Total coliforms, *Escherichia coli, Clostridium perfringens,* and *Staphylococcus aureus.*

3.2.2.1 *Buffered peptone water*

Buffered peptone water (Merck C134) was used as a diluent.

The composition of the buffered peptone water used to achieve a 2% w/v of peptone water was as follows:

Peptone	10g
Sodium Chloride (NaCl)	5g
Disodium hydrogenorthophosphate dodecahydrate ($Na_2HPO_4.12H_2O$)	9g
Potassium dihydrogenorthophosphate (KH_2PO_4)	1.5g
Distilled water	1000ml

The buffer was prepared as follows:

The already premixed powder was dissolved in the distilled water by boiling. Then 10mls measured buffer quantities were prepared for swabbing as well as 9ml quantities for dilutions. The buffered peptone water was sterilised for 15 min at

121°C. The 10ml buffered peptone water was sterilised in Schott bottles whereas the 9ml buffered peptone water was sterilised in test tubes with aluminium caps. An Everlight vertical type autoclave TA-630 was used for the sterilisation of the buffer as well as the other media. The consecutive dilutions in the test tubes were made by diluting 1ml of each sample in 9ml sterile buffered peptone water.

Summaries of the dilutions are as follows:

2 swabs in 20 ml buffered peptone water	Homogenate
1ml in 9ml	(1/10 or 10^{-1})
1ml in 9ml	(1/100 or 10^{-2})
1ml in 9ml	(1/1000 or 10^{-3})

Every dilution was made after mixing of the sample in the test tube using a Vortex (Vortex mixer VM-300 from Gemmy Industrial Corporation).

3.2.2.2 *Aerobic plate count*

Nutrient agar (Merck C1) was used to determine the aerobic plate count of organisms in the samples. The medium was prepared as follows:

Nutrient agar (23g) was suspended in 1 litre of distilled water and brought to boil. This was done using a hot plate and stirred with a magnetic stirrer. The agar was then sterilised at 121°C for 15 min in sealed Schott bottles. The sterilised agar was left to cool to 50°C before plating.

Pour plates were made by mixing 1ml of 10^{-3} diluent sample with about 20ml of nutrient agar in sterile plastic petri dishes. These petri dishes were then incubated at 30°C for 48 hours before evaluation (Nortjé, Jordaan, Badenhorst, Goedhart, Holzapfel & Grimbeek (1990a). Incubation done at 30°C for 48 hours tended to yield higher counts of both the mesophiles and psychrotrophs (Nortjé, Nel, Jordaan, Naude, Goedhart, Holzapfel, & Grimbeeek, 1990b).

3.2.2.3 *Staphylococci species*

Baird Parker agar (Merck C41), a highly selective medium for the isolation and counting of coagulase-positive *staphylococci* was used.

The *Staphylococcus* species media was prepared according to the instructions by Merck. Baird-Parker agar base (63g) was suspended in 1 litre of distilled water and dissolved by boiling on a hot plate. Portions of 90 ml of the prepared agar were then dispensed into Schott bottles. The agar was sterilised at 121^{0}C for 15 minutes and cooled to about 50^{0}C.

At this stage the following were added:

i. 1ml of 1% sterile potassium tellurite (Bx15). This is a suspension of potassium-tellurite in distilled water and the ration is 0.5g: 49.5g respectively.

ii. 5ml 50% egg yolk emulsion (Bx14). This is a composition of 50ml-egg yolk added to 50ml sterile saline solution (0.85% NaCl in water)

The BX14 and BX15 were purchased already prepared.

The final medium contained Lithium Chloride and Tellurite, which inhibit the growth of accompanying microbial flora like coliforms, whereas pyrovate and glycine selectively stimulate the growth of *staphylococci*. *Staphylococci* show two characteristic features when grown on this opaque medium:

i. Characteristic opaque zones and clear rings outside the opaque zone are formed in the opaque medium as a result of the proteolysis (lecithin production) and lypolysis (lipase production) of the egg yolk in the medium, and

ii. Reduction of the tellurite to telluride by *Staphylococcus aureus* produces a black colouration of the colony.

Duplicate pour plates were made by surface plating the 0.2 ml of 10^{-2} diluent sample on the 20ml of the Baird-Parker agar in each sterile plastic petri dish. The plates were then incubated aerobically at 37^{0}C for 24hrs.

