

# ANALYTICAL MICROBIOLOGY

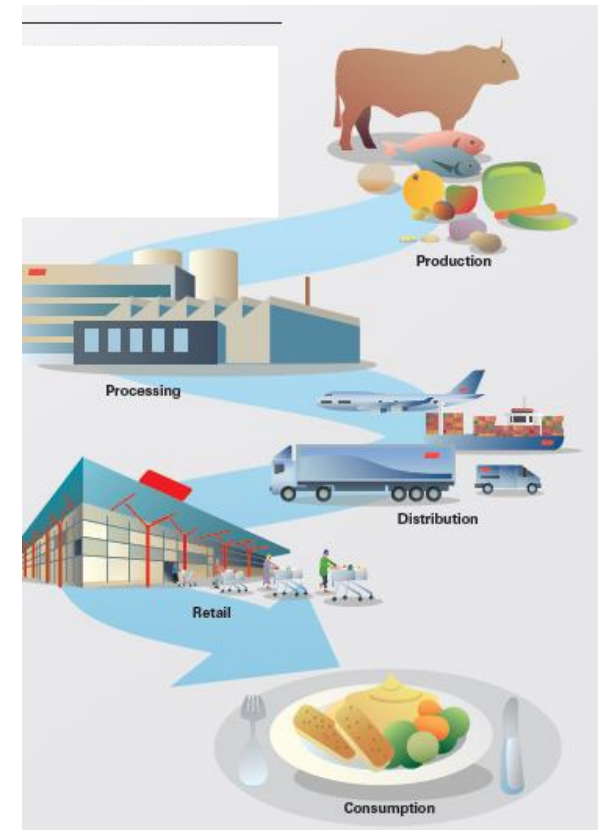
## QUALITY CONTROL IN MICROBIOLOGICAL LABORATORY

1. Microbiological specification and regulations
2. Local and international approaches to obtaining safe food
3. Management and quality assurance in the microbiology laboratory

Lecturer: Miss Elemba, O. M.

# Microbiological specification and regulations

- ❖ **Microbiological Criteria:** Microbiological criteria are used at any stage in the food chain to assess the acceptance of lots of raw material or finished product.
- ❖ They are based on the absence / presence of certain microorganisms or quantitative limits of these microorganisms, per unit(s) of mass, volume, area or lot.



# Three types of Microbiological criteria

**1. Microbiological Standards:** Mandatory microbiological criteria which are written into law or government regulations and specified by government to protect public health

**2. Microbiological Guidelines:** Microbiological criteria which provide advice to food manufacturers about acceptable or expected microbial levels when the food production process is under control when applying best practices.

**3. Microbiological Specifications:** Microbiological criteria established between buyers and producers that define product quality and safety attributes required by the buyer.

- a detailed description of requirements

Regulatory agencies and industrial assurance personnel regularly examine foods or ingredients for microorganisms or their metabolic products that may indicate

1. The presence of a pathogen or harmful toxin
2. The possibility that faulty practices occurred during production, processing, storage and distribution
3. The suitability of a food or ingredient for a desired purpose.

- Setting goals for public health is the right and responsibility of governments.
- These goals may specify the maximum number of harmful bacteria that may be present in a food.
- Where possible, the determination of this number should be based on scientific and societal factors.
- Costs may include industry costs for reformulation and changes in processing, consumer costs due to increased prices, or reduced availability of certain products, and regulatory costs in terms of surveillance.

## **Bodies with roles to play in food safety**

### **Government bodies**

#### **(a). The Federal Ministry of Health (FMOH)**

- ❖ The Federal Ministry of Health is responsible for the formulation of national policies, guidelines and regulations on food safety including monitoring and evaluation.
- ❖ It is also responsible for the assessment of the nutritive value of food, environmental sanitation, food environment and handlers, control of food borne disease, quality of public water from taps, as well as national and international matters relating to food.

## **(b) The National Agency For Food And Drug Administration and Control (NAFDAC)**

- ❖ NAFDAC is responsible for the regulation and control of the importation, exportation, manufacture, advertisement, distribution, sale and use of food, drug, cosmetics, medical devices, chemicals, packaged water and detergent at Federal and State levels in Nigeria.
- ❖ Appropriate tests are conducted and compliance with standard specifications for the effective control of the quality of food, bottled water and the raw materials as well as their production processes in factories and other establishments is ensured.
- ❖ The Agency undertakes appropriate investigations into production premises and raw materials for food and establishes relevant quality assurance systems including certification of the production sites and the regulated products and pronounces on the quality and safety of food, bottled water and chemicals.
- The role of the Agency also includes the inspection of imported food facilities to ascertain relevant quality assurance systems necessary for certification of the imported food product.

