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Original Research Article

Statistical Process Control in the Evaluation of Microbiological Surface Cleanliness Quality and Spotting the Defects in Clean Area of Pharmaceutical Manufacturing Facility

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Abstract: Regular environmental monitoring (EM) system is indispensable routine activity in assessing the microbiological quality of the manufacturing environment surfaces for the production of medicinal products. However, the release of the full potential of such regular activity will not be achieved nor the elucidation for points of defects and improvements cannot be appropriately investigated unless systematic analysis of the results can be ensured and performed periodically. In the current study, statistical process control (SPC) and six sigma tools were used in the study of the quality of microbiological surface cleanliness in clean rooms in pharmaceutical manufacturing plant during ten months period on weekly basis using contact plates. Initial evaluation of data distribution showed that ten out of 11 partitions of the manufacturing area followed distributions other than the Gaussian ones which required logarithmic transformation to approach normalization for further statistical analysis. When control charts were constructed for transformed data, material followed by personnel airlocks showed the greatest risk of microbial excursions then the corridor with the overall risk of failure 99.5% suggesting that there was a state of significant imbalance between cleaning program and its frequency with the work type and load in these sections. The present work provided insight for the area of defects that could not be observed using conventional data trending and provided focus on defined sections that could impact the overall microbiological quality. This analysis provided a promising mean for designing quantitative risk assessment for microbiological quality control in pharmaceutical, human consumables manufacturing and other healthcare industries.

Keywords: environmental monitoring, statistical process control, contact plates, control charts, airlocks.

INTRODUCTION

Controlling measures for quality control of pharmaceutical manufacturing facility begin from the initial design of the industrial plant [1]. Controlled area is basically devised as utilitarian units for particular objectives by providing and maintaining environment with specific quality. A properly-laid out clean room is built with articles and items that take into consideration the facilitation of decontamination and disinfection. Seamless and smooth floor to wall intersections in addition to readily reachable niches are part of good design practice when clean area construction is considered. Floors, area-divisions, and roofs are built of smooth and hard covers that can be readily sanitized according to CDER, 2003 [2,3].

Effectively isolated regions for manufacturing activities are a crucial need in contamination control. In order to sustain appropriate environment quality of in compartments of higher classification, it is essential to attain an appropriate airflow and a relatively higher pressure in comparison with the surrounding regions of lower degree of classification. Chambers of greater air quality ought to have a considerable positive pressure differential in respect to adjoining rooms of lower air cleanliness. For instance, a positive pressure differential of no less than 12.5 Pascals are required to be kept up at the interface between classified and non-classified regions higher air cleanliness should have a substantial positive pressure differential relative to adjacent rooms of lower air cleanliness. This requirement has been addressed by FDA, 2002 [4].

Practical surface monitoring is mandated by USP <797> so as to evaluate the accomplishment of the cleaning program in keeping surfaces free of microbial defilement. While USP <797> commands regular surface examining, it doesn't determine an authoritative

time frame outline for inspecting. A plausible choice is for surface examining to occur in the meantime as air inspecting. The contact plate system is suggested when quantitative information are looked for from level, non-porous surfaces. Contact plates are filled so that the media shapes a vault. The supplement medium utilized as a part of the contact plate might likewise contain a substances that neutralize antimicrobial chemicals. The surface of the media is squeezed against the surface being inspected. The subsequent inspected zones for a 50 mm diameter plates are roughly 25 cm^2. The plates are incubated for the required measure of time, and the visualized microbial colonies, if present, are then numbered [5,6,7].

The current study was designed to analyze bioburden results of surfaces of class C manufacturing facility using Shewhart control charts derived from microbiological counts from contact plates. The present work aimed to use statistical process control and six sigma tools to assess the quality, identify the distribution of microbial count, locate regions of defects, demonstrate the trend of data and the correlation between the compartments.

MATERIAL and METHODS

Surface samples using contact plates were taken from pharmaceutical manufacturing plant using methods and limits described by Eissa, 2014 [8]. The total number of samples was 94 different surface samples from class C. All microbiological culture media and chemicals were purchased from OXOID (Basingstoke, Hampshire) and Sigma-Alrich (St. Louis, MO 63103), respectively. All prepared cultures were sterilized by high pressure saturated steam in autoclave (steam sterilizer) (FEDEGARI FOB3, Fedegari Autoclavi SpA, SS 235 km 8, 27010 Albuzzano (PV), Italy). Contact 55 mm, sterile plastic plates were obtaineded from Sterilin Limited (solaar house, 19 mercers row, Cambridge, UK). Results of samples were obtained from the microbiology laboratory in the quality control department after incubation in Series BD 115 Incubators with natural convection (BINDER GmbH, Im Mittleren, Ösch 5, 78532 Tuttlingen, Germany). Sampling of surface bioburden and personnel monitoring activities were done using contact plates (surface area of approximately 25 cm^2) filled with an appropriate recovery medium (supplemented with Tween 80 and lecithin) [9-12]. All statistical analysis and six sigma tools along with their criteria were used as detailed by Kastango and Douglass, 2001 and Eissa *et al.*, 2015 [13,14]. Culture media used in surface monitoring were subjected to growth promotion tests according to the methods and specifications by USP <61>, 2015 and passed the acceptance criteria [15]. Microbial enumeration was performed using digital colony counter (Digital Colony Counter Model: 361, Laxman Mahtre Rd. Navagaon, Dahisar West, Mumbai). Illustrations of generated data and calculations were performed using Microsoft Office Excel 2007. Control charts, cause enumeration diagram (Cause–and–Effect Diagram) and Pareto diagram were constructed using Minitab® v17.1.0. GraphPad Prism v6.01 for windows was used for statistical analysis at $\alpha = 0.05$.

RESULTS and DISCUSSION

Microbial count from surface samples using *Replicate Organism Detection and Counting* (RODAC) system in class C production facility followed distributions for discrete values as shown in Table 1. Trends of data during ten month period showed that the bioburden distribution of most compartments followed or approximate Poisson followed by geometric and negative binomial distribution. Table 2 demonstrated statistical comparisons between untransformed and $\log_{10}$ transformed results of each section in the area. With the exception of semisolid material airlock all original data did not pass D'Agostino & Pearson omnibus normality test, while the transformation normalized all data. In addition, skewness and kurtosis values had been significantly improved to approach a Gaussian distribution. Moreover, the process of transformation significantly reduced numbers of outliers from 38 to two and from 25 to zero values only when using Robust regression and Outlier removal (ROUT) analysis at less strict (Q = 10.00 %) and moderately strict (Q = 1.00 % (recommended by the program)) threshold for defining excursions [16]. On the other hand, a graphical summary of the distribution of the samples illustrating the samples' central tendencies and variability were demonstrated in Figure 1 for both normalized and raw data. The default interval plot displays a mean symbol with a 95% confidence interval (CI) bar. Interval plots are especially useful for comparing groups. It should be noted that data scatter around the mean were minimum for logarithmically transformed in comparison with original untransformed counterparts [17].

The overall cleaning efficiency of the class C production area met and exceeded the 6 Sigma (σ) level with defects much less than 3.4 part per million (PPM) [18] as illustrated in Figure 2. The results as such revealed that the net microbiological cleanliness of the area was apparently ideal and achieved the target level of the microbial quality. The upper control limit (UCL) of 10 CFU/25 cm^2 may be used as a general alarming alert threshold for bioburden trend of the surfaces from the control chart. Similar concept, but in a different way was demonstrated by other researchers [19]. There were no signs of out-of-control states observed on the control charts. On the same line, the time-series-plot of the each partition in the manufacturing facility showed the microbial trend in Figure 3. Trending of the results as such could reveal little information about the microbial quality of surfaces in each zone. An empirical cumulative distribution function (CDF) plot was used to show the fitness of the normal distribution to data,

estimate percentiles and compare different sample distributions [20] as illustrated in Figure 4.

Interestingly, different locations showed wide variation in the bioburden control. The descending order of microbiological surface cleanliness control was in the following order: Suppositories Room = Liquid Preparation Compartment (UCL = 11 CFU) > Ointment Preparation area (UCL = 16 CFU) > Ointment Filling Division (UCL = 17 CFU) > Liquid Filling Room (UCL = 20 CFU) > Liquid Preparation Material Airlock = Main Corridor (UCL = 21 CFU) > Personnel Airlock (UCL = 25 CFU) > Liquid Preparation Personnel Airlock (UCL = 30 CFU) > Semisolid Material Airlock (UCL = 49 CFU) > Liquid Filling Material Airlock (UCL = 50 CFU), based on UCL. The last four areas showed that the sanitization processes were not able to meet the standard requirement as the upper specification limit (USL) was ≤ UCL of the affected locations [21]. These findings were evident from Figures 5 to 15. The quantitative risk of microbiological failures (defects) in Figure 16 demonstrated the major contributors of the failure risk – expressed as defects in PPM - based on the previously illustrated Process Capability SixPack Reports using Pareto chart [22]. Interestingly, more than 80% of the total microbiological surface cleanliness defects could be attributed to semisolid and liquid filling material airlocks which are directly exposed to the main corridor and this finding requires further investigations.

The relation between surface microbial counts in different locations in class C are expressed in Table 4 by Pearson correlation matrix. The magnitude of the association between the bioburden of different compartments were mostly of low values but significant correlation existed between the main corridor and the surrounding area which ranged from very weak to moderate with notably that liquid preparation room and airlocks - which are physically isolated and distant from the main corridor and the other manufacturing sites - did not demonstrate any significant correlation. In addition, there was no appreciable relation between surface bioburden and aerially distributed microbes of the same compartments collected in other studies during the same periods [17]. Fishbone diagram showing the degree of cross contamination between the main corridor and the surrounding compartments based on the coefficient of determination (R^2) is demonstrated in Figure 17.

