# Application Note

Institute for Reference Materials and Measurements

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# IRMM-351

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## 1. Presence / absence test

For application in presence/absence tests, analyse at least two vials of the CRM. Plate and incubate material spheres as explained on the certificate under instructions for use. Count colony forming units (cfu) per plate and evaluate results based on individual cfu values per analysed vial. The test has been passed if, for each material sphere, the result is within the 95% confidence interval specified for the CRM (4 ± 2). The test failed if the obtained cfu values are not within these limits.

## 2. Method validation

If this CRM is used for method validation or testing of media, a similar approach as for certification of the batch should be applied. This requires the measurement of an appropriate number of CRM vials, minimum 15 in agreement with the number of CRM vials analysed during the characterisation study (section 5.2 of the certification report). Conclusions should be based upon patterns (histograms) of the results obtained in the laboratory and during certification rather than on mean cfu values. The histogram obtained in the laboratory is compared with the hypergeometric distribution obtained for the homogeneity and batch characterisation data (fig.1) and a chi square value is calculated. The success of the validation is assessed from this chi square value with respect to critical limits. lab falls short of the critical value, it failed in method validation. A detailed explanation on the statistics can be found in the certification report.

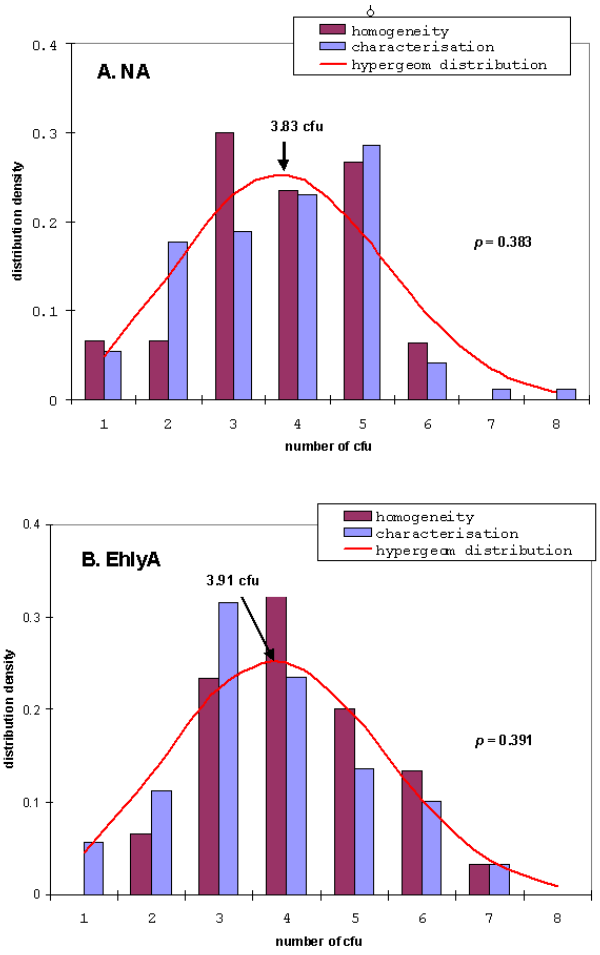


*Fig. 1: Representation of observed (histograms) and expected (hypergeometric distribution) cfu values obtained for homogeneity and batch characterisation by colony counting on NA and EhlyA. Mean cfu values are indicated by arrows.*