การดูแลรักษาและการควบคุมเชื้อจุลินทรีย์ในระบบน้ำ

รองศาสตราจารย์ ภารุณี ถนอมเกียรติ

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จุฬาลงกรณ์มหาวิทยาลัย

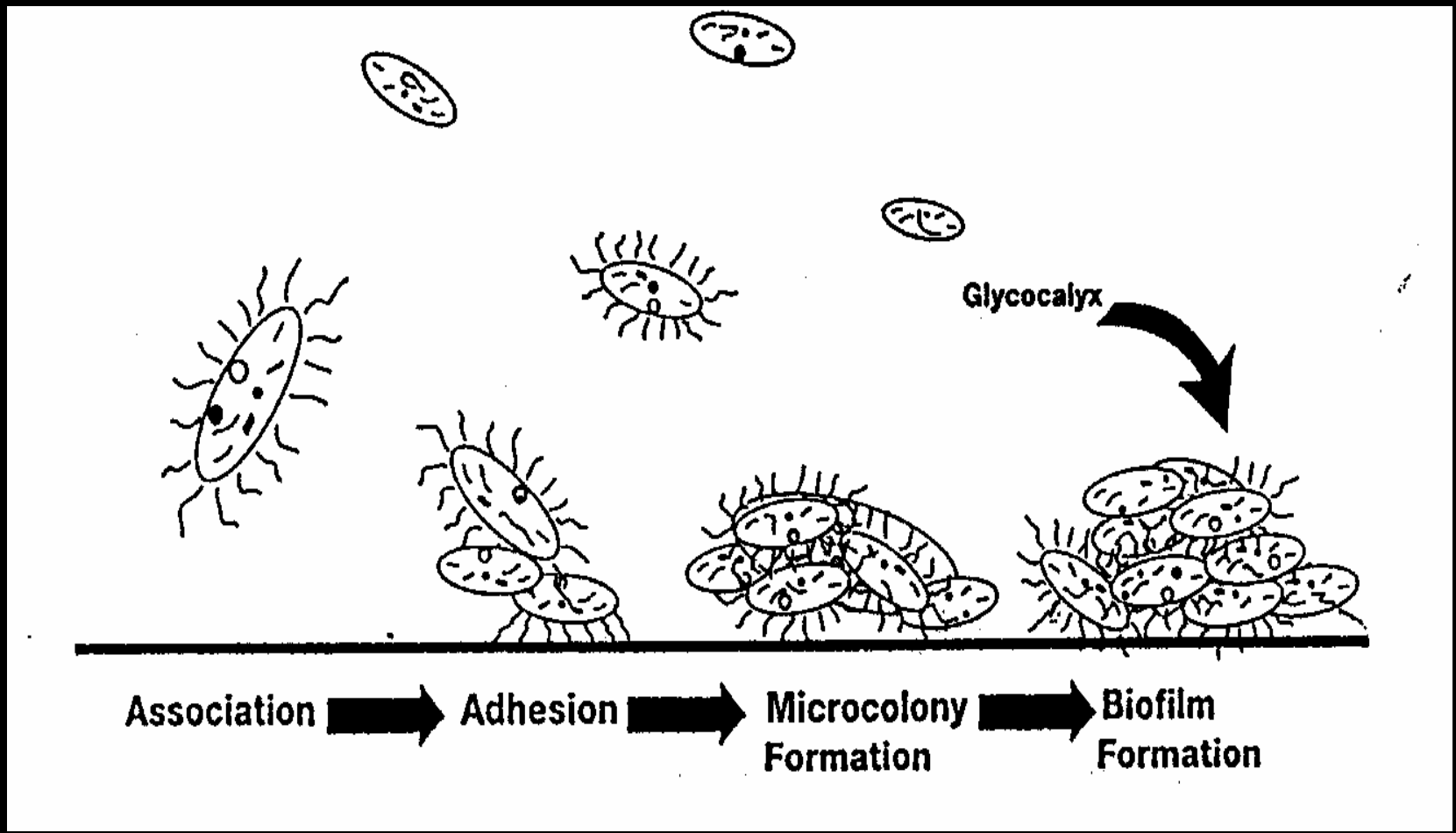
Microorganism in high purity water

- Planktonic microorganism
  - Free-floating microorganisms whose movements are control by water movement (not attached to surfaces)
- Benthic (Sessile) microorganism => Biofilm
  - Microorganisms that attached to solid surface

Microorganism in high purity water

- Microbial contamination can arise as a result of colonization of surfaces and stagnant areas by aquatic bacteria with the formation of biofilm
- Sample of flowing water are only indicative of the concentration of planktonic microorganism

BIOFILM FORMATION



BIOFILM BACTERIA

- A very common biofilm bacteria is *Pseudomonas aeruginosa*
- Cell of *Psedomonas* are rod shapeed and approximately 0.3-0.8 microns wide x 1.0-1.2 microns long
- Biofilm are generally present in a greater numbers and are the source of planktonic population
- Microorganisms in biofilms represent a continuous source of contamination and are difficult to sample and quantify

BIOFILM BACTERIA

- Biofilm is an adaptive response by certain microorganisms to survive in a low nutrient environment

Factors that affect biofilm attachment and grow

- Surface smoothness
- Flow velocity

Good design to prevent biofilm

- Smooth surfaces
- Moving water
- No dead spots

Surface finish

- Smoothness of stainless steel surface
- Internal surface finish
  - Purified water system
    - 180 grit (Ra = 30-40)
  - Water for Injection system
    - 240 grit (Ra = 10-15) often with electropolish

Relationship between Grit, RMS, and Ra for Stainless Steel Tubing

Grit Size	RMS (microinch)	Ra (microinch)
120	58	40-50
180	34	20-30
240	17	15
320	14	12

Grit finish is use with mechanical polishing and refer to the number of grit lines per inch of abrasive

RA – 'roughness average' or the arithmatic average deviation from the center line of the surface