Most importantly, fishbone (characteristic) diagram - in Figure 18 - was used to determine all probable causes of microbiological excursions [23]. The association of single compartment with the whole surrounding production area is demonstrated by the bar chart in Figure 19. The descending order of relation based on R^2 was as the following: main corridor, ointment filling area, ointment preparation room, suppositories room, personnel airlock, semisolid material airlock, liquid filling area, liquid filling material airlock, liquid preparation personnel airlock, liquid preparation material airlock and liquid preparation area.

Finally, the present study provided an evidence that there is inadequate quality of microbiological cleanliness of the surfaces with special attention should be brought to the airlocks, while the microbial counts of contact plates were apparently met the specifications in most of the parts of the control charts. This finding could be attributed to insufficient nor appropriate sanitization frequency and/or procedure - provided that disinfectant used was validated - if compared with the type and/or frequency of the activities performed by personnel. The magnitude of out-of-control conditions would be aggravated by non-compliance to good manufacturing practice (GMP) rules.

Table 1: Goodness-of-fit for data of microbiological surface cleanliness in clean area of class C.

Area	Distri-bution Test	Distribution									
		Poisson		Binomial or Neg. Binomial		D. Uniform		Geometric		Logarithmic	
		Statistic	Rank	Statistic	Rank	Statistic	Rank	Statistic	Rank	Statistic	Rank
Ointment Preparation	Kolmogorov Smirnov	0.19	1	0.34	2	0.35	3	0.42	4	NA	NA
	Anderson Darling	2.44	1	4.72	2	18.52	4	7.00	3	NA	NA
Ointment Filling	Kolmogorov Smirnov	0.24	2	0.30	3	0.23	1	0.35	4	NA	NA
	Anderson Darling	2.26	1	3.77	2	12.03	4	6.70	3	NA	NA
Personnel Airlock	Kolmogorov Smirnov	0.22	1	0.34	4	0.25	2	0.31	3	NA	NA
	Anderson Darling	5.00	3	4.57	2	20.24	4	3.36	1	NA	NA
Suppositori-es Room	Kolmogorov Smirnov	0.22	1	0.38	3	0.31	2	0.38	4	NA	NA
	Anderson Darling	2.46	1	7.83	3	13.87	4	6.35	2	NA	NA
Semisolid Corridor	Kolmogorov Smirnov	0.19	1	0.24	2	0.25	3	0.34	4	NA	NA
	Anderson Darling	2.53	2	2.15	1	10.93	4	5.14	3	NA	NA
Liquid Filling	Kolmogorov Smirnov	0.16	1	0.39	4	0.23	2	0.37	3	NA	NA
	Anderson Darling	2.08	1	8.13	3	15.17	4	6.31	2	NA	NA
Liquid Preparation	Kolmogorov Smirnov	0.30	2	0.31*	3	0.20	1	0.44	4	NA	NA
	Anderson Darling	3.18	1	3.87*	2	14.80	4	9.47	3	NA	NA
Liquid Filling Mat. Airlock	Kolmogorov Smirnov	0.23	3	0.21	2	0.15	1	0.25	4	NA	NA
	Anderson Darling	7.37	3	2.47	1	15.01	4	3.46	2	NA	NA
Liquid Prep. Personnel Airlock	Kolmogorov Smirnov	0.26	2	NA	NA	0.29	3	0.22	1	NA	NA
	Anderson	9.34	2	NA	NA	16.04	3	1.79	1	NA	NA
Liquid Prep. Material Airlock	Kolmogorov Smirnov	0.21	1	NA	NA	0.29	3	0.24	2	NA	NA
	Anderson Darling	5.90	2	NA	NA	14.44	3	2.42	1	NA	NA
Semisolid Mat. Airlock	Kolmogorov Smirnov	0.24	3	0.18	2	0.12	1	0.24	4	0.38	5
	Anderson Darling	8.84	4	1.92	1	7.83	3	4.15	2	10.50	5

* = Followed Binomial distribution while all non-marked data followed negative binomial distribution.

Table 2: Descriptive statistics, confidence intervals and Gaussian distribution tests of the original untransformed and transformed data of the microbial counts on contact plates for each sector in the class C manufacturing facility.

Column Statistics	Ointment Preparation	Ointment Filling	Personnel Airlock	suppositories room	Semisolid Corridor	Liquid Filling	Liquid Prep. Personnel Airlock	Liquid Preparation	Liquid Prep. Material Airlock	Semisolid Mat. Airlock	Liquid Filling Mat. Airlock	Ointment Preparation (T)	Ointment Filling (T)	Personnel Airlock (T)	Suppositories Room (T)	Semisolid Corridor (T)	Liquid Filling (T)	Liquid Prep. Personnel Airlock (T)	Liquid Preparation (T)	Liquid Prep. Material Airlock (T)	Semisolid Mat. Airlock (T)	Liquid Filling Mat. Airlock (T)
Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.12	0.0	0.12	0.0	0.10	0.0	0.0	0.30
25% Percentile	1.0	1.0	1.0	1.0	2.0	2.0	0.5	2.0	0.5	3.0	4.0	0.3	0.3	0.3	0.2	0.5	0.4	0.2	0.4	0.2	0.6	0.7
Median	2.0	2.0	2.0	2.0	3.0	3.0	2.0	2.0	2.0	6.0	8.0	0.48	0.48	0.48	0.43	0.54	0.60	0.48	0.51	0.48	0.85	0.95
75% Percentile	3.5	3.0	4.0	3.5	6.0	4.5	5.0	4.0	3.5	11	13	0.6	0.6	0.7	0.7	0.8	0.7	0.8	0.7	0.7	1.1	1.2
Maximum	12	9.0	16	10	15	11	17	7.0	18	17	17	1.1	1.0	1.2	1.1	1.2	1.1	1.3	0.92	1.3	1.3	1.3
10% Percentile	1.0	0.0	0.2	0.0	1.0	1.0	0.0	1.0	0.0	1.2	2.0	0.2	0.0	0.1	0.1	0.3	0.3	0.0	0.2	0.0	0.3	0.5
90% Percentile	6.8	5.8	10	4.8	8.0	7.0	10	5.0	8.0	15	17	0.87	0.80	1.0	0.75	0.95	0.90	1.0	0.79	0.95	1.2	1.3
Mean	2.9	2.7	3.4	2.4	4.1	3.5	3.5	2.7	3.1	6.8	8.7	0.49	0.46	0.53	0.47	0.61	0.59	0.49	0.52	0.47	0.80	0.91
Std. Deviation	2.4	2.0	3.7	2.0	3.1	2.6	4.3	1.6	4.0	4.6	5.2	0.24	0.25	0.31	0.24	0.25	0.23	0.38	0.20	0.35	0.31	0.28
Std. Error of Mean	0.37	0.32	0.58	0.31	0.48	0.40	0.67	0.24	0.63	0.71	0.81	0.04	0.04	0.05	0.04	0.04	0.04	0.06	0.03	0.06	0.05	0.04
Lower 95% CI of mean	2.2	2.0	2.2	1.7	3.1	2.6	2.1	2.2	1.8	5.4	7.1	0.42	0.38	0.43	0.39	0.53	0.52	0.37	0.45	0.36	0.70	0.82
Upper 95% CI of mean	3.6	3.3	4.6	3.0	5.0	4.3	4.8	3.2	4.4	8.3	10	0.57	0.54	0.62	0.54	0.69	0.67	0.61	0.58	0.58	0.90	1.0
Lower 95% CI of median	2.0	2.0	1.0	1.0	2.0	2.0	1.0	2.0	1.0	4.0	6.0	0.40	0.40	0.30	0.30	0.48	0.48	0.30	0.44	0.30	0.70	0.85
Upper 95% CI of median	3.0	3.0	3.0	3.0	5.0	4.0	3.0	3.0	3.0	8.0	12	0.54	0.54	0.60	0.64	0.74	0.70	0.60	0.60	0.60	0.95	1.1
D'Agostino & Pearson omnibus normality test																						
K2	27	14	25	22	18	15	24	6.3	34	3.3	8.3	1.6	0.39	1.2	2.3	0.20	0.87	2.0	0.26	0.94	5.1	3.3
P value	< 0.0001	0.0011	< 0.0001	< 0.0001	0.0001	0.0004	< 0.0001	0.0423	< 0.0001	0.1940	0.0161	0.4488	0.8210	0.5497	0.3115	0.9064	0.6476	0.3683	0.8791	0.6253	0.0791	0.1908
Passed normality test (alpha=0.05)?	No	No	No	No	No	No	No	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
P value summary	****	**	****	****	***	***	****	*	****	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P value summary	****	****	****	***	***	***	****	****	****	*	ns	ns	ns	ns	ns	*	ns	*	ns	*	ns	ns
Coefficient of variation	81.46%	75.98%	109.75%	83.88%	75.95%	74.48%	124.71%	57.37%	129.16%	67.01%	59.48%	48.73%	54.78%	58.98%	51.49%	40.97%	38.20%	76.93%	37.78%	75.33%	38.84%	30.57%
Skewness	1.9	1.2	1.9	1.5	1.5	1.4	1.9	0.90	2.3	0.5	0.3	0.5	-0.2	0.4	0.2	0.2	0.3	0.3	-0.1	0.3	-0.8	-0.6
Kurtosis	4.6	2.1	3.4	4.1	2.7	1.8	3.3	0.57	5.9	-0.7	-1.2	-0.0	-0.1	-0.1	-0.8	-0.1	-0.3	-0.7	-0.4	-0.3	0.4	-0.6
Sum	119	109	139	97	167	142	142	111	128	280	356	20	19	22	19	25	24	20	21	19	33	37

Table 3: Robust regression and Outlier removal (ROUT) method showing the difference between both raw and transformed data.