## **Standards Organization of Nigeria (SON)**

- The Standards Organisation of Nigeria is responsible for the formulation and enforcement of set standards on the composition of imported and locally manufactured food.
- responsible for investigating the quality of facilities, materials and products in Nigeria. They also establish a quality assurance system, including certification of factories, products and laboratories and *to promote consumer confidence and global competitiveness of Nigerian products and services through standardisation and quality assurance*
- The mandate of the Organisation includes preparation of Standards relating products, measurements, materials, processes and services amongst others and their promotion at National, Regional and International levels; certification of products, assistance in the production of quality goods and services; improvement of measurement accuracies and circulation of information relating to standards



- **The Federal Ministry of Agriculture and Rural Development (FMA&RD)**
- The Federal Ministry of Agriculture and Rural Development is responsible for formulating policies on primary agricultural production and practices which cover plants, animals, pests and diseases etc.; supervising and overseeing its departments and parastatals i.e. research institutes, colleges of agriculture, colleges of fisheries etc.

# Regulatory strategies USED BY NAFDAC

- ❖ The National Agency for Food & Drug Administration & Control (NAFDAC) is the regulatory authority in Nigeria with the mandate to regulate and control the manufacture, importation, exportation, advertisement, distribution, sale and use of food, drug, cosmetics, medical devices, chemicals, detergents and packaged water often referred to as regulated products.
- ❖ NAFDAC is the lead Agency for food safety and quality.

## **Regulatory strategies**

- a. **Product Registration** : The product registration process is one of the regulatory strategies of NAFDAC.

The Agency uses product registration to establish and monitor the ownership and/or distributorship of the products it regulates, generally known as regulated products (i.e. food, drug, cosmetics, medical devices, chemicals, detergents and packaged water); their safety; quality; labelling; claims etc.

NAFDAC employs a structured and systematic Regulation and Enforcement of Legislation on Food Safety in Nigeria at the end of which the product is assigned a NAFDAC Registration Number which is an attestation to the safety, quality and appropriateness for its intended use.

The registration process involves:

**1. Documentation:** Documents are required such as:

- ❖ Power of Attorney from the manufacturer authorizing an applicant to speak for his principal on all matters relating to the latter's specialties;
- ❖ Certificate of Manufacture and Free Sale which is an evidence that the product is manufactured and freely sold in the country of origin;
- ❖ Certificate of Incorporation of the representative company in Nigeria;
- ❖ Evidence of Trade Mark registration; Comprehensive Certificate of Analysis of the batch of product to be registered.
- ❖ The permit to import samples for registration purposes is issued if documentation is satisfactory.

**2. Labelling:** Labels should be informative, clear and accurate;

- ❖ Indicate the name of product;
- ❖ Name and address of the manufacturer, packer, distributor, importer, exporter, or vendor;
- ❖ Make provision for NAFDAC Registration Number; batch number, manufacturing date and expiry or best before date; net content,
- ❖ Ingredients list in metric weight in case of solids, semi solids and aerosols and metric volume in case of liquids.

**3. Inspection:** Good Manufacturing Practice (GMP) inspection of the production facility is carried out prior to registration of the product.

**4. Product Approval Committee Meetings:** A three (3) tier product approval meeting is held to consider the documentation, laboratory reports, GMP inspection reports, product labels etc. of a product prior to its registration.

- Once a product is satisfactory, it is assigned a NAFDAC Registration Numbers and can be freely sold or marketed within the country.

**b. Consultative Meetings:** NAFDAC encourages sectoral groups, small and medium scale entrepreneurs etc. to form umbrella associations. E.G.

- Association of Food, Beverage and Tobacco Employers (AFBTE);
- National Association of Small Scale Industrialists (NASSI);
- Association of Table Water Producers (ATWAP),
- Association of Fast Foods and Confectionaries

### **c. Public Enlightenment Campaigns:**

- The Agency organizes public enlightenment campaigns on topical and emerging issues using the electronic media, print media and physical presence .
- The Agency also uses television advertisements and radio jingles to inform and educate the public.



