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

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Foreword

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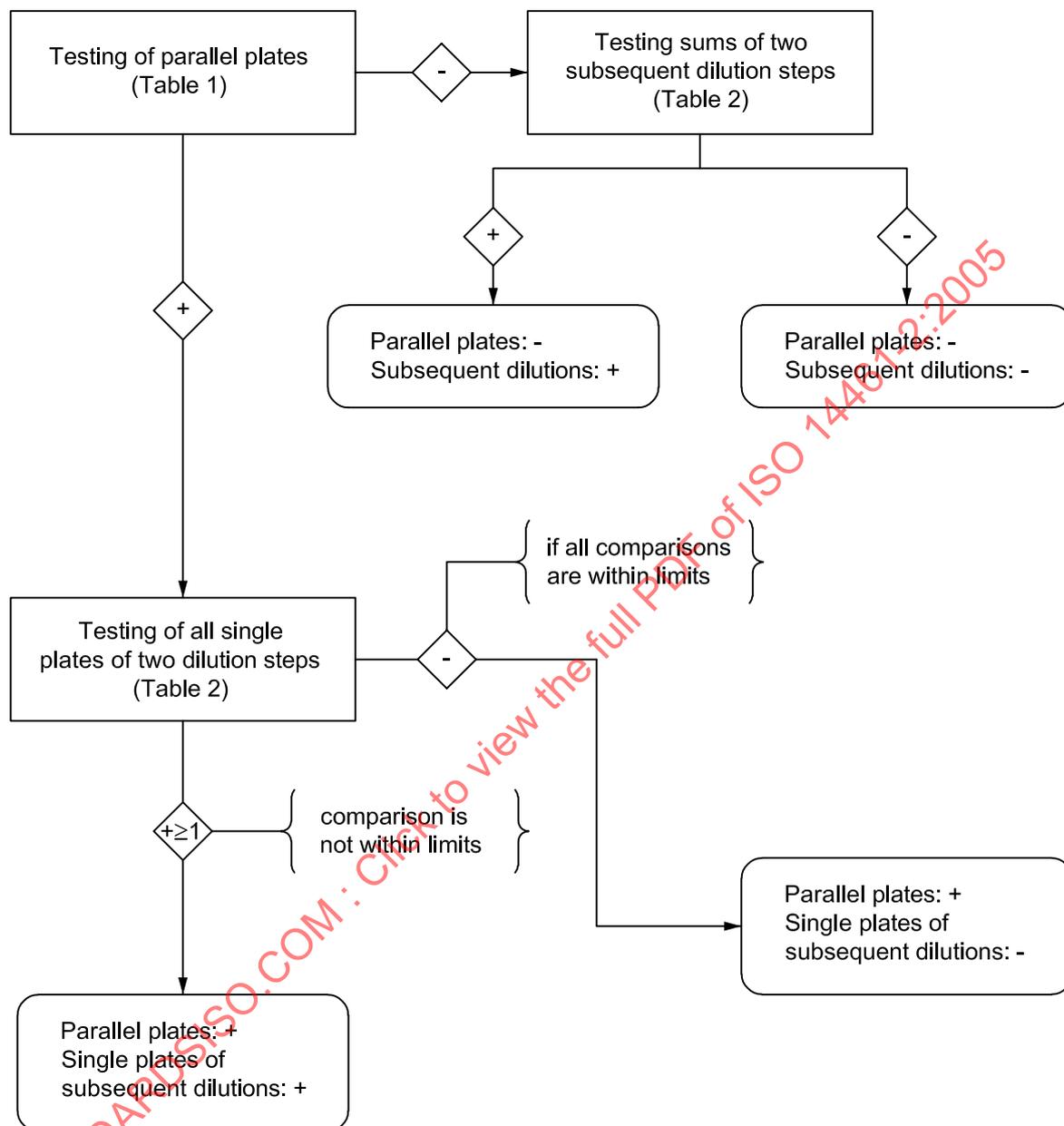
NOTE   A minus sign (–) indicates an acceptable result within limits. A plus sign (+) indicates a result that is out of limits, and is an indication of technical problems.

