NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF APPLIED BIOLOGY AND BIOCHEMISTRY

INDUSTRIAL INTERNSHIP REPORT

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ABSTRACT

This report documents the practical and theoretical experience that was attained during the internship period at Irvine's Zimbabwe-ZIMVET Laboratory. ZIMVET is the only ISO/IEC 17025 Accredited private veterinary laboratory in Zimbabwe. It boasts of five departments that are Bacteriology, Feed Analysis, Serology, Post Mortem and Hygiene. Departments attached to were Bacteriology, Feed Analysis and Serology for three months each. In Bacteriology tests conducted included Bacterial culture and identification, antimicrobial sensitivity tests, fungal isolation and 5 day *Salmonella* testing. Feed Analysis conducts chemical and biochemical analyses on raw materials and finished poultry feed. Serology conducts serological tests on blood sera to monitor the effectiveness of vaccination as well as to check on viral disease exposure to poultry. Tests conducted included ELISA for diseases such as Newcastle Disease virus and Avian Influenza. Rapid Plate Agglutination test was also carried out for *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and *Salmonella gallisepticum*. The Hygiene department was yet to be visited. It however conducted microbiological tests that indicate the effectiveness of cleaning and hygiene related services. The internship was greatly beneficial and was an eye-opener. It enabled the student to have a vital feel of the real on-hands practical aspect in industry. It also improved personal attributes like good communication skills, punctuality, organization, and appreciation of the need for teamwork.

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CHAPTER 1

1.1 ORGANIZATION BRIEF AND HISTORY

ZIMVET laboratory is a department of Irvine's Zimbabwe located along Huxton road in Waterfalls Harare. It is a Veterinary pathology and diagnostic laboratory. It was established on the 11th of February 1993 and has amassed a wealth of experience in laboratory testing for over twenty-one years that it has been operating. It is the only ISO/IEC 17025 accredited private veterinary laboratory in Zimbabwe. ZIMVET was accredited by SANAS (South African National Accreditation System) to ISO 17025 In 2008 and re-assessed in 2013. It complies with the Council of Veterinary Surgeons of Zimbabwe requirements and is registered by Council through annual inspections.

ZIMVET's standard of service is characterized by its quality objectives:

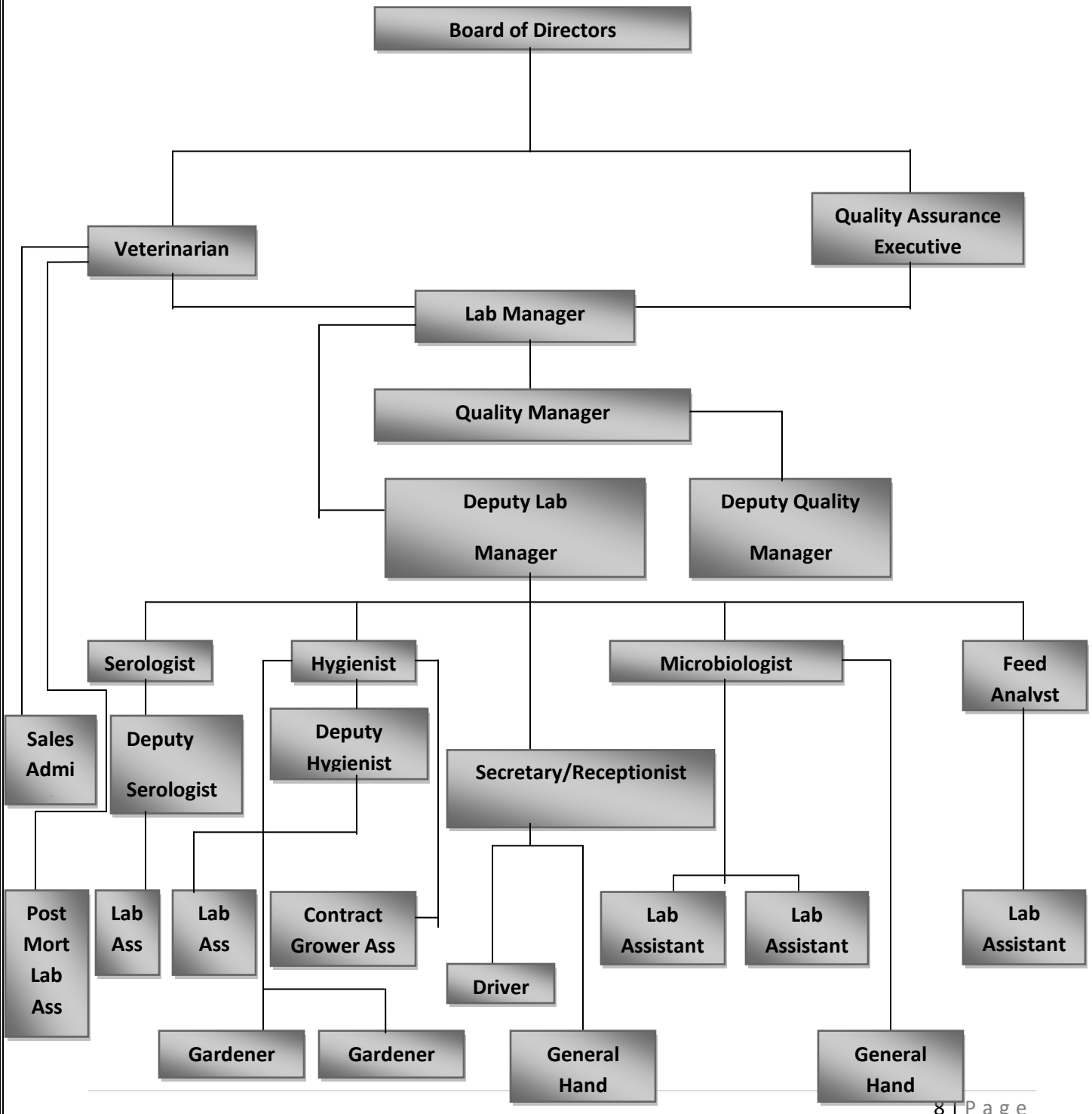
- To make available reliable and accurate results to customers through the use of validated test methods and competent personnel only at all times.
- To meet the specified turnaround time for all test work requested by customers.
- To operate in compliance with the Veterinary Surgeons Regulations.
- To maintain SANAS accreditation status.
- To ensure that all staff in relevant areas of operations is continually trained in the management system in accordance with scheduled programmes

ZIMVET provides Veterinary Technical services and diagnostic facilities to its parent company Irvine's Zimbabwe. Irvine's Zimbabwe, the parent company of ZIMVET is a leading poultry company which produces chickens, commercial table eggs, day old Cobb 500 chicks and H & N layers. Irvine's Zimbabwe started as a backyard project in 1957. It is Zimbabwe's sole producer of the world's leading broiler, the Cobb 500, which it breeds under franchise from the United States, based Cobb breeding company.

