Microbial Status and Sampling of Pharmaceutical Water Systems

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Determining and Ensuring the Microbiological Integrity of HIGH PURITY Product Water

Types of Bacteria in DI Water

- 12 broad groups identified including pseudomonas, Moraxella, Morganella, Flexibacter, Caulobacter and other stalked forms, gram positive Pleomorphic rods, and Sphaerotilus
- Many unidentified gram+ were present too.[5]

- Fishkill 43 types (25 pseudomonad)
- Pilot plant -only 9 (7 pseudomonad) [6]

Non-viable Vs Viable Ratios

- RO inlet 41 : 1
- RO product 3 : 1
- UV effluent 67000 : 1
- MB outlet 461 : 1
- Point of distribution 15 : 1

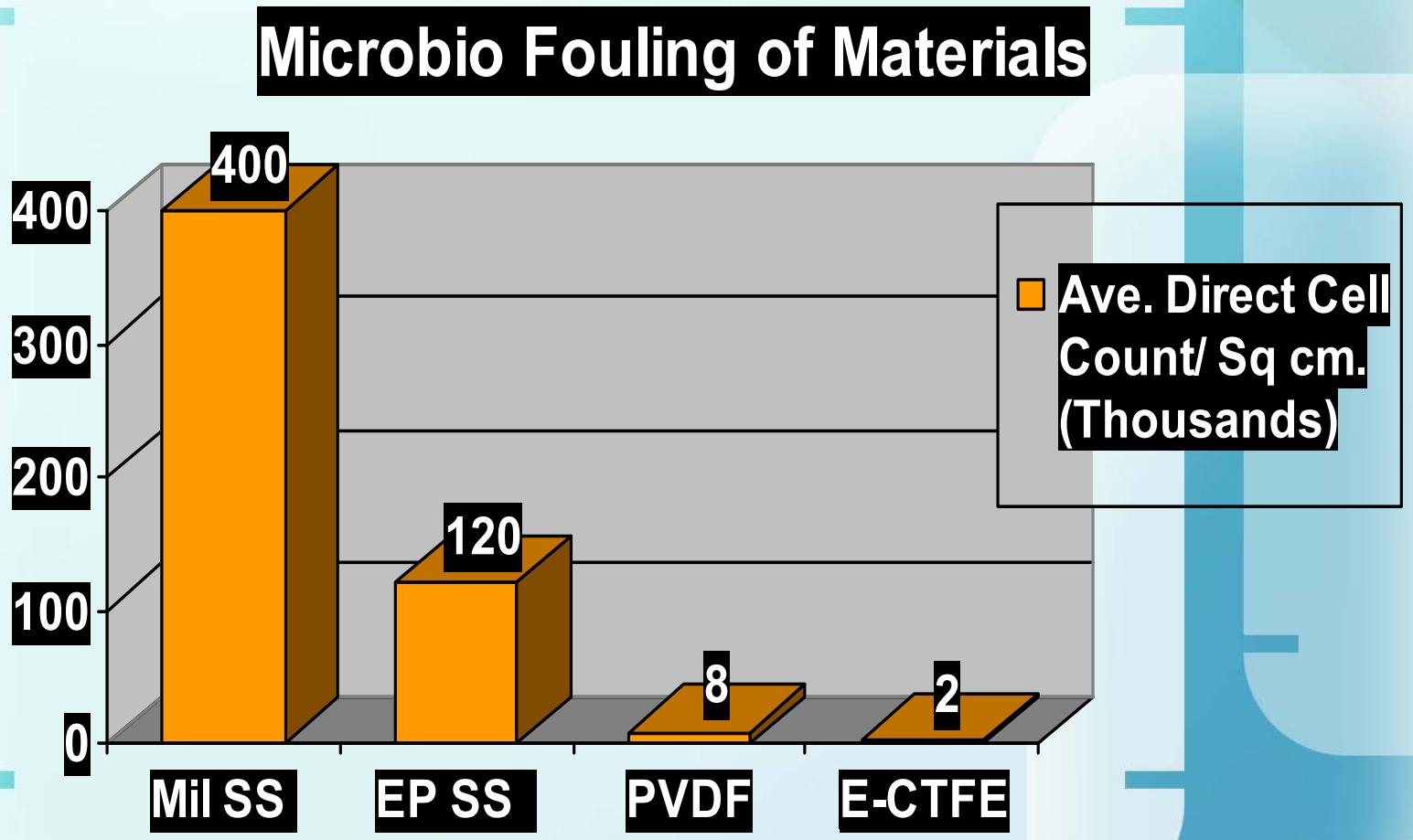
Velocity, Reynolds # and Biofilm

- DI water velocities of 5-7 ft/sec is erroneously considered required for microbio control
- Biofilms are thinner than viscous boundary layer, even in turbulent water
  - It is believed turbulent flow may support biofilm growth due to better nutrient transport