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			Colony count			Colony count		
Upper	**Lower**	Sum	**Upper**	**Lower**	Sum	**Upper**	**Lower**	Sum
10	**2**	12	**54**	**31**	85	**98**	**66**	164
11	**3**	14	**55**	**32**	87	**99**	**67**	166
12	**3**	15	**56**	**32**	88	**100**	**67**	167
13	**4**	17	**57**	**33**	90	**101**	**68**	169
14	**4**	18	**58**	**34**	92	**102**	**69**	171
15	**5**	20	**59**	**35**	94	**103**	**70**	173
16	**5**	21	**60**	**36**	96	**104**	**71**	175
17	**6**	23	**61**	**36**	97	**105**	**71**	176
18	**6**	24	**62**	**37**	99	**106**	**72**	178
19	**7**	26	**63**	**38**	101	**107**	**73**	180
20	**7**	27	**64**	**39**	103	**108**	**74**	182
21	**8**	29	**65**	**39**	104	**109**	**75**	184
22	**9**	31	**66**	**40**	106	**110**	**76**	186
23	**9**	32	**67**	**41**	108	**111**	**76**	187
24	**10**	34	**68**	**42**	110	**112**	**77**	189
25	**11**	36	**69**	**43**	112	**113**	**78**	191
26	**11**	37	**70**	**43**	113	**114**	**79**	193
27	**12**	39	**71**	**44**	115	**115**	**80**	195
28	**12**	40	**72**	**45**	117	**116**	**81**	197
29	**13**	42	**73**	**46**	119	**117**	**81**	198
30	**14**	44	**74**	**46**	120	**118**	**82**	200
31	**14**	45	**75**	**47**	122	**119**	**83**	202
32	**15**	47	**76**	**48**	124	**120**	**84**	204
33	**16**	49	**77**	**49**	126	**121**	**85**	206
34	**16**	50	**78**	**50**	128	**122**	**86**	208
35	**17**	52	**79**	**50**	129	**123**	**86**	209
36	**18**	54	**80**	**51**	131	**124**	**87**	211
37	**19**	56	**81**	**52**	133	**125**	**88**	213
38	**19**	57	**82**	**53**	135	**126**	**89**	215
39	**20**	59	**83**	**54**	137	**127**	**90**	217
40	**21**	61	**84**	**54**	138	**128**	**91**	219
41	**21**	62	**85**	**55**	140	**129**	**91**	220
42	**22**	64	**86**	**56**	142	**130**	**92**	222
43	**23**	66	**87**	**57**	144	**131**	**93**	224
44	**24**	68	**88**	**58**	146	**132**	**94**	226
45	**24**	69	**89**	**58**	147	**133**	**95**	228
46	**25**	71	**90**	**59**	149	**134**	**96**	230
47	**26**	73	**91**	**60**	151	**135**	**96**	231
48	**27**	75	**92**	**61**	153	**136**	**97**	233
49	**27**	76	**93**	**62**	155	**137**	**98**	235
50	**28**	78	**94**	**62**	156	**138**	**99**	237
51	**29**	80	**95**	**63**	158	**139**	**100**	239
52	**29**	81	**96**	**64**	160	**140**	**101**	241
53	**30**	83	**97**	**65**	162	**141**	**102**	243

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Colony count			Colony count			Colony count		
Upper	Lower	Sum	Upper	Lower	Sum	Upper	Lower	Sum
142	102	244	188	142	330	234	182	416
143	103	246	189	143	332	235	183	418
144	104	248	190	144	334	236	184	420
145	105	250	191	144	335	237	185	422
146	106	252	192	145	337	238	186	424
147	107	254	193	146	339	239	186	425
