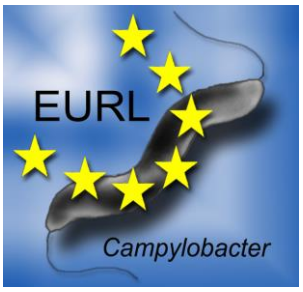

EURL-*CAMPYLOBACTER*

REPORT

PROFICIENCY TEST NUMBER 27

Detection and species identification of *Campylobacter* spp.

Contents

Abbreviations

C.	*Campylobacter*
CAT	cefoperazone amphotericin teicoplanin
cfu	colony forming units
EU	European Union
EURL	European Union reference laboratory
FP	false positive
ISO	International Organization for Standardization
LOD_{50}	level of detection for which 50 % of tests give a positive result
log_{10}	logarithm to base 10 (common logarithm)
MALDI-TOF MS	matrix-assisted laser desorption ionization–time of flight mass spectrometry
mCCD	modified charcoal cefoperazone deoxycholate
MS	Member State (of the European Union)
MS-NRL	Member State national reference laboratory
NRL	national reference laboratory (in this report also used for a laboratory with a similar function in a non EU Member State)
PCR	polymerase chain reaction
PT	proficiency test
spp.	species

Summary of proficiency test number 27, 2020

The EU reference laboratory for *Campylobacter* organised proficiency test (PT) number 27 on detection and species identification of *Campylobacter* in March 2020. The PT included detection and species identification of *Campylobacter* spp. in 10 samples of chicken caecal content with vials with or without freeze-dried *Campylobacter*. The samples were composed to mimic samples pooled from up to 30 chicken caeca, according to the procedure for pooling contents from whole poultry caeca described in ISO 6887-6. The objective was to assess the performance of the national reference laboratories (NRLs) to detect and identify *Campylobacter* species in chicken caecal content.

Thirty-one NRLs in 25 EU Member States and in the United Kingdom, Iceland, Norway, and Switzerland had registered for and received the proficiency test. Due to the Covid-19 pandemic, four laboratories were unable to carry out and report part of or the complete PT. Twenty-nine NRLs reported results for detection and and 27 NRLs for species identification within the specified timeframe.

Twenty-eight of the 29 NRLs used the recommended method ISO 10272-1:2017 for analysing the samples, and at least 27 of them the recommended procedure C with direct plating on modified charcoal cefoperazone deoxycholate (mCCD) agar. Seven NRLs used an enrichment protocol as the only method or in addition to the direct plating.

All 29 participating NRLs (24 Member State NRLs, MS-NRLs) fulfilled the criterion for excellent or good performance in detection of *Campylobacter* (sensitivity in detection), and 27 (22 MS-NRLs) for excellent or good performance in detecting *Campylobacter* positive and negative samples (accuracy). No NRL scored below the acceptable limits for sensitivity in detection or accuracy. This is a better result than for the primary production samples analysed in PT number 24, 2019 (sock samples) and in PT number 22, 2018 (chicken feacal swabs).

Of the 27 NRLs reporting results for species identification, 24 (20 MS-NRLs) fulfilled the criterion for excellent or good performance in identification of *Campylobacter* spp., and one scored below the acceptable limit.

Although the Covid-19 pandemic prevented some NRLs from performing PT 27 in time, the participating NRLs reported high-level results. Most NRLs met the criteria for excellent or good performance in both detection and species identification of *Campylobacter*, and only one scored below the acceptable limit for species identification. Thus, the *Campylobacter* NRLs are well meeting the requirements of being NRLs.

Introduction

The voluntary proficiency test (PT) number 27 on detection and species identification of *Campylobacter* was organised by the EU reference laboratory (EURL) for *Campylobacter* in March 2020. Thirty-one national reference laboratories (NRLs) in 25 EU Member States (some Member States have more than one NRL) and in the United Kingdom, Iceland, Norway, and Switzerland had registered for and received the proficiency test. Due to the Covid-19 pandemic, four laboratories were unable to carry out part of or the complete PT within the specified timeframe. This report only includes the results generated and reported before the deadline. Twenty-seven NRLs (23 MS-NRLs) reported results for both detection and species identification and two NRLs only reported results for detection, i.e. 29 NRLs (24 MS-NRLs) participated in PT 27.

