PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Isolation and Identification of Microorganisms	**Effective Date:**
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1. **Purpose:** To lay down the procedure for Isolation and Identification of Microorganisms.

2. **Scope:** This Standard Operating Procedure is applicable at Microbiology section of Quality Control department.

3. **References & Annexures:**

   3.1 **References:**

   3.1.1 NA

   3.2 **Annexures:**

   3.2.1 Annexure-1 : Identification of Microbial Isolates

   3.2.2 Annexure-2 : Preservative of Isolates Record

   3.2.3 Annexure-3 : Growth Promotion Test of Isolates

   3.2.4 Annexure-4 : Colony Morphology, Shape & Cell Arrangement of Microorganism

4. **Responsibilities:**

   4.1 **Microbiologist / Executive - Quality control.**

   4.1.1 Responsible for the isolation and identification of microorganisms.

   4.1.2 To maintain all the records as per SOP.

   4.2 **Quality Control (QC) Head.**

   4.2.1 Ensure proper control and compliance of the SOP.

   4.3 **Quality Assurance (QA) Department:**

   4.3.1 To review the SOP.

   4.3.2 To ensure the implementation of SOP.

   4.4 **Regulatory Affairs, Quality Head and Plant Head :**

   4.4.1 To review and approve new or revised SOP's.

5. **Distribution:**

   5.1 **QC**

   5.2 **QA**

6. **Abbreviations and Definition of Terms:**

   6.1 **Abbreviations :**

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6.1.1 CC : Change Control
6.1.2 cfu : Colony forming units
6.1.3 ºC : Degree Celsius
6.1.4 H_2S : Hydrogen Sulphide
6.1.5 IPA : Isopropyl alcohol
6.1.6 KOH : Potassium Hydroxide
6.1.7 LAF : Laminar Air Flow
6.1.8 MR : Methylene Red
6.1.9 NA : Not Applicable
6.1.10 No. : Number
6.1.11 QA : Quality Assurance
6.1.12 QC : Quality Control
6.1.13 SCDA : Soyabean Casein Digest Agar
6.1.14 SDA : Sabouraud Dextrose Agar
6.1.15 SOP : Standard Operating Procedure
6.1.16 TSI : Triple Sugar Iron
6.1.17 VP : Voges Proskauer
6.1.18 v/v : Volume by Volume
6.1.19 % : Percentage
6.1.20 +ve : Positive
6.1.21 -ve : Negative

6.2 **Definition of Terms :**

6.2.1 **Standard Operating Procedure (SOP):** A written authorized procedure, which gives instructions for performing operations.

6.2.2 **Colony forming unit (cfu):** Visible outcome of growth of micro-organisms arising from a single or multiple cells.

7. Procedure:

7.1 **Isolates from Water and Environment**

7.1.1 Isolate all the morphologically different colonies and identify them up to the genus preferably up to species level. Create a database (if required) for the cultures obtained from the water and Environment.

7.1.2 Update the data base as and when a new isolate is identified.

7.1.3 Identify the predominant flora when the total microbial counts exceed alert and action limits in water or Environment. The characterization of the predominant flora shall be useful in investigating the excursions.

7.1.4 Due to the physiological stress on the isolates, the organisms inherently may show up slightly different morphology. In this context, if an isolate is found morphologically different, the isolate shall be picked, and take a photograph of colony for colony shape, elevation and Edge refer Annexure-4 (but not limited to) and identified. In case this organism is already identified (repeat isolate), then the database shall be updated accordingly, but the culture need not be preserved.

7.1.5 Verify the growth promotion ability of the media using the isolates initially and record in Annexure- 3.

7.1.6 The identified isolates shall be preserved for a period of one year using the seed lot technique and maintain the Annexure-2.

7.1.7 Allot the number of seed lots of environment and water isolates as below.

ISLCYYXXX

Where:

I – Isolates

SLC – Seed Lot Culture

YY- Last Two Digits of current year

XXX- Serial No. (Start from 001)

For Example: ISLC14001

7.2 **Isolates from the non-sterile products (Oral Solid Dosage Forms)**

7.2.1 Isolate and identify the colonies if the total microbial counts exceed the action limits (Out of Specification results) in case of the finished products.

7.2.2 Evaluate the isolates, summarize the identification profile and the recommendations to the Quality Assurance through an addendum to the OOS document.

7.2.3 In case any of the isolates is identified as Specified Microorganisms, notify the Quality Assurance and Production department.

7.2.4 Verify the growth promotion ability of the media using the Isolate initially and record in Annexure- 3.

7.2.5 The identified isolates shall be preserved for a period of one year using the seed lot technique and maintain in Annexure- 2.

7.2.6 Allot the numbers of isolates as per point no. 7.1.7

7.3 In case of fungi obtained from monitoring, these isolates may not be identified always. However, the fungal isolates shall be used to verify the growth promotion ability of the media used for monitoring.

7.4 **Isolation procedure**

7.4.1 Media, Chemicals and Reagents.

(A) Media:

- Nutrient broth
- Simon's Citrate agar
- Nitrate broth
- Urea Agar Base (Christensen)
- Triple Sugar Iron Agar
- Phenol Red Lactose Broth
- Phenol Red Mannitol Broth
- Phenol Red Sucrose Broth
- Phenol Red Glucose Broth
- Starch Agar
- Nutrient Gelatin Medium
- Skim Milk Agar

(B) Chemicals and Reagents:

- Gram Staining Kit
- Malachite Green Staining Reagent
- Kovac's Reagent
- Methyl Red Solution
- Alpha napthol, followed by 1 ml of 40% KOH
- Sulphanilic Acid and Alphanaphthalamine
- Zinc

- 3.0 % Hydrogen peroxide
- Oxidase disc
- Lacto Phenol Cotton Blue/Grocott Silver Stain/Hematoxylin/Eosin India ink

7.4.2 Pick the individual colonies from the previously incubated plates in which the growth is observed.

7.4.3 Prepare the suspension of colony in a 10ml of sterile peptone water.

7.4.4 Take a loopful of prepared suspension and streak on the pre incubated SCDA Plate.

7.4.5 Streak the suspension in such a way to get a single isolated pure colony with its original phenotypic expression. Inoculate the culture in to a slant also for seed lots preparation.

