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# Evaluation of Microbiological Cleanliness of Machines/Equipment through Rinse Technique Using Statistical Process Control

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## Abstract

To guarantee that patients receive safe therapy with predictable and acceptable medicinal properties, monitoring the quality standards in the healthcare sector and the pharmaceutical business, in particular, is essential. Medicinal products are no exception from these crucial characteristics and hence mitigation of the sources of microbial contamination is a mandatory strategy to avoid harming already-ill populations. Machines, equipment and tools that are used in the industry must be appropriately cleaned to ensure that they will not contaminate the product under processing. The study herein aimed to establish an evaluation system for cleaning efficiency through the rinse technique using Statistical Process Control (SPC) methodologies. A database was established and created for the recorded cleaning process over 20 months of the monitoring period. The control charts were constructed and evaluated from processed data using SPC software. A rare event control chart was used to track the cleaning process intervals with event probability estimated to be 0.080. Concerning the monitoring of the bioburden of rinse samples, the most appropriate fitting attribute chart is U type with Laney modification of over-dispersion to correct for tight control limits that increase alarming false points. Understanding the inspection property is inevitable for the right interpretation of trends.

**Keywords**

Laney correction, U chart, over-dispersion, control limits, SPC, event probability

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## INTRODUCTION

The establishment and dissemination of the quality concept are crucial for any reputable organization that strives to survive and grows in the modern competitive world (Kanji *et al.*, 1999; Zakuan *et al*., 2012). Total Quality Management (TQM) is a vision and perspective of management that focuses on long-term success through customer satisfaction (Mehra and Ranganathan, 2008). In a TQM initiative, every employee of a firm takes part in enhancing their workplace's processes, goods, and services. One of the important statistical tools is the process-behavior diagram which is used in the control and monitoring of a specific process and/or certain inspection characteristic.

One of the critical quality aspects in the healthcare industry, specifically the pharmaceutical field is the consistent safety of the delivered service or product to the final customer (Marucheck *et al*., 2011). Microbiological quality and safety are among the hot topics, especially when considering that medicinal goods are intended to be administered to populations that are sick and with health problems in most situations. This is a critical aspect to avoid any adverse events that could be stemmed from inappropriate product handling and processing.

During manufacturing and processing, medicinal products may be getting exposed to microbial contamination through the processing machines, equipment and tools themselves. Thus, it will be crucial to monitor and control their bioburden quality to ensure stability, predictability and diminishing level of contamination. The investigation herein focuses on the examination of the microbiological cleanliness of the machine/equipment that is used in the preparation of non-sterile medicinal products through rinse samples using Statistical Process Control (SPC).

## MATERIALS AND METHODS

The establishment of a measurable monitoring and control system for the assessment of the microbiological cleanliness of the machines and/or equipment that are used in the medicinal sector would be crucial for microbial safety of the manufactured products to be safe for consumption by minimization of the product risk of contamination. The Total Viable Count (TVC) was used as an estimate for microbiological cleanliness which was determined following the path of previous works (Eissa, 2018; Eissa *et al*., 2022).

**Study principle and goal**

Evaluation of cleaning quality for specific items is usually conducted through swabbing and/or rinse methods to monitor and control the efficacy of the standard practice established in the firm chemically and microbiologically (Schmitt and Moerman, 2016). Herein, the present investigation will focus on the method used to investigate a segment of this practice which is the microbiological quality of the rinse samples that were collected from equipment/machines after cleaning according to Standard Operating Procedure (SOP). Since bioburden count is examined in this case, the monitoring was performed using an attribute control chart (Eissa *et al*., 2021). The microbial count is expressed as Colony Forming Unit (CFU) which belongs to a discrete type of dataset and quantification is usually stated as CFU/mL or CFU/100 mL and the maximum allowable limit was set at 100 CFU/100 mL (Walters, 2008; Eissa *et al*., 2022). There should be an appropriate surveillance procedure over machinery and tools to maintain control over the TVC of microbes and hence protect the processed product from exposure to high microbial populations that could be transferred to the patients or terminal users (Roesti, 2019).