All 29 participating NRLs reported that they were accredited for detection of *Campylobacter* and 21 of them were also accredited for enumeration of *Campylobacter*.

The PT included detection and species identification of *Campylobacter* spp. in 10 samples mixed with vials with or without freeze-dried *Campylobacter* (Table 1). The objective was to assess the performance of the NRLs to detect and identify *Campylobacter* species in chicken caecal content.

Table 1. Bacteria in the vials in proficiency test No. 27 (2020).

Vial No.	Bacterial species in vial	Batch No.	Level *C.*[a] (log_{10} cfu/vial)	Level *E. coli*[b] (log_{10} cfu/vial)
11	*Campylobacter jejuni*[c]	SVA021	4.28 (low)	–
12	–		–	–
13	*Escherichia coli*	SVA045	–	4.74
14	*Campylobacter jejuni*[c] + *Escherichia coli*	SVA041	4.78 (low)	5.14
15	*Campylobacter jejuni*[c]	SVA036	5.52 (high)	–
16	*Campylobacter coli*	SVA033	4.85 (low)	–
17	*Campylobacter lari*	SVA044	4.77 (low)	–
18	*Campylobacter coli* + *Escherichia coli*	SVA043	5.39 (high)	4.61
19	*Campylobacter jejuni*[c]	SVA039	4.79 (low)	–
20	*Campylobacter coli*	SVA037	4.32 (low)	–

[a] Total quantity of *Campylobacter* in each vial. The low and high levels were based on a LOD_{50} of 6.1 log_{10} cfu per test portion of 10 µl (one direct streaked loop), according to ISO 10272-1:2017. The theoretical content per test portion varied from 3 to 12 × LOD_{50} at the low level and was above 40 × LOD_{50} at the high level.

[b] Total quantity of *Escherichia coli* in each vial.

[c] All *Campylobacter jejuni* strains were hippurate positive.

Terms and definitions

- *Campylobacter* spp.: Thermotolerant *Campylobacter* spp., i.e. which are able to grow at 41.5 °C, foremost (but not exclusively) *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*.
- Detection of *Campylobacter* spp.: Determination of the presence or absence of *Campylobacter* spp.
- Confirmation of *Campylobacter* spp.: Microorganisms suspected to be *Campylobacter* spp. are confirmed as such by biochemical tests and/or by molecular methods.
- Species identification of *Campylobacter*: Identification of thermotolerant *Campylobacter* species with biochemical tests and/or molecular methods.

Outline of the proficiency test

The PT contained 10 samples of chicken caecal content. Each sample was composed to mimic a sample pooled from up to 30 chicken caeca, according to the procedure for pooling contents from whole poultry caeca described in ISO 6887-6:2013. The participants were instructed to mix the content of the vial with the corresponding sample. This resulted in six samples with a low content of *Campylobacter*, two samples with a high content of *Campylobacter* and two samples without *Campylobacter* (Table 1). The theoretical level of contamination in the test portions of the samples with low levels was estimated to be between 3 and 12 times LOD_{50} (according to ISO 10272-1:2017, annex C) and the high level approximately 10 times higher than the low level.

Preparation of the chicken ceacal content

The caecal material used as matrix in the PT was obtained from a broiler producer that had not delivered any *Campylobacter*-positive flocks to slaughter for more than six months. The broilers were slaughtered at a slaughterhouse with a very low level of flocks positive for *Campylobacter* (2.9 % during 2019) and no positive flocks at all for three months before taking out and sending caeca to the EURL. Chicken skin and caecal samples from the broiler flock tested negative for presence of *Campylobacter*. The caeca were freeze-stored until production of the samples.

Six days before dispatch, freeze-stored caeca were thawed, cut, placed in a stomacher bag and mixed with Buffered Peptone Water to produce a caecum suspension. Six ml of the suspension were added to a plastic tube, one for each sample. The plastic tubes with caecal content to be mixed with the vials were freeze-stored until dispatch.

Production and quality control of bacterial cultures

The vials with freeze-dried bacterial cultures used in the PT were produced and tested for homogeneity and stability by the EURL (all samples containing bacteria) or the Swedish Food Agency (only negative samples).

