# Measurement uncertainty evaluation of microbial enumeration test for medicines

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## INTRODUCTION

Microbial quality is an important aspect that should be assessed to guarantee the efficacy and safety of medicines. Despite of all efforts to ensure the reliability of microbial enumeration test, there will always be some uncertainty associated with the measured value, particularly when is expected a reduced microbial load [1]. The aim this work was to evaluated measurement uncertainty of microbial enumeration test for medicines using a bottom-up approach.

## MATERIALS AND METHODS

Ten different medicines were intentionally contaminated with three levels ($10^3$, $10^4$, and $10^5$ CFU/mL) of bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) and fungal (*Aspergillus brasiliensis* and *Candida albicans*). Aliquots of 10 mL of contaminated medicines was subjected to decimal serial dilutions (1:10, 1:100, and 1:1000) and aliquots of 1 mL of each dilution were transferred to Petri plates (three replica per dilution for each microorganism). Portions of 15-20 mL of tryptic soy agar (TSA) and Sabouraud dextrose agar (SDA) were placed into the plates for bacteria and fungal, respectively. Plates containing TSA were incubated at 30-35 ºC for 2-3 days, while plates containing SDA were incubated at 20-25 ºC for 5-7 days.

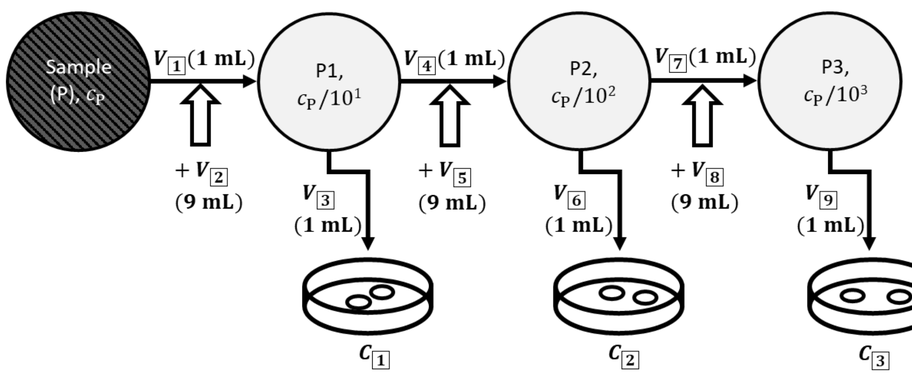


**Figure 1.** Schematic representation of the measurement procedure.

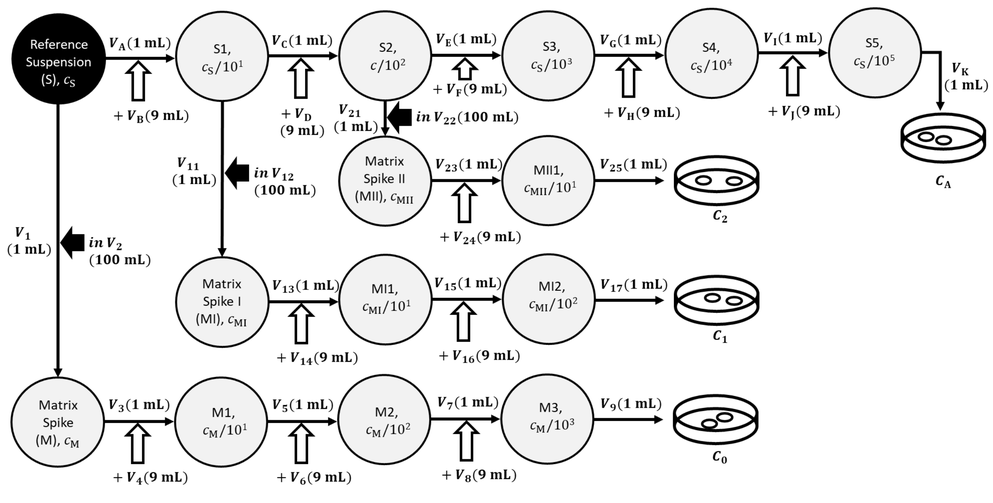


**Figure 1.** Schematic representation of the measurement performance evaluation.

## RESULTS AND DISCUSSION

Monte Carlo method and Poisson-lognormal regression [2] were used to assess how measurement uncertainty is related to the microbial load. Uncertainties from dilution factors, repeatability between plate microbial counts, and recovery of microbial counts in comparison to microbial counts of reference material were considered.

