Mostafa Essam Eissa

HIKMA Pharma, Egypt.

E-mail Id:
mostafaessameissa@yahoo.com

Application of Control Charts in Quality Characteristics Evaluation of Microbiological Media

Abstract

Microbiological culture media are the backbone of any microbiology laboratory. However, appropriate monitoring of their quality characteristics is crucial to ensure accuracy and validity of the results derived from them. The microbiologist should be aware and well-focused on these quality attributes and not only the activities performed with them. Consistent and controlled shipment, storage, handling and preparation conditions and methods are critical for reproducible performance of the media. The application of Shewhart charts to trend the quality attributes of culture media batches provides internal monitoring for important characteristics of these media in a microbiology laboratory. Two approaches can be used, variable or attribute control charts. Both can be customized according to the nature of laboratory activity in terms of number and frequency of the prepared and tested-culture media. Variable control charts can be used for a limited number of measurable properties of media such as thickness of the solid media, gel strength and pH, while attribute charts are suitable for monitoring of defects for microbiological culture media. Some of the quality characteristics may have certain specification limit (SL) values such as growth promotion (GP) failure rate, the depth of the agar medium in the culture plate, gel strength, contaminated fraction from total batch and pH. Thus, the statistical process control (SPC) may be useful in the assessment, control, investigation-on-failure and prediction of the performance of microbiological culture media. Shewhart control charts provide the microbiologist with a tool for quality monitoring and improvement of the prepared culture media.

Keywords: Culture media, Quality control, Control limits, Specification limit, Statistical process control.

Introduction to Microbiological Culture Media

History of microbiological culture media can be dated back up to the nineteenth century where animal and plant tissues were used mainly as a source of nutrient for microbial proliferation. This work was developed principally by Robert Koch and his team. Another major breakthrough was made by Fanny Hesse in Koch's laboratory through the discovery of agar as a solidifying agent for microbiological media. Virtually any extracts of plant and animal organs were considered for use in the preparation of these early media. Then, their broth could be mixed with agar to give different varieties of culture media. Till nowadays, some classical infusions, such as beef heart, calf brains and beef liver are still being used as a part of key ingredients in conventional culture media like Brain Heart Infusion Agar and Liver Broth. However, recent attempt of replacing animal components of culture media with materials of plant origin have been made. This change offered advantage on the number of commercially available media in the market.[1] Data and results accuracy issued from the microbiology laboratory are largely dependent upon the quality of the microbiological media in use. Different quality characteristics, including growth promotion test (GPT) should be inspected systematically to judge on the validity of the culture media for subsequent testing

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activities.[2]

Since many components of microbiological media are from natural sources, challenges of obtaining consistent quality characteristics of these media are significantly high.

Culture media are grouped into different main types based on the purpose, including *selective* and *nutritive.* Selective media include certain antimicrobial substances that inhibit most of the bioburden of the inoculated samples, but allow for growth of specific microbial species. On the other hand, nutritive (non-selective) media are formulated to promote the growth of diverse types of bacteria and fungi.[3] Another type of media that may be selective or not, is called *differential*. This type of media can differentiate between closely related microorganisms due to the presence of certain dyes or compounds. Nutritive media can be *enriched* with highly nutritive components like blood, serum or yeast extract for the purpose of cultivating fastidious (slowly growing) microorganisms.[4]

Inspection Characteristics of Microbiological Culture Media

In-house quality control on either ready-made or internally prepared microbiological culture media is mandatory activity that should be regularly performed in a microbiology laboratory to ensure precision of results obtained from microbiological analysis and testing. This task is performed by the laboratory microbiologist and includes inspection of physical, chemical and biological characteristic quality attributes. The overall visual aspects of the culture media usually provide an indication of its quality.

Visual and Physical Characteristics of Culture Media

Microbiological media should be garbled based on physical appearance such as the presence of air bubbles or observed pits and non-uniform depth or height of agar media in the plates.[5] Signs of freezing, presence of crystals and/ or cracks in the culture media must be inspected by the naked eye of the technically experienced microbiologist.

However, in case of inspecting unequally levelled filled plates, depth of agar media can be measured at four sides. The locations of the four measurements are determined at the edges by drawing hypothetical two perpendicular diameter lines. The depth at the four ends is recorded and the mean thickness is calculated and documented as an average height of the medium in the culture plate, which should be between 3.8 and 4.2 mm.[6]

The degree of acidity or alkalinity (pH value) of the microbiological culture medium is another vital physical character that should be checked regularly.[7] It is a good practice to record pH during media preparation before autoclaving and after terminal sterilization. The pH meter must be accurately calibrated before measuring media pH, using standard buffers that cover the measuring range.[2]

Gel strength provides a quantitative measure for the degree of solidification of agar media and can detect abnormalities of consistencies in solid media. The solidification of the gel can be evaluated by a simple device composed of a support stand which holds a rod at its center. This rod transfers the applied pressure to the agar surface. The lower end of the pressure rod that comes into contact with the agar has a rounded surface with known surface area.

