INTERNATIONAL STANDARD

ISO 14461-2

IDF 169-2

First edition
2005-04-01

Milk and milk products — Quality control in microbiological laboratories —

Part 2: Determination of the reliability of colony counts of parallel plates and subsequent dilution steps

Lait et produits laitiers — Contrôle de qualité en laboratoire microbiologique —

Partie 2: Détermination de la fiabilité des comptages de colonies en boîtes parallèles et des dilutions décimales suivantes

Reference numbers
ISO 14461-2:2005(E)
IDF 169-2:2005(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 14461-2 | IDF 169-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

ISO 14461 | IDF 169 consists of the following parts, under the general title *Milk and milk products — Quality control in microbiological laboratories*:

— *Part 1: Analyst performance assessment for colony counts*

— *Part 2: Determination of the reliability of colony counts of parallel plates and subsequent dilution steps*

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO and AOAC International in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the National Committees casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights.

ISO 14461-2 | IDF 169-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Statistics of analytical data*, of the Standing Committee on *Quality assurance, statistics of analytical data and sampling*, under the aegis of its project leaders, Dr. H. Glaeser (EU) and Prof. Dr. H. Weiss (DE).

This edition of ISO 14461-2 | IDF 169-2, together with ISO 14461-1 | IDF 169-1, cancels and replaces IDF 169:1994, which has been technically revised.

ISO 14461 | IDF 169 consists of the following parts, under the general title *Milk and milk products — Quality control in microbiological laboratories*:

— *Part 1: Analyst performance assessment for colony counts*

— *Part 2: Determination of the reliability of colony counts of parallel plates and subsequent dilution steps*

Introduction

Every microbiological method consists of several steps that are followed in a specific sequence (sub-sampling, diluting, plating and counting). The final result has a margin of uncertainty that is determined by the variability of all the steps involved.

In order to obtain results with a margin of uncertainty not much larger than what can be expected from the correct application of the method, it is necessary to follow the rules of Good Laboratory Practice (GLP).

The three most important factors in obtaining a correct plate count are

— the homogeneity of the sample material,

— the exactness with which the dilutions are performed, and

— the technique of inoculation and/or counting of the plates.

By homogenizing a sample material very well, making multiple dilution series, and inoculating several plates from the same dilution, it is possible to assess how well a laboratory can perform the colony-count technique, taking into account the expected variability of the method.

Too large a variability indicates that at least one of the steps in the performance of the method is out of control. The identification of those steps is carried out by comparison of the replicate inoculations, the different dilution levels and the dilution series. When the steps with excessive variability have been identified, necessary measures should be taken to bring these steps under control.

Milk and milk products — Quality control in microbiological laboratories —

Part 2:
Determination of the reliability of colony counts of parallel plates and subsequent dilution steps

1 Scope

This part of ISO 14461|IDF 169 describes a routine procedure for the evaluation of results of the enumeration of microorganisms using colony-count methods with subsequent 10-fold dilution steps and one plate or two parallel plates within each dilution step.

This routine procedure is applied regularly in each laboratory performing colony counts. It provides criteria for the acceptability of differences between the results from parallel plates and subsequent dilution steps, as follows.

a) The results (colony counts) obtained from parallel plates are compared with tabulated limits for given colony counts. If these limits are exceeded, a technical problem when performing the parallel determinations may be indicated.

b) The results (sums of colony counts) of two parallel plates of two subsequent 10-fold dilution steps are compared with tabulated limits for given sums of colony counts. If these limits are exceeded, a technical problem when performing the dilutions may be indicated.

c) If the limits mentioned above are exceeded in more cases than expected, this indicates that the test procedure lacks reliability.

NOTE The formulae for calculating the values in Table 1 and 2 are given and explained in Clause 7.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 14461-1|IDF 169-1, *Milk and milk products — Quality control in microbiological laboratories — Part 1: Analyst performance assessment for colony counts*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply

3.1
colony count
number of microorganisms found, as determined by the method specified in ISO 14461-1|IDF 169-1

NOTE The number of microorganisms is expressed per gram or per millilitre of test sample.