ZIMVET laboratory has a staff compliment of 23, consisting of one Quality Assurance Executive; one Veterinary Surgeon; one Lab Manager; one Quality Manager; one Deputy Quality Manager; four Technologists; five Lab Assistants, one Sales Administrator; one secretary; one Gardener; two General hands; one driver and one contract grower assistant.

ZIMVET has five departments which are Bacteriology, Hygiene, Serology, Feed Analysis and Post Mortem. Each department is headed by a Head of Department, who is also referred to as a Technologist. The Bacteriology department is responsible for the surveillance of disease at Irvine's Zimbabwe and other areas where Irvine's Zimbabwe products are produced. The Hygiene department conducts microbiological tests that indicate the effectiveness of cleaning and hygiene related activities. The Serology department offers serological tests on blood sera to monitor routine vaccination programmes as well as disease surveillance, that is, to check on viral disease exposure to poultry. The Feed Analysis department performs chemical analyses on raw materials and finished poultry feed. The Post Mortem department offers veterinary technical services of a veterinary doctor for consultations and anatomical pathological examination of organs, tissues and bodies.

Figure 1: **ZIMVET ORGANOGRAM**



1.2 **DEPARTMENTS AND FUNCTIONS**

ZIMVET'S department's are as follows:

1. Bacteriology
2. Hygiene
3. Feed Analysis
4. Serology
5. Post Mortem

Bacteriology Department

The department is headed by a Microbiologist (Head of Department). The Microbiologist is responsible for:

- Carrying out bacterial cultures (e.g. *Salmonella, Staphylococcus, Escherichia coli);*
- Performs method validation in the bacteriology department and modification methods.
- Supervise assistant (s) and students in bacteriology.
- Monitoring of quality of reagents and media.
- Reporting of, sending out accurate bacteriology results to relevant
- Overall charge of autoclaving process.
- Preparing the company's *Salmonella* report for the government.
- Staining of slides mainly using Ciemsa Stain, Quick Diff and Gram Stain.

The Bacteriology department is mainly responsible for the surveillance of Salmonella at Irvine's Zimbabwe. The department also cooperates with the Post Mortem department in the diagnosis of diseases by performing bacterial culture and isolation from organs, swabs, and tissues. The department also performs Antimicrobial sensitivity tests aimed at the selection of effective antimicrobial drugs.

Hygiene Department

The department is headed by a Hygienist (Head of Department). It is responsible for monitoring and maintaining hygiene standards at Irvine's Zimbabwe. It conducts tests that indicate the effectiveness of cleaning and hygiene related activities. Tests conducted by the department include Total Viable Counts, Total Coliform Counts, Total *E. Coli* counts and Total *S. aureus* counts in foods. Total Viable Count tests are also conducted on cleaned surfaces and personnel hand swabs. Water testing for Total Viable Counts, Total Coliform Counts and Total *E. Coli* counts is also done.

Serology Department

The department is headed by a Serologist (Head of Department). The department monitors the effectiveness of vaccination as well as check on viral disease exposure to poultry. It achieves this by conducting serological tests on blood sera.

The department performs Enzyme Linked Immunosorbent Assay (ELISA) for:

- Newcastle Disease (NDV)
- Infectious Bronchitis (IBV)
- Infectious Bursal Disease (IBD)
- Reovirus (REO)
- *Mycoplasma gallisepticum* (Mg)
- Infectious Laryngotracheitis (ILT)
- Egg Drop Syndrome (EDS)
- Avian Pneumovirus (APV)
- Chicken Anaemia Virus (CAV)
- Avian Influenza (AI)
- *Mycoplasma synoviae* (Ms)

- *Salmonella enteritidis* (Se)

The department also performs Rapid Plate Agglutination (RPA) for *Mycoplasma gallisepticum* (Mg), *Mycoplasma synoviae* (Ms) and *Salmonella gallinarium* (Sg).

Feed Analysis Department

The department is headed by a Feed Analyst (Head of Department). It conducts chemical analyses on raw materials and finished poultry feed. These chemical analyses ensure that all feed and raw materials procured or manufactured contain essential nutritional and chemical components which are vital for the high productivity of poultry. Tests conducted include:

- Crude Protein
- Fat
- Moisture
- Ash
- Calcium
- Phosphorous
- Salt (Sodium)
- Urease Activity

Post Mortem Department

The Post Mortem department is headed by a Veterinary Surgeon who performs diagnosis of diseases based on the examination of organs, tissues and bodies. The Post Mortem also relies on lab analysis of bodily fluids such as the Serology blood sera tests and the Bacteriology bacterial culture of Post Mortem organs. The department also recommends medication, corrective action and preventative measures to curb the disease.

Sales Department

The department is responsible for the procurement of animal health products such as vaccines from international suppliers and distributing them to Irvine's Zimbabwe's poultry farms and also selling them to external customers.

1.3 **NATURE OF THE INDUSTRY**

Veterinary Laboratory diagnostic services are of great relevance to the livestock and poultry industry. The productivity of food-producing animals is highly dependent on their health and welfare. The veterinary services aids in the diagnosis, monitoring, surveillance and treatment of diseases. ZIMVET Laboratory has continued to provide quality diagnostic services to its parent company Irvine's Zimbabwe and Irvine's Zimbabwe contract growers. ZIMVET offers both Anatomical veterinary pathology and clinical veterinary pathology. The former focuses on the diagnosis of diseases based on the examination of organs, tissues and bodies. The latter is aimed at the diagnosis of diseases based on laboratory analyses of bodily fluids such as urine, blood or serum.

The Veterinary Laboratory industry has benefited internationally from scientific research on properties and characteristics of microorganisms and viruses. This has resulted in the production of diagnostic kits and media that are highly effective in the detection and enumeration of microorganisms. The demand for quality in this industry has increased immensely. This has prompted laboratories to demonstrate their competence in performing tests in order to retain and lure more customers. ZIMVET in this respect boasts of an ISO/IEC accreditation. It is the only private veterinary laboratory accredited to ISO/IEC 17025:2005 by SANAS (South African National Accreditation System) for a number of Microbiology (Hygiene), Serology, and Chemistry tests.

ZIMVET participates in Proficiency Testing (PT) with other international ISO/IEC accredited laboratories. Proficiency testing determines the performance of individual laboratories for specific tests and is used to monitor laboratories' continuing performance. In proficiency testing one or more artifacts are sent around a number of participating laboratories. Each laboratory measures the artifacts according to a given set of instructions and reports its results to the administrator. These results are compared to a reference value. ZIMVET also participates in inter-laboratory comparison tests with local laboratories such as Tobacco Research Board (TRB), Aglabs and Central Veterinary Laboratory (CVL)

1.4 **PRODUCTS AND SERVICE MARKETS**

ZIMVET offers timely and reliable poultry diagnostic services to its parent company Irvine's Zimbabwe in its Post Mortem, Serology, Bacteriology, Feed Analysis and Hygiene departments.

Table 1: **Services offered**

DEPARTMENT	PRODUCT/ SERVICE
Post Mortem	• Post Mortem Examination and disease diagnosis. • Technical Consultations by Veterinarian.
Serology	• ELISA tests for poultry diseases e.g. Avian Influenza. • Rapid Agglutination Tests.