## d. Training and Publications

- NAFDAC organizes international, national and in-house capacity building training programmes consistently for staff , the industry and the general public.
- There are also collaborations and exchange programmes with credible regulatory authorities and international bodies such as the United States Food and Drug Administration (USFDA), US Department of Agriculture (USDA), International Atomic Energy Agency (IAEA), World Health Organization (WHO), Directorate General for Health and Consumers (DG SANCO) of the European Commission, African Union/Interafrican Bureau for Animal Resources (AU/IBAR) etc.
- The Agency produces informative news bulletins, pamphlets, magazines etc; such as the:
- “Consumer Safety” Magazine which not only offers technical information to the general public but also has a catch-them-young programme for schools through the Consumer Safety Club where NAFDAC educates members of the club on food safety issues and
- organizes annual essay competitions on selected food safety topics for member schools



Table 1: Guideline levels for determining the microbiological quality of ready-to eat foods

Test	Microbiological result (cfu/g unless otherwise stated)			
	Good	Acceptable	Unsatisfactory	Potentially hazardous
Standard Plate Count				
Category A	<10 <sup>4</sup>	<10 <sup>5</sup>	≥10 <sup>5</sup>	N/A
Category B	<10 <sup>6</sup>	<10 <sup>7</sup>	≥10 <sup>7</sup>	N/A
Category C	N/A	N/A	N/A	N/A
Indicators				
<i>Enterobacteriaceae</i>	<10 <sup>2</sup>	10 <sup>2</sup> to <10 <sup>4</sup>	≥10 <sup>4</sup>	N/A
<i>E. coli</i>	<3	3 to <10 <sup>2</sup>	≥10 <sup>2</sup>	N/A
Pathogens				
Coagulase +ve staphylococci	<10 <sup>2</sup>	10 <sup>2</sup> to <10 <sup>3</sup>	10 <sup>3</sup> to <10 <sup>4</sup>	≥10 <sup>4</sup>
<i>C. perfringens</i>	<10 <sup>2</sup>	10 <sup>2</sup> to <10 <sup>3</sup>	10 <sup>3</sup> to <10 <sup>4</sup>	≥10 <sup>4</sup>
<i>B. cereus</i>	<10 <sup>2</sup>	10 <sup>2</sup> to <10 <sup>3</sup>	10 <sup>3</sup> to <10 <sup>4</sup>	≥10 <sup>4</sup>
<i>V. parahaemolyticus</i>	not detected in 25 g	If detected then as per below		
	<3	3 to <10 <sup>2</sup>	10 <sup>2</sup> to <10 <sup>4</sup>	≥10 <sup>4</sup>
<i>Campylobacter</i> spp	not detected in 25 g			detected in 25 g
<i>Salmonella</i> spp.	not detected in 25 g			detected in 25 g
<i>L. monocytogenes</i>				
Food Group 1	not detected in 25 g			detected in 25 g
Food Group 2	not detected in 25 g	detected but <10 <sup>2</sup>		≥10 <sup>2</sup>
Food Group 3	not detected in 25 g	detected but <10 <sup>2</sup>		≥10 <sup>2</sup>

potential action for each of the categories of microbiological quality is presented in Table 2.