**Control charts selection**

From this perspective, C or U charts may deem the most appropriate types for this type of analysis (Eissa, 2018). Nevertheless, previous experience in the trending and collection of this type of data rarely follows the prerequisite of Poisson distribution for proper interpretation of these types of trending charts (Laney, 2002). This challenge could lead to misleading reading and interpretation of data due to incorrect control limits with subsequent false out-of-control alarms. Thus, if the initial diagnostic probability plot test failed, a correction procedure might be necessary to adjust for data dispersion (Rinaman, 2018). With this respect, Laney's approach was sought as a reasonable means to solve this issue based on previous experience. It will be plausible to conduct a preliminary examination and visualization of the process rate by using time-interval type of chart i.e. G chart which is known as a rare event process behavior chart to determine the frequency of the cleaning operation.

**Programs and software application**

Bioburden data of Equipment/Machine rinse was collected and processed in the Microsoft Excel database after chronological arrangement based on dates. The study coverage period embraces 20 months which is equivalent to about 91 weeks or 635 days. The generated dataset was then analyzed using Minitab version 17.1.0 (Ryan *et al*., 2013; Rinaman, 2018). G control chart was drawn from the “Stat” tab then going to “Control Charts” and selecting “Rare Events Charts”. The chart that illustrates the number of opportunities or days between events i.e. G chart was selected. Since the sample sizes (rinses) were variable and greater than unity, the U chart was the option of choice in this case and was selected from the same tab till reaching “Attribute Charts”. The first step here is to run a diagnostic test to determine whether to use an ordinary U or Laney trending chart. The second step is to execute the run of the most appropriate chart based on the fit to the Poisson probability plot (Ryan et al., 2013; Rinaman, 2018). The U chart monitors the average defects per unit which are herein corresponding to the TVC per 100 mL rinse or microbial count per mL may be equivalent to a Defect per Unit (DPU) using the “Assistant” tab help scheme for “Control Charts”.

## RESULTS AND DISCUSSION

This study was established with the aim to disseminate the concept of the implementation of SPC techniques and methodologies to monitor, investigate, correct and improve the bioburden quality – and hence safety – in the healthcare field with a specific scope that focused on the rinse as a marker for the efficacy in the cleaning process from the microbiological point of view. The examination of microbial count data did not return any sign of out-of-specifications results with none of the rinse samples exceeding 100 CFU/mL (Steyaert, 2021). The details of the usability and the outcome of the Shewhart charts will be discussed in the following sections based on the software analysis results.

### G process behavior chart output

The average interval between machine and equipment rinses is about one week. Figure 1 shows a rare event control chart that demonstrates an interval timeline of chronologically arranged machines' rinse record.