Bacteriology	• Bacterial culture and identification. • Antimicrobial sensitivity tests for poultry drug development. • Fungal isolation. • Salmonella isolation.
Feed Analysis	• Chemical Analyses of finished and raw material poultry feed for Crude Protein, Fat, Moisture, Ash, Calcium, Phosphorous, Sodium and Urease.
Hygiene	• Food Tests (Total Viable and coliform counts) • Water tests (Total *E. coli* counts, TVC)

CHAPTER 2

PROCESSES

2.1 **BACTERIOLOGY DEPARTMENT PROCESSES**

The Bacteriology department together with the Hygiene department falls under Microbiology. It is responsible for the following tests:

- Isolation of *Salmonella*
- Salmonella Serotyping
- Identification of *Aspergillus*
- Media preparation

Samples that are tested by the bacteriology department include eggs, fluff, rectal swabs, milk, finished feed, raw materials, meconium, litter, chick liners, neck skins, post mortem organs, egg pulp and meat. These samples are from different sections (of Irvine's Zimbabwe) such as processing plants, mill, layers and broilers section. Some samples come from contract growers, National Foods, Pro-Feeds, and other Laboratories such as Central Veterinary Laboratory (CVL) for inter-lab testing.

2.1.1 ISOLATION OF SALMONELLA (BACT 01)

The major test done in the bacteriology department is the Isolation of Salmonella. Detection of Salmonella before contaminated foods/feed can be consumed by man and animals is an essential feature of safeguarding public and animal health and incidentally preserving the reputations and fortunes of manufacturers and processors. Isolation of Salmonella is a five-day procedure

consisting of stages: Pre-enrichment, Enrichment, Selection, Reading and Biochemical testing and Slide Agglutination

PRE-ENRICHMENT (DAY 1)

Pre-enrichment media is used at day 1 to resuscitate and/ or repair injured and damaged cells to a sound physiological condition before subjecting them to the severity of selective enrichment media. Buffered Peptone Water (BPW) is a pre-enrichment medium used for increasing the multiplication and recovery of small numbers of *Salmonella and* resuscitation of *Salmonella* that have been sub-lethally damaged. Sub- lethal injury to *Salmonella* may occur due to food preservation techniques involving heat, desiccation, high osmotic pressure, preservatives or pH changes. Factors that affect the recovery of *Salmonella* species include sample rehydration, period of pre-enrichment, incubation, media composition and the relative merits of pre-enrichment and direct selective enrichment

Buffered Peptone Water contains proteose peptone as a source of Carbon, Nitrogen, Vitamins and minerals. Sodium chloride maintains the osmotic balance and phosphate buffer system prevents bacterial damage that may arise due to changes in the pH of the medium. Other media that can be used for pre-enrichment is Universal pre-enrichment broth.

Samples are incubated at 37± 2 degrees Celsius for 21± 3 hours. This temperature is the optimum temperature to the growth of *Salmonella* cells. The volume of Buffered Peptone Water added to the sample is proportional to the weight of the sample enabling full development and growth of bacterial cells and delaying the stationary and death phases of bacterial growth before the enrichment (day 2).

Original samples are kept at ambient temperature for retests and are only disposed after the samples have been confirmed to be *Salmonella* free and reported to their customers. Perishable samples such as eggs, neck skins and chicken portions are stored in the cold room in clean tied

plastics (2 degrees Celsius to 8 degrees Celsius). Controls are also set up (Positive, Negative and blank).

ENRICHMENT (DAY 2)

Samples from enrichment are transferred from Buffered Peptone Water into Rappaport Vassiliadis (RV). Rappaport Vassiliadis is an enrichment media which selectively permits the growth of *Salmonellae*, while inhibiting the growth of other bacteria. It is used for the enrichment and selective isolation of *Salmonella* species. It contains malachite green which inhibits other bacteria, including other enteric bacteria but enriches *Salmonella* species. Magnesium chloride which is also in Rappaport vassiliadis raises the osmotic pressure in the medium and potassium phosphate acts as a buffer, maintaining a low pH (acidic). This inhibits other competing organisms (bacteria) which may produce *Salmonella*-like colonies, while selecting for the highly resistant *Salmonella* species.

Each sample (100µl) is aseptically inoculated into 10±1 ml of Rappaport Vassiliadis broth using a micropipette and sterile tips. The samples in universal bottles are incubated at 42± 1 degrees Celsius for 21± 3 hours. Over-incubation will overpopulate the bacterial cells, leading to accumulation of waste and toxic products. This will slow growth and lead to an early death stage of bacteria. Little incubation time lead to inadequate selectivity and enrichment of *Salmonella* spp. Incubation of Rappaport Vassiliadis at 42 degrees Celsius increases the selectivity of this enrichment media for *Salmonella*. All samples from enrichment in Buffered Peptone Water (BPW) are disposed into bins except neck skins, drag swabs and meconium.

SELECTION (DAY 3)

Day 3 of the isolation of *Salmonella* involves the transfer of samples incubated in Rappaport Vassiliadis onto solid Xylose Lysine desoxycholate agar. The required number of XLD plates are removed from the refrigerator and dried in the dryer. This is done by placing the plates upside down in the dryer. The XLD plates are divided into 4 quarters, a procedure known as "quarter plating" using a marker pen and each quarter is labeled with a different sample laboratory number. Quarter plating is done in order to reduce costs of media and plates. Each quadrant is inoculated with a loopful of the respective sample and streaked. The XLD plates are incubated at 37± 2 degrees Celsius for 21± 3 hours. *Salmonella* species have an optimum temperature of 37 degrees Celsius. The Rappaport Vassiliadis bottles are sent to wash up for disposal. Decontamination of Rappaport Vassiliadis sample bottles prevents contamination and spread of *Salmonellae* within the laboratory.

PRINCIPLE OF SALMONELLA REACTIONS ON XLD

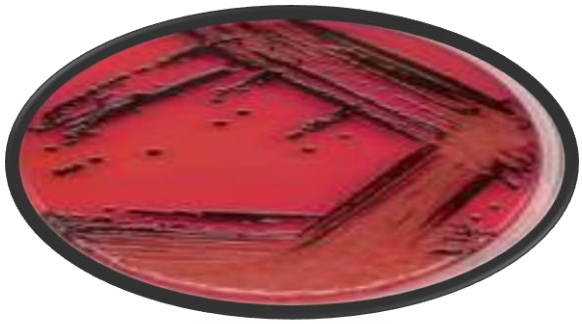
Xylose Lysine desoxycholate is a selective solid medium which contains the fermentable sugars: xylose, lactose and sucrose. The major carbohydrate source is xylose which is fermented by most enteric bacteria except for *Shigella* species. The selective agent in XLD Agar is Sodium desoxycholate, which inhibits gram-positive organisms. It also contains other substrates like lysine and chemicals for detecting hydrogen sulphide production and bile salts. XLD has a pH of approximately 7.4, leaving it with a bright pink or red appearance due to the indicator phenol red. *Salmonella* rapidly ferments xylose and exhausts the supply to produce acid. This lowers the pH and the phenol red indicator registers this by changing to yellow. After exhausting the xylose supply, *Salmonella* colonies will decarboxylate lysine by the enzyme lysine decarboxylase to form amines, increasing the pH once again to alkaline and mimicking the red *Shigella* colonies. *Shigella* colonies cannot ferment xylose but ferments lactose and sucrose which are in excess in XLD and therefore colonies remain red. The addition of sodium thiosulphate and ferric ammonium citrate as a sulfur source and indicator, respectively, allows hydrogen sulfide forming

organisms to produce colonies with black centers, under alkaline conditions. Organisms which ferment xylose, are lysine decarboxylase-negative, and do not ferment lactose and sucrose cause an acid pH in the medium, and form yellow colonies. Examples of such organisms are *Citrobacter*, *Proteus* species and *Escherichia.*