RMS - 'root mean square' of the deviations from the center line of the surface

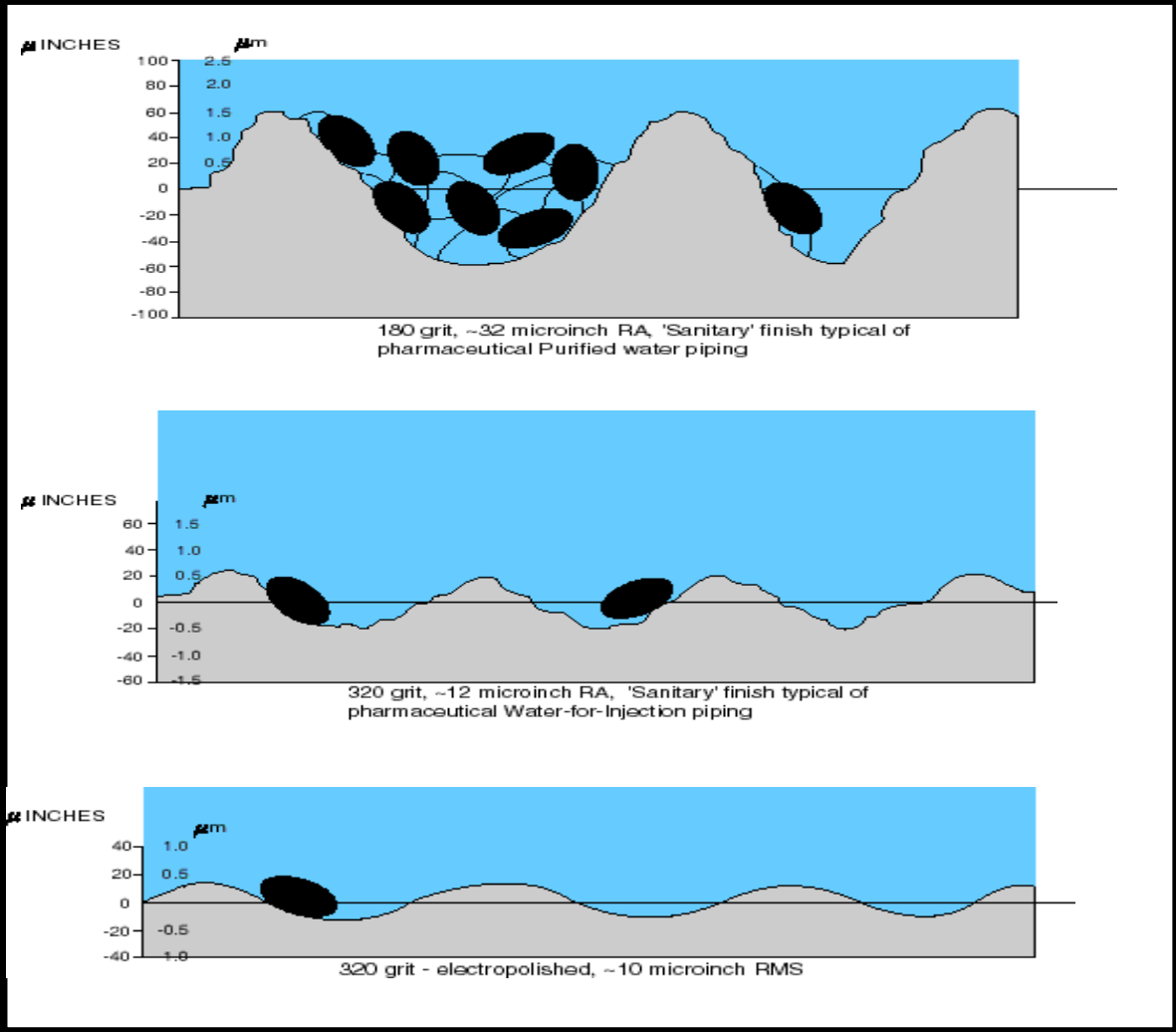
RA x 1.11 = RMS

Surface finish and cell size

180 grit

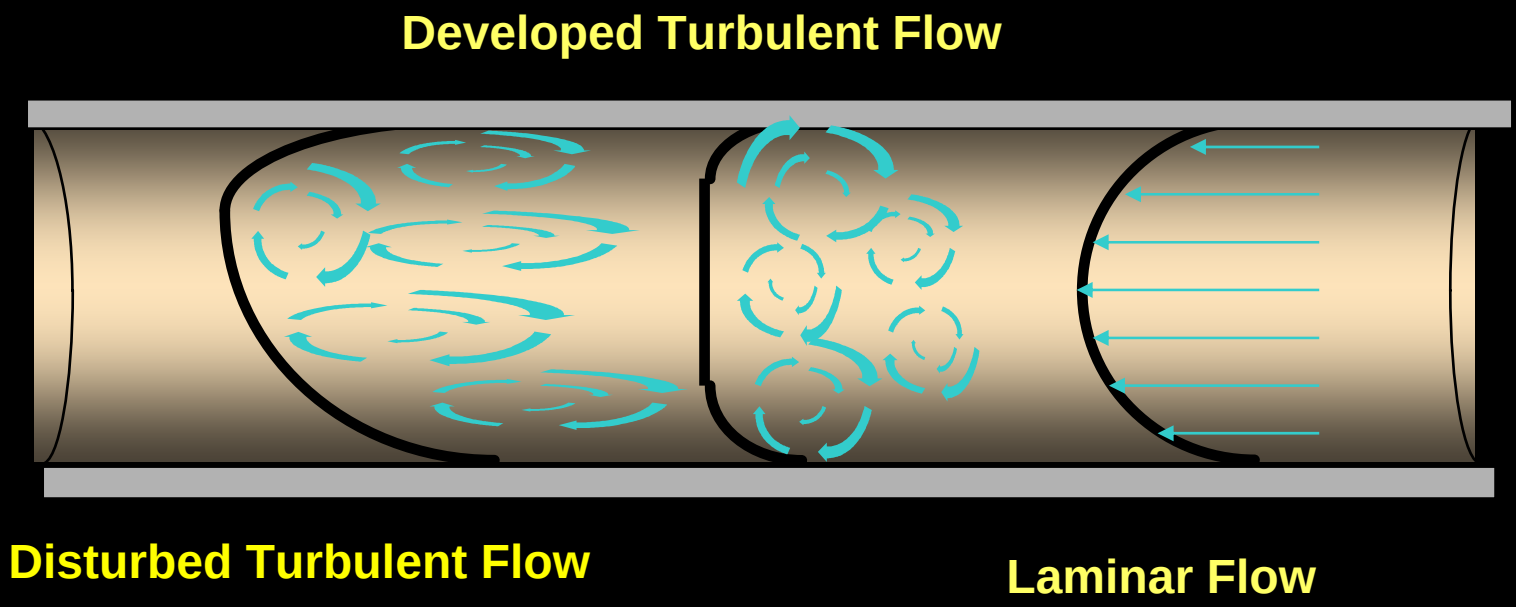
320 grit

320 grit
Electropolish



Turbulent flow

- Maintenance of continuous turbulent flow circulation within water distribution systems reduces the propensity for the formation of biofilm



DEAD LEGS

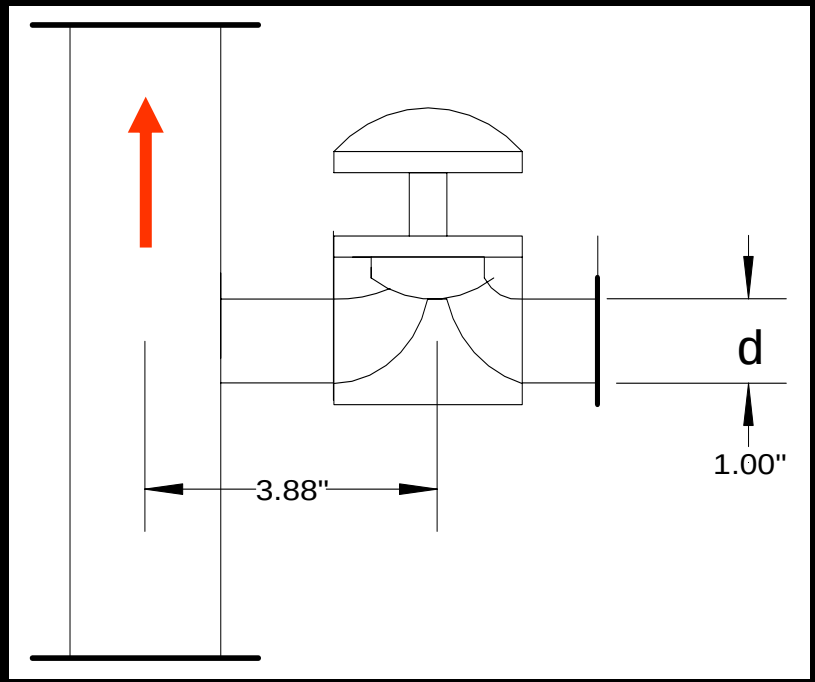
- Dead legs are stagnant areas where there is no water flow. Stagnant areas allow microbial contamination as a result of colonization of surface with the formation of biofilm