Each combination of vial and matrix was prepared and tested according to ISO 10272-1:2017, procedure C (direct plating), at least three times: before dispatch, just after dispatchi and four days after dispatch, i.e. at the last time for start of the analysis by the participants. Before dispatching, also enrichment procedures with Preston and Bolton broth were tested.

Distribution of the proficiency test

The PT samples were distributed from the EURL 9th of March, 2020. The samples were placed in foam boxes along with freezing blocks. The foam boxes were packed in cardboard boxes for transport and were sent from the EURL using courier service.

Each participant received a package containing 10 numbered vials, each containing freeze-dried material with or without *Campylobacter* spp., and 10 numbered samples with 6 ml chicken ceacal material in plastic tubes, one for each of the 10 vials. A Micro-T-Log was included in each shipment to record the temperature every second hour during transport.

The PT analyses were recommended to be started as soon as possible after the arrival and at the latest 13th of March, 2020. All results had to be reported in the Questback Essentials system by 20th of April, 2020. Instructions for preparation of the samples from the vials and matrix were included in the packages, and were also sent out by e-mail a few days before the PT distribution. Until start of analysis, samples with chicken caecal material and vials were recommended to be stored at cold temperature (between 1 °C and 8 °C). If the analysis was started more than 24 hours after the arrival, the vials were recommended be stored at –20 °C.

Methods for analysis

The NRLs were recommended to follow ISO 10272-1:2017, procedure C (direct plating procedure) for performing the PT but were allowed to use another method if their standard laboratory procedure followed a different method.

Campylobacter spp. should be incubated in a microaerobic atmosphere, with oxygen content of 5% ± 2%, and carbon dioxide 10% ± 3%. The appropriate microaerobic atmosphere can be obtained by using commercially available microaerobic incubators, commercial gas-generating kits, or by using gas-jars, filled with the appropriate gas mixture prior to incubation. Of the 29 participating NRLs, 15 reported using gas-generating kits, 10 microaerobic incubators, five the Anoxomat® system and two other methods (zip-lock bags filled with gas and jars filled with gas mixture). Some of the NRLs used more than one system.

Assessment of performance in detection and identification

The NRLs' performance in sensitivity in detection, sensitivity in identification, and accuracy in detection of *Campylobacter* positive and negative samples were calculated and categorized on a five-level grading scale for each performance parameter.

The **sensitivity** was calculated based on the NRLs ability to correctly detect *Campylobacter* spp. and identify *Campylobacter* species in the samples containing *Campylobacter*. Correct detection of all *Campylobacter*-positive samples resulted in a sensitivity in detection of 100%. Correct identification of all *Campylobacter* species in positive samples in which *Campylobacter* spp. were detected resulted in a sensitivity in identification of 100%. The cut-off for good performance of detection/identification of *Campylobacter* species was set to 85.0%.

The **accuracy** was also calculated, giving an overall performance of the results of correct detection of *Campylobacter* spp. in samples with *Campylobacter* and correct identification of samples without *Campylobacter* as non-*Campylobacter* samples. The accuracy was calculated as total number of correct detection results divided by total number of samples. The cut-off for good performance was set to 90%.

Since there were only two *Campylobacter*-negative samples in each set of results for which the performance assessment was done, the specificity was not assessed.

Results

Detection and species identification of *Campylobacter*

Of the 29 participating NRLs, 23 received the test one day after dispatch and the remaining 6 received it two days after dispatch.

According to the instructions, analysis of the samples should be started as soon as possible after arrival and no later than four days after dispatch. Eight NRLs started the analysis the day after the samples were dispatched from the EURL, fourteen NRLs two days after, four NRLs three days after, one NRL four days after, one NRL seven days after, and one NRL two weeks after.

Of the 29 NRLs, 28 followed ISO 10272-1:2017 for detection of *Campylobacter* spp., and one NRL used another method with enrichment in and plating on CampyFood® media. Also, 27 NRLs used the recommended procedure of direct plating (procedure C), seven NRLs used an enrichment protocol (one NRL as the only method and six NRLs in addition to direct plating) and one NRL did not report which procedure was used. The NRLs performing an enrichment procedure used Preston broth (4), Bolton broth (1), CampyFood® broth (1), or Exeter broth (1). Twenty-eight NRLs did the plating on modified charcoal cefoperazone deoxycholate (mCCD) agar, and 22 plated on at least one additional medium. Other media used for plating were CampyFood® agar (5), Karmali agar (4), Skirrow agar (4), Butzler agar (2), CASA® agar (2), CHROMagar™ Campylobacter (2), Preston agar (1), Brilliance™ CampyCount agar (1), and cefoperazone amphotericin teicoplanin (CAT) agar (1).