READING AND BIOCHEMICAL TESTING (DAY 3)

The Xylose Lysine Desoxycholate plates are removed from the incubator after 21± 3 hours. The plates are checked for *Salmonella* suspect colonies. Typical colonial appearance of *Salmonella* on XLD agar plate: pink colonies with a black centre. The colony can be entire black or entire pink.

Figure 2: **Typical *Salmonella* colonial appearance**



Further confirmation tests are performed on plates with suspect colonies (S). These tests are termed Biochemical Tests. Urea agar, Lysine Iron agar (LIA), Kligler Iron agar (KIA) and Nutrient agar (NA) are the media used for biochemical tests. A Suspect colony is picked up using a straight wire from XLD culture plate and inoculated onto the four media. On Urea agar, the inoculums is streaked on the surface of the slant, for LIA and KIA, the butt of the media are stabbed and then streaked on their surfaces and finally on NA plates, the suspect is inoculated and streaked onto the agar. Sets of Urea agar slope, LIA slope, and KIA slope are inoculated with *Salmonella Dublin* and *Proteus vulgaris* as positive and negative controls respectively. These samples are incubated at 37± 2 degrees Celsius for 21± 3 hours.

After reading and biochemical tests, XLD plates are decontaminated in the autoclave at 121 degrees Celsius at 110 Kpa for 15 minutes. This ensures that pathogenic bacteria like *Salmonella* do not spread throughout the laboratory.

Table 2: **Interpretation of biochemical tests**

BACTERIA	**UREA AGAR**	**KIA**		**LIA**	
		Slope	*Butt*	*Slope*	*Butt*
Salmonella	Orange	*Pink/Red*	*Yellow*	*Purple*	*Purple*
Proteus	*Pink*	*Pink/Red*	*Yellow*	Brick red	Yellow

Summary of key precautions to note on the isolation of *Salmonella*

- To use sterile equipment when culturing bacteria.
- To disinfect the workbench before and after working all the time.

- To disinfect any spillages immediately and wash hands after handling spillage.
- Never use hot materials when culturing bacteria.
- To ensure left over Buffered Peptone Water (BPW) is re-sterilized before use.

2.1.2 SALMONELLA SEROTYPING AND SLIDE AGGLUTINATION

The principle of the serological identification of *Salmonella* involves mixing cultures of organisms identified as *Salmonella* by biochemical features with antiserum containing specific *Salmonella* antibodies. The bacteria will agglutinate (clump) in the presence of homologous antiserum after a short incubation period.

Polyvalent antisera ("O" (somatic) and "H" (flagella)) are used for initial sero-grouping. Specific identification of "O" antigens is then achieved using monovalent specific "O" antisera. The serotype of the *Salmonella* isolates is then determined by the use of polyvalent and monovalent "H" (flagella) antisera.

2.1.3 IDENTIFICATION OF ASPERGILLUS

a) Sample Testing

Samples for which this test applies include litter (hay, wood shavings and cotton seed hulls), fluff, post mortem tissues and post mortem swabs. Sabouraud dextrose Agar (SAB) is used for culturing *Aspergilllus* species. The *Aspergillus* is rapidly growing moulds with septate hyphae. Sabourand dextrose agar has a pH of 5.6 which inhibits the growth of other bacteria while supporting the growth of fungi that are acid tolerant. Peptic digest of animal tissue or peptone special is the source of nitrogenous growth factors while dextrose provides the energy source for growth of microorganisms. SAB plates are incubated at 37± 2 degrees Celsius for 46± 2 hours.

Plates without growth or moulds are re-incubated. Controls are also prepared and they consist of an Open plate, Cotton swab stick and the Blank control. The open plate is placed on the bench away from the samples being tested and left open throughout the process of testing the samples. The cotton swab stick plate is prepared by swabbing a sterile cotton swab stick across the entire surface of an SAB plate. The Blank control is left unopened.

Table 3: **Colonial Morphology of Aspergillus**

***ASPERGILLUS* SPECIES**	**APPEARANCE ON SAB AGAR**
1.) *A. fumigatus*	White fluff colony when it first appears and bright in color when mature. Smoky green colonies
2.) *A. flavus*	Yellow colonies at first, A green colony with a sugary texture when mature.
3.) *A. niger*	White colony when it first appears that develops a black pepper effect

b) Microscopic Identification of *Aspergillus*

Involves the use of the microscope to view and identify the *Aspergillus* species. A small drop of methylene blue stain is placed on a clean microscope slide. Approximately 5 cm of transparent adhesive tape is cut and held with both hands with the adhesive side facing down. The tape is gently pressed on the surface of a mould. The part of the tape with parts of the mould is then stuck onto the drop of the methylene blue on the microscope slide and viewed under the light microscope.

Table 3**: Microscopic Identification of *Aspergillus***

***ASPERGILLUS* SPECIES**	**MICROSCOPIC APPEARANCE**
1.) *A. fumigatus*	Conidiophores are moderate in length and have a characteristic foot cell at their bases. The vesicles are doom shaped and the upper one half bear's phalides from which long chain of spiny green conidia are produced.
2.) *A. fllavus*	The vesicles are round with sporulation over the entire surface.
3.) *A. niger*	Very large fruiting bodies that look like black balls.