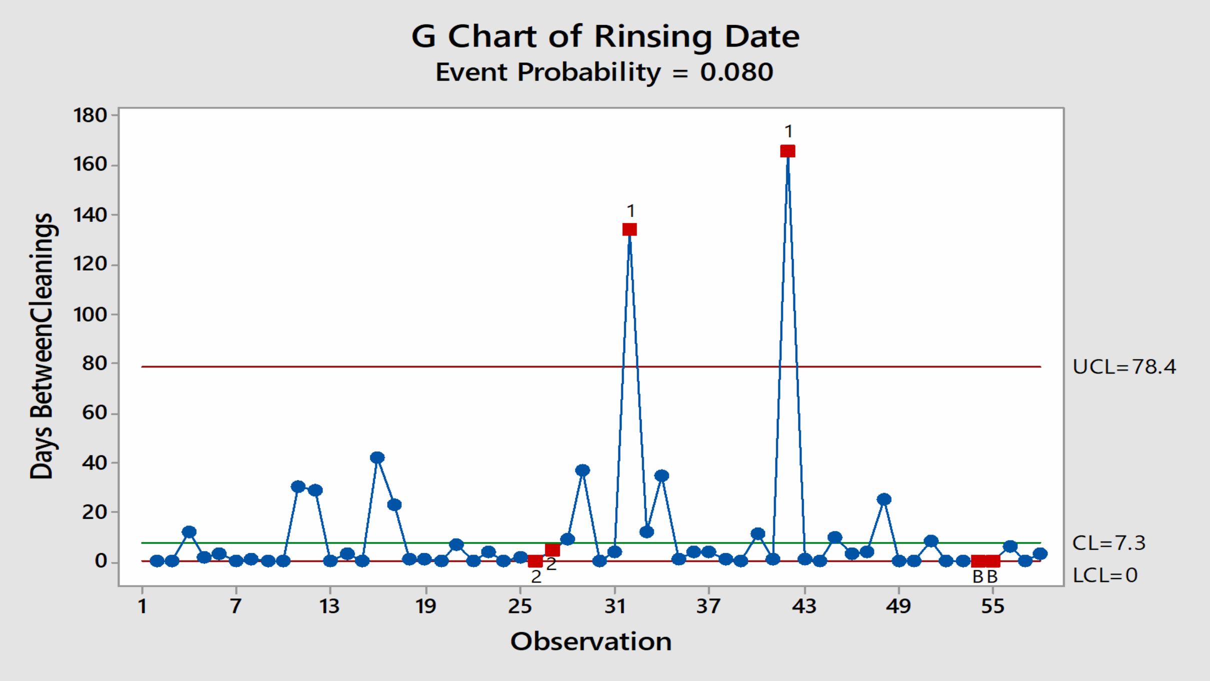



**Figure 1:** Rare event control chart illustrating the intervals and frequency of the machine and equipment cleaning process.

The event probability is 0.080. The event probability is the chance of an event occurring on any given day. For the machine and equipment cleaning data, the rinse process occurring on a given day is 8.0% (Park and Wang, 2022). Two points failed the Benneyan "B" test (three points in a row are equal to zero), which indicates that three or more cleaning and rinsing procedures were recorded on the same day. Alarm points "1" are showing higher than usual intervals between event dates with a "K" value greater than three standard deviations ($\sigma$) from the center line. In the same line, the red dots "2" indicate nine points on the same side of the mean line.

**Checklist for reporting U trending chart**

Assessment of the fulfillment of the Laney U (prime) attribute process behavior chart criteria were subjected for brief screening as the following (Minitab® Statistical Software: The Assistant, 2022):

**Stability**

The number of CFUs per 100 mL of rinse might not be stable. Ten (27%) subgroups are out of control (0.7% of out-of-control subgroups might be observed by chance, even when the process is stable). Out-of-control subgroups should be investigated to decide whether to omit those with special causes from the calculations.

**Number of subgroups**

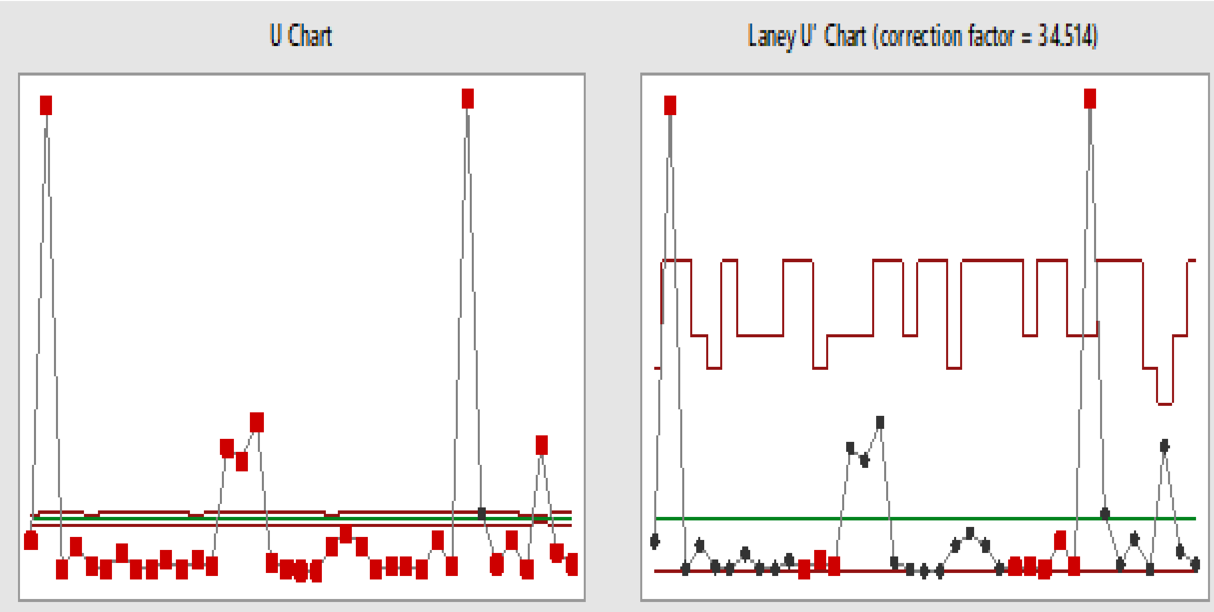
The precision of the control limits would not be of concern because more than seven subgroups are included in the calculations.