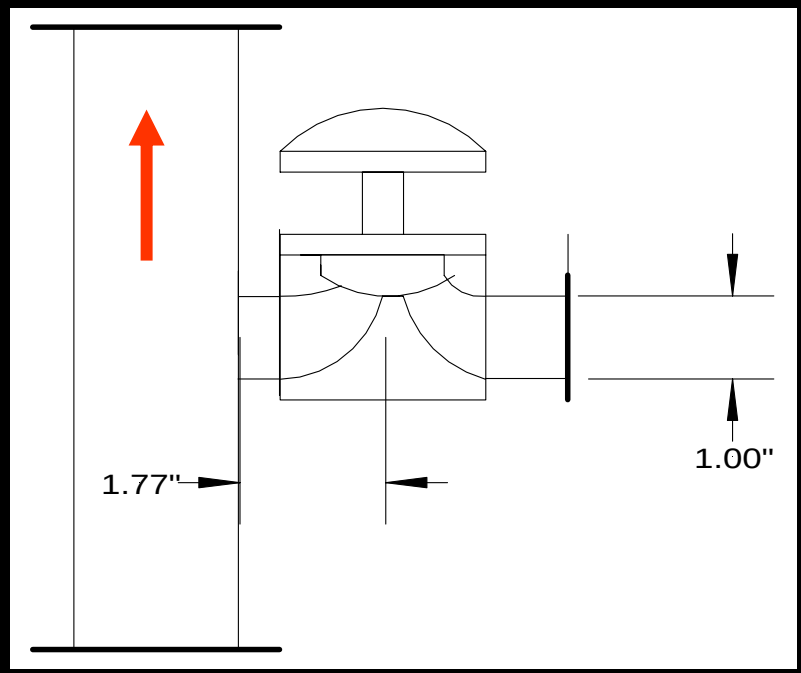
DEAD LEGS

FDA Guide to Inspections of High Purity Water Systems

- "One common problem with piping is that of 'dead-legs'. The proposed LVP regulations defined dead-legs as not having an unused portion greater in length than six diameters of the unused pipe measured from the axis of the pipe in use. It should be pointed out that this was developed for hot 75-80◦C circulating systems. With colder systems (65-75◦C), any drops or unused portion of any length of piping has the potential for the formation of a biofilm and should be eliminated if possible or have special sanitizing

Distribution Loops - Dead legs





< 6 D

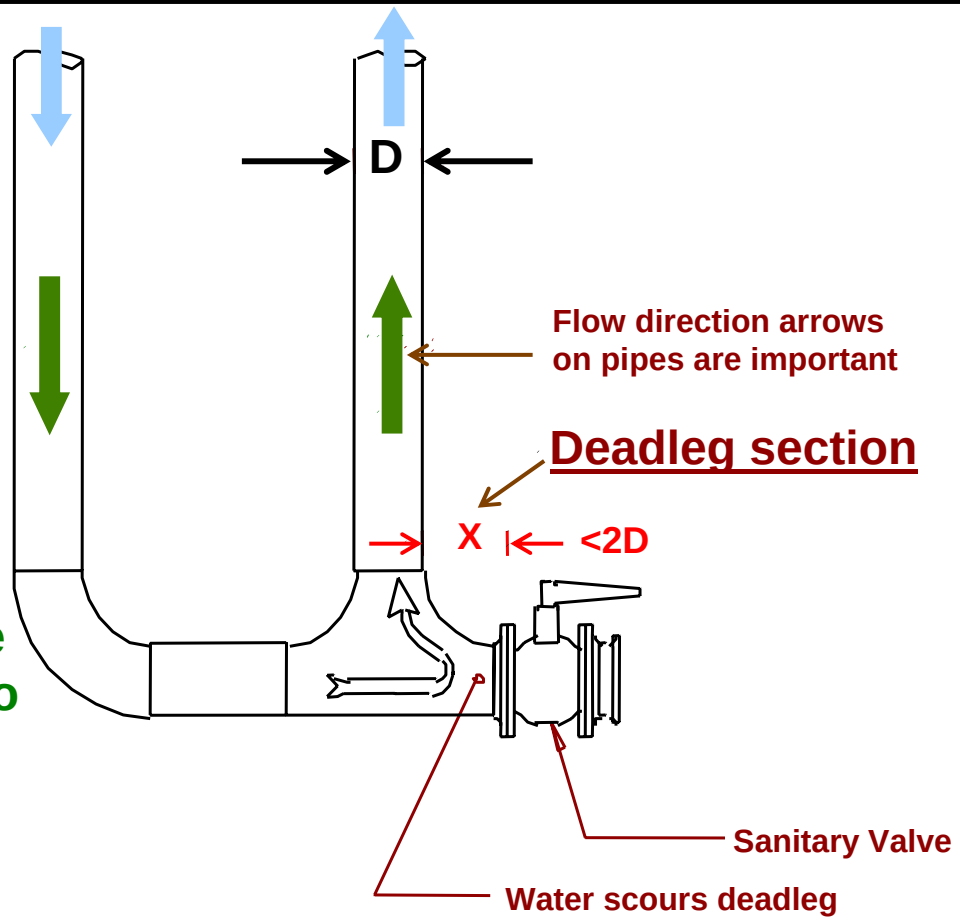
< 2 D

DEAD LEGS

USP 28 <1231>Water for Pharmaceutical Purposes

- Dead legs and low-flow conditions should be avoided, and valve tie-in points should have length-to diameter ratio of 6 or less.

DEAD LEGS



If D=25 mm & distance X is greater than 50 mm, we have a dead leg that is too long.