7.4.6 Incubate the plate at 30-35°C for 18-72 hours. In case of fungal isolates, incubate the plate for 5 days.

7.4.7 Plating of peptone water used for suspension should be done to confirm the sterility of the medium.

7.4.8 Note down the colony characters of the isolate after sub-culturing as per the specimen format given in Annexure-1.

7.4.9 **Identification of Bacterial Isolates (Morphological):**

A. Gram Staining:

- Conduct the orientation checks like Gram nature and the shape and arrangement of the cells on the isolate. Perform the Gram staining as below.
  - Prepare a thin smear of suspension on a clear, dry glass slide.
  - Allow it to dry and fix by gentle heat.
  - Flood with Gram's Crystal Violet for 1 minute. Wash with tap water.
  - Flood the smear with Gram's Iodine and allow it to remain for 1 minute.
  - Decolourize with Gram's Decolourizer until the blue dye no longer flows from the smear. Wash with tap water.
  - Counter stain with 0.5% w/v Safranin. Allow it to remain for 1 minute. Wash with tap water.
- Allow the slide to air dry or blot dry and examine under oil immersion objective.

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- Gram positive organisms should stain bluish purple colour.
- Gram negative organisms should stain pinkish red colour.
- Run the positive control during the Gram staining. For the positive control, use a reference culture of known Gram reaction.

B. Spore Staining:

- Take clean and dry glass slide and make it grease free by passing it through the flame for 3-4 times.
- Take the growth of organisms to be identified by means of sterile nichrome wire loop/pre sterile loop on the clean glass slide and add a drop of purified water on agar culture.
- Spread evenly on the centre of slide to prepare a smear.
- Allow to dry in air and gently pass it through flame 2-3 times for fixation of smear.
- Cover the smear with malachite green staining reagent.
- Heat the reagent gently by placing the slide on a warm hot plate or by exposing it to the flame of a Bunsen burner or waterbath.
- Allow the reagent to evaporate for 2 to 3 min, more reagent may be added to prevent drying.
- Gently rinse the slide with purified water and spread the smear with 0.5 % Safranine for 30 second.
- Wash the smear with tap water, and blot dry gently prior to microscopic examination

C. Shape:

Shape of bacteria may be of the following types.

- **Coccus.**
  - Cocci : Single spherical.
  - Diplococcic : Cocci that remain in pairs.
  - Streptococci : Remain in chains of cells.
  - Tetrad : Cocci remain in groups of four forming squares.
  - Sarcinae : Cocci that remain in groups of eight forming cubes.
  - Staphylococci : Cocci that remain in amorphous sheets or clumps.
- Bacillus.

- Bacilli: Single rods, various shaped bacteria like rod, tapered rod, staff, cigar, oval, curved.
- Diplobacilli (Paired rods) : Bacilli that remain in pairs.
- Streptobacilli : Bacilli that remain in chains of cells.
- Coccobacilli (Ambiguous designation) : A short Bacilli that nearly looks like a cocci.

■ Vibrio:

- Comma Shape.

■ Spiral:

- Spiral Shape.

D. Motility:

- Take a cover slip and put a small amount of petroleum jelly with the help of Sterile loop on each corner of the cover slip.
- Place one or two loop full of a 24 hours old broth culture in the center of the cover slip with the help of inoculating loop.
- The depression slide is placed on the cover slip with the depression over the drop of fluid and quickly inverts the slide/cover slip.
- Examine with low power focus (10X and 40 X) on the edge of the drop (Use a minimum amount of light with unstained specimens).

7.4.10 **Biochemical Identification.**

A. Indole Production.

- Prepare 1.0 % peptone water in test tubes and sterilize them by autoclaving at 121°C and 15 psi. for validated time.
- Aseptically inoculate the tube with the culture to be identified and incubate at 30-35°C for 48 hours.
- After incubation take the tube and add 1 ml Kovac's reagent (p-dimethyl aminobenzaldehyde) and allow to stand for one minute.
- Upon examination if a Pink or Red colour ring appears in the top layer of medium it indicates a positive test with the breakdown of Tryptophan to Indole formation and no formation of colour indicates the negative test.

B. Methyl Red Test (MR)

- Prepare a nutrient broth in test tubes as per SOP on media preparation and sterilize by autoclaving at 121°C and 15 psi. for validated time.

- Aseptically inoculate the tube with the culture to be identified and incubate at 30-35°C for 48 hours.
- After incubation take the tube and add few drops of Methyl Red solution to the culture tube and observe the result.
- Medium producing red colour indicates the MR positive test and no formation of red colour indicates the MR negative test.

C. Voges Proskauer Test

- Prepare buffered glucose broth in test tubes as per SOP and sterilize by autoclaving at 121°C and 15 psi. for validated time
- Inoculate tube with the culture to be identified and incubate at 30-35°C for 48 hours.
- After incubation, add approximately 3 ml of Alpha Napthol, followed by 1 ml of 40% KOH to the tube.
- Mix well and allow to stand for 15 minutes.
- Medium producing brick red colour indicates the positive VP test and no colour change indicate the negative VP test.

D. Citrate Utilization

- Prepare the Simmon's Citrate agar slants as per the SOP of media preparation.
- Streak Simmon’s Citrate agar slants with culture to be identified.
- Incubate plates at 30-35°C for 24-48 hours and observe after incubation.
- If initial green colour of media turns deep blue so it indicates the citrate utilization.