2.1.4 MEDIA PREPARATION

Buffered Peptone Water and Rappaport Vassiliadis were the two media trained on their preparation.

a) Preparation and storage of Buffered peptone water

BPW is a non-selective pre-enrichment medium used for increasing the recovery of *Salmonella*. On preparation of the media, BPW was weighed as per manufacture's instruction and the appropriate amount of distilled water was measured using a measuring cylinder. The BPW used required 30 grams to be added to 1000ml of distilled water. The media was then dissolved by use of a magnetic stirrer. This was followed by autoclaving and subsequent cooling of the media until it was safe to touch with bare hands. The pH of the media was then measured using a pH meter. The bottles were then labeled by putting a sticker written BPW, date prepared, batch number and expiry date. The expiry date of BPW was three months from the date of preparation. The media was then sent for quality control tests where sample bottles are added 10 ml aliquots of control organisms: *Salmonella Dublin, E. coli* and *Proteus vulgaris.* If the media fails quality

control tests, new media has to be prepared. The media was stored at ambient temperature in a cupboard for up to three months.

b) Preparation and storage of Rappaport Vassiliadis

Rappaport vassiliadis was recommended for enrichment of *Salmonella* under conditions of low pH and high temperature (42± 1 degrees Celsius. On preparation of the media, RV was weighed as per manufacture's instruction and the appropriate amount of distilled water was measured using a measuring cylinder. The manufacture's RV used required 30 grams to be added to 1000ml of distilled water. The media was then dissolved by use of a magnetic stirrer and a hot plate, and then 10±1 ml of broth was then dispensed into universal bottles. The bottles are closed and autoclaved then cooled at ambient temperature until it was safe to touch with bare hands. One bottle was selected randomly and its pH measured using a pH meter according to the manufacture's instruction. The media was then labeled by putting a sticker written RV, Batch number date prepared and expiry date. The expiry date was three months from the date of expiry. Two bottles are randomly selected and sent for quality control tests. The media was then sent for quality control tests where sample bottles are added 10 ml aliquots of control organisms: *Salmonella Dublin*, *E. coli* and *Proteus vulgaris.* If the media fails quality control tests, new media has to be prepared. The media was stored at ambient temperature in a cupboard for up to three months.

2.1.5 RELATION OF BACTERIOLOGY PROCESSES TO APPLIED BIOLOGY AND BIOCHEMISTRY

The Bacteriology processes are highly related to Applied biology, particularly in the following courses: Microbiology and Food Chemistry. Salmonella Isolation, Media preparation, use of incubators, balances, measuring cylinders, micropipettes are fundamental aspects in

Microbiology. Some skills were learnt orally at school and more practical and skills were practiced, refined and developed at ZIMVET. Some of these skills include: bacterial culture, streaking, food preservation, sterilization, decontamination and media preparation.

2.2 FEED ANALYSIS DEPARTMENT PROCESSES

The Feed analysis department is responsible for the following tests on Stock Feeds and Raw materials:

- Determination of Crude Protein
- Determination of Moisture
- Determination of Fat
- Determination of Calcium by AAS
- Determination of Phosphorous
- Determination of Crude Ash
- Determination of Urease activity in Soya

*Sample preparation, Balance calibration and Glassware cleaning are also other non-testing but vital activities in Feed Analysis.

2.2.1 CLEANING AND CARE OF GLASSWARE

a) General Glassware

Glassware is soaked in detergent (HC7) for at least 30 minutes. A Scotch pad or a wire brush is then used to clean inside the glassware. A scotch pad is used to clean outside glassware. This is followed by a thorough rinsing of glassware with a lot of running tap water in order to remove all

the detergent. Glassware is then rinsed once in distilled water and placed on the drying rack or in a hot air oven. The cleaning of glassware is simplified by rinsing in tap water immediately after use.

b) Fat Extraction Glassware

Flat bottomed flasks used for fat extraction are first rinsed with at least 50 ml of recycled petroleum ether for at least an hour. The flasks are swirled so that the petroleum ether can reach the neck of the flask and remove any fats sticking on the walls. The petroleum ether is then discarded into a holding container for further use. The flasks are then rinsed thoroughly with tap water and then soaked in detergent (HC7) for at least 30 minutes. A scotch brush or wire brush is used to clean inside the glassware. A scotch brush is used to clean outside glassware in the detergent bucket, and then rinsed thoroughly with running tap water.

c) Pipettes

Pipettes are first soaked in detergent (HC7) for at least 30 minutes and then are rinsed thoroughly with a lot of running tap water to remove all the detergent. Distilled water is used to rinse them once before placing them on a drying rack.

2.2.2 SAMPLE PREPARATION

In feed analysis procedures, it is crucial to carefully select a sample whose composition represents that of the feed being analyzed and to ensure that its composition does not change significantly prior to analysis. Solid feeds are finely ground and then carefully mixed to facilitate the choice of a representative sample. Grinding of samples facilitate the homogenization of samples and also reduces particle size of granular samples thereby increasing the reaction rates. Before carrying out an ash analysis, samples that are high in moisture are often dried to prevent

spattering during ashing. Other possible problems include contamination of samples by minerals in grinders, glassware or crucibles which come into contact with the sample during the analysis. For the same reason, it is recommended to use de-ionized water when preparing samples.

2.2.3 BALANCE CALIBRATION

Balance calibrations were done each and every morning before tests to ensure that the balances were in the specified ranges. This ensured that accurate weights were measured as well as to ensure that precise, credible results were obtained. Corner loading checks were also done once every week by placing standard weights at each corner and recording the weights on appropriate forms. Balance calibration is related to Applied Biology particularly Analytical biochemistry which involves various tests that require the weighing of samples and/ reagents.

2.2.4 FEED ANALYSIS TESTS

DETERMINATION OF CRUDE PROTEIN

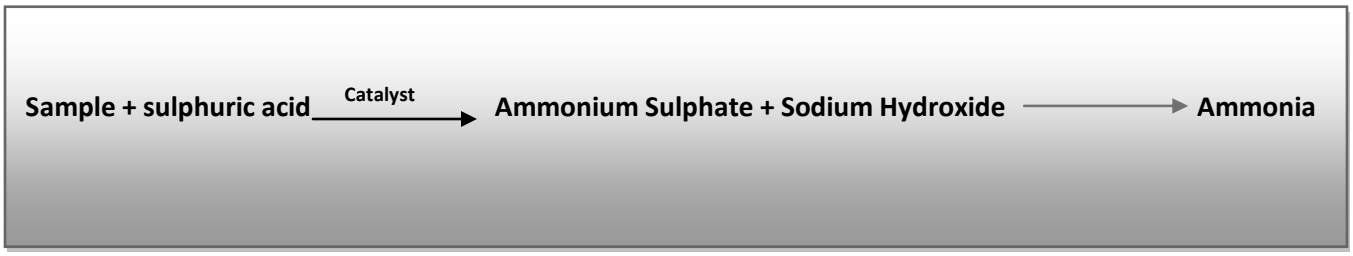
The Kjeldahl analysis is the method used at ZIMVET. It is an analytical method to quantitatively determine the nitrogen in certain organic compounds. The method was developed in 1833 by Johan Kjeldahl, a Danish Chemist. The analysis involves three main steps: digestion, distillation and titration. The principle of kjeldahl nitrogen analysis is as follows:

- The feed sample is digested to convert the nitrogen in protein and other nitrogen-containing substances into ammonium sulfate;
- The ammonium sulfate is reacted with an alkali (NaOH), thus releasing the ammonia; the ammonia is distilled and received by an acid (Boric Acid);

- The receiving solution containing the NH_3 is titrated to quantify the Nitrogen. The amount of nitrogen is finally converted to crude protein using a factor 6.25 since most feed proteins contain 16% nitrogen.