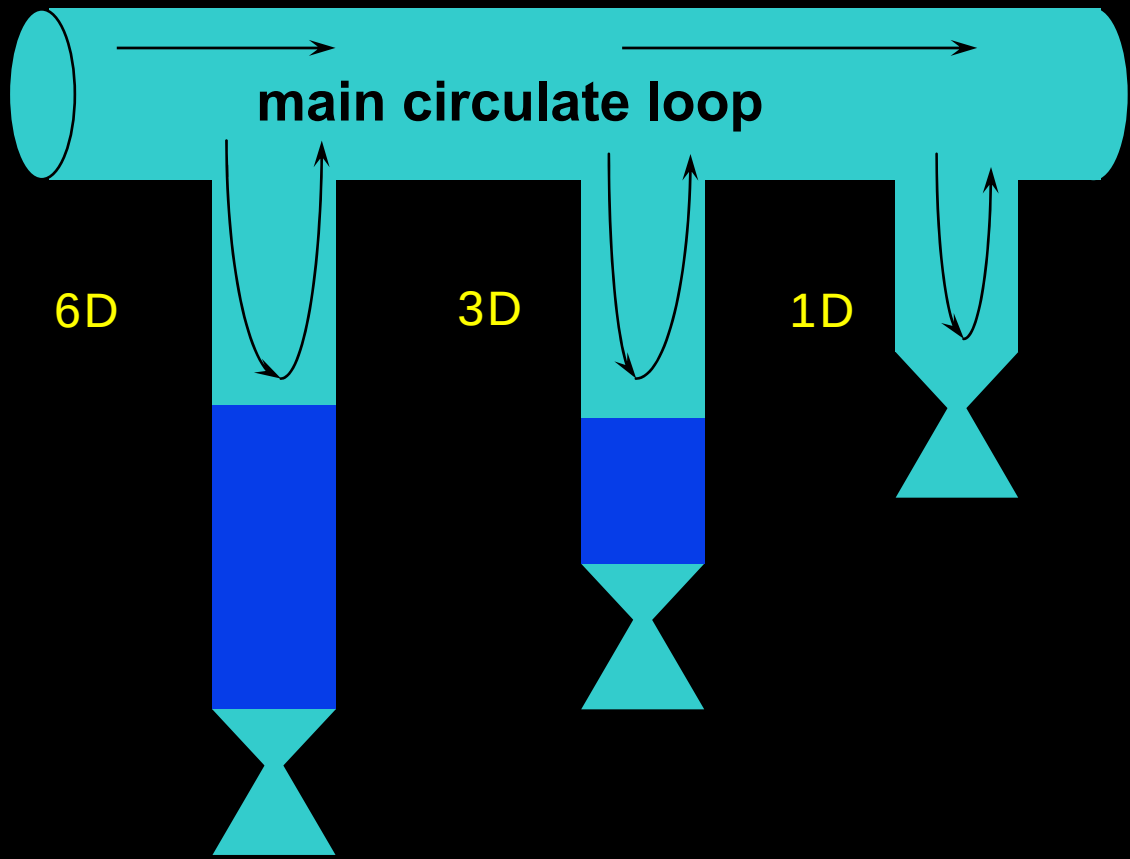
DEAD LEGS

- Dead legs in the pipework installation greater than 1.5 times the branch diameter should be avoid.

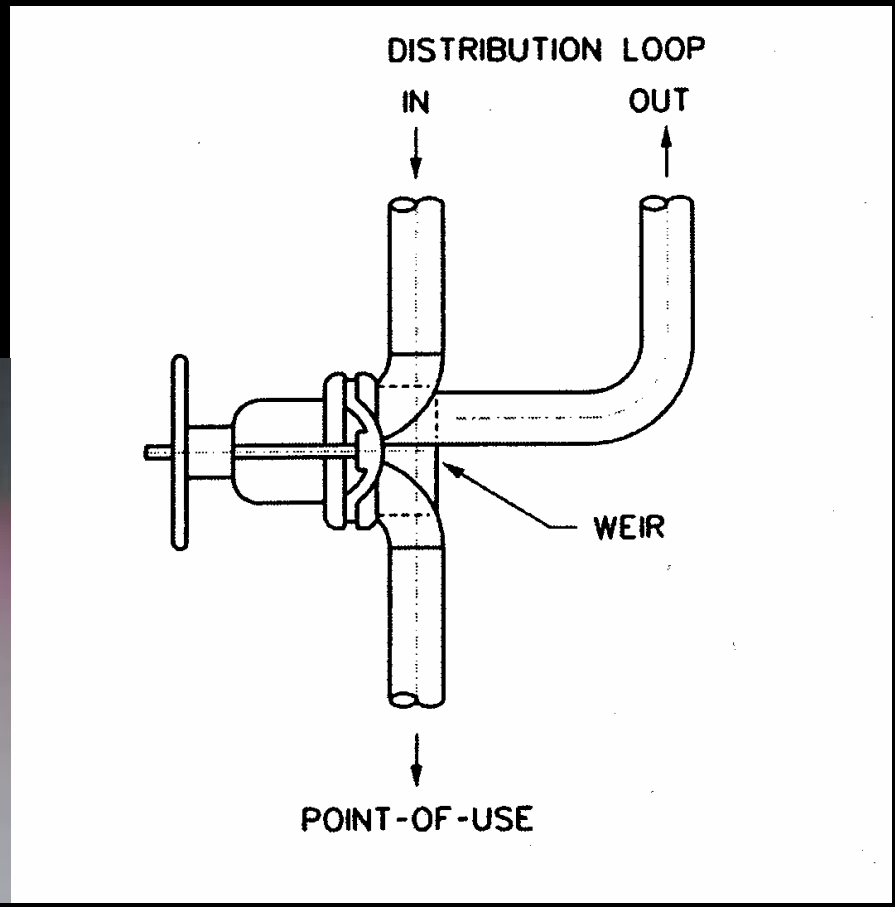
WHO TRS 929,2005

WHO Good Manufacturing Practices : water for pharmaceutical use

DEAD LEGS



Zero Dead Leg Valve



Alert and Action Levels

- Water systems should be microbiologically monitored to confirm that they continue to operate within their design specifications and produce water of acceptable quality.
- The use of process parameters and product specifications is the establishment of Alert and Action Level, which signal a shift in process performance.
- Alert and Action Level are use for monitoring and control rather than accept or reject decision.

Alert Levels

- Levels or ranges that, when exceeded, indicate that a process may have drifted from its normal operating condition.
- Constitute a warning and do not necessarily require a corrective action

Action Levels

- Levels or ranges that, when exceeded, indicate that a process has drifted from its normal operating range.
- Exceeding an Action Level indicates that corrective action should be taken to bring the process back into its normal operating range.

Alert and Action Levels

- Established with process and product specification tolerances
- Based on a combination of technical and product-related consideration

Alert and Action Levels

Technical consideration

- Review of equipment design specification to ensure that the purification equipment is capable of achieving the required level of purity
- Sample should be collected and analyzed over a period of time to develop data reflecting normal quality trends
- Historical or statically based levels can be established using these data

Alert and Action Levels

product-related consideration

- Alert and Action Level should represent both product-quality concern and ability to effective manage the purification process
- Alert and Action Level are typically base on a review of process data and an assessment of product sensitivity to chemical and microbiological contamination
- The levels set should be such that, when it is exceeded, product quality is not compromised.