148	107	255	194	147	341	240	187	427
149	108	257	195	148	343	241	188	429
150	109	259	196	149	345	242	189	431
151	110	261	197	150	347	243	190	433
152	111	263	198	151	349	244	191	435
153	112	265	199	151	350	245	192	437
154	113	267	200	152	352	246	193	439
155	113	268	201	153	354	247	194	441
156	114	270	202	154	356	248	194	442
157	115	272	203	155	358	249	195	444
158	116	274	204	156	360	250	196	446
159	117	276	205	157	362	251	197	448
160	118	278	206	158	364	252	198	450
161	119	280	207	158	365	253	199	452
162	119	281	208	159	367	254	200	454
163	120	283	209	160	369	255	201	456
164	121	285	210	161	371	256	202	458
165	122	287	211	162	373	257	202	459
166	123	289	212	163	375	258	203	461
167	124	291	213	164	377	259	204	463
168	125	293	214	165	379	260	205	465
169	125	294	215	165	380	261	206	467
170	126	296	216	166	382	262	207	469
171	127	298	217	167	384	263	208	471
172	128	300	218	168	386	264	209	473
173	129	302	219	169	388	265	210	475
174	130	304	220	170	390	266	210	476
175	131	306	221	171	392	267	211	478
176	131	307	222	172	394	268	212	480
177	132	309	223	172	395	269	213	482
178	133	311	224	173	397	270	214	484
179	134	313	225	174	399	271	215	486
180	135	315	226	175	401	272	216	488
181	136	317	227	176	403	273	217	490
182	137	319	228	177	405	274	218	492
183	138	321	229	178	407	275	218	493
184	138	322	230	179	409	276	219	495
185	139	324	231	179	410	277	220	497
186	140	326	232	180	412	278	221	499
187	141	328	233	181	414	279	222	501

Colony count			Colony count			Colony count		
Upper	**Lower**	Sum	**Upper**	**Lower**	Sum	**Upper**	**Lower**	Sum
280	**223**	503	**309**	**249**	558	**338**	**275**	613
281	**224**	505	**310**	**250**	560	**339**	**276**	615
282	**225**	507	**311**	**251**	562	**340**	**277**	617
283	**226**	509	**312**	**251**	563	**341**	**278**	619
284	**226**	510	**313**	**252**	565	**342**	**278**	620
285	**227**	512	**314**	**253**	567	**343**	**279**	622
286	**228**	514	**315**	**254**	569	**344**	**280**	624
287	**229**	516	**316**	**255**	571	**345**	**281**	626
288	**230**	518	**317**	**256**	573	**346**	**282**	628
289	**231**	520	**318**	**257**	575	**347**	**283**	630
290	**232**	522	**319**	**258**	577	**348**	**284**	632
291	**233**	524	**320**	**259**	579	**349**	**285**	634
292	**234**	526	**321**	**260**	581	**350**	**286**	636
293	**234**	527	**322**	**260**	582	**351**	**287**	638
294	**235**	529	**323**	**261**	584	**352**	**287**	639
295	**236**	531	**324**	**262**	586	**353**	**288**	641
296	**237**	533	**325**	**263**	588	**354**	**289**	643
297	**238**	535	**326**	**264**	590	**355**	**290**	645
298	**239**	537	**327**	**265**	592	**356**	**291**	647
299	**240**	539	**328**	**266**	594	**357**	**292**	649