The isolated *Campylobacter* spp. were identified by biochemical tests and/or molecular methods, mostly matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) or polymerase chain reaction (PCR). The biochemical tests included detection of catalase, hippurate hydrolysis, indoxyl acetate hydrolysis, and sensitivity to nalidixic acid and cephalotin. One NRL reported to have performed a hydrogen sulphide test in addition to other biochemical tests.

Fifteen of the 27 NRLs reporting identification results used MALDI-TOF MS for the species identification, in six cases in combination with other techniques. Nine NRLs used one or more PCR assays, in eight cases in combination with other techniques. Four NRLs reported to have used or adapted the multiplex PCR assay published by Wang et al. (2002), and two NRLs used the PCR protocol by Denis et al. (1999). Fourteen NRLs used biochemicals tests (at least detection of catalase), in eight cases in combination with MALDI-TOF MS and/or PCR.

Seventeen NRLs used one technique only (a set of biochemical tests regarded as one technique), nine NRLs combined two techniques, and one NRL used three techniques for the species identification.

Of the 29 NRLs, 21 reported correct results of detection, i.e. correct identification of the eight samples **with** *Campylobacter* and the two samples **without** *Campylobacter* (Figure 1). Two false positive results were reported, of sample No. 12 and 13. Eighteen of the 27 NRLs reported correct species in all eight samples that had been inoculated with *Campylobacter* spp., and 23 NRLs correct species in all inoculated samples where *Campylobacter* spp. had been detected. Eighteen NRLs reported correct results of both detection and species identification.

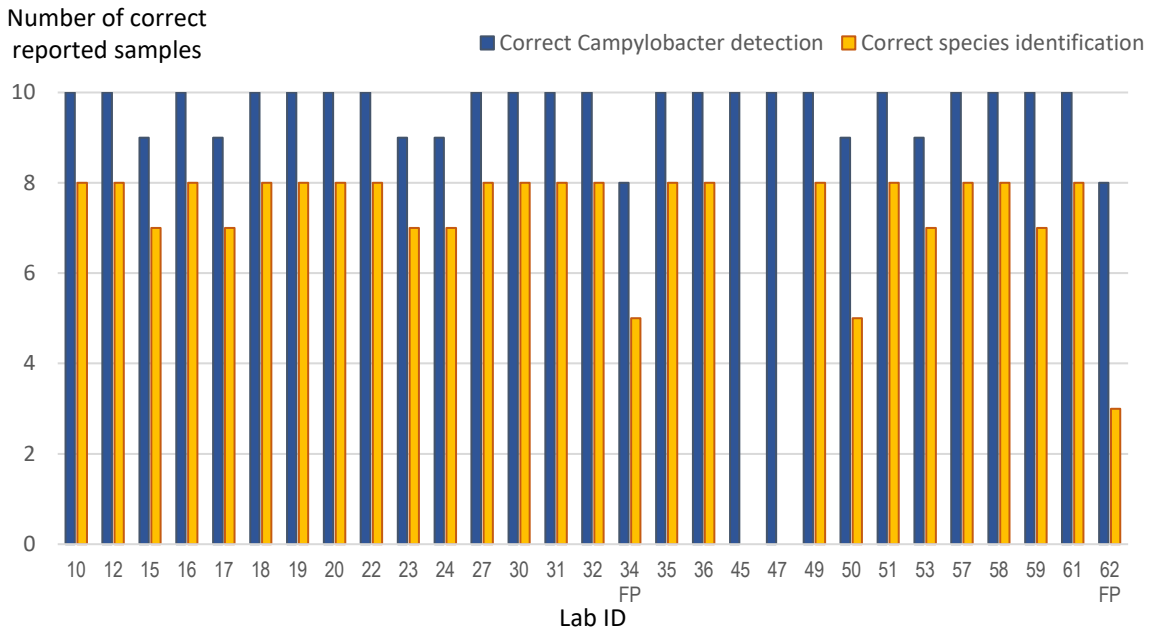

Figure 1. Distribution of correct results by 29 NRLs participating in proficiency test in proficiency test No. 27 (2020) in the detection and species identification of *Campylobacter* spp. in chicken caecal content. In total, 29 NRLs performed detection and 27 NRLs species identification. Two false positive results are marked with FP.