E. Nitrate Reduction

- Prepare the nitrate broth tubes as per the SOP of media preparation
- Take the sterile tubes of nitrate broth and inoculate with loop full of culture to be identified.
- Incubate tubes for 5 days at 30-35°C and observe after incubation.
- After incubation add 1 ml of Sulphanilic acid and 1 ml of Alphanaphtalamine in each nitrate broth culture tube.
- Reduction of nitrate to nitrite indicates by production of a red colour in culture tube.

- After addition of Sulphanilic acid and alphanaphtalamine, if there is no production of red colour, this indicates negative test.
- Negative test is confirmed by adding a few particles of Zinc; the appearance of a red colour indicates that nitrate is still present and not reduced by the organism. If the solution does not change colour the organism has reduced the nitrate through nitrite to nitrogen gas.

F. Urease Test

- Prepare Christensen's urea agar slant tubes as per the SOP for preparation and use of Microbiological Media.
- Take the sterile tubes of Christensen's urea agar slant and inoculate with an inoculating needle by streaking the slant.
- Incubate tubes at 30-35°C for 24 hours and observed after incubation.
- Positive organisms utilize urea and make the media alkaline, producing a pink-red colour.
- In case of negative test, urea agar slant and butt remains light orange.

G. Triple Sugar Iron Agar (TSI)

- Prepare the Triple Sugar Iron Agar slants for stabbing as per the SOP of Preparation and use of microbiological media.
- Take the sterile tubes of TSI and inoculate with an inoculating needle by first stabbing the butt and then streaking the surface of slant.
- Incubate these tubes at 30-35°C for 24 hours and observe after incubation.
- If the butt colour changes to yellow and slant colour remains red, indicates Glucose fermentation.
- If butt and slant colour changes to yellow this indicates Glucose, Lactose and/or Sucrose fermentation.
- If butt and slant colour remain yellow, indicates that no fermentation has taken place.
- H_2S production is indicated by the presence of a black precipitate in butt.

H. Lactose Fermentation

- Prepare the Phenol Red Lactose broth tubes as per the SOP for preparation and use of microbiological media.
- Take the sterile tubes of Phenol Red Lactose broth suspended with Durham’s tube.

- Inoculate Phenol Red Lactose broth tube with a loop full of culture to identify.
- Incubate these tubes at 30-35°C for 24 hours and observe after incubation.
- Lactose fermentation indicates if acid end product is produced and phenol red lactose turns yellow.
  (i) If gas is produced along with the acid, it collects in the Durham's tube as a gas bubble.
  (ii) If the Phenol Red Lactose broth remains unchanged and no acid or gas is produced, indicates no lactose fermentation has taken place.

I. Mannitol Fermentation

- Prepare the Phenol Red Mannitol broth tubes as per the SOP for preparation and use of microbiological media.
- Take the sterile tubes of Phenol Red Mannitol broth suspended with Durham's tube.
- Inoculate Phenol Red Mannitol broth tube with a loop full of the culture to identify.
- Incubate these tubes at 30-35°C for 24 hrs and observed after incubation.
- Mannitol fermentation indicates if acid end product is produced and Phenol Red Mannitol turns yellow.
- If gas is produced along with the acid, it collects in the Durham tube as a gas bubble.
- If the Phenol Red Mannitol broth remains unchanged and no acid or gas is produced, indicates no Mannitol fermentation has taken place.

J. Sucrose Fermentation

- Prepare the Phenol Red Sucrose broth tubes as per the SOP of preparation and use of microbiological media.
- Take the sterile tubes of Phenol Red Sucrose broth suspended with Durham's tube.
- Inoculate Phenol Red Sucrose broth tube with the culture to identify.
- Incubate tubes at 30-35°C for 24 hours and observe after incubation.
- Sucrose fermentation indicates if acid end product is produced and Phenol Red Sucrose turns yellow.
  (i) If gas is produced along with the acid, it collects in the Durham's tube as a gas bubble.

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(ii) If the Phenol Red Sucrose broth shall remain unchanged and no acid or gas is produced, indicates no Sucrose fermentation has taken place.

K. Glucose Fermentation

- Prepare the Phenol Red Glucose broth tubes as per the SOP of preparation and use of microbiological media.
- Take the sterile tubes of Phenol Red Glucose broth suspended with Durham's tube.
- Inoculate Phenol Red Glucose broth tube with a loop full of the culture to identify.
- Incubate tubes at 30-35°C for 24 hours and observe after incubation.
- Glucose fermentation indicates if acid end product is produced and Phenol Red Glucose turns yellow.

(i) If gas is produced along with the acid, it collects in the Durham tube as a gas bubble.

(ii) If the Phenol Red Glucose broth shall remain unchanged and no acid or gas is produced, indicates no Glucose fermentation has taken place.

L. Catalase Test

- Using a loop, pick a small growth of culture from a 24 hours old colony.
- Place the culture on a clean glass slide.
- Using a Sterile pipette or a dropper, place a drop of 3% Hydrogen peroxide (H_2O_2) over the organism on the slide and observe for immediate formation of bubble.
- In the positive reaction Catalase test gives immediate bubbles if no bubble formation has taken place it means negative reaction for Catalase.

M. Starch Hydrolysis

- Prepare the Starch agar plates as per the SOP of preparation and use of microbiological media.
- Take the sterile Starch agar plates and inoculate with the culture to identify by streaking line on the agar surface.
- Incubate tubes at 30-35°C for 24 hours and observe after incubation.
- After incubation, add iodine to the surface of growth and observe hydrolysis of starch.

- A positive reaction of starch hydrolysis indicates a yellow zone around the bacterial growth. If starch has not been hydrolyzed, the agar shall remain dark brown or blue/black in color.

