Sterility Test

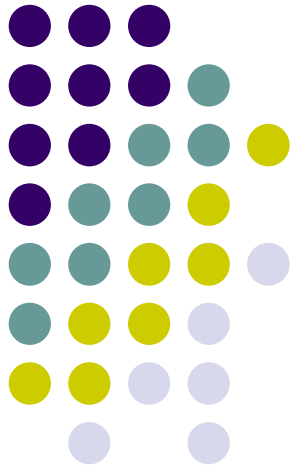
By

Dr. Shantaram Nagpure

Sr. Manager - Quality,

***Fresenius Kabi Oncology Ltd.*– Baddi**

(Formerly **Dabur Pharma Ltd.)**

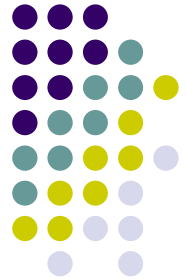


Sterility test

Sterility:

- A specimen said to be sterile only when there is complete absence of viable microorganisms from it.
- However, this absolute definition cannot currently be applied to an entire lot of finished compendial articles because of limitations in testing.
- sterility test suffers from significant statistical limitations and this contributes to the low probability of detecting anything less than gross contamination. However, these limitations can be reduced considerably by performance of the test under conditions that optimize the recovery of micro-organisms.

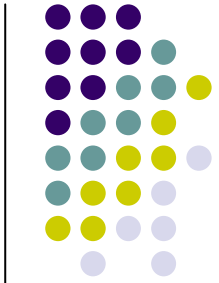
Sterility test



Approach

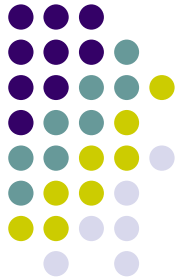
- A method and apparatus for testing of injectables / any sterile preparations, to determine the presence of microorganisms, in which the prepared solution is flowed through a microporous membrane filter.

Sterility test



- State of Sterility Assurance must be established by appropriate validation of test equipments and testing and manufacturing process.
- Includes……
- Critical control equipments (to prepare test accessories)
- Testing environment
- Test method

Sterility test



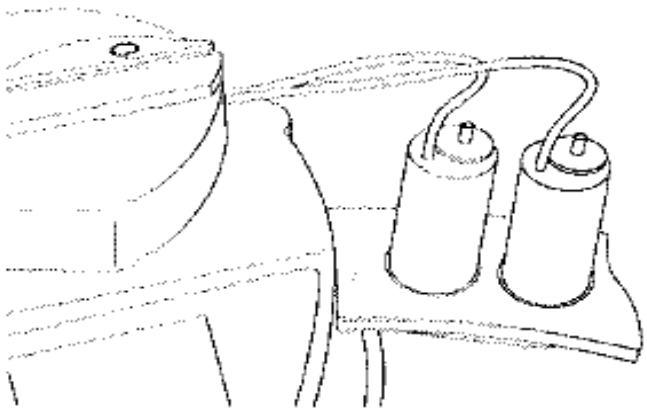
- Method of Sterilization for the accessories to be used
- Steam sterilization
  - (121°C, 15 lbs Pressure for 15 min)
- Dry Heat sterilization
  - (170°C/1 Hr or 140°C /3 hrs or Suitable temp. & times)
- Gas sterilization
  - (Ethylene Oxide, Chlorine Dioxide)
- Sterilization by Radiation
  - (Gamma sterilization, UV sterilization)
- By filtration
  - (Membrane filtration & Cartridge filtration)

Sterility test

- Selection of Test Method

- Direct inoculation
- Membrane filtration method

  - Open funnel method (Manifold method)
  - Closed method



Sterility test

Minimum Sample Quantity to be Used for Each Medium

Liquids

S.No.	Quantity per Container	Minimum Quantity to be Used (unless otherwise justified and authorized)
1	Less than 1 mL	The whole contents of each container
2	1–40 mL	Half the contents of each container, but not less than 1 mL
3	Greater than 40 mL, and not greater than 100 mL	20 mL
4	Greater than 100 mL	10% of the contents of the container, but not less than 20 mL
5	Antibiotic liquids	1 mL
6	*Other preparations soluble in water or in isopropyl myristate*	The whole contents of each container to provide not less than 200 mg
7	*Insoluble preparations, creams, and ointments to be suspended or emulsified*	Use the contents of each container to provide not less than 200 mg