The organic material is digested in boiling concentrated sulphuric acid in the presence of a catalyst (Copper sulphate or titanium oxide).The nitrogen evolved is in the form of Ammonia and it combines with the sulphate ions to form ammonium sulphate, which in the presence excess alkali and Zinc catalyst further dissociates to liberate ammonia. As the Ammonia distills over into the receiver, it is trapped in a solution of boric acid in which form it may be determined. Ammonia (Total Nitrogen Content) is converted to crude protein by multiplying by the conventional factor 6.25

Chemical Equation



DETERMINATION OF PHOSPHOROUS

Colorimetric methods are used to determine the concentration of a wide variety of different minerals including Phosphorous. A weighed portion (Test portion) of a sample is wet oxidized with a mixture of Sulphuric Acid and nitric acid. An aliquot portion of the acid solution is mixed with molybdovanadate reagent and the absorbance of the yellow solution obtained is measured at a wavelength of 430nm. Molybdovanadate is often used as a colorimetric reagent because it

changes color when it reacts with minerals. The color intensity is proportional to the phosphorous content in a sample. The colored complex (yellow-orange) formed with phosphorous can be quantified by measuring the absorbance of the solution at 430nm with a UV-VIS Spectrophotometer.

DETERMINATION OF FAT

A Soxhlet unit is used for fat extraction at ZIMVET. Petroleum ether is continuously volatized, condensed and allowed to soak through the sample, extracting the ether soluble component. The extract is collected for 5 hours at a condensation rate of 6-8 drops.

Summary of key precautions

- Avoid inhaling ether vapours.
- Make sure all ether is evaporated from the beakers before placing them in the oven to avoid a fire or explosion.
- Peroxides can accumulate in open containers of ether. These are explosive and shock sensitive. Check each container opened for more than 30 days for peroxides. Ether containing peroxides must be disposed of with special techniques.

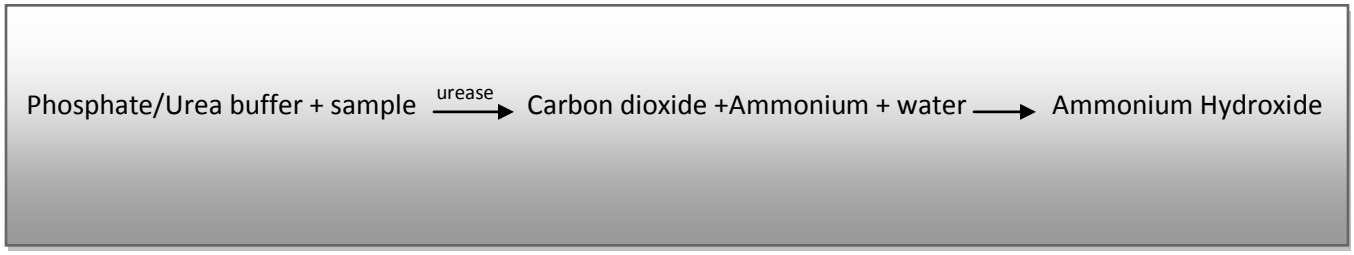
DETERMINATION OF UREASE ACTIVITY

In the urease determination test, 0.2± 0.05g of sample is weighed into two tubes: one labeled T (Test) and the other B (Blank). Then 10 ml of phosphate buffer is added into a tube labeled B whilst Urea buffer is added into the tube labeled T. The tubes are incubated in a water bath set at 30 degrees Celsius for 30 minutes. Tubes are mixed at every 5 minute interval. Samples are then removed from the water bath and allowed to stand at ambient temperature for another 5 minutes.

The urease activity is determined by reading the pH of the samples and then subtracting the pH of the two tubes.

PH (Test) – pH (Blank) = pH difference (Urease Activity)

Chemical Equation



Full Fat Soya Bean (FFSB) contains anti-nutritional factors, the most important being the proteases (trypsin and chymotrypsin) inhibitors and haemoglutonins/lectins. These make it unfit for feeding to monogastric and immature ruminant animals. Processing of the raw FFSB by means of heat and mechanical treatment destroys the anti-nutrients which are heat labile. Only an optimum level of heat treatment will produce maximal availability of the amino acids to the animal, thus securing the best animal performance. Insufficient heating, i.e. under processing of the FFSB limits amino acid availability due to only partial destruction of the anti-nutritional factors (ANFs). On the other hand, excessive heating (over-processing) decreases amino acid availability as a result of the Maillard reaction that occurs between the aldehyde groups of sugars and free amino groups. The main objective of heat processing of FFSB is to achieve an optimum balance between degradation of ANFs and maintenance of amino acid availability. The aim of processed FFSB quality control is to establish if that balance has been achieved.

DETERMINATION OF CRUDE ASH

The ash content is a measure of the total amount of minerals present within a feed. It is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. The organic matter is decomposed by incineration at not less than 550^0C by an incineration Furnace. Ash obtained is weighed and expressed as a percentage by mass. At ZIMVET, dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures of between 500 and 600^oC. Water and other volatile materials are vaporized and organic substances are burnt in the presence of the oxygen in air to CO_2, H_2O and N_2. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates. Although most minerals have fairly low volatility at these high temperatures, some are volatile and may be partially lost, *e.g.*, iron, lead and mercury.

Determination of the ash and mineral content of foods is important for a number of reasons:

- *Nutritional labeling.* The concentration and type of minerals present must often be stipulated on the label of a food/feed.
- *Quality.* The quality of many foods/feeds depends on the concentration and type of minerals they contain, including their taste, appearance, texture and stability.
- *Microbiological stability.* High mineral contents are sometimes used to retard the growth of certain microorganisms.
- *Nutrition.* Some minerals are essential to a healthy diet (*e.g.*, calcium, phosphorous, potassium and sodium) whereas others can be toxic (*e.g.*, lead, mercury, cadmium and aluminum.

DETERMINATION OF MOISTURE

It is also referred to as the dry matter content. Samples are subjected to a specific temperature range (103 ± 1 degrees Celsius) sufficient to remove free moisture by evaporation until a constant weight is obtained on reweighing. Besides moisture, other volatile compounds, such as ammonia and volatile fatty acids, disappear during drying.

DETERMINATION OF CALCIUM BY ATOMIC ABSORPTION SPECTROSCOPY

Atomic spectroscopy (absorption and emission) is used to perform trace elemental analysis. Atomic spectroscopy has high sensitivity high selectivity due to the presence of extremely narrow spectral lines. In atomic spectroscopy, a substance is vaporized and decomposed into gaseous atoms in a flame, furnace, or plasma (a gas hot enough to contain ions and free electrons). The aqueous sample is nebulized into the flame and as the aerosol enters the flame, the liquid is rapidly evaporated and the remaining solid is atomised by the flame. The element will absorb specific wavelengths of light (from a hollow cathode lamp). The sample is subjected to a high-energy thermal environment in order to produce excited-state atoms However, since the excited state is unstable, the atoms spontaneously return to the "ground state" and emit light. Concentrations of atoms are measured by absorption or emission of specific wavelengths of radiation. For sodium *Flame emission spectroscopy* (FES) is used. The principle is to measure the intensity of molecular bands or atomic or ionic lines emitted by excited molecules, excited atoms or even by excited ions. For Calcium *Flame atomic absorption spectroscopy* (FAAS) is used. The principle is to let the fire a light beam with such a wavelength that can be absorbed by ground state atoms and thus we measure the decrease of light intensity.

