MAURITIUS RESEARCH COUNCIL

ISOLATION AND CHARACTERISATION OF FOODBORNE BACTERIA

Final Report

Year 2012

MAURITIUS RESEARCH COUNCIL

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Isolation and Characterisation of Foodborne Bacteria

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Summary

Start date: Project officially approved March 2009

The official approval of the project was received in March 2009. A transfer of Rs 380 000 was made in April 2009 from MRC to the UoM account. A second transfer of Rs 93, 465 was made in November 2010. The rest was transferred in 2012 after submission of the report.

Ms Zaheera Kurreemun worked as part-time research assistant for two months, November and December 2009. She performed sampling and isolation from different sources. Mr Deepak Ramjeawon worked on the first activity.

March 2010- March 2011

Mr Deepak Ramjeawon left in May 2010 because of his other commitments in his employment.

Ms Shital Boodhoo appointed since June 2010. The contract was renewed in November 2010 and again in February 2011 until August 2011. A total of 15 months of appointment. Ms Boodhoo has been working full-time and made good progress since her appointment. A significant part of the sampling and laboratory work has been completed.

Ms Vidusha Tilhoo was appointed from September- December 2011. She left while the work was not yet complete.

Y Jaufeerally-Fakim and S Santchurn supervised the work of the research assistants.

Acknowledgements
The authors wish to express their kind thanks to the Mauritius Research Council for having funded this work and the University of Mauritius for the support in implementing the project.

We also wish to acknowledge the contribution of the technical staff of the molecular biology laboratory of the Faculty in particular Mrs N Sahebally and Mr Olivier Sheik Amamuddy as well as those in the finance section.

Abstract

Meat samples were collected from different suppliers in several districts of Mauritius between 2009 and 2011. They were brought to the laboratory for isolation and characterization.

ISO methods were used for the isolation of the bacterial colonies and this was followed by appropriate biochemical tests. About 40 % of the samples had confirmed Salmonella and about 77 % contained *Campylobacter jejuni*. Presumptive *E coli* was also widely detected in 55% of the samples.

All isolates were tested for their susceptibility to a battery of antibiotics.

PCR amplification was done to confirm the species and obtain products for sequencing. All sequences obtained so far are still being analysed.

Introduction

Salmonella, Campylobacter, Escherichia coli, and *Listeria* are among the most commonly encountered food-borne bacterial contaminants. Together they form a major public health burden, and the economic costs to the agricultural and food producing industries are very significant. Worldwide, zoonoses are responsible for various forms of human intestinal diseases. In the United States alone, food borne illnesses account for over **20 million** reported cases yearly and about 10 billion US dollars of medical care. Even countries with well established food safety management systems, have significantly high numbers of such cases. Occurrence of food-borne contaminants is more important in developing countries where the risks are higher mainly because of the fact that food safety systems are either not in place or the monitoring is not adequate. In many countries of the world, bacterial food-borne zoonotic infections are the most common cause of human intestinal disease. *Salmonella* and *Campylobacter* account for over **90%** of all reported cases of bacteria-related food poisoning world-wide

Setting up of appropriate control strategies requires precise information on the dissemination pathways of the microorganisms thus allowing the targeting of key areas. Surveillance mechanisms have become priority issues ever since the increasing concern for the risks of transfer of bacterial food contaminants from animal to humans. In many instances, it has been shown that there is an overlap between bacterial isolates from humans and those of veterinary sources, pointing to the latter as an important reservoir of pathogenic organisms. Presence of microorganisms in foods has been associated with food spoilage, economic losses, shortage of foods but also food borne disease. Hence, dietary guidelines and microbiological criteria, good manufacturing practices, food standards and legislation have been devised.

One of the fundamental objectives of food laws is to ensure food safety. The microbiological criteria give guidance on the acceptability of foodstuffs and their manufacturing handling and distribution processes. Foodstuffs should not contain micro-organisms or their toxins or metabolites in quantities that present an unacceptable risk for human health. The safety of foods is ensured through the adoption of good manufacturing and hygiene practices and application of processes based on hazard analysis and critical control point principles.

Food safety criteria have been established in order to define the acceptability of a marketable product. The Commission of the European Communities has established microbiological criteria for foodstuffs in November 2005 EC No. 2073/2005.

Reports on food borne contaminants are sparse in Mauritius. The data available in 2012 dates back to 2006 and reports of a number of 78 cases of food poisoning. The Food Technology Laboratory (FTL) of the Ministry of Agro Industry and Food Security, has the mandate to carry out the microbiological testing of foods, feeds and water. No report is available at the time of writing (i.e August 2012). Health inspectors play a crucial role in the monitoring of quality of food on sale in Mauritius. Samples are sent to for analysis and the Ministry of Health apply contraventions if the regulations have not been adhered to.

Export of food products require that strict norms are respected. This is particularly important in the fish sector in Mauritius. Many countries have seen their products banned entry into EU because of inadequate measures for ensuring microbial safety (Neeliah et al, 2011). Systems of control rely on efficient detection methods for microorganisms. The development of molecular approaches have brought a large array of techniques for, not only detecting, but also quantifying any known microbial organism which can be present in a food sample. They have revolutionised the screening and surveillance procedures and are presently widely used in many countries. Such systems are however costly to set up and monitor, but are of high resolution.

Polymerase chain reaction (PCR) detection has become the norm in bacterial screening and is more sensitive than other methods. Real-Time PCR (RT-PCR) has also replaced conventional PCR for a large number of applications in food safety as it provides a good indication of the level of contamination. RT-PCR relies on specific fluorescent labeling of primers and probes that can anneal to particular DNA sequences thus allowing the quantification of amplicon that is produced during the reaction. Such approach is particularly useful for microorganisms that are difficult to culture in the laboratory, such *Campylobacter jejuni*. (Chengbo et al. 2004).

For more accurate epidemiological studies, it is essential that strains of bacteria be typed so that their differences are highlighted. This can be achieved through the application of either antibody-based methods (serology) or DNA-based methods. Early DNA-typing methods have been replaced by Pulse-field gel-electrophoresis (PFGE). This is an approach that relies on the separation of large fragments of DNA that have been obtained through specific restriction enzyme digestion of the microbial DNA and comparison of the banding patterns. It is a type of DNA fingerprinting for bacteria. The Center for Disease Control (CDC) in the US has a database of such patterns for food borne bacteria that can be readily accessed (http://www.cdc.gov/pulsenet/). The objectives of this program are :

1. Rapid detection of food-borne disease cases by PFGE
2. Enable communication with health authorities both national and international

3. Track main source of contaminated food

The system has resulted in the tracking of contaminated nuts with *E coli* O175H7 because the samples from patients had a different DNA **PFGE** pattern and therefore was a new strain. The source of the nuts was immediately identified and further consumption prevented.

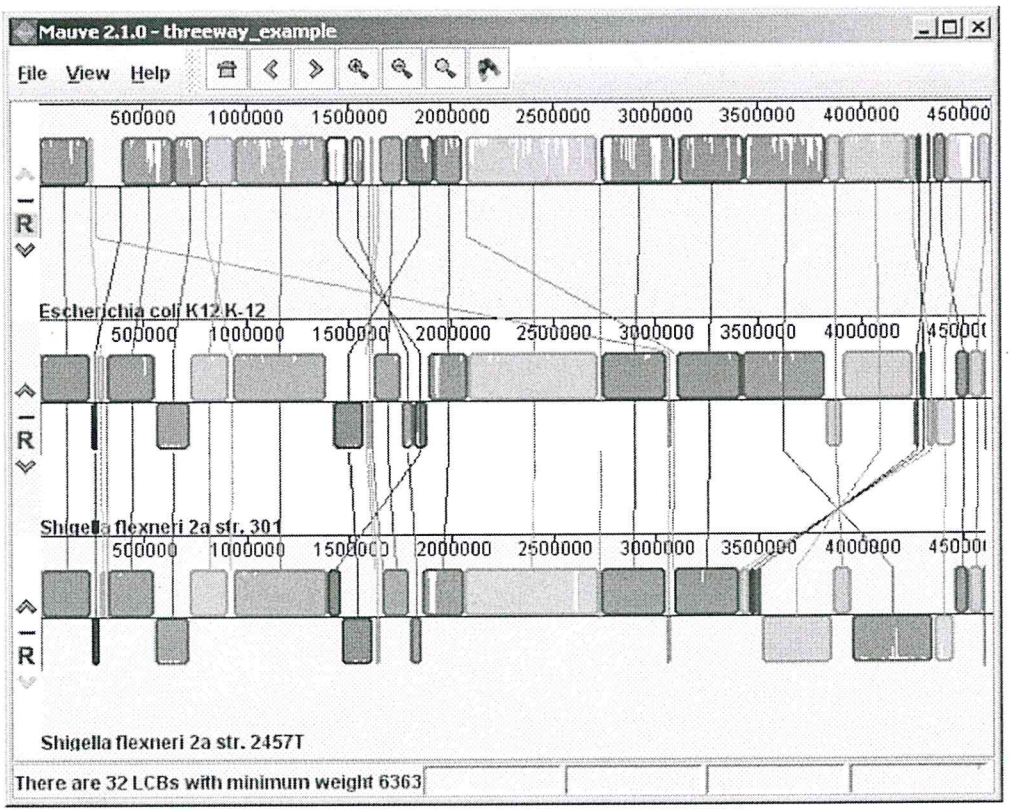
Other methods for molecular typing of bacterial strains include the multi-locus sequence type (MLST) and is obtained through the sequencing of a given set of genes. Each gene has several alleles, which are distinguished by different nucleotide sequences. **MLST** databases host all the alleles that have been reported and users submit their sequences for the identification of profiles for each gene. Seven housekeeping genes are the usual targets:

For Salmonella (Foley et al, 2006) : the genes are :glnA, manB, pduF, the 16S rRNA gene, pefB, hila and flmH

For Campylobacter (Miller et al, 2005) : the genes targeted are: adk; asp A; glnA; gltA; glyA; tkt; pgm;

MLST databases allow rapid profiles comparison and identification of strains. The resolution is more advanced than with other methods as the number and combination of alleles are high.

Recent advances in sequencing methods have also change the approach for typing with the advent of Next-Generation Sequencing. This has meant that bacterial genomes can be sequenced in a very short time and at low costs. Bioinformatics applications for whole genomes comparisons have followed and allow users to map regions which are different in strains of a given species. Artemis Comparison Tool (ACT) and MAUVE are two such tools for visualizing alignments of multiple bacterial genomes. The figure below indicates regions that are common in the three genome and are depicted by colour coded segments.



The work described here was directed towards a specific study of isolates of Salmonella, Campylobacter and *E coli* obtained from meat samples across Mauritius. The bacterial cultures were obtained through ISO methods and cultured in their appropriate media. Stock cultures were kept in aliquots at -80° C and thawed whenever required. DNA was extracted and kept at -20° C. The bacterial cultures were tested for their biochemical activities as per current procedures. The species identification was confirmed through specific PCR amplification using species specific primers.

Finally the genetic diversity was assessed through amplification and sequencing of selected genes used for MLST. Not all seven genes could be obtained or sequenced. The sequences were aligned and compared.

Aims and Objectives

The main aim of this project was to assess samples of meat for possible contamination by some common food-borne bacteria – *Salmonella spp* , *Campylobacter spp*, *Escherichia coli* at different levels of the food chain in Mauritius.

The specific objectives were:

1 Isolation of the bacterial contaminants from food samples on selective media
2 Morphological characterization and biochemical characterization of isolated bacteria and the serotyping using established procedures
3 Molecular typing using appropriate methods for a given organism (genome-wide and specific PCR)
4 Determination of DNA profiles and comparison with reference strains.
5 Assessing susceptibility to antimicrobials
6 Amplification and sequencing of genes responsible for antibiotic resistance.
7 Data analysis for understanding the nature and phylogenetic relationship of the isolated organisms.

Activities completed

The following activities, as specified in the initial proposal, have been performed.