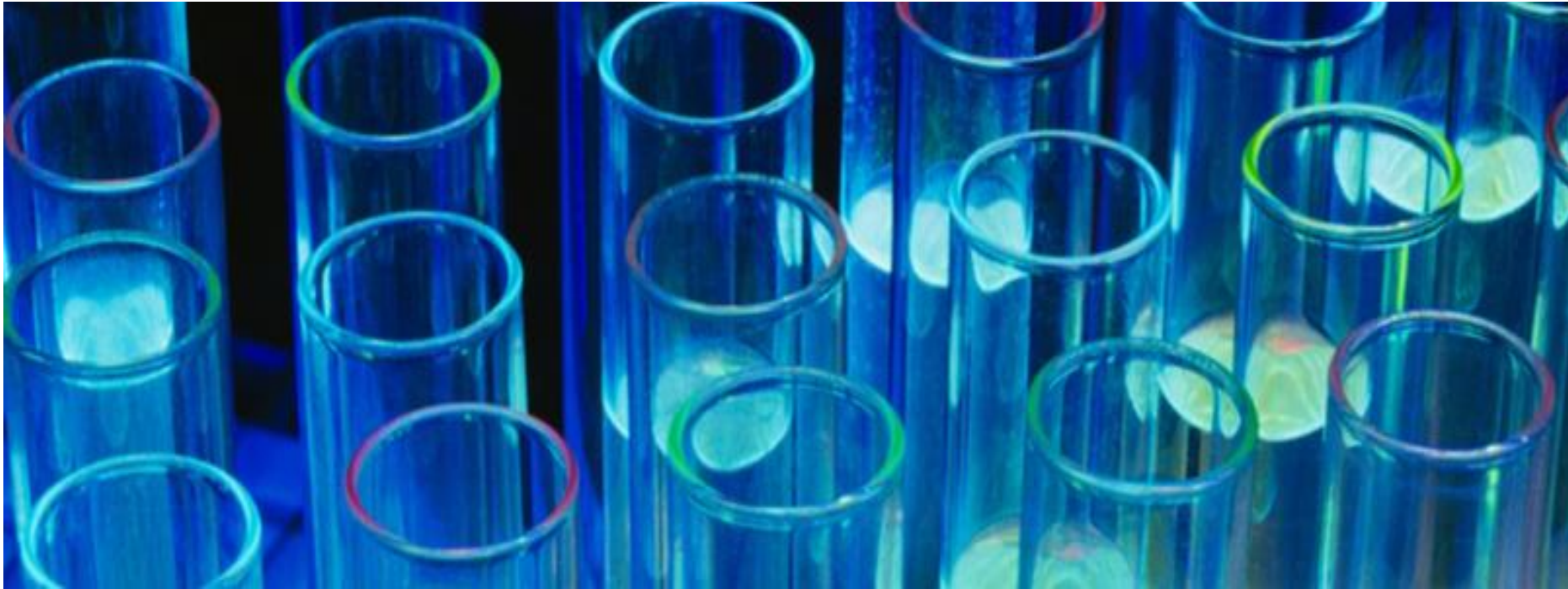
**Table 2: Potential action based on the microbiological category of ready-to-eat foods**

	<b>Result category</b>	<b>Interpretation</b>	<b>Action</b>
<b>Pass</b>	Good	Results are within expected microbiological levels for this type of product (lower range) and present no food safety concern	None
	Acceptable	Results are within expected microbiological levels for this type of product (upper range) and present no food safety concern	None
<b>Fail</b>	Unsatisfactory	Results are outside the expected microbiological levels for this type of product, present no food safety concern, but might indicate poor food handling practices	Further samples are taken for testing. If these return good or acceptable results no action is taken. If these return unacceptable results the business is inspected to determine if food handling controls and hygiene practices are adequate. A product withdrawal may be considered while further testing occurs.
	Potentially hazardous	Results are outside of the expected microbiological levels for this type of product and present a potential food safety concern	Inspect supplier to determine if food handling controls and hygiene practices are adequate; consider withdrawal of the product from sale

- Generally, Salmonella and E. coli O157 should not be present in any ready-to-eat product

# method used to estimate the risk of foodborne illness

- In many countries, governments rely on disease and food surveillance data in combination with expert advice on epidemiology, food microbiology and food technology to evaluate which types and numbers of harmful microorganisms in foods will cause disease. The level of risk can be expressed in a qualitative way (e.g., high, medium or low risk), or when possible, as the number of cases of foodborne disease per number of people per year. Particularly in developing countries, disease surveillance data are limited or not available at all. In such instances, estimates of the risk level have to be based on clinical information available (e.g., how many stool samples have been found to contain salmonellae) in combination with results from microbiological surveys of foods, evaluations of the types of foods that are produced, how they are produced and how they are stored, prepared and used. A few countries may use scientific techniques such as Quantitative Microbiological Risk Assessment (QMRA) to estimate the risk of illnesses using detailed knowledge of the relationship between the number of microorganisms in foods and the occurrence of foodborne diseases.
- Whatever method is used to estimate the risk of foodborne illness, the next step is to decide whether this risk can be tolerated or needs to be reduced. The level of risk a society is willing to accept is referred to as the “Appropriate Level Of Protection” (ALOP)



# Management and quality assurance in the microbiology laboratory

**LECTURER: Miss Elemba, O. M.**

**MCB 406**

**DEPT. OF BIOLOGY/MICROBIOLOGY/BIOTECHNOLOGY  
FEDERAL UNIVERSITY NDUFU ALIKE IKWO. EBONYI STATE**

# WHAT IS QUALITY?

## QUALITY ASSURANCE?

Quality means meeting the pre-determined requirements of users for a particular substance or service.