## Subgroup size

The accuracy of the investigated control limits would be acceptable because all subgroups have at least one unit.

## Expected variation

The present data have more variation than expected, a condition known as over-dispersion. This causes the control limits on a U chart to be too narrow for the examined data, resulting in an increased number of false alarms (Figure 2). The Laney U′ chart corrects the control limits to account for the over-dispersion. The ratio of observed variation to the expected variation was found to be 2711.3%. 95% upper limit for ratio if the process mean is constant and its value is 140.2%. Using a U chart might result in an elevated false alarm rate. Thus, the use of the Laney U′ chart could be considered instead of the conventional one. It should be noted that the upper limit depends on the number of subgroups and the process mean.



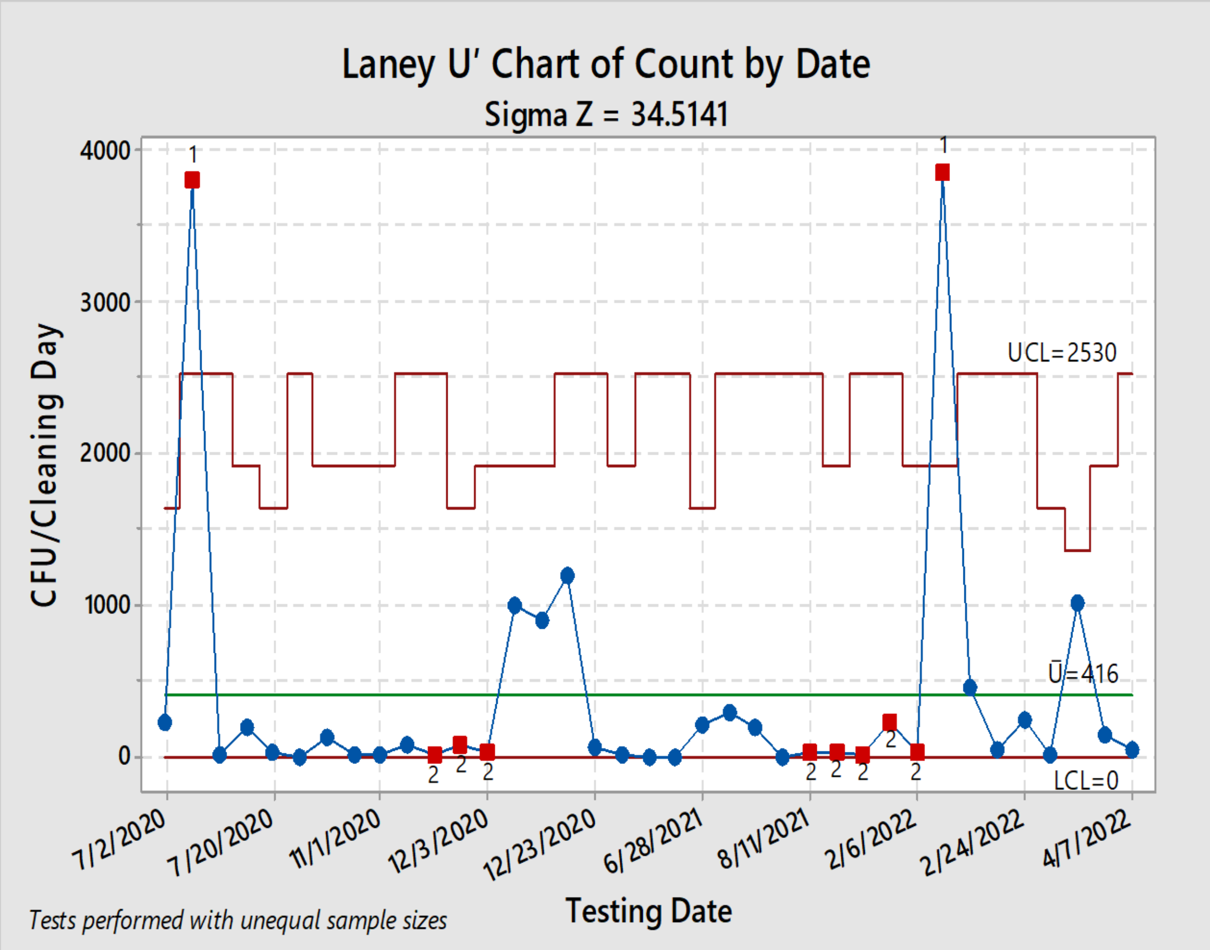


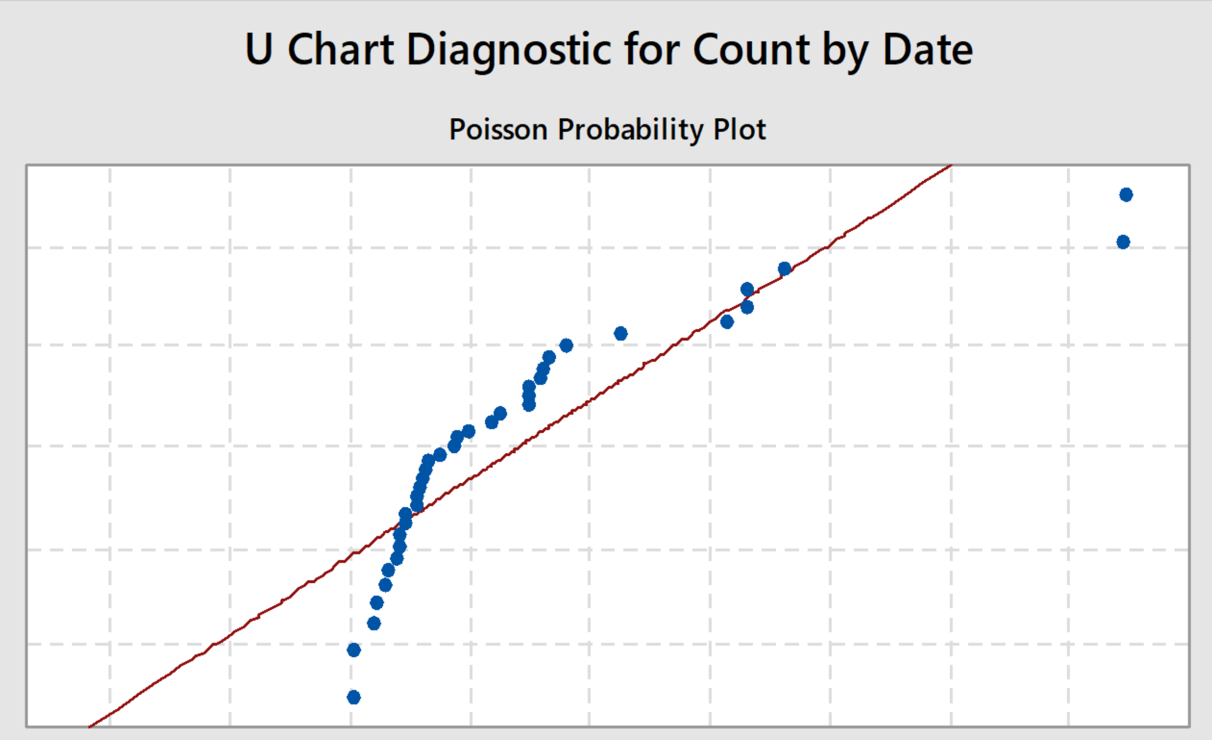

**Figure 2:** Analysis of the deviation of the dataset scattering from the assumed Poisson distribution and the effect on the extrapolation of the control chart.

The statistical process software assesses the observed variation as a percentage of expected variation to determine which chart should be use used. The current microbiological rinse data have considerably more variation than expected (over-dispersion), which can result in an increased number of false alarms. Accordingly, the Laney U′ chart was used, which corrects this condition (Pharma Focus Asia (FPA), 2022). The use of an ordinary U chart would lead to excess variation which results in control limits that are too narrow for the existing data, which can cause an elevated false alarm rate.

**Implementation of Laney U′ (prime) control chart**

Laney U′ Chart imposes modification for adjustment of the excessive variations due to over-dispersion of the dataset results (correction factor (Σ Z) = 34.514). The Laney U′ chart corrects the control limits to account for the excess variation. Hence, the chart should signal appropriately (Minitab® 20 Support, 2022). Assessing the stability of the defects per unit in the investigated process and looking for patterns could help quality officer investigators to distinguish between common and special causes. Typically, a process that exhibits only common causes has a constant defect rate. However, global trends or cyclical patterns may also be common causes. Other patterns, such as shifts and drifts, might be due to special causes. This chart has variable sample sizes which are reflected on the Upper Control Limit (UCL) as could be found in Figure 3 where it is not a fixed straight line as that for the C chart.