For five of the ten samples all 29 NRLs reported correct results of detection (Figure 2, Table 2). Three of the five samples containing *Campylobacter* (No. 11, 15 and 19) correctly detected by all NRLs were also correctly identified by all 27 NRLs performing species identification, all three as *C. jejuni*. Six NRLs failed to detect *Campylobacter* spp. in sample No. 14, which consisted of *E. coli* and a low level of *C. jejuni*.

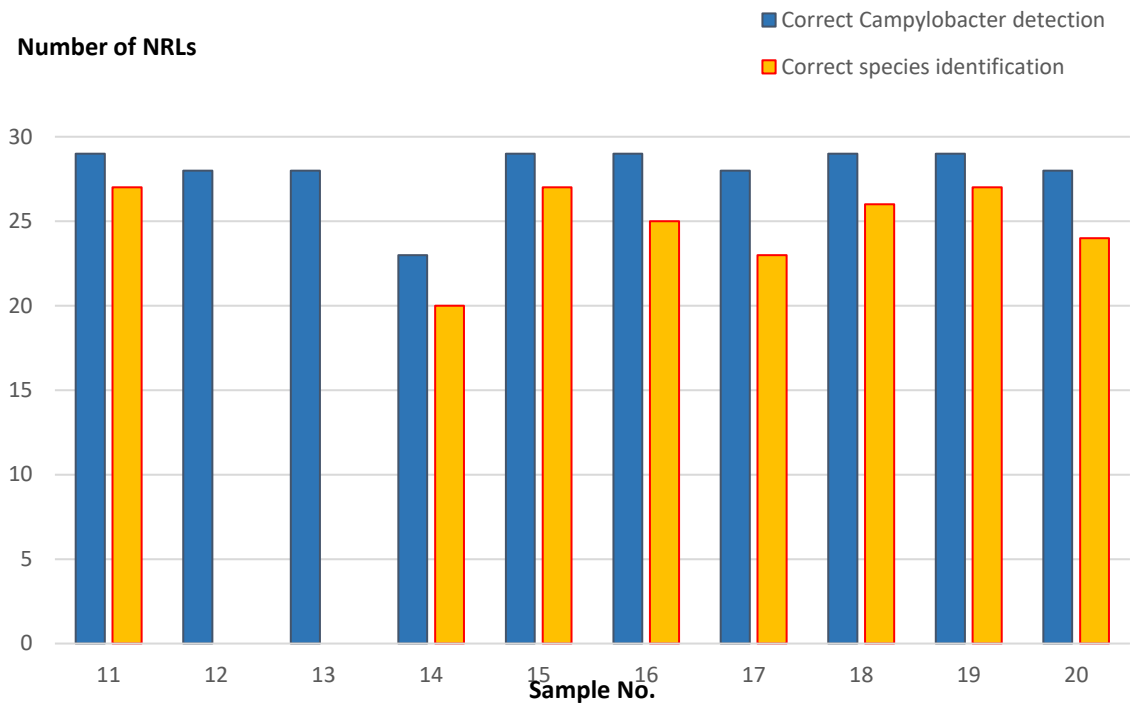

Figure 2. Number of NRLs participating in proficiency test No. 27 (2020) that correctly reported results in the detection and species identification of *Campylobacter* in 10 samples of chicken caecal content. In total, 29 NRLs performed detection and 27 NRLs species identification.

Table 2. Results of detection and species identification in 10 samples of chicken caecal content in proficiency test No. 27 (2020). In total 27 NRLs performed species identification.

Sample No.	**Bacterial species in vial**	*C. jejuni*	*C. coli*	*C. lari*	*Other Campylobacter* species detected	Growth of other, not *Campylobacter*	No growth at all
11	*Campylobacter jejuni*	27					
12	–	1				12	14
13	*Escherichia coli*				1	24	2
14	*Campylobacter jejuni* + *E. coli*	20			1	6	
15	*Campylobacter jejuni*	27					
16	*Campylobacter coli*		25	2			
17	*Campylobacter lari*	1	2	23			1
18	*Campylobacter coli* + *E. coli*		26		1		
19	*Campylobacter jejuni*	27					
20	*Campylobacter coli*	1	24	1			1

Performance in detection and species identification of *Campylobacter* spp.