The upper part of this rod is designed to hold standard weights on it. The rounded part of this central rod is placed over the solid culture medium and the standard weights are added gradually on the upper portion at increasing increments and the effect of adding weights is observed for some time after each addition. The weight at which the agar cracks under the pressure of the rod is recorded. The force imparted by the rod on the agar surface can be obtained from the equation: $(W_t–W_r)/p.r^2$ where, W_t=total weight of the rod and the standard weights on the rod platform at which agar becomes broken, W_r=weight of the central rod, r=radius of the rounded part at the lower portion of the pressure rod and p=3.14. Gel strength of about 300-500 dynes/cm^2 will yield acceptable results.[2]

Chemical Composition of Microbiological Culture Media

Tests for the determination of chemical composition of the microbiological culture media are considered additional monitoring tests that are not normally conducted by the laboratory.[8] These extra checks may be conducted using high performance liquid chromatography (HPLC) to determine major components such as sugars.[9] Since chemical analysis of microbiological culture media is not considered part of the routine work of the microbiology laboratory, it will not be subjected for further discussion in this article.

Microbiological Quality of Culture Media

Sterility check, growth promotion test (GPT), indicative and/ or growth inhibition test (GInT) (if it was found applicable for the selective media) are three important properties of the microbiological culture media that should be adequately performed and controlled by a qualified microbiologist.[3] GInT is usually a test for those media with selective characteristics to test their inhibitory effect on unwanted microbes. Both in-house prepared and ready-made purchased culture media are subjected and must pass these tests to be used for further activities of the microbiology laboratory. Each one of them is detailed in the following sections.

Sterility check is one of the important quality control monitoring criteria that is required to expose any evidence of accidental contamination during transportation not visible by visual examination. Contaminated culture media should be discarded, reported, and communicated to the supplier. Table 1 provides detailed guidelines of sample size for sterility checking of culture media that is stemmed from chapter <71> of the *United States Pharmacopeia (USP).*[10] Apart from 100% full inspection for the containers of microbiological culture media that should be performed by the microbiologist, pre-incubation step is usually performed for the whole batch prior to the use. This step is done for a short period of time in order to detect any signs of gross contamination as suggested by some companies. Moreover, USP Chapter <1117> recommends special precautions for culture media that are used for microbiological environmental monitoring (EM) of critical-to-monitor areas. The risk of "sporadic adventitious contamination" which may interfere with results' interpretation requires that articles which are neither terminally sterilized nor protected by double-warp should be totally pre-incubated and 100% inspected thoroughly.[11] In addition, some authors recommended the performance of a sterility check at 30-35°C for not less than three days.[3] In conclusion, whether culture media are readily-prepared or in-laboratory prepared both should be inspected for physical integrity, signs of piercing or cracks and sterility before conducting any type of microbiological activity with them.

Table 1.Number of Samples that are required to be Incubated for Sterility Check Procedure Based on the Campaign Size according to USP <71>[10]

Number of Articles per Batch	Number of Samples for Sterility Testing
<100 units	10% of the total number of units or 4 units, whichever is less
>100-<500 units	10 units
>500 units	2% of the total number of units or 20 units, whichever is less

There has been great debate among experts in the microbiology field (especially in the clinical and quality-control laboratories) about the necessity of performing GP tests for the standard microbiological media. There are some significant concerns as to the need for GP testing of standard media. The crux on which this argument stands is that GP test offers little further information about the quality of the growth characteristics and abilities of the media when all conditions of their preparation are met and in-control. Moreover, the examination of the final prepared culture media for any unusual observable physical defects can provide safeguard against accidental use of microbiologically invalid media. Thus, the consumed time and the wasted labor effort outweigh any significant data that could be obtained from the test.[12]

In addition, there are a few microbiological culture media that require extensive testing for their GP capabilities. The reason for that is that they contain sensitive ingredients. However, most other media are stable and can be excluded from the in-house testing based on the manufacturer's testing data.[13] The support of this opinion came from two major survey studies performed in 1973 and in the early 1980s. The earlier study was performed by Nagel and Kunz on purchased prepared media. GP test was performed on 350,000 articles from 46 types of different media. The rate of failure was 17 lots (approximately 1.9%) per 900 batches of the tested commercial culture media. This failure came from specialized culture media that contains labile components.[14] The later survey study confirmed the former one with pooled results from 1164 different laboratories. However, even the standard M22-A2 "Quality Assurance for Commercially Prepared Microbiological Culture Media" does not obligate testing of the most commonly used culture media in clinical microbiology laboratory in the United States.[13]

In the opposite side, the pharmaceutical field was more conservative about the previously discussed approach. A prominent example of this can be exemplified by Trypticase Soya (TS) media. While there is no persuasive rational proof that justify the need for GP testing of this media, yet compendial guidelines put the testing of the growth-promoting capabilities of TS media as a requirement. Both sterility and microbial limit testing chapters specify that this medium must pass the test using predetermined spectrum of compendial test microorganisms.[10,15,16] The test may include microbial

isolates that have been isolated from past samples as common environmental monitoring (EM) isolate or sterility test contaminant along with pharmacopeial organisms required for the GP tests.

