INTERNATIONAL STANDARD

ISO 14461-2

IDF 169-2

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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

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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

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Foreword

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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.

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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.

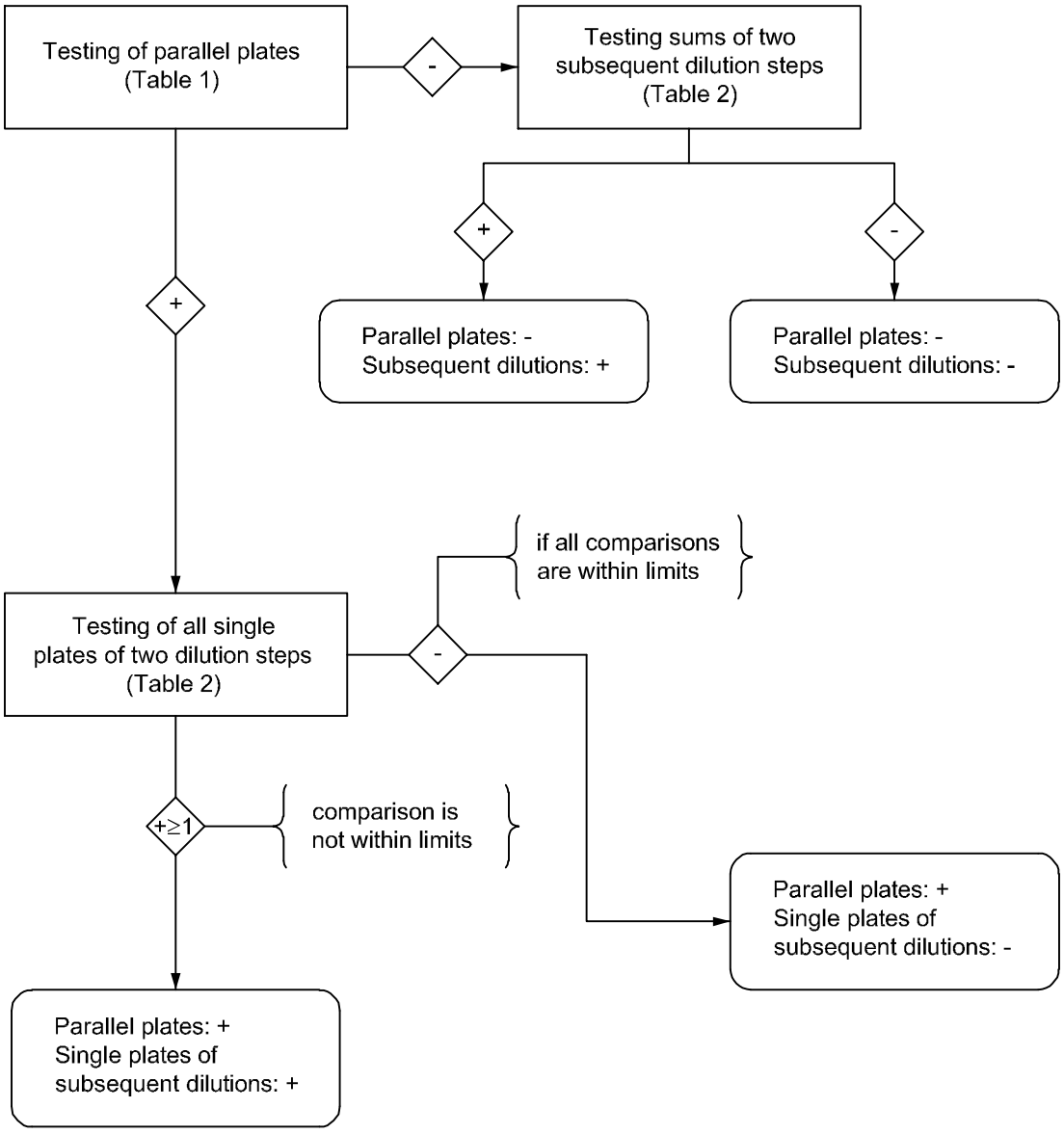
Observed sums outside these limits indicate that the ratio of the colony counts obtained over two 10-fold dilution steps deviates significantly from the expected ratio.