N. Oxidase Test

- Take a sterile forceps and hold a test paper disc of the Oxidase (containing Tetramethyl-p-phenylenediamine dihydrochloride).
- With the help of forceps touch the test paper onto an area of heavy growth to be identified.
- Observe the result immediately. Positive Oxidase test shows change of colour from colourless to purple.
- If the colour change takes place with in 10 seconds test is positive, if colour change takes place with in 10 - 60 seconds it is delayed positive and more than 60 seconds it is negative.

O. Gelatin Liquefaction Test

- Prepare sterile Nutrient Gelatin stabbing tubes.
- Inoculate the tubes by stabbing of culture with the help of an inoculating needle.
- Incubate tube at 20-22°C and alternatively, incubate at 30-35^{0}C and then transfer the tube to a refrigerator or into cold water before observation.
- In positive test liquefaction of Nutrient Gelatin medium takes place and in negative test medium remains solid.

P. Protein Hydrolysis (Casein)

- Prepare the Skim Milk agar plates as per the SOP of preparation and use of microbiological media.
- Inoculate Skim Milk agar plates with the culture using an inoculating needle.
- Incubate these plates at 30-35°C for 48 hrs and observed after incubation.
- If casein is hydrolyzed, there shall be a clear zone around the bacterial growth.
- If casein is not hydrolyzed, the agar shall remain white and opaque.

7.4.11 Note down the observations as per the specimen format given in the Annexure-1.

7.4.12 Identify the isolate using a suitable identification method.

7.4.13 Preserve the culture on a slant or plate till the identification is obtained.

7.4.14 Record the results of identification in Annexure-1.

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7.5 **Identification of Fungal Isolates**

7.5.1 Note down colony characteristics of fungal colonies developed on the media plates.

7.5.2 Perform staining of the colonies using Lactophenol Cotton Blue as below:

- Place a drop of Lactophenol Cotton Blue reagent on a clean and dry slide.
- By using an inoculating wire, carefully tease the fungal culture in to a thin preparation.
- Place a cover slip on the preparation and wait for 5 minutes.
- Observe under microscope with low power for hyphal arrangement and morphology.

7.5.3 Based on the Microscopy, identify the organism up to genus level by comparing it with characteristics given in the Annexure-4 (but not limited to).

7.5.4 Record the results of identification in Annexure-1.

7.6 **Extent of Characterization of Isolates**

7.6.1 In all the above cases, the microbial isolates shall be identified based on the phenotypic characteristics as listed below.

- Culture- Colony morphology, colour, shape and size, pigment production
- Morphology- Cellular morphology, Gram reaction.

7.6.2 In case of any of the below cases, the isolates shall be identified by genotypic methods based on the investigation need.

7.6.3 Any market complaint related to microbial quality of the product

7.6.4 Significant adverse trends in environmental and water monitoring.

7.6.5 To support any investigation related to the product quality.

7.7 **Maintenance of Isolates**

7.7.1 Follow the procedure detailed under ‘Seed lot preparation’ in the current version of BSQM/032 for revival and maintenance of the seed lots.

7.7.2 Revive seed lots of environmental and water isolates as and when required.

7.8 **Frequency**

7.8.1 Identification of environmental and water isolates on Half yearly basis and if any new isolate is observed, GPT shall be conducted.

7.9 In case any of the isolates is identified as Specified Microorganisms, notify the Quality Assurance and Engineering department.

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Note: After isolation and identification by biochemical method, prepared the slant and send to other authorized laboratory for the species level study.

Annexure -1

IDENTIFICATION OF MICROBIAL ISOLATES

A. Organism details:

Source	
Location	
Date of Isolation	
Date of Testing	
Laboratory code No.	

B. Preliminary Observation:

Test	Detail of observation
Name of Media	
Growth Temperature	
Colony color	
Colony shape	
Colony size	
Fluorescence	
Surrounding zone	
Other	

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C. Photograph

Plate:-
Colony:-

D. Identification Details of Bacteria.

1.0 Morphological characteristics:

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Test	Detail of observation
Gram's staining	
Spore staining	
Cell shape	
Cell size	
Arrangement	
Motility	
Mycelium	
Cell Photograph	

2.0 Biochemical characteristics:

S.No.	Test	Results
1	Indole	
2	Methyl Red (MR)	
3	Vogus Proskauer (VP)	
4	Citrate utilization	
5	Glucose fermentation	
6	Lactose fermentation	
7	Sucrose fermentation	

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S.No.	Test	Results
8	Mannitol fermentation	
9	Nitrate reduction	
10	Urease	
11	Triple sugar Iron	
12	Catalase	
13	Starch utilization	
14	Oxidase	
15	Casein utilization	
16	Gelatin utilization	

2.1 Indole Production

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

2.2 Methyl Red (MR)

Date of Testing_____

Media used _____Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

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2.3 Vogues Proskauer (VP)

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results:_____

2.4 Citrate utilization

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

2.5 Glucose fermentation

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

2.6 Lactose fermentation

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

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2.7 Sucrose fermentation

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

2.8 Mannitol fermentation

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

2.9 Nitrate reduction

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

2.10 Urease production

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

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2.11 Triple Sugar Iron Agar

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

2.12 Catalase

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

2.13 Starch utilization

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

2.14 Oxidase

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

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Results :_____

2.15 Casein utilization

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

2.16 Gelatin utilization

Date of Testing_____

Media used_____ Media Lot No._____

Incubation: Time : From_____ To_____ Temp_____

Incubator Used ID No.:_____

Observation:_____

Results :_____

D. Conclusion:

Analysed By
(Sign/ Date)

Checked By
(Sign./ Date)