300	**241**	541	**329**	**267**	596	**358**	**293**	651
301	**242**	543	**330**	**268**	598	**359**	**294**	653
302	**243**	545	**331**	**269**	600	**360**	**295**	655
303	**243**	546	**332**	**269**	601	**361**	**296**	657
304	**244**	548	**333**	**270**	603	**362**	**297**	659
305	**245**	550	**334**	**271**	605	**363**	**297**	660
306	**246**	552	**335**	**272**	607	**364**	**298**	662
307	**247**	554	**336**	**273**	609	**365**	**299**	664
308	**248**	556	**337**	**274**	611	**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}	$10^{-(x+1)}$			10^{-x}	$10^{-(x+1)}$		
Observed	Expected			Observed	Expected		
Sum/ Count	**Lower limit**	Sum/ Count	**Upper limit**	Sum/ Count	**Lower limit**	Sum/ Count	**Upper limit**
10	**0**	1,0	**4**	51	**1**	5,1	**12**
11	**0**	1,1	**5**	52	**1**	5,2	**12**
12	**0**	1,2	**5**	53	**1**	5,3	**12**
13	**0**	1,3	**5**	54	**1**	5,4	**12**
14	**0**	1,4	**5**	55	**1**	5,5	**13**
15	**0**	1,5	**6**	56	**1**	5,6	**13**
16	**0**	1,6	**6**	57	**1**	5,7	**13**
17	**0**	1,7	**6**	58	**1**	5,8	**13**
18	**0**	1,8	**6**	59	**1**	5,9	**13**
19	**0**	1,9	**6**	60	**1**	6,0	**13**
20	**0**	2,0	**7**	61	**1**	6,1	**14**
21	**0**	2,1	**7**	62	**1**	6,2	**14**
22	**0**	2,2	**7**	63	**1**	6,3	**14**
23	**0**	2,3	**7**	64	**2**	6,4	**14**
24	**0**	2,4	**7**	65	**2**	6,5	**14**
25	**0**	2,5	**8**	66	**2**	6,6	**14**
26	**0**	2,6	**8**	67	**2**	6,7	**14**
27	**0**	2,7	**8**	68	**2**	6,8	**15**
28	**0**	2,8	**8**	69	**2**	6,9	**15**
29	**0**	2,9	**8**	70	**2**	7,0	**15**
30	**0**	3,0	**8**	71	**2**	7,1	**15**
31	**0**	3,1	**9**	72	**2**	7,2	**15**
32	**0**	3,2	**9**	73	**2**	7,3	**15**
33	**0**	3,3	**9**	74	**2**	7,4	**16**
34	**0**	3,4	**9**	75	**2**	7,5	**16**
35	**1**	3,5	**9**	76	**2**	7,6	**16**
36	**1**	3,6	**9**	77	**2**	7,7	**16**
37	**1**	3,7	**10**	78	**2**	7,8	**16**
38	**1**	3,8	**10**	79	**2**	7,9	**16**
39	**1**	3,9	**10**	80	**2**	8,0	**16**
40	**1**	4,0	**10**	81	**2**	8,1	**17**
41	**1**	4,1	**10**	82	**2**	8,2	**17**
42	**1**	4,2	**11**	83	**2**	8,3	**17**
43	**1**	4,3	**11**	84	**2**	8,4	**17**
44	**1**	4,4	**11**	85	**3**	8,5	**17**
45	**1**	4,5	**11**	86	**3**	8,6	**17**
46	**1**	4,6	**11**	87	**3**	8,7	**17**
47	**1**	4,7	**11**	88	**3**	8,8	**18**
48	**1**	4,8	**11**	89	**3**	8,9	**18**
49	**1**	4,9	**12**	90	**3**	9,0	**18**
50	**1**	5,0	**12**	91	**3**	9,1	**18**

10^{-x}	$10^{-(x+1)}$			10^{-x}	$10^{-(x+1)}$		
Observed	Expected			Observed	Expected		
Sum/ Count	**Lower limit**	Sum/ Count	**Upper limit**	Sum/ Count	**Lower limit**	Sum/ Count	**Upper limit**
92	**3**	9,2	**18**	136	**6**	13,6	**24**
93	**3**	9,3	**18**	137	**6**	13,7	**24**
94	**3**	9,4	**18**	138	**6**	13,8	**25**