Quality includes the following :

- (a) Total Quality Management (TQM)
- (b) Continuous Quality Improvement (CQI)
- (c) Quality Assurance (QA)

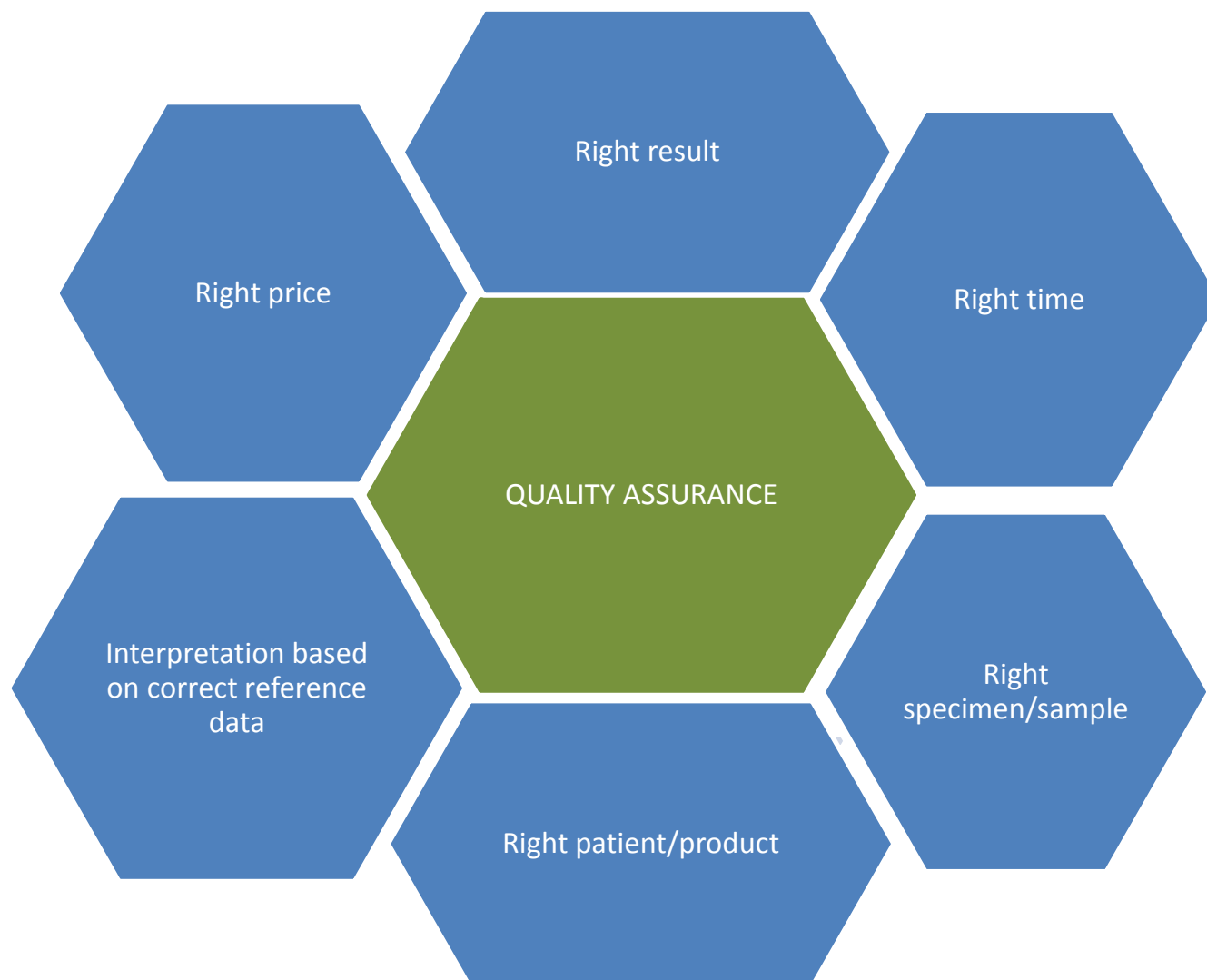
**Quality assurance** has been defined by WHO as:

“The total process whereby the quality of laboratory reports can be guaranteed.”