Sterility test

Minimum Sample Quantity to be Used for Each Medium

Solids

S.No.	Quantity per Container	Minimum Quantity to be Used (unless otherwise justified and authorized)
1	Less than 50 mg	The whole contents of each container
2	50 mg or more, but less than 300 mg	Half the contents of each container, but not less than 50 mg
3	300 mg–5 g	150 mg
4	Greater than 5 g	500 mg

Sterility test

Minimum Sample Quantity to be Used for Each Medium

Devices

S.No.	Quantity per Container	Minimum Quantity to be Used (unless otherwise justified and authorized)
1	Catgut and other surgical sutures for veterinary use	3 sections of a strand (each 30-cm long)
2	Surgical dressing / cotton / gauze (in packages)	100 mg per package
3	Sutures and other individually packaged single-use material	The whole device
4	Other medical devices	The whole device, cut into pieces or disassembled

Sterility test

Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch

Parenteral preparations

S.No.	Number of Items in the Batch	Minimum No. of Items to be Tested for Each Medium (unless otherwise justified)
1	Not more than 100 containers	10% or 4 containers, whichever is the greater
2	More than 100 but not more than 500 containers	10 containers
3	More than 500 containers	2% or 20 containers, whichever is less
4	For large-volume parenterals	2% or 10 containers, whichever is less

Sterility test

Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch

Antibiotic solids

S.No.	Number of Items in the Batch	Minimum No. of Items to be Tested for Each Medium (unless otherwise justified)
1	Pharmacy bulk packages (<5 g)	20 containers
2	Pharmacy bulk packages (5 g)	6 containers
3	Bulks and blends	According to Bulk solid products

Sterility test

Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch

Ophthalmic and other noninjectable preparations

S.No.	Number of Items in the Batch	Minimum No. of Items to be Tested for Each Medium (unless otherwise justified)
1	Not more than 200 containers	5% or 2 containers, whichever is the greater
2	More than 200 containers	10 containers

Sterility test

Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch

Devices

S.No	Number of Items in the Batch	Minimum No. of Items to be Tested for Each Medium (unless otherwise justified)
1	Catgut and other surgical sutures for veterinary use	2% or 5 packages, whichever is the greater, up to a maximum total of 20 packages
2	Not more than 100 articles	10% or 4 articles, whichever is greater
3	More than 100, but not more than 500 articles	10 articles
4	More than 500 articles	2% or 20 articles, whichever is less

Sterility test

Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch

Bulk solid products

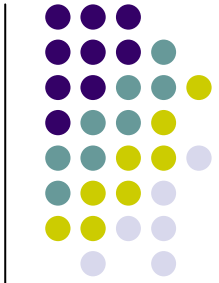
S.No.	Number of Items in the Batch	Minimum No. of Items to be Tested for Each Medium (unless otherwise justified)
1	Up to 4 containers	Each container
2	More than 4 containers, but not more than 50 containers	20% or 4 containers, whichever is greater
3	More than 50 containers	2% or 10 containers, whichever is greater

Sterility test

Media types

- The media used should be in accordance with the pharmacopoeial method followed.
- Soya-bean casein digest (SCD) media and fluid thioglycollate media (FTM) should normally be used.
- Alternative media are permitted and may be appropriate if the nature of the product or method of manufacture could result in the presence of fastidious organisms (e.g. vaccines, blood products, etc).
- Validation studies should demonstrate that alternative media are capable of supporting the growth of a wide range of micro-organisms.
- Eg: Alternative Thioglycollate media for Direct inoculation method.
- Rinse fluid shall be selected based on the nature of the product to be tested.

Sterility test



Incubation period

- All test containers should be incubated at temperatures specified by the pharmacopoeial method for each test media for at least 14 days, regardless of whether filtration or direct inoculation test methodology is used.

- Test containers should be inspected at intervals during the incubation period and these observations recorded.

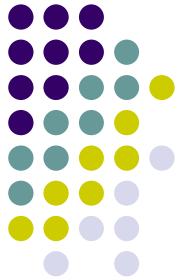
- If the product produces a suspension, flocculation or deposit in the media, suitable portions (e.g. 2-5 percent) of the contents of the containers should be transferred to fresh media under clean room conditions, after 14 days, and re-incubated for a further 7 days.

Sterility test

Test – *(Product Sterility)*

- ☺ Product or simulated product of known or undoubted sterility that is tested during the same test session as the product test samples.

