



Proficiency test SPIL-2 (2025)

**Nitrogen parameters in wastewater
(untreated sewage)**

**Quality Documentation
July 2025**

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1 INTRODUCTION

A proficiency test on the analysis of nitrogen parameters in wastewater was conducted on 15 May 2025. The proficiency test was organised by Eurofins Miljø A/S.

The present report contains Eurofins' documentation for the quality of the proficiency test. Results of the proficiency test including data from participating laboratories and statistical analysis of these data were issued in a report to all participants /1/ on 19 June 2025.

2 FEATURES OF THE PROFICIENCY TEST

Participants in the proficiency test were a total of 35 laboratories from Denmark, Spain and Sweden.

The closing date for submission of results was 30 May 2025. All participants except laboratory no. 4 had submitted their results before the deadline.

2.1 Sample preparation

The parameters covered in the proficiency test are listed in Table 2 as are the abbreviations used in this report.

Four samples were dispatched for the proficiency test. The samples were sample pairs covering the parameters as described in Table 1. The matrix of the samples represented wastewater, in this case untreated sewage. Sample preparation is described in Appendix A.

Table 1 Samples in the proficiency test

Sample name	Parameters
A1/B1	TN, NH ₄ , NO ₂₊₃ , NO ₃ , γ ₂₅
A2/B2	pH

2.2 Statistical analysis of participants' data

A split-level design was used. The data analysis was performed in accordance with ISO 5725: "Accuracy (trueness and precision) of measurement methods and results" (2019) /2/, ISO 13528:2022 /3/ and as described in detail in Spliid (1992) /4/. A short introduction to the statistics and a list of symbols and abbreviations used is given in Eurofins document "Schedule for a proficiency test", which is available at Eurofins' home page /5/

The statistical model used is based on the assumption that the variances for the two samples in a sample pair are identical. The assumption was tested (F-test, 95% confidence level) and the result was that the two variances may be assumed to be identical for all parameters.

2.3 Assigned and spike value

An overview of the concentrations in the samples (the assigned values) and the difference in concentration between the two samples of a sample pair (spike value) are shown in Table 2 compared to the range of concentrations normally encountered in untreated sewage. The table also gives the expanded uncertainty of the assigned values. Assigned values, spike values and uncertainty of the assigned values were calculated in accordance to ISO 13528:2022 /3/. The Uncertainty of the assigned values are the expanded uncertainty with coverage factor, $k = 2$.

Table 2 Assigned and spike value

Parameter	Abbreviation	Unit	Typical Range	Assigned value	Uncertainty of assigned value	Spike value
Total nitrogen	TN	mg/L N	30 - 80	66	2.2	10
Ammonium	NH ₄	mg/L N	20 – 50	48.0	0.74	6.4
Nitrite+nitrate	NO ₂₊₃	mg/L N	< 5	0.69	0.074	0.17
Nitrate	NO ₃	mg/L N	< 5	0.97	0.075	0.17
Conductivity	γ ₂₅	mS/m	50 – 2000	116.2	0.51	122.4
pH			6 – 9	7.07	0.032	0

2.3.1 Assigned and spike values

The content of each parameter in each sample is given an assigned value for the sample with the lower content and a spike value, the spike value being the difference in concentration between the two samples of the sample pair.

In order to ensure optimal use of the data, the assigned value is calculated as the average of the median for both samples in the sample pair after subtraction of the spike value. The spike values are calculated from sample preparation except for conductivity and pH, where the spike value is the difference between median values for the two samples in the sample pair.

The assigned value for TN is operationally defined and is a consensus value based upon the median for method no. 1-5 + lab 34 (method no. 9). A list of method identification numbers is found in the report to participants /1/.

Assigned values for NH₄, NO₂₊₃, NO₃, conductivity and pH are consensus values for all laboratories based on the median.

2.3.2 Test of spike values

A comparison was made (t-test, 95% confidence level) between the spike value and the difference in concentration between the two samples in the sample pair found from the laboratories' results, see Appendix B.

The test showed no significant difference between the two for NH₄, NO₂₊₃, conductivity and pH. The test revealed a significant difference between the two for TN and NO₃. However, the difference is numerically small and has insignificant influence on the general quality of analyses estimated from the data as well as on the evaluation of accuracy of participating laboratories.

2.3.3 Test of assigned values

The assigned value and the average of the results obtained from all laboratories were also compared (t-test, 95% confidence level), see Appendix C. The test showed no significant difference between the two and the control of assigned value at Eurofins confirmed the value (Appendix D).