ROUT	Ointment Preparation	Ointment Filling	Personnel Airlock	suppositories room	Semisolid Corridor	Liquid Filling	Liquid Prep. Personnel Airlock	Liquid Preparation	Liquid Prep. Material Airlock	Semisolid Mat. Airlock	Liquid Filling Mat. Airlock	Ointment Preparation	Ointment Filling	Personnel Airlock	suppositories room	Semisolid Corridor	Liquid Filling	Liquid Prep. Personnel Airlock	Liquid Preparation	Liquid Prep. Material Airlock	Semisolid Mat. Airlock	Liquid Filling Mat. Airlock
	Raw untransformed data											Transformed microbial count to log_{10} (CFU+1)										
Analyzed	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41
Outliers (Q = 10.00%)	8	2	5	1	2	5	8	1	6	0	0	0	0	0	0	0	0	0	0	0	2	0
Outliers (Q = 1.000%)	5	2	5	0	1	3	6	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0

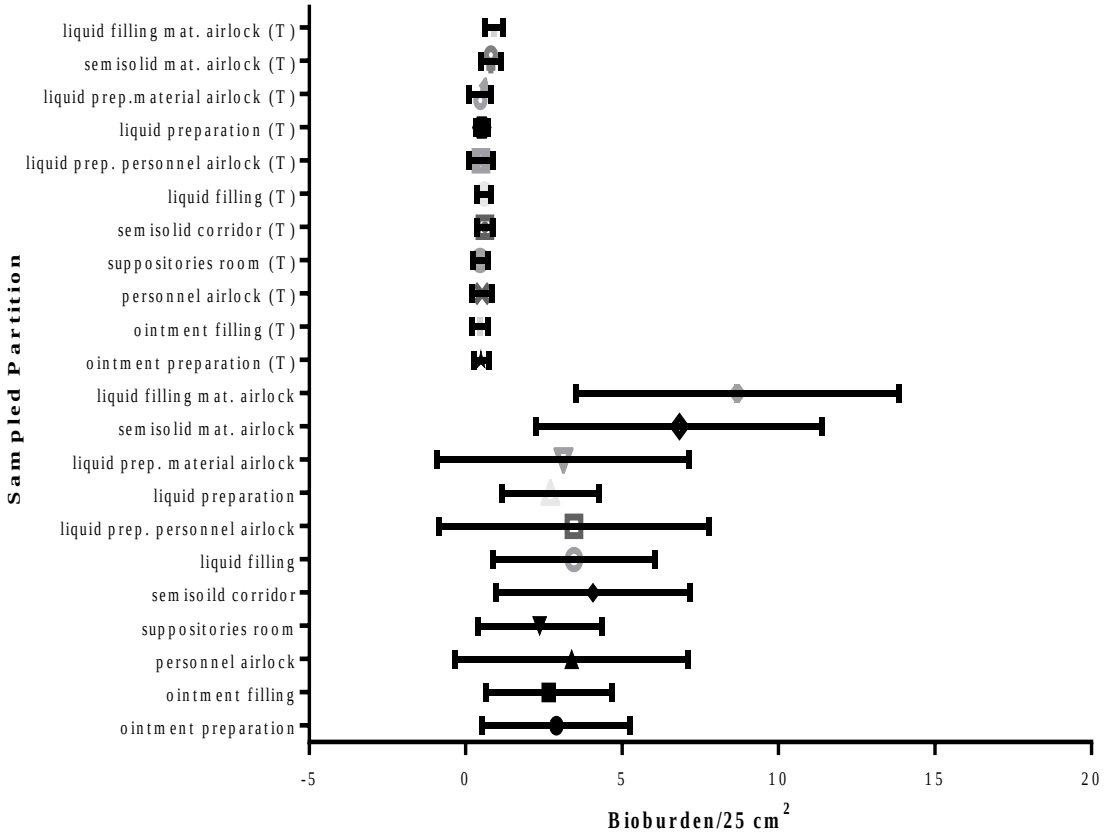

Fig-1: Interval plot showing the means and the confidence intervals (CI) for the raw and transformed surface bioburden in each zone of class C manufacturing facility.

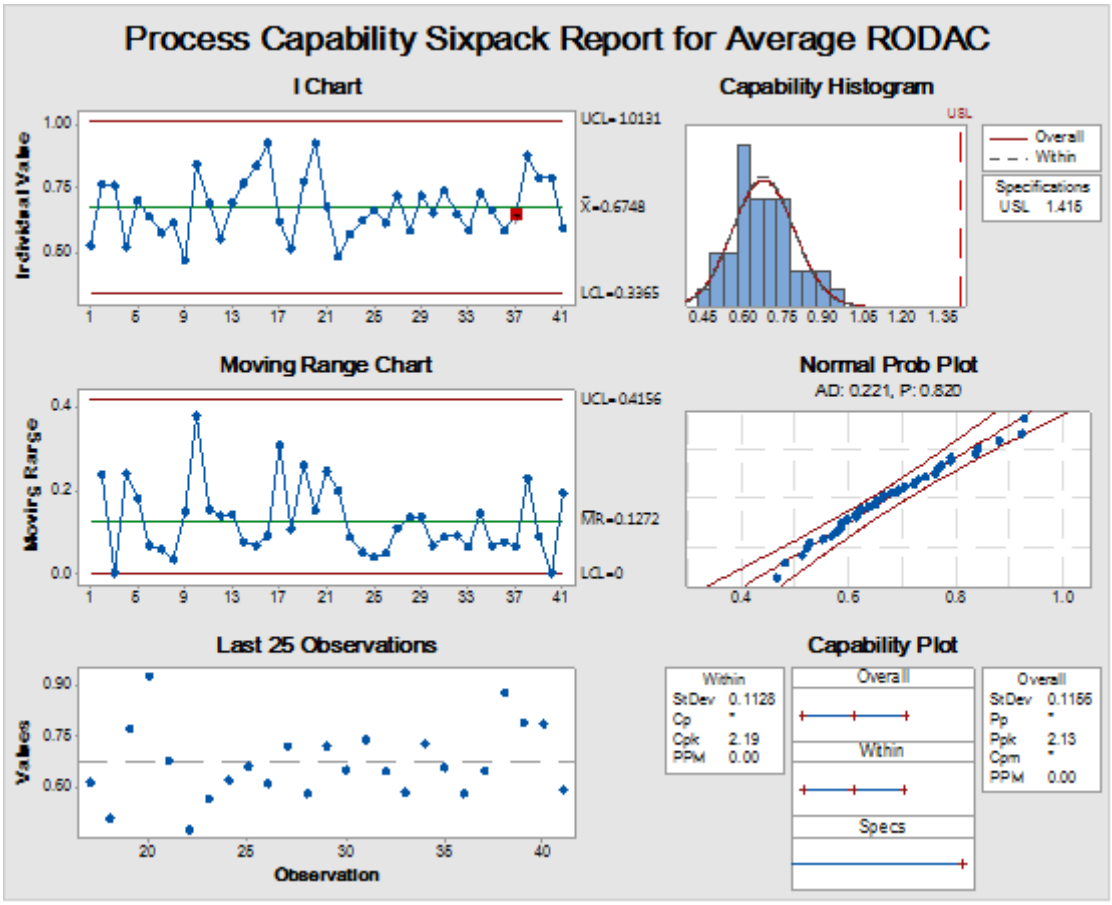

Fig-2: Assessment of the process stability and capability for the overall averaged and transformed contact plates microbiological counts in addition to the normality of data when it is assumed to follow normal distribution.

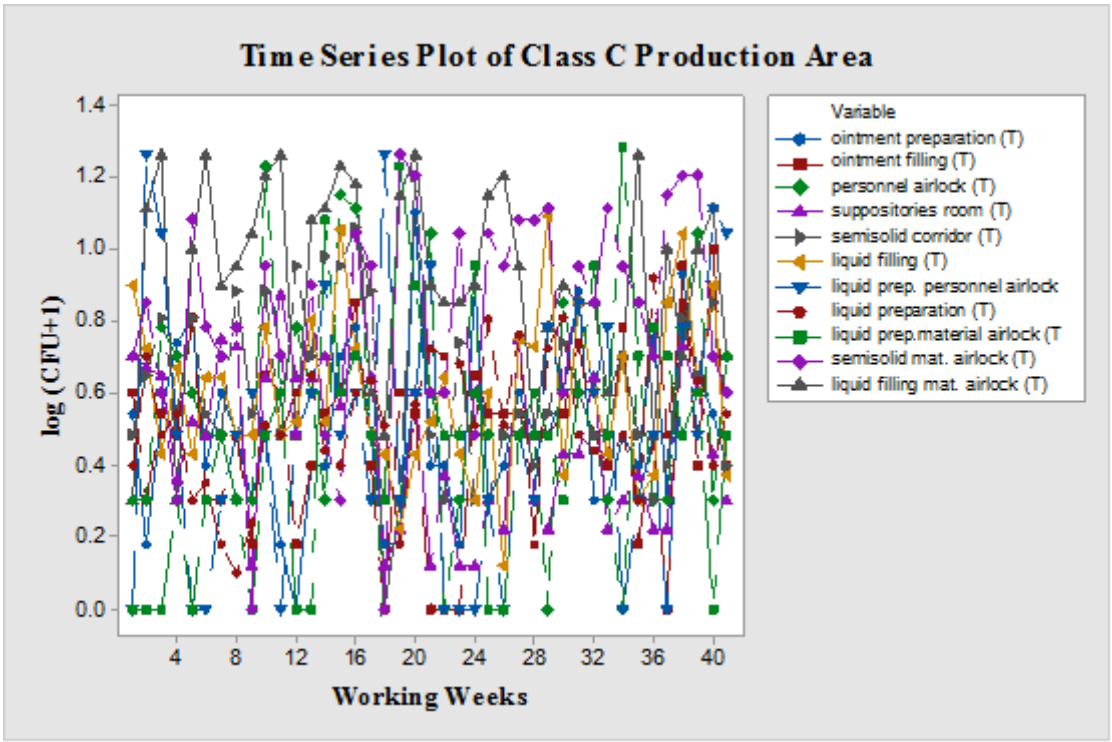

Fig-3: Chronologically plotted transformed microbiological surface bioburden count per 25 cm^2 approximately in different manufacturing partitions of class C.