2.3 RELATION OF FEED ANALYSIS PROCESSES TO APPLIED BIOLOGY AND BIOCHEMISTRY

A number of processes in feed analysis were immensely related to Applied biology and biochemistry. Balance calibration, pH meter calibration, and feed analysis tests were related to Applied Biology particularly these courses: Analytical biochemistry, Principles of Nutrition and Chemistry of Biomolecules. Analytical Biochemistry Course introduces students to practical techniques used in biochemical research as well as terms that are the language of the practising biochemist. Topics covered include measurement of pH, extraction of biomolecules and techniques used in elucidating their structures (e.g. Centrifugation, Chromatography, Electrophoresis, Spectrophotometry, Radioisotope technique). The Determination of Phosphorous test is also highly related to Analytical Biochemistry in that the UV/IS Spectrophotometer (Spectrophotometry) is used, which is a common equipment in that course. The determination of urease activity applies the vast knowledge of Applied Biology of enzymes, optimum temperatures of enzymes and also the effect of high or low urease in soya feeds, the correlation of urease to trypsin inhibitors and anti-nutritional factors are all born out of applied biology research. This test also applies to the Enzymology Course. Calcium determination by AAS is also related to Analytical Biochemistry and applies modern equipment like the AAS. The use of fume extractors, acid disposal and effluent disposal in Feed Analysis were all aspects of Environmental Science course.

2.4 SEROLOGY DEPARTMENT PROCESSES

The department of Serology is responsible for the following tests on blood sera:

1. Enzyme Linked Immunosorbent Assay (ELISA) for:

- Newcastle Disease (NDV)

- Infectious Bronchitis (IBV)
- Infectious Bursal Disease (IBD)
- Reovirus (REO)
- *Mycoplasma gallisepticum* (Mg)
- Infectious Laryngotracheitis (ILT)
- Egg Drop Syndrome (EDS)
- Avian Pneumovirus (APV)
- Chicken Anaemia Virus (CAV)
- Avian Influenza (AI)
- *Mycoplasma synoviae* (Ms)
- *Salmonella enteritidis* (Se)

2. Rapid Plate Agglutination (RPA) for:

- *Mycoplasma gallisepticum* (Mg)
- *Mycoplasma synoviae* (Ms)
- *Salmonella gallinarium* (Sg).

*Sample preparation, Temperature reading and washing of blood tubes are also other non-testing but vital activities in Serology.

2.3.1 TEMPERATURE READING AND MONITORING

It is important to monitor temperatures of freezers and fridges used to store sera and ELISA kits to avoid spoilage of preserved sera. If the temperatures are not monitored, the freezers and the fridges maybe out of range causing the spoilage of sera and

decomposition of ELISA kits which will make them unsuitable for carrying out tests. If bad kits are used wrong results will be obtained and this will result in the customer giving the chickens wrong medication. Test components are allowed to sit at room temperature for 2–3 hours, including the plates which should be sealed in their original bags with desiccants included.

2.3.2 SAMPLE PREPARATION

Blood is centrifuged at 5000 rpm for 5 minutes in order to separate the serum from the blood. The serum is collected into ependorf tubes. Reagents to be used for tests are removed from the refrigerator and allowed to sit at room temperature for 2–3 hours, including the plates which should be sealed in their original bags with desiccants included. Frozen sera are shook on a micro plate shaker for 30 minutes. Five microlitres of serum sample are added into microtitre plates followed by dilution with 120 microlitres of sample diluents. The plate is then shaken on a micro plate shaker to mix sample and diluents.

2.3.3 SEROLOGY TESTS

a) ELISA FOR AI, NDV, IBD and MG

Principle

The ELISA assay is designed to measure the levels of antibodies against NDV, AI, MG and IBD. 96-well antigen-coated polystyrene plates are used. The function of the solid phase is to immobilize antibodies in the sample, as they bind to the solid phase. After incubation, the plates are washed to remove any unbound material. An anti-species globulin conjugate is then added to the plate wells and allowed to incubate. The conjugate consists of an enzyme-

labeled anti-species. The enzyme portion of the conjugate enables detection. The plates are washed again and an enzyme substrate (hydrogen peroxide and a chromogen) is added and allowed to incubate. Color develops in the presence of bound enzyme and the optical density is read with an ELISA plate reader. The addition of an enzyme substrate-chromogen reagent causes color to develop. This color is directly proportional to the amount of bound sample antibody. The more antibodies present in the sample, the stronger the color development in the test wells.

Fig 3: **ELISA plate reader and software reading interface**

ELISA Sample Testing

Ninety-five micro litres (95µl) of sample diluents are added to corresponding antigen coated wells. One hundred micro litres (100µl) of corresponding positive and negative controls are added into their respective wells. Five micro litres (5µl) of diluted samples are transferred into their corresponding 95µl diluents-containing wells. The plate is then tapped on the sides to mix

the contents and incubated for 30 minutes. The plate is then washed by a semi-automated washer with distilled water. One hundred micro litres (100µl) of conjugate is added into each well and incubation is allowed for 30 minutes at room temperature. The plate is then washed again before adding 100µl of a substrate-chromogen reagent. This time 15 minutes is allowed for incubation before adding 100µl of stop solution which stops the enzyme-substrate reaction and, thereby, the color development.

b) RAPID PLATE AGGLUTINATION

This test is used as a cheap, rapid and effective flock-screening serological test for infections with Mycoplasma gallisepticum (Mg) and Mycoplasma synoviae (Ms) in chicken serology. A white porcelain tile is used. Forty micro litres (40µl) of Mg antigen is added onto a tile for 15-20 samples discarding pipette tips. A positive and negative control is included. Forty micro litres (40) µl of serum is added onto one drop of 40µl Antigen, mixed and the tips are discarded into a waste bucket. The tile is rocked and read after 2 minutes.

Principle

Mycoplasma synoviae is a killed and purple stained suspension of the strain WVU 1853 (ATCC). Mycoplasma gallisepticum antigen is a suspension of killed and blue stained Mg organisms–strain S6 of Adler (USA). On mixing the antigen with serum containing Mg antibodies agglutination takes place. If no antibodies are present no agglutination takes place.

2.3.4 RELATION OF SEROLOGY PROCESSES TO APPLIED BIOLOGY AND BIOCHEMISTRY

All processes in serology were related and of high relevance to applied biology. The major applications were in the course Introduction to Enzymology and Immunology. Enzyme Linked Immuno-sorbent Assay (ELISA) is one of the practical techniques in Analytical Biochemistry course. The use of the centrifuge done in Serology is also one of the practical techniques in that course. The principles of ELISA and Rapid Plate Agglutination are from Enzymology and Immunology course as vast knowledge of vaccination; antibody-antigen reactions and immune-response are applied. Other aspects were however much more aligned to the Medical side of industry like handling of blood tubes and storage of blood (serum) in ependorf tubes and in freezers.

