Inoculation Loops - Certificate of Calibration

Colorimetric Evans Blue Dye Procedure[1,2]

1. **a) Preparation of stock dye solution**
2. Date the solution to expire 6 months after preparation.
3. Prepare stock dye solution by adding 0.75 g of Evans Blue dye to 100 ml of distilled water.
4. After the dye is dissolved, filter the solution through a no. 4 Whatman filter paper.

Store the filtered dye in a tightly closed dark bottle at room temperature.

b) Procedure

1. Prepare accurate 1:500, 1:1000, 1: 2000, and 1:4000 dilutions of the stock dye solution distilled water. Measure the absorbance of each dilution a spectrophotometer at wavelength of 600nm.
2. Using these data, prepare a calibration curve by plotting the optical density (vertical scale) against the four different concentrations of dye (horizontal scale). Draw a single straight line that most closely fits all four points. This is your calibration "curve".
3. I)The four dilution standards (1:500 to 1: 4000) may be saved for 6 months provided they are stored in tightly closed dark bottles at room temperature.
   II)If the absorbance reading of any of the dilution standards differs from its' previous reading by more than 3%, the entire dilution series must be prepared fresh.
4. The calibration of the 1µl (0.001 ml) loop can be tested by using the loop to transfer 10 loopfuls of the stock dye solution to 10 ml of distilled water. The loop must be rinsed in a separate flask of distilled water after each of the 10 transfers. After thorough mixing, measure the absorbance of the test solution. The absorbance of the 10 ml solution should correspond to that of the 1: 1000 dilution on the calibration curve, +/- 20%. If the margin of error is greater than +/- 20% the loop is rejected. Quantitative loops for semi-quantitative cultures, e.g. urine cultures, are generally allowed a +/- 20% inaccuracy. 20 loops are randomly sampled during the manufacture of each individual production lot of loops. If any of the 20 loops sampled from any production lot show an error of greater than +/- 20% then that lot is withheld and quarantined for detailed inspection.
5. The calibration of the 10µl (0.01 ml) loop can be tested by using the loop to transfer 10 loopfuls of the stock dye solution to 100 ml of distilled water as above. After thorough mixing, measure the absorbance of the test solution. The absorbance of the 100 ml solution should correspond to that of the 1: 1000 dilution. On the calibration curve, +/- 20%. If the margin of error is greater than +/- 20% the loop is rejected. Quantitative loops for semi-quantitative cultures, e.g. urine cultures, are generally allowed a +/- 20% inaccuracy. 20 loops are randomly sampled during the manufacture of each individual production lot of loops. If any of the 20 loops sampled from any production lot show an error of greater than +/- 20% then that lot is withheld and quarantined for detailed inspection.

Date:	14TH SEPTEMBER '17
Microbiology Supervisor:	Paroni Roberto
Loop size (µl):	10 UL SOFT
Mould ref. :	7257
Lot number:	1715292
Inaccuracy %:	Acceptable tolerance +/- 20% : 18%
Acceptable:	(YES) / NO

STERILIZED BY IONIZING IRRADIATION

References:

1. Clarridge, J. E., M. T. Pezzlo, and K. L. Vosti., 1987. Cumitech 2A, Laboratory Diagnosis of Urinary Tract Infections. Corordinating ed., A.S. Weissfeld. American Socity for Microbiology, Washington, DC.
2. Isenberg HD (Editor in Chief),1992. Calibration of Quantitative Loops, 12.17.10 -12.17.12, Clinical Microbiology Procedures Handbook. American Society for Microbiology, Washington, DC,