3 **HOMOGENEITY AND STABILITY OF SAMPLES**

The homogeneity and stability of samples were tested using the following parameters as indicators:

NH ₄	Combined homogeneity and stability test
NO ₃	Combined homogeneity and stability test
pH	Combined homogeneity and stability test

The results of control measurements are shown in Appendix E. The appendix also gives the results of the statistical evaluation of the control data. The data are analysed by analysis of variance (ANOVA) giving:

1. the standard deviation/variance for replicates (the contribution from analytical variability),
2. the between bottle standard deviation/variance (the contribution from heterogeneity) and
3. the between days concentration difference (the contribution from instability).

Homogeneity is evaluated by comparing the between bottle variance to $0.3 \cdot \hat{\sigma}$ specified by the Danish EPA /6/, whereas the stability is evaluated by comparing the concentration change of the samples to $0.3 \cdot \hat{\sigma}$ or $0.3 \cdot \hat{\sigma} + 2\sqrt{u_x^2 + u_y^2}$ where the precision of the measurement method contribute to the inability to meet the criterion. This test ensures that heterogeneity and instability will not have negative influence on the evaluation of participant performance /3/.

The appendix also shows the standard deviation within and between laboratories from the proficiency test to allow comparison between tests performed and average quality from participating laboratories.

The tests for stability and homogeneity show that the samples are stable and homogeneous.

4 CONCLUSION

The quality control performed, including test of sample stability and homogeneity as well as test of recovery of spike and assigned values, shows that the samples and their assigned values are suitable for testing the proficiency of the participating laboratories for all parameters. The results are also suitable for estimation of the general quality of analyses among all participating laboratories.

For TN and NO₃ the participants could not recover the spike value. The difference between the calculated spike value and that found by the participants is small and the influence on evaluation of participant performance or estimation of general quality of analyses is insignificant.

5 REFERENCES

- /1/ Eurofins A/S, *Proficiency test SPIL-2 (2025)*, Report to participants, June 2025.
- /2/ ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*, 2019.
- /3/ ISO 13528, *Statistical methods for use in proficiency testing by interlaboratory comparison*, 2022.
- /4/ Spliid, H., *Procedure and analysis of data for proficiency tests and environmental analyses*, Report to Danish Environmental Protection Agency, 1994 (in Danish).
- /5/ Eurofins A/S, *Schedule for a proficiency test*, document may be downloaded from www.eurofins.dk/proficiencytest.
- /6/ The Ministry of the Environment and Gender Equality's Statutory Order 811 of 19/06/2024 on quality requirements for environmental measurements (in Danish).

A N N E X E S

APPENDIX A SAMPLE PREPARATION

Stock solution	Prepared from	Concentration
Stock TN	50.0012 g Disodium edetate, 2H ₂ O milli-Q water up to 1000.1 g	TN: 376.3 mg/kg N
Stock NH ₄	15.000 g Ammonium chloride (NH ₄ Cl) milli-Q water up to 1000.2 g	NH ₄ : 392.8 mg/kg N
Stock NO ₃	2.9994 g Potassium nitrate (KNO ₃) milli-Q water up to 1000.2 g	NO ₃ : 41.6 mg/kg N

Sample	Sample prepared from	TN mg/L N	NH ₄ mg/L N	NO ₃ mg/L N
A1	500.1g stock TN 199.99 g stock NH ₄ 200.02 g stock NO ₃ Filtered water from Holsted sewage treatment plant up to 120.01 kg	a+58.92	b+37.55	c+1.02
B1	50.0004 g stock TN 100.0002 g stock NH ₄ 25.0038 g stock NO ₃ Sample A1 up to 60.0 kg	0,997· (a+58.92) + 9.65	0.997· (b+37.55) + 6.41	0.997· (c+1.02) + 0.17

Sample	Sample prepared from	pH
A2/B2	Filtered untreated sewage from Holsted sewage treatment plant	d

ANNEX B CONTROL OF SPIKE VALUES

Total Nitrogen, mg/L N

Control of differences within sample pairs

Laboratory	Difference AB	Outlier
1	-0.5	
2	3.3	
3	-0.1	
5	1.1	
6	4.1	
8	0.3	
9	-0.8	
10	2.0	
11	1.6	
12	1.9	
13	2.4	
14	0.6	
15	-0.1	
16	1.1	
17	3.8	
18	0.5	
19	0.6	
20	1.1	
21	0.3	
22	1.1	
23	1.0	
24	-14.1	C
25	0.8	
27	1.7	
28	-2.8	
29	0.8	
30	4.4	
31	1.0	
33	-2.2	
34	4.3	
35	-0.5	