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

Colony count		
Upper	**Lower**	Sum
280	**223**	503
281	**224**	505
282	**225**	507
283	**226**	509
284	**226**	510
285	**227**	512
286	**228**	514
287	**229**	516
288	**230**	518
289	**231**	520
290	**232**	522
291	**233**	524
292	**234**	526
293	**234**	527
294	**235**	529
295	**236**	531
296	**237**	533
297	**238**	535
298	**239**	537
299	**240**	539
300	**241**	541
301	**242**	543
302	**243**	545
303	**243**	546
304	**244**	548
305	**245**	550
306	**246**	552
307	**247**	554
308	**248**	556

Colony count		
Upper	**Lower**	Sum
309	**249**	558
310	**250**	560
311	**251**	562
312	**251**	563
313	**252**	565
314	**253**	567
315	**254**	569
316	**255**	571
317	**256**	573
318	**257**	575
319	**258**	577
320	**259**	579
321	**260**	581
322	**260**	582
323	**261**	584
324	**262**	586
325	**263**	588
326	**264**	590
327	**265**	592
328	**266**	594
329	**267**	596
330	**268**	598
331	**269**	600
332	**269**	601
333	**270**	603
334	**271**	605
335	**272**	607
336	**273**	609
337	**274**	611

Colony count		
Upper	**Lower**	Sum
338	**275**	613
339	**276**	615
340	**277**	617
341	**278**	619
342	**278**	620
343	**279**	622
344	**280**	624
345	**281**	626
346	**282**	628
347	**283**	630
348	**284**	632
349	**285**	634
350	**286**	636
351	**287**	638
352	**287**	639
353	**288**	641
354	**289**	643
355	**290**	645
356	**291**	647
357	**292**	649
358	**293**	651
359	**294**	653
360	**295**	655
361	**296**	657
362	**297**	659
363	**297**	660
364	**298**	662
365	**299**	664
366	**300**	666

6.2 Examples of testing parallel plates

6.2.1 Example 1

If (24, 12) is an observed pair of colony counts, then 24 forms the upper limit with 10 as the lower limit.

Since $12 \geqslant 10$, these colony counts can be considered.

6.2.2 Example 2

If (97, 65) is an observed pair of colony counts, then 97 forms the upper limit with 65 as the lower limit.

Since 65 = 65, these colony counts can be considered.

6.2.3 Example 3

If (193, 142) is an observed pair of colony counts, then 193 forms the upper limit with 146 as the lower limit.

Since $142 < 146$, these colony counts cannot be considered.

6.3 Examples of testing sums of two subsequent dilution steps

6.3.1 Example 1

If 232 is an observed sum/count on dilution step 10^{-x}, then 12 is the lower limit for the sum/count on dilution step $10^{-(x+1)}$ and 37 is the upper limit for the sum/count on dilution step $10^{-(x+1)}$. If 15 is the observed sum/count on dilution step $10^{-(x+1)}$, then both sums/counts on both dilution steps can be considered, since $12 < 15 < 37$.

6.3.2 Example 2

If 357 is an observed sum/count on dilution step 10^{-x}, then 21 is the lower limit for the sum/count on dilution step $10^{-(x+1)}$. If 18 is the observed sum/count on dilution step $10^{-(x+1)}$, then neither sums/counts on the dilution steps can be considered, since $18 < 21$.

6.3.3 Example 3

If 151 is an observed sum/count on dilution step 10^{-x}, then 26 is the upper limit for the sum/count on dilution step $10^{-(x+1)}$. If 31 is the observed sum/count on dilution step $10^{-(x+1)}$, then neither sums/counts on both dilution steps can be considered, since $31 > 26$.

Table 2 — Limits of agreement for sums of colony counts of two parallel Petri dishes or colony counts from one Petri dish per dilution step over two 10-fold dilution steps
(with a probability of 99 % per comparison)

10^{-x}	$10^{-(x+1)}$		
Observed	Expected		
Sum/ Count	**Lower limit**	Sum/ Count	**Upper limit**
10	**0**	1,0	**4**
11	**0**	1,1	**5**
12	**0**	1,2	**5**
13	**0**	1,3	**5**
14	**0**	1,4	**5**
15	**0**	1,5	**6**
16	**0**	1,6	**6**
17	**0**	1,7	**6**
18	**0**	1,8	**6**
19	**0**	1,9	**6**
20	**0**	2,0	**7**
21	**0**	2,1	**7**
22	**0**	2,2	**7**
23	**0**	2,3	**7**
24	**0**	2,4	**7**
25	**0**	2,5	**8**
26	**0**	2,6	**8**
27	**0**	2,7	**8**
28	**0**	2,8	**8**
29	**0**	2,9	**8**
30	**0**	3,0	**8**
31	**0**	3,1	**9**
32	**0**	3,2	**9**
33	**0**	3,3	**9**
34	**0**	3,4	**9**
35	**1**	3,5	**9**
36	**1**	3,6	**9**
37	**1**	3,7	**10**
38	**1**	3,8	**10**
39	**1**	3,9	**10**
40	**1**	4,0	**10**
41	**1**	4,1	**10**
42	**1**	4,2	**11**
43	**1**	4,3	**11**
44	**1**	4,4	**11**
45	**1**	4,5	**11**
46	**1**	4,6	**11**
47	**1**	4,7	**11**
48	**1**	4,8	**11**
49	**1**	4,9	**12**
50	**1**	5,0	**12**