The stability of the microbial count in the rinse samples showed that the number of CFUs per 100 mL of the rinse may not be stable. Ten (27.0%) subgroups are out of control. Keeping in mind that 0.7% out-of-control subgroups might be found by chance, even when the process is stable. Nevertheless, the microbial count data has a single-side specification criterion towards the UCL. Hence, there is no specific lower limit as the bioburden level devolves to zero, it becomes better (Vargas *et al*., 2022). Accordingly, the reported shift in the TVC below the average line was found to be insignificant with a minor shift - as could be found from the trending chart - and a decision was made to cancel these alarms that have been spotted by the program. Thus, the significant excursions were actually two and the rate of the out-of-control bioburden level would be 5.4%.



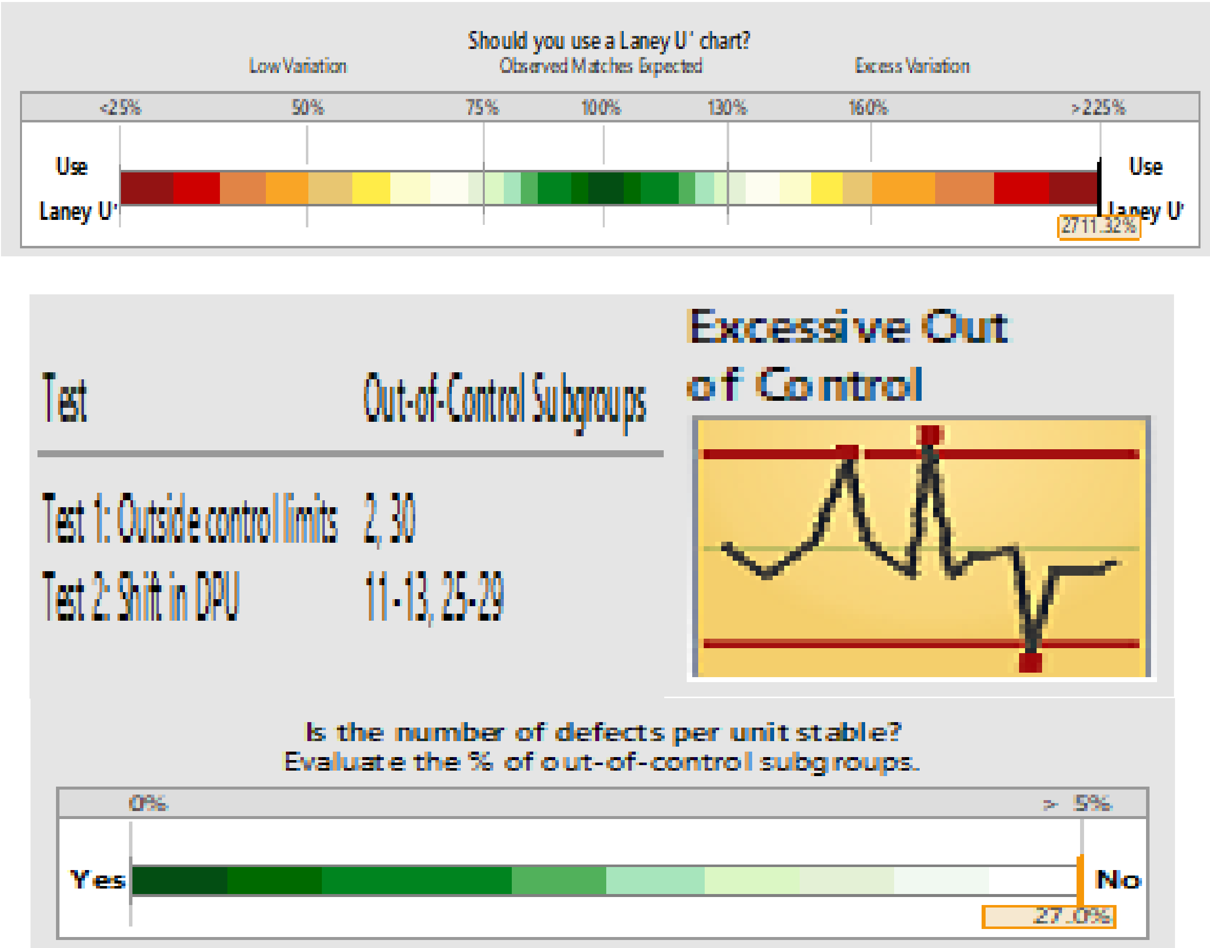



**Figure 3:** Laney corrected attribute chart showing the trending chart (with control limits), correction factor for over-dispersion, detected out-of-control rinse points and the defect rate.