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Annexure -2

PRESERVATIVE OF ISOLATES RECORD

S.No.	Name of Isolate	Source of Isolate	No. of Isolates	Date of Isolate	Preserve on	Use before	Done By	Checked By

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Annexure -3

GROWTH PROMOTION TEST OF ISOLATES

Date of Testing	
Name of Isolates	
Name of Media	
Media Sterilization Cycle No	
No. of Cells Inoculated (10-100 cfu)	
Temprature of Incubation	
Incubator ID	
Test Performed By	
Compled on	

Results

Plate I	Plate II	Average	% Recovery

Remarks:

Performed By:
Date:

Checked By:
Date:

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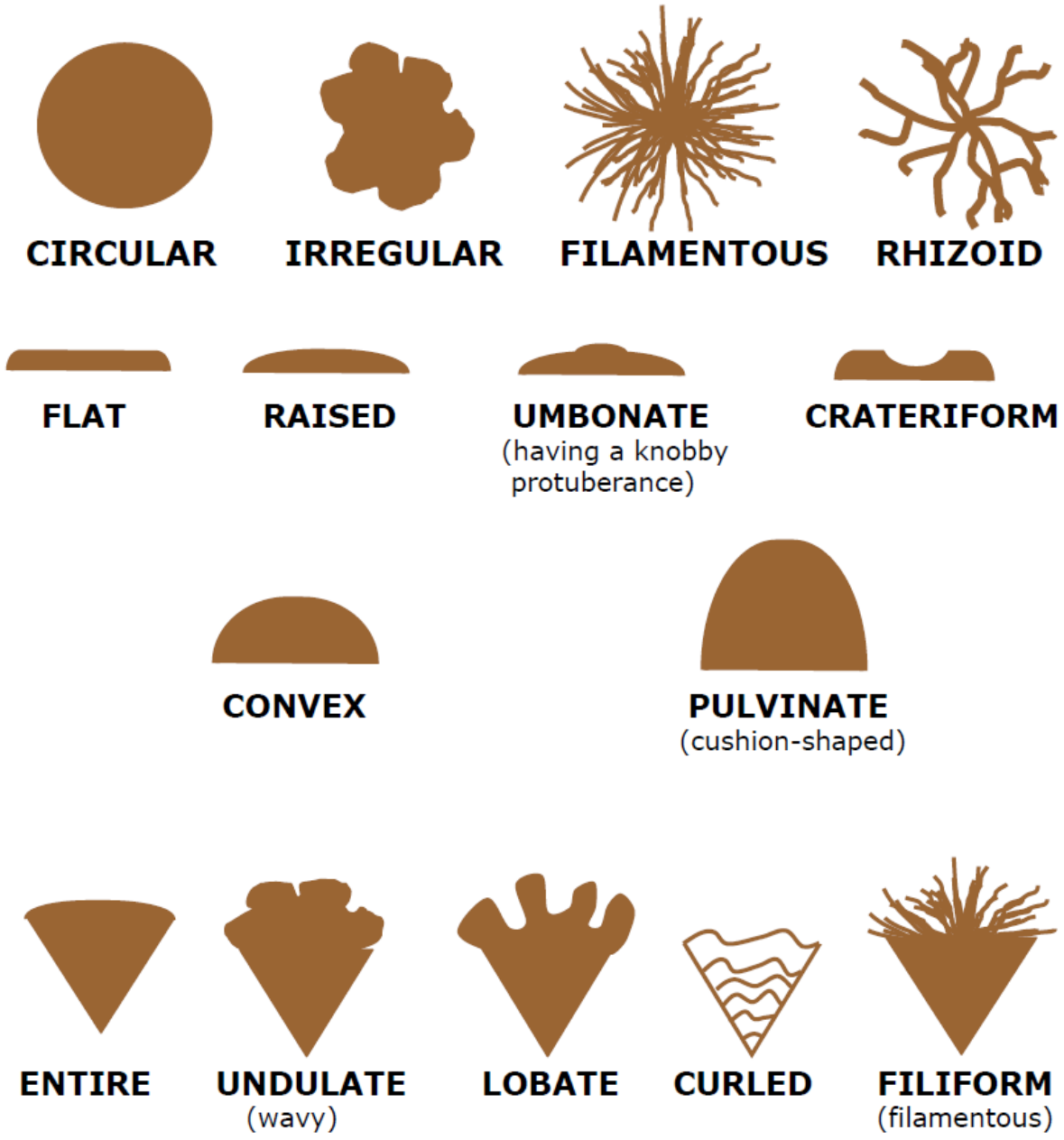
Annexure -4