10^{-x}	$10^{-(x+1)}$		
Observed	Expected		
Sum/ Count	**Lower limit**	Sum/ Count	**Upper limit**
51	**1**	5,1	**12**
52	**1**	5,2	**12**
53	**1**	5,3	**12**
54	**1**	5,4	**12**
55	**1**	5,5	**13**
56	**1**	5,6	**13**
57	**1**	5,7	**13**
58	**1**	5,8	**13**
59	**1**	5,9	**13**
60	**1**	6,0	**13**
61	**1**	6,1	**14**
62	**1**	6,2	**14**
63	**1**	6,3	**14**
64	**2**	6,4	**14**
65	**2**	6,5	**14**
66	**2**	6,6	**14**
67	**2**	6,7	**14**
68	**2**	6,8	**15**
69	**2**	6,9	**15**
70	**2**	7,0	**15**
71	**2**	7,1	**15**
72	**2**	7,2	**15**
73	**2**	7,3	**15**
74	**2**	7,4	**16**
75	**2**	7,5	**16**
76	**2**	7,6	**16**
77	**2**	7,7	**16**
78	**2**	7,8	**16**
79	**2**	7,9	**16**
80	**2**	8,0	**16**
81	**2**	8,1	**17**
82	**2**	8,2	**17**
83	**2**	8,3	**17**
84	**2**	8,4	**17**
85	**3**	8,5	**17**
86	**3**	8,6	**17**
87	**3**	8,7	**17**
88	**3**	8,8	**18**
89	**3**	8,9	**18**
90	**3**	9,0	**18**
91	**3**	9,1	**18**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
92	**3**	9,2	**18**
93	**3**	9,3	**18**
94	**3**	9,4	**18**
95	**3**	9,5	**19**
96	**3**	9,6	**19**
97	**3**	9,7	**19**
98	**3**	9,8	**19**
99	**3**	9,9	**19**
100	**3**	10,0	**19**
101	**3**	10,1	**19**
102	**3**	10,2	**20**
103	**4**	10,3	**20**
104	**4**	10,4	**20**
105	**4**	10,5	**20**
106	**4**	10,6	**20**
107	**4**	10,7	**20**
108	**4**	10,8	**20**
109	**4**	10,9	**21**
110	**4**	11,0	**21**
111	**4**	11,1	**21**
112	**4**	11,2	**21**
113	**4**	11,3	**21**
114	**4**	11,4	**21**
115	**4**	11,5	**21**
116	**4**	11,6	**22**
117	**4**	11,7	**22**
118	**4**	11,8	**22**
119	**4**	11,9	**22**
120	**5**	12,0	**22**
121	**5**	12,1	**22**
122	**5**	12,2	**22**
123	**5**	12,3	**23**
124	**5**	12,4	**23**
125	**5**	12,5	**23**
126	**5**	12,6	**23**
127	**5**	12,7	**23**
128	**5**	12,8	**23**
129	**5**	12,9	**23**
130	**5**	13,0	**24**
131	**5**	13,1	**24**
132	**5**	13,2	**24**
133	**5**	13,3	**24**
134	**5**	13,4	**24**
135	**5**	13,5	**24**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
136	**6**	13,6	**24**
137	**6**	13,7	**24**
138	**6**	13,8	**25**
139	**6**	13,9	**25**
140	**6**	14,0	**25**
141	**6**	14,1	**25**
142	**6**	14,2	**25**
143	**6**	14,3	**25**
144	**6**	14,4	**25**
145	**6**	14,5	**26**
146	**6**	14,6	**26**
147	**6**	14,7	**26**
148	**6**	14,8	**26**
149	**6**	14,9	**26**
150	**6**	15,0	**26**
151	**6**	15,1	**26**
152	**7**	15,2	**27**
153	**7**	15,3	**27**
154	**7**	15,4	**27**
155	**7**	15,5	**27**
156	**7**	15,6	**27**
157	**7**	15,7	**27**
158	**7**	15,8	**27**
159	**7**	15,9	**27**
160	**7**	16,0	**28**
161	**7**	16,1	**28**
162	**7**	16,2	**28**
163	**7**	16,3	**28**
164	**7**	16,4	**28**
165	**7**	16,5	**28**
166	**7**	16,6	**28**
167	**8**	16,7	**29**
168	**8**	16,8	**29**
169	**8**	16,9	**29**
170	**8**	17,0	**29**
171	**8**	17,1	**29**
172	**8**	17,2	**29**
173	**8**	17,3	**29**
174	**8**	17,4	**29**
175	**8**	17,5	**30**
176	**8**	17,6	**30**
177	**8**	17,7	**30**
178	**8**	17,8	**30**
179	**8**	17,9	**30**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
180	**8**	18,0	**30**
181	**8**	18,1	**30**
182	**9**	18,2	**31**
183	**9**	18,3	**31**
184	**9**	18,4	**31**
185	**9**	18,5	**31**
186	**9**	18,6	**31**
187	**9**	18,7	**31**
188	**9**	18,8	**31**
189	**9**	18,9	**31**
190	**9**	19,0	**32**
191	**9**	19,1	**32**
192	**9**	19,2	**32**
193	**9**	19,3	**32**
194	**9**	19,4	**32**
195	**9**	19,5	**32**
196	**10**	19,6	**32**
197	**10**	19,7	**32**
198	**10**	19,8	**33**
199	**10**	19,9	**33**
200	**10**	20,0	**33**
201	**10**	20,1	**33**
202	**10**	20,2	**33**
203	**10**	20,3	**33**
204	**10**	20,4	**33**