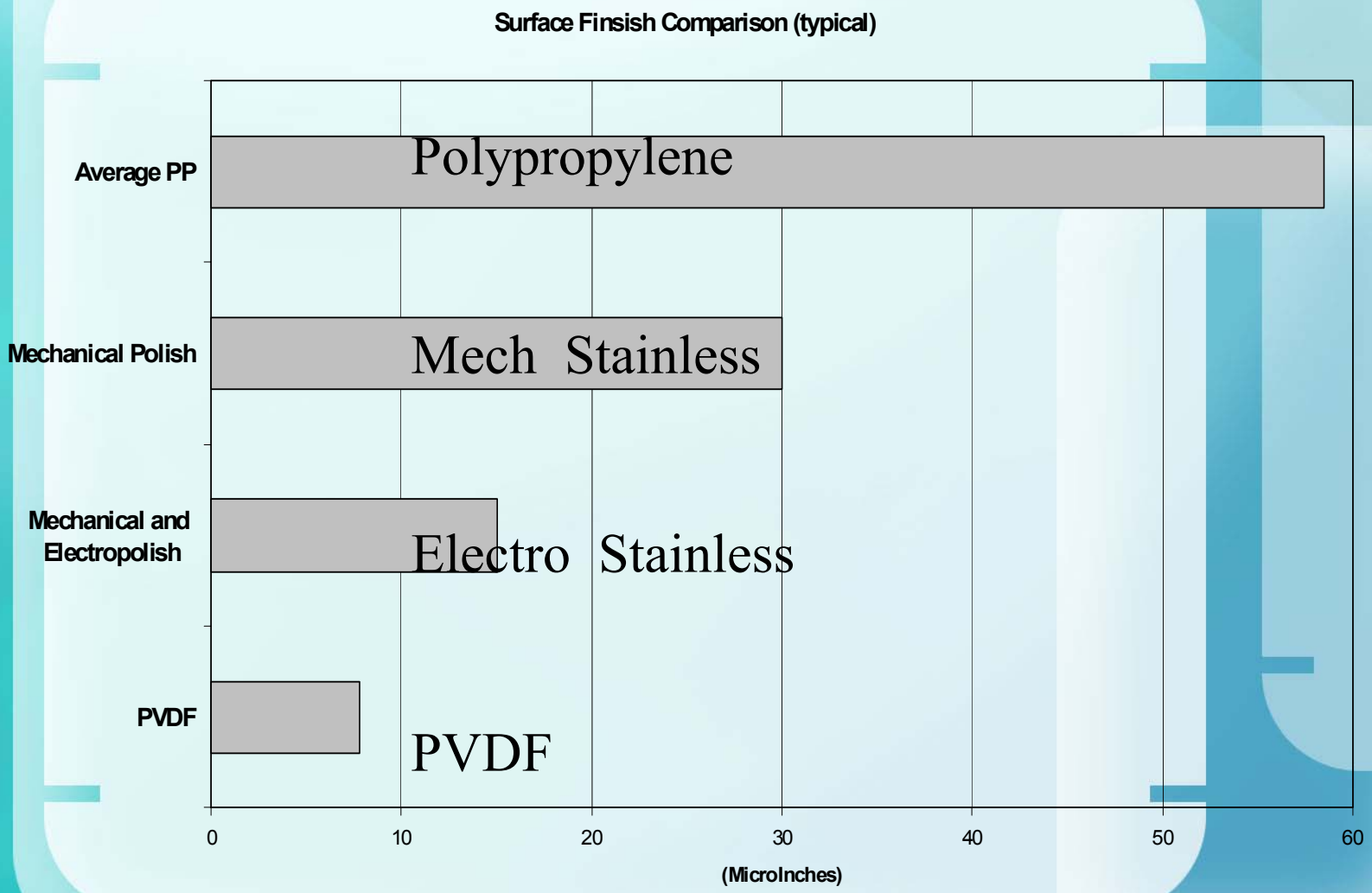
Stainless Steel vs. Thermoplastic Piping

- Husted demonstrated Thermoplastics had consistently less biofouling than stainless steel. [i]
- Stainless Steel was generally concluded to allow more extensive biofilm growth than plastics.[4]

Biofouling by Materials



Surface Roughness for Piping



Biofilm Control Strategies

- Sanitization.
  - System sanitization is a procedure that addresses failure to control microbiology.
- Do not sanitize unless you reach a predetermined action limit (1 cfu/ 100 mL).
- You should only reach that action limit if something is wrong.