## Defects and bioburden concept in control charts

Table 1 summarizes the outcome of Figures 1 and 3 by showing the number of failed sequences from the time series order number. Benneyan alarm is unique for the G chart and showed two successive points.

**Table 1:** Comparison between two types of the trending charts implemented in the rinse study.

| **Comparison** | **Process Behavior Chart** | |
|---|---|---|
| | **G Chart** | **Laney U (Prime) Chart** |
| **Chart Type** | Rare event | Attribute |
| **Failed Test Points:** | | |
| 1- One Point > 3 $\sigma$ from CL: "1" | 32, 42 | 2, 30 |
| 2- Three points in a row equal to zero: "Benneyan" | 54, 55 | NA |
| 3- Nine points in a row on same side of CL: "2" | 26, 27 | 11, 12, 13, 25, 26, 27, 28, 29 |

$\sigma$: Standard deviation  CL: Control Limit  NA: Not Applicable  U (Prime) = U′

On the other hand, the type 1 and 2 warnings were found in both kinds of graphs. The first one indicates abnormal excursion while the second one is related to the drift in the monitored means of the inspection characteristics. Logically, the attention should only be drawn to the aberrant high level of the microbial count while the very low alarming values could provide an opportunity for improvements in the present study. In the current case, two out-of-control points – (i.e. number “1” alarms) are present in each graph with an indication of unusually extended time intervals and excessive microbial count, respectively (JMP Statistical Discovery, 2022). While the frequency of cleaning might be more related to the usage of machines or equipment (and obviously can be adjusted when needed, the abnormally high microbial density could be attributed to the goodness of the cleaning process. With this respect, an investigation would be executed to determine the source of the abnormally high TVCs, even if there are no Out-Of-Specification (OOS) to avoid any adverse outcome in the future as they provide early warning signs for underlying problem(s) to be corrected and prevented through CAPA plan. Table 2 centralizes the main conceptual difference between the industrial and non-industrial inspection characteristics. By common sense, the industrial use of the U chart primarily focuses on the number of Defects per Unit (DPU) and hence the Defect per Million Opportunity (DPMO). The story is different in microbiology as there is an appreciably permissible level of the microbial count which has a specification criterion of 10000 CFU/100 mL (Moharram *et al.*, 2014). Although the aim is to maintain bioburden density as low as possible, yet the concept of industrial defect would not apply and this could be exemplified by analogous parameters Count per Milliliter (CPM) and Count per Million Opportunity (CPMO) that correspond to DPU and DPMO (PPM).

**Table 2:** Evaluation summary of the Laney U prime chart for the equipment and machine rinse.

| Subgroup Parameters | Record of | Rinse Bioburden Level |
|---|---|---|
| Number of subgroups: 37 | Total Rinses: 63 | CFU£ per mL (CPM¥): 4 |
| Average subgroup size: 1.703 | Total CFU£: 26239 | PPM§ (CPMO€): 4164921 |

£ Colony Forming Unit, § Part Per Million, ¥ Count Per Milliliter, € Count Per Million Opportunity

## CONCLUSION

The present work showed the applicability of the trending charts in the control and monitoring of the microbiological cleanliness of the machines using the rinse technique. The pattern and excursions could be spotted and identified within the investigated time series of the process-behavior charts. While the implementation of the rare event (G) and attribute (Laney-corrected U) charts seems to be convenient for the present study, the classical understanding of the industrial defects cannot be applied herein when considering microbial bioburden data in the non-sterile applications in the medical field. A solid comprehension is mandatory for understanding and interpretation of the trending charts by quality experts and this could be projected in the consideration of the alarming points. In the present case, Laney U′ Chart is related to the quality of cleaning microbiologically while the G chart is more likely to be linked to the operation practice in the plant which is associated with the activity and workload. The present work opens the gates for a further investigation that includes Pareto analysis to spot the major contributors in this microbial load in terms of products and machines with the possible development of a quantitative risk analysis tool that is based on the bioburden density and the frequency of the occurrence that could be extracted from the Pareto investigation.

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