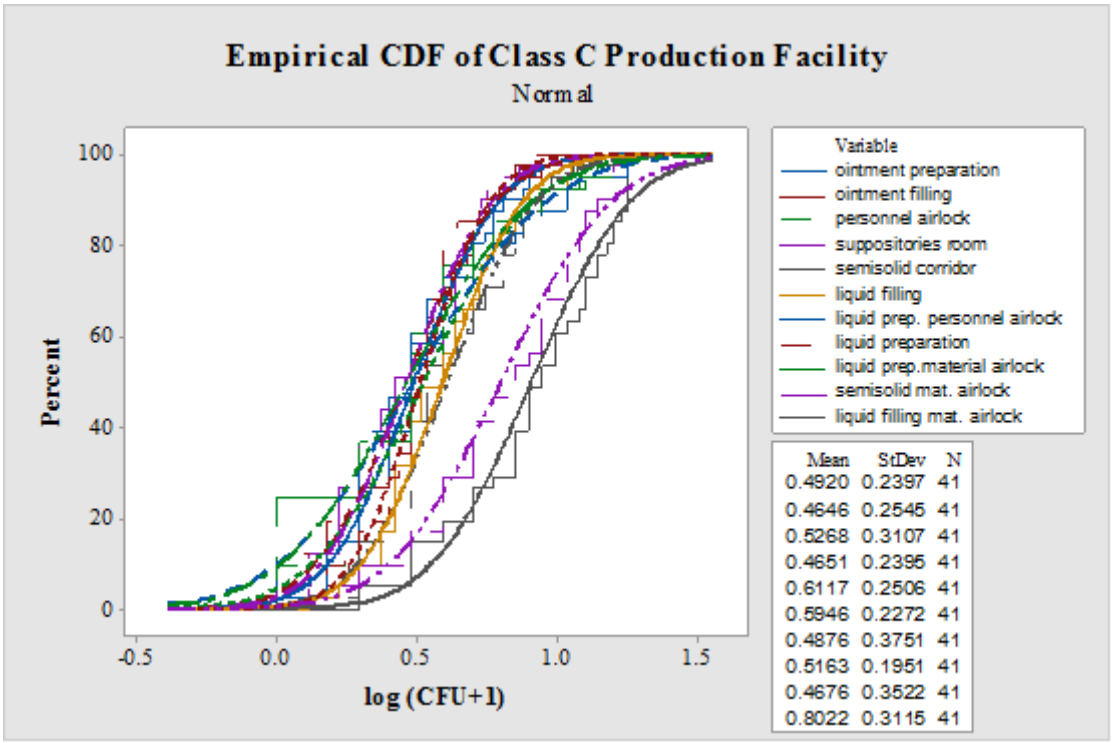

Fig-4: Cumulative distribution function (CDF) of the 11 partitions of the pharmaceutical manufacturing area showing the relative abundance, distribution and pattern of microbial surface bioburden based on replicate organism detection and counting (RODAC) samples.

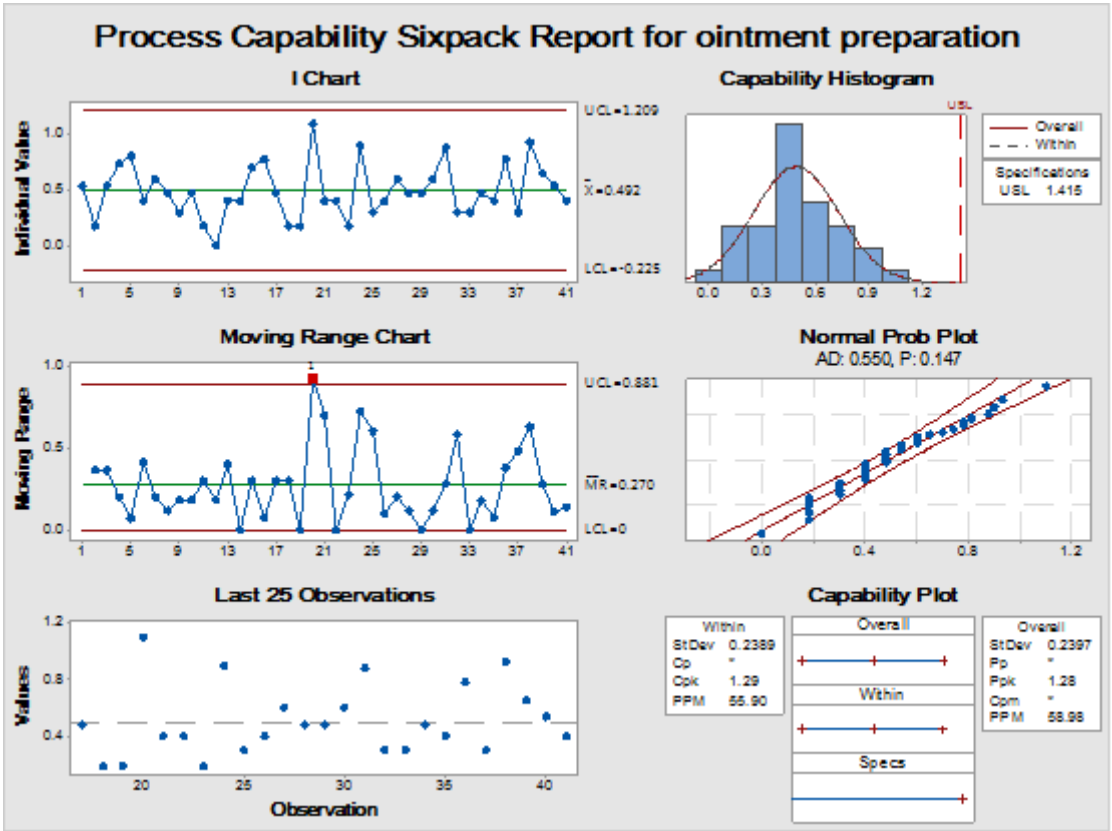

Fig-5: Assessment of the process stability and capability for the averaged and transformed contact plates microbiological counts of ointment preparation area in addition to the normality of data when it is assumed to follow normal distribution.

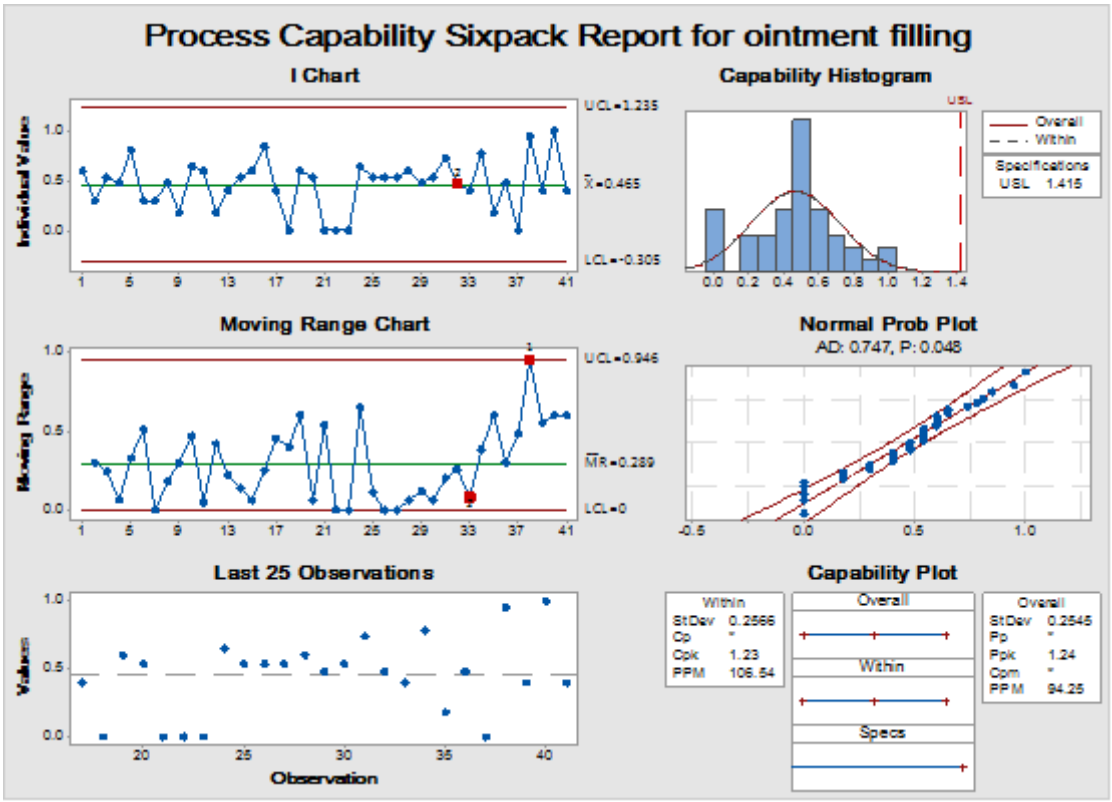

Fig-6: Assessment of the process stability and capability for the averaged and transformed contact plates microbiological counts of ointment filling room in addition to the normality of data when it is assumed to follow normal distribution.

