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# DYNAMICS OF WATER PURIFICATION SYSTEMS AND MICROBIAL ANALYSIS IN PHARMACEUTICAL INDUSTRY

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## ABSTRACT

*Water purification system and contamination free water plays a key role in pharmaceutical parenteral drug product manufacturing. The water used as excipient in drug manufacturing undergoes several purification procedures and qualitative checks for identification of microbial contamination. To identify the efficiency of water purification systems and water quality, microbial trending is performed by collecting and correlating the microbial analysis results. Trend evaluation identifies the level of microbial contamination in pure water and water for injection grades. Based on the outcome of trend, corrective and preventive measures are implemented to reduce the recurrence of microbial contamination. After implementation of corrective and preventive measures, microbial results trending is performed. Trending results shows that the microbial recurrence and contamination levels are reduced. The complete study is an evident that the frequent trending of microbial results helps in reducing the microbial contamination and improves the water quality in pharmaceutical parenteral drug manufacturing industry.*

## KEY WORDS

*Water purification system, sampling points, microbial analysis results, trend data, corrective and preventive measures.*

## INTRODUCTION

Microbial contamination of water is the most common and unavoidable phenomenon identifying commonly in pharmaceutical Parenteral (1) manufacturing industry. For the degree of excellence and assurance of quality, water at various stages of its processing is subjected for examination to identify microbial contamination at different grades of water such as Source water (2), Potable water (2), pure water (2) and water for Injection (2). Source water and potable water is further processed to achieve pure water and water for injection. Pure water is regularly used in pharmaceutical industry for several activities such as equipment washing, Surface and floor cleaning, machinery cleaning, generation of pure steam. The final stage of water, water for Injection is mixed with Excipients (3) and Active Pharmaceutical Ingredient (4) (API) to obtain finished drug product, which is further prescribed to patient as Intravenous or Intramuscular injection. Hence availability of non-contaminated pure water is one of the major essential requirements for maintaining quality and efficacy in pharmaceutical industry and patient safety. Failure to maintain qualitative and contamination free water systems can cause the product recalls and audit failures for parenteral manufacturing companies. Along with Parenteral manufacturing industry, the oral dosage forms such as tablets, capsules and syrups manufacturing facilities also need to maintain the quality water systems to ensure manufactured drug quality, safety and efficacy. This indicates that, the water used in pharmaceutical industry should subject to

several purification and filtration steps to control the contamination at every step of purification. Considering the importance of water purification systems, a study was performed to evaluate the microbial burden present in the water system. Based on the outcome of study the corrective and preventive measures are established to reduce the recurrence of microbial contamination in water systems, which ensure the manufacture of high quality medicines for patient safety and recovery.

## MATERIALS AND METHODS:

The following materials and methods were used for the study:

- Apparatus for Pour plate method to perform source water Bio Burden identification
- Apparatus for Membrane filtration method for Potable, pure and water for injection analysis.
- Gram staining kit
- Spore staining kit
- Lacto phenol cotton blue mount kit
- Apparatus for Biochemical tests
- VITEK 2 (Figure 2) compact identification system
- Incubation chambers for Bacteria and fungi Microorganisms

**Water Purification Procedure:**

The purification process contains multiple steps from conversion of Source water to water for Injection. The pictorial representation of water purification system is represented in figure 1.

**Water purification steps (5):**

**Portable water generation system**

Consists of multi system grade sand filters, ultra-filtration, softener system, HSRO (Hot water sanitizable Reverse Osmosis)

1. **Borewell water (Source Water) (5)**

↓

Chlorinated by NaCl (Done by inline dosing pumps)

↓

Continuous circulation

↓

Chlorine sensor fitted to analyze FRC level
(Free residual chlorine).

