Sources of variations, repeatability and reproducibility of microbial count data

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INTRODUCTION

Implementation of effective quality control protocols requires a clear understanding of a process including a thorough evaluation of possible sources of variation. While it may not be possible to remove all sources of variation completely, eliminating special causes of variation and reducing random variability remains key to process improvement.

OBJECTIVE

The main objective of the study was to evaluate the relative contribution of technician effects to the total process variability, and to compare these contributions at different sub-processes.

MATERIALS & METHODS

Design of Experiment:

Experiment-1: Two lots of a raw material were used to prepare three batches of media. Each of the three technicians measured 5 independent samples of a microbial product and worked on their own samples till the end of the process: dilution, plating, and counting. The experiment was run over a 10-day period.

Experiment -2: Each technician weighed and diluted three independent samples per day, over three days. Samples were then shared between technicians during plating and counting. Each data point contained information on technicians who did the weighing & dilution (Wtech) , plating (Ptech), and counting(Ctech).

Data Analysis : Count data were converted to log10 values and analyzed using MIXED procedure of SAS. Models used were,

Expt.-I: *Y = Mean + Lot + batch + technician + day +sample(lot*batch*day*technician) + error*
Expt.-II: *Y = Mean + day+ Wtech + Ptech + Ctech+ sample(day*wtech*ptech*ctech) +error*

RESULTS & DISCUSSION

- Technician-A recorded the lowest mean count (Table 1) and CV values ranged between 1.0% and 1.17%.
- Sampling variance in expt-1 accounted for 35% of the total variance followed by a 33% contribution of technician effects (Figure 1).
- The overall repeatability standard deviation of count data was 0.08.
- Reproducibility standard deviation of measurements made by different technicians on the same day was 0.12.
- Reproducibility standard deviation of microbial count by the same technician at different days was 0.10.

Table 1. Basic stats for experiment-I

Technician	Mean	Min	Max	STD
A	10.99	10.67	11.37	0.11
B	11.16	10.79	11.65	0.12
C	11.14	10.79	11.47	0.13

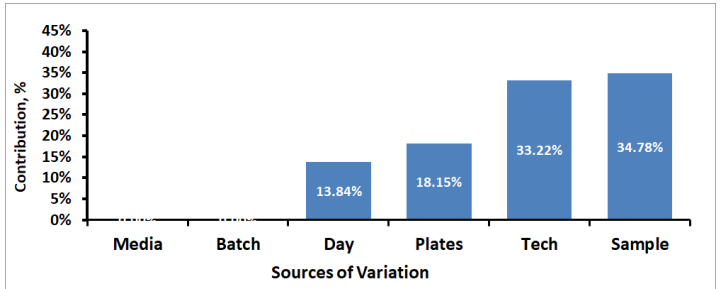

Figure 1. Percentage contribution to total variability Expt-1

- Contribution of sampling variance to the total variability remained the highest in expt-2 (39%, Figure 2).
- Variance due to technician effect was all due to technician differences during weighing-dilution step.
- The overall repeatability standard deviation of count data in expt-2 was 0.09.
- Reproducibility standard deviation of measurements made by different technicians on the same day was 0.14.
- Reproducibility standard deviation of measurements made by the same technician on the different days was 0.11.

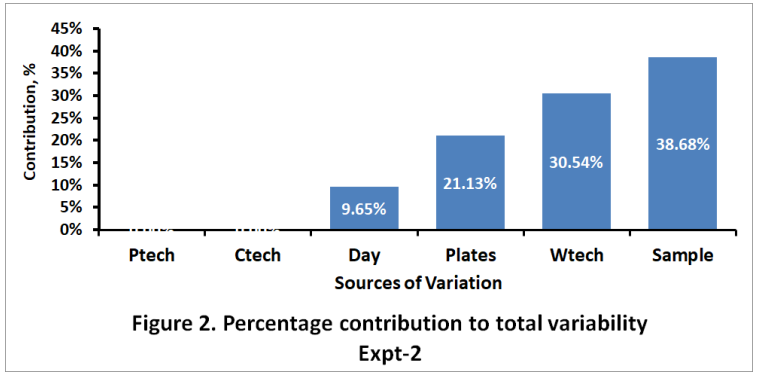

Figure 2. Percentage contribution to total variability Expt-2

CONCLUSION

- The relatively large contribution of sample-to-sample variance suggests a major improvement in the precision of count data may be obtained through additional samples rather than increasing number of plates per sample.

- Technician training can further reduce variation and improve consistency in sample weighing and dilution.