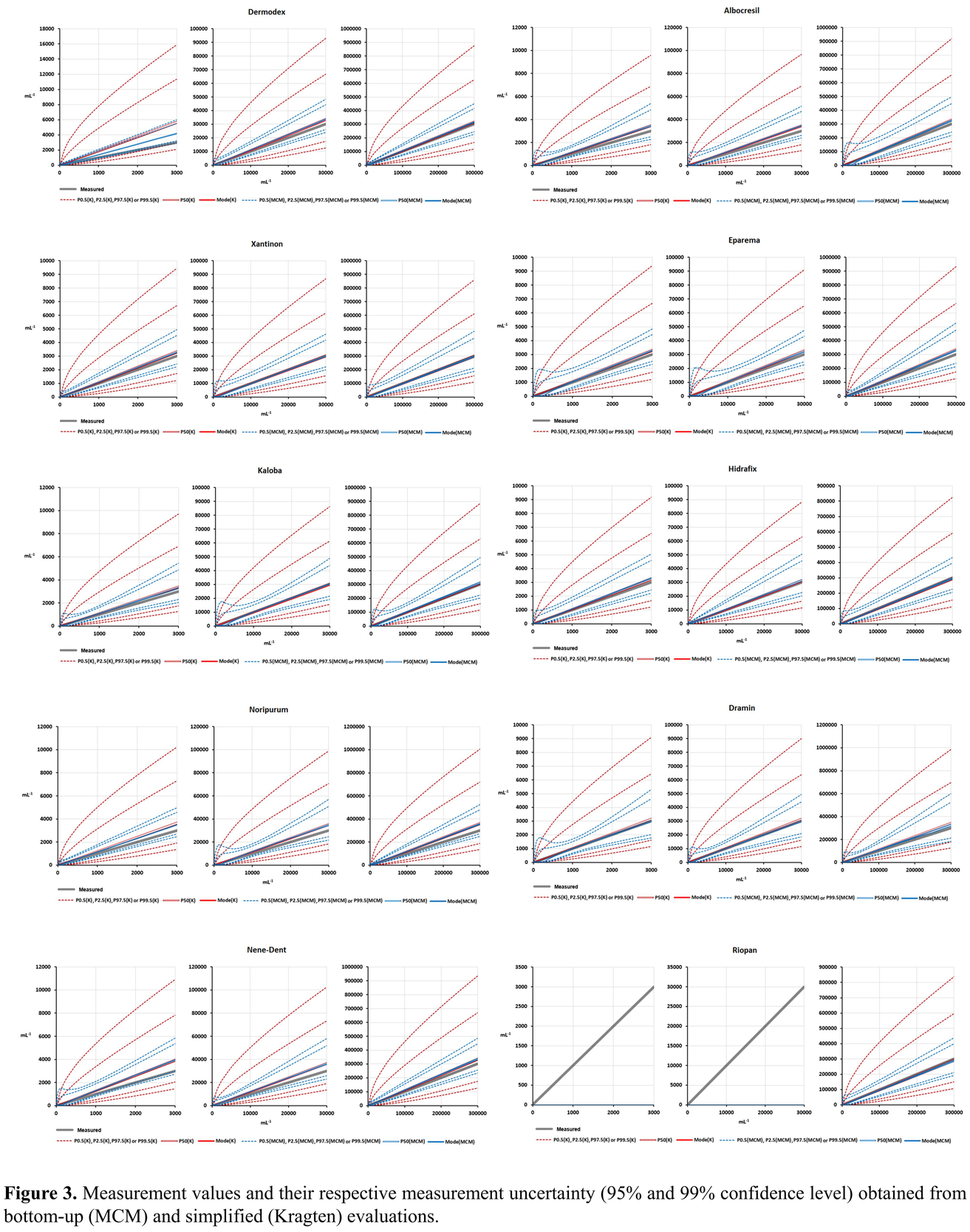


**Figure 3.** Measurement values and their respective measurement uncertainty (95% and 99% confidence level) obtained from bottom-up (MCM) and simplified (Kragten) evaluations.

Measurement uncertainty is important to guarantee the reliability and quality of enumeration test results and it should be taken into account in order to decide whether a medicine batch is compliant or non-compliant to the specification limits.

## References

[1] U. Gonzales-Barron, M. Kerr, J.J. Sheridan, F; Butler, *International Journal of Food Microbiology*, **2010**, *136*, 268-277.

[2] U. Gonzales-Barron, F. Butler, *Food Control*, **2011**, *22*, 1268-1278.

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