No of labs, p	30
No of repl, n	2
d	1.0916
s ²	2.9668
s	1.7224
t	3.4712 **
Sign. level 99.9%	3.6594
Sign. level 99%	2.7564
Sign. level 95%	2.0452

** denotes that there is a significant difference (t-test, 1 %-level)

C denotes a Cochran outlier

Ammonium, mg/L N

Control of differences within sample pairs

Laboratory	Difference AB	Outlier
1	0.50	
2	-0.60	
3	0.90	
4	-2.32	
5	0.50	
6	1.00	
8	-0.70	
9	-0.00	
11	-0.60	
12	-2.00	
13	-1.00	
14	-0.70	
15	-0.34	
17	-1.90	
18	-1.40	
19	0.00	
21	-8.80	C
22	-0.90	
24	0.20	
25	-0.20	
27	1.00	
29	-2.00	
30	-4.00	
31	-0.10	
32	0.87	
33	2.90	
34	0.30	
35	-0.58	

No of labs, p	27
No of repl, n	2
d	-0.4139
s ²	1.8053
s	1.3436
t	-1.6006
Sign. level 99.9%	3.7066
Sign. level 99%	2.7787
Sign. level 95%	2.0555

No test statistics were found to be significant

C denotes a Cochran outlier

Nitrite+Nitrate, mg/L N

Control of differences within sample pairs

Laboratory	Difference AB	Outlier
12	0.010	
15	0.030	
27	-0.040	
29	-0.037	
32	0.166	
35	-0.020	

No of labs, p	6
No of repl, n	2
d	0.0182
s ²	0.0060
s	0.0775
t	0.5755
Sign. level 99.9%	6.8688
Sign. level 99%	4.0321
Sign. level 95%	2.5706

No test statistics were found to be significant

Nitrate, mg/L N

Control of differences within sample pairs

Laboratory	Difference AB	Outlier
1	0.035	
2	0.115	
3	-0.006	
5	0.060	
9	0.050	
11	0.080	
12	0.010	
14	-0.050	
15	0.033	
17	0.029	
18	0.040	
21	0.050	
22	-0.020	
24	0.081	
27	-0.040	
30	0.086	
31	0.040	
33	0.060	
34	0.090	G

No of labs, p	18
No of repl, n	2
d	0.0363
s ²	0.0019
s	0.0441
t	3.4861 **
Sign. level 99.9%	3.9651
Sign. level 99%	2.8982
Sign. level 95%	2.1098

** denotes that there is a significant difference (t-test, 1 %-level)

G denotes a Grubbs outlier

Conductivity, mS/m

Control of differences within sample pairs

Laboratory	Difference AB	Outlier
2	-0.20	
3	-3.00	C
4	0.28	
5	0.00	
6	0.20	
7	-0.10	
8	1.00	
10	-1.40	
12	0.20	
14	0.10	
18	0.10	
20	-0.30	
22	1.20	
24	0.80	G
28	0.20	
29	0.30	
30	-0.80	
31	0.30	
34	-0.10	

No of labs, p	17
No of repl, n	2
d	0.0576
s ²	0.3457
s	0.5880
t	0.4042
Sign. level 99.9%	4.0150
Sign. level 99%	2.9208
Sign. level 95%	2.1199

No test statistics were found to be significant

C denotes a Cochran outlier

G denotes a Cochran outlier

pH

Control of differences within sample pairs

Laboratory	Difference AB	Outlier
1	-0.130	
2	0.013	
3	0.080	
5	-0.030	
6	-0.010	
7	0.010	
8	0.020	
9	-0.030	
10	0.040	
11	-0.020	
12	0.000	
13	-0.010	
14	0.035	G
15	0.050	
16	0.000	
17	0.030	
18	-0.020	
20	0.040	
21	-0.240	C
22	0.010	
23	0.061	
24	0.116	
27	0.010	
28	0.020	
29	-0.010	
30	-0.020	
31	0.000	
32	-0.060	
33	-0.190	C
34	0.010	
35	0.040	

No of labs, p	28
No of repl, n	2
d	0.0075
s ²	0.0021
s	0.0453
t	0.8764
Sign. level 99.9%	3.6896
Sign. level 99%	2.7707
Sign. level 95%	2.0518