1. Literature survey
2. Problem identification, survey and collection of samples
3. Purchase of consumables
4. Optimisation of methods for bacterial isolation and culture
5. Collection of samples
6. Isolation of *Salmonella, Campylobacter, E .coli* from meat and poultry originating from outlets
7. Biochemical and serological tests
8. Antibiotic sensitivity tests
9. Confirmation of Salmonella species by PCR

10. Isolation of presumptive *Campylobacter jejuni* from poultry using two methods
11. PCR testing for Salmonella using primers : MALO and OMPC
12. PCR testing for Campylobacter using primers: CJ F and CJ R (hipO gene)
13. Amplification of Salmonella with glnA, manB, pduF, the 16S rRNA
14. Amplification of Campylobacter DNA with adk; asp A; glnA; gltA; glyA; tkt.
15. Sequencing of amplicons from 13 and 14 above.
16. Editing of the sequences, assembly of contigs from forward and reverse sequences
17. Alignment of the sequences
18. Determination of phylogenetic tree

2. 0 Methodology

2.1.1 Isolation of bacterial isolates from meat samples

Samples were collected from different parts of the island according to the sampling plan (Appendix 1). They were brought to the lab before 12 noon and the procedure for the isolation started immediately. The protocols were according to the ISO standards. (Appendix 2 and 3).

Tables 1, 2 and 3 list the details of the samples used during the study. Samples of retail food products included raw and processed poultry products, chicken eggs, raw beef, mutton, goat, dear, pork meat from a total of 164 retail outlets of the nine districts of Mauritius during the period 29.03.2010 to 24.05.2011. Poultry feed was also analyzed. The sampling period covered both winter and summer seasons.

2.1.2 Biochemical tests

Biochemical tests are performed to determine what species of bacteria are on the plates. The results of the tests are then combined to give a biochemical profile which is associated with a given species.

Method used to isolate Campylobacter: ISO 10272-1:2006 and Simplate method (optional)

The ISO method includes an enrichment in Bolton Broth (incubation at 42°C for 48H under microaerophillic conditions) which is used to obtain information on absence/presence of campylobacter and is ideal for the detection of low numbers. The simplate method is an MPN method used to enumerate the number of campylobacter in a food sample. It has no enrichment step and is used to obtain a count of the number of campylobacter present per g of food sample. However it may not be ideal when dealing with low counts of campylobacter which might be reported as absence.

The steps below are common for both **ISO and Simplate methods**.

1. X g of food is measured using aseptic techniques and transferred to a stomacher bag/ 'Zip n Seal lock' bag.

2. 9X ml of BPW is measured and poured aseptically in the stomacher bag/ 'Zip n Seal lock' bag. This represents a 1:10 dilution and is known as the rinse method.
3. The stomacher bag is masticated in a stomacher for 1min. The 'Zip n Seal lock' bag should not be put in stomacher. All air should be excluded, then sealed and be masticated using the hands for about 5min and homogenised by frequent pressing. This is known as the **rinse method**.

After these steps, we can either choose ISO or Simplate method or both.

ISO 10272-1:2006 method (cont.)

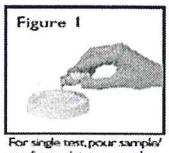
4. 20 ml (±2 ml) of the BPW masticated with the food sample is transferred to a 50ml centrifuge tube (Autoclavable Corning tubes) containing 20 ml of sterile 2X Bolton broth (with supplement).
5. The tube contents are mixed by inversion and when in an upright position, the caps are loosened slightly to be incubated in an anaerobic jar with CampyGen (under microaerophillic conditions) at 42 °C for 48H. The anaerobic jar containing the CampyGen is first placed in a refrigerator for 15min and then transferred to the incubator at 42°C since the reaction is very exothermic and the jar might explode if too much of heat is generated. The jar is always maintained in an upright position. Care is taken so as not to spill the tube contents after the caps are loosened before and after incubation.
6. After 48H, the mCCDA or Karmali plates on which the enrichment broth will be streaked must be dried inverted (agar top and plate cover on the plates) for 5-15min to remove excess moisture. Else, campylobacter colonies will be spread and no longer look as isolated colonies. Plates are labelled at the bottom.
7. The anaerobic jar is removed from incubator, opened and all caps of tubes are tightened.
8. Each enrichment broth is mixed by inversion before being opened and streaked on mCCDA or Karmali using aseptic techniques. Use a 1/3 streak method.
9. Plates are incubated as inverted in the anaerobic jar using CampyGen (under microaerophillic conditions) at 42 °C for 48H. Again, the jar with CampyGen

is first cooled in a refrigerator for 15min and then transferred to the incubator at 42°C.

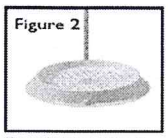
10. After 48H, campylobacter colonies appear as small 1-2mm grey/translucent isolated colonies on mCCDA or Karmali. **(Not white/cream!) Note:** Campylobacter dies very rapidly in normal atmospheric conditions at room temperature. They should be conserved in glycerol quickly to keep the isolate for long term storage or DNA extraction should be done quickly if only DNA is required.
11. 40 ml of the BPW masticated with the food sample is transferred to a sterile 50ml centrifuge tube (Autoclavable Corning tubes). This is referred to as the **food sample**.
12. The Simplate procedure is kept unchanged and is quoted below :

Test Procedure for homogenized food sample

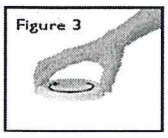
a) Aseptically resuspend the powdered medium (tube containing white powder is provided) with 9.5 mL of sterile deionized water. Add 0.5 mL of the food sample. This constitutes 1:2 dilution of sample.
b) Aseptically add 0.025 mL (25µl) of rifampicin additive solution and 0.040 mL (40µl) of hemin additive solution to each container using the 20-200µl pipettor. The final volume of liquid in the media/sample mixture container should be 10 ± 0.2 mL. Mix well by inversion.
c) Remove the lid from the SimPlate device and pour the sample/medium mixture onto the center of the plate. Immediately replace the lid. See Figure's 1-5 Below.
d) Gently swirl to distribute the sample/medium mixture into all the wells. The plate may be held with both hands and tilted slightly to help distribute the liquid into the wells.
e) If necessary, tap the SimPlate device GENTLY on a hard surface to remove any air bubbles which may have become trapped in the wells. Do not be concerned if partially filled wells are present. Wells containing partial volume of liquid will turn positive in the presence of viable *Campylobacter*.
f) Pour off excess medium by holding the lid against the plate on either side of the sponge cavity. Tip the plate toward you to allow liquid to drain into the sponge (Figure 5).
g) DO NOT invert the SimPlate device. Incubate in the dark for 48-52 h at 42°C in a microaerophilic (5% O_2, 10% CO_2, and 85% N_2) environment. We will use a Campy-Gen envelope to create the microaerophilic condition.



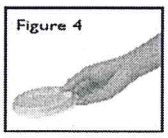
For single test, pour sample/ medium mixture onto the center of the plate.



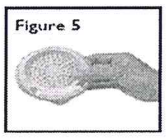
For multiple tests, pipette sample onto center of plate. Add rehydrated medium to make a final volume of 10 ± 0.2 mL.



Cover plate, gently swirl to distribute the sample into all of the wells.



Tap plate GENTLY on a hard surface to remove air bubbles.



Holding the cover, tip the plate toward you to allow liquid to drain.

Reading and Interpretation of Results

a) After incubation, observe for any red-colored liquid in the wells. Observe and record the background color of the wells. Background is defined as the color of the sample/medium mixture inside the wells.

b) Count all the red wells (the intensity of the color in the wells may vary). Some wells may be encircled by a red ring or contain red particles at the bottom of the well. This is acceptable. **All red wells** are presumptive positive for *Campylobacter*.

c) Count the number of red wells that fluoresce blue by holding UV light (366 nm wavelength) approximately 5 cm (2 inches) above the SimPlate device. Occasionally, there may be wells that are not red but fluoresce. DO NOT count these since they are not presumptive for *Campylobacter*. **NOTE:** Some meat samples can cause the medium to fluoresce slightly. If this occurs, repeat test with a lower sample volume or count only the fluorescent wells where the intensity of the fluorescent reaction significantly exceeds the background reaction.

d) To determine the total Campylobacter/gram, perform the following calculations:

1) Count the number of red wells on the plate.
2) Subtract the number of fluorescent (fluorescent wells are confirmed negative results) red wells from the total number of red wells to determine confirmed positive results for *Campylobacter*.
3) Use the SimPlate Conversion Table to determine the total number of microorganisms per plate.

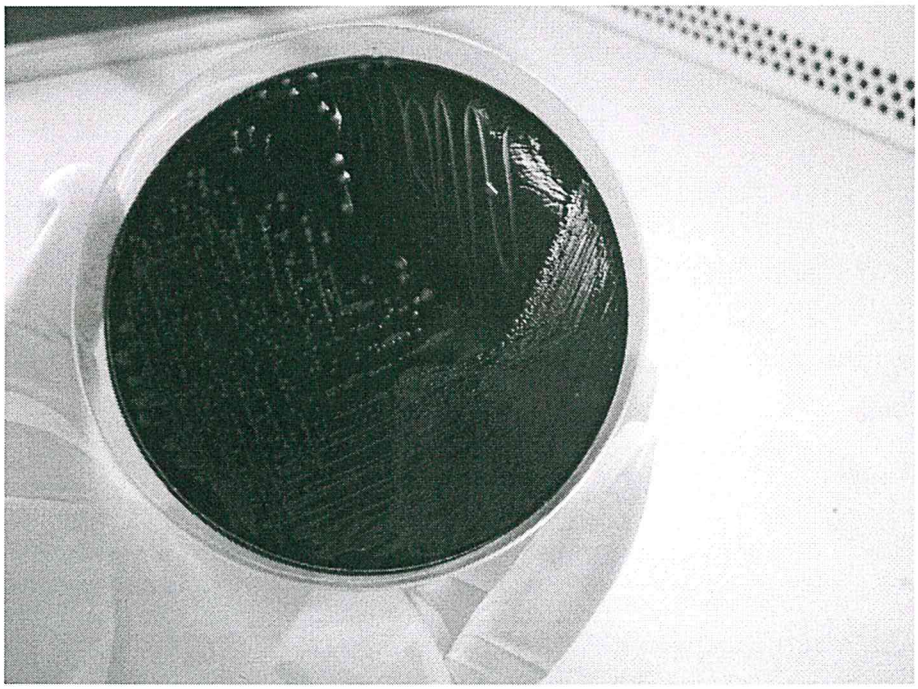
   Note: The SimPlate method does not allow colonies of Campylobacter to be obtained directly.

How to obtain colonies of Campylobacter from the Simplates

Once a non-fluorescent red well is obtained from the Simplate, these are marked and the suspected campylobacter from that well is streaked on mCCDA or Karmali plate. A fine loop (flamed until red hot and cooled) is immersed in the well and the red material at the bottom is carefully taken on the loop and streaked on the plate. The plates are incubated inverted at 42°C for 48H under microaerophillic conditions using

CampyGen. Same is done with another positive wells (if available) to get a duplicate colony of campylobacter from the 2nd well.

Again, campylobacter colonies appear as small 1-2mm grey/translucent isolated colonies on mCCDA or Karmali. (**Not white/cream!)**



Photos of Campylobacter spp. on Karmali plate taken in Microbiology lab at FOA, UOM, Mauritius.

Method used to isolate *Salmonella spp.*: ISO 6579-1:2002(E)

1. A portion of food sample (Xg) is diluted with 9X of Buffered Peptone Water (BPW), the pre-enrichment broth and incubated at 37°C for 24H.

2.(i) 1.0ml of the pre-enrichment broth is transferred to the enrichment broth Mueller Kauffman tetrationate (MKttn), vortexed and incubated at 37°C for 24H.

2.(ii) 0.1ml of the pre-enrichment broth is transferred to enrichment broth Rappaport Vassiliadis (RVS), vortexed and incubated at 41.5° C for 24H.

3. Both enrichment broths (MKttn and RVS) are then plated/ streaked on XLD and BGA and incubated at 37°C for 24H.

On BGA Salmonella colonies appear as pink colonies under a red medium. On XLD, they appear as red colonies with a black centre. These are presumptive Salmonella colonies.