It includes group of activities carried out in order to ensure the validity of test results



It has been summarized as the: right result, at the right time, on the right specimen, from the right patient, with the result interpretation based on correct reference data, and at the right price



# Quality Management

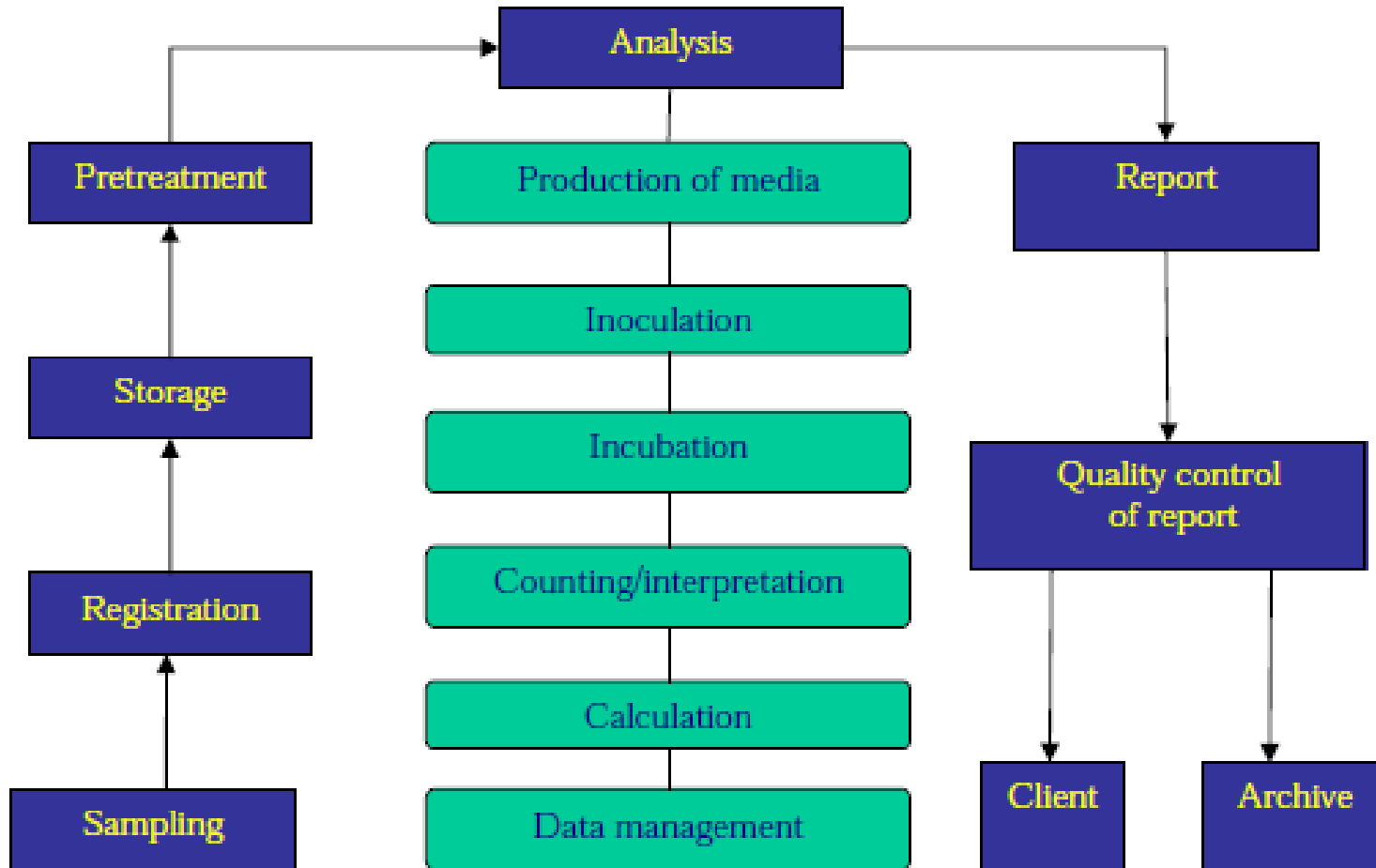
- ❖ Quality management is defined in ISO 9000: as coordinated activities to direct and control an organisation with regard to quality.
- ❖ Quality management generally includes establishment of a quality policy with quality objectives, and quality planning, quality control, quality assurance and quality improvement.
- ❖ It describes both management requirements and technical requirements.

## Approach to quality management in microbiology laboratories

A common approach for implementation of a practical QA/QC system is “the 5D’s”.

1. **Decide** where it is relevant to perform quality management
2. **Describe** who does what, how and when
3. **Do** what is decided and described
4. **Document** what has actually been done
5. **Deem** whether procedures and practices give the desired results and make improvement, if necessary

## 1. Decide



Example of a flowchart describing the steps of a microbial analysis.