No test statistics were found to be significant

C denotes a Cochran outlier

G denotes a Cochran outlier

ANNEX C CONTROL OF RECOVERY

Total Nitrogen, mg/L N

Control of recovery, average of results

Laboratory	Sample pair AB	Outlier
1	67.4	
2	56.5	
3	60.7	
5	64.8	
6	60.0	
8	68.6	
9	60.7	
10	68.9	
11	68.2	
12	67.8	
13	59.4	
14	65.0	
15	63.9	
16	69.2	
17	65.0	
18	71.7	
19	66.9	
20	64.7	
21	68.9	
22	66.4	
23	64.7	
24	54.2	C
25	65.5	
27	65.9	
28	69.0	
29	65.9	
30	61.4	
31	67.5	
33	67.6	
34	64.6	
35	67.8	

No of labs, p	30
No of repl, n	2
m	65.4699
s ²	11.9718
s	3.4600
Assigned value, μ	66
Recovery, %	99.2
t	-0.8391
Sign. level 99.9%	3.6594
Sign. level 99%	2.7564
Sign. level 95%	2.0452

No test statistics were found to be significant

C denotes a Cochran outlier

Ammonium, mg/L N

Control of recovery, average of results

Laboratory	Sample pair AB	Outlier
1	49.55	
2	46.90	
3	44.30	
4	50.67	
5	48.05	
6	49.00	
8	48.65	
9	45.80	
11	46.80	
12	48.80	
13	50.90	
14	45.65	
15	47.07	
17	48.45	
18	49.40	
19	48.40	
21	46.70	C
22	46.85	
24	45.00	
25	48.30	
27	47.90	
29	50.20	
30	42.40	
31	46.35	
32	48.79	
33	44.55	
34	49.45	
35	47.86	

No of labs, p	27
No of repl, n	2
m	47.6312
s ²	4.2392
s	2.0589
Assigned value, μ	48.0
Recovery, %	99.2
t	-0.9309
Sign. level 99.9%	3.7066
Sign. level 99%	2.7787
Sign. level 95%	2.0555

No test statistics were found to be significant

C denotes a Cochran outlier

Nitrite+Nitrate, mg/L N

Control of recovery, average of results

Laboratory	Sample pair AB	Outlier
12	0.705	
15	0.865	
27	0.600	
29	0.714	
32	-0.041	
35	0.680	

No of labs, p	6
No of repl, n	2
m	0.5872
s ²	0.1020
s	0.3193
Assigned value, μ	0.69
Recovery, %	85.1
t	-0.7889
Sign. level 99.9%	6.8688
Sign. level 99%	4.0321
Sign. level 95%	2.5706

No test statistics were found to be significant

Nitrate, mg/L N

Control of recovery, average of results

Laboratory	Sample pair AB	Outlier
1	0.888	
2	0.575	
3	0.937	
5	1.080	
9	0.828	
11	1.070	
12	0.705	
14	1.255	
15	0.856	
17	0.935	
18	1.130	
21	1.075	
22	1.030	
24	0.891	
27	0.600	
30	0.953	
31	0.970	
33	0.970	
34	2.945	G

No of labs, p	18
No of repl, n	2
m	0.9304
s ²	0.0310
s	0.1761
Assigned value, μ	0.97
Recovery, %	95.9
t	-0.9542
Sign. level 99.9%	3.9651
Sign. level 99%	2.8982
Sign. level 95%	2.1098

No test statistics were found to be significant

G denotes a Grubbs outlier

Conductivity, mS/m

Control of recovery, average of results

Laboratory	Sample pair AB	Outlier
2	116.20	
3	109.20	C
4	115.45	
5	116.50	
6	116.30	
7	118.35	
8	115.70	
10	113.00	
12	116.90	
14	116.95	
18	116.75	
20	116.25	
22	116.40	
24	93.80	G
28	116.20	
29	113.45	
30	117.40	
31	115.15	
34	116.25	

No of labs, p	17
No of repl, n	2
m	116.0706
s ²	1.6872
s	1.2989
Assigned value, μ	116.2
Recovery, %	99.9
t	-0.4108
Sign. level 99.9%	4.0150
Sign. level 99%	2.9208
Sign. level 95%	2.1199

No test statistics were found to be significant

C denotes a Cochran outlier

G denotes a Grubbs outlier

pH

Control of recovery, average of results

Laboratory	Sample pair AB	Outlier
1	7.225	
2	6.977	
3	7.150	
5	7.045	
6	7.015	
7	7.045	
8	7.120	
9	6.955	
10	7.010	
11	7.080	
12	7.100	
13	7.285	
14	6.688	G
15	7.225	
16	7.010	
17	7.085	
18	7.180	
20	7.170	
21	6.690	C
22	7.185	
23	7.219	
24	7.124	
27	7.075	
28	7.020	
29	7.015	
30	7.010	
31	7.060	
32	6.970	
33	7.255	C
34	7.015	
35	7.050	