Although pharmacopoeias specify quality control tests to be performed batch-wise, a logical and scientific approach may be implemented based on the application of the risk assessment carefully to justify minimized or limited testing of culture media. In case of in-house prepared culture media, the frequency of testing can be reduced to testing each shipment batch of powder medium. This can be taken when the autoclaving cycles of the autoclave are adequately validated and the sterilizer has passed all its calibration tests. Otherwise, with each preparation and sterilization, culture media must be subjected to GPT. Also, the microbiology laboratory must provide documented proof that prepared media is valid till the assigned expiry date. In case of commercially prepared media, the origin of the manufacturer can provide evidence for expecting the media quality. For example, commercially prepared media in the United States are put under control as medical devices through the regulatory bodies which include the Department of Health and Human Services and the Food and Drug Administration (FDA). Accordingly, extensive testing is already being conducted by the manufacturers of culture media and certificates of analysis (CoA) are being issued for each manufactured batch and can be easily available to the customers. However, it is possible to inspect the qualification of the manufacturer through what is called "company-sponsored vendor audits." Moreover, storage conditions at which culture media are shipped and stored must be rigorously controlled with strict procedures that should be ensured to be followed. However, the decision of reducing the GPT frequency-which bears significant financial burden on the laboratory budget-is a case-by-case thorough long-term (usually one-year period) study that should be performed by each laboratory depending on the vendor, the media type and its intended use. After gathering sufficient data, statistical analysis should be performed to justify reduced testing program. Thus, scientific approach through risk evaluation can provide a mean of reducing cost and time consumed for testing without compromising the quality.[3]

A rationally important specification that has been set by the National Committee on Clinical Laboratory Standards (NCCLS), "Quality Control for Commercially Prepared Microbiological Culture Media," Document M22-A3 recommends QC testing in batch-wise manner of culture medium that shows a percentage of failed units greater than 0.5%.[17] The culture media with lower failure rate trends may be subjected to reduced and limited testing (for example, the first three incoming batches during a one-year period) and may be even omitted in sometimes. This reference document was formerly discussed by the USP *Pharmacopeial Forum* which concluded that GPT may not be conducted routinely by the end users for manufacturers of most culture media who follow the NCCLS Standard M22-A3.[18] Fastidious culture media demonstrate greater chance of failure in supporting microbial growth. However, most of the other media maintain their ability to support microbial growth provided that storage conditions were followed strictly as recommended by the manufacturers. Figure 1 demonstrates some examples based on survey by the NCCLS, M22-A3 2001 on culture media.[3]

Another important topic related to GP test is the suitability of the test design. The test is simply based on comparison between new batches of media with the previous valid ones. Since the inherited variability of the microbiological data is relatively high, caution in proper selection of the statistical analysis and design must be carefully approached. Basically, conventional microbiological culture media are differentiated into two main types-solid agar and liquid broth-and each group has signs of microbial growth that are demonstrated by a totally different means.[19] There are several techniques and methods through which microbiological capabilities of culture media can be assessed. When considering statistical analysis that involves colony-forming units (CFU), special statistical tools for Poisson distribution must be used. Otherwise, microbial count should be transformed to approximate normality in order to use ordinary statistical tools such as Student's T-Test.[20] Testing of solid media involves several commonly known techniques, namely, spread plates or pour plates, Miles-Misra (drop count) and ecometric methods.[11,15,21,22] Figure 2 illustrates two of these methods for GPT of agar media.[12,22,26] On the other hand, broth media have different types of tests that include: copious growth, end-point, most probable number (MPN) and kinetic parameters methods.[12,15,23-25] Whatever the chosen technique, the microbiology laboratory expert committee can design a suitable testing based on media type and the activity done with it. But, it is important to establish strict procedure to be followed by the microbiologist to ensure the validity, accuracy and reproducibility of data obtained from testing.

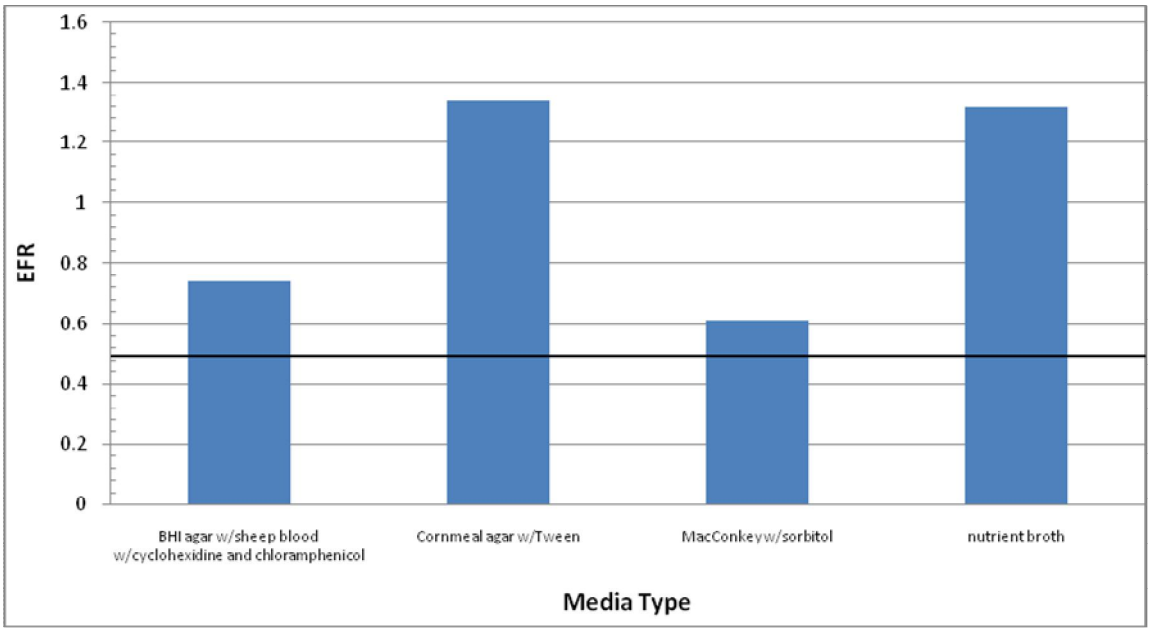


Figure 1.Extrapolated Failure Rate (EFR) of Some Labile Media. The Black Line Shows the Cut-Off Value of EFR Set by NCCLS Standard M22-A3[3]

