

# Precise Reference Materials for Microbiology

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## >> Summary

Precise reference materials that have been certified to the ISO34 standard are now commercially available. Flow cytometry is used to dispense precise numbers of cells into a droplet of fluid that is processed to form a small freeze dried ball. The freeze dried ball, known as a BioBall™, contains a precise number of viable cells and can be tipped into a sample or onto an agar plate with no losses of the cells.

## >> Introduction

### Problems with traditional microbiological QC

- No quantification
- Lack of precision
- Lack of harmonization of results
- False positive results

## >> Methods - How are BioBall™ made?

1. Bacterial cultures are grown for maximum freeze drying recovery.
2. A modified flow cytometer is used to count and sort bacteria into a single droplet.
3. The droplet is frozen in liquid nitrogen and placed into a vial.
4. BioBall™ are freeze dried, sealed under vacuum and crimped.

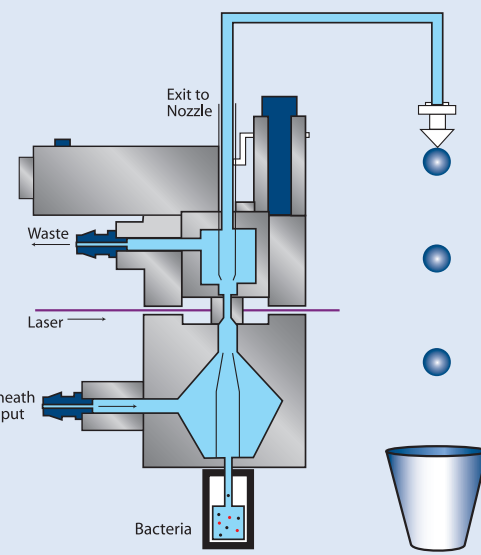


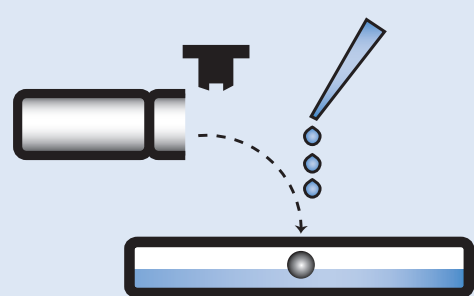
Fig 1: Bacteria sorted and dispensed into liquid nitrogen by modified Flow Cytometry.

## >> Results



BioBall™ is ready for use.

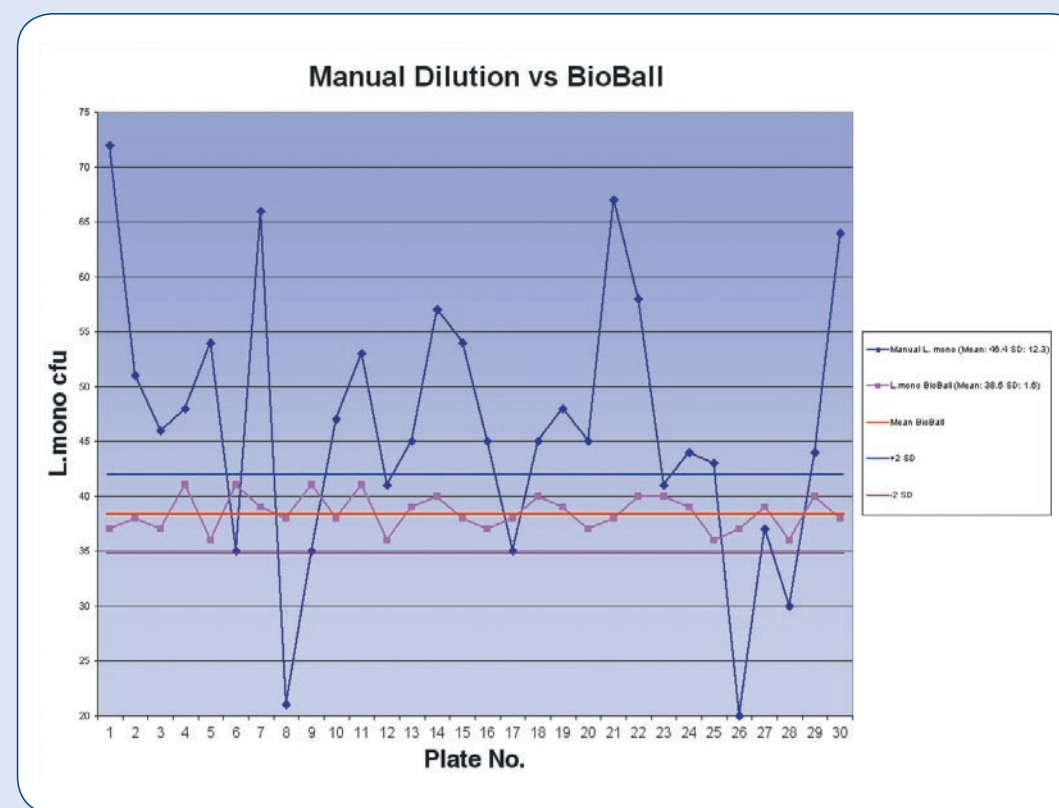
Plate out the BioBall™ by rehydrating with sterile saline solution.