Parameters **not** Effecting Biofilm Growth

- **Light**
  - **Run Water system in a dark room ??**
- **Velocity of Water**
  - Velocity of water has been shown to have little or no effect on microbiology and biofilm formation
- **Dissolved Gases ??**
  - **Insufficient information**

Parameters Effecting Biofilm Growth

- **Prevent Colonization of Loop by Bacteria.**
  - **System Design Philosophy**
- **Heat**
- **Materials of Construction**
  - **metals may provide scarce limiting nutrients**
- **Nutrient Deprivation**
  - **In extreme nutrient deficient environments, bacteria will not attach to surfaces. If they do not attach, cannot form biofilm. -Costerton[i]**

Purified Water is not good enough

- 10 - 50 cfu/ml cannot be reliably controlled
  - Bugs can spike OOS for a variety of reasons
- Purified Water is not of sufficient microbial quality for most pharmaceutical applications

HP Purified Water is secretly desired

- HP (Highly Purified) Water requires
  - <10 cfu/ 100 ml bioburden
  - < 0.25 EU Endotoxin

  due to popular demand
- Low microbial HP Purified Water is required for diverse application

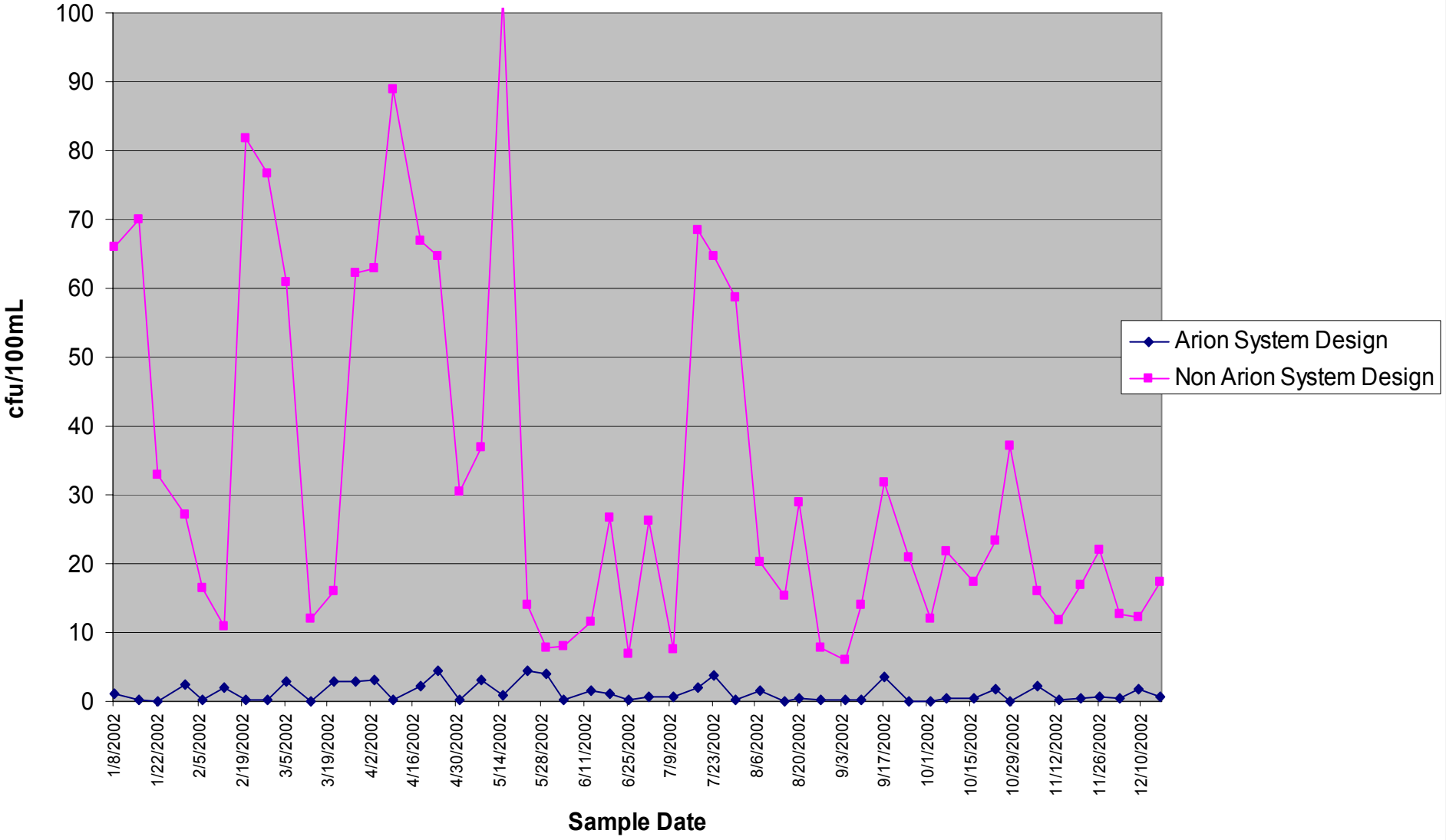
It's is better than you think

- The microbial quality of the water is distribution is typically better than the microbio test results indicate
- The difficulty is getting the water out of the distribution system without contamination