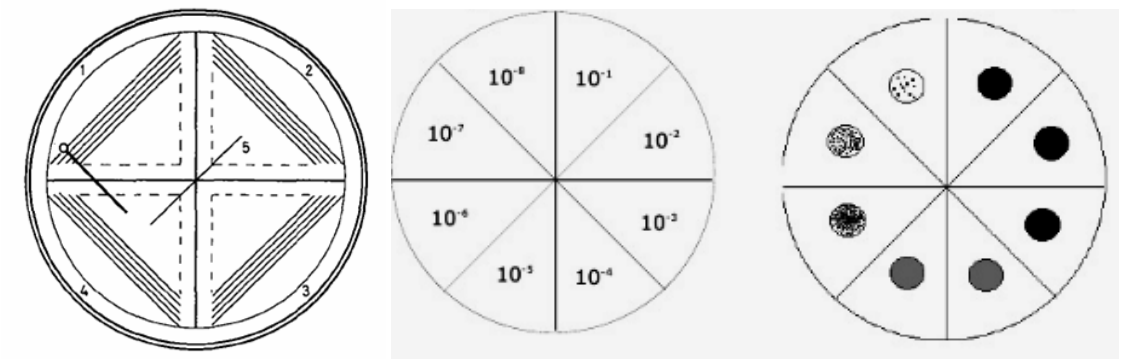
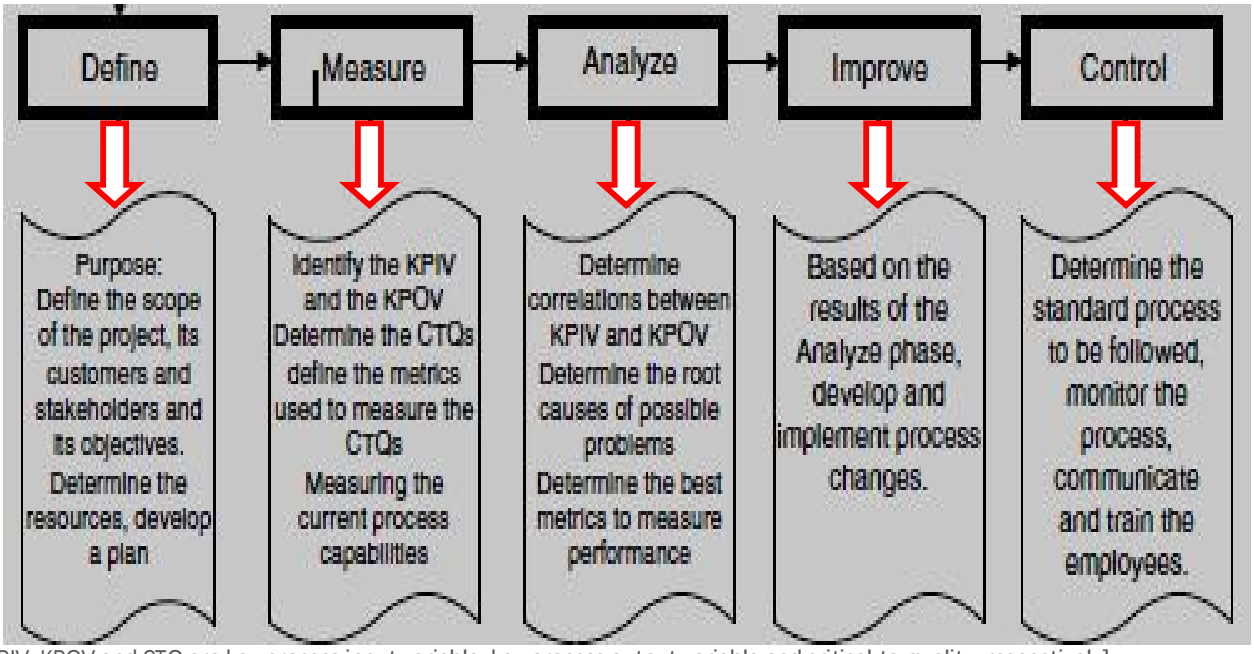


Figure 2.Ecometric-Type of GPT (Left Image),[12,22] Miles-Misra (Drop Count)-Type of GPT (Right Image)[26]

Quality Control Charts: History and Purpose

The application of the statistics in the field of management generally and QC specifically has begun to play prominent rule since more than 90 years. Thus, the concept of the statistical process control (SPC) has been introduced long before the *Six Sigma* term became popular. Historically, Walter Shewhart was the first to introduce SPC in the Bell Laboratories in 1924. While emerging of the science of statistics during this era was at its infancy, Shewhart was able to use his invention, i.e., control charts to determine variation in the manufacturing process which were reflected on products' quality and accordingly necessary adjustments could be performed at right time to keep production process under control. The great breakthrough in statistics was achieved during the last century and its application in QC testing has become extensive to assess the quality accurately and quantitatively in addition to minimizing defects and rework parallel to improvement in the productivity.[27]