COLONY MORPHOLOGY, SHAPE & CELL ARRANGMENT OF MICROORGANISM



PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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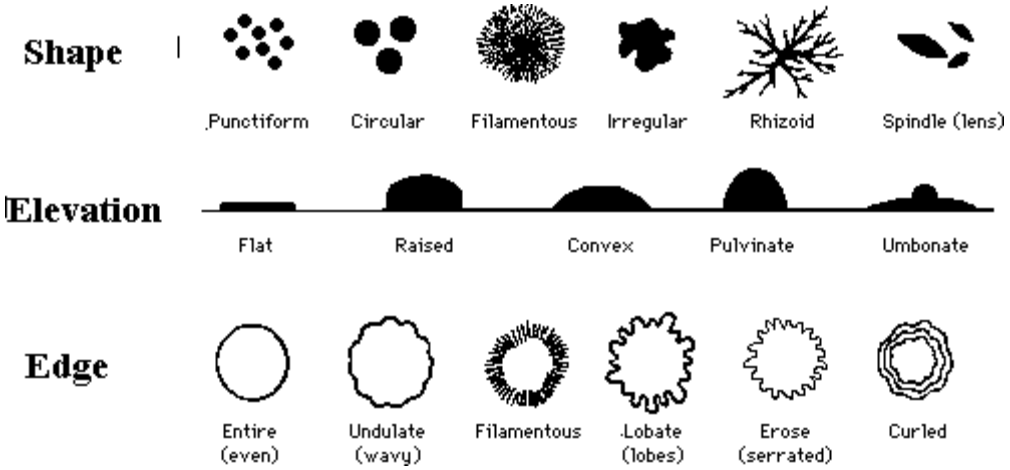
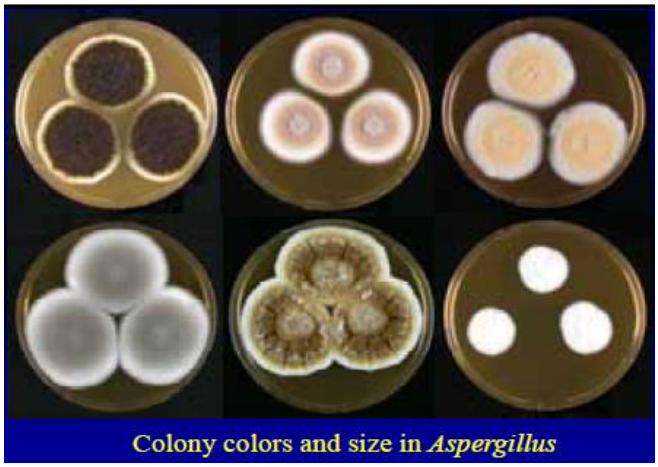
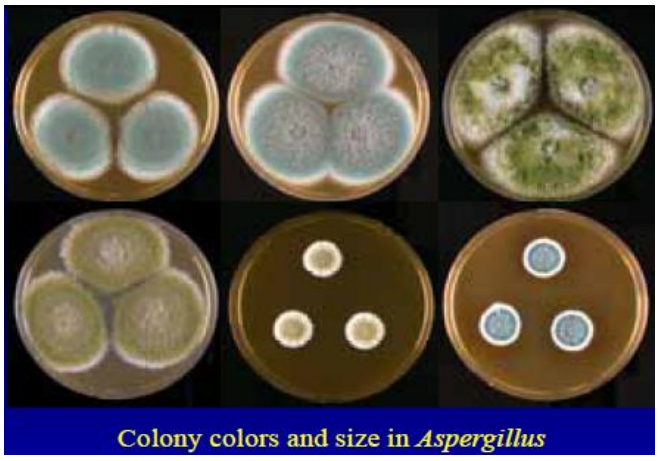
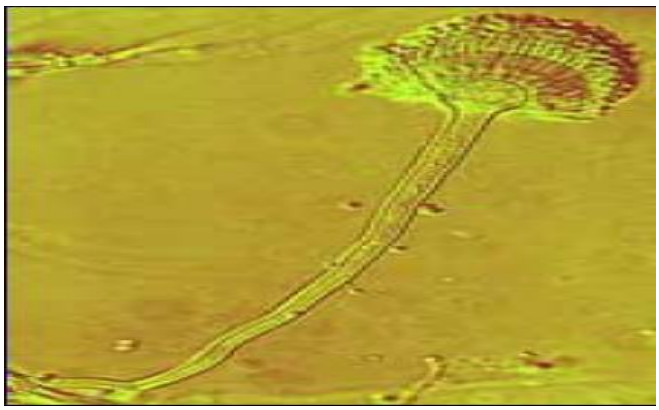