Staphylococcus aureus gave black, glossy, convex colonies (diameter 1-1.5 mm), with a narrow opaque margin surrounded by a clear zone, 2-5mm broad. Confirmation of the colonies was done using staphytect plus test DR 850 as follows:

1. The plates with the black glossy, convex colonies indicating presence of *Staphylococcus aureus* were tested.
2. A drop of the test latex was placed within one circle on the test card
3. A loop full of the colonies were removed and placed on the medium
4. The culture and the test latex was then mixed thoroughly and the test card gently rocked

Only agglutination, which was observed within a maximum of two minutes, was considered as positive presence of *Staphylococcus aureus.* Lack of agglutination indicates presence of other *Staphylococcus* species. Agglutination was an indication of the presence of clumping factor, protein A or MRSA capsular polysaccharides.

3.2.2.4 *Total coliforms*

Violet Red Bile agar (VRB) (Biolab CM107) was used for the enumeration of coliform organisms in the sample. The selectivity of the medium is due to the presence of bile salts and crystal violet. The presence of the coliform organisms is indicated by the red colour produced by the acid products of lactose fermentation. Lactose fermenters produce red or purple colonies often surrounded by a halo of the same colour.

The VRB agar was prepared as prescribed by Biolab (Code CM 107). VRB agar (40g) was suspended in 1 litre of water. It was then boiled until dissolved using a hot plate. The agar was then sterilised at 121^{0}C for 15 minutes in sealed Schott bottles. The sterilised agar was left to cool to 50^{0}C before using.

Duplicate pour plates were made by mixing the 1ml of 10^{-1} diluent sample with about 20 ml of the VRB agar in each sterile plastic petri dish. The plates were then incubated at 37^{0}C for 24hrs before evaluation.

3.2.2.5 *Escherichia coli*

Eosin Methylene Blue agar (Oxoid CM69) was used for the isolation of *Escherichia coli* in the samples.

The medium is made selective by the use of methylene blue and eosin, which in the presence of acid produced by *Escherichia coli* forms an amide bond giving the distinct metallic sheen. Other coliforms do not produce enough acid to cause this reaction. Eosin inhibits most gram-positive organisms.

The medium was prepared according to the method of Oxoid as follows: Eosin methylene blue agar (37.5g) was suspended in 1 litre of distilled water and brought to boil. This was done using a hot plate with a magnetic stirrer in place. The agar was then sterilised at 121°C for 15 minutes in sealed Schott bottles. The sterilised agar was left to cool to about 60°C. The media was then shaken in order to ensure uniform distribution of the flocculant, which is an essential part of this medium.

Duplicate pour plates were made by mixing the 1ml of 10^{-1} diluent sample with about 20ml of Eosin Methylene Blue in each sterile plastic petri dish. The plates were then incubated aerobically at 37^{0}C for 24hrs. Metallic green colonies were counted as positive *Escherichia coli* colonies.

3.2.2.6 *Clostridium perfringens,*

Perfringens, agar (Oxoid CM543) supplements type A (Oxoid SR076E) and type B (Oxoid SRO77E) was used for the cultivation and counting of *Clostridium perfringens.*

Being an anaerobic microorganism, the anaerobic conditions conducive for its growth was created by sealing the 90mm triple vented petri dishes with the activated Anaerocult A sachet (Merck 1.13827) moistened with water in an air tight jar. Anaerotest indicator (Merck 1.15112) was used to confirm anaerobic conditions. The blue reaction zone of the indicator was moistened, then introduced into the jar. Confirmation of the anaerobic environment was noted when the zone turned white. Anaerocult contains an oxygen-binding reagent mixture, which also produces carbondioxide.

The medium was prepared according to the instructions from Oxoid. Perfringens, agar (50g) was suspended in 500ml of distilled water and dissolved by bringing to boil on a hot plate. The agar was then sterilised at 121^{0}C for 15 minutes. It was then cooled to about 60^{0}C.

At this stage a vial of the following were added:

i. Perfringens (OPSP) Selective Supplement A (Oxoid SR076E)
ii. Perfringens (OPSP) Selective Supplement B (Oxoid SR077E)

A vial of Perfringens (OPSP) Selective Supplement A contains 50mg of sodium sulphadiazine, while a vial of Perfringens (OPSP) Selective Supplement B is composed of Oleondomycin Phosphate (0.25mg) and Polymyxin B sulphate (5000 I.U). To each of these vials 2 ml of sterile distilled water was aseptically added and mixed gently to dissolve. These were then added to 500ml of Perfringens agar base (OPSP) (CM543) mixed well and then let to cool to 50^{0}C. Duplicate pour plates were then made by mixing the 1ml of 10^{-1} diluent sample with about 20ml of the perfringens, agar in each 90ml sterile vented plastic petri dish. Once the petri dishes were dry they were overlayed with the agar. The plates were then incubated at 37^{0}C under anaerobic conditions (CO_2 atmosphere) for 18-24hrs.

