



# DO MICROBIOLOGICAL REFERENCE MATERIALS MATCH THEIR ASSOCIATED “CERTIFICATES OF ANALYSIS”?

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## INTRODUCTION & DISCUSSION

Ready to use microbiological “reference materials” (RM’s) are used more and more often as microbiological positive controls during pharmaceutical manufacturing Quality Control including growth promotion testing and method validations. These RM’s are each supplied with an empirically made “Certificate of Analysis” (COA). The COA usually contains qualitative and quantitative information about the batch including, strain, designation, and the mean & standard deviation of the Colony Forming Units (CFU) in each dose. These quantitative numbers are important for use as a reference point to determine if our methods and media are proficient for their purpose.

We set out to challenge if these numbers on the COA of reference materials can be reproducible.

## MATERIALS AND METHODS

### MATERIALS

Six different readily available reference materials were tested in a single or multi dose format:

**Single Dose:** BIOBALL® SingleShot.

**MultiDose:** BIOBALL® MultiShot 550 • EZAccu Shot™ • EZ-CFU™ One Step • EZ-CFU™ • Quanti-Cult Plus™.

**Strain designation:** All products used were from a *Pseudomonas aeruginosa* strain directed in the pharmacopoeias, either the NCTC strain or the ATCC strain.

**Growth Agar:** The products were all plated on to a single batch of Trypticase soy agar (TSA, Oxoid, lot#, exp. Date).

### METHODS

All products were prepared and plated according to the manufacturer’s instructions. Ten replicates were used for the single dose products and 25 replicates for the multi-dose products (i.e. 5 replicates from 5 doses/pellets/ balls). Plates were incubated at 37°C for 24hrs and colonies manually counted by a single operator. The results from all the products were summarized and a mean average CFU count for each product was determined. The mean average for each product from the study was compared with the mean average stated on the corresponding product “Certificate of Analysis”. We also analysed the repeatability of achieving the published mean by looking at the percentage of results that were within 10 CFU of the published mean.

## SUMMARY

Abbreviation	BB-SS	BB-MS	EZ-AC	EZ-OS	EZ	QCP
Name	BIOBALL® SingleShot	BIOBALL® MultiShot	EZ-Accu Shot™	EZ- CFU™ One Step	EZ- CFU™	Quanti-Cult Plus™
Strain designation	<i>P. aeruginosa</i> NCTC12924	<i>P. aeruginosa</i> NCTC12924	<i>P. aeruginosa</i> ATCC 9027	<i>P. aeruginosa</i> ATCC 9027	<i>P. aeruginosa</i> ATCC 9027	<i>P. aeruginosa</i> ATCC 9027
Supplier	bioMérieux	bioMérieux	Microbiologics	Microbiologics	Microbiologics	Thermo Fisher
Product Code	56040	56017	0484A	0484Z	0484C	R4715210
Batch #	4556	4544	484-910	484-887	484-890	155804
Published mean replicate	30.23 CFU	50.28 CFU	76 CFU	58 CFU	90 CFU	50 CFU
Measured mean replicate	29.9 CFU	47.04 CFU	54.88 CFU	39.64 CFU	45.2 CFU	38.92 CFU
Difference PUB VS ACT mean	1%	6%	28%	32%	50%	22%
Results within 10 CFU of PUB mean	100%	92%	12%	8%	0%	28%