95	**3**	9,5	**19**	139	**6**	13,9	**25**
96	**3**	9,6	**19**	140	**6**	14,0	**25**
97	**3**	9,7	**19**	141	**6**	14,1	**25**
98	**3**	9,8	**19**	142	**6**	14,2	**25**
99	**3**	9,9	**19**	143	**6**	14,3	**25**
100	**3**	10,0	**19**	144	**6**	14,4	**25**
101	**3**	10,1	**19**	145	**6**	14,5	**26**
102	**3**	10,2	**20**	146	**6**	14,6	**26**
103	**4**	10,3	**20**	147	**6**	14,7	**26**
104	**4**	10,4	**20**	148	**6**	14,8	**26**
105	**4**	10,5	**20**	149	**6**	14,9	**26**
106	**4**	10,6	**20**	150	**6**	15,0	**26**
107	**4**	10,7	**20**	151	**6**	15,1	**26**
108	**4**	10,8	**20**	152	**7**	15,2	**27**
109	**4**	10,9	**21**	153	**7**	15,3	**27**
110	**4**	11,0	**21**	154	**7**	15,4	**27**
111	**4**	11,1	**21**	155	**7**	15,5	**27**
112	**4**	11,2	**21**	156	**7**	15,6	**27**
113	**4**	11,3	**21**	157	**7**	15,7	**27**
114	**4**	11,4	**21**	158	**7**	15,8	**27**
115	**4**	11,5	**21**	159	**7**	15,9	**27**
116	**4**	11,6	**22**	160	**7**	16,0	**28**
117	**4**	11,7	**22**	161	**7**	16,1	**28**
118	**4**	11,8	**22**	162	**7**	16,2	**28**
119	**4**	11,9	**22**	163	**7**	16,3	**28**
120	**5**	12,0	**22**	164	**7**	16,4	**28**
121	**5**	12,1	**22**	165	**7**	16,5	**28**
122	**5**	12,2	**22**	166	**7**	16,6	**28**
123	**5**	12,3	**23**	167	**8**	16,7	**29**
124	**5**	12,4	**23**	168	**8**	16,8	**29**
125	**5**	12,5	**23**	169	**8**	16,9	**29**
126	**5**	12,6	**23**	170	**8**	17,0	**29**
127	**5**	12,7	**23**	171	**8**	17,1	**29**
128	**5**	12,8	**23**	172	**8**	17,2	**29**
129	**5**	12,9	**23**	173	**8**	17,3	**29**
130	**5**	13,0	**24**	174	**8**	17,4	**29**
131	**5**	13,1	**24**	175	**8**	17,5	**30**
132	**5**	13,2	**24**	176	**8**	17,6	**30**
133	**5**	13,3	**24**	177	**8**	17,7	**30**
134	**5**	13,4	**24**	178	**8**	17,8	**30**
135	**5**	13,5	**24**	179	**8**	17,9	**30**

10^{-x}	$10^{-(x+1)}$			10^{-x}	$10^{-(x+1)}$		
Observed	Expected			Observed	Expected		
Sum/ Count	Lower limit	Sum/ Count	Upper limit	Sum/ Count	Lower limit	Sum/ Count	Upper limit
180	8	18,0	30	224	11	22,4	36
181	8	18,1	30	225	12	22,5	36
182	9	18,2	31	226	12	22,6	36
183	9	18,3	31	227	12	22,7	36
184	9	18,4	31	228	12	22,8	36
185	9	18,5	31	229	12	22,9	37
186	9	18,6	31	230	12	23,0	37
187	9	18,7	31	231	12	23,1	37
188	9	18,8	31	232	12	23,2	37
189	9	18,9	31	233	12	23,3	37
190	9	19,0	32	234	12	23,4	37
191	9	19,1	32	235	12	23,5	37
192	9	19,2	32	236	12	23,6	38
193	9	19,3	32	237	12	23,7	38
194	9	19,4	32	238	12	23,8	38
195	9	19,5	32	239	13	23,9	38
196	10	19,6	32	240	13	24,0	38
197	10	19,7	32	241	13	24,1	38
198	10	19,8	33	242	13	24,2	38
199	10	19,9	33	243	13	24,3	38
200	10	20,0	33	244	13	24,4	39
201	10	20,1	33	245	13	24,5	39
202	10	20,2	33	246	13	24,6	39
203	10	20,3	33	247	13	24,7	39