Figure 7

Standard Plate Count for Ambient Temperature Purified Water System

No Sanitizations Performed Since Start-up (May 2000)
Average of 6 samples collected weekly
8 out of 300 samples were discarded



Sanitization may not help

- Hot Water and Chemical Sanitization of problem water systems may exacerbate problems
- TNTC spikes are common after sanitization
  - This is likely the recolonization of biofilms

Problem Water Systems

- Allow stabilization or equilibration of microbial results
- If numbers remain excessive, look for ways to reduce nutrients (TOC)
- If quality is not acceptable, the water system design is likely at fault

The Microbiological Quality of Water in Distribution

- If higher quality Purified Water is required, 100 ml sample size is statistically too small
- To demonstrate

  < 1 cfu/ 100 ml quality, one liter sample sizes are required

If Better Microbio Results are Required

- Reliable HP Purified water system design is required
- Point of Use and Sample valve configuration is critical
- Properly designed and operated distribution loop is required

If Better Micro Results are Required

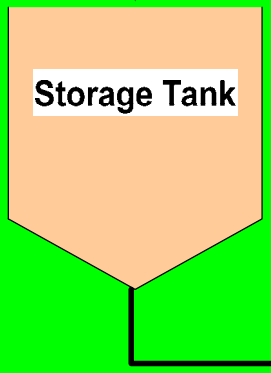
- Sampling method is critical
- Max velocity rinse is required
  - Both before use and before sampling

Microbiological Quality of Water in Distribution

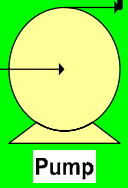
2) Point of Return
The quality of water the piping system distributes

Large sample volume (1 Liter) required

1) Point of Distribution
The quality of water the system design can produce.
Large sample volume (1 Liter) required



Post treatment



3) Points of Use
The quality of water procured from the use point configurations and sample points.
Large volume sampling is difficult

Microbio Quality of Product Water

- **Point of Distribution (PoD)**
  - **the quality of water the system can produce. If it is not in spec, the water system design is at fault.**
- **PoD must be sampled at high volume**
  - **(1L minimum) for statistical relevance.**

Micro Bio Quality of Return

- Point of Return (PoR)
  - The quality of water the distribution system can deliver. If not in spec; the use points or distribution piping design is suspect.
- PoR must also be sampled in large volume
  - 1 Ltr sample size

Point of Use Configuration

- Point of Use (PoU) – The quality of water procured from the use point configurations and the sample points
- The PoU bacteria results are often significantly poorer than the water in distribution

Point of Use Valve Design

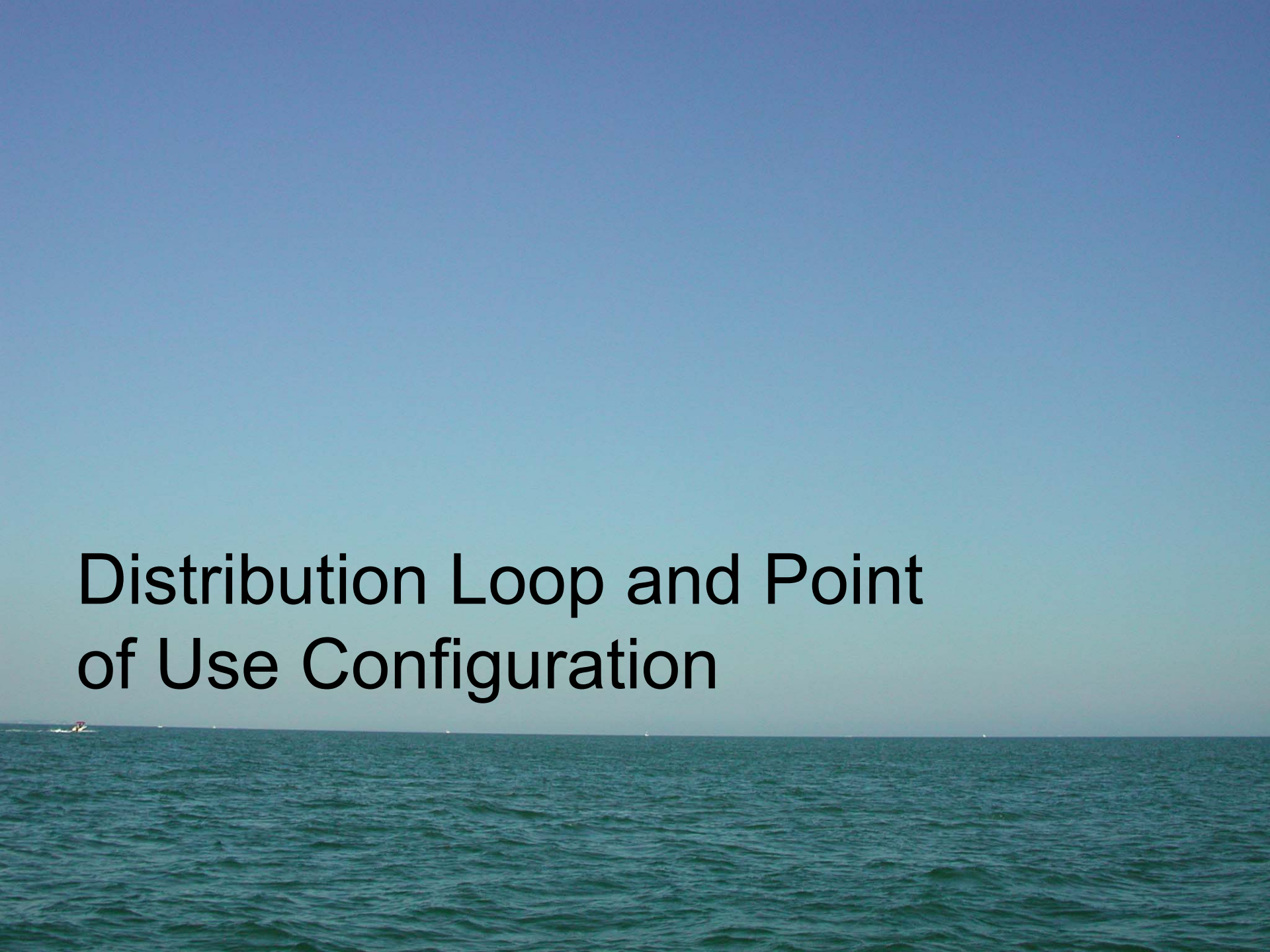
(best to worst)