↓

FRC analyzer is linked to electronic inline dosing system to maintain required level

↓

2. **Multi Grade sand Filter (MGSF): [suspended particles and turbidity removal] (5)**

↓

Water passes from top to bottom through different grade of filters such as pebbles and gravel (Supporting $15m^3$/hour)

↓

3. Ultra-filtration: [Colloidal Matter Removal]

(Used to separate macromolecules and colloids based on their molecular weight and also ensures removal of large organic bacteria, pyrogens, colloidal silica, iron and emulsified oils) Water recovery of about 90-95% by back flushing for every hour

↓

4. **Softener system (5):**[Used to remove total hardness by using ion-exchange resign]
(Filtered water then led to sodium softener. $Ca^{++}$and $Mg^{++}$ present in water will be replaced by $Na^{++}$)

↓

5. **Cartridge filter (5): [**colloidal particles Removal**]**
(5µ filter e SS 316L housing provided along drain and air vent)

↓

6. **Dechlorination** (5): $Na^{-}$ meta bisulphate dosing

↓

7. Anti-succulent dosing system: [Prevents organic and inorganic fouling of RO and polymer based neutral chemicals].(Present prior to cartridge filter for RO)

↓

8. pH correction by dosing system: Maintain pH after softener

↓

9. HSRO: connected e conductivity meter and TOC

↓

10. Potable water

↓

11. Storage tank

↓

12. Pre-jacketed storage tank and steam is passed to heat up water provided i.e vent filter and spray balls

↓

13. UV dosing Module: [Fed to purified water level to provide high degree of quality assurance]
(UV dose of 130000 MW/$cm^2$)

↓

14. RO system

↓

15. Chemical cleaning and hot water generation tank

↓

16. RO water collection tank

↓

17. RO water transfer pump and UV dosing module

18. Pure water/ water for Injection as required.

**Figure 1 provides the pictorial representation of water purification system use commonly in pharmaceutical industries.**

**Source water/Feed water** (5) (Raw water): Source water represents the water derived from a variety of sources including a public water utility, a private water supply (e.g. tube well/ bore-well) or a combination of both sources.

**Potable water (Drinking water)** (5): Water meeting the requirements of the national primary drinking water regulations (NPDWR) (40 CFR 141) issued by the U.S Environmental protection agency (EPA) or the drinking water regulations of the European Union or Japan, Bureau of Indian standards (BIS) (100500) or the world Health Organization (WHO) drinking water guidelines. This water is used for the production of purified water.

**Purified water** (5): Water used for operations such as cleaning of equipment, clean room floors, walls, glass panels and non-product- contact components. Purified water is obtained after potable water purification steps such as deionization, ion exchange, reverse osmosis filtration, or other suitable purification procedures.

**Water for Injection** (5): Water used as an excipient in the manufacturing of finished drug product and several other sterile operations. The microbial contamination and endotoxin levels in water for injection are very important and should be controlled.

Rationale for selection of sampling point:

Sampling locations/ points (6) are selected based on selection criteria, and these sampling points are covering all stages of water purification as explained above. Water sampling points (7) are identified based on sampling criteria and water grade utilization frequency (8). From these sampling points water for analysis is collected regularly on rotational basis as per standard operating procedures. The analysis is mainly performed through two techniques named as pour plate method (9) and membrane filtration method [9]. Pour plate method is preferred for source water and membrane filtration method is for other grades of water.

Establishment of specification Limits:

To evaluate the water purification system a study was conducted for a period of 12 months from January 2015 to January 2016 by monitoring all sampling locations of all grades of water. Based on water monitoring trend data (10) after initial qualification of water systems and regulatory guidelines such as International Conference of Harmonization (ICH), United States Pharmacopeia (USP) and British Pharmacopeia (BP), the established monitoring limits for water analysis is explained in Table 1.