Microbial Alert and Action Levels

Levels established

- Necessarily linked to the monitoring method chosen
- USP Recommended methodologies
  - Drinking Water 500 CFU/ml
  - Purified Water 100 CFU/mL
  - WFI 10 CFU/100 mL

Suggested bacterial limits (CFU /mL)

Sampling location	Target	Alert	Action
Raw water	200	300	500
Post multimedia filter	100	300	500
Post softener	100	300	500
Post activated carbon filter	50	300	500
Feed to RO	20	200	500
RO Permeate	10	50	100
Point of Use	1	10	100

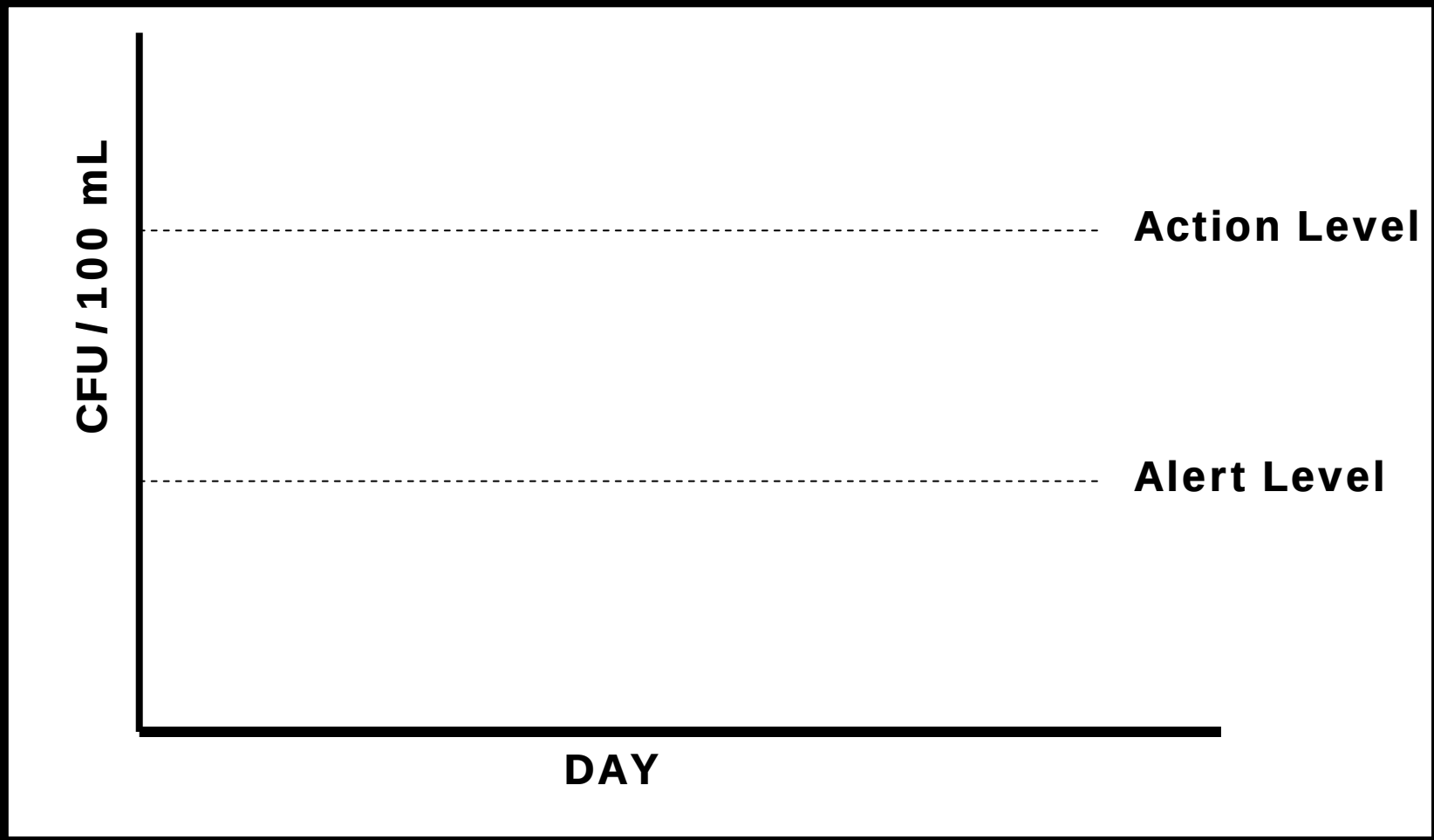
Trend analysis

- Monitoring data should be analyzed on an ongoing basis to ensure that the process continue to perform within acceptable limits.
- An analysis of data trends is often used to evaluate process performance.
- Can be used to predict departures from established operating parameters, thereby signaling the need for appropriate preventive maintenance.