- Zero dead-leg diaphragm
- Diaphragm
- Ball Valve (Not allowed in loop)
- Needle Valve
- Goose Neck (Dead-leg)
- Re-circulating goose neck (Restricted flow)

Point of Use Valve Multiplier (estimated)

(distribution bacteria = 1X)

- **Zero dead-leg diaphragm (1.5x)**
- **Diaphragm (3 x)**
- **Ball Valve (5 X)**
- **Needle Valve (10X)**
- **Goose Neck (20 X)**
- **Hoses or Tubing (30 X)**


Distribution Loop and Point
of Use Configuration

Use Point Configuration

- The number and size of the use points must be in realistic proportion to the size of the pipe
- PoU valves must not exceed ½ the size of the distribution pipe size

Size and # of Use Points

Pipe Size	Use Point	Max #
1.0”	¼ - 1/2”	15- 10
1.5”	3/8” – ¾”	30- 20
2.0”	½” – 1.0”	40 - 20
3.0”	¾” – 1.5”	50 - 20
4.0”	1 ” – 2 ”	60 - 20
5.0”	1 ” – 3 ”	70 - 20

Distribution Loop Design

- Instantaneous water demand should not exceed 75% of the flow in distribution
- In critical applications, max water demand should not exceed 50% of the flow in distribution

Distribution Piping

Pipe Size	Flow rate	Length
1.0”	12 gpm	400 ft
1.5”	30 gpm	~1,500 ft
2.0”	60 gpm	~3,000 ft
3.0”	140 gpm	~4,000 ft
4.0”	200 gpm	~6,000 ft
5.0”	350 gpm	~8,000 ft

Distribution Loop Design

- Do not disrupt flow
- Flow surges and pressure fluctuations in the distribution loop scour pipe and dislodge biofilm

Do not disrupt flow

- Only distribution pumps need be on back up power supply
- If distribution is disrupted, await one or two loop recirculation volumes to be assured of a return to equilibrium quality