Sterility test



Negative controls -

- Negative product controls, which are similar in type and packaging to the actual product under test, should be included

- These controls facilitate the interpretation of test results, particularly when used to declare a test invalid because of contamination in the negative product controls.

- The negative control contamination rate should be calculated and recorded.

Sterility test

Positive test controls

Test Organisms

Strains of the test Microorganisms Suitable for Use in the Growth Promotion test and the Validation

Staphylococcus aureus	ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518*
Bacillus subtilis	ATCC 6633, CIP 52.62, NCIMB 8054
Pseudomonas aeruginosa	ATCC 9027, NCIMB 8626, CIP 82.118
Clostridium sporogenes	ATCC 19404, CIP 79.3, NCTC 532 or ATCC 11437
Candida albicans	ATCC 10231, IP 48.72, NCPF 3179
Aspergillus niger	ATCC 16404, IP 1431.83, IMI 149007

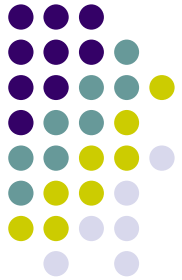
*National Collections of Industrial, Marine and Food Bacteria

Sterility test

Growth promotion test

- Challenge organism strains that are used to verify the fertility of each batch of standard test media should be selected from those reference strains specified by the pharmacopoeial method. Environmental or fastidious organisms may be used.
- The media should be inoculated with 10-100 CFU of challenge organisms.
- Growth promotion challenge organisms should show clearly visible growth in the test media within 3 days for bacteria and 5 days for fungi.

Sterility test



Positive test controls –

Validation (bacteriostasis and fungistasis) test

- The test methodology should be validated by inoculation with 10-100 CFU of challenge organism strains to the media /product container.

- The preferred validation method involves addition of challenge organisms directly to the product prior to direct inoculation or membrane filtration.

- The test is declared invalid if validation challenge organisms do not show clearly visible growth of bacteria within 3 days, and fungi within 5 days in the test media containing product.

Sterility test

Positive test controls – Stasis test

- It is not mandatory, but it is recommended that a stasis test be performed when antibiotics, inherently antimicrobial or preserved products are tested.
- The test is performed by inoculation of 10-100 CFU of challenge organisms
- Stasis test challenge organisms should show clearly visible growth in the test medium within 3 days for bacteria and 5 days for fungi, otherwise the test is invalid.
- It is recommended that the stasis test is repeated at least every 12 months on the relevant categories of products.

Sterility test

Results and interpretation

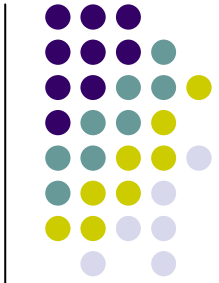
- Test validity will determined and considered when the negative control & Test shows negative and positive control shows positive.
- A test may be repeated only when it can be demonstrated that the test was invalid for causes unrelated to the product being examined.
- The data of the microbiological monitoring of the sterility testing facility show a fault.
- A review of the testing procedure used during the test in question reveals a fault.
- Microbial growth is found in the negative controls.
- After determination of the identity of the micro-organisms isolated from the test the growth of this species or these species may be ascribed unequivocally to faults with respect to the material and/or the technique used in conducting the sterility test procedure.

Sterility test

Results and interpretation

- If contamination, which is established to be unrelated to the product, occurs in the original test, the test may be repeated with the same number of test samples as used in the original test, with negative product controls tested concurrently.

- If contamination is detected in the repeat test performed on the same number of test samples, the product does not comply with the test for sterility and the entire batch should be rejected.



STERILITY TEST FACILITIES

❖ Clean room design

- ☺ Classification:
  - ☺ The sterility test should be conducted within a class A laminar airflow cabinet located within a class B clean room, or in an isolator.
- ☺ Air supply:
  - ☺ Air supplied to the environment should be provided through terminal HEPA filters,
  - ☺ There should be a pressure differential of not less than 10 to 15 Pascals (guidance value) between each of the areas, i.e. ambient/airlock and airlock/test room.

STERILITY TEST FACILITIES

❖ Airlock and aseptic gowning

☺ *Airlock conditions:*

- ☺ Entry to the clean room should be via an airlock in which operators are required to change into clean room garments.
- ☺ Movement of the operator between the relatively unclean and clean areas of the room
- ☺ The airlock should contain: a full-length wall mirror; gowning instructions; hand washing, disinfection and drying facilities.