3.2.2.7 *Salmonella*

Malthius analyzer (Malthius 2000 incubator) was used for the detection of *Salmonella*. The Malthius 2000 incubator is a bench mounted, water filled incubator

comprising control and scanning for the automatic electrical detection of microbial growth. The incubator is used in conjunction with a cooler unit, microcomputer and software, printer and Malthius cells. The full procedure is illustrated in Figure 3.6. The method uses a modification of Easter and Gibson's medium and Ogden's medium for the detection of *Salmonella* by conductance measurement (Easter & Gibson, 1985; Gibson, 1987; Ogden & Cann, 1987). It consists of the following stages:

- Pre-enrichment
- Growth and
- Confirmation

3.2.2.7.1 Pre-enrichment Broth (Glucose/Lysine Buffered Peptone Water)

Malthius *salmonella* Pre-enrichment Broth (30g; CM 509) was mixed well with 1 litre of distilled water according to the manufacturer's instruction. The broth (9ml quantities) was then dispensed into test tubes and then sterilised by autoclaving at 121°C for 15 minutes. Pre-enrichment broth is composed of:

Buffered peptone water		Biolab C134 Merck	20 g/l
L-Lysine	($C_6H_2ClN_2O_2$)	5700	5,0 g/l
D-glucose	($C_4H_{12}O_6$)	Univar 2676020	5,0 g/l

3.2.2.7.2 Growth medium

The detection mediums were prepared as follows:

Modified Easter and Gibson's method

Bacteriological peptone (Oxoid L37)	5.0g
Disodium hydrogen orthophosphate dihydrate ($Na_2HPO_4.2H_2O$) (Unilab 5043800)	10.0g
Dulcitol ($CH_2(OH).(CH.OH)_4.CH_2OH$) (Unilab 195600)	5.0g

Trimethylamine-N-oxide dihydrate ($C_3H_9NO.2H_2O$) (Sigma T-0514)	5.6g
Sodium Biselenite $C_6H_{12}N_2O_4S_2$ (Oxoid L121)	4.0g
L-cystine solution	10ml
Distilled water	1000ml
pH 7.2 ± 0.2	

Ogden's Lysine medium

Lactalbumin hydrolysate (Oxoid L48)	5.0g
D(+) glucose $C_6H_{12}0_6$ (BDH 10117)	10.0g
L-lysine monohydrochloride $C_6H_{15}ClN_2O_2$ (Sigma L5626)	10.0g
Sodium biselenite (Oxoid L121)	4.0g
L-cystine solution	10ml
Distilled water	1000ml
pH 7.0 ± 0.2	

All the ingredients of the specific media except the cystine solution were dissolved in water and heated. Overheating was avoided as this results in the production of an orange-red precipitate. The media is then cooled immediately and the cystine solution added to it. The cystine solution was prepared as follows: 0.1g L-cystine (Sigma C8755) was added to 15ml, 1 molar sodium hydroxide and diluted to 100ml with distilled water.

The Trimethylamine-N-oxide dihydrate (T.M.A.O) is important as the *Salmonella* reduces it to Trimethylamine (T.M.A) increasing the conductivity of the medium which can be detected and measured by the conductance/impedance Malthius equipment. During the incubation stage both positive and negative organism controls and negative media controls were included with each batch of sample.