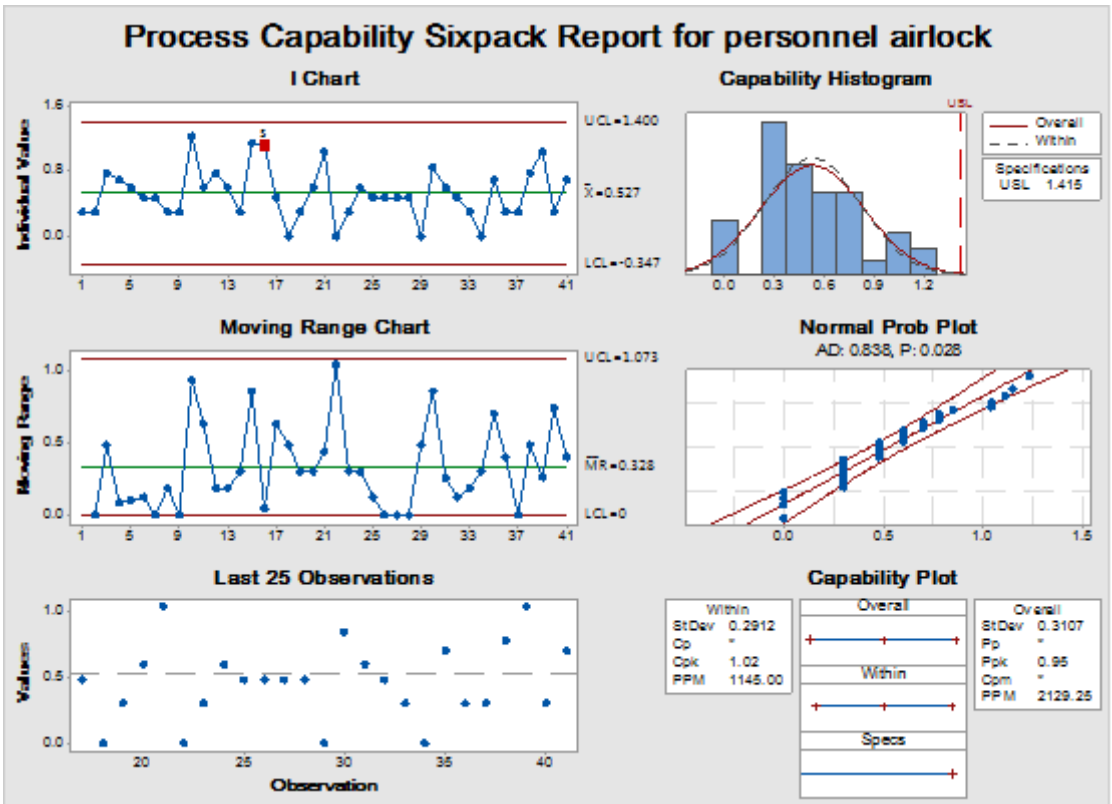

Fig-7: Assessment of the process stability and capability for the averaged and transformed contact plates microbiological counts of personnel airlock in addition to the normality of data when it is assumed to follow normal distribution.

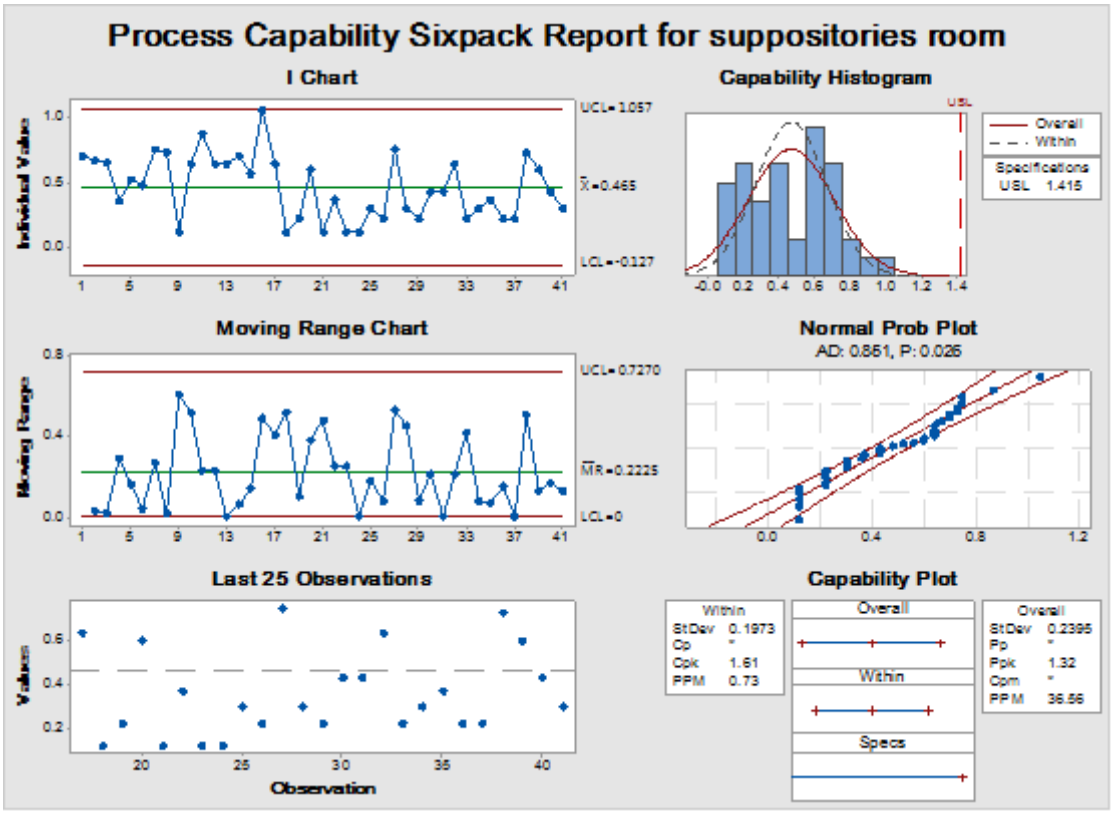

Fig-8: Assessment of the process stability and capability for the averaged and transformed contact plates microbiological counts of suppositories room in addition to the normality of data when it is assumed to follow normal distribution.

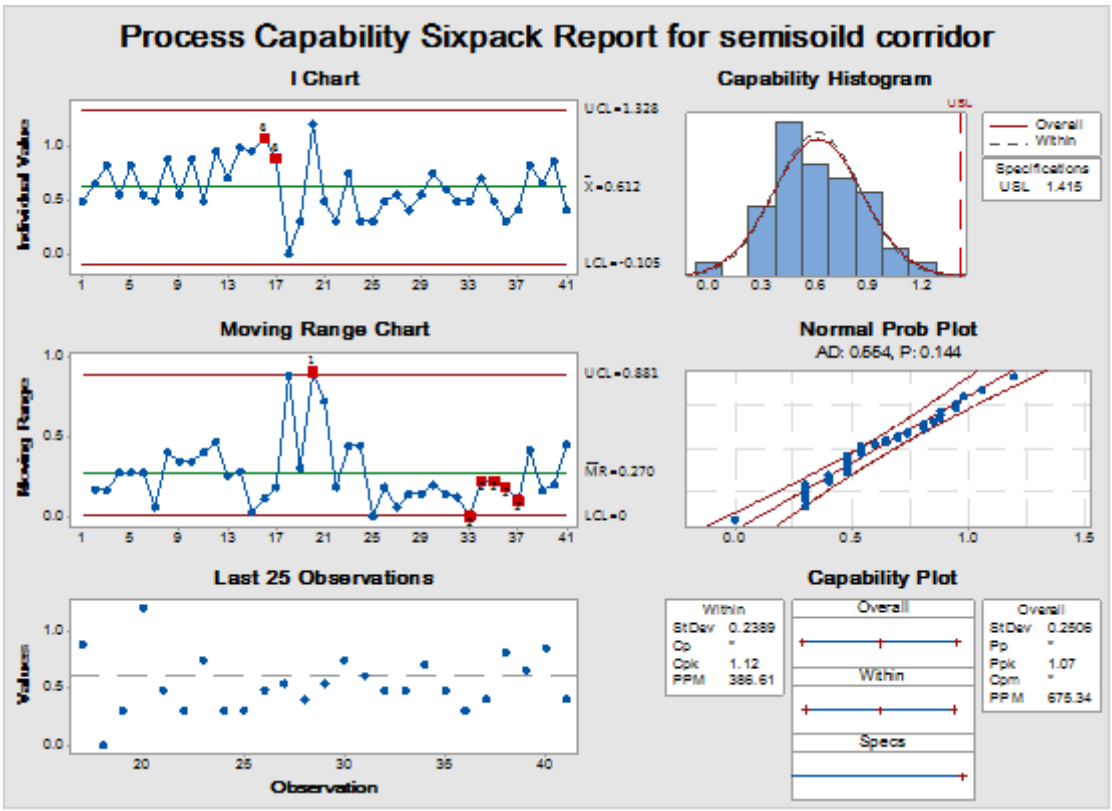

Fig-9: Assessment of the process stability and capability for the averaged and transformed contact plates microbiological counts of semisolid corridor in addition to the normality of data when it is assumed to follow normal distribution.