## 2. Describe

- ❖ When the steps requiring an operational procedure have been decided, the procedures should be written as short and clear as possible without missing any points
  
- ❖ It is suggested that the procedures follow a common format, which includes at least the following:
  1. A unique title
  2. The purpose of the procedure
  3. The process
  4. Responsibilities
  5. Name of the author and the approving person
  6. Date of approval, date of expiry and edition

### **3. Do**

- This part is quite simple: You just have to do what was decided and described.

### **4. Document**

- Document what has actually been done. This requirement is included for at least four reasons:
  1. It provides a tool for identifying errors and thereby preventing the same errors to take place in the future work.
  2. It enables the company to perform internal audit to verify that the actions to be taken were actually taken.
  3. It enables audit to be done by an independent third party, if necessary.
  4. In case of complaints, or if unusual results have been obtained, the laboratory can control and prove that the quality of the analysis is sufficient and the results are reliable.

# Phases of Quality Assurance.

- Quality assurance has been defined by WHO as the total process whereby the quality of laboratory reports can be guaranteed.
- Laboratory quality assurance: A group of activities carried out in order to ensure the validity of test results

## Quality control (QC)

- The term QC covers that part of QA, which primarily concerns the control of errors in the performance of tests and verification of test results.
- QC must cover all aspects of every procedure within the department.
- It must be *practical, achievable, and affordable*.
- *All materials* equipment and procedures must be adequately controlled.
- Culture media must be tested for sterility and performance ion is increasingly alarming in some sections of the community,
- In summary, QC is Continual monitoring of working practices, equipment & reagents so as to detect & correct defects

- Microbiological investigations are important in the diagnosis, treatment, and surveillance of infectious diseases and policies regarding the selection and use of antimicrobial drugs.
- It is, therefore, essential that test reports are relevant, reliable, timely, and interpreted correctly.
- High cost of culture media and reagents,
- lack of rational approach to the selection and use of microbiological investigations, and
- A shortage of trained technical staff and microbiologists are important factors in preventing the establishment of essential quality control in developing country laboratories

## Standard operating procedures (SOPs)

- Each laboratory must have SOPs, sometimes referred to as the local laboratory bench manual. It is required for the following reasons:

a) to improve and maintain the quality of laboratory services and identify problems associated with poor work performance.

b) to provide laboratory staff with written instructions on how to perform tests consistently to an *acceptable standard in the laboratory*.

c) to help avoid short-cuts being taken when performing tests.

d) to provide written standardized techniques for use in the training of laboratory personnel.

e) to facilitate the preparation of a list and inventory of essential reagents, chemicals and equipment.

f) to promote safe laboratory practice.



- The QC/QA program must ensure optimization and result integrity throughout the 3 stages/phases/processes:

1. Pre-analytical

2. Analytical

3. Post-analytical

# 1. Pre-analytical

## (a) Specimen collection:

- The material must be from the **actual site of infection**.
- **It** should be properly collected with minimum chances of contamination.
- It should be in adequate sized sterile container. E.g. Pus should be collected from the inflamed area near the margins of the abscess and should not be collected from the centre of the abscess where the dead and necrotic material is likely to be there.
- **Optimal time of Collection of sample must be established to provide the best** chance of recovering the causative micro-organism from the specimen. E.g. In typhoid fever the blood culture is recommended to be done in the first week of fever.
- The WIDAL test should be done in the end of second week of fever. The specimen should be collected before the administration of any antibiotic. If the patient is on antibiotics then the specimen should be collected before the next dose of antibiotic is administered.

## **(b) Specimen container**

- **Appropriate collection devices and specimen containers should be used for the collection of specimen.**
- All containers used for collection of culture specimen should be sterile.
- The handling of the containers, while collection of the specimen, should also be such that the sterility of the container is maintained at all times.
- Labeling of the specimen should be proper to ensure there is no mixing up of specimen.

## **(c) Culture media**

- Proper selection of culture media should be made to ensure that the pathogenic organisms are isolated from the specimen.
- Fastidious organisms like Streptococci and Meningococci may require blood agar and chocolate agar to be used for Isolation.

**(d) Specimen transportation:** The primary objective of the transport of diagnostic Specimen is to maintain the sample in as near its original state as possible.

- If prolonged delay is expected before the specimen can be processed, it is generally preferable to freeze the specimen at  $-70^{\circ}\text{C}$ .
- Freezing at  $-20^{\circ}\text{C}$  may be used for many specimens if the period of storage is brief.
- **Transport media:** Some transport media's are available for microbiology specimen e.g. Stuart's media, Cary-Blair media.
- **Specimen receipt and Preliminary observations:** Initial observation and handling of specimen should be performed carefully.