STERILITY TEST FACILITIES

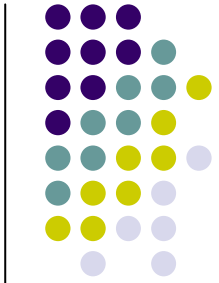
- **Airlock and aseptic gowning**

- *Aseptic gowning:*

  - The sterility test operator should change into sterile clean room garments consisting of a one-piece coverall suit, head cover, beard cover (if applicable), overshoes, gloves and mask.

  - Records should be kept of the sterilisation or sanitisation of the garments.

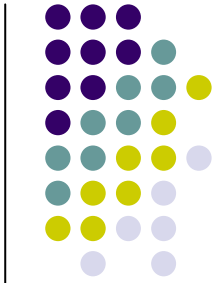
  - Each operator should be trained and certified in gowning procedures with training records maintained.



STERILITY TEST FACILITIES

❖ Clean room fittings and surfaces

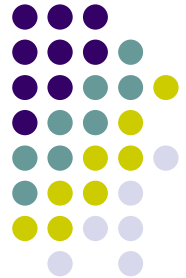
- ☺ All fittings, such as power outlets and light fittings should be flush with the wall or ceiling surfaces.
- ☺ Surfaces should be smooth and impervious.
- ☺ The joints between ceiling/walls/floor should be coved.
- ☺ If supplied, intercom or communication systems should be designed to allow hands-free use.
- ☺ Chairs, storage cabinets and trolleys should be designed to facilitate cleaning.
- ☺ Ultraviolet lamps may be fitted within pass-through cabinets only. Where there is more than one parallel tube.



STERILITY TEST FACILITIES

❖ CLEANING, SANITISATION AND DISINFECTION

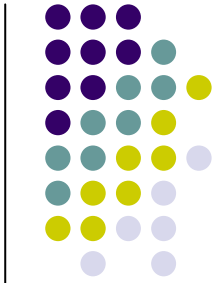
- ☺ Outer surfaces of samples and equipment entering the testing suite should be disinfected, preferably with a sporicidal agent.
- ☺ Surfaces and operators' gloved hands should be disinfected regularly during the test session.
- ☺ There should be protocols to cover all daily, weekly, and periodic cleaning, sanitisation, disinfection and fumigation procedures
- ☺ If an isolator is used, the method of disinfection or sterilisation should be specified.



STERILITY TEST FACILITIES

❖ CLEANING, SANITISATION AND DISINFECTION

- ☺ Prior to implementation, all cleaning, sanitising and disinfecting procedures should be validated from a microbiological perspective with respect to minimum disinfectant contact times and efficacy.
- ☺ Cleaning and disinfecting agents should be purchased to agreed and documented specifications/
- ☺ Records should be retained in respect of routine preparation of cleaning and disinfecting agents, directions for their use, and validation of their efficacy.



Environmental Monitoring

- Environmental microbiological monitoring should include a combination of air and surface sampling methods,

- Such as:

  - Active air sampling
  - Settle (exposure) plates
  - Surface contact (RODAC) plates,
  - Swabs or flexible films
  - Operators' gloved hand plates.
  - Non-viable particulate monitoring