4 Principle

The counting results obtained are compared with tabulated limits for given colony counts. Decisions are based on the way the limits are exceeded. The tabled values are calculated and explained.

5 Procedure

5.1 General

The procedure shall be applied routinely in laboratories carrying out colony counts. A standardized method for performing colony counts must be applied in any case.

If the applied method is not in accordance with an International Standard or another accepted standard, a detailed description of the method shall be available and followed precisely.

In the case that a method is followed with only one plate per dilution step, the procedure described in 5.2 shall be carried out with a certain minimum frequency (e.g. once per hundred sample units tested).

5.2 Counting results of two parallel plates

Compare the results (colony counts) of two parallel plates with the limits tabulated in Table 1.

Compare the upper and lower colony counts of an observed pair of results with the corresponding colony counts given in Table 1. Use the upper colony count given in Table 1 as basis for the comparison. Then compare the lower colony count given in Table 1 with the observed lower count.

A lower observed count below the lower colony count of Table 1 indicates that the difference between the colony counts obtained with the two parallel plates is unacceptably high. (See the results of the first dilution step in Examples 1 and 2 in Clause 7.)

A lower observed count, which is at least equal to the lower colony count, indicates that the difference is acceptable. (See the results of the second dilution step in Examples 1 and 2 in Clause 7.)

5.3 Sum of counting results of subsequent dilution steps

5.3.1 Use for the following test the colony counts from the two sets of parallel plates that passed the test in 5.2. Compare the sums of colony counts from parallel plates over two 10-fold dilution steps with the tabulated limits in Table 2.

For an observed sum with dilution step 10^{-x}, compare the sum obtained with dilution step $10^{-(x+1)}$ with the tabulated lower limit for the sum. Observed sums for the dilution step $10^{-(x+1)}$ within the ranges given in Table 2 are acceptable.

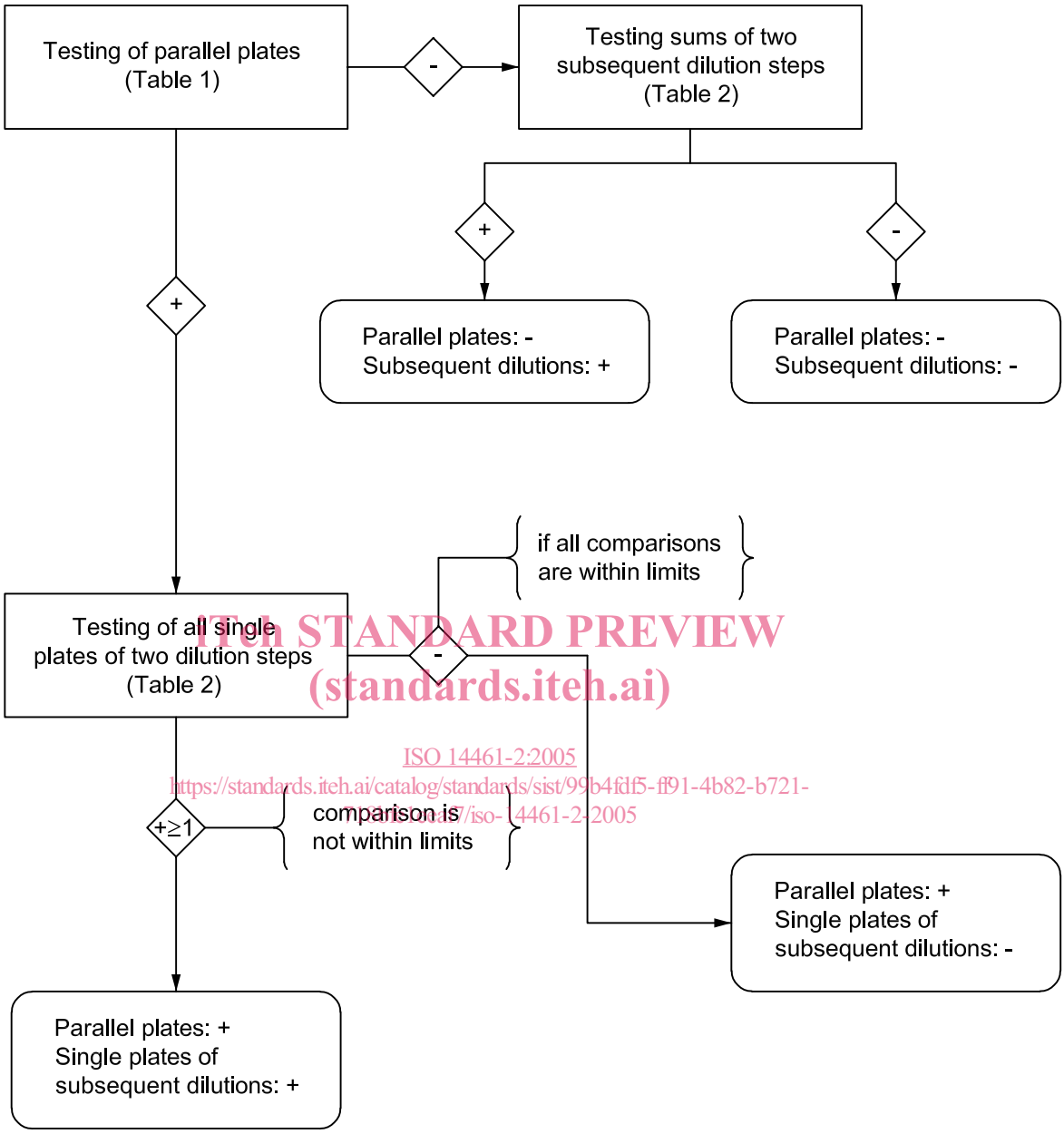
Observed sums outside these limits indicate that the ratio of the colony counts obtained over two 10-fold dilution steps deviates significantly from the expected ratio.