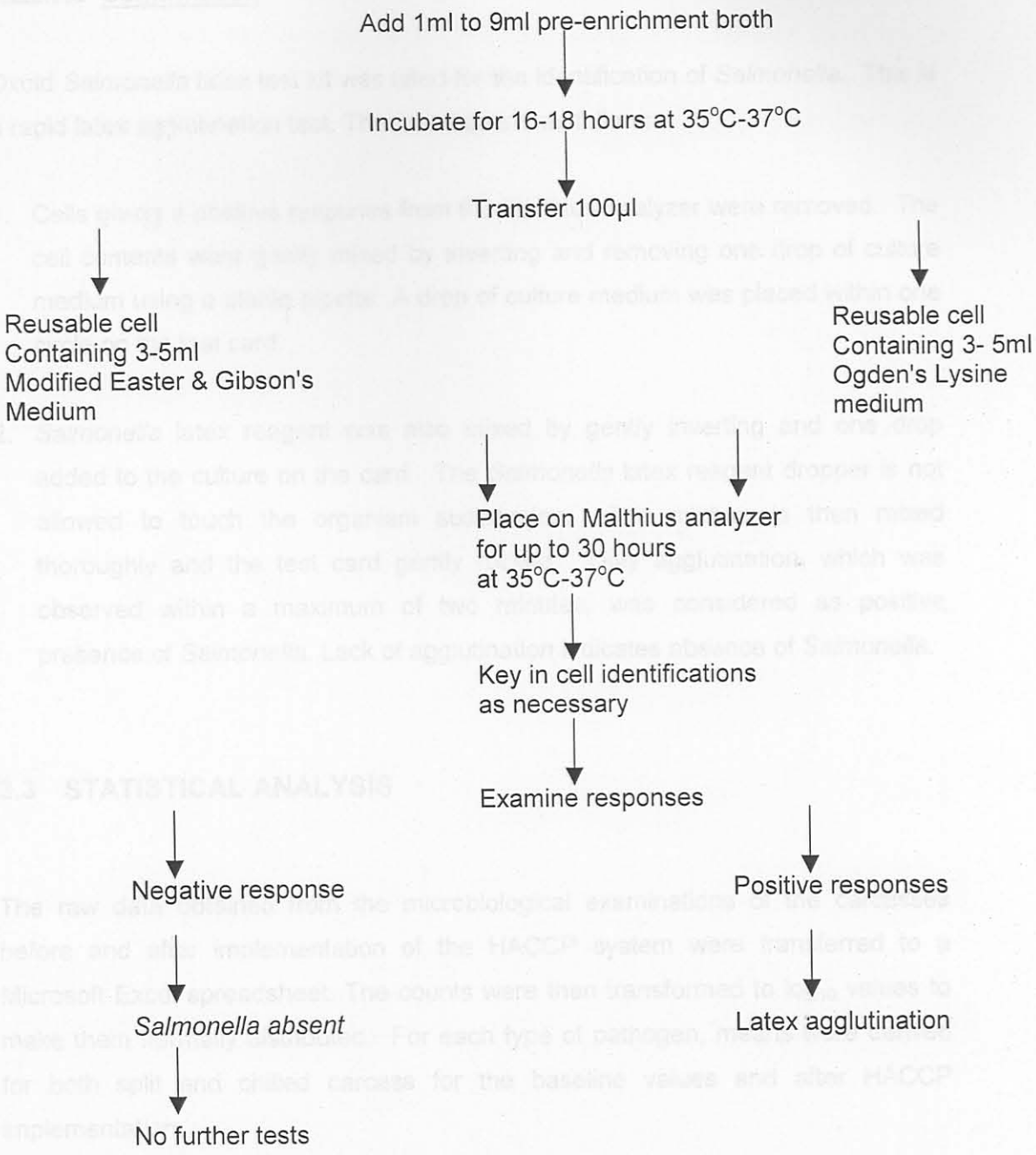

Figure 3.6: Malthius procedure for the detection of *Salmonella.*

3.2.2.7.3 Confirmation

Oxoid *Salmonella* latex test kit was used for the identification of *Salmonella*. This is a rapid latex agglutination test. The procedure is as follows:

1. Cells giving a positive response from the Malthius analyzer were removed. The cell contents were gently mixed by inverting and removing one drop of culture medium using a sterile pipette A drop of culture medium was placed within one circle on the test card.

2. *Salmonella* latex reagent was also mixed by gently inverting and one drop added to the culture on the card. The *Salmonella* latex reagent dropper is not allowed to touch the organism suspension. The mixture is then mixed thoroughly and the test card gently rocked. Only agglutination, which was observed within a maximum of two minutes, was considered as positive presence of *Salmonella*. Lack of agglutination indicates absence of *Salmonella*.

3.3 STATISTICAL ANALYSIS

The raw data obtained from the microbiological examinations of the carcasses before and after implementation of the HACCP system were transferred to a Microsoft Excel spreadsheet. The counts were then transformed to $\log_{10}$ values to make them normally distributed. For each type of pathogen, means were derived for both split and chilled carcass for the baseline values and after HACCP implementation.

A comparison of means for microbial populations aerobic plate count, total coliform count, *Escherichia coli* and *Staphylococcus aureus* was then done as follows:

- Between splitting and overnight chilling at baseline and after HACCP
- Before HACCP and after HACCP.

All comparisons were made using the Tukey's Honestly Significant Difference (HSD) test option at 5% level of significance using the Statistica programme (StaSoft, Inc., 1995) for windows. Statistical analysis -0.5 $\log_{10}$cfu/cm^2 was entered for samples in which *Escherichia coli* and C*lostridium perfringens,* was not detected (Gill, Badoni, Macginns, 1999; Gill & Jones, 1998).