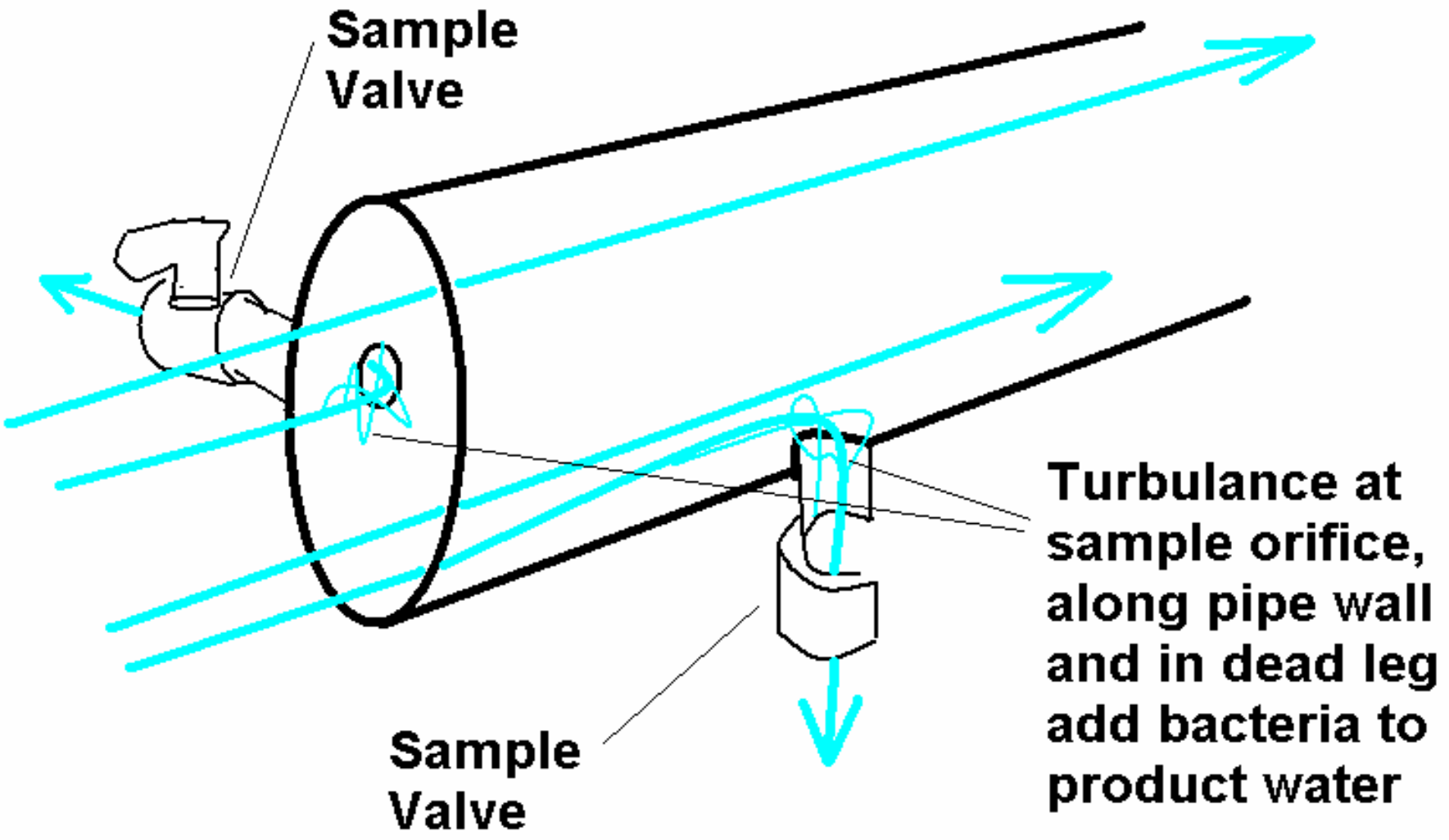

Water Sampling

Water Sampling

- Sampling still remains problematic
- Sampling should reflect the way water is used
- Do not use alcohol or long rinse regimes
- Require Max velocity rinse for 3-5 seconds prior to use or sampling