205	**10**	20,5	**34**
206	**10**	20,6	**34**
207	**10**	20,7	**34**
208	**10**	20,8	**34**
209	**10**	20,9	**34**
210	**10**	21,0	**34**
211	**11**	21,1	**34**
212	**11**	21,2	**34**
213	**11**	21,3	**35**
214	**11**	21,4	**35**
215	**11**	21,5	**35**
216	**11**	21,6	**35**
217	**11**	21,7	**35**
218	**11**	21,8	**35**
219	**11**	21,9	**35**
220	**11**	22,0	**35**
221	**11**	22,1	**36**
222	**11**	22,2	**36**
223	**11**	22,3	**36**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
224	**11**	22,4	**36**
225	**12**	22,5	**36**
226	**12**	22,6	**36**
227	**12**	22,7	**36**
228	**12**	22,8	**36**
229	**12**	22,9	**37**
230	**12**	23,0	**37**
231	**12**	23,1	**37**
232	**12**	23,2	**37**
233	**12**	23,3	**37**
234	**12**	23,4	**37**
235	**12**	23,5	**37**
236	**12**	23,6	**38**
237	**12**	23,7	**38**
238	**12**	23,8	**38**
239	**13**	23,9	**38**
240	**13**	24,0	**38**
241	**13**	24,1	**38**
242	**13**	24,2	**38**
243	**13**	24,3	**38**
244	**13**	24,4	**39**
245	**13**	24,5	**39**
246	**13**	24,6	**39**
247	**13**	24,7	**39**
248	**13**	24,8	**39**
249	**13**	24,9	**39**
250	**13**	25,0	**39**
251	**13**	25,1	**39**
252	**14**	25,2	**40**
253	**14**	25,3	**40**
254	**14**	25,4	**40**
255	**14**	25,5	**40**
256	**14**	25,6	**40**
257	**14**	25,7	**40**
258	**14**	25,8	**40**
259	**14**	25,9	**40**
260	**14**	26,0	**41**
261	**14**	26,1	**41**
262	**14**	26,2	**41**
263	**14**	26,3	**41**
264	**14**	26,4	**41**
265	**14**	26,5	**41**
266	**15**	26,6	**41**
267	**15**	26,7	**41**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
268	**15**	26,8	**42**
269	**15**	26,9	**42**
270	**15**	27,0	**42**
271	**15**	27,1	**42**
272	**15**	27,2	**42**
273	**15**	27,3	**42**
274	**15**	27,4	**42**
275	**15**	27,5	**42**
276	**15**	27,6	**43**
277	**15**	27,7	**43**
278	**15**	27,8	**43**
279	**16**	27,9	**43**
280	**16**	28,0	**43**
281	**16**	28,1	**43**
282	**16**	28,2	**43**
283	**16**	28,3	**43**
284	**16**	28,4	**44**
285	**16**	28,5	**44**
286	**16**	28,6	**44**
287	**16**	28,7	**44**
288	**16**	28,8	**44**
289	**16**	28,9	**44**
290	**16**	29,0	**44**
291	**16**	29,1	**44**
292	**16**	29,2	**45**
293	**17**	29,3	**45**
294	**17**	29,4	**45**
295	**17**	29,5	**45**
296	**17**	29,6	**45**
297	**17**	29,7	**45**
298	**17**	29,8	**45**
299	**17**	29,9	**45**
300	**17**	30,0	**46**
301	**17**	30,1	**46**
302	**17**	30,2	**46**
303	**17**	30,3	**46**
304	**17**	30,4	**46**
305	**17**	30,5	**46**
306	**18**	30,6	**46**
307	**18**	30,7	**46**
308	**18**	30,8	**47**
309	**18**	30,9	**47**
310	**18**	31,0	**47**
311	**18**	31,1	**47**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
312	**18**	31,2	**47**
313	**18**	31,3	**47**
314	**18**	31,4	**47**
315	**18**	31,5	**47**
316	**18**	31,6	**48**
317	**18**	31,7	**48**
318	**18**	31,8	**48**
319	**19**	31,9	**48**
320	**19**	32,0	**48**
321	**19**	32,1	**48**
322	**19**	32,2	**48**
323	**19**	32,3	**48**
324	**19**	32,4	**49**
325	**19**	32,5	**49**
326	**19**	32,6	**49**
327	**19**	32,7	**49**
328	**19**	32,8	**49**
329	**19**	32,9	**49**
330	**19**	33,0	**49**
331	**19**	33,1	**49**
332	**20**	33,2	**50**
333	**20**	33,3	**50**
334	**20**	33,4	**50**
335	**20**	33,5	**50**
336	**20**	33,6	**50**
337	**20**	33,7	**50**
338	**20**	33,8	**50**
339	**20**	33,9	**50**
340	**20**	34,0	**51**
341	**20**	34,1	**51**
342	**20**	34,2	**51**
343	**20**	34,3	**51**
344	**20**	34,4	**51**
345	**20**	34,5	**51**
346	**21**	34,6	**51**
347	**21**	34,7	**51**
348	**21**	34,8	**52**
349	**21**	34,9	**52**
350	**21**	35,0	**52**
351	**21**	35,1	**52**