Biochemical tests

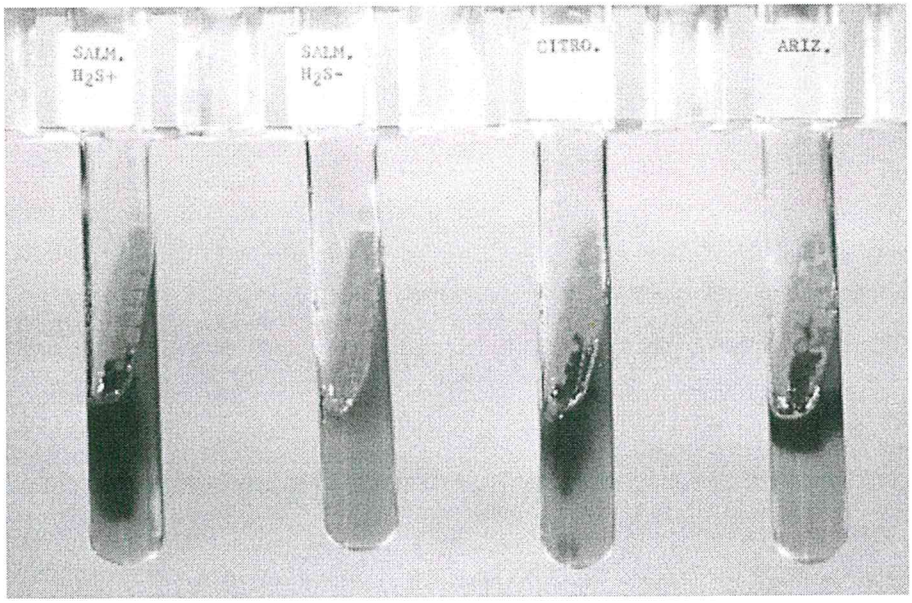
Biochemical tests that were done on presumptive Salmonella were: TSI (Triple Sugar Iron), Urease test (on Urea agar slant), L-Lysine, Indole test.

TSI (Triple Sugar Iron)

7 ml of pre-molten TSI (Triple Sugar Iron) agar is dispensed into test tubes with loosened caps and autoclaved at 121°C for 15 min. When removed and still hot, the caps are tightened and test tubes are inclined at about 10° (angle) to the table surface level to create the slants in the agar. When solidified, TSI slants are ready for use.

A presumptive colony of Salmonella is stabbed in the center of the butt of the agar and when it is removed, the agar slant surface is streaked. Test tubes are incubated at 37°C for 24H.

A typical Salmonella colony will give a yellow butt, black in the middle and red at the top of the slant. Can be presence of gas (disruption of agar). Note: yellow colour can be masked by black colour. Black colour can be absent if the Salmonella is H_2S negative.



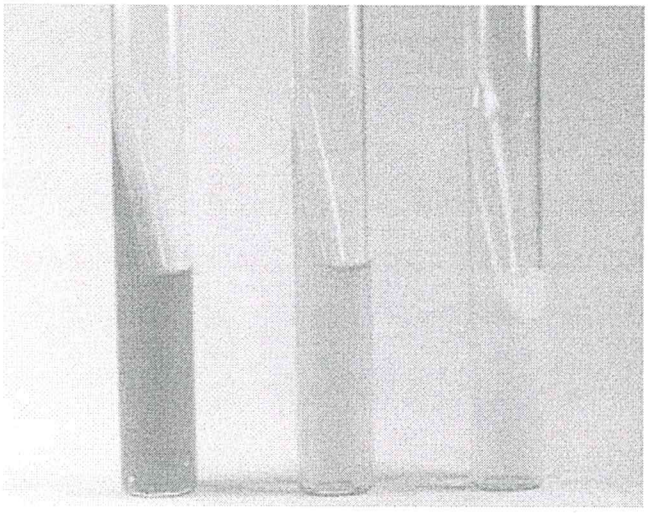
Various Salmonella reactions compared with C*itrobacter* and *Salmonella arizonae*.

Urease test

7 ml of pre-molten Urea agar is dispensed into test tubes with loosened caps and autoclaved at 115°C for 20 min. When removed and still hot, the caps are tightened and test tubes are inclined at about 10° (angle) to the table surface level to create the slants in the agar. When solidified, Urea agar slants are ready for use.

A presumptive colony of Salmonella is stabbed in the center of the butt of the urea agar and when it is removed, the agar slant surface is streaked. Test tubes are incubated at 37°C for 24H.

A typical Salmonella colony will give urease negative reaction, i.e a yellow/orange butt and slant. Positive reactions are bright pink/purple.



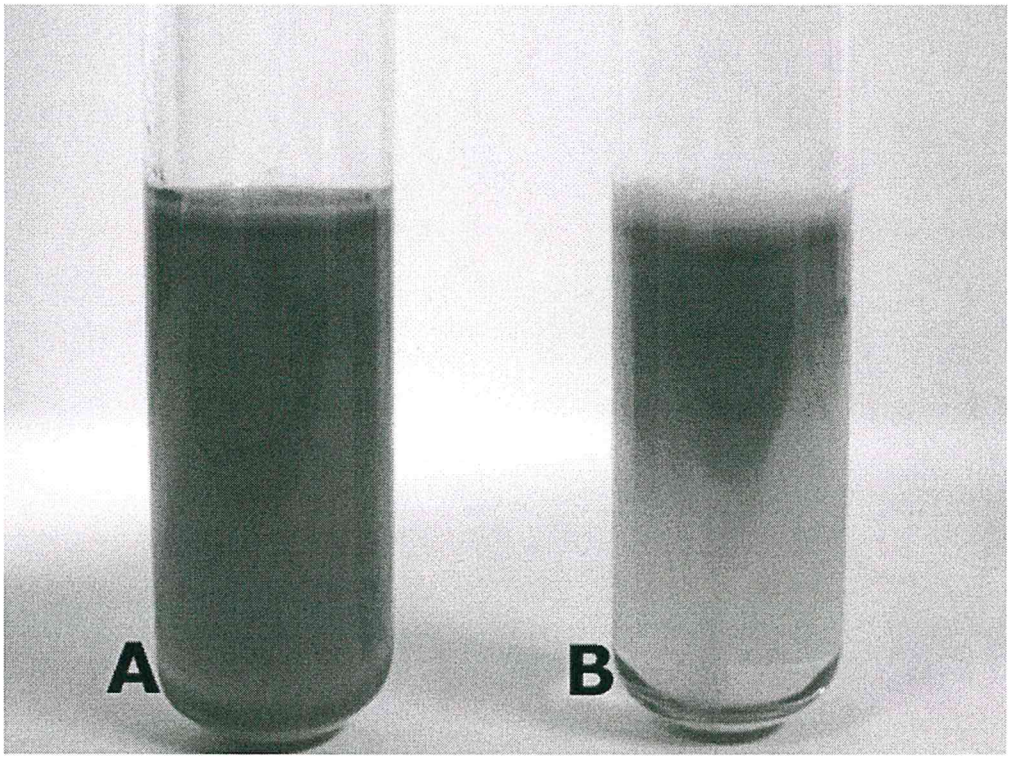
1st: Positive reaction. 2nd and 3rd : Negative reaction.

L-Lysine

3ml of L- Lysine decarboxylase broth is dispensed in test tubes with loosened caps and autoclaved at 121°C for 15 min. When cooled, the test tubes are ready for use.

A presumptive colony of Salmonella is taken with a loop and inoculated into the broth. The test tube cap is tightened, vortexed briefly and incubated at 37°C for 24H.

A typical Salmonella colony will give a positive lysine reaction, i.e a purple/grey purple colour reaction. A negative reaction is a yellow coloured broth.



A: positive reaction. B: negative reaction.

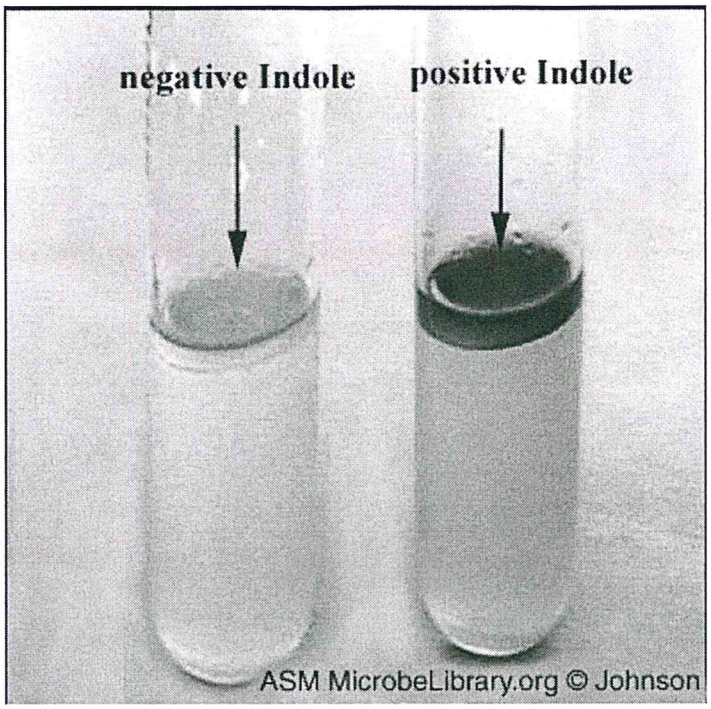
Indole test

3ml of Trytophan broth is dispensed in test tubes with loosened caps and autoclaved at 121°C for 15 min. When cooled, the test tubes are ready for use.

A presumptive colony of Salmonella is taken with a loop and inoculated into the broth. The test tube cap is tightened, vortexed briefly and incubated at 37°C for 24H.

1-2 drops of Kovac's reagent is added into the Trytophan broth. A presumptive colony of Salmonella will give a negative reaction: yellow ring with Kovac's reagent.

A positive reaction is a cherry-red ring with Kovac's reagent.



Positive (Right) and Negative indole reaction

These tests together with serological tests were used to confirm whether the isolates were *Salmonella spp.* or not. Polyvalent O Antisera, Polyvalent H Antisera and Polyphase 1&2 Antisera were used for serological testing to detect antigens O, H and 1&2. The level of agglutination was noted. (strong, medium, weak or none)

Method used to isolate *E.coli*: ISO 16649-1:2001(E)

A portion of food sample (Xg) is diluted with 9X of Buffered Peptone Water (BPW). 1.0ml of the BPW is spread homogeneously on a Mineral Modified Glutamate Agar (MMGA) plate containing a sterile 8.5mm cellulose membrane acetate paper and incubated at 37°C for 4H. The cellulose membrane acetate paper is then removed using sterile forceps onto a Tryptone Bile X-Glucuronide (TBGA) plate and incubated at 44°C for 24H. Characteristic colonies appear as blue/blue green colonies and then can be enumerated on a colony counter.

We use a chromogenic agar in the method used to isolate E.coli. Therefore no further biochemical test is required for confirmation. A characteristic blue colony is already confirmatory for E.coli.

DNA Extraction

DNA extraction of Salmonella, *E.coli* and Campylobacter have been carried out using *Zymo Research* Fungal/Bacterial DNA MiniPrep™ Extraction kit. All the isolates need to be suspended in broth or growing overnight in the broth after inoculation. The bacteria is pelleted in an eppendorf tube containing 1.5ml of inoculated broth in a centrifuge at 10 000 rpm for 4min. The supernatant is discarded. If the pellet size is too small, an additional 1.5ml of the inoculated broth is centrifuged again. The supernatant is discarded again and the pellet is resuspended in 200µl of sterile distilled water using a pipette.

The procedure for DNA extracted is followed as per the manual of instruction given in the DNA extraction kit, with only 1 amendment at the last step: The final volume of DNA elution buffer added is 75µl instead of 100µl. Note: Vortex was used to lyse the bacterial cells for 10min since Disruptor Genie was not available.

Bacterial DNA is conserved at -20°C in 1.5ml eppendorf tubes.

Long term storage of cultures in Glycerol.

All isolates of Salmonella, *E.coli* and Campylobacter are conserved for long term storage in glycerol at -80 °C in 1.5ml eppendorf tubes.

For *E.coli* and Salmonella, the broth used was Nutrient broth and the final percentage of glycerol used was 15%.

For Campylobacter, the broth used was Brucella broth and the final percentage of glycerol used was 20%.

Note: The eppendorf tubes should **not** be thawed. For a re culture, a sterile needle is used to scrap off some of the frozen culture from the eppendorf and it is placed on the agar (Nutrient agar for *E.coli* and Salmonella; Karmali for Campylobacter). When the ice melts, the agar/plate is streaked and incubated.

Note: The viability of the Campylobacter isolates are for about 1 year only.

RESULTS

A. Salmonella and *E coli* Isolation

Only fresh meat and mainly chicken products were sampled and isolation rates in fresh poultry are very high according to literature.