**Table 1: Specification Limits of Water Monitoring**

| Grade of Water | Method | Testing sample volume | Limit |
|---|---|---|---|
| Source water | Pour Plate method | 1.0mL | 500cfu/mL |
| Potable water | Membrane filtration method | 1.0mL | 500cfu/mL |
| Pure water | Membrane filtration method | 1.0mL | 100cfu/mL |
| Water for injection | Membrane filtration method | 100mL | 10cfu/100mL |

**Sampling Methodology:**

Water sampling (11) is one of the most skilled process need to be performed in pharmaceutical industry. As the number of sampling points is high for all types of water grades from Source water to Water for Injection, a rationale is proposed for sampling the water (12). Based on the rationale a weekly schedule is prepared for all sampling points, where the specified set of sampling points were selected from all grades of water and analyzed on daily basis (12) in which all sampling points are covered in cyclic basis for daily monitoring. Through this rational water samples are collected and tested, to identify the presence of microbial contamination.

**Procedure of sample collection:**

A sterilized/depyrogenated glass bottle with screw cap is used for water sampling. After completion of depyrogenation (13) the sampling bottle is covered with aluminum foil and carried to sampling location through SS transfer bin. During collection of sample, aluminum foil is removed and open the cap of bottle, then slowly

releases the sampling valve/dead leg (12) to collect the water. After opening the valve, water is allowed to drain for some time as the initial water is prone for microbial presence due to environmental factors and contact surfaces of valves (14) after draining rinse the sampling bottle for twice and collect required quantity of water for analysis. After completion of sampling, bottle cap is replaced and cover with aluminum foil and the bottle is transferred to laboratory for analysis (14) through SS transfer bin.

**Source water testing by Pour plate method**:

Aseptically 1mL water sample was transferred into the two different labeled Petri plates. Approximately 20-25mL of R2A agar maintained at 45° to 50° was poured in both the plates and rotated clockwise and anti-clockwise to mix contents uniformly and allowed to solidify (9).

Negative control: R2A agar media was poured in the pre-sterilized plates and allowed to solidify.

Positive control: Growth promotion test was performed with *Pseudomonas Aeruginosa* on R2A media.

Interpretation:

Mean of two plates was calculated and results interpreted as CFU/mL of water sample. Positive plates are observed for presence of growth. In positive plates the CFU's observed must not differ by a factor greater than 2 from the standardized inoculums value (organism suspension' count)

Negative plates should not show growth.

**Membrane filtration method for Potable, Pure and Water for Injection:**

For Potable and pure water, approximately 25 mL of sterile Fluid of peptone is aseptically poured in to the sterile filtration funnel and then 1 mL of water sample was aseptically added into the filtration funnel and filtered through a 0.45 µm membrane (15) filter by applying vacuum. Filter was rinsed with 1X 100mL of sterile fluid peptone and transferred to pre-labeled R2A plate (9). In case of Water for injection 200 mL of sample was filtered through 0.45µm membrane and rinsed with 1X 100mL sterile fluid peptone and transferred to pre-labeled R2A plate (9). Plates are incubated in upright position at 30°-35° for 48 hours.

Negative control: 100 mL of sterile fluid peptone was filtered through 0.45µm membrane filter. After filtration the membrane was transferred to R2A plate.

Positive control: 200 mL of any one representative sample was filtered with 1X100mL of sterile fluid peptone containing 0.1 mL of *Pseudomonas aeruginosa*[9] having 10-100 CFU was filtered through 0.45µm membrane filter and transferred to R2A plate.

Interpretation:

Plates are observed after completion of the incubation period and results interpreted as CFU/mL and CFU/200 mL.

Negative plates should show no growth

After completion of sampling the plates are carefully removed by closing the lid and transferred to incubation chamber for incubation in inverted position at 20° to 25° for 72 hours and further at 30° to 35° for 48 hours. After completion of incubation plates at respective intervals, plates are examined physically for growth and results were recorded appropriately.