204	10	20,4	33	248	13	24,8	39
205	10	20,5	34	249	13	24,9	39
206	10	20,6	34	250	13	25,0	39
207	10	20,7	34	251	13	25,1	39
208	10	20,8	34	252	14	25,2	40
209	10	20,9	34	253	14	25,3	40
210	10	21,0	34	254	14	25,4	40
211	11	21,1	34	255	14	25,5	40
212	11	21,2	34	256	14	25,6	40
213	11	21,3	35	257	14	25,7	40
214	11	21,4	35	258	14	25,8	40
215	11	21,5	35	259	14	25,9	40
216	11	21,6	35	260	14	26,0	41
217	11	21,7	35	261	14	26,1	41
218	11	21,8	35	262	14	26,2	41
219	11	21,9	35	263	14	26,3	41
220	11	22,0	35	264	14	26,4	41
221	11	22,1	36	265	14	26,5	41
222	11	22,2	36	266	15	26,6	41
223	11	22,3	36	267	15	26,7	41

10^{-x}	$10^{-(x+1)}$			10^{-x}	$10^{-(x+1)}$		
Observed	Expected			Observed	Expected		
Sum/	**Lower limit**	Sum/	**Upper limit**	Sum/	**Lower limit**	Sum/	**Upper limit**
Count		Count		Count		Count	
268	**15**	26,8	**42**	312	**18**	31,2	**47**
269	**15**	26,9	**42**	313	**18**	31,3	**47**
270	**15**	27,0	**42**	314	**18**	31,4	**47**
271	**15**	27,1	**42**	315	**18**	31,5	**47**
272	**15**	27,2	**42**	316	**18**	31,6	**48**
273	**15**	27,3	**42**	317	**18**	31,7	**48**
274	**15**	27,4	**42**	318	**18**	31,8	**48**
275	**15**	27,5	**42**	319	**19**	31,9	**48**
276	**15**	27,6	**43**	320	**19**	32,0	**48**
277	**15**	27,7	**43**	321	**19**	32,1	**48**
278	**15**	27,8	**43**	322	**19**	32,2	**48**
279	**16**	27,9	**43**	323	**19**	32,3	**48**
280	**16**	28,0	**43**	324	**19**	32,4	**49**
281	**16**	28,1	**43**	325	**19**	32,5	**49**
282	**16**	28,2	**43**	326	**19**	32,6	**49**
283	**16**	28,3	**43**	327	**19**	32,7	**49**
284	**16**	28,4	**44**	328	**19**	32,8	**49**
285	**16**	28,5	**44**	329	**19**	32,9	**49**
286	**16**	28,6	**44**	330	**19**	33,0	**49**
287	**16**	28,7	**44**	331	**19**	33,1	**49**
288	**16**	28,8	**44**	332	**20**	33,2	**50**
289	**16**	28,9	**44**	333	**20**	33,3	**50**
290	**16**	29,0	**44**	334	**20**	33,4	**50**
291	**16**	29,1	**44**	335	**20**	33,5	**50**
292	**16**	29,2	**45**	336	**20**	33,6	**50**
293	**17**	29,3	**45**	337	**20**	33,7	**50**
294	**17**	29,4	**45**	338	**20**	33,8	**50**
295	**17**	29,5	**45**	339	**20**	33,9	**50**
296	**17**	29,6	**45**	340	**20**	34,0	**51**
297	**17**	29,7	**45**	341	**20**	34,1	**51**
298	**17**	29,8	**45**	342	**20**	34,2	**51**
299	**17**	29,9	**45**	343	**20**	34,3	**51**
300	**17**	30,0	**46**	344	**20**	34,4	**51**
301	**17**	30,1	**46**	345	**20**	34,5	**51**
302	**17**	30,2	**46**	346	**21**	34,6	**51**
303	**17**	30,3	**46**	347	**21**	34,7	**51**
304	**17**	30,4	**46**	348	**21**	34,8	**52**
305	**17**	30,5	**46**	349	**21**	34,9	**52**
306	**18**	30,6	**46**	350	**21**	35,0	**52**
307	**18**	30,7	**46**	351	**21**	35,1	**52**
308	**18**	30,8	**47**	352	**21**	35,2	**52**