102 samples were taken from various locations (Table 2) and 40.1 % were positive for *Salmonella.* Most of the salmonella positive samples (60%) were from poultry products. The others were from beef, goat, mutton, fish, deer, and pork products. None of the poultry feed samples analyzed tested positive for Salmonella.

102 samples were taken from various locations (Table 1) and 55% were positive for *E.coli.* Most of the *E.coli* positive samples were from poultry products. The others were from beef, goat, mutton, fish, deer, and pork products. None of the poultry feed samples analyzed tested positive for *E.coli*.

Table 2. Sampling for Salmonella and *E coli*.

Source	Date collected	District	Site
Beef meat	29.03.10	Plaine-Wilhems	Rose-Hill market & supermarket
Beef burger	28.09.10	MMA	Abbattoir
Beef burger	14.09.10	Flacq	Winners Flacq
Beef meat	27.07.10	Port Louis	Port Louis market
Beef meat	03.08.10	Plaine-Wilhems	Curepipe market
Beef meat	12.10.10	Rose-Hill	Rose-Hill market
Beef meat	12.10.10	Quatre Bornes	Quatre Bornes market
Beef meat	26.04.10	Port Louis	Port Louis market & supermarket
Beef meat	21.09.10	MMA	Abbattoir
Beef meat	28.09.10	MMA	Abbattoir
Chicken BBQ sausage	17.08.10	Triolet	Winners Triolet & Chantefrais Triolet
Chicken burger	12.04.10	Plaine-Wilhems	Rose-Hill market & supermarket
Chicken burger	10.08.11	Moka	Winners St Pierre & Chantefrais Moka
Chicken egg	21.06.10	Riviere du Rempart village	Meat shop & Chantefrais
Chicken Ham	07.06.10	Gentilly, St Pierre	St Pierre Chantefrais & supermarket
Chicken ham	28.06.11	Flacq + Medine	Chantefrais, Winners
Chicken ham	06.07.11	Black River	Chicken meat shop, Winners Bambous, Medine mar
Chicken ham	20.07.10	Grand Port	Rose Belle Winners, meat shop & Chantefrais
Chicken ham	14.09.10	Flacq	Winners Flacq

Chicken ham	05.10.11	Grand Port	Winners Rose Belle
Chicken ham with olives	31.08.12	Black River	Winner's Bambous
Chicken Liver	14.07.10	Tyack, Riviere des Anguilles, Grand Bois	Chantefrais Tyack & Riviere des Anguilles & Grand Bois
Chicken liver	20.07.10	Grand Port	Rose Belle Winners, meat shop & Chantefrais
Chicken Liver	10.08.10	Moka	Winners St Pierre & Chantefrais Moka
Chicken Liver	31.08.10	Black River	Winner's Bambous
Chicken Liver	14.09.10	Flacq	Winners Flacq
Chicken Liver	05.10.10	Grand Port	Winners Rose Belle
Chicken Liver	17.08.10	Triolet	Winners Triolet & Chantefrais Triolet
Chicken Liver	24.08.10	Riviere du Rempart village	Carmen meat shop & Chantefrais Rempart
Chicken Liver	05.10.10	Riviere des Anguilles	Chantefrais Riviere des Anguilles
Chicken meat	29.03.10	Plaine-Wilhems	Rose-Hill market & supermarket
Chicken meat	21.06.10	Riviere du Rempart village	Meat shop & Chantefrais
Chicken meat	28.06.10	Flacq, Medine	Chantefrais, Winners
Chicken meat	06.07.10	Black River	Chicken meat shop, Winners Bambous, Medine mar
Chicken meat	14.07.10	Tyack, Riviere des Anguilles, Grand Bois	Chantefrais Tyack & Riviere des Anguilles & Grand Bois
Chicken meat	20.07.10	Grand Port	Rose Belle Winners, meat shop & Chantefrais
Chicken meat	27.07.10	Port Louis	Port Louis market
Chicken meat	03.08.10	Plaine-Wilhems	Curepipe market
Chicken meat	05.10.10	Riviere des Anguilles	Chantefrais Riviere des Anguilles
Chicken meat	12.10.10	Rose-Hill	Rose-Hill market
Chicken meat	12.10.10	Quatre Bornes	Quatre Bornes market
Chicken meat	09.11.10	Beau Climat	Innodis Abbattoir
Chicken meat	09.11.10	Beau Climat	Innodis Abbattoir
Chicken meat	09.11.10	Beau Climat	Innodis Abbattoir
Chicken meat	09.11.10	Beau Climat	Innodis Abbattoir
Chicken meat	07.06.10	Gentilly, St Pierre	St Pierre Chantefrais & supermarket
Chicken meat	14.06.10	Pamplemousses	Chantefrais Triolet
Chicken meat	10.08.10	Moka	Winners St Pierre & Chantefrais Moka
Chicken meat	17.08.10	Triolet	Winners Triolet & Chantefrais Triolet
Chicken meat	24.08.10	Riviere du Rempart village	Carmen meat shop & Chantefrais Rempart
Chicken meat	31.08.10	Black River	Winner's Bambous
Chicken meat	14.09.10	Flacq	Winners Flacq
Chicken meat	05.10.10	Grand Port	Winners Rose Belle
Chicken meat	09.11.10	Beau Climat	Innodis Abbattoir
Chicken meat (raw)	26.04.10	Port Louis	Port Louis market & supermarket
Chicken sausage	07.06.10	Gentilly, St Pierre	St Pierre Chantefrais & supermarket
Chicken sausage	14.06.11	Pamplemousses	Chantefrais Triolet
Chicken sausage	21.06.11	Riviere du Rempart village	Meat shop & Chantefrais
Chicken sausage	10.08.10	Moka	Winners St Pierre & Chantefrais Moka
Chicken sausage	17.08.11	Triolet	Winners Triolet & Chantefrais Triolet
Chicken sausage	24.08.10	Riviere du Rempart village	Carmen meat shop & Chantefrais Rempart
Croquette poulet raw	05.10.10	Grand Port	Winners Rose Belle
Deer meat	03.08.10	Plaine-Wilhems	Curepipe market

Deer meat	20.07.10	Grand Port	Rose Belle Winners, meat shop & Chantefrais
Egg	12.04.10	Plaine-Wilhems	Rose-Hill market & supermarket
Egg	26.04.10	Port Louis	Port Louis market & supermarket
Egg	14.06.10	Pamplemousses	Chantefrais Triolet
Egg	28.06.10	Flacq + Medine	Chantefrais, Winners
Egg	06.07.10	Black River	Chicken meat shop, Winners Bambous, Medine mar
Egg	14.07.10	Tyack, Riviere des Anguilles, Grand Bois	Chantefrais Tyack & Riviere des Anguilles & Granc Bois
Egg	20.07.10	Grand Port	Rose Belle Winners, meat shop & Chantefrais
Egg	27.07.10	Port Louis	Port Louis market
Egg	03.08.10	Plaine-Wilhems	Curepipe market
Egg	24.08.11	Riviere du Rempart village	Carmen meat shop & Chantefrais Rempart
Egg	05.10.10	Riviere des Anguilles	Chantefrais Riviere des Anguilles
Egg	12.10.10	Quatre Bornes	Quatre Bornes market
Fish	26.04.10	Port Louis	Port Louis market & supermarket
Fish	06.07.12	Black River	Chicken meat shop, Winners Bambous, Medine mar
Fish (cateau)	12.04.10	Plaine-Wilhems	Rose-Hill market & supermarket
Goat meat	27.07.10	Port Louis	Port Louis market
Goat meat	21.09.10	MMA	Abbattoir
Goat meat	28.09.10	MMA	Abbattoir
Goat meat	28.09.10	MMA	Abbattoir
Goat meat	21.09.10	MMA	Abbattoir
mutton meat	29.03.10	Plaine-Wilhems	Rose-Hill market & supermarket
Mutton meat	28.09.10	MMA	Abbattoir
Pork meat	27.07.10	Port Louis	Port Louis market
Pork meat	21.09.10	MMA	Abbattoir
Pork meat	31.08.13	Black River	Winner's Bambous
Pork meat	28.09.10	MMA	Abbattoir
Pork meat	28.09.10	MMA	Abbattoir
Poultry feed	12.04.10	Plaine-Wilhems	Rose-Hill market & supermarket
Poultry feed	26.04.10	Port Louis	Port Louis market & supermarket
Poultry feed	09.11.10	Beau Climat	Innodis Abbattoir
Poultry feed	09.11.11	Beau Climat	Innodis Abbattoir
Poultry feed	09.11.12	Beau Climat	Innodis Abbattoir
Poultry feed	09.11.13	Beau Climat	Innodis Abbattoir
Poultry feed	09.11.14	Beau Climat	Innodis Abbattoir
Process chicken meat	26.04.10	Port Louis	Port Louis market & supermarket
Roti de boeuf	10.08.10	Moka	Winners St Pierre & Chantefrais Moka
Roulade de poulet	17.08.11	Triolet	Winners Triolet & Chantefrais Triolet

B. Campylobacter spp isolation

Campylobacter species are quite susceptible to growth conditions and therefore difficult to isolate compared to Salmonella and *E coli*. Two methods are being used for this purpose, namely the ISO method and a SIM Plate method. The SIM plate

allows for the detection and quatification of Campylobacter. Prepared samples are dispensed into the plates which have wells and allowed to incubate at 42° C for 48 hours in a microaerophilic environment (low oxygen). Those wells that turn red are counted. A UV light is then used to assess for fluorescence. The wells that are red and do not fluoresce are presumptive Campylobacter spp. The ISO method starts with an enrichment step whereby low numbers can be detected.

63 food samples were taken from various locations (Table 3) and 77% were positive for *Campylobacter*. Most of the *Campylobacter* positive samples were from raw poultry and meat products.

Number of isolates: 13 isolates were obtained from the poultry samples while 13 others from street foods. These have yet to be tested by PCR.

No.	***Sampling region***		***Results***		
	District	***Location***	***Salmonella***	***E. coli***	***Campylobacter***
1*	Plaine Wilhems	Rose-Hill	**Suspected presence in beef, chicken and mutton meat**	**Confirmed presence in beef, chicken and mutton meat**	NA
2*	Plaine Wilhems	Rose-Hill	Absence of *Salmonella spp.*	**Confirmed presence in fish and chicken burger**	NA
3*	Port-Louis	Port-Louis	**Suspected presence in chicken meat and fish**	**Confirmed presence in chicken, beef meat and fish**	NA
4	Moka	St Pierre	Absence of *Salmonella spp.*	**Confirmed presence in chicken meat and chicken ham**	NA
5	Pamplemousses	Triolet	Absence of *Salmonella spp.*	**Confirmed presence in chicken meat**	NA
6	Riviere du Rempart	Riviere du Rempart	**Suspected presence in chicken meat**	**Confirmed presence in chicken meat**	NA
7	Flacq	Medine+ Flacq	Absence of *Salmonella spp.*	**Confirmed presence in chicken meat**	NA
8	Black River	Bambous	**Suspected presence**	**Confirmed presence**	NA

			in chicken meat	**in chicken meat**	
9	Savanne	Riviere des Anguilles, Tyack, Grand Bois	**Suspected presence in chicken meat and chicken liver**	**Confirmed presence in chicken meat and chicken liver**	NA
10	Grand Port	Rose-Belle	**Suspected presence in chicken meat and chicken liver**	**Confirmed presence in chicken meat, chicken liver and deer meat**	NA
11	Port Louis	Port-Louis market	**Confirmed presence in beef meat and pork meat. Suspected presence in chicken meat and goat meat**	**Confirmed presence in chicken meat, beef meat and pork meat**	NA
12	Plaine wilhems	Curepipe market	**Confirmed presence in chicken meat, beef meat and deer meat**	**Confirmed presence in chicken meat and deer meat**	NA
13	Moka	Moka, St Pierre	**Confirmed presence in chicken liver**	**Confirmed presence in chicken meat, chicken liver and chicken burger**	NA
14	Pamplemousses	Triolet	Absence of *Salmonella spp.*	**Confirmed presence in chicken meat, chicken liver and chicken BBQ sausage**	NA
15	Riviere du Rempart	Riviere du Rempart	Absence of *Salmonella spp.*	**Confirmed presence in chicken meat, chicken liver and chicken sausage**	NA
16	Black River	Bambous	**Confirmed presence in chicken liver**	**Confirmed presence in chicken meat and chicken liver**	NA
17	Flacq		**Confirmed presence in chicken**	**Confirmed presence in chicken meat,**	NA

			liver	**chicken liver and beef burger**	
18	Savanne		**Confirmed presence in chicken meat**	**Confirmed presence in chicken meat and chicken liver**	NA
19	Grand Port		**Confirmed presence in croquette de poulet(raw)**	**Confirmed presence in chicken meat, chicken liver and croquette de poulet(raw)**	NA
20	Abbatoir Roche bois	MMA	**Confirmed presence in goat meat and pork meat**	**Confirmed presence in goat meat,beef meat and pork meat**	NA
21	Abbatoir Roche bois	MMA	**Confirmed presence in goat meat, beef meat and mutton meat**	**Confirmed presence in goat meat, pork meat and mutton meat**	NA
22	Quatre Bornes	Market	**Confirmed presence in chicken meat and beef meat**	**Confirmed presence in chicken meat and beef meat**	NA
23	Rose-Hill	Market	**Confirmed presence in beef meat**	**Confirmed presence in chicken meat and beef meat**	NA
24	Beau Climat		**Confirmed presence in 4 out of 5 chicken samples**	**Confirmed presence in all 5 samples of chicken meat**	NA

Between 01 June 2010 to Aug 2011, 92 samples were analysed.