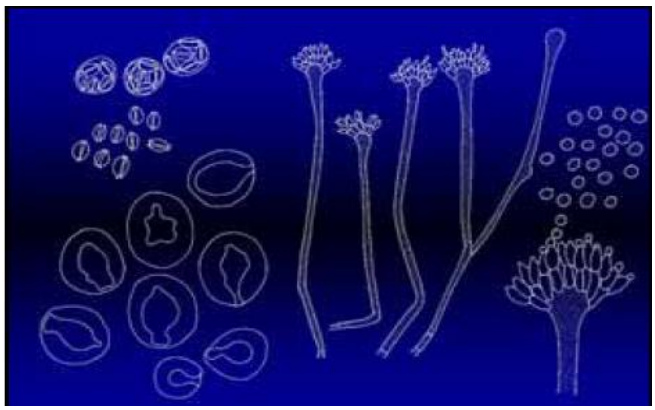


Colony colors and size in *Aspergillus*



Colony colors and size in *Aspergillus*





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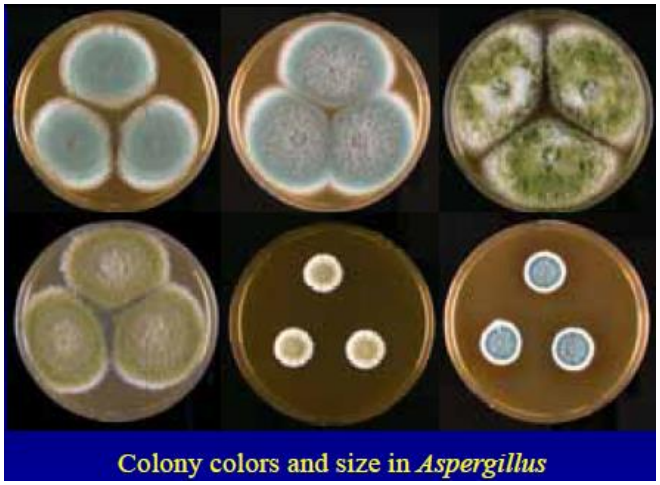
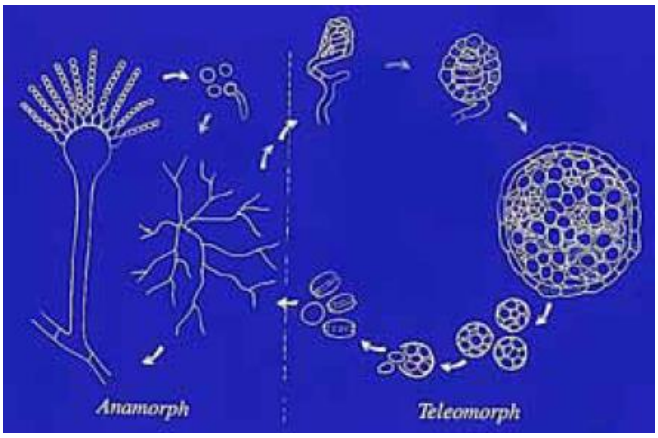
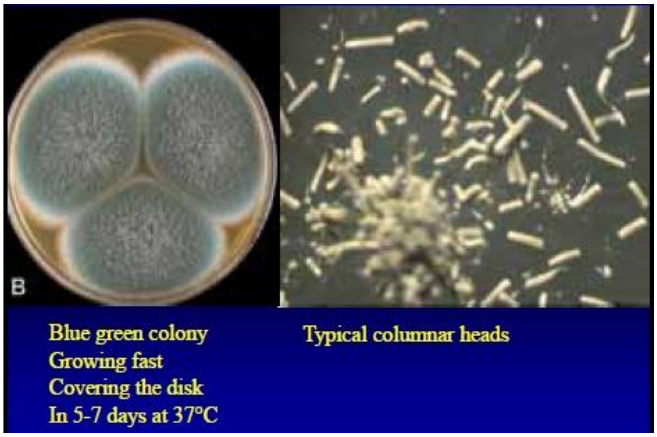
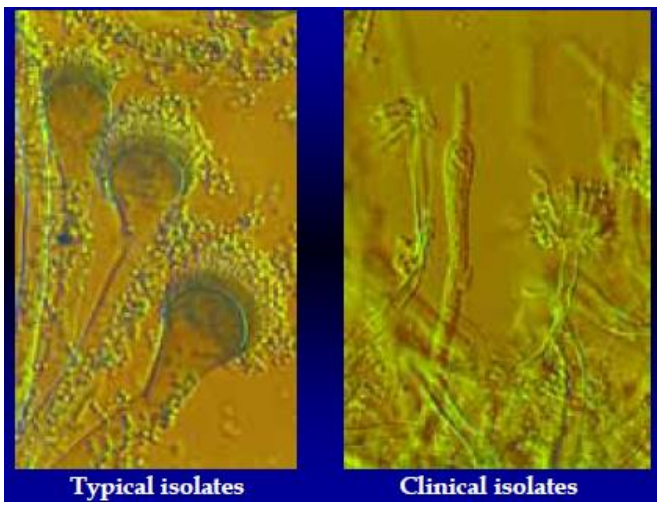
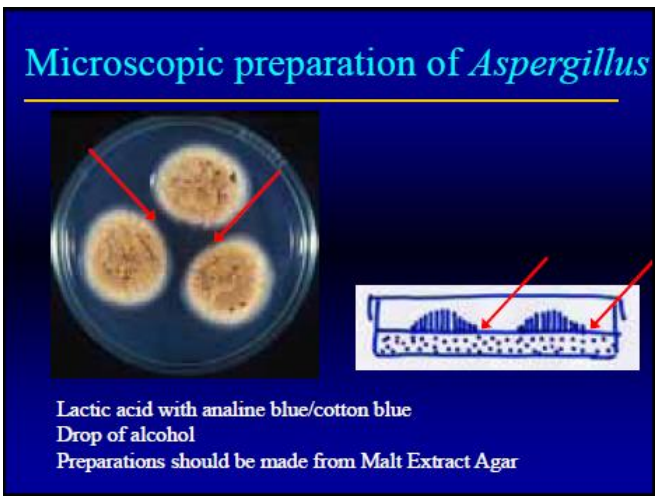


Colony colors and size in *Aspergillus*









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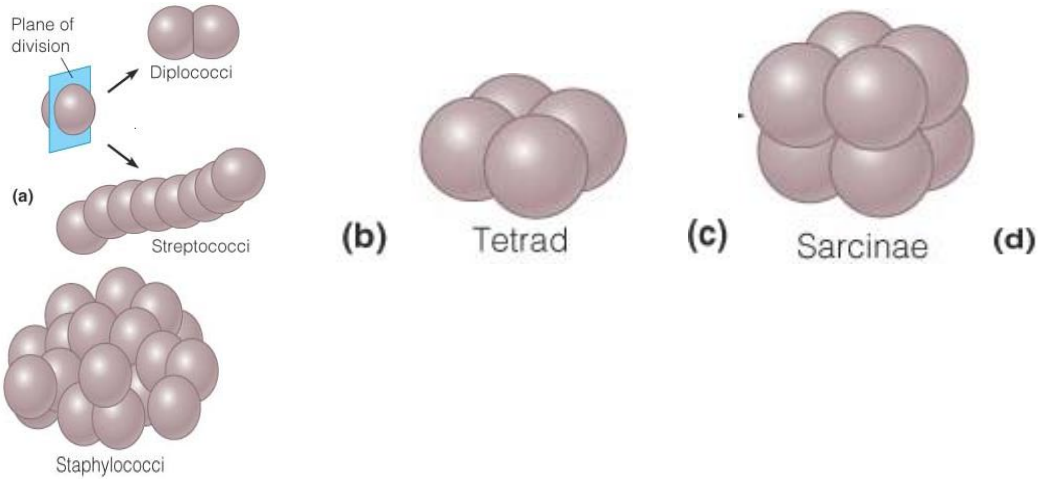
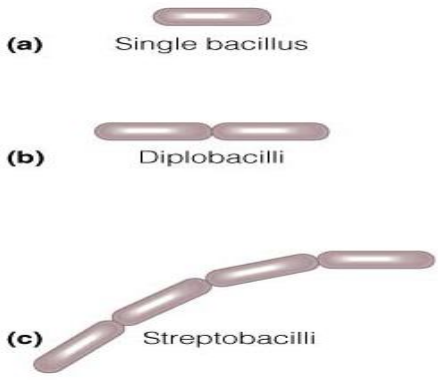
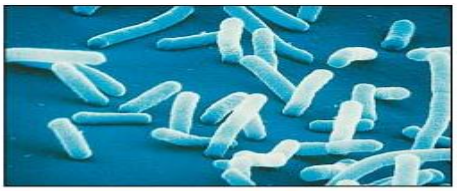
- Coccus (spherical) :