- While handling the specimen universal safety precautions should be observed at all times. Personal protective equipment like gloves and masks should be worn whenever necessary.

The acceptance of specimens includes the following:-

- Documentation of essential data in a log book
- Visual examination of the specimen for adequacy.
- Samples which do not meet the acceptance criteria should be rejected.
- For example saliva is rejected when sputum sample is supposed to be collected.
- A well formed stool is not the proper sample for hanging drop preparation to look for darting motility of suspected *Vibrio cholera* Bacteria.

- Rejection of inappropriate specimen includes:
- Submission of contaminated specimens
- Delay in specimen delivery
- Viral culture without transport media
- Collection of specimens from inappropriate body sites
- specimen container leaking,
- Specimen in wrong medium, Non-sterile container for culture

## 2. Analytical

- Analytical phase includes the following:-

**(a) Training and re-training of the staff: The quality system is only as good as the staff who** actually work with it. No matter how good the quality system is on paper, if the theory cannot be translated into practice, quality cannot be achieved.

- Training of the staff is essential to achieve the goals of the quality system.
- The training must include an understanding of the importance of quality.
- Post training support is also essential to ensure continued competence of the staff.

**(b) Microscopic examination of specimen: The microscopic examination of** the clinical specimen is done to assess the presence of pathogenic bacteria,. It may also be used to assess the suitability of the specimen for acceptance or rejection.

**(c) Processing of specimen:** The proper processing of microbiology specimen includes:

- The proper selection of culture media,
- maintaining the optimal temperature and atmosphere of incubation and
- proper characterization of the isolated pathogen by appropriate biochemical reactions and antibiotic sensitivity testing.

**(d) Monitoring and evaluation:** The laboratory management must develop and implement quality indicators to systematically monitor and evaluate laboratory's contribution to the patient care.

- Assessment of quality through audits (Internal or External) is a must.
- The laboratory must participate in an External quality assurance program.
- It is also possible to do inter – laboratory comparisons of test results. Internal quality is also essential to evaluate the technician competence and the performance of automated equipment.



- The following should be incorporated in the microbiological SOPs covering the analytical stage:
  - a) Detailed procedure for examining different specimens.
  - b) Staining techniques and QC of stains.
  - c) Aseptic techniques and safe handling of infectious material.
  - d) Preparation and QC of culture media and preservation of stock strains.
  - e) Inoculation of liquid and solid media.
  - f) Reading and interpretation of cultures.
  - g) Techniques used to identify pathogens.
  - h) Antimicrobial sensitivity testing and QC of procedures and antibiotic discs.
  - i) Cleaning and QC of equipment used in microbiology laboratory.
  - j) Immunologic techniques and QC of antigen and antibody reagents.
  - k) Safe working practices.
  - l) Disposal of specimens and cultures.
  - m) Cleaning of glassware, plastic ware, etc.
  - n) Sterilization procedures and their control. *Control of stains and reagents* All stains and reagents must be clearly labelled, and dated

### 3. Post - analytical

**a) Reporting of results:** Reports of microbiology culture results should be issued as soon as useful information becomes available.

- Each laboratory must establish those results that will be considered “Urgent” or “critical”.
- In addition some results may be considered as important but not necessarily urgent.

**b) Analysis of results:** It is incumbent on the laboratory Head to provide feedback to the clinician on some parameters of laboratory performance.

- Studies on the Turn around time (TAT) and anti microbial susceptibility patterns is helpful to the clinicians.

The terminology and format used in reporting should be standardized and agreed between laboratory personnel and clinicians.

- Any preliminary report must be followed by a full written report.

- All reports must be checked for correctness and clarity and signed by head of the department.
- Report distribution and delivery systems must be efficient and urgent reports should be telephoned at all the significant stages
- Appropriate steps must be taken to ensure confidentiality of reports both in the laboratory and during transfer.
- Those receiving the reports should consult the laboratory when any part of the report is not clear.
- There must be effective communication between those requesting tests and laboratory staff.
- Microbiologist should be prepared to give advice on the type of investigations that might be helpful in the diagnosis and be prepared to advise on antibiotic
- treatment.

## **Benefits of Quality assurance programs include the following**

- Production of quality products and reliable services.
- Motivation factor for the staff to work better.
- Creation of good reputation for the laboratory.
- Prevention of legal suits and associated complications