Presumptive positive salmonella	% positive out of 92	Presumptive positive E.coli	% positive out of 92
29 samples	31.5%	27 samples	58.7 %

At least 1 isolate (max 3) of salmonella were obtained from each positive sample tested as salmonella positive.

Generally, 2 isolates of *E.coli* were obtained from each positive sample tested as *E.coli* positive, except when only 1 colony of *E.coli* was obtained.

Results of Campylobacter spp. Isolation

Table 3. Food samples used for isolation of *Campylobacter*

Source	Date collected	District	Site
Alouda	07.03.11		
Beef ham Ile de France	07.03.11	Plaine Wilhems	Trianon Shoprite
Beef meat	05.04.11	Plaine Wilhems	Curepipe market
Beef meat	24.05.11	Port Louis	Port Louis market
Blanc de poulet	10.05.11	Pamplemousses	Chantefrais & Winners, Triolet
Blanc de poulet Dore	10.05.11	Plaine Wilhems	Jumbo, Phoenix
Chicken	07.03.11		
Chicken	07.03.11		
Chicken briani	07.03.11		
Chicken brochette	07.03.11		
Chicken brochette lemon herb	15.03.11	Plaine Wilhems	Trianon Shoprite
Chicken brochette sweet chilli	15.03.11	Plaine Wilhems	Trianon Shoprite
Chicken croquettes raw	22.03.11	Grand Port	Winner's Rose-Belle
Chicken Gesier	29.03.11	Black River	Winner's Bambous, Chantefrais Bambous
Chicken grillade	07.03.11		
Chicken grilled	07.03.11		
Chicken ham	07.03.11	Phoenix	Jumbo
Chicken ham St Michel	07.03.11	Plaine Wilhems	Trainon Shoprite
Chicken kebab	07.03.11		
Chicken kebab spicy roasted	07.03.11		
Chicken liver	08.03.11	Plaine Wilhems	Way Rose-Hill
Chicken liver	15.03.11	Plaine Wilhems	Trianon Shoprite
Chicken liver	22.03.11	Grand Port	Winner's Rose-Belle
Chicken liver	05.04.11	Plaine Wilhems	Curepipe market
Chicken liver	12.03.11	Riviere du Rempart	Riviere du Rempart
Chicken liver	17.05.11	Savanne	Riviere des Anguilles Chantefrais
Chicken liver	24.05.11	Port Louis	Port Louis market
Chicken Mortadella		Phoenix	Jumbo

Chicken Mortadella + olives Chiko		Plaine Wilhems	Trainon Shoprite
Chicken Mortadella St Michel		Plaine Wilhems	Trainon Shoprite
Chicken sausage	01.03.11	Moka	Chantefrais Moka
Chicken thigh	01.03.11	Moka	Chantefrais Moka
Chicken thigh	08.03.11	Plaine Wilhems	Way Rose-Hill
Chicken thigh	15.03.11	Plaine Wilhems	Trianon Shoprite
Chicken thigh	22.03.11	Grand Port	Winner's Rose-Belle
Chicken thigh	22.03.11	Grand Port	Winner's Rose-Belle
Chicken thigh	29.03.11	Black River	Winner's Bambous, Chantefrais Bambous
Chicken thigh	29.03.11	Black River	Winner's Bambous, Chantefrais Bambous
Chicken thigh	05.04.11	Plaine Wilhems	Curepipe market
Chicken thigh	05.04.11	Plaine Wilhems	Curepipe market
Chicken thigh	12.03.11	Riviere du Rempart	Riviere du Rempart
Chicken thigh	12.03.11	Riviere du Rempart	Riviere du Rempart
Chicken thigh	26.04.11	Flacq	Flacq Winners
Chicken thigh	10.05.11	Triolet	Chantefrais & Winners
Chicken thigh	17.05.11	Savanne	Riviere des Anguilles Chantefrais
Chicken thigh	24.05.11	Port Louis	Port Louis market
Chicken tikka	07.03.11		
Chicken tikka	07.03.11		
Chicken wing	01.03.11	Moka	Chantefrais Moka
Chicken wing	15.03.11	Plaine Wilhems	Trainon Shoprite
Chicken wing	22.03.11	Grand Port	Winner's Rose-Belle
Chicken wing	22.03.11	Grand Port	Winner's Rose-Belle
Chicken wing	29.03.11	Black River	Winner's Bambous, Chantefrais Bambous
Chicken wing	05.04.11	Plaine Wilhems	Curepipe market
Chicken wing	05.04.11	Plaine Wilhems	Curepipe market
Chicken wing	12.03.11	Riviere du Rempart	Riviere du Rempart
Chicken wing	26.04.11	Flacq	Flacq Winners
Chicken wing	17.05.11	Savanne	Riviere des Anguilles Chantefrais
Chicken wing	24.05.11	Port Louis	Port Louis market
Pork ham		Phoenix	Jumbo
Pork ham Charcuterie Francaise		Plaine Wilhems	Trainon Shoprite
Pork Mortadella		Phoenix	Jumbo
Turkey sausage	10.05.11	Triolet	Chantefrais & Winners

Week	Region	Food sample	Result Simplate (NFR*)	Result ISO method
-	-	C. jejuni +ve	+ve	+ve **

		control	ALL	
-	-	C. coli +ve control	+ve	+ve
1	Moka	Chicken wing	1	+ve
1	Moka	Chicken thigh	0	+ve
1	Moka	Chicken sausage	0	-ve
2	Rose-Hill	Chicken liver	48	+ve
2	Rose-Hill	Chicken thigh	5	+ve
2	Rose-Hill	Chicken roulade	0	-ve
2	Rose-Hill	Chicken galantine	0	-ve
3	Trianon	Chicken brochette lemon herb	0	+ve
3	Trianon	Chicken brochette sweet chilli	0	+ve
3	Trianon	Chicken liver	2	+ve
3	Trianon	Chicken thigh	0	+ve
3	Trianon	Chicken wing	0	+ve
4	Rose-belle	Chicken liver W	All-10	+ve
4	Rose-belle	Chicken thigh M	17	+ve
4	Rose-belle	Chicken wing M	0	+ve
4	Rose-belle	Chicken thigh W	all	+ve
4	Rose-belle	Chicken wing W	10	+ve

4	Rose-belle	Chicken croquettes W	0	+ve? To be confirmed by PCR

C. Antibiotic Resistance Tests

All Salmonella and *E coli* isolates have been tested for their susceptibility to the antibiotics.

After OD values for cell cultures, were adjusted to between 0.08 and 0.13 inclusive at 625nm, the solutions of isolates were inoculated on Mueller Hinton agar, antibiotic discs were applied under aseptic conditions and the plates were incubated inverted. The methodology used for Antibiotic resistance tests were adapted from EUCAST disk diffusion methods.

All Salmonella isolates were resistant to Clindamycin10µg (inhibition zone diameter= 0mm*). At first sight, from raw data values, some of the isolates (12) were resistant (inhibition zone diameter= 0mm*) to Tetracycline 30µg, 4 were resistant (inhibition zone diameter= 0mm*) to Erythromycin 30 µg and 2 was resistant (inhibition zone diameter= 0mm*) to chloramphenicol both 30 and 50 µg.

24 isolates of *E.coli* were tested for antibiotic resistance. All *E.coli* isolates were resistant to Clindamycin10µg (inhibition zone diameter= 0mm*) except for 4 isolates which gave some zones of inhibition of size 13, 21, 22 and 10 mm respectively. 9 of the isolates were resistant (inhibition zone diameter= 0mm*) to Tetracycline 30µg. 4 were resistant to Nalidixic acid 30µg, 3 were resistant to Erythromycin 30µg , Ampicillin 30µg and Amoxicillin 30µg, 1 was resistant to Streptomycin 25µg.

*0mm means growth of bacteria on the disc too and hence no inhibition zone.

D. DNA Extraction

DNA extraction is completed for all Salmonella spp. and *E.coli* isolated. The extracted DNA was viewed on agarose gel (1% w/v). 6µl of undiluted DNA + 3 µl of gel loading dye.

Pictures of the gel viewed under UV light are available.

PCR Amplification

In order to confirm the nature of the isolates PCR amplifications are done with primers that are specific for species.

1. Salmonella

Several sets of primers were used for testing the Salmonella isolates. Some are genus-specific others are species-specific.

Primer sets **ENT** and **TYPH** are specific for *Salmonella enteriditis* and *S typhymurium* species respectively.

OMPC and **Malo** primers are specific for all *Salmonella spp.* Both primer sets identified all the presumptive Salmonella isolates. These were further tested with the ENT and TYPH primers to identify the *Salmonella enteriditis* and *S typhymurium* species. Ten strains of Salmonella of known serotypes were used as controls. These included *Salmonella enteriditis, S typhymurium, S Newport* and *S*

Sample code	species
2155.1	-
2020.1	*S. enteritidis*
2093.1	-
1671.2	-
1502.1	-
1502.2	-
1931.1	S. typhimurium
850.1	-
1106.1	S. typhimurium
2154.1	S. typhimurium
1848.4	-
721.5	*S. typhimurium*
1671.1	S. typhimurium
112	*S. typhimurium*
2297.3	-
3066.1	*S. enteritidis*
2667.1	-
3065.1	*S. enteritidis*
2018.1	-
2092.1	-
1565.1	*S. enteritidis*
2153.1	-
2226.5	*S. typhimurium*
719.1	*S. typhimurium*
720.1	*S. typhimurium*
1932.5	-
2094.1	*S. typhimurium*
2661.1	-
1749.1	*S. enteritidis*
2746.2	*S. typhimurium*
1502.5	-
719.2	*S. typhimurium*
2824.1	-
2662.1	*S. typhimurium*
2091.1	-
2827.1	*S. typhimurium*
2747.1	*S. enteritidis*
3069.1	*S. enteritidis*
2571.1	*S. typhimurium*
2825.1	-
2664.1	*S. typhimurium*
2749.1	*S. enteritidis*
2573.1	*S. typhimurium*

Total no. of isolates = 43

S.enteritidis isolates = 8
S. typhimurium isolates = 17

Lane	Gel3 ID	Sample code	species
1	16	3066.1	*S. enteritidis*
2	59		
3	17	2667.1	-
4	60		
5	18	3065.1	*S. enteritidis*
6	61		
7	19	2018.1	-
8	62		
9	20	2092.1	-
10	63		
11	21	1565.1	*S. enteritidis*
12	64		
13	22	2153.1	-
14	65		
15	23	2226.5	*S. typhimurium*
16	66		
17	ladder		
18	TYPH +ve		
19	ENT +ve		
20			

2. Campylobacter

Primer **23S** is specific for all *Campylobacter spp.* and some other bacteria as well such as *Helicobacter pylori* and *Arcobacter* etc. But these bacteria would not be isolated on Karmali and mCCDA agar as these are selective for Campylobacter.

Primers **CC** and **CJ** are specific for *Campylobacter coli* and *Campylobacter jejuni* species respectively.

Results of PCR amplification for Salmonella and Campylobacter

Gel photos are in Appendix 2. All details of the primer used and samples are provided.