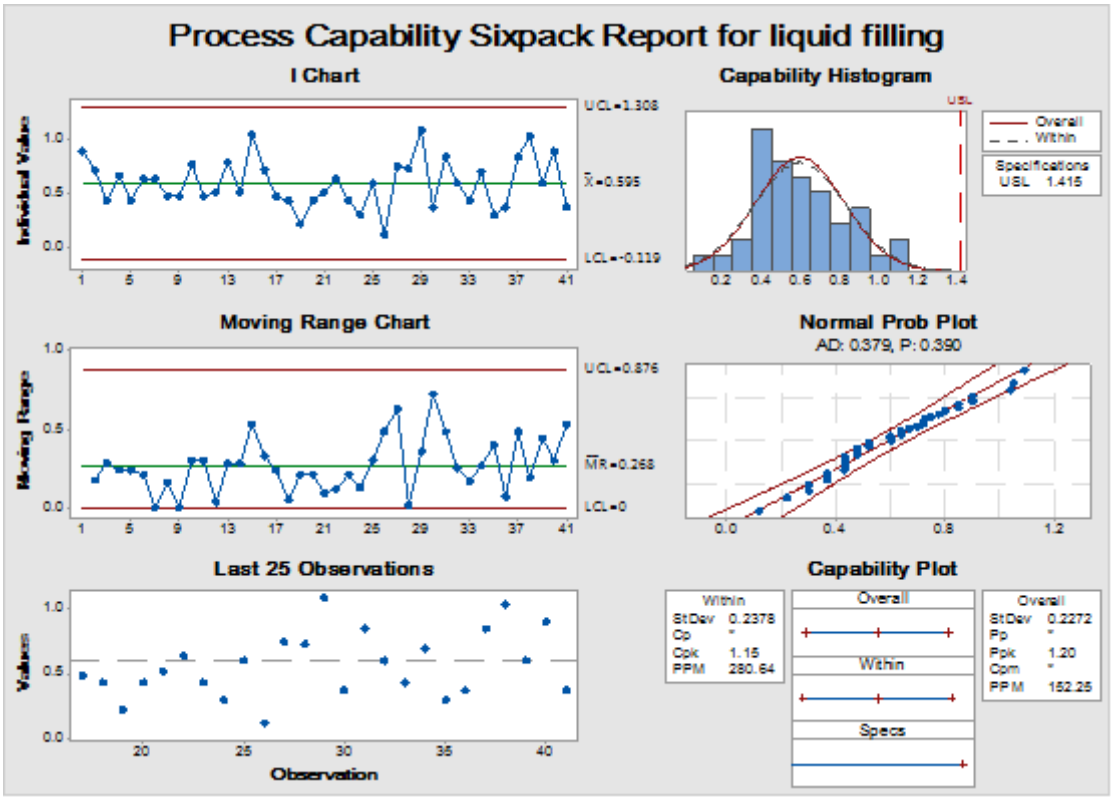

Fig-10: Assessment of the process stability and capability for the averaged and transformed contact plates microbiological counts of liquid filling compartment in addition to the normality of data when it is assumed to follow normal distribution.

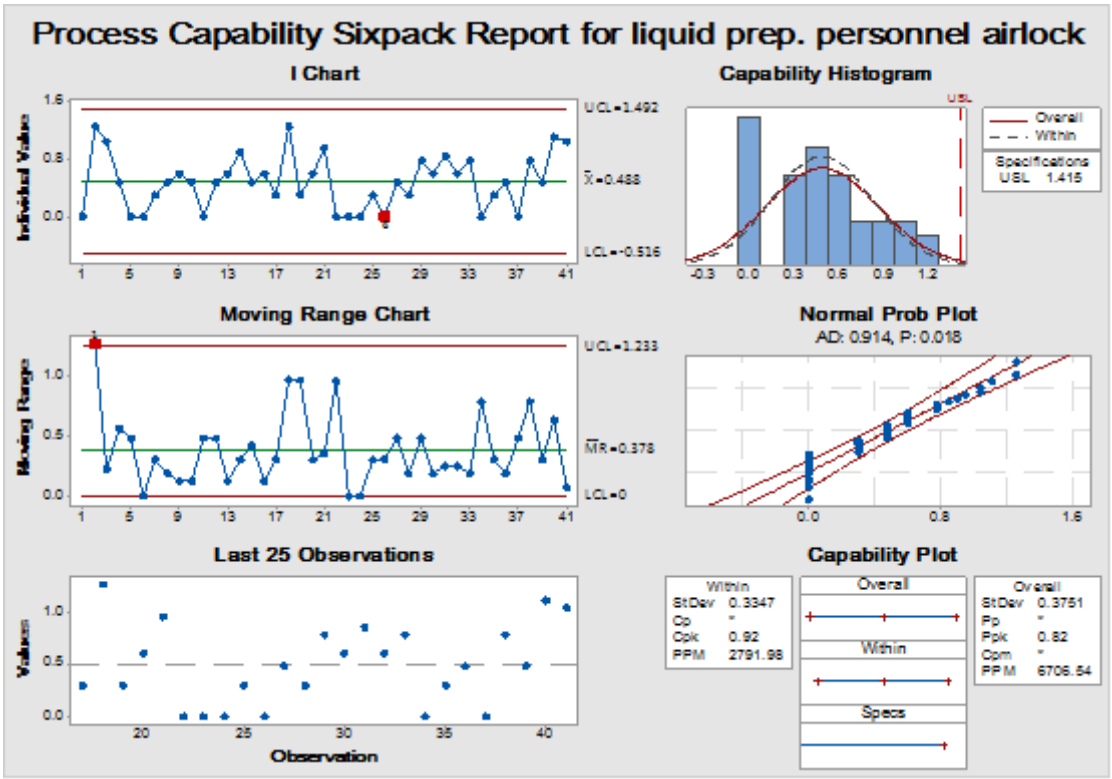

Fig-11: Assessment of the process stability and capability for the averaged and transformed contact plates microbiological counts of liquid preparation personnel airlock in addition to the normality of data when it is assumed to follow normal distribution.

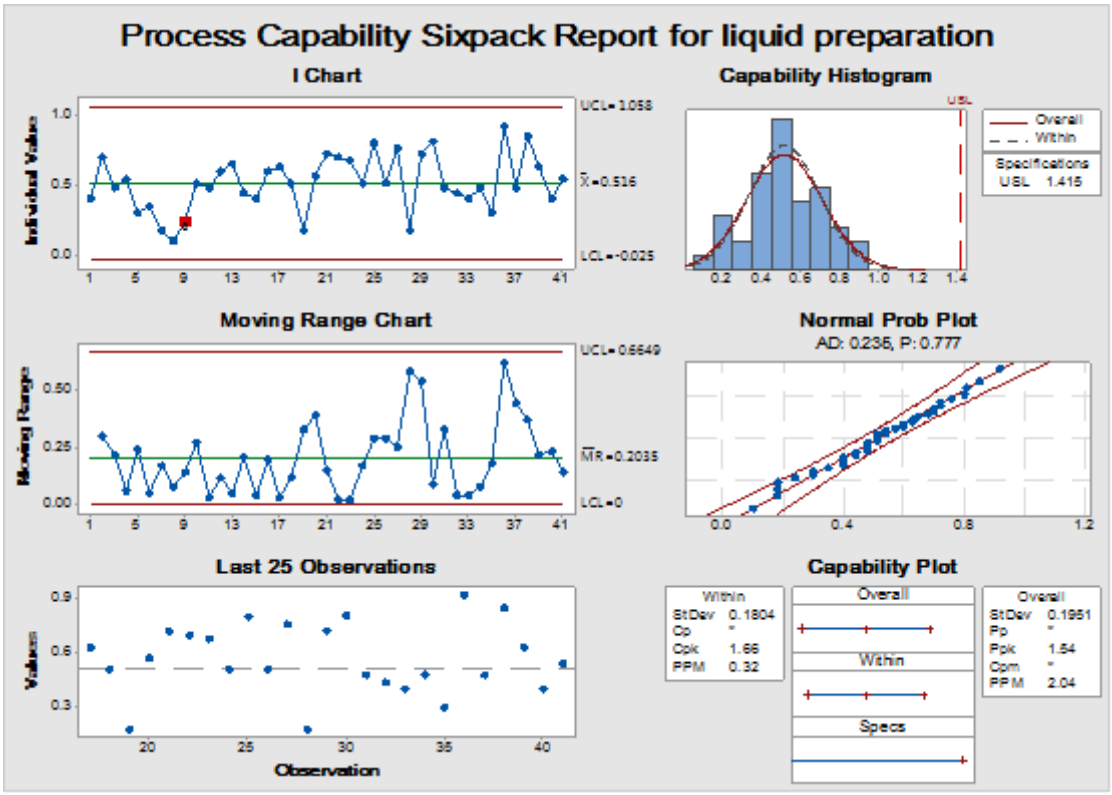

Fig-12: Assessment of the process stability and capability for the averaged and transformed contact plates microbiological counts of liquid preparation partition in addition to the normality of data when it is assumed to follow normal distribution.

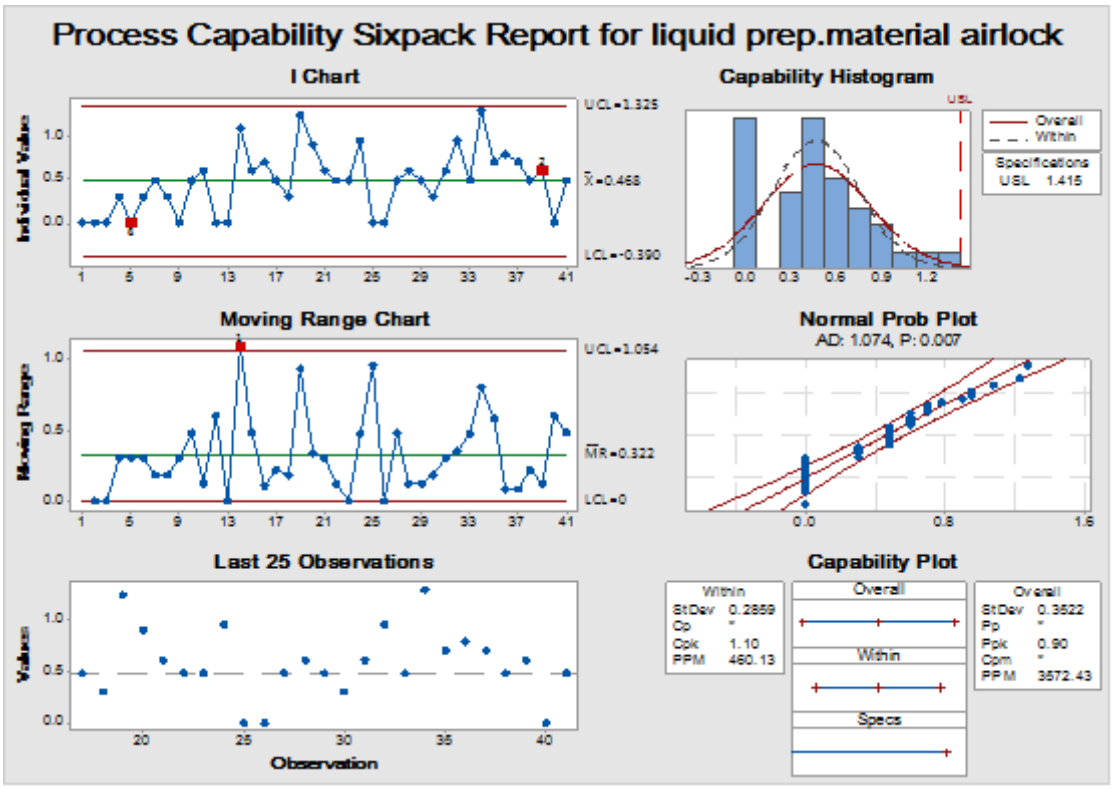

Fig-13: Assessment of the process stability and capability for the averaged and transformed contact plates microbiological counts of liquid preparation material airlock in addition to the normality of data when it is assumed to follow normal distribution.