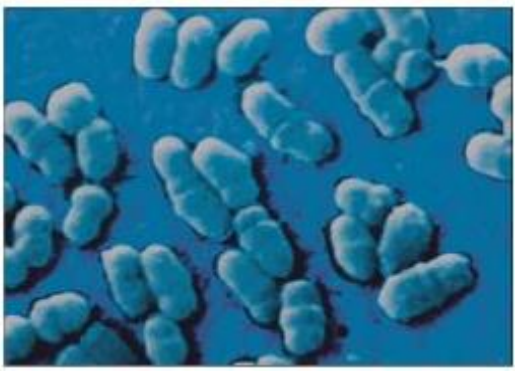
- Bacillus (rod like)





Most bacilli appear as single rods. Diplobacilli appear in pairs after division

Streptobacilli appear in chains after division



Some bacilli are so short and fat that they look like cocci and are referred to as coccobacilli

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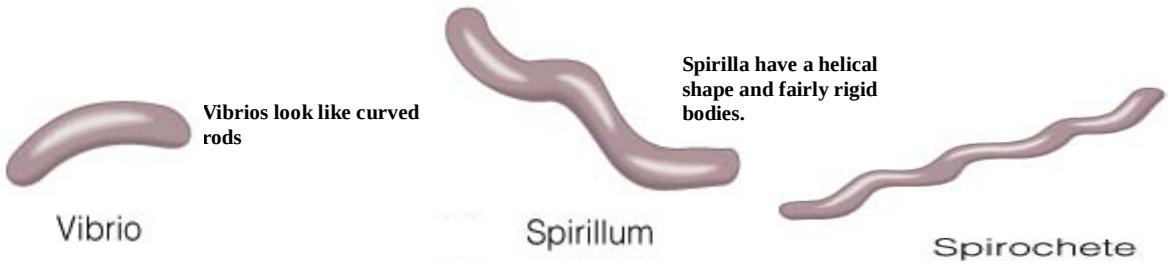
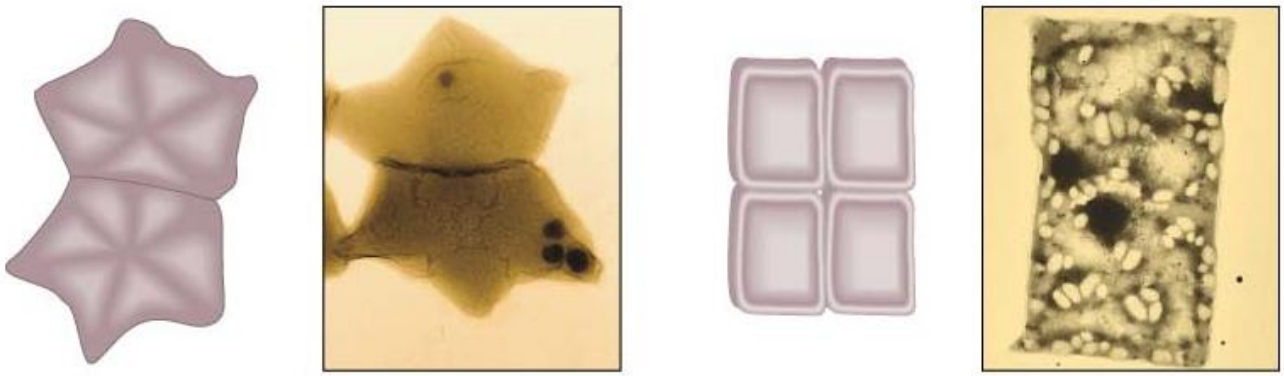


Other shapes



Star-shaped bacteria

Rectangular bacteria

- Bacilli

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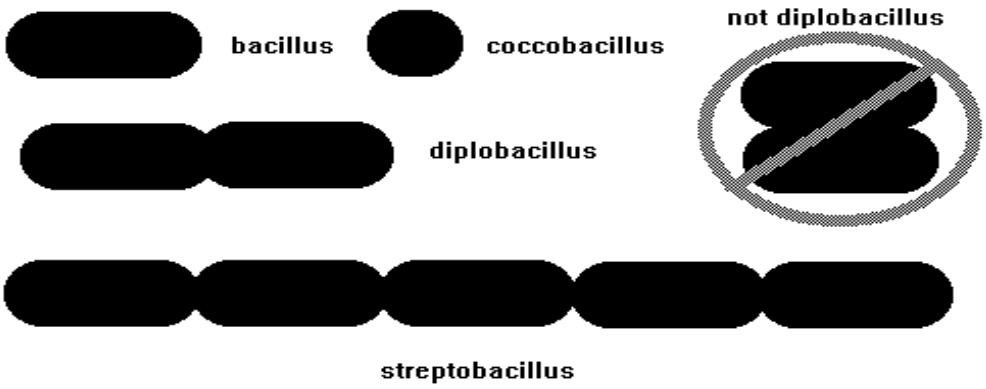

8. History:

Revision No	Effective Date	Revision Details	CC No.
		1.	