309	**18**	30,9	**47**	353	**21**	35,3	**52**
310	**18**	31,0	**47**	354	**21**	35,4	**52**
311	**18**	31,1	**47**	355	**21**	35,5	**52**

10^{-x}	$10^{-(x+1)}$			10^{-x}	$10^{-(x+1)}$		
Observed	Expected			Observed	Expected		
Sum/ Count	Lower limit	Sum/ Count	Upper limit	Sum/ Count	Lower limit	Sum/ Count	Upper limit
356	21	35,6	53	400	25	40,0	58
357	21	35,7	53	401	25	40,1	58
358	22	35,8	53	402	25	40,2	58
359	22	35,9	53	403	25	40,3	58
360	22	36,0	53	404	25	40,4	58
361	22	36,1	53	405	25	40,5	58
362	22	36,2	53	406	25	40,6	59
363	22	36,3	53	407	25	40,7	59
364	22	36,4	53	408	25	40,8	59
365	22	36,5	54	409	25	40,9	59
366	22	36,6	54	410	26	41,0	59
367	22	36,7	54	411	26	41,1	59
368	22	36,8	54	412	26	41,2	59
369	22	36,9	54	413	26	41,3	59
370	22	37,0	54	414	26	41,4	60
371	23	37,1	54	415	26	41,5	60
372	23	37,2	54	416	26	41,6	60
373	23	37,3	55	417	26	41,7	60
374	23	37,4	55	418	26	41,8	60
375	23	37,5	55	419	26	41,9	60
376	23	37,6	55	420	26	42,0	60
377	23	37,7	55	421	26	42,1	60
378	23	37,8	55	422	27	42,2	61
379	23	37,9	55	423	27	42,3	61
380	23	38,0	55	424	27	42,4	61
381	23	38,1	56	425	27	42,5	61
382	23	38,2	56	426	27	42,6	61
383	23	38,3	56	427	27	42,7	61
384	24	38,4	56	428	27	42,8	61
385	24	38,5	56	429	27	42,9	61
386	24	38,6	56	430	27	43,0	62
387	24	38,7	56	431	27	43,1	62
388	24	38,8	56	432	27	43,2	62
389	24	38,9	57	433	27	43,3	62
390	24	39,0	57	434	27	43,4	62
391	24	39,1	57	435	28	43,5	62
392	24	39,2	57	436	28	43,6	62
393	24	39,3	57	437	28	43,7	62
394	24	39,4	57	438	28	43,8	62
395	24	39,5	57	439	28	43,9	63
396	24	39,6	57	440	28	44,0	63
397	25	39,7	58	441	28	44,1	63
398	25	39,8	58	442	28	44,2	63
399	25	39,9	58	443	28	44,3	63

10^{-x}	$10^{-(x+1)}$			10^{-x}	$10^{-(x+1)}$		
Observed	Expected			Observed	Expected		
Sum/ Count	**Lower limit**	Sum/ Count	**Upper limit**	Sum/ Count	**Lower limit**	Sum/ Count	**Upper limit**
444	**28**	44,4	**63**	488	**32**	48,8	**68**
445	**28**	44,5	**63**	489	**32**	48,9	**69**
446	**28**	44,6	**63**	490	**32**	49,0	**69**
447	**29**	44,7	**64**	491	**32**	49,1	**69**
448	**29**	44,8	**64**	492	**32**	49,2	**69**
449	**29**	44,9	**64**	493	**32**	49,3	**69**
450	**29**	45,0	**64**	494	**32**	49,4	**69**
451	**29**	45,1	**64**	495	**32**	49,5	**69**
452	**29**	45,2	**64**	496	**32**	49,6	**69**
453	**29**	45,3	**64**	497	**33**	49,7	**70**
454	**29**	45,4	**64**	498	**33**	49,8	**70**
455	**29**	45,5	**65**	499	**33**	49,9	**70**
456	**29**	45,6	**65**	500	**33**	50,0	**70**
457	**29**	45,7	**65**	501	**33**	50,1	**70**
458	**29**	45,8	**65**	502	**33**	50,2	**70**
459	**29**	45,9	**65**	503	**33**	50,3	**70**
460	**30**	46,0	**65**	504	**33**	50,4	**70**
461	**30**	46,1	**65**	505	**33**	50,5	**70**
