

**Report by the National Public Health Service for Wales for the AOAC
Research Institute
on the NISSUI Compact Dry Total Count method**

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1.0 Introduction

This repeatability study compared the performance of the NISSUI Compact Dry Total Count plate with the reference method, which in this case was the AOAC *Official Method 966.23* (pour plate) for the enumeration of aerobic bacteria in raw ground beef. Three different levels of aerobic bacteria were tested with replicate (n=5) samples. The method is detailed in Appendix 6.

Compact Dry utilises dehydrated medium coated onto the base of a small, plastic Petridish. There is no preparation of reagents or setting time required.

2.0 Methods

Table 1 lists the sample and aerobic colony count (ACC) details of the study. The method for the Compact Dry TC plates was as recommended by the manufacturer. The method for the comparison was the AOAC *Official Method 966.23*, performed exactly as specified, with no deviations or alterations.

All the media (excluding the Compact Dry TC plates) was tested prior to usage with the standard accredited laboratory quality control procedures prior to usage. All the media tested passed all relevant tests.

Table 1 Compact Dry repeatability study overview

Matrix	Expected ACC level	Replicates
Raw Ground Beef	10 ⁴ cfu/g (low)	5
	10 ⁶ cfu/g (medium)	5
	10 ⁸ cfu/g (high)	5

2.1 Sample description, preparation and examination

2.1.1 Sample Preparation

The sample was frozen, raw ground beef purchased from the local supermarket. Three packets of the same batch (same brand, same pack size, same producer, same use-by date) were purchased on the same day. Samples were transported immediately to the laboratory in the frozen state, and on receipt at the laboratory were placed directly into a freezer for storage.

2.1.2 Sample preparation

To achieve the required aerobic colony counts, the three samples were prepared as follows:

1. Batch 1 was incubated unopened from frozen at 22°C for 28 hours, then examined. Expected count level was 10^8 cfu/g.
2. Batch 2 was incubated unopened from frozen at 22°C for 19 hours, then examined. Expected count level was 10^6 cfu/g.
3. Batch 3 was examined directly from frozen. Expected count level was 10^4 cfu/g.

2.1.3 Sample Examination

Sample preparation and examination for the replicate tests were carried out as follows:

1. Each replicate was given a unique identification code that allowed the sample to be traced, but that could not identify the sample in terms of expected count.
2. Each replicate was examined in duplicate using the methods mentioned above, with no deviations or alterations. Plates were incubated at 35°C for 48 hours.

3. Plates were counted within the range 30-300 colony forming units (cfu).

4. Overall log counts were calculated (cfu/g).

Note: The coding, homogenisation, diluting, plating and counting of each sample was carried out by a separate technician, under the supervision of the Food Scientist.

3.0 Results

3.1 Summary of results

A summary of results is listed in Table 2. The indigenous level of bacteria (pre-aging) was as detailed in Table 2. The mean level was 4.37 log cfu/g (using AOAC method 966.23).

Table 2 Summary of results for raw ground beef

Estimated Level (log cfu/g)	Replicate	AOAC Official method 966.23 (log cfu/g)	Compact Dry TC (log cfu/g)	Difference (log cfu/g)
4	1	4.40	4.18	0.22
	2	4.39	3.98	0.41
	3	4.30	4.04	0.26
	4	4.45	4.04	0.41
	5	4.32	4.02	0.30
6	1	6.11	6.20	-0.09
	2	6.43	6.22	0.21
	3	6.25	6.27	-0.02
	4	5.99	6.04	-0.05
	5	6.22	6.27	-0.05
8	1	7.94	7.97	-0.03
	2	8.05	8.10	-0.05
	3	8.11	8.22	-0.11
	4	8.02	8.08	-0.06
	5	8.20	8.28	-0.08