352	**21**	35,2	**52**
353	**21**	35,3	**52**
354	**21**	35,4	**52**
355	**21**	35,5	**52**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
356	**21**	35,6	**53**
357	**21**	35,7	**53**
358	**22**	35,8	**53**
359	**22**	35,9	**53**
360	**22**	36,0	**53**
361	**22**	36,1	**53**
362	**22**	36,2	**53**
363	**22**	36,3	**53**
364	**22**	36,4	**53**
365	**22**	36,5	**54**
366	**22**	36,6	**54**
367	**22**	36,7	**54**
368	**22**	36,8	**54**
369	**22**	36,9	**54**
370	**22**	37,0	**54**
371	**23**	37,1	**54**
372	**23**	37,2	**54**
373	**23**	37,3	**55**
374	**23**	37,4	**55**
375	**23**	37,5	**55**
376	**23**	37,6	**55**
377	**23**	37,7	**55**
378	**23**	37,8	**55**
379	**23**	37,9	**55**
380	**23**	38,0	**55**
381	**23**	38,1	**56**
382	**23**	38,2	**56**
383	**23**	38,3	**56**
384	**24**	38,4	**56**
385	**24**	38,5	**56**
386	**24**	38,6	**56**
387	**24**	38,7	**56**
388	**24**	38,8	**56**
389	**24**	38,9	**57**
390	**24**	39,0	**57**
391	**24**	39,1	**57**
392	**24**	39,2	**57**
393	**24**	39,3	**57**
394	**24**	39,4	**57**
395	**24**	39,5	**57**
396	**24**	39,6	**57**
397	**25**	39,7	**58**
398	**25**	39,8	**58**
399	**25**	39,9	**58**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
400	**25**	40,0	**58**
401	**25**	40,1	**58**
402	**25**	40,2	**58**
403	**25**	40,3	**58**
404	**25**	40,4	**58**
405	**25**	40,5	**58**
406	**25**	40,6	**59**
407	**25**	40,7	**59**
408	**25**	40,8	**59**
409	**25**	40,9	**59**
410	**26**	41,0	**59**
411	**26**	41,1	**59**
412	**26**	41,2	**59**
413	**26**	41,3	**59**
414	**26**	41,4	**60**
415	**26**	41,5	**60**
416	**26**	41,6	**60**
417	**26**	41,7	**60**
418	**26**	41,8	**60**
419	**26**	41,9	**60**
420	**26**	42,0	**60**
421	**26**	42,1	**60**
422	**27**	42,2	**61**
423	**27**	42,3	**61**
424	**27**	42,4	**61**
425	**27**	42,5	**61**
426	**27**	42,6	**61**
427	**27**	42,7	**61**
428	**27**	42,8	**61**
429	**27**	42,9	**61**
430	**27**	43,0	**62**
431	**27**	43,1	**62**
432	**27**	43,2	**62**
433	**27**	43,3	**62**
434	**27**	43,4	**62**
435	**28**	43,5	**62**
436	**28**	43,6	**62**
437	**28**	43,7	**62**
438	**28**	43,8	**62**
439	**28**	43,9	**63**
440	**28**	44,0	**63**
441	**28**	44,1	**63**
442	**28**	44,2	**63**
443	**28**	44,3	**63**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
444	**28**	44,4	**63**
445	**28**	44,5	**63**
446	**28**	44,6	**63**
447	**29**	44,7	**64**
448	**29**	44,8	**64**
449	**29**	44,9	**64**
450	**29**	45,0	**64**
451	**29**	45,1	**64**
452	**29**	45,2	**64**
453	**29**	45,3	**64**
454	**29**	45,4	**64**
455	**29**	45,5	**65**
456	**29**	45,6	**65**
457	**29**	45,7	**65**
458	**29**	45,8	**65**
459	**29**	45,9	**65**
460	**30**	46,0	**65**
461	**30**	46,1	**65**
462	**30**	46,2	**65**
463	**30**	46,3	**65**
464	**30**	46,4	**66**
465	**30**	46,5	**66**
466	**30**	46,6	**66**
467	**30**	46,7	**66**
468	**30**	46,8	**66**
469	**30**	46,9	**66**
470	**30**	47,0	**66**
471	**30**	47,1	**66**
472	**31**	47,2	**67**
473	**31**	47,3	**67**
474	**31**	47,4	**67**
475	**31**	47,5	**67**
476	**31**	47,6	**67**
477	**31**	47,7	**67**
478	**31**	47,8	**67**
479	**31**	47,9	**67**
480	**31**	48,0	**68**
481	**31**	48,1	**68**
482	**31**	48,2	**68**
483	**31**	48,3	**68**
484	**31**	48,4	**68**
485	**32**	48,5	**68**
486	**32**	48,6	**68**
487	**32**	48,7	**68**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
488	**32**	48,8	**68**
489	**32**	48,9	**69**
490	**32**	49,0	**69**
491	**32**	49,1	**69**
492	**32**	49,2	**69**
493	**32**	49,3	**69**
494	**32**	49,4	**69**
495	**32**	49,5	**69**
496	**32**	49,6	**69**
