

Inter-laboratory collaborative trial of real-time PCR method phase 1: homogeneity data

On completion of each sample type preparation and its verification against the standard curves as described above, homogeneity was assessed for each sample type by analysis of ten randomly-selected units under repeatability conditions.

The data are provided in Table 4.

The mean and standard deviation were calculated for each sample type and no Grubbs' outliers were detected, so all data were included. At the higher preparation levels of 10% w/w and 3% w/w, the RSD values ranged 5.06 – 11.0% RSD. At the mid-level preparation of 1% w/w, the RSD values ranged 6.39 – 14.5%. At the lower preparation levels of 0.5% w/w and 0.1% w/w, the RSD values ranged 13.6 – 43.7%. The processed pork generally had the lower RSD values, and the raw pork samples generally had the higher RSD values.

As the preparation level reduces in concentration of target species, the RSD increases. This finding is in keeping with the general trend in analytical (bio)chemistry of increasing variance at lower concentrations. The higher preparation levels were used to establish fitness for purpose prior to the lower preparation levels being sequentially diluted from them. Hence, there is a high degree of confidence that the lower preparation levels derive from a well-prepared stock sample.

The repeatability and reproducibility RSD values from the collaborative trial itself are included on Table 4. In all cases, the homogeneity RSD values are in keeping with the trial RSD values. The main exception is the raw pork sample at 0.1% w/w in which the homogeneity RSD (43.7%) exceeds the reproducibility RSD of 35.8%. Since the trial reproducibility RSD is consistently about 35% across all three sample types at the 0.1% w/w level, the high homogeneity RSD remains unexplained and may be an artefact of challenging repeatability rather than it having an effect on the trial.

The overall homogeneity data, including the verification against standard curves, provides sufficient confidence in the preparation of the samples as being fit for the purpose of the collaborative trial.

Processed horse

Sample number	10% w/w	3% w/w	1% w/w	0.5% w/w	0.1% w/w
Sample 1	14.1	3.29	ND	0.652	0.135
Sample 2	14.4	2.97	1.56	0.846	0.207
Sample 3	13.4	3.25	1.74	0.842	0.182

Sample number	10% w/w	3% w/w	1% w/w	0.5% w/w	0.1% w/w
Sample 4	12.7	3.56	1.66	0.726	0.209
Sample 5	14.0	3.77	1.80	0.925	0.185
Sample 6	13.5	3.05	1.53	0.468	0.159
Sample 7	14.6	2.69	1.47	0.633	0.115
Sample 8	12.1	3.06	1.65	0.915	0.172
Sample 9	12.5	2.92	1.63	0.698	0.092
Sample 10	13.7	3.10	1.55	0.872	0.129

Data	10% w/w	3% w/w	1% w/w	0.5% w/w	0.1% w/w
Mean, % w/w	13.5	3.17	1.62	0.758	0.158
SD, % w/w	0.836	0.316	0.104	0.148	0.0395
RSD, %	6.19	9.98	6.39	19.5	25.0
CT RSDr, %	10.1	5.77	6.90	10.4	19.9
CT RSDR, %	12.5	11.02	21.2	21.4	34.9

Processed pork

Sample number	10% w/w	3% w/w	1% w/w	0.5% w/w	0.1% w/w
Sample 1	5.67	1.84	1.13	0.622	0.144
Sample 2	5.78	1.82	1.06	0.481	0.130

Sample number	10% w/w	3% w/w	1% w/w	0.5% w/w	0.1% w/w
Sample 3	5.93	1.96	1.02	0.533	0.152
Sample 4	5.19	1.82	1.39	0.556	0.127
Sample 5	5.47	1.93	0.965	0.600	0.0826
Sample 6	6.06	2.12	1.49	0.701	0.133
Sample 7	5.50	2.08	1.42	0.531	0.153
Sample 8	6.01	1.85	1.22	0.449	0.128
Sample 9	5.83	2.03	1.29	0.659	0.144
Sample 10	6.06	1.96	1.24	0.598	0.185

Data	10% w/w	3% w/w	1% w/w	0.5% w/w	0.1% w/w
Mean, % w/w	5.75	1.94	1.22	0.573	0.138
SD, % w/w	0.291	0.111	0.179	0.0782	0.0260
RSD, %	5.06	5.71	14.6	13.6	18.8
CT RSDr, %	10.8	6.96	16.3	11.9	16.2
CT RSDR, %	17.3	17.5	28.4	19.9	32.6

Table 4: homogeneity data (% w/w) for 10 units for each sample type. SD is observed standard deviation of the 10 units, RSD is relative standard deviation (observed of the 10 units). CT RSDr is the collaborative trial repeatability RSD, CT RSDR is the collaborative trial reproducibility RSD.

Raw Pork

Sample number	10% w/w	3% w/w	1% w/w	0.5% w/w	0.1% w/w
Sample 1	9.59	3.29	1.20	0.610	0.109
Sample 2	10.5	3.31	1.05	0.457	0.141
Sample 3	9.86	3.38	1.08	0.471	0.158
Sample 4	7.62	2.92	1.07	0.652	0.100
Sample 5	8.17	2.96	1.08	0.601	0.103
Sample 6	7.89	2.73	1.22	0.497	0.0703
Sample 7	7.92	2.97	1.22	0.418	0.107
Sample 8	9.72	2.78	0.875	0.513	0.0701
Sample 9	9.00	2.51	1.31	0.485	0.0387
Sample 10	8.72	3.26	0.962	0.646	0.0316

Data	10% w/w	3% w/w	1% w/w	0.5% w/w	0.1% w/w
Mean, % w/w	8.89	3.01	1.11	0.535	0.0928
SD, % w/w	0.981	0.292	0.131	0.0846	0.0406
RSD, %	11.0	9.69	11.8	15.8	43.7
CT RSDr, %	8.76	8.60	19.2	21.0	24.0
CT RSDR, %	9.27	17.4	22.7	25.4	35.8

Table 4 (continued): homogeneity data (% w/w) for 10 units for each sample type. SD is observed standard deviation of the 10 units, RSD is relative standard deviation (observed of the 10 units). CT RSDr is the collaborative trial repeatability RSD, CT RSDR is the collaborative trial reproducibility RSD.