5.3.2 Use for the following test the colony counts from the parallel plates that did not pass the test in 5.2 or those colony counts from one plate per dilution step. Compare the colony counts from plates over two subsequent 10-fold dilution steps with the limits tabulated in Table 2.

For an observed colony count with dilution step 10^{-x}, compare the colony count obtained with dilution step $10^{-(x+1)}$ with the tabulated lower limit for the count. Observed colony counts for dilution step $10^{-(x+1)}$ within the ranges given in Table 2 are acceptable.

Observed colony counts outside these limits indicate that the ratio of the colony counts obtained over two 10-fold dilution steps deviates significantly from the expected ratio. (Two comparisons of results are given in Examples 1 and 2 in Clause 7).

See Figure 1 for a flowchart of the procedure.



NOTE A minus sign (–) indicates an acceptable result within limits. A plus sign (+) indicates a result that is out of limits, and is an indication of technical problems.

Figure 1 — Flowchart describing the testing of colony-counting procedure and its evaluation

6 Evaluation

6.1 Tables of results

For each test described in 5.3.1 and 5.3.2, the results outside the limits specified in Table 1 or Table 2 shall not occur more often than once in a 100 cases. If results outside these limits occur more often, the microbiological test procedure shall be scrutinized.

Table 1 — Limits of agreement for colony counts of two parallel Petri dishes
(with a probability of 99 % per comparison)

Colony count		
Upper	**Lower**	Sum
10	**2**	12
11	**3**	14
12	**3**	15
13	**4**	17
14	**4**	18
15	**5**	20
16	**5**	21
17	**6**	23
18	**6**	24
19	**7**	26
20	**7**	27
21	**8**	29
22	**9**	31
23	**9**	32
24	**10**	34
25	**11**	36
26	**11**	37
27	**12**	39
28	**12**	40
29	**13**	42
30	**14**	44
31	**14**	45
32	**15**	47
33	**16**	49
34	**16**	50
35	**17**	52
36	**18**	54
37	**19**	56
38	**19**	57
39	**20**	59
40	**21**	61
41	**21**	62
42	**22**	64
43	**23**	66
44	**24**	68
45	**24**	69
46	**25**	71
47	**26**	73
48	**27**	75
49	**27**	76
50	**28**	78
51	**29**	80
52	**29**	81
53	**30**	83