497	**33**	49,7	**70**
498	**33**	49,8	**70**
499	**33**	49,9	**70**
500	**33**	50,0	**70**
501	**33**	50,1	**70**
502	**33**	50,2	**70**
503	**33**	50,3	**70**
504	**33**	50,4	**70**
505	**33**	50,5	**70**
506	**33**	50,6	**71**
507	**33**	50,7	**71**
508	**33**	50,8	**71**
509	**33**	50,9	**71**
510	**34**	51,0	**71**
511	**34**	51,1	**71**
512	**34**	51,2	**71**
513	**34**	51,3	**71**
514	**34**	51,4	**72**
515	**34**	51,5	**72**
516	**34**	51,6	**72**
517	**34**	51,7	**72**
518	**34**	51,8	**72**
519	**34**	51,9	**72**
520	**34**	52,0	**72**
521	**34**	52,1	**72**
522	**35**	52,2	**73**
523	**35**	52,3	**73**
524	**35**	52,4	**73**
525	**35**	52,5	**73**
526	**35**	52,6	**73**
527	**35**	52,7	**73**
528	**35**	52,8	**73**
529	**35**	52,9	**73**
530	**35**	53,0	**73**
531	**35**	53,1	**74**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
532	**35**	53,2	**74**
533	**35**	53,3	**74**
534	**36**	53,4	**74**
535	**36**	53,5	**74**
536	**36**	53,6	**74**
537	**36**	53,7	**74**
538	**36**	53,8	**74**
539	**36**	53,9	**75**
540	**36**	54,0	**75**
541	**36**	54,1	**75**
542	**36**	54,2	**75**
543	**36**	54,3	**75**
544	**36**	54,4	**75**
545	**36**	54,5	**75**
546	**36**	54,6	**75**
547	**37**	54,7	**75**
548	**37**	54,8	**76**
549	**37**	54,9	**76**
550	**37**	55,0	**76**
551	**37**	55,1	**76**
552	**37**	55,2	**76**
553	**37**	55,3	**76**
554	**37**	55,4	**76**
555	**37**	55,5	**76**
556	**37**	55,6	**77**
557	**37**	55,7	**77**
558	**37**	55,8	**77**
559	**38**	55,9	**77**
560	**38**	56,0	**77**
561	**38**	56,1	**77**
562	**38**	56,2	**77**
563	**38**	56,3	**77**
564	**38**	56,4	**77**
565	**38**	56,5	**78**
566	**38**	56,6	**78**
567	**38**	56,7	**78**
568	**38**	56,8	**78**
569	**38**	56,9	**78**
570	**38**	57,0	**78**
571	**39**	57,1	**78**
572	**39**	57,2	**78**
573	**39**	57,3	**79**
574	**39**	57,4	**79**
575	**39**	57,5	**79**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
576	**39**	57,6	**79**
577	**39**	57,7	**79**
578	**39**	57,8	**79**
579	**39**	57,9	**79**
580	**39**	58,0	**79**
581	**39**	58,1	**79**
582	**39**	58,2	**80**
583	**40**	58,3	**80**
584	**40**	58,4	**80**
585	**40**	58,5	**80**
586	**40**	58,6	**80**
587	**40**	58,7	**80**
588	**40**	58,8	**80**
589	**40**	58,9	**80**
590	**40**	59,0	**81**
591	**40**	59,1	**81**
592	**40**	59,2	**81**
593	**40**	59,3	**81**
594	**40**	59,4	**81**
595	**41**	59,5	**81**
596	**41**	59,6	**81**
597	**41**	59,7	**81**
598	**41**	59,8	**81**
599	**41**	59,9	**82**
600	**41**	60,0	**82**
601	**41**	60,1	**82**
602	**41**	60,2	**82**
603	**41**	60,3	**82**
604	**41**	60,4	**82**
605	**41**	60,5	**82**
606	**41**	60,6	**82**
607	**42**	60,7	**83**
608	**42**	60,8	**83**
609	**42**	60,9	**83**
610	**42**	61,0	**83**
611	**42**	61,1	**83**
612	**42**	61,2	**83**
613	**42**	61,3	**83**
614	**42**	61,4	**83**
615	**42**	61,5	**83**
616	**42**	61,6	**84**
617	**42**	61,7	**84**
618	**42**	61,8	**84**
619	**43**	61,9	**84**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
620	**43**	62,0	**84**
621	**43**	62,1	**84**
622	**43**	62,2	**84**
623	**43**	62,3	**84**
624	**43**	62,4	**85**
625	**43**	62,5	**85**
626	**43**	62,6	**85**
627	**43**	62,7	**85**
628	**43**	62,8	**85**
629	**43**	62,9	**85**
630	**43**	63,0	**85**
631	**43**	63,1	**85**
632	**44**	63,2	**85**
633	**44**	63,3	**86**
634	**44**	63,4	**86**
635	**44**	63,5	**86**
636	**44**	63,6	**86**
637	**44**	63,7	**86**
638	**44**	63,8	**86**
639	**44**	63,9	**86**
640	**44**	64,0	**86**
641	**44**	64,1	**87**
642	**44**	64,2	**87**
643	**44**	64,3	**87**