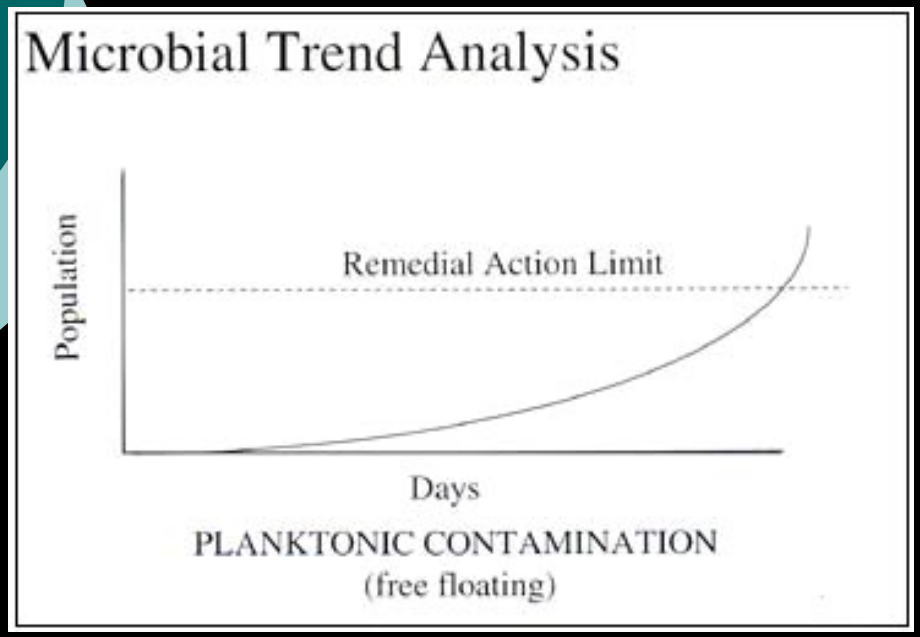
Trend analysis

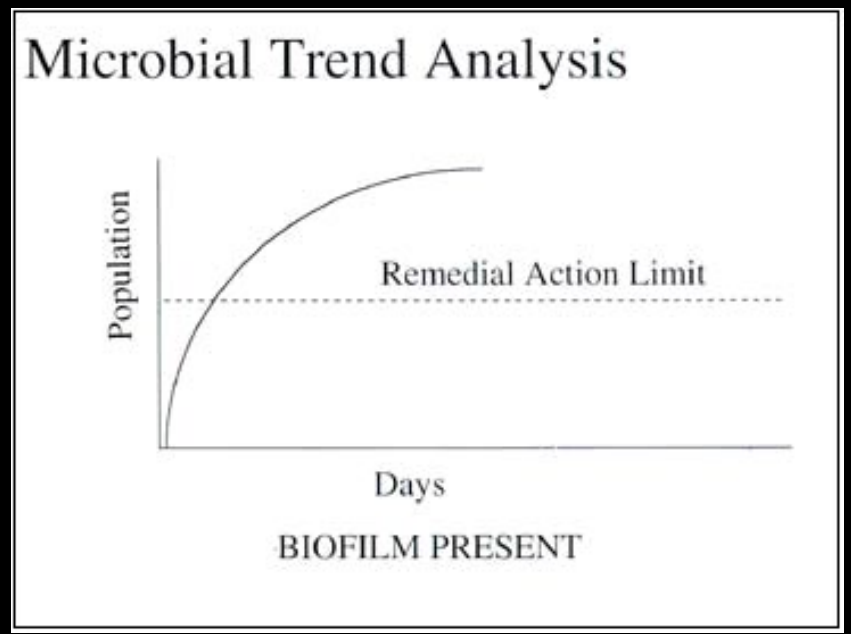
- Plot ค่าจำนวนเชื้อ บน Control chart เพื่อดูแนวโน้มว่า มีการเพิ่มขึ้นมากน้อยอย่างไร เพื่อจะได้ทราบว่าจะต้องเฝ้าระวังอย่างไร หรือจะดำเนินการอย่างไรเพื่อไม่ให้จำนวนของเชื้อเพิ่มขึ้นอีกจนเกินขีดจำกัดที่กำหนด

Trend analysis

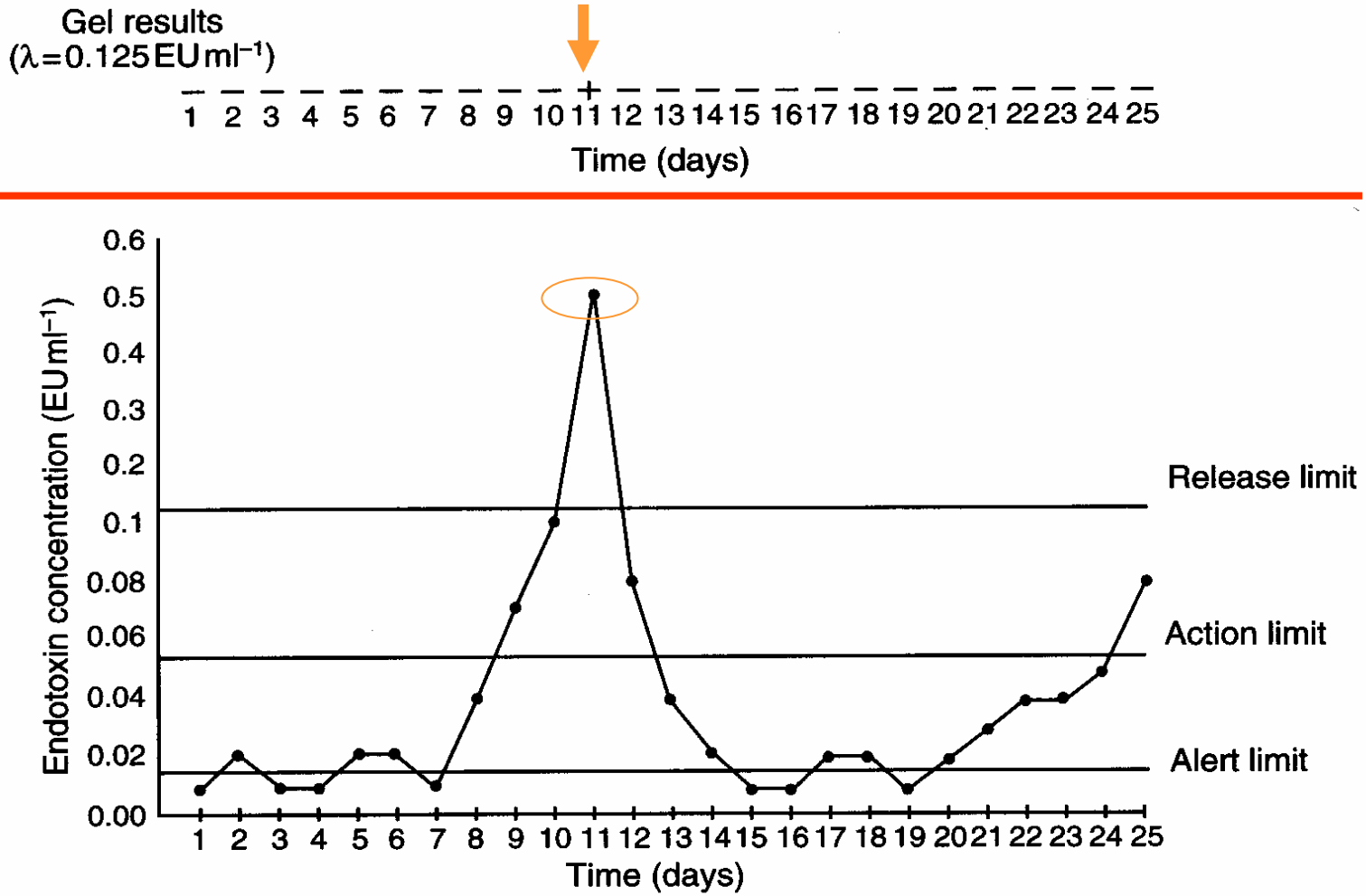


Trend analysis





Endotoxin Trending



Trend analysis

- The consistent appearance of elevated planktonic levels is usually an indication of advanced biofilm development in need of remedial control
- System control and sanitization are key in controlling biofilm formation and consequent planktonic population

The growth of microorganisms can be inhibited by

- UV radiation sources in pipework
- Maintaining the system heated (guidance T 70-80^{o}C)
- Sanitizing the system periodically using hot water (guidance T >70^{o}C)
- Sterilizing or sanitizing the system periodically using superheated hot water or clean steam
- Routine chemical sanitization using ozone or other suitable chemical agents.

WHO GMP : water for pharmaceutical use , WHO TRS 929,2005

SANITIZATION

- Thermal approaches
  - Most popular – nothing added to system
  - If system continuously hot, all but eliminates concerns about sanitization
- Chemical methods
  - Must ensure that cleaning chemicals are completely removed from system
  - Rinsing post-sanitization time and cost intensive
- UV light

SANITIZATION

Thermal approaches

- Periodic or continuously circulating hot water and the use of steam
- Control biofilm development
- Not effective in removing established biofilm