4.0 Observations

In general terms, the Compact Dry system was easier and quicker than the conventional pour plate technique. The Compact Dry was timed at approximately 25 seconds, on average, for a skilled technician to take the sample from the diluent bottle, remove the lid, inoculate and close the lid again. The pour plate took longer, bearing in mind the extra time required to pour the agar and swirl to mix. There was also the time required to prepare the agar (preparation, melting and tempering) and to allow the agar to set before the plates could be stacked and stored away. The Compact Dry system required no preparation or setting time. Homogenisation and dilution of samples took the same time for each method. Reading the plates was faster with the Compact Dry system, with the TTC indicator speeding up counting. It was observed that food particles, when present, did not appear to absorb the indicator, although at high colony counts (>150 colonies) the relative size of the Compact Dry plate made counting slower than the pour plate. Food particles in the pour plate made reading plates and counting colonies relatively more difficult. The Compact Dry system would require a lot less training than the pour plate technique. The instructions on the use of the Compact Dry were clear and unambiguous.

Benefits to all laboratories would be quicker examination time for foodstuffs and ease of use of the system, when compared to the conventional technique (pour plate) assessed, but also presumably to other methods such as the spread plate and spiral plate methods. It is clear that the Compact Dry system would also bring benefits in reduced storage space, waste disposal and required incubator space. The long shelf life of the product also has benefit compared to ready prepared agar, which has a limited shelf life and therefore requires more logistical planning.

Appendix

Raw data

Raw ground beef- AOAC Official method 966.23. Results in bold used for colony count.

Estimated Level (cfu/g)	Replicate	Count –1 dilution (cfu)		Count –2 dilution (cfu)		Count –3 dilution (cfu)		Count –4 dilution (cfu)	
		10⁴	1	TNTC	TNTC	235	273	29	24
	2	TNTC	TNTC	247	246	20	32	4	2
	3	TNTC	TNTC	214	190	23	25	2	1
	4	TNTC	TNTC	264	295	24	20	3	2
	5	TNTC	TNTC	204	210	32	21	1	1

Estimated Level (cfu/g)	Replicate	Count –4 dilution (cfu)		Count –5 dilution (cfu)		Count –6 dilution (cfu)		Count –7 dilution (cfu)	
		10⁶	1	123	137	17	18	1	2
	2	129	138	14	17	0	4	0	0
	3	178	*	21	27	3	6	0	0
	4	98	98	10	9	1	2	0	0
	5	164	172	28	18	4	1	0	0

Estimated Level (cfu/g)	Replicate	Count –6 dilution (cfu)		Count –7 dilution (cfu)		Count –8 dilution (cfu)		Count –9 dilution (cfu)	
		10⁸	1	88	85	9	7	1	0
	2	97	126	8	18	1	2	0	1
	3	126	130	13	14	0	2	0	0
	4	108	100	17	13	3	1	0	0
	5	170	147	19	26	3	2	0	0

* Plate damaged during incubation-no count available

Raw ground beef-Compact Dry. Results in bold used for colony count.

Estimated Level (cfu/g)	Replicate	Count -1 dilution (cfu)	Count -2 dilution (cfu)	Count -3 dilution (cfu)	Count -4 dilution (cfu)
10⁴	1	TNTC	153	21	0
	2	TNTC	96	12	3
	3	TNTC	109	9	2
	4	TNTC	111	13	0
	5	TNTC	106	15	2

Estimated Level (cfu/g)	Replicate	Count -4 dilution (cfu)	Count -5 dilution (cfu)	Count -6 dilution (cfu)	Count -7 dilution (cfu)
10⁶	1	159	15	3	0
	2	166	17	0	0
	3	187	24	4	3
	4	111	17	1	0
	5	185	24	0	2

Estimated Level (cfu/g)	Replicate	Count -6 dilution (cfu)	Count -7 dilution (cfu)	Count -8 dilution (cfu)	Count -9 dilution (cfu)
10⁸	1	93	11	1	0
	2	125	11	20	0
	3	164	24	3	0
	4	120	13	3	1
	5	190	30	2	0

Appendix 6

Method Comparison Flowchart