The main roadmap for a successful *Six Sigma* project in quality improvement and hence cost reduction is called (DMAIC), which is abbreviation of clearly defined scientific and statistical tools for process improvement, viz., Define, Measure, Analyze, Improve and Control. Figure 3 summarizes each step of DMIAC process.[27] The main types of control charts are: X-bar/Range ($\bar{x}$-R), X-bar/Standard Deviation ($\bar{x}$-S), X (Individual)/Moving Range (I-MR), p, np, c and u charts all are grouped into a class named control charts. Control charts are important tools in the last four steps, i.e., Measure, Analyze, Improve and Control phases. Although the reasons for using a control chart are usually the same for each of these charts, when to use them varies with the situation. Typical uses for a control chart are included in Table 2.[28]



[KPIV, KPOV and CTQ are key process input variable, key process output variable and critical-to-quality, respectively]

Figure 3.Basic Roadmap of Six Sigma (DMAIC)[27]

Control chart is essentially a useful tool in the last four steps of DMAIC, i.e., measure, analyze, improve and control.[28]

The basic and important applications of control charts where each one is dependent on the other step sequentially include the following.[29]

1. **Measure:** Evaluation of the existing process efficiency and its statistical stability and predictability. Initiating a trial control chart is an essential first step.
2. **Analyze:** Refinement of the process using control charts. Studies must be made to regain process stability. Then, eliminate the sources of special-cause variability.
3. **Improve:** Common-cause sources of variation should be neutralized. Achieving target value mean and minimizing the variability in the process to an acceptable limit must be attained for improvement.
4. **Control:** Process monitoring for any signs of instability and holding the gain. Moreover, statistical control of the process should be maintained with proper identification and removal of any sources of unusual variability.

Control charts provide very handy tools for quality monitoring and improvement. Many sources (including online references) provide simple description on their types, uses and their construction on computer using commercially available software packages such as Minitab.[30-32]

Applications of Control Charts in Quality Monitoring of Microbiological Culture Media

The first step in the application of control charts is the determination of the best type of chart that fits the quality characteristics to be monitored. Broadly speaking, there are two basic types of Shewhart charts: variable and attribute charts. The quality characteristic to be monitored in the first type is quantitative and measurable, while the second is a count of the number of defective units or number of defective quality characteristics examined in a sample from production lot. Table 2 summarizes the most commonly used control charts in terms of type, application and examples for use in quality monitoring of microbiological culture media.[29,33,34]

When the rate of occurrence of certain type of observable defect is very low, a special type of attribute control chart called *g* chart is used.[35] Another less commonly used attribute control chart, called *t* or time between-control chart (time between events). On the other hand, other types of less commonly used variable control charts include: Analysis of Means Control Chart (ANOM), Cumulative Sum Control Chart (Cusum), Exponentially Weighted Moving Average Control Chart (EWMA), Levey Jennings Control Chart and Moving Average Control Chart.[36] A graphical presentation of control charts that can fit different types of inspection characteristics is shown in Figs. 4, 5 and 6. Upper control limit (UCL) and lower control limit (LCL) show the

boundaries of the process or the character being inspected over time or successive batches.[37] However, when sample size is not equal, the control chart will have variable UCL and LCL.

In Figs. 5 and 6, p and u-charts respectively show variable CLs, each depending on the sample size for each point in the chart. This will introduce difficulty when comparing voice-of-process (VOP) to the voice-of-customer (VOC).[38,39] CLs are compared to specification limits (SLs) to determine if the quality characteristics inspected are meeting users' requirements (or more appropriately in this case regulatory bodies).[40,41]

Microbiology laboratory processes of media handling and preparation can get benefits from constructing of flow charts (FCs) to monitor system of media flow till consumption. Accordingly, check sheets can be constructed to fit the quality characteristics being inspected and they are useful tool in data collection, if they were designed carefully for convenient interpretations. They are of different forms and types including: process, defect, stratified defect, defect location and cause and effect diagram check sheets.[42]

Table 2.Control Charts with Selected Examples and Criteria of Suitable Application[29,33,34]

Type of Control Chart*	Probability Distribution	Suitability of Application Situation(s)	Examples in Microbiological Media Quality Monitoring
Variable	Normal (Bell-shaped)	Continuous data with Gaussian distribution. Choice of chart type depends on the available subgroup size for charting. The order of preference of chart type is as following: $\bar{x}$-S > $\bar{x}$-R > I-MR.	1- Culture media pH. 2- Culture media thickness. 3- Gel strength of solid agar media.
np (Plot number of total defective units in sample)	Binomial	Total number of rejected units per sample group. Sample size of each plotted point is constant in the chart.	1- Sterility failure of microbiological media in batch. 2- Failed units of culture media in GInT and indicative properties.
p (Plot Fraction of defective units from total sample)		Fraction of single dichotomous outcome (i.e. go/ not-to-go, pass/ fail) from total sample inspected. Sample size of each plotted point may be variable in the chart.	1- Proportion failed media in sterility check from batch. 2- Fraction of physically rejected media from total number of prepared units.
c (Plot number of defects per subgroup)	Poisson	The units in a sample may possess several quality attributes to be inspected. Sample size of each plotted point is constant in the chart.	1- Number of defective visual inspection characteristics in agar plate media sample. 2- Number of failed quantitative tests within solid media sample.
u (Plot number of defects per unit)		The rate of errors or defects per unit in inspected sample. Sample size of each plotted point may be variable in the chart.	The same as c chart but with average number of defects per unit of the sample media.
g (Plot count between events)	Geometric	Alternative to the p-chart. Useful in rarely occurring events with very low frequency (e.g., rate < 0.01)	1- Total number of units from commercial media until specific visual defect is observed. 2- Total number of batches passing GPT for specific media till lot failing meeting acceptance criteria of the test.