10^{-x}	$10^{-(x+1)}$		
Observed Sum/ Count	Expected		
	Lower limit	Sum/ Count	**Upper limit**
644	**45**	64,4	**87**
645	**45**	64,5	**87**
646	**45**	64,6	**87**
647	**45**	64,7	**87**
648	**45**	64,8	**87**
649	**45**	64,9	**87**
650	**45**	65,0	**88**
651	**45**	65,1	**88**
652	**45**	65,2	**88**
653	**45**	65,3	**88**
654	**45**	65,4	**88**
655	**45**	65,5	**88**
656	**46**	65,6	**88**
657	**46**	65,7	**88**
658	**46**	65,8	**89**
659	**46**	65,9	**89**
660	**46**	66,0	**89**
661	**46**	66,1	**89**
662	**46**	66,2	**89**
663	**46**	66,3	**89**
664	**46**	66,4	**89**
665	**46**	66,5	**89**
666	**46**	66,6	**89**

7 Calculation formulae and examples

7.1 In Table 1

The probability of compliance, P, with the limit C_{lower} and C_{upper} is approximately

$$P\left(\chi_1^2\right) = P\left\{2\left[C_{\text{lower}} \cdot \ln\left(\frac{C_{\text{lower}}}{\left(C_{\text{lower}} + C_{\text{upper}}\right)/2}\right) + C_{\text{upper}} \cdot \ln\left(\frac{C_{\text{upper}}}{\left(C_{\text{lower}} + C_{\text{upper}}\right)/2}\right)\right]\right\}$$

$$\approx 0{,}01 \ (\geqslant 0{,}01)$$

where

C_{lower} is the lower colony count of parallel plates;

C_{upper} is the upper colony count of parallel plates.

7.2 In Table 2

The probability of compliance, P, between the observed sum/count S_1 on dilution step 10^{-x} and the chosen S_{lower} on dilution step $10^{-(x+1)}$ or between the observed sum/count S_1 on dilution step 10^{-x} and the limit S_{upper} on dilution step $10^{-(x+1)}$ is approximately

$$P\left(\chi_1^2\right) = P\left\{2\left[S_1 \cdot \ln\left(\frac{S_1}{10{,}0 \cdot \left(S_1 + S_{\text{lower}}\right)/11}\right) + S_{\text{lower}} \cdot \ln\left(\frac{S_{\text{lower}}}{1{,}0 \cdot \left(S_1 + S_{\text{lower}}\right)/11}\right)\right]\right\}$$

$$\approx 0{,}01 \ (\geqslant 0{,}01)$$

or

$$P\left(\chi_1^2\right) = P\left\{2\left[S_1 \cdot \ln\left(\frac{S_1}{10{,}0 \cdot \left(S_1 + S_{\text{upper}}\right)/11}\right) + S_{\text{upper}} \cdot \ln\left(\frac{S_{\text{upper}}}{1{,}0 \cdot \left(S_1 + S_{\text{upper}}\right)/11}\right)\right]\right\}$$

$$\approx 0{,}01 \ (\geqslant 0{,}01);$$

where

S_1 is the sum of both counts of parallel plates or the result of a colony count from one plate on dilution level 10^{-x};