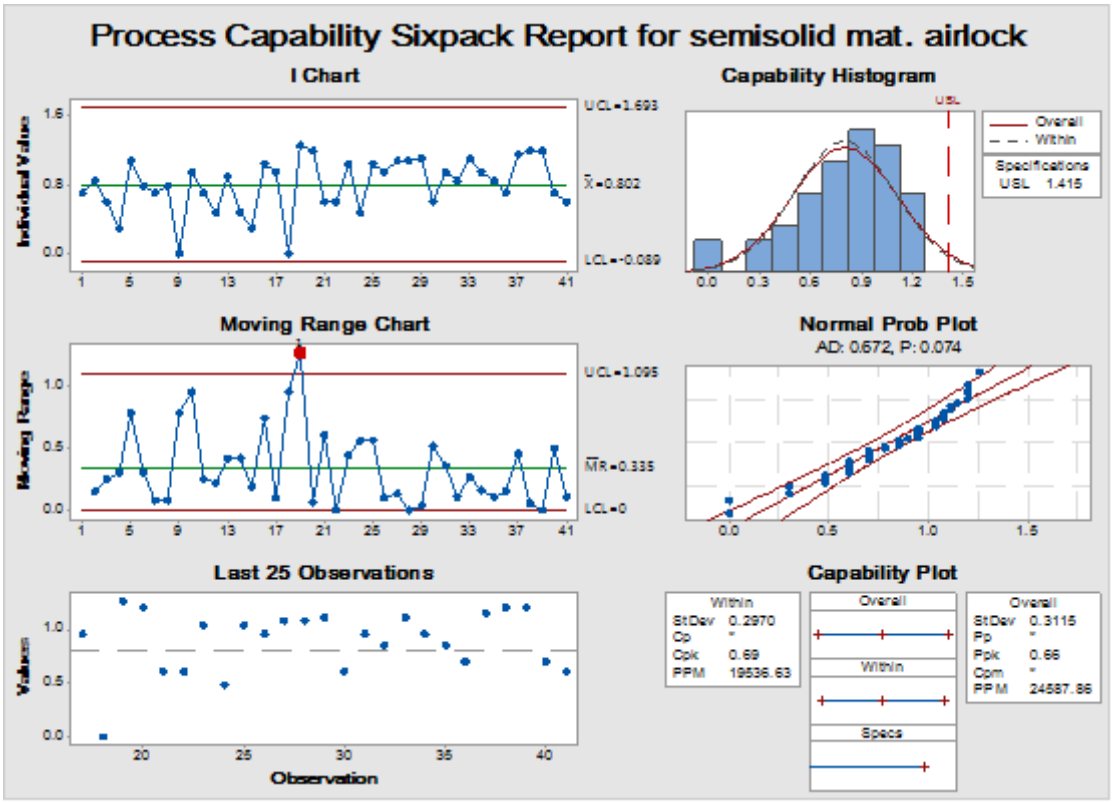

Fig-14: Assessment of the process stability and capability for the averaged and transformed contact plates microbiological counts of semisolid material airlock in addition to the normality of data when it is assumed to follow normal distribution.

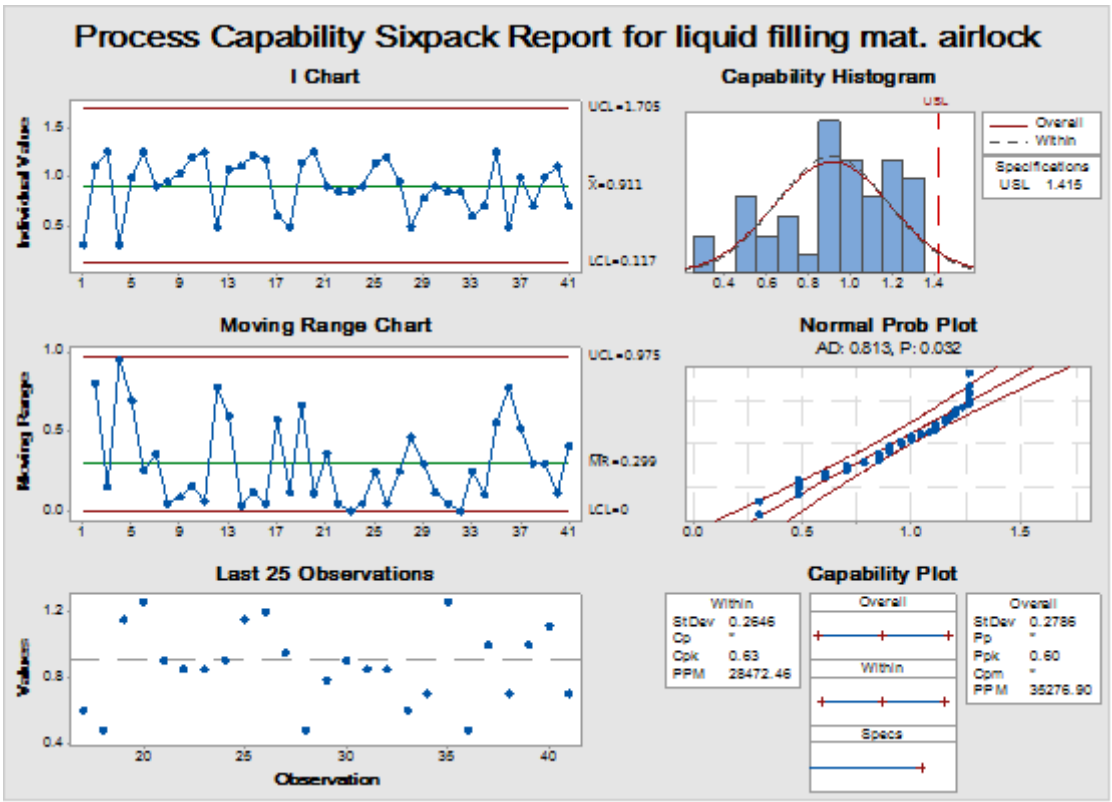

Fig-15: Assessment of the process stability and capability for the averaged and transformed contact plates microbiological counts of liquid filling material airlock in addition to the normality of data when it is assumed to follow normal distribution.

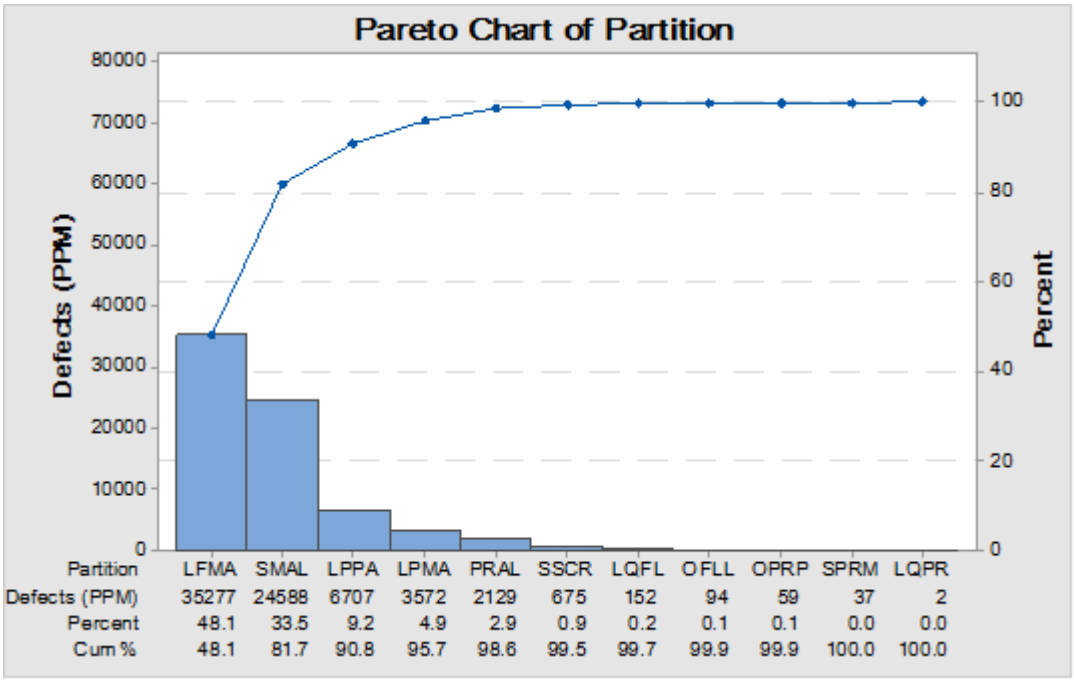

Fig-16: Pareto chart of the manufacturing zones in class C pharmaceutical plant displaying defects of microbiological surface quality in decreasing magnitude of frequency in order to determine the area of priority for improvements

. LFMA = Liquid Filling Material Airlock, SMAL = Semisolid Material Airlock, LPPA = Liquid Preparation Personnel Airlock, LPMA = Liquid Preparation Material Airlock, PRAL = Personnel Airlock, SSCR = Semisolid Corridor, LQFL = Liquid Filling area, OFLL = Ointment Filling room, OPRP = Ointment Preparation room, SPRM = Suppositories Room and LQPR = Liquid Preparation Room.