462	**30**	46,2	**65**	506	**33**	50,6	**71**
463	**30**	46,3	**65**	507	**33**	50,7	**71**
464	**30**	46,4	**66**	508	**33**	50,8	**71**
465	**30**	46,5	**66**	509	**33**	50,9	**71**
466	**30**	46,6	**66**	510	**34**	51,0	**71**
467	**30**	46,7	**66**	511	**34**	51,1	**71**
468	**30**	46,8	**66**	512	**34**	51,2	**71**
469	**30**	46,9	**66**	513	**34**	51,3	**71**
470	**30**	47,0	**66**	514	**34**	51,4	**72**
471	**30**	47,1	**66**	515	**34**	51,5	**72**
472	**31**	47,2	**67**	516	**34**	51,6	**72**
473	**31**	47,3	**67**	517	**34**	51,7	**72**
474	**31**	47,4	**67**	518	**34**	51,8	**72**
475	**31**	47,5	**67**	519	**34**	51,9	**72**
476	**31**	47,6	**67**	520	**34**	52,0	**72**
477	**31**	47,7	**67**	521	**34**	52,1	**72**
478	**31**	47,8	**67**	522	**35**	52,2	**73**
479	**31**	47,9	**67**	523	**35**	52,3	**73**
480	**31**	48,0	**68**	524	**35**	52,4	**73**
481	**31**	48,1	**68**	525	**35**	52,5	**73**
482	**31**	48,2	**68**	526	**35**	52,6	**73**
483	**31**	48,3	**68**	527	**35**	52,7	**73**
484	**31**	48,4	**68**	528	**35**	52,8	**73**
485	**32**	48,5	**68**	529	**35**	52,9	**73**
486	**32**	48,6	**68**	530	**35**	53,0	**73**
487	**32**	48,7	**68**	531	**35**	53,1	**74**

10^{-x}	$10^{-(x+1)}$			10^{-x}	$10^{-(x+1)}$		
Observed	Expected			Observed	Expected		
Sum/ Count	Lower limit	Sum/ Count	Upper limit	Sum/ Count	Lower limit	Sum/ Count	Upper limit
532	35	53,2	74	576	39	57,6	79
533	35	53,3	74	577	39	57,7	79
534	36	53,4	74	578	39	57,8	79
535	36	53,5	74	579	39	57,9	79
536	36	53,6	74	580	39	58,0	79
537	36	53,7	74	581	39	58,1	79
538	36	53,8	74	582	39	58,2	80
539	36	53,9	75	583	40	58,3	80
540	36	54,0	75	584	40	58,4	80
541	36	54,1	75	585	40	58,5	80
542	36	54,2	75	586	40	58,6	80
543	36	54,3	75	587	40	58,7	80
544	36	54,4	75	588	40	58,8	80
545	36	54,5	75	589	40	58,9	80
546	36	54,6	75	590	40	59,0	81
547	37	54,7	75	591	40	59,1	81
548	37	54,8	76	592	40	59,2	81
549	37	54,9	76	593	40	59,3	81
550	37	55,0	76	594	40	59,4	81
551	37	55,1	76	595	41	59,5	81
552	37	55,2	76	596	41	59,6	81
553	37	55,3	76	597	41	59,7	81
554	37	55,4	76	598	41	59,8	81
555	37	55,5	76	599	41	59,9	82
556	37	55,6	77	600	41	60,0	82
557	37	55,7	77	601	41	60,1	82
558	37	55,8	77	602	41	60,2	82
559	38	55,9	77	603	41	60,3	82
560	38	56,0	77	604	41	60,4	82
561	38	56,1	77	605	41	60,5	82
562	38	56,2	77	606	41	60,6	82
563	38	56,3	77	607	42	60,7	83
564	38	56,4	77	608	42	60,8	83
565	38	56,5	78	609	42	60,9	83
566	38	56,6	78	610	42	61,0	83
567	38	56,7	78	611	42	61,1	83
568	38	56,8	78	612	42	61,2	83
569	38	56,9	78	613	42	61,3	83
570	38	57,0	78	614	42	61,4	83
571	39	57,1	78	615	42	61,5	83
572	39	57,2	78	616	42	61,6	84
573	39	57,3	79	617	42	61,7	84
574	39	57,4	79	618	42	61,8	84
575	39	57,5	79	619	43	61,9	84