The performance (sensitivity and accuracy) of detection and identification of *Campylobacter* spp. in chicken caecal content for all participators are presented in Table 3.

Table 3. The performance (sensitivity and accuracy) in detecting *Campylobacter* and non-*Campylobacter* spp. and the performance (sensitivity) in identification of *Campylobacter* spp. of the 29 NRLs participating in proficiency test No. 27 (2020). Coloured cells indicate performance below 100%. Green shadowed cells indicate grades Good (bright green) and Acceptable (pale green). Red shadowed cells indicate grades below the acceptable limit.

Lab id	Sensitivity in detection	Accuracy in detection	Sensitivity in species identification
10	100%	100%	100%
12	100%	100%	100%
15	88%	90%	100%
16	100%	100%	100%
17	88%	90%	100%
18	100%	100%	100%
19	100%	100%	100%
20	100%	100%	100%
22	100%	100%	100%
23	88%	90%	100%
24	88%	90%	100%
27	100%	100%	100%
30	100%	100%	100%
31	100%	100%	100%
32	100%	100%	100%
34	88%	80%	71%
35	100%	100%	100%
36	100%	100%	100%
45	100%	100%	–
47	100%	100%	–
49	100%	100%	100%
50	88%	90%	71%
51	100%	100%	100%
53	88%	90%	100%
57	100%	100%	100%
58	100%	100%	100%
59	100%	100%	88%
61	100%	100%	100%
62	88%	80%	43%

All 29 participating NRLs (24 MS-NRLs) fulfilled the criterion for excellent or good performance in detection of *Campylobacter* and none scored below the acceptable limit (Table 4). Twenty-four NRLs (20 MS-NRLs) fulfilled the criterion for excellent or good performance in identification of *Campylobacter* spp., and one scored below the acceptable limit (Table 5). Regarding accuracy, 27 laboratories (22 MS-NRLs) fulfilled the criterion for excellent or good performance, and none scored below the acceptable limit (Table 6).

Table 4. Overall performance of NRLs' sensitivity in correct detection of *Campylobacter* spp. in proficiency test No. 27 (2020).

Performance in detection of *Campylobacter* spp.			
Grade	**Sensitivity**	**Number of NRLs (%) All NRLs, n=29**	**Number of NRLs (%) MS-NRLs, n=24**
Excellent	95.1–100%	21 (72%)	16 (67%)
Good	85.0–95.0%	8 (28%)	8 (33%)
Acceptable	70.0–84.9%	0 (0%)	0 (0%)
Needs improvement	57.0–69.9%	0 (0%)	0 (0%)
Poor	<57.0%	0 (0%)	0 (0%)

Table 5. Overall performance of NRLs' sensitivity in correct species identification of *Campylobacter* in proficiency test No. 27 (2020).

Performance in identification of *Campylobacter* spp.			
Grade	**Sensitivity**	**Number of NRLs (%) All NRLs, n=27**	**Number of NRLs (%) MS-NRLs, n=23**
Excellent	95.1–100%	23 (85%)	19 (83%)
Good	85.0–95.0%	1 (4%)	1 (4%)
Acceptable	70.0–84.9%	2 (7%)	2 (9%)
Needs improvement	57.0–69.9%	1 (4%)	0 (0%)
Poor	<57.0%	0 (0%)	1 (4%)

Table 6. Overall performance of NRLs' accuracy in correctly detecting *Campylobacter* positive and negative samples in proficiency test No. 27 (2020).

Performance in detection of *Campylobacter* positive and negative samples			
Grade	**Accuracy**	**Number of NRLs (%) All NRLs, n=29**	**Number of NRLs (%) MS-NRLs, n=24**
Excellent	95.1–100%	21 (72%)	16 (67%)
Good	90.0–95.0%	6 (21%)	6 (25%)
Acceptable	80.0–89.9%	2 (7%)	2 (8%)
Needs improvement	70.0–79.9%	0 (0%)	0 (0%)
Poor	<70.0%	0 (0%)	0 (0%)

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