SANITIZATION

Chemical methods

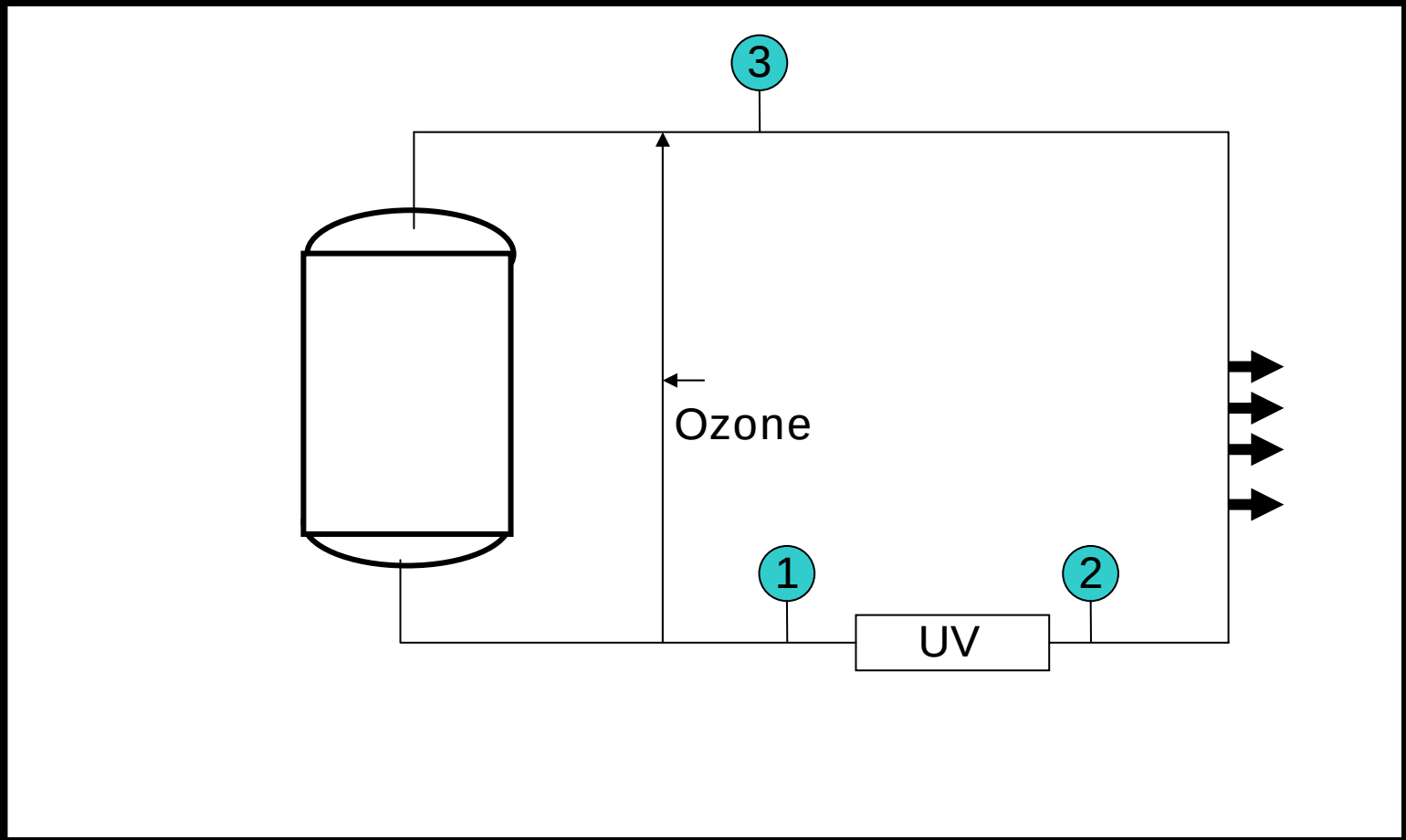
- Oxidizing agent
  - Halogenated compounds
  - Ozone
  - Hydrogen peroxide
  - Peracetic acid

SANITIZATION

Ozone

- Low capital and operating cost compared to hot water generation and storage
- Has the added value of reducing TOC
- Remove by UV light at 254 nm, reducing the ozone to oxygen
- Dose level 10-50 mg/L contact time <1 hr.

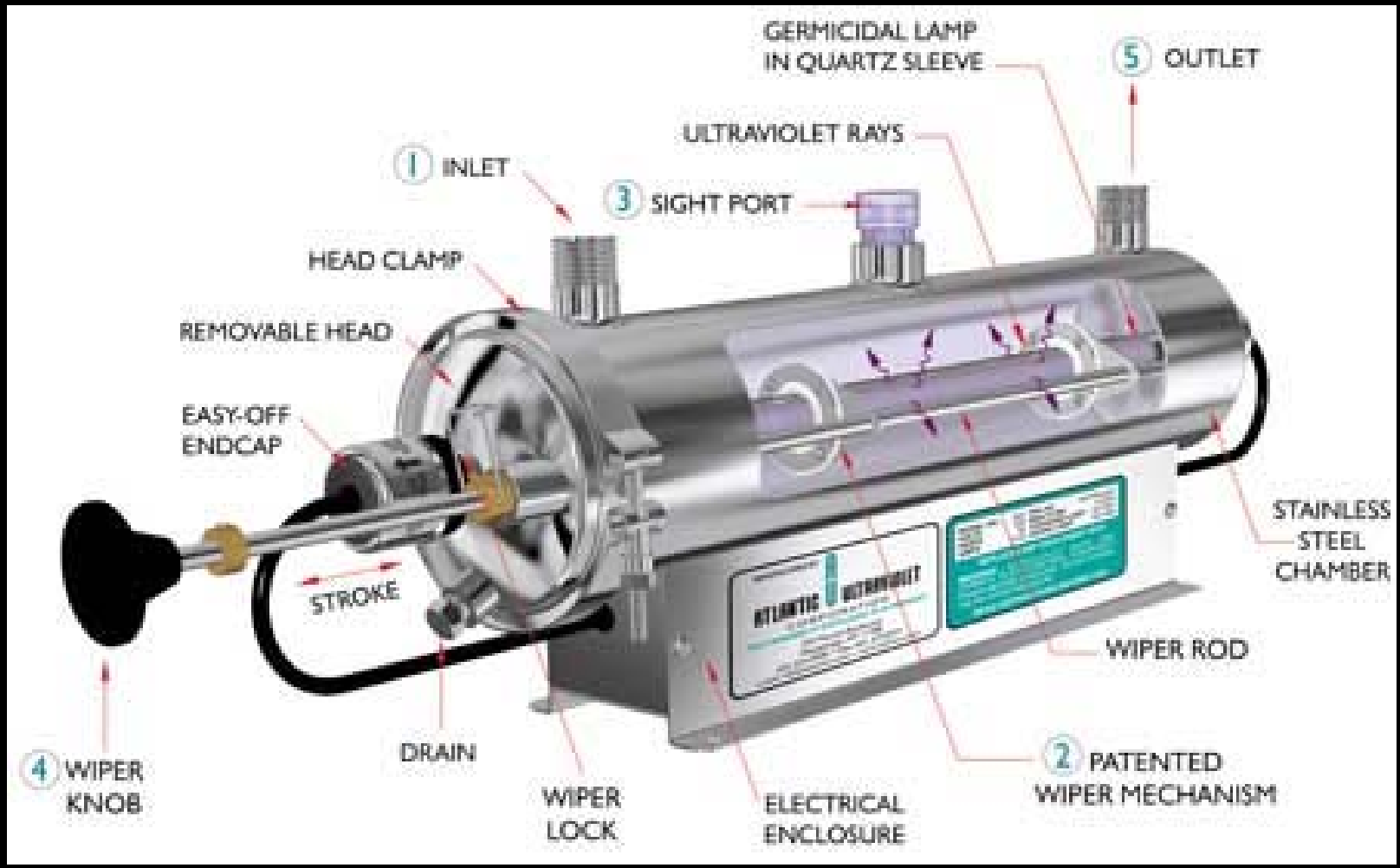
Ozone Sanitization Procedure



Ultraviolet

- Removal of Microorganisms and Dissolved Organics
- Design Considerations
  - Sized by flow rate
  - Ultraviolet sterilizers emitting:
    - 185 nm UV light with the proper contact time can reduce TOC levels in water.
    - 254 nm UV light with proper contact time will “Sterilize” the water
  - Ultraviolet sterilizers for pharmaceutical waters must be equipped with a monitor that measures the UV irradiance and they should be of sanitary design.