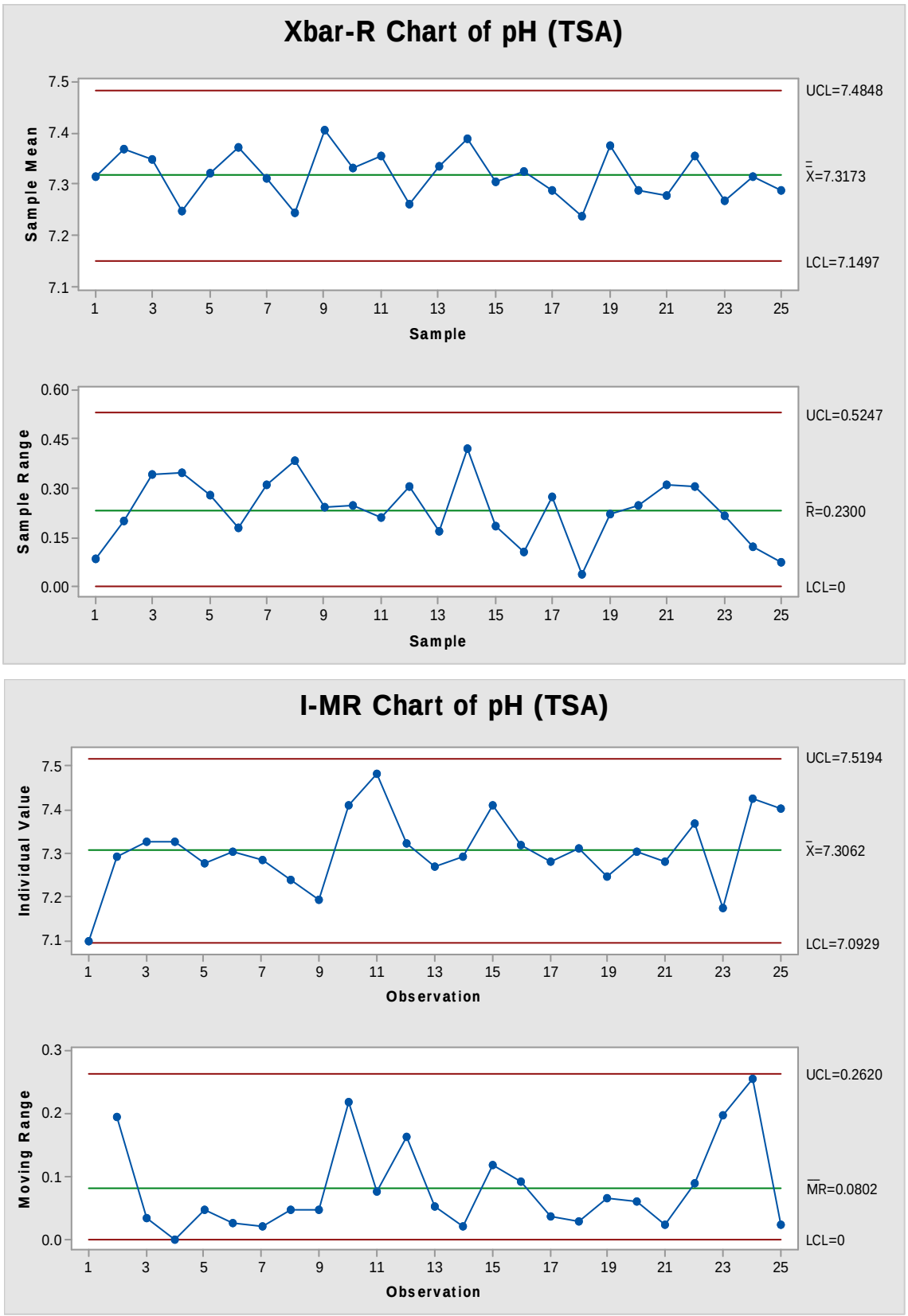


Figure 4.Variable Control Charts Showing Two Types of Them Used for Monitoring of pH Trends of In-House Prepared Tryptone Soya Agar (TSA) [Generated Using Minitab® Version 17.1.0]