**RESULTS AND DISCUSSION:**

After analysis of all sampling points through these methods throughout the time period of 12 months, surprisingly the CFU above the specified limits were identified in Pure water and Water for Injection. However numerous microbes were identified in source water and potable water but the identified CFU were complies with the specification limits. The main approach of this study is to trend the identified samples which are exceeding the established specification limits. The identification and trending of out of specification is extended to the sample location, water grade, criticality of sampling point and category of microorganisms identified. However, the microbes identified at initial stages of water is further subjected to several further purification steps as described above which will ultimately reduce the microbial burden of water. In microbial trending the first important point for consideration is identifying the colonies which exceed speciation limits on colony counter (16). If the colonies are found out of specification then they were subjected for further assessment and comparison with previous water monitoring results and trend history. The identification procedure is explained below.

- Incubated plates which showed growth above the specification limit were observed for colony characteristics and stored in refrigerated condition till the identification procedure was completed.
- Gram staining, spore staining, lacto phenol cotton blue mount and biochemical tests (16) were performed on identified colonies to identify the phenotypic morphology of microorganisms.

- Further identification of the microbial colony upto genomic level was conducted by using VITEK 2 identification system [17]. VITEK 2 compact identification system is a fully automated system which performs bacterial identification by biochemical analysis using advanced colorimetry. This VITEK 2 compact system is highly automated and allows for the rapid, accurate identification of bacterial strains. In addition to being able to identify bacteria, the VITEK 2 compact system is able to identify multiple species of yeast also.
- After completion of identification, the identification number was assigned to each source of isolate in the format of XX/YY/ZZ. Where XX represents the date, YY represents month and ZZ represents year for better clarity and traceability [10].

**Results of Pure Water analysis:**

Pure water is the grade of water used mostly for several activities such as cleaning of equipment's and floors, generation of pure steam, washing of personnel gowns. Hence contamination in pure water is a major threat for pharmaceutical quality management systems. By interpreting the results of one-year trend, two outbreaks with out of specification limits were identified in samples collected for pure water. Upon complete investigation the colonies were identified as ***Kocuria kristinae*** (16) and ***Acinetobacter calcoaceticus*** (16). The detailed description and characteristic of these identified species is explained in table 2. Figure 3 represents the colony morphology of *Kocuria kristinae* and Figure 4 represents *Acinetobacter calcoaceticus.*

**Table 2: Microorganisms Identified Out of Specification in Pure Water Trend During the Period of January 2015 To December 2015**

| **Microorganism** | *Kocuria kristinae* | *Acinetobacter calcoaceticus* |
|---|---|---|
| **Domain** | Bacteria | Bacteria |
| **Phylum** | Actinobacteria | Proteobacteria |
| **Order** | Actinomycetales | Psuudomonadales |
| **Family** | Micrococcaceae | Moraxellaceae |
| **Morphology** | Saprophyte, circular, convex, opaque, shiny pink smooth colonies | Saprophyte, coccobacilli with smooth, round mucoid white or creamy colonies |
| **Metabolism** | Strictly aerobic | Strictly aerobic |
| **Gram staining** | Gram positive | *Gram Negative* |
| **Catalase** | Positive | *Positive* |
| **Oxidase** | Positive | *Negative* |
| **Normal Flora** | Wide spread in water and Found on skin of human and other animals. | Widespread, Found in soil, food and drinking water. |

**Table 3: Microorganisms Identified Out of Specification in Water for Injection Trend During The Period Of January 2015 To December 2015**

| | |
|---|---|
| **Microorganism** | *Alicyclobacillus acidocaldarius*[16] |
| **Domain** | *Bacteria* |
| **Division** | *Firmicutes* |
| **Class** | *Bacilli* |
| **Order** | *Bacillales* |
| **Family** | *Alicyclobacillus* |
| **Metabolism** | *Strictly aerobic* |
| **Morphology** | Large circular, mucoid creamy, white colonies |
| **Gram staining** | Gram positive |
| **Catalase** | Positive |
| **Oxidase** | Negative |
| **Normal Flora** | Wide spread Found in food and fruit spoilage. |