S_{lower} is the acceptable lower limit on dilution level $10^{-(x+1)}$;

S_{upper} is the acceptable upper limit on dilution level $10^{-(x+1)}$.

7.3 Examples

7.3.1 Example 1

Dilution step	Plate 1 Colony count	Plate 2 Colony count
10^{-x}	100	200
$10^{-(x+1)}$	5	9

$$P\left(\chi_1^2\right) = P\left\{2\left[100 \cdot \ln\left(\frac{100}{(100+200)/2}\right) + 200 \cdot \ln\left(\frac{200}{(100+200)/2}\right)\right]\right\}$$

$$\approx P(33{,}98) < 0{,}001$$

This is not acceptable; please compare the values with Table 1.

$$P\left(\chi_1^2\right) = P\left\{2\left[5 \cdot \ln\left(\frac{5}{(5+9)/2}\right) + 9 \cdot \ln\left(\frac{9}{(5+9)/2}\right)\right]\right\}$$

$$\approx P(1{,}16) = 0{,}28 > 0{,}01$$

This is acceptable; please compare the values with Table 1.

$$P\left(\chi_1^2\right) = P\left\{2\left[100 \cdot \ln\left(\frac{100}{10{,}0 \cdot (100+5)/11}\right) + 5 \cdot \ln\left(\frac{5}{1{,}0 \cdot (100+5)/11}\right)\right]\right\}$$

$$\approx P(2{,}84) = 0{,}09 > 0{,}01$$

This is acceptable; please compare the values with Table 2.

$$P\left(\chi_1^2\right) = P\left\{2\left[200 \cdot \ln\left(\frac{200}{10{,}0 \cdot (200+9)/11}\right) + 9 \cdot \ln\left(\frac{9}{1{,}0 \cdot (200+9)/11}\right)\right]\right\}$$

$$\approx P(7{,}07) = 0{,}008 < 0{,}01$$

This is not acceptable; please compare the values with Table 2.

Therefore, the colony count of 200 on dilution step 10^{-x} is not comparable with the parallel result 100 and the parallel results 5 and 9 on dilution step $10^{-(x+1)}$.

7.3.2 Example 2

Dilution step	Plate 1 Colony count	Plate 2 Colony count
10^{-x}	50	90
$10^{-(x+1)}$	10	20

$$P\left(\chi_1^2\right) = P\left\{2\left[50 \cdot \ln\left(\frac{50}{(50+90/2)}\right) + 90 \cdot \ln\left(\frac{90}{(50+90)/2}\right)\right]\right\}$$

$$\approx P(11{,}59) < 0{,}001$$

This is not acceptable; please compare the values with Table 1.

$$P\left(\chi_1^2\right) = P\left\{2\left[10 \cdot \ln\left(\frac{10}{(10+20)/2}\right) + 20 \cdot \ln\left(\frac{20}{(10+20)/2}\right)\right]\right\}$$

$$\approx P(3{,}40) = 0{,}07 > 0{,}01$$

This is acceptable; please compare the values with Table 1.

$$P\left(\chi_1^2\right) = P\left\{2\left[50 \cdot \ln\left(\frac{50}{10{,}0 \cdot (50+10)/11}\right) + 10 \cdot \ln\left(\frac{10}{1{,}0 \cdot (50+10)/11}\right)\right]\right\}$$

$$\approx P(3{,}42) = 0{,}06 > 0{,}01$$

This is acceptable; please compare the values with Table 2.

$$P\left(\chi_1{}^2\right) = P\left\{2\left[90 \cdot \ln\left(\frac{90}{10{,}0 \cdot (90+20)/11}\right) + 20 \cdot \ln\left(\frac{20}{1{,}0 \cdot (90+20)/11}\right)\right]\right\}$$

$$\approx P(8{,}76) = 0{,}003 < 0{,}01$$

This is not acceptable; please compare the values with Table 2.

The colony count of 90 on dilution step 10^{-x} is not comparable with the parallel result 50. The parallel results 10 and 20 on dilution step $10^{-(x+1)}$ are comparable, 50 and 90 with 10^{-x} are comparable, but 50 and 90 are not acceptable results compared with 20. Report all single results.

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