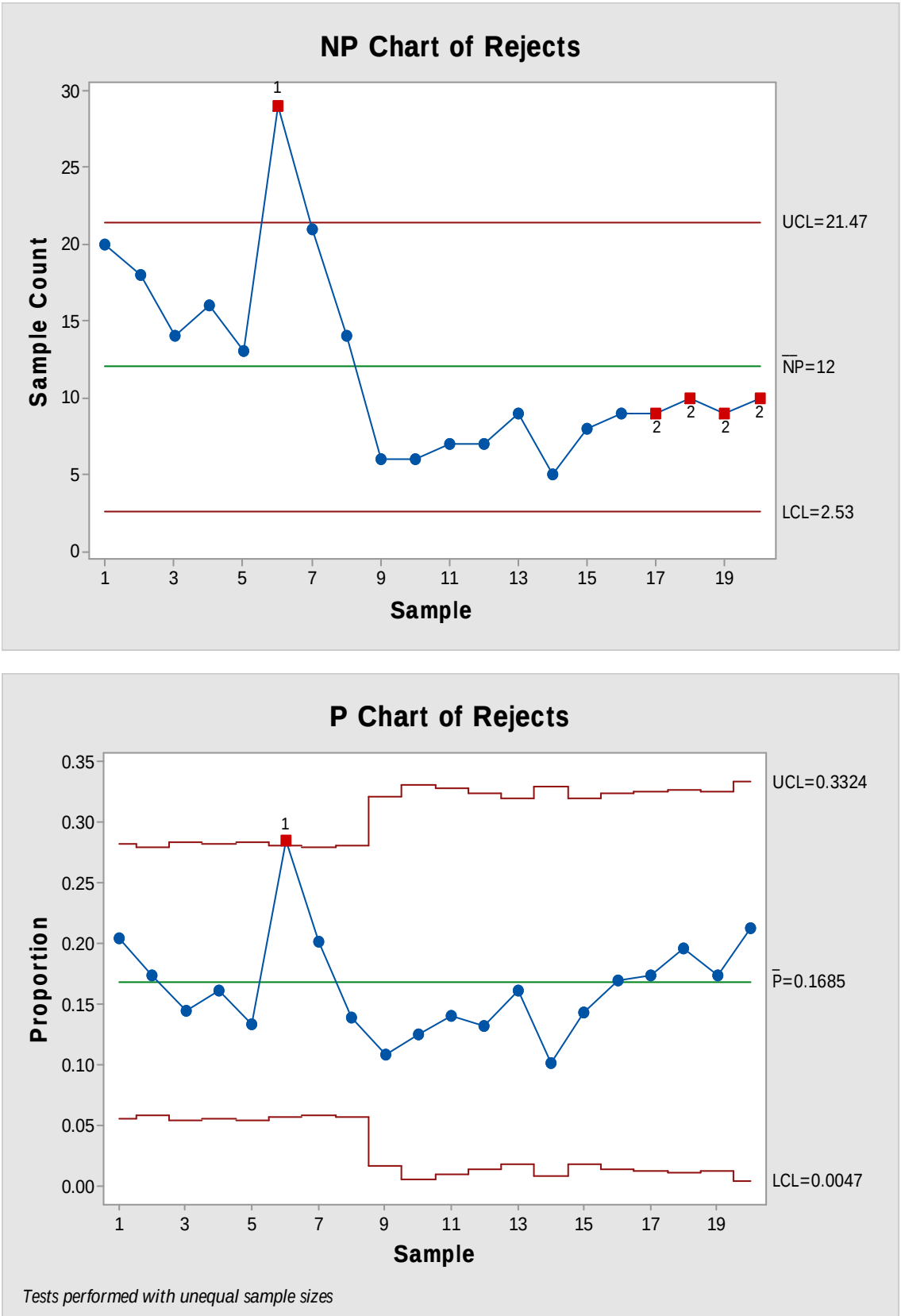


Figure 5.np-Chart and p-Chart for Number of Rejected Units per Fixed Sample Size and Fraction Defective Units from Variable Sample Size [Generated using Minitab® Version 17.1.0]

