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Homogeneity of reference material Dw 2020:A

Homogeneity is evaluated by use of some different measures, both decisive ones and other to understand the result distribution. Duplicate analyses from 10 vials have been used.

Test 1 is a numerical check to see if the cfu results *within the vials* are randomly distributed as expected by a Poisson distribution. The ratio between variance/mean is used as the test value for an "Index of dispersion". This value is compared with what is expected from a χ^2 distribution. If the value is bigger than expected there is an over dispersion, if the value is less than expected there is an under dispersion (**I_1**).

Test 2 is similar to test 1 but here the "Index of dispersion" compares the results *between vials* (**I_2**). ***[Decisive]***

Test 3 is a one way analysis of variance, ANOVA (**AN**), to *check if the variance* (= the squared standard deviation) *is larger between vials than within*. If there is strong under dispersion in test 1 (unusually small variation due to chance) the variation between vials may, relatively seen, be large. This will result in a high F-value even when variation between vials is not unusually large. *This test is a traditional one in e.g. chemistry.*

Test 4 is a separate test for reproducibility *between vials* (**T** according to the RIVM Report 250935001/2003) that has the property to be insensitive to the cfu values (in contrast to I_2, where high cfu results will more easily be deemed inhomogeneous than low cfu results). ***[Decisive]***

The homogeneity is judged as satisfactory when the two decisive criteria, I_2 and T, not simultaneously have values larger than 2.

Mean values and measures of homogeneity for RM Dw 2020:A [1]

Analysis	**Volume**	**Mean value**		**CV** [2]	**Homogeneity** [4]			
	(*ml*)	(*cfu*)	√(*cfu*)	(*%*)	I_1	AN [3]	I_2	T
Coliform bacteria, 37 °C [5]	5	82	9.047	4.5	0.47	NS	0.84	1.23
E. coli, 37 °C [5]	5	47	6.822	5.1	0.39	NS	0.57	1.24
E. coli, 44 °C [6]	5	36	6.00	8.0	1.08	NS	0.70	1.31
Intestinal enterococci, 37 °C	5	85	9.236	8.9	2.20	NS	3.24	1.46
Pseudomonas aeruginosa, 37 °C	5	36	6.039	8.1	0.88	NS	1.04	1.41
Clostridium perfringens, 44 °C	5	33	5.723	8.1	0.79	NS	0.88	1.39
Culturable microorg., 37 °C, 2d	1	39	6.240	6.8	0.73	NS	0.69	1.30
Culturable microorg., 22 °C, 3d	1	38	6.179	7.7	1.03	NS	0.75	1.31

Notes are given overleaf

1 Results from 10 vials in duplicate dissolved in **400** ml diluent 6 weeks after freeze-drying. For more information see INSTRUCTION.

2 Coefficient of variation (CV) is SD as per cent of the mean (square-root transformed values), where SD is the pooled standard deviation from the ANOVA with a degree of freedom of 19.

3 ANOVA: NS: not significant F-test; *, **, ***: F-test significant on the levels 5, 1 and 0.1%, respectively.

4 The decisive tests of homogeneity, I_2 and T, together indicate homogeneity.

5 *C. freundii* and *E. coli* are separately counted from the same plates with Chromocult Coliform Agar (CCA) – no confirmation.

6 *E. coli* is counted from m-FC Agar – no confirmation.