Sterility test

Environmental Monitoring

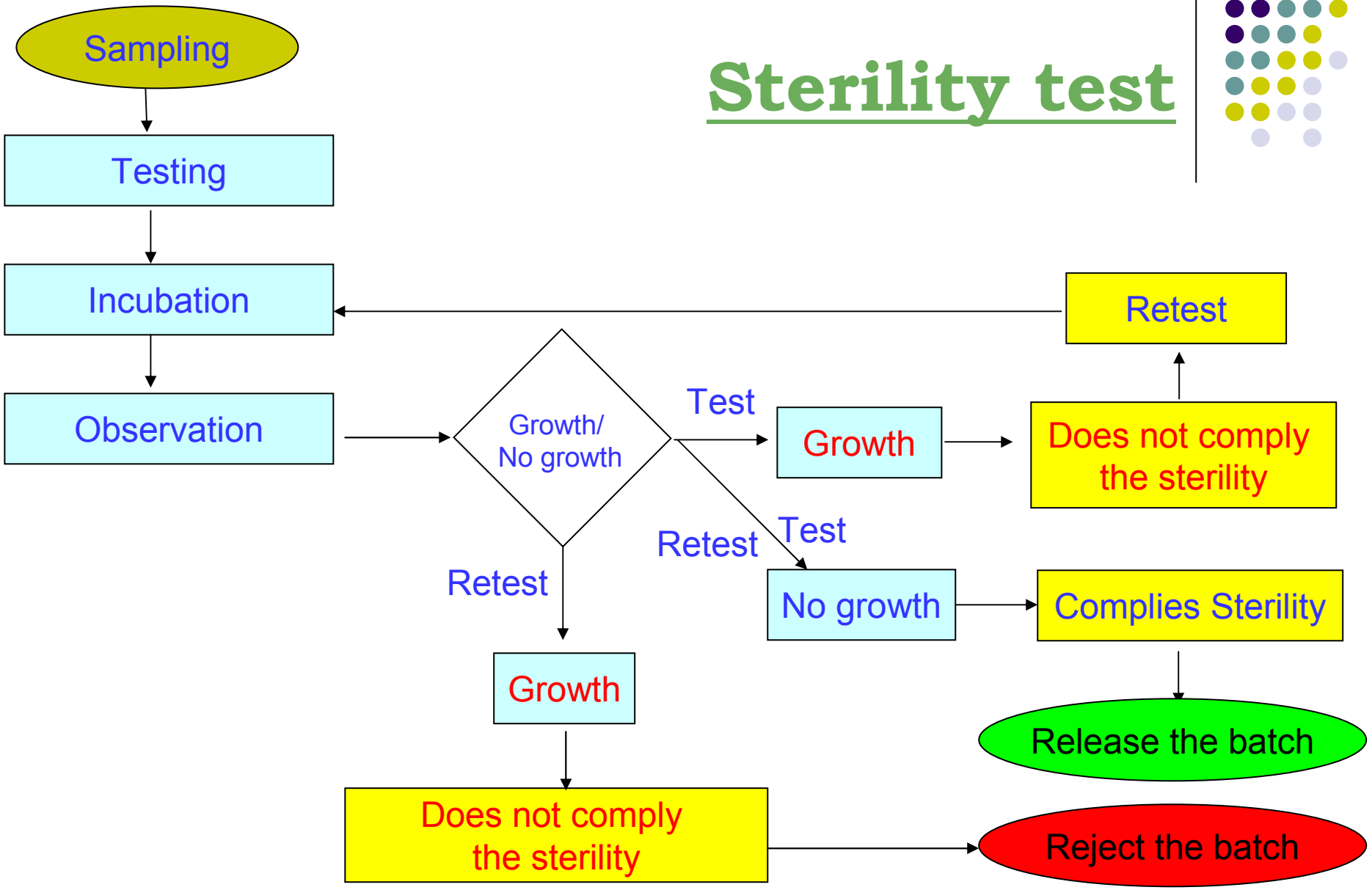
- Environmental monitoring should be performed under operational (dynamic) conditions either within the isolator or in the laminar airflow and associated background areas.
- Location maps, exposure duration, and frequency of all types of microbiological environmental monitoring should be specified in written procedures.
- The recovery of micro-organisms on the chosen media should be validated.
- There should be written specifications, including appropriate alert and action limits for microbial contamination.
- Records should be maintained of the numbers and type of organisms isolated and results presented in a format that facilitates early detection of trends.

Sterility test

TRAINING

- Sterility testing should only be performed by personnel who have been trained, qualified and certified to perform the various tasks and procedures related to sterility testing.
- The examination of test and control containers during and at the end of the incubation period should be included as part of the operator training program.
- Supervisors should ensure that all personnel are monitored and follow Standard Operating Procedures (SOPs).
- Personnel should undergo periodic re-certification, particularly when problems are detected during the course of routine environmental and negative control monitoring, or when operators perform the test infrequently.
- Personnel training should be documented and records maintained

Sterility test



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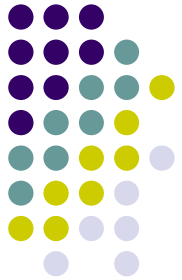
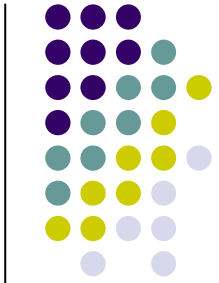


Bibliography

- ☺ Documents referred are .....

- ☺ United States Pharmacopoeia (USP)
- ☺ British Pharmacopoeia (BP)
- o Health Sciences authority GUIDE-MQA-004-005
- o PIC - PIC/S Annex to the Guide to Good Manufacturing Practice for Medicinal Products - Manufacture of Sterile Medicinal Products.
- o PIC/S documents PE 001/1 : Recommendations on Sterility Testing



Thank you