PIONEERING DIAGNOSTICS

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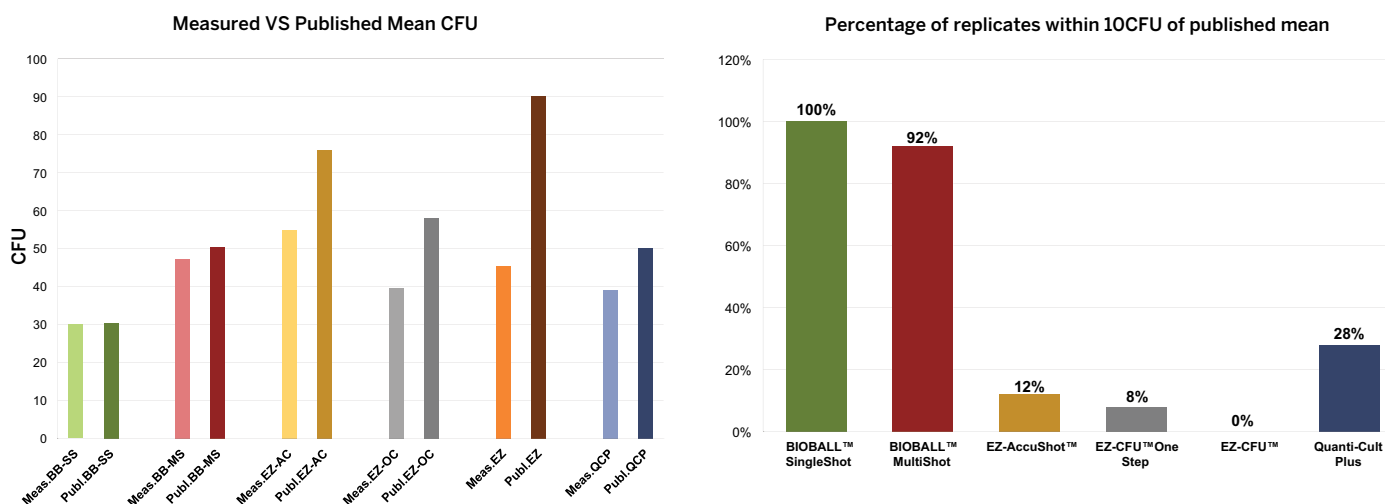
## RESULTS

Although the products had different preparation steps and incubation periods all aliquots had growth and no contamination was found on any plates. There was some large variances in the results from the published CFU VS measured CFU, ranging from as small as 1% to as large as 50% difference.

The BIOBALL measured CFU mean was noticeably closer to the published “certificate of analysis” CFU mean compared to all other products. As well as the percentage of results within 10 CFU of the mean was substantially higher with the BIOBALL product ranges.

BIOBALL SingleShot & Multishot measured results showed a difference of 1% & 6% from the stated Certificate of Analysis results whereas EZ-AccuShot™, EZ-CFU™ One Step EZ-CFU™ and Quanti-Cult Plus™ showed a difference of 28%, 32% 50% and 22% respectively.

BIOBALL Singleshot and Multishot were within 10CFU of the published mean 100% and 92% of the time. Whereas EZ-Accu Shot™, EZ-CFU™ One Step EZCFU™ and Quanti-Cult Plus were only within 10CFU of the published mean 12%, 8% 0% and 28% respectively.



## CONCLUSION & CONSIDERATIONS

The majority of the reference materials did not show recoveries that matched the mean CFU number that is shown on the “Certificate of Analysis” issued with the batch. The BIOBALL product range showed results that were close to the published “certificate of analysis” results and considerably closer than other ready to use reference materials.

**BIOBALL Multishot measured results showed a difference of 6% from the stated Certificate of Analysis results whereas EZ-AccuShot™, EZ-CFU™ One Step EZ-CFU™ and Quanti-Cult Plus showed a difference of 28%, 32% 50% and 22% respectively.**

**The BIOBALL Singleshot and Multishot were within 10CFU of the published mean 100% and 92% of the time. Whereas EZ-AccuShot™, EZ-CFU™ One Step EZ-CFU™ and Quanti-Cult Plus were only within 10CFU of the published mean 12%, 8% 0% and 28% respectively.**

The positive BIOBALL outcomes are likely due to the BIOBALL manufacturing process which uses flow cytometry to count and dispense cells accurately and precisely. The other products do not utilize flow cytometry to dispense cells. The BIOBALL Single-Shot was the only SingleShot product included in the study.

*Testing with a Ready-to-Use Reference Material has the advantage of providing an independent and precise external calibration point. It is very important that you are able to trust and verify your results using this calibration. With BIOBALL you can be assured that you will have a precise and accurate calibration result similar to that of the associated batch “Certificate of Analysis”.*