Table 4: Pearson correlation matrix with Two-tailed P at 95% CI and α = 0.05 for microbiological surface cleanliness of Class C productions partitions.

Pearson Correlation Matrix	Corridor	Ointment Preparation	Ointment Filling	Personnel Airlock	Suppositories Room	Liquid Filling	Liquid Prep. Personnel	Liquid Preparation	Liquid Prep. Material Airlock	Semisolid Mat. Airlock	Liquid Filling Mat. Airlock
Corridor		0.33*	0.39*	0.43*, ¥	0.59*, ¥	0.22	0.13	0.01	-0.04	0.18	0.31*
Ointment Preparation	0.33*		0.56*, ¥	0.33*	0.21	0.21	-0.01	0.13	0.19	0.17	0.01
Ointment Filling	0.39*	0.56*, ¥		0.22	0.39*	0.26	0.03	-0.06	0.10	0.30	0.12
Personnel Airlock	0.43*, ¥	0.33*	0.22		0.38*	0.05	0.09	0.12	-0.05	0.03	0.29
Suppositories Room	0.59*, ¥	0.21	0.39*	0.38*		0.31*	0.06	-0.02	-0.08	0.17	0.24
Liquid Filling	0.22	0.21	0.26	0.05	0.31*		0.15	0.15	-0.10	0.13	-0.11
Liquid Prep. Personnel	0.13	-0.01	0.03	0.09	0.06	0.15		0.18	-0.16	-0.25	-0.03
Liquid Preparation	0.01	0.13	-0.06	0.12	-0.02	0.15	0.18		-0.07	0.12	-0.15
Liquid Prep. Material Airlock	-0.04	0.19	0.10	-0.05	-0.08	-0.10	-0.16	-0.07		0.24	0.03
Semisolid Mat. Airlock	0.18	0.17	0.30	0.03	0.17	0.13	-0.25	0.12	0.24		0.19
Liquid Filling Mat. Airlock	0.31*	0.01	0.12	0.29	0.24	-0.11	-0.03	-0.15	0.03	0.19	

* = Significant correlation. ¥ = Moderate correlation while all the other showed either weak or very weak association.

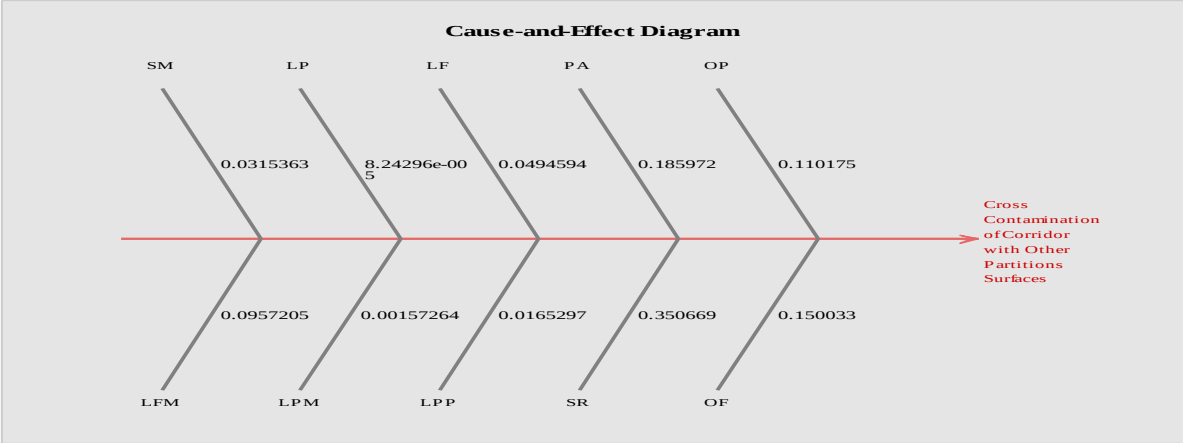

Fig- 17: Ishikawa Diagram showing the degree of the relation between surface bioburden of the main corridor and the other manufacturing partitions using coefficient of determination (R^2).

SM = Semisolid Material Airlock, LP = Liquid Preparation room, LF = Liquid Filling area, PA = Personnel Airlock, OP = Ointment Preparation room, LFM = Liquid Filling Material Airlock, LPM = Liquid Preparation Material Airlock, LPP = Liquid Preparation Personnel Airlock, SR = Suppositories Room and OF = Ointment Filling Area.

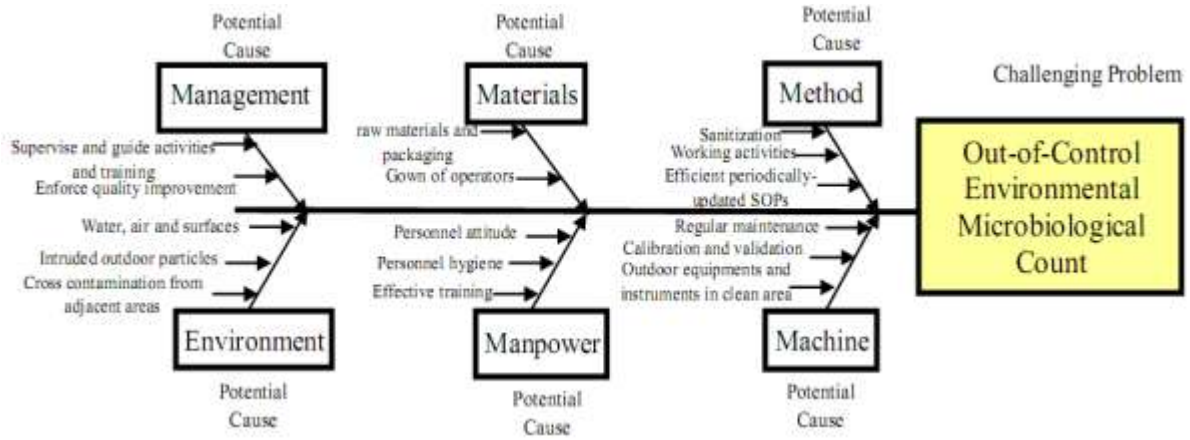

Fig-18: Ishikawa Diagram showing all the possible causes of microbiological out-of-control states.

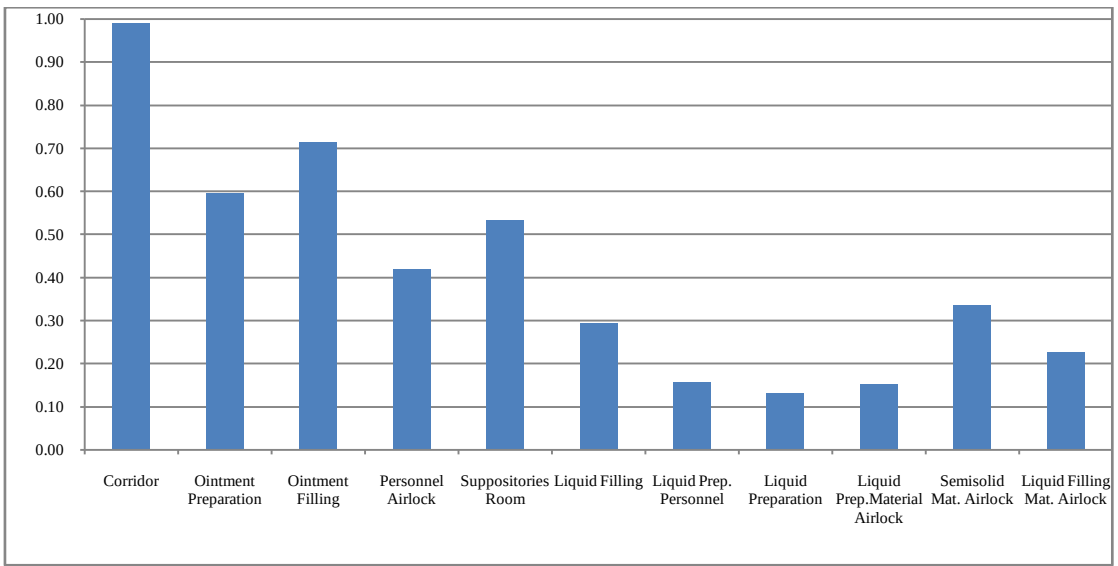

Fig-19: Total coefficient of determination (R^2) of each manufacturing location in class C.

CONCLUSION

Airlocks - especially for material transfer - are locations of high risk of failure in microbiological quality for pharmaceutical facility. A balance between sanitization practice and the activity encountered in specific production area should be established. An optimization between the two events could be determined using statistical process control as demonstrated in the current study. In addition, the use of control charts could take the routine microbiological environmental monitoring beyond just pass or fail to meet acceptance criteria. Six Sigma tools provide insight of the state of control, identifying defective spots and assisting in the investigation of any excursion in microbiological results. At the same time, the applied methodology is simple not expensive yet effective in supporting quality control (QC)/quality assurance (QA) tasks with the promising ability to apply quantitative risk analysis for production area that could impact the final microbiological quality of pharmaceutical products and bioburden of the medicinal drugs.

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