Ultraviolet



SANITIZATION

The frequency of sanitization

- Dictated by the results of system monitoring.
- Conclusion derived from the trend analysis of the microbial data should be used as the alert mechanism for maintenance.
- The frequency of sanitization established should be such that the system operates in a state of microbiological control and does not exceed Alert Levels.

Operation, Maintenance, and Control

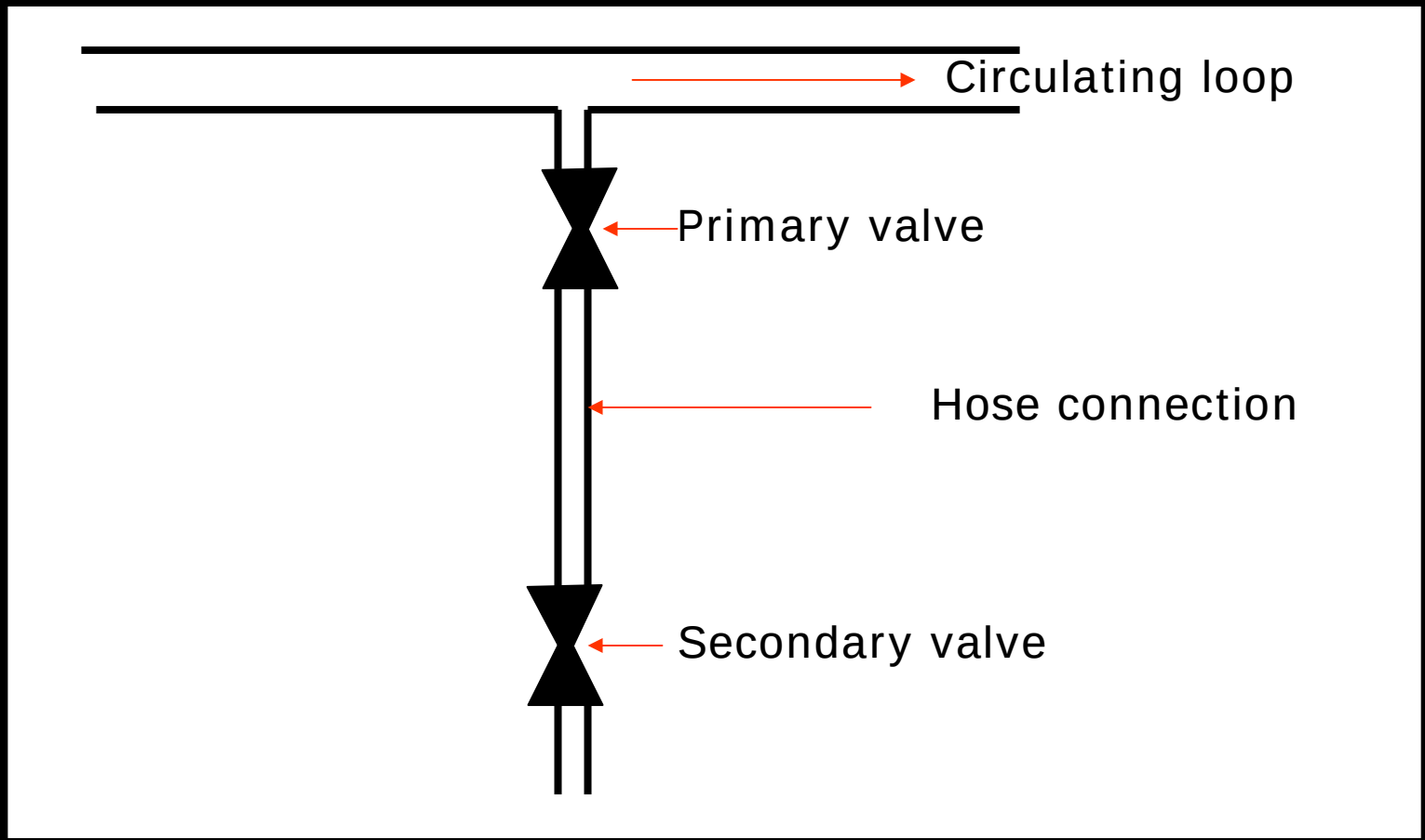
- Preventive maintenance program should be established to ensure that the water system remains in a state of control.
- The program should include
  1. procedures to operate the system
  2. monitoring programs for critical quality attributes and operating conditions, including calibration of critical instruments
  3. a schedule for periodic sanitization
  4. preventive maintenance of components
  5. control of changes to the mechanical system and to operating conditions

Operating Procedure

- Written procedure
  - operating the water system
  - performing routine maintenance
  - corrective action
- Procedure
  - detail the function of each job
  - assign who is responsible for performing the work
  - describe how the job is to be conducted

Operating Procedure

- Typical problem



Monitoring program

- Critical quality attributes and operating parameters should be documented and monitoring
- The program may include
  - a combination of in-line sensors or recorders (:- a conductivity meter and recorder)
  - manual documentation of operational parameter (:- carbon filter pressure drop)
  - laboratory test (:- total microbial counts)
  - frequency of sampling
  - the requirement for evaluating test results
  - the necessity for initiating corrective action

Sanitization

- Depending on system design and selected units of operation
- Routine periodic sanitization may be necessary to maintain the system in state of microbial control

Preventive maintenance

- The program should establish
  - what preventive maintenance is to be performed
  - the frequency of maintenance work
  - how the work should be document

Change control

- The mechanical configuration and operating conditions must be controlled
  - Proposed changes should be evaluated for their effects on the whole system
  - The need to requalify the system after changes are made should be determined
- Following a decision to modify a water system, the affected drawings, manuals and procedures should be revised.

Sampling considerations

- Sampling ports should be sanitized and thoroughly flushed before a sample are taken
- Sample containing chemical sanitizing agents require neutralization prior to microbiological analysis
- Sample for microbiological analysis should be tested immediately or suitably protectd to preserve the sample until analysis can begin

Sampling considerations

- Monitoring frequency enough
- Taken from representative location within processing & distribution system
- Sampling frequencies should be based on system validation data & should cover critical areas
- Unit-operation sites might be sampled less frequently than point-of-use sites
- Sampling plan should take into consideration the desired attributes of the water :- WFI more rigorous sampling frequency

Inspection of water systems

- A sampling and monitoring plan with a drawing of all sample points
- The setting of monitoring alert and action levels
- Monitoring results and evaluation of trends
- Inspection of the last annual system review
- Review of any changes made to the system since the last audit and check that the change control has been implemented
- Review of deviations recorded and their investigation
- General inspection of system for status and condition
- Review of maintenance, failure and repair logs
- Checking calibration and standardization of critical instruments

PI 009-2

AIDE MEMOIRE

INSPECTION OF UTILITIES

คำถาม?