DISCUSSION

Few reports on the microbiological food safety of meat and poultry in Mauritius have been published. The increasing demand of consumers for safe food emphasizes the need for such studies to be conducted. A number of incidents where individuals have suffered severe consequences after consuming contaminated food over the past few years, underlines the real threats to public health. With the advent of highly sensitive molecular methods for detecting microorganisms and assessing their genetic diversity, epidemiological studies can be carried out with high precision. Sources of contamination can be accurately traced back, thus allowing remedial measures to be taken. Several public databases (www.mlst.net; www.pulsenet.com) have been set up to classify microbial strains of bacterial species that are important human pathogens on the basis of their molecular typing. Currently both pulse-field gel electrophoresis (PFGE) and Multi-Locus Sequence Typing (MLST) are used for finding profiles of DNA types associated with different strains of a given species. Thus if a new virulent strain appears it can be rapidly detected and eliminated.

The aim of this study was to assess the food safety of meat and poultry available at several outlets in different parts of the island. This was done by buying the materials from retailers and testing them for the presence of commonly found bacteria. The samples were collected and brought to the laboratories for isolation and identification. Morphological characterization on selective media is an adequate approach for a first screening of food samples. Further confirmation requires the testing for the presence of specific enzymes which can metabolise specific substrates. Biochemical profiles are reliable indication of the nature of bacterial species while additional molecular tests using specific primers in PCR amplification form another step towards the identification at species level. The most precise identification relies of obtaining DNA sequences of specific regions of the bacterial genome through DNA sequencing strategies. Many regions of a genome can be associated to the nature of the species and various alleles can be identified. Combinations of alleles reveal a profile or genotype of the strain. This approach relies on availability of the technical expertise to run the method and analyse the data. Sequences from the same region of different isolates are then aligned using an appropriate alignment tool to obtain a dendrogram or a phylogenetic tree.

The results show that a significant number of samples of meat and poultry (31 %) carried Salmonella and an even higher number (45 %) had Campylobacter. From most of the samples *E coli* could be obtained on the culture plates. These observations are indicative of the poor hygienic practices in the outlets. However it cannot be confirmed whether the contaminations originated from the producers or from the meat handling in the shops. This would require a much larger sampling procedure and analysis. The state of many retail shops and markets where meat and poultry are handled, are not appropriate for ensuring their safety. Most do not comply with regulations as stipulated in the Food Act (1998).

The Salmonella isolates were confirmed with the biochemical tests and PCR. *S typhimurium* and *S enteritidis* could be differentiated with the specific primers ENT and TYPH. These have been sent to PulseNet for further analysis by PFGE. Extensive serological testing for the identification of other serotypes was not performed due to the very high costs of sera that are required. However, ten isolates (101-110) which had been typed abroad, were obtained from a donor. They were used as controls in the PCR. ATCC samples of the different species were also available as controls for the other tests.

The PCR approach used was reliable in identifying the Salmonella isolates to the genus level and for two serotypes, *S typhimurium* and *S enteritidis.* Similarly for Campylobacter, after the culture with the Simplate method, the colonies were further identified with specific primers. Some samples produced a lot more colonies on the plates than others. This could be indicative of higher levels of the bacteria.

All isolates were tested for their susceptibility to several antibiotics. Most were resistant to Clindamycin and few to other antibiotics. Patterns of resistance usually result from clinical or environmental exposure to the anti-microbial agent. Genes that are implicated are carried on plasmids or on the chromosome. Their presence on extra-chromosomal DNA contributes to their horizontal dissemination across the strains and species. Such pressure is known to accelerate the rate at which mutations accumulate in those genes and in the presence of the agent there is a positive selection for the resistant isolates.

The primers used could identify the respective species of *S typhymurium* and *S enteritidis* for Salmonella. Similarly the primers CJ F and CJ R, targeting the hipO gene, amplified the suspected Campylobacter isolates.

Amplification of Salmonella with glnA, manB, pduF, the 16S rRNA ws done as well as amplification of Campylobacter DNA with adk; asp A; glnA; gltA; glyA; tkt.

The amplicons for some of the products were sent for sequencing. Many of the sequences have been received and are still being analysed. They will be aligned in order to identify which ones are more related to each other and find the diversity among the isolates. These results are still being processed and will be submitted when ready.

Results of PFGE for Salmonella are still being awaited from PulseNet, South Africa.

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PCR sheet for *Campylobacter*

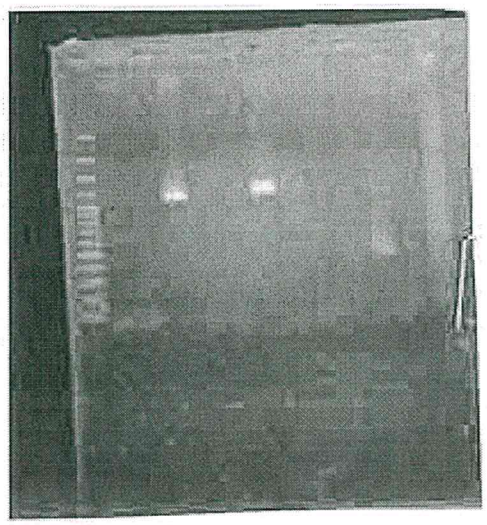
Date : 23.11.2011

Primer : aspA A1 & A2

Expected band size : bp

Annealing temperature : 59°C

Lane no.	Tube no.	Sample code	Region amplified
1		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	
2	C21	1.1/1	-
3	C22	1.1/2	-
4	C23	1.2/1	1200 bp
5	C24	2.1/1	-
6	C25	2.1/2	-
7	C26	2.2/1	1200 bp
8	C27	2.2/2	1200 bp
9	C28	2.3/1	-
10	C29	2.4/1	-
11	C30	2.5/1	-
12	C31	-ve control	-
13		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	



Component	Stock conc.	Final conc.	Volume/tube	11 tubes + 1 (12)
Buffer	10X	1X	3 µl	36 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	28.8 µl
aspA A1	10 µM	20 pmoles	2.0 µl	24 µl
aspA A2	10 µM	20 pmoles	2.0 µl	24 µl
Taq	5U	1U	0.2 µl	2.4 µl
DNA	-	-	1 µl	-
Water	-	-	19.4 µl	232.8 µl
			Total= 30.0 µl	

PCR sheet for *Campylobacter*

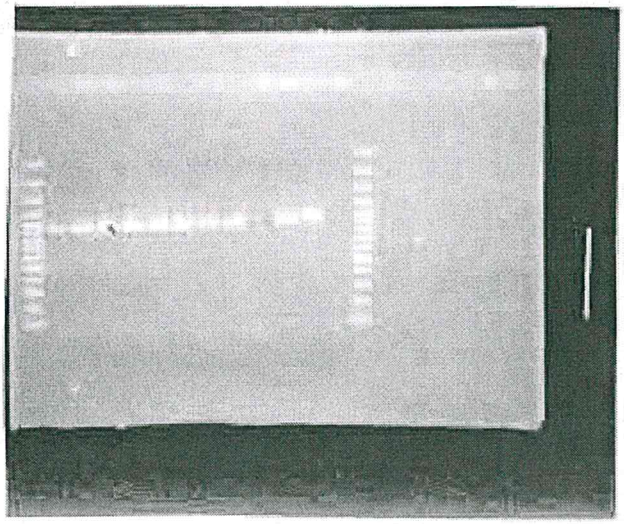
Date : 02.12.2011

Primer : aspA A9 & A10

Expected band size : bp

Annealing temperature : 55°C

Lane no.	Tube no.	Sample code	Region amplified
1	[redacted]	O'GeneRuler 100bp DNA Ladder Plus, Fermentas	[redacted]
2	C118	*C. jejuni* (2) (+ve control)	950 bp
3	C119	6.2/1	
4	C120	6.3/1	
5	C121	6.4/1	
6	C122	6.5/1	
7	C123	7.1/1	
8	C124	7.2/1	
9	C125	7.3/1	
10	C126	7.4/1	-
11	C127	8.1/1	950 bp
12	C128	8.2/1	
13	C129	-ve control	-
14	[redacted]	O'GeneRuler 100bp DNA Ladder Plus, Fermentas	[redacted]



Component	Stock conc.	Final conc.	Volume/tube	12 tubes + 1 (13)
Buffer	10X	1X	3 µl	39 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	31.2 µl
aspA A9	10 µM	20 pmoles	2.0 µl	26 µl
aspA A10	10 µM	20 pmoles	2.0 µl	26 µl
Taq	5U	1U	0.2 µl	2.6 µl
DNA	-	-	1 µl	-
Water	-	-	19.4 µl	252.2 µl
			Total= 30.0 µl	

PCR sheet for *Campylobacter*

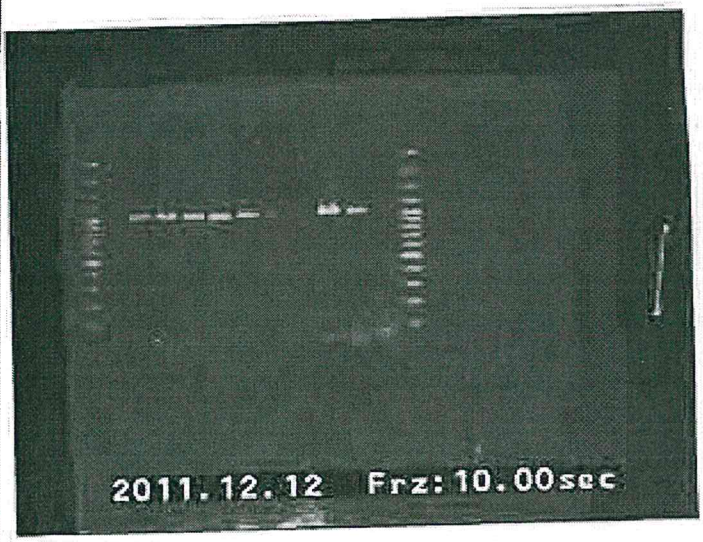
Date : 09.12.2011

Primer : glyA

Expected band size : bp

Annealing temperature : 55°C

Lane no.	Tube no.	Sample code	Region amplified
1		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	
2	C187	*C. jejuni* (2) (+ve control)	1050 bp
3	C188	6.2/1	
4	C189	6.3/1	
5	C190	6.4/1	
6	C191	6.5/1	
7	C192	7.1/1	
8	C193	7.3/1	
9	C194	7.4/1	-
10	C195	8.1/1	1050 bp
11	C196	8.2/1	
12	C197	-ve control	-
13		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	



Component	Stock conc.	Final conc.	Volume/tube	11 tubes + 1 (12)
Buffer	10X	1X	3 µl	36 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	28.8 µl
glyA F	10 µM	20 pmoles	2.0 µl	24 µl
glyA R	10 µM	20 pmoles	2.0 µl	24 µl
Taq	5U	1U	0.2 µl	2.4 µl
DNA	-	-	1 µl	-
Water	-	-	19.4 µl	232.8 µl
			Total= 30.0 µl	

PCR sheet for *Campylobacter*

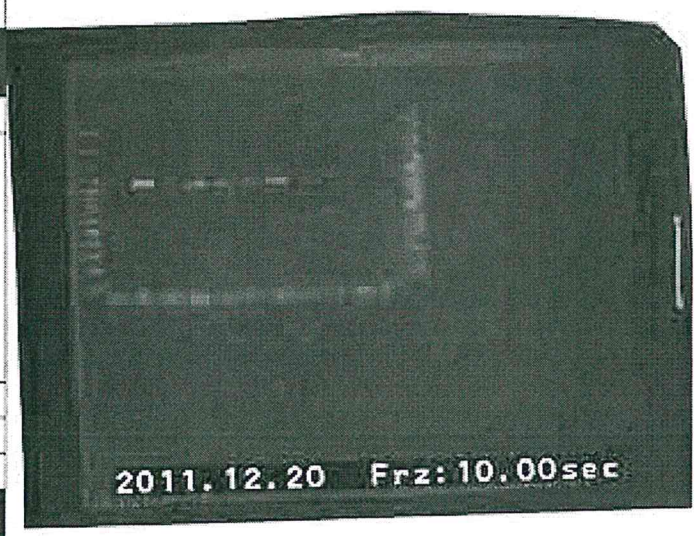
Date : 20.12.2011

Primer : gltA

Expected band size : bp

Annealing temperature : 50°C

Lane no.	Tube no.	Sample code	Region amplified
1		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	
2	C274	*C. jejuni* (2) (+ve control)	-
3	C275	9.2/1	1050 bp
4	C276	10.1/1	
5	C277	10.2/1	
6	C278	10.3/1	
7	C279	11.1/1	
8	C280	11.2/1	
9	C281	11.3/1	
10	C282	720.1 E.coli (-ve control)	-
11	C283	105 Salmo (-ve control)	-
12	C284	-ve control	-
13		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	