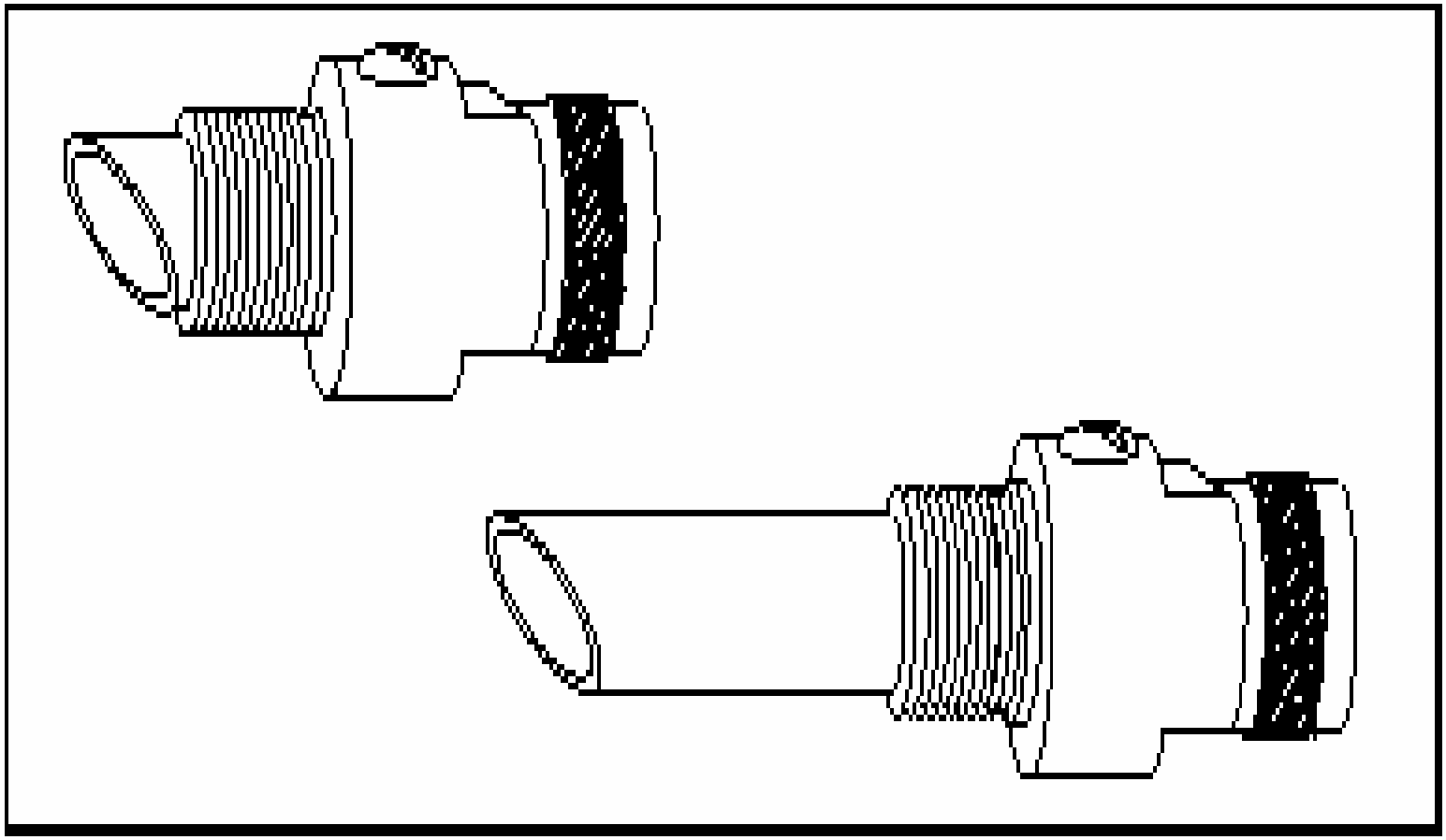
Rinse at Maximum Velocity

- Use points must be capable of max velocity rinse
  - Do not place in ceiling or pointed at electrical panels
- Use points over 1.5” cannot be rinsed at max velocity
  - Must add a hose or a sample valve