Exact number of bacterial colonies are enumerated every time.

## >> Comparative and Quantitative results

Manual dilution data compared to BioBall™ data for *Listeria monocytogenes*.



## >> Within and between batch variation

- BioBall™ has a within batch variation of <3 standard deviations.
- BioBall™ has a between batch variation of <2 standard deviations.

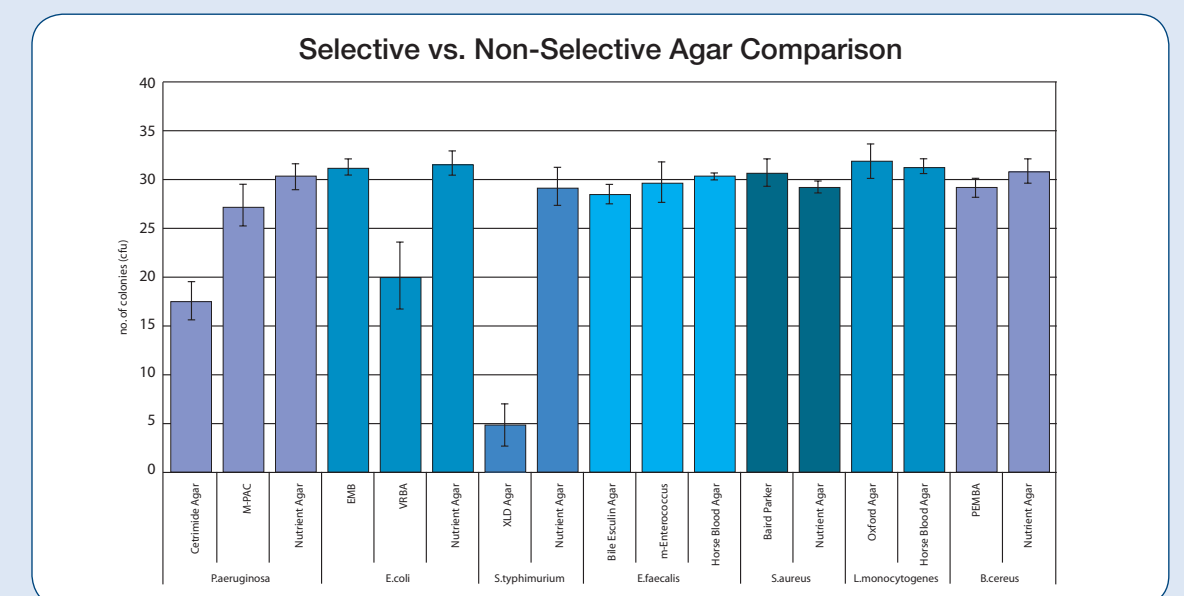
Organism	Mean within batch								Between batch mean	SD
<i>B.cereus</i>	31.2	29.6	29.4	29.3	31.7	30.2	29.9		30.2	1.0
<i>B.subtilis</i>	32.8	29.7	31	31.2	32.8	30.3	29.1	32.8	31.2	1.5
<i>L.innocua</i>	29.1	30.1	29.6	29.6	30				29.7	0.4
<i>L.monocytogenes</i>	28.8	29.7	29.2	30.3	30.3				29.7	0.7
<i>E.coli</i>	32.4	30.9	30.5	29.2	32.6	31.2	29.3	31.8	31.0	1.3
<i>S.typhimurium</i>	31.9	28.4	29	29					29.8	1.9
<i>E.faecalis</i>	32.1	30.5	28.4						30.3	1.9
<i>S.abaetetuba</i>	31.2	30.4							30.8	0.6
<i>E.aerogenes</i>	31.4	31.8							31.6	0.3

## >> Culture media Quality Control

- Significant differences seen for some selective agar
- Choosing a selective agar for routine use is made easier
- More precision from media QC = better control over media production

## >> Selective vs. Non-Selective media

No significant difference from non-selective	Significant difference from non-selective
m-PAC	Cetrimide agar
EMB	XLD agar
Bile Esculin agar	VRBA
m-Enterococcus agar	
Baird Parker agar	
Oxford agar	
PEMBA	



## >> Applications for the technology

To use a reference standard for quality control in:

- Media production
- Batch testing
- Method validations
- Polymerase chain reaction (PCR) analysis
- Harmonising results across the industry

## >> Fluorogenic strains

Internal controls  
Easily identifiable QC strain



## >> Measurement of Uncertainty Solution

Quantified reference control to enable precise measurements  
Reduce the uncertainty in the spike = Reduced measurement of uncertainty

## >> 10,000 cfu BioBall™

Precise high spike loads for whole method validations

## >> PCR Quantification

Precise copies of DNA within a BioBall™ amplification control within your PCR process

## >> Conclusions

BioBall™ provides solutions to problems associated with traditional methods of microbiological QC.

BioBall™ provides:

- Precision: an exact number of cfu or DNA template
- Harmonisation of results due to consistency of batches
- Quantification of QC results allowing laboratory performance to be accurately monitored
- Traceability: Use of fluorogenic strains to identify QC organisms
- Stability: freeze dried microorganisms without a loss in viability
- Simplicity: simple to use, saving on set up time and labour costs