Component	Stock conc.	Final conc.	Volume/tube	11 tubes + 1 (12)
Buffer	10X	1X	3 µl	36 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	28.8 µl
gltA F	10 µM	20 pmoles	2.0 µl	24 µl
gltA R	10 µM	20 pmoles	2.0 µl	24 µl
Taq	5U	1U	0.2 µl	2.4 µl
DNA	-	-	1 µl	-
Water	-	-	19.4 µl	232.8 µl
			Total= 30.0 µl	

PCR sheet for *Campylobacter*

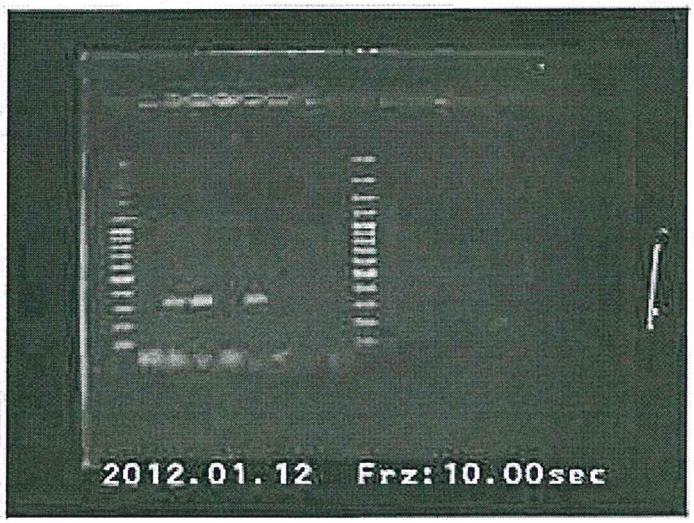
Date : 12.01.2012

Primer : CJ F & CJ R

Expected band size : 323 bp

Annealing temperature : 53°C

Lane no.	Tube no.	Sample code	Region amplified
1		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	
2	C293	*C. jejuni* (2) (+ve control)	-
3	C294	1.2/1	323 bp
4	C295	2.2/1	
5	C296	5.1/1	-
6	C297	6.1/1	323 bp
7	C298	720.1 E.coli (-ve control)	-
8	C299	109 Salmo (-ve control)	-
9	C300	-ve control	-
10		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	



Component	Stock conc.	Final conc.	Volume/tube	8 tubes + 1 (9)
Buffer	10X	1X	3 µl	27 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	21.6 µl
CJ F	10 µM	20 pmoles	2.0 µl	18 µl
CJ R	10 µM	20 pmoles	2.0 µl	18 µl
Taq	5U	1U	0.2 µl	1.8 µl
DNA	-	-	1 µl	-
Water	-	-	19.4 µl	174.6 µl
			Total= 30.0 µl	

PCR sheet for *Campylobacter*

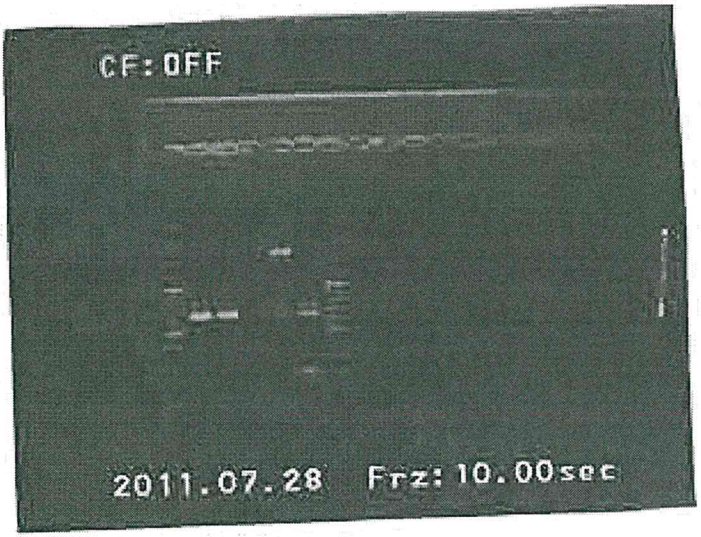
Date : 28.07.2011

Primer : 23S

Expected band size : 650 bp

Annealing temperature : 48°C

Lane no.	Tube no.	Sample code	Region amplified
1		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	
2	1	Campy 1.2/1	650 bp
3	2	Campy 2.1/1	
4	3	Campy 2.2/1	-
5	4	Campy 2.3/1	650 bp
6	5	Campy 2.4/1	
7		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	



Component	Stock conc.	Final conc.	Volume/tube	5 tubes + 1 (6)
Buffer	10X	1X	3 µl	18 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	14.4 µl
23S F	10 µM	30 pmoles	3.0 µl	18 µl
23S R	10 µM	30 pmoles	3.0 µl	18 µl
Taq	5U	1U	0.2 µl	1.2 µl
DNA	-	-	1.0 µl	-
Water	-	-	19.4 µl	104.4 µl
			Total= 30.0 µl	

PCR sheet for *Salmonella*

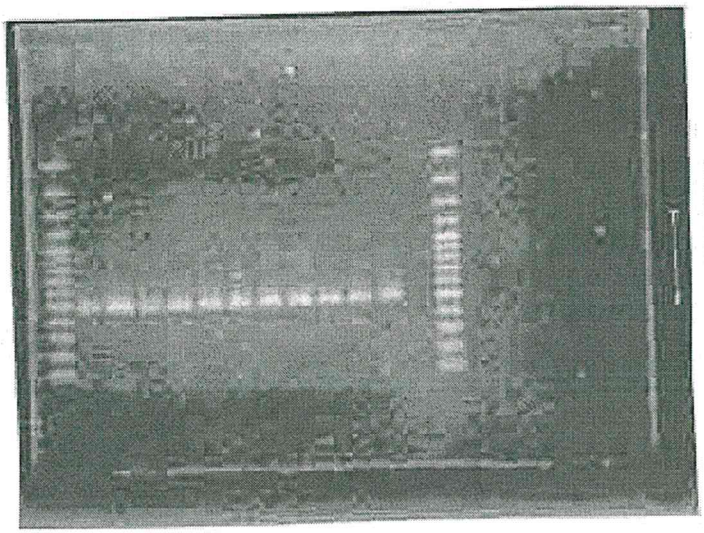
Date : 08.11.2011

Primer : gluA

Expected band size : 270 bp

Annealing temperature : 55°C

Lane no.	Tube no.	Sample code	Region amplified
1		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	
2	600	102	500 bp
3	601	105	
4	602	106	
5	603	107	
6	604	108	
7	605	109	
8	606	110	
9	607	112	
10	608	719.1	
11	609	720.1	
12	610	721.5	
13	611	-ve control	-
14		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	



Component	Stock conc.	Final conc.	Volume/tube	12 tubes + 1 (13)
Buffer	10X	1X	3 µl	39 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	31.2 µl
gluA F	10 µM	20 pmoles	2.0 µl	26 µl
gluA R	10 µM	20 pmoles	2.0 µl	26 µl
Taq	5U	1U	0.2 µl	2.6 µl
DNA	-	-	1 µl	-
Water	-	-	19.4 µl	252.2 µl
			Total= 30.0 µl	

PCR sheet for *Salmonella*

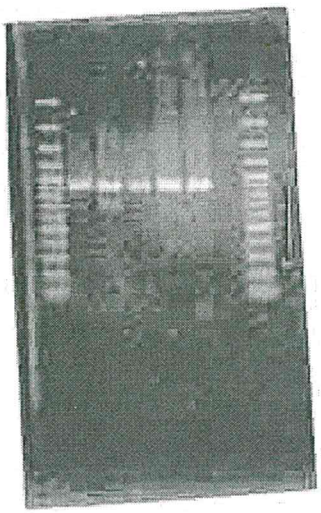
Date : 28.10.11

Primer : manB

Expected band size : 660 bp

Annealing temperature : 55°C

Lane no.	Tube no.	Sample code	Region amplified
1		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	
2	501	104	900 bp
3	502	105	
4	503	107	
5	504	110	
6	505	112	
7	506	-ve control	-
8		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	



Component	Stock conc.	Final conc.	Volume/tube	6 tubes + 1 (7)
Buffer	10X	1X	3 µl	21 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	16.8 µl
manB F	10 µM	20 pmoles	2.0 µl	14 µl
manB R	10 µM	20 pmoles	2.0 µl	14 µl
Taq	5U	1U	0.2 µl	1.4 µl
DNA	-	-	1 µl	-
Water	-	-	19.4 µl	135.8µl
			Total= 30.0 µl	

PCR sheet for *Salmonella*

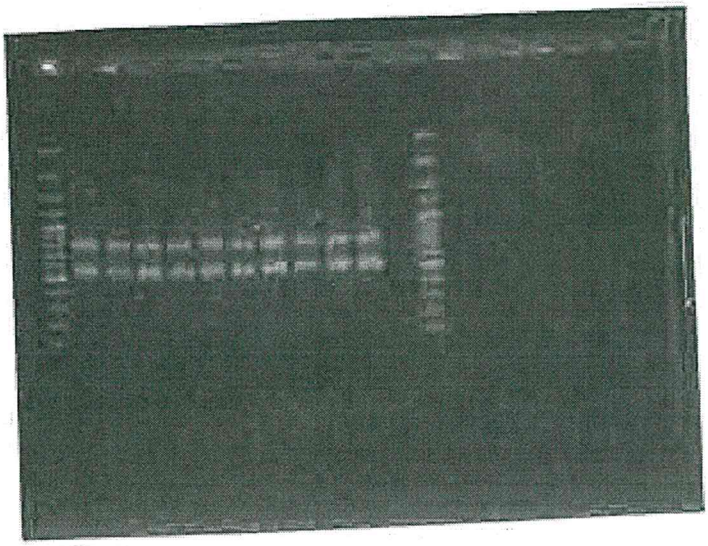
Date : 16.11.2011

Primer : pduF

Expected band size : 210 bp

Annealing temperature : 60°C

Lane no.	Tube no.	Sample code	Region amplified
1		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	
2	716	108 (+ve control)	500 bp & 750 bp
3	717	721.5	
4	718	1106.1	
5	719	1671.1	
6	720	1848.3	
7	721	1848.5	
8	722	2020.1	
9	723	2093.1	
10	724	2094.1	
11	725	2154.1	
12	726	-ve control	-
13		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	



Component	Stock conc.	Final conc.	Volume/tube	11 tubes + 1 (12)
Buffer	10X	1X	3 µl	36 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	28.8 µl
pduF F	10 µM	20 pmoles	2.0 µl	24 µl
pduF R	10 µM	20 pmoles	2.0 µl	24 µl
Taq	5U	1U	0.2 µl	2.4 µl
DNA	-	-	1 µl	-
Water	-	-	19.4 µl	232.8 µl
			Total= 30.0 µl	

PCR sheet for *Salmonella*

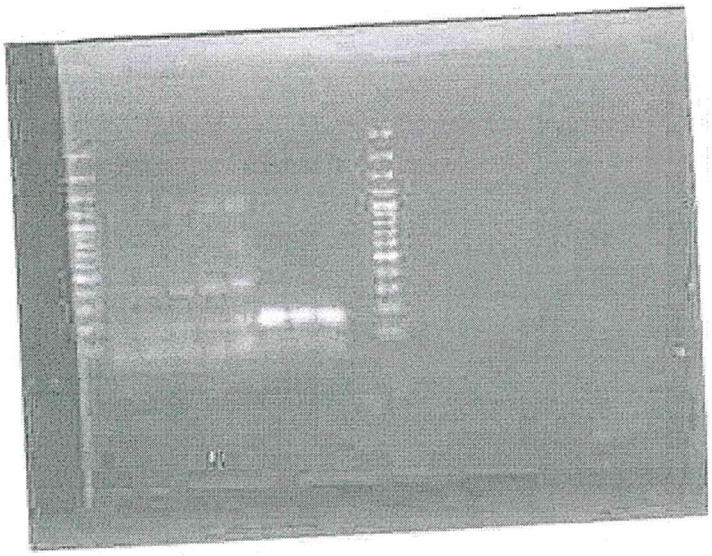
Date : 19.10.2011

Primer : S18 & S19

Expected band size : 159 bp

Annealing temperature : 60°C

Lane no.	Tube no.	Sample code	Region amplified
1		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	
2	313	101	159 bp
3	314	102	
4	315	106	
5	316	108	
6	317	109	
7	318	719.1	
8	319	720.1	
9	320	721.5	
10	321	-ve control	-
11		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	