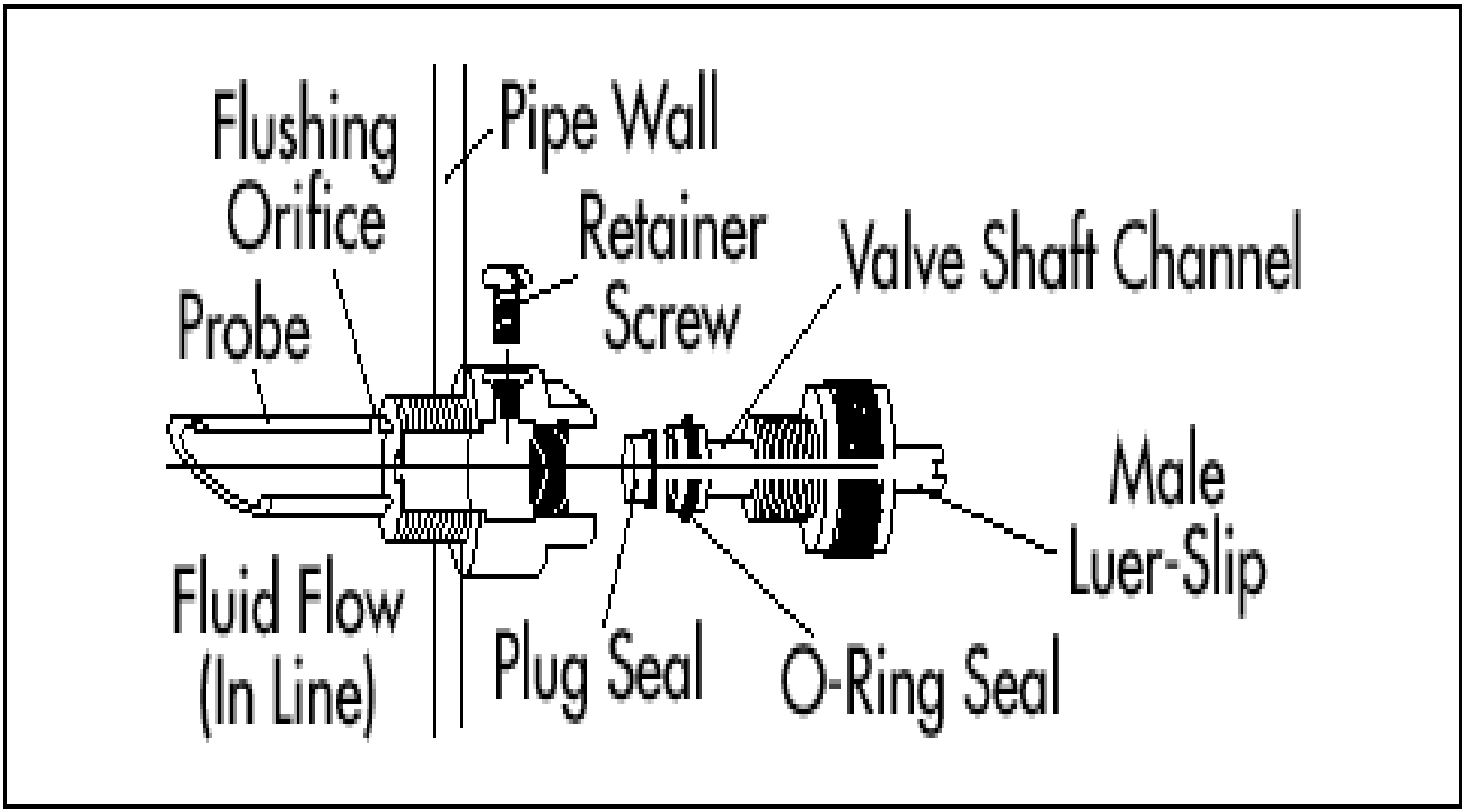


Standard sample cocks typically add bacteria

Sanitary Sampling Valve



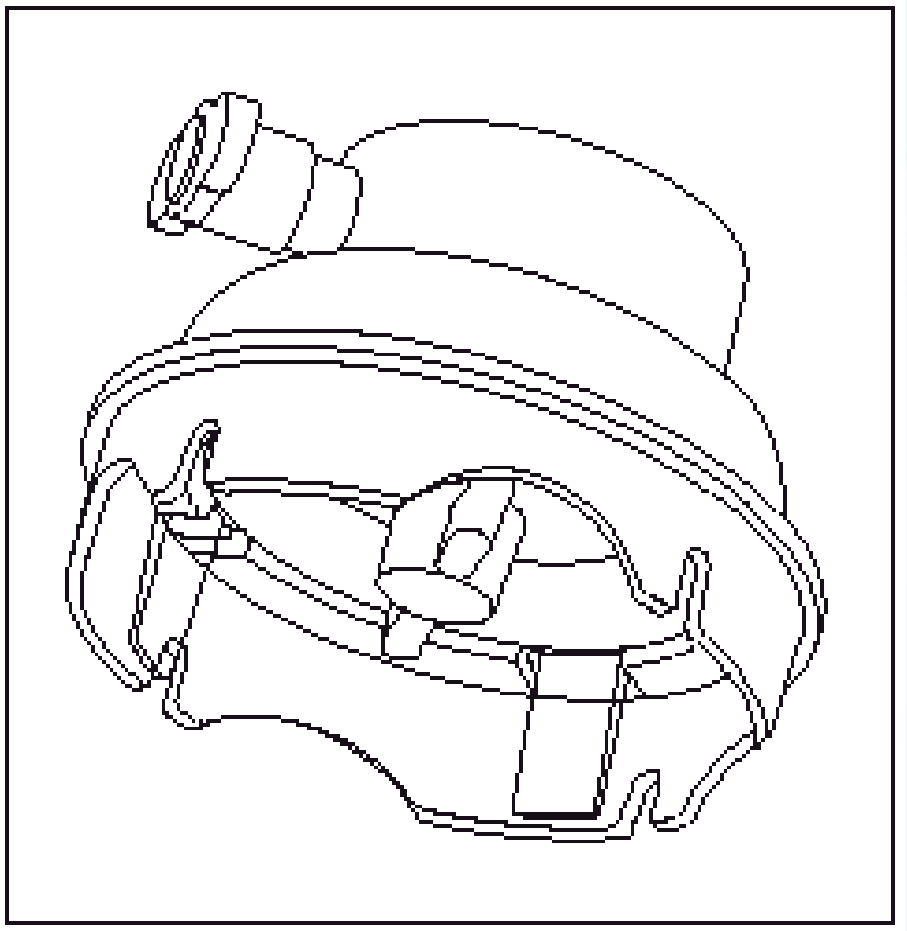
Pitot style sample valve



Pitot Style sample valve

- Sample water from the bulk phase of the water in distribution
- Sample water away from the pipe wall which can contaminate the sample
- Very small sample bore permits high velocity rinse
- Adapter permits use of large volume SPC bacteria tester for Liter samples

MicropreSure® In-line Filtration Monitor



Sample valve and configuration

- Sample valves must be at least less than ½ the distribution pipe size
- The smaller the valve, the higher the rinse velocity
- Hit max velocity (wide open) for 3-5 seconds prior to use or sampling

Minimum SPC Microbial Sample Volumes

- Purified Water = 100 ml
- HP Purified = 100-300 ml*
- WFI Water =100- 300 ml*

  * 250 ml in ISPE Baseline Guide
- Incubate at room temperature and read at 3 and 7 days


Thank You
Questions?
Contact Us:
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