**Table 4: Water Analysis Results During Period of March 2016 to March 2017**

| | |
|---|---|
| **Source water** | Complies with specification limit |
| **Potable water** | Complies with specification limit |
| **Pure water** | Complies with specification limit |
| **Water for injection** | Complies with specification limit |

**Fig 1: Multi Grade sand Filter image (MGSF)**

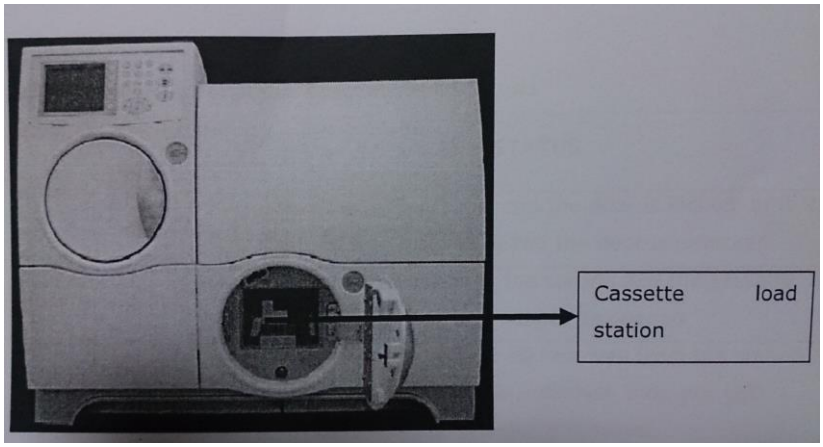


**Fig 2: VITEK 2 compact identification system**

**Fig 3: Colonies morphology of *Kocuria kristinae***

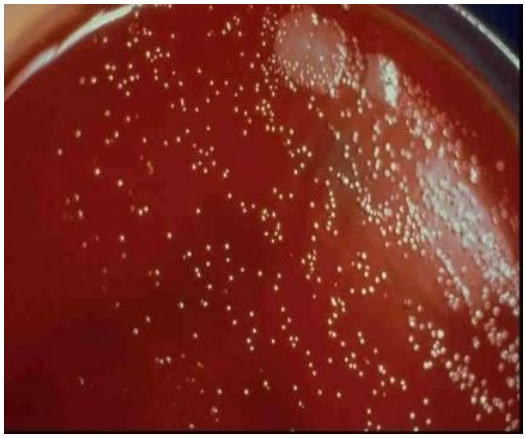

**Fig 4: Colonies morphology of *Acinetobacter calcoaceticus***

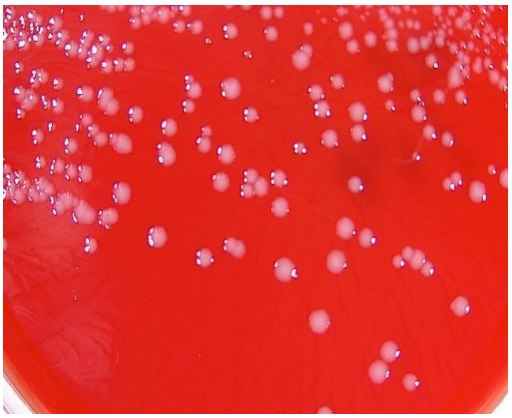

**Fig 5: Colonies morphology of Alicyclobacillus *acidocaldarius***

**RESULTS OF WATER FOR INJECTION ANALYSIS:**

Water for injection is the final grade of water, which will directly use in the manufacturing of finished drug product and also prescribed as a re-constitution fluid for many lyophilized injections. Hence the chance of contamination in this grade of water is highly problematic. Accordingly, the stringent specification limit is established as mentioned in Table 1. However, an outbreak of out of specification limit was identified in this study. The detailed description of identified organism ***Alicyclobacillus acidocaldarius*** is detailed in table 3 and figure 5 represents the colonies morphology**.**

The common characters identified in all the three organisms are, these are wide spread in water, Catalase positive and most commonly found in food and water. As these organisms are not present in previous stages source water and potable water, indicating that the purification process such as free radical chlorination, Multi grade sand filter, ultra-filtration, softener, Cartridge filter, De chlorination, Anti succulent dosing system, pH correction dosing system and UV dosing Module are working effectively for removal and control the microbial contamination. Based on the investigation the following possibilities are identified for microbial contamination.