Component	Stock conc.	Final conc.	Volume/tube	9 tubes + 1 (10)
Buffer	10X	1X	3 µl	30 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	24 µl
S18	10 µM	20 pmoles	2.0 µl	20 µl
S19	10 µM	20 pmoles	2.0 µl	20 µl
Taq	5U	1U	0.2 µl	2.0 µl
DNA	-	-	1 µl	-
Water	-	-	19.4 µl	194 µl
			Total= 30.0 µl	

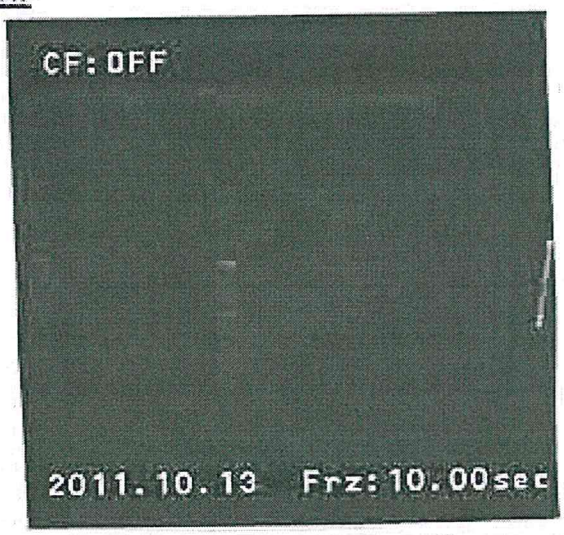
PCR sheet for *Salmonella*

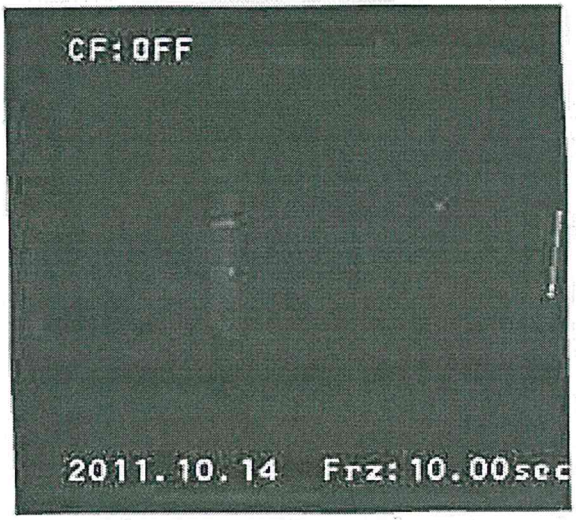
Date : 13.10.11 & 14.10.11 & 03.11.11

Primer : 16S RNA

Expected band size : 350 bp

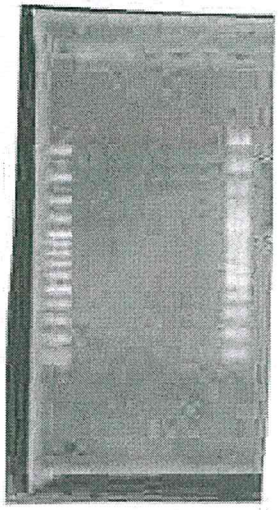
Annealing temperature : 55°C & 48°C & 42°C





Lane no.	Tube no.	Sample code	Region amplified
1		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	
2	401	104	-
3	402	105	-
4	403	107	-
5	404	110	-
6	405	112	-
7	406	-ve control	-
8		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	

Component	Stock conc.	Final conc.	Volume/tube	6 tubes + 1 (7)
Buffer	10X	1X	3 µl	21 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	16.8 µl
16S RNA F	10 µM	20 pmoles	2.0 µl	14 µl
16S RNA R	10 µM	20 pmoles	2.0 µl	14 µl
Taq	5U	1U	0.2 µl	1.4 µl
DNA	-	-	1 µl	-
Water	-	-	19.4 µl	135.8µl
			Total= 30.0 µl	



PCR sheet for *Salmonella*

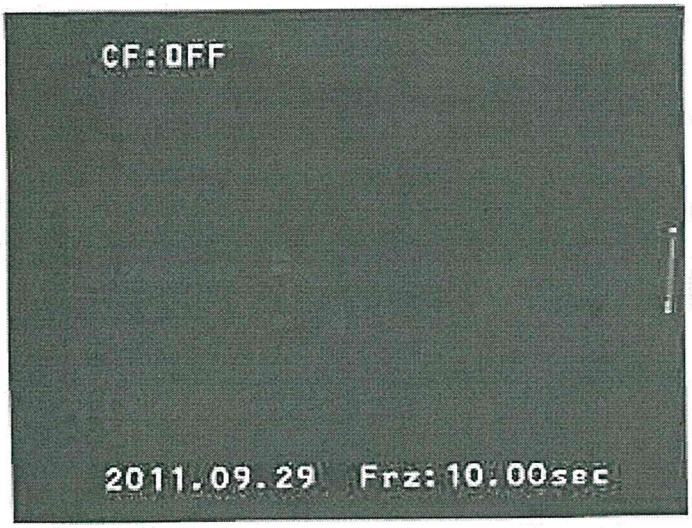
Date : 29.09.2011

Primer : Malo

Expected band size : 373 bp

Annealing temperature : 55°C

Lane no.	Tube no.	Sample code	Region amplified
1		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	
2	223	101 (+ve control)	373 bp
3	224	1671.1	
4	225	1671.2	
5	226	1749.1	-
6	227	1848.3	373 bp
7	228	1848.4	-
8	229	1848.5	373 bp
9	230	1931.1	-
10	231	1932.5	-
11	232	2018.1	-
12	233	-ve control	-
13		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	



Component	Stock conc.	Final conc.	Volume/tube	11 tubes + 1 (12)
Buffer	10X	1X	3 µl	36 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	28.8 µl
Malo F	10 µM	20 pmoles	2.0 µl	24 µl
Malo R	10 µM	20 pmoles	2.0 µl	24 µl
Taq	5U	1U	0.2 µl	2.4 µl
DNA	-	-	1 µl	-
Water	-	-	19.4 µl	232.8 µl
			Total= 30.0 µl	

PCR sheet for *Salmonella*

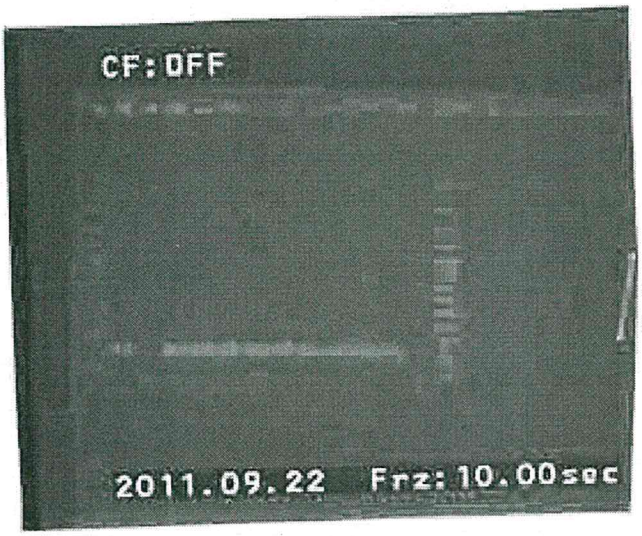
Date : 22.09.2011

Primer : OMPC

Expected band size : 204 bp

Annealing temperature : 48°C

Lane no.	Tube no.	Sample code	Region amplified
1		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	
2	45	104 (+ve control)	204 bp
3	46	2297.3	-
4	47	2400.1	204 bp
5	48	2505.1	
6	49	2571.1.1	
7	50	2573.1	
8	51	2661.1	
9	52	2662.1	
10	53	2664.1	
11	54	2667.1	
12	55	2746.2	
13	56	-ve control	-
14		O'GeneRuler 100bp DNA Ladder Plus, Fermentas	



Component	Stock conc.	Final conc.	Volume/tube	12 tubes + 1 (13)
Buffer	10X	1X	3 µl	39 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	31.2 µl
OMPC F	10 µM	20 pmoles	2.0 µl	26 µl
OMPC R	10 µM	20 pmoles	2.0 µl	26 µl
Taq	5U	1U	0.2 µl	2.6 µl
DNA	-	-	1 µl	-
Water	-	-	19.4 µl	252.2 µl
			Total= 30.0 µl	

PCR sheet for *Salmonella*

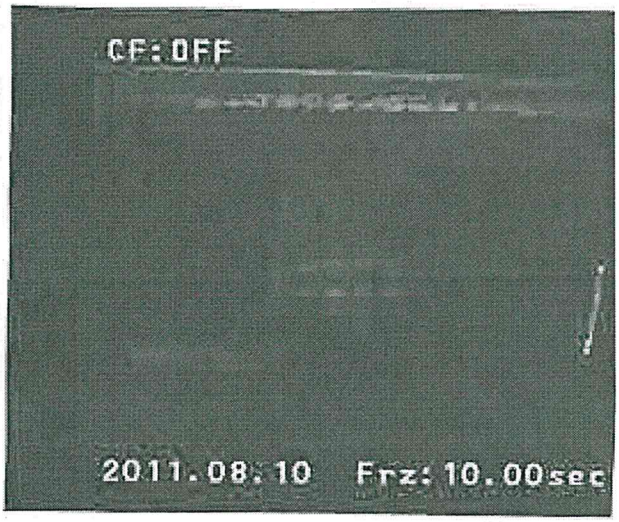
Date : 10.08.2011

Primer : ttr6 & pduF

Expected band size : bp for ttr6 & 210 bp for pduF

Annealing temperature : 48°C

Lane no.	Tube no.	Sample code		Region amplified
1		O'GeneRuler 100bp DNA Ladder Plus, Fermentas		
2	1	Salmo +ve control	ttr6	-
3	2	101		100 bp
4	3	719.2		
5	4	1671.2		
6	5	-ve control		-
7		O'GeneRuler 100bp DNA Ladder Plus, Fermentas		
8	6	719.2	pduF	550 bp
9	7	101		
10	8	Salmo +ve control		
11	9	1671.2		
12	10	-ve control		-



Component	Stock conc.	Final conc.	Volume/tube	5 tubes + 1 (6)
Buffer	10X	1X	3 µl	18 µl
$MgCl_2$	20 mM	2 mM		
dNTPs	2.5 mM	0.2 mM	2.4 µl	14.4 µl
Primer F	10 µM	20 pmoles	2.0 µl	12 µl
Primer R	10 µM	20 pmoles	2.0 µl	12 µl
Taq	5U	1U	0.2 µl	1.2 µl
DNA	-	-	1.0 µl	-
Water	-	-	19.4 µl	116.4 µl
			Total= 30.0 µl	

The train of mass 0.45 kg was moving at a speed v when it hit the buffer. The train was brought to rest by the spring as it compresses 12 mm.

(i) Show that the work done compressing the spring is 1.8×10^{-3} J.

[2]

(ii) Calculate the speed of the train just before it hits the buffer. Assume that the work done compressing the spring is equal to the initial kinetic energy of the train.

speed = ... $m s^{-1}$ **[3]**

(c) State and explain the effect on the compression of the spring if the speed of the train is doubled.

.....

.....

..... **[2]**

[Total: 8]

8 **(a)** A car of mass 850 kg is travelling at a constant speed of 16 m s^{-1} on a dry, level road when the driver reacts to a problem ahead. The car travels 12 m before the driver applies the brakes. The car then stops in a braking distance of 28 m.

(i) Calculate the average deceleration of the car when braking.

deceleration = ... m s^{-2} **[2]**

(ii) Calculate the average braking force required to bring the car to rest.

braking force = ... N **[2]**

(b) **(i)** Explain how the road surface enables a car to come to rest when braking.

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..... **[2]**

(ii) Hence explain why the braking distance of a car increases if the road is wet.

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..... **[1]**

[Total: 7]

END OF QUESTION PAPER