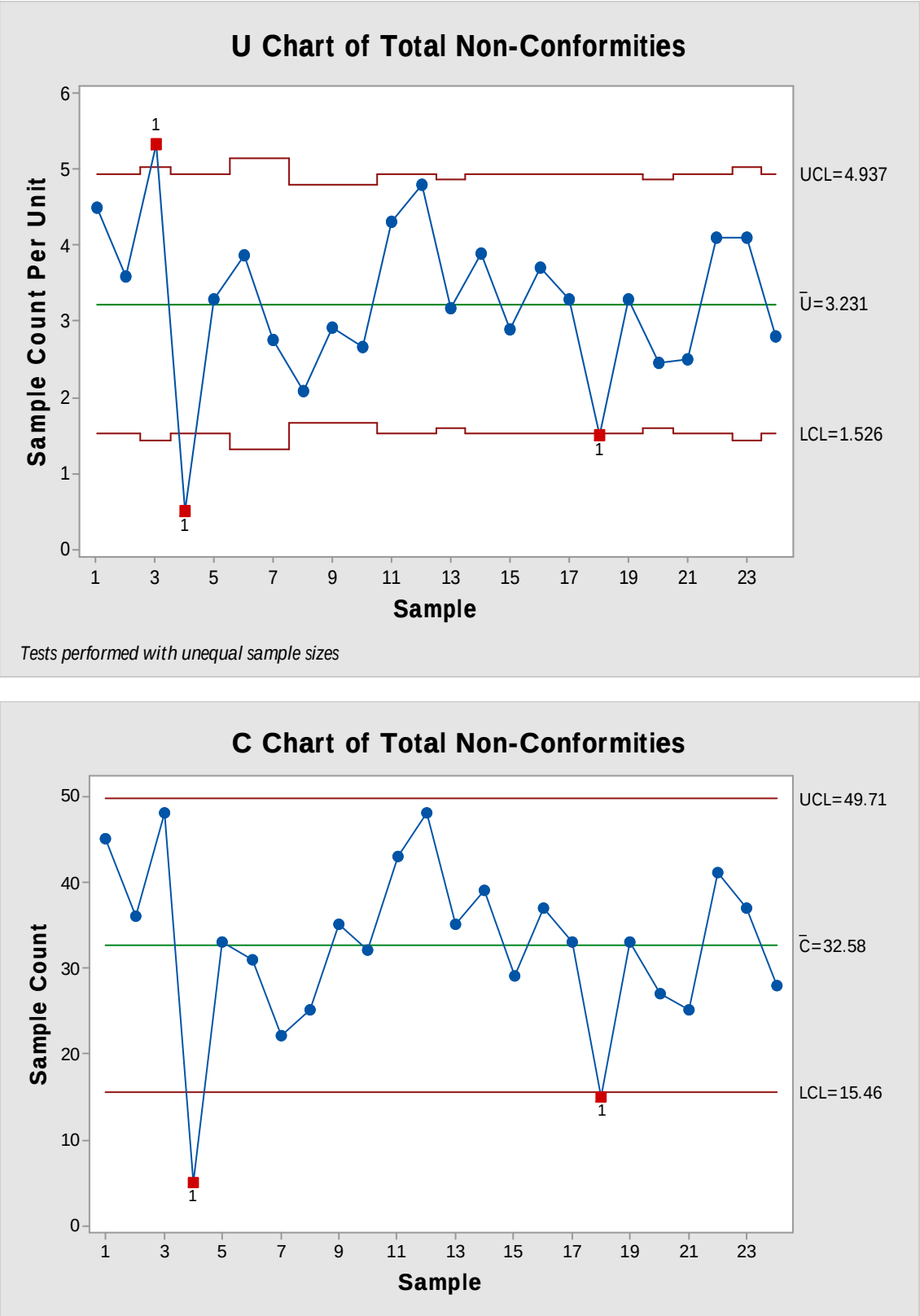


Figure 6.u-Chart and c-Chart Monitoring the Number of Defects per Unit and Subgroup, Respectively [Generated Using Minitab® Version 17.1.0]

Table 3 shows an example of a check sheet that contains simple instructions for the microbiology laboratory analysts as well as areas for filling of data by each by applying the method. This type of sheets is not useful only for simple and handy data gathering but it is also important for investigating out-of-control or abnormal outcomes of inspection and monitoring of quality for microbiological culture media.

Table 3.Example of Stratified Defect Check Sheet that Shows Defect Distribution Tallies for Quality Inspection Characteristics of Solid Culture Media Prepared in the Laboratory

Defective Check Sheet for Solid Media

Instructions for data filling by employee:

- Enter mark (/) in the corresponding area for each culture media plate (horizontal row) showing defect in the inspection quality characteristics (IQC) in the left vertical column.
- Primary data (name, date of preparation and inspection, sample size of batch that is tested or inspected, ...etc.) should be filled by each operator and inspector.
- When no defect is observed at all, the cell must be filled with nd = not detected.
- When quality characteristic does not fit media type, na = not applicable is written down.

MediaPrimary data	TSA	SDA	EMB	CTA	BCA
Microbiologist	A. A. AAAA	B. B. BBBB	C. C. CCCC	B. B. BBBB	A. A. AAAA
Date of preparation	xx/yy/zzzz	xx/yy/zzzz	xx/yy/zzzz	xx/yy/zzzz	xx/yy/zzzz
Inspector	D. D. DDDD	E. E. EEEE	D. D. DDDD	E. E. EEEE	D. D. DDDD
Date of inspection	xx/yy/zzzz	xx/yy/zzzz	xx/yy/zzzz	xx/yy/zzzz	xx/yy/zzzz
Sample size	M	N	O	P	Q
IQC:					
1- Gel strength	/	////	//	nd	/
2- Out-of-range pH	nd	/	nd	nd	///
3- Air bubbles	nd	//	~~////~~ /	/	nd
4- Precipitation	nd	nd	///	nd	nd
5- Darkening	nd	~~////~~ ~~////~~ ///	nd	nd	nd
6- Abnormal color	~~////~~ /	nd	nd	nd	//
7- Toxicity	/	~~////~~ ~~////~~ ///	nd	//	///
8- Poor growth	//	////	~~////~~	~~////~~ ~~////~~ ~~////~~	~~////~~ ~~////~~ /
9- Poor selectivity	na	na	///	/	//

TSA: Tryptone Soya Agar. SDA: Sabouraud Dextrose Agar.
EMB: Eosin Methylene Blue Agar. CTA: Cetrimide Agar.
BCA: Burkholderia Cepacia Selective Agar

Conclusion

Culture media are the backbone of most microbiology laboratories. Rigorous control on their quality will ensure the quality of derived data from them. GPT and GInT can be restricted on culture media with highly sensitive and labile components based on appropriate approach for risk assessment, provided that most other common media were preserved in strictly controlled conditions. Initial screening of visual and physical characteristics of media can give early warning for any defects in the batch of culture media that helps in investigating the root cause and prevent it in the future. Control charts on the other hand provide a greatly supportive tool for assessing the trend media quality and stability of its properties. SPC provides great help in determining differences in suppliers' quality or commercially prepared media and lot-to-lot variation in internally made media. Moreover, a provision of any abnormalities can be easily visualized to allow for rapid corrections of the causes of the assignable sources of unwanted variations on the process and product quality.

Conflict of Interest: Nil

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