- Pure water and water for injection are stored in large water storage tanks and transferred to desired locations through water system loops and dead legs, which are sterilizing for once in 15 days.
- The water samples of out of specification results are located in the areas where frequent activity such as manufacturing and cleaning is not occurring. However, the sample collection and testing is performed on rotational basis as per the schedule.
- The excursion (out of specification) sampling points are located in the area where collection of water sample is more critical to handle for sample collecting person in comparison with other sampling locations.
- The water sampling procedure and handling practices.

Based on the possibilities of contamination the following preventive measures were implemented to control the microbial contamination in water systems.

- The first preventive measure implemented is dead legs/sampling valves are physically verified for any leakages or damages, which may cause contamination to the water.
- The sampling points, where the regular activity is not occurring is scheduled to drain the water at frequent intervals of time, which will avoid the water storage at sampling point for longer time.
- The main control and preventive measure implemented in water systems is, the steam sterilization frequency of water loops by temperature 121$^{o}$ is revised from once in 15 days to once in a week (18).
- Personal training sessions are conducted for water sampling techniques and procedures (18).

After implementation of above discussed preventive measures a study of water sample results trending was carried out for a period of 12 months form March 2016 to March 2017 for all types of water grades as per sampling points monitoring schedule. In this study, no out of specifications results were identified in all grades of water samples, but on a few occasions microbial counts were observed, although within the specified limits. The basic investigation was performed with the obtained microorganisms and the colonies identified are Gram negative, Gram positive and aerobic species. The results of water monitoring trend is represented in Table 4.

## CONCLUSION:

Based on 12 months water system trend data review form January 2015 to January 2016 commonly three organisms were identified as being out of specification limits, *viz*. ***Kocuria kristinae*** and ***Acinetobacter calcoaceticus*** in pure water and ***Alicyclobacillus acidocaldarius*** in water for injection. Upon investigation of these organisms, it is identified that the microorganisms are wide spread as water and food contaminants. Even though the exact cause of contamination in water systems is unknown, it may have occurred due to possibility of human interactions during sampling procedure and several external environmental factors which are unavoidable. This could have been aided by the fact that the organisms are common contaminants.

The study was mainly focused to establish the preventive measures in water systems which will help to reduce the microbial contamination and possible reoccurrence of same organisms identified earlier. As is evident the trend data review studied in the period of March 2016 to march 2017 indicates that the contamination was reduced and no recurrence of previous identified microorganisms occurred.

Out of several preventive measures tested the most important in reduction of microbial contamination was sterilization of loops and dead legs in water system. The sterilization efficiency was checked and validated by utilization of steam resistant biological indicator *Bacillus stearothermophilus*. It may be noted that the organisms isolated and identified during trend was not steam resistant. No recurrence of organisms has occurred in trend period of march 2016 to march 2017 which shows that the revision sterilization frequency (18) of water system loops form once in 15 days to once in week is efficient in reducing the possibility of contamination. Accordingly, the microbial contamination is minimized and the organisms are not identified in the corresponding trending study as explained in Table 4.

Upon complete interpretation of work it is evident that water monitoring results trending is very effective in controlling and preventing the microbial contamination in water systems of pharmaceutical industry (20). Also the implementation of sterilization frequency on weekly basis will ensure the reduction of microbial growth, by reducing the prolonged incubation period of microorganisms in water systems.

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