Colony count		
Upper	**Lower**	Sum
54	**31**	85
55	**32**	87
56	**32**	88
57	**33**	90
58	**34**	92
59	**35**	94
60	**36**	96
61	**36**	97
62	**37**	99
63	**38**	101
64	**39**	103
65	**39**	104
66	**40**	106
67	**41**	108
68	**42**	110
69	**43**	112
70	**43**	113
71	**44**	115
72	**45**	117
73	**46**	119
74	**46**	120
75	**47**	122
76	**48**	124
77	**49**	126
78	**50**	128
79	**50**	129
80	**51**	131
81	**52**	133
82	**53**	135
83	**54**	137
84	**54**	138
85	**55**	140
86	**56**	142
87	**57**	144
88	**58**	146
89	**58**	147
90	**59**	149
91	**60**	151
92	**61**	153
93	**62**	155
94	**62**	156
95	**63**	158
96	**64**	160
97	**65**	162

Colony count		
Upper	**Lower**	Sum
98	**66**	164
99	**67**	166
100	**67**	167
101	**68**	169
102	**69**	171
103	**70**	173
104	**71**	175
105	**71**	176
106	**72**	178
107	**73**	180
108	**74**	182
109	**75**	184
110	**76**	186
111	**76**	187
112	**77**	189
113	**78**	191
114	**79**	193
115	**80**	195
116	**81**	197
117	**81**	198
118	**82**	200
119	**83**	202
120	**84**	204
121	**85**	206
122	**86**	208
123	**86**	209
124	**87**	211
125	**88**	213
126	**89**	215
127	**90**	217
128	**91**	219
129	**91**	220
130	**92**	222
131	**93**	224
132	**94**	226
133	**95**	228
134	**96**	230
135	**96**	231
136	**97**	233
137	**98**	235
138	**99**	237
139	**100**	239
140	**101**	241
141	**102**	243

Colony count		
Upper	Lower	Sum
142	102	244
143	103	246
144	104	248
145	105	250
146	106	252
147	107	254
148	107	255
149	108	257
150	109	259
151	110	261
152	111	263
153	112	265
154	113	267
155	113	268
156	114	270
157	115	272
158	116	274
159	117	276
160	118	278
161	119	280
162	119	281
163	120	283
164	121	285
165	122	287
166	123	289
167	124	291
168	125	293
169	125	294
170	126	296
171	127	298
172	128	300
173	129	302
174	130	304
175	131	306
176	131	307
177	132	309
178	133	311
179	134	313
180	135	315
181	136	317
182	137	319
183	138	321
184	138	322
185	139	324
186	140	326
187	141	328

Colony count		
Upper	Lower	Sum
188	142	330
189	143	332
190	144	334
191	144	335
192	145	337
193	146	339
194	147	341
195	148	343
196	149	345
197	150	347
198	151	349
199	151	350
200	152	352
201	153	354
202	154	356
203	155	358
204	156	360
205	157	362
206	158	364
207	158	365
208	159	367
209	160	369
210	161	371
211	162	373
212	163	375
213	164	377
214	165	379
215	165	380
216	166	382
217	167	384
218	168	386
219	169	388
220	170	390
221	171	392
222	172	394
223	172	395
224	173	397
225	174	399
226	175	401
227	176	403
228	177	405
229	178	407
230	179	409
231	179	410
232	180	412
233	181	414

Colony count		
Upper	Lower	Sum
234	182	416
235	183	418
236	184	420
237	185	422
238	186	424
239	186	425
240	187	427
241	188	429
242	189	431
243	190	433
244	191	435
245	192	437
246	193	439
247	194	441
248	194	442
249	195	444
250	196	446
251	197	448
252	198	450
253	199	452
254	200	454
255	201	456
256	202	458
257	202	459
258	203	461
259	204	463
260	205	465
261	206	467
262	207	469
263	208	471
264	209	473
265	210	475
266	210	476
267	211	478
268	212	480
269	213	482
270	214	484
271	215	486
272	216	488
273	217	490
274	218	492
275	218	493
276	219	495
277	220	497
278	221	499
279	222	501