CHAPTER 3

3.1 QUALITY ASSURANCE

ZIMVET's management is committed to providing quality diagnostic and testing service to its customers through good professional practice. Its management system is based on the international standard, ISO/IEC 17025. The management system aims to meet the requirements of regulatory bodies and South African National Accreditation Body (SANAS). All personnel concerned with testing activities within the laboratory familiarize themselves with the quality documentation and implement the policies and procedures in their work. The management system ensures that there is continual assessment of personnel and internal audits are done regularly as per schedule. ZIMVET selects and purchases services and supplies from approved providers only. The quality management system is continually improved through the use of:

a) Quality policy
b) Quality objectives
c) Audit results
d) Analysis of data
e) Corrective and preventative actions
f) Management review
g) Customer Feedback

ZIMVET's standard of service is characterized by its quality objectives:

- To make available reliable and accurate results to customers through the use of validated test methods and competent personnel only at all times.
- To meet the specified turnaround time for all test work requested by customers.
- To operate in compliance with the Veterinary Surgeons Regulations.
- To maintain SANAS accreditation status.
- To ensure that all staff in relevant areas of operations is continually trained in the management system in accordance with scheduled programmes.
- To continually improve the effectiveness of the management system through the improvement procedure, refresher training of staff and implementation of corrective action of audit findings.
- To ensure that the laboratory continues to meet changing national and international regulatory requirements and customer needs through active participation in laboratory associations and analysis of customer feedback.

3.2 LABORATORY WORK

The student carried out tests in all departments attached to under supervision by HOD and/or Deputy HOD. Worksheets done by the student were countersigned by the Head of Department to indicate supervision. In Bacteriology, the student was trained and assessed for competence in Salmonella testing Pre-Enrichment, Enrichment stages and Selection. Based on the assessment, the student was deemed competent and authorized to carry out Pre-enrichment, Enrichment and Selection in the Salmonella. (See attached competence Forms)

Table 4: **Summary of Internship activities**

DEPARTMENT	TESTS CARRIED OUT BY STUDENT	EQUIPMENT USED
Bacteriology (3 months)	-Salmonella Isolation -Aspergillus testing	
Feed Analysis (3 Months)	-Crude Protein -Calcium by AAS -Moisture -Fat -Urease -Phosphorous -Crude Ash	-Kjeldatherm -Atomic Absorption Spec (AAS) -Desiccator -Soxhlet Extraction Unit -PH Meter -UV/VIS Spectrophotometer
Serology (3 Months)	-ELISA for Avian Influenza -Rapid Plate Agglutination	- Vortex Mixer, Elisa Plate Reader & Plate-Strip Washer - Centrifuge

The student was also assessed on equipment and apparatus use by the Laboratory Equipment Officer. Equipment assessed included the incubator, balance, measuring cylinder and micropipettes. Based on these assessments, the student was deemed competent and authorized to use the equipment.

3.3 TRAINING COURSES ATTENDED

ZIMVET has an annual training schedule in place which is prepared by the Deputy Quality manager. Training needs are identified and set based on ISO/IEC 17025 standard, ZIMVET procedures, policies and Management review findings.

Environmental conditions and good housekeeping training

The training was focused on lab assistants and students making them aware how the environmental conditions are monitored, controlled and recorded and on how to ensure good housekeeping. Good housekeeping focused on cleaning and waste disposal activities. Key areas and activities under scope were:

- Recording of serology room, incubators, water baths, fridges, freezers, cold room and oven temperatures.
- Notifying the Equipment Officer and HOD if equipment temperature is out of range. Adjusting the temperature setting and monitoring temperature of the cold room.
- Switching of the air conditioner if the room temperature is out of range.
- Disinfecting area where Salmonella has been isolated as indicated by drag swab, boot swab results and Rodac swab index.
- Restriction of entry to departments to authorized personnel only.
- General routine cleaning of premises (offices, floors, shelves, corridors, toilets).
- Routine fogging of the Microbiology Laboratory
- Disposal of wastes
- Cleaning of Laboratory apparatus

CHAPTER 5

CONCLUSIONS

The whole internship period has been a success. It gave the opportunity of a hands-on experience in industrial laboratory work, more befittingly in an ISO/IEC 17025: 2005 accredited laboratory/institution. The student benefited much in learning new methods not taught at University, in using sophisticated equipment not available at the university and on an exposure to a more professional and hierarchical work environment. Modern equipment like the Atomic Absorption Spectrophotometer (AAS), the ELISA reader and washer, the Soxhlet fat extraction unit, the UV/VIS spectrophotometer, centrifuges, Kjedaltherms and sterilizers were some of the equipment that the student got the opportunity to operate in running tests. The student got the chance to appreciate how quality assurance is implemented through ISO accreditation, internal and external audits, and training. Personally, the student gained and developed good communication skills, better organization, punctuality and a team-work oriented mindset. The student wishes to acknowledge ZIMVET management on allowing student internship programmes despite the high unemployment rate in the country that has forced many companies to suspend such programmes.

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RECOMMENDATIONS

1. My first recommendation is to my institution (NUST). I am recommending an inter-company rotation schedule for students on attachment whereby a student will not only be attached at a single company but will rotate on a number of companies that are in different fields. This will improve students' exposure to various industrial fields. For example one student could rotate from Parirenyatwa (Medical Institution), Nestle (Food Manufacturing), SIRDC (Research Institution) and Irvine's Zimbabwe (Agricultural Company-Poultry). The rotation schedule would be drafted by the school authority in association with the respective companies.

2. Installation of a perchloric acid fume hood/ Acid resistant hood

I am recommending for the installation of a specialized perchloric acid hood to handle hazardous reagents like perchloric acid and sulphuric acid. This is to try and minimize perchloric acid vapors that settle on duct work, resulting in the deposition of perchlorate crystals on surfaces which could to detonate on contact causing serious injury to personnel.

3. Inoculating Cabinet (Safety Hood)

I recommend for the installation of a safety hood in the bacteriology department for purposes of handling of specimens and cultures. The design of the cabinet should be such that air is drawn through the cabinet to a filter and then to an outlet funnel (by means of an extractor fan). The flow of air has the purpose of carrying infected particles and aerosols away from the environment of the operator to the outside atmosphere where they are at once diluted and destroyed by the ultraviolet light in the sun's rays. This will help in creating a bacterial and fungal-free atmosphere.

`Figure 4: **Inoculating cabinet**

4. Use of specific conversion factors

I recommend for the use of specific conversion factors rather than use of 6.25 for all feed types. The international conversion factor (6.25) is based on the average composition of amino acids in proteins in feedstuffs, found to be about16. The true factor can therefore vary between the individual feedstuffs. F = 6.25 for all forages, feeds and mixed feeds, F = 5.70 for wheat grains, and F = 6.38 for milk and milk products

5. Fat Reagent Blank

In the determination of fat, a reagent blank can be included in each run, to improve on quality control and the Quality control sample for fat determination can be changed every 6 months due to instability of fats.