No of labs, p	28
No of repl, n	2
m	7.0864
s ²	0.0079
s	0.0889
Assigned value, μ	7.07
Recovery, %	100.2
t	0.9760
Sign. level 99.9%	3.6896
Sign. level 99%	2.7707
Sign. level 95%	2.0518

No test statistics were found to be significant

C denotes a Cochran outlier

G denotes a Grubbs outlier

APPENDIX D CONCENTRATION LEVEL

Parameter	Unit	Sample	Bottle no.	I	II	Bottle Average	Sample	Assigned	Spike	
							Average	value	Measured	Assigned
Total nitrogen	mg/L N	A1	8	65,3	65,2	65,3	66,8	66	9,5	10
			22	68,0	68,1	68,1				
			44	67,1	67,1	67,1				
		B1	3	76,9	77,0	77,0	76,3	76		
			43	77,1	77,1	77,1				
			55	74,8	74,8	74,8				
Ammonium	mg/L N	A1	8	47,8	48,0	47,9	49,0	48	7,2	6,4
			22	49,8	49,8	49,8				
			44	49,0	49,6	49,3				
		B1	3	55,4	55,0	55,2	56,2	54,4		
			43	55,0	55,6	55,3				
			55	58,2	58,2	58,2				
Nitrite+nitrate / nitrate	mg/L N	A1	8	1,19	1,20	1,20	1,19	0,97	0,06	0,17
			22	1,20	1,19	1,20				
			44	1,18	1,17	1,18				
		B1	3	1,26	1,26	1,26	1,25	1,14		
			43	1,26	1,25	1,25				
			55	1,26	1,25	1,25				
pH		A2	3	7,02	7,04	7,03	7,04	7,07	0,03	0
			33	7,04	7,05	7,05				
			44	7,04	7,05	7,05				
		B2	4	7,08	7,08	7,08	7,07	7,07		
			21	7,04	7,06	7,05				
			52	7,07	7,07	7,07				

APPENDIX E HOMOGENEITY AND STABILITY

PT: SPIL-2
Parameter: NH ₄
Unit: mg/L N
Sigma: 10,8

Responsible for tests: S7MS/DHBP
Approval of controltest FYE3

Homogeneity test Date: 2025-04-29

Sample	x(a)	x(b)	aver- age	sd	sd^2
1	57	57	56,7	0,000	0,000
10	57	58	57,4	0,141	0,02
13	57	57	57,0	0,141	0,020
18	57	57	56,9	0,071	0,005
22	58	58	58,0	0,141	0,020
28	58	58	57,7	0,000	0,000
35	56	57	56,5	0,071	0,005
40	56	56	55,7	0,141	0,020
47	56	56	56,2	0,000	0,000
51	57	57	56,8	0,141	0,020
57	58	58	57,8	0,000	0,000
64	58	58	57,5	0,000	0,000

For homogeneity

General average (x) 57,01
Sample average sd (s_x) 0,696
Within-sample sd (s_w): 0,096
Between-samples sd (ss): 0,6924
S_L in the Proficiency Test: 1,94
S_R in the Proficiency Test: 2,172

Stability test Date: 15/5-25 DHBP

Sample	x(a)	x(b)
3	55,4	55
43	55	55,6
55	58,2	58,2

For stability

General average (y): 56,23333
/x-y/ = 0,775

Conclusions

ss = 0,692 0.3*sigma= 3,24
/x-y/ = 0,775

Analytical Is s_w < 0,15*sigma
quality **YES**

Homogeneity: Is ss < 0.3*sigma?
 YES

Stability: /x-y/ < 0.3*sigma?
 YES

PT: SPIL-2 Parameter: NO ₃ Unit: mg/L N Sigma: 3,6
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Responsible for tests: S7MS/DHBP
 Approval of control-test: FYE3

Homogeneity test Date: 2025-04-29

Sample	x(a)	x(b)	average	sd	sd^2
1	1,2	1,2	1,2	0,007	0,000
10	1,2	1,21	1,2	0,000	0,000
13	1,2	1,2	1,2	0,000	0,000
18	1,2	1,21	1,2	0,000	0,000
22	1,2	1,24	1,2	0,000	0,000
28	1,2	1,18	1,2	0,000	0,000
35	1,2	1,21	1,2	0,007	0,000
40	1,3	1,23	1,2	0,014	0,000
47	1,2	1,22	1,2	0,000	0,000
51	1,2	1,21	1,2	0,007	0,000
57	1,2	1,25	1,2	0,007	0,000
64	1,3	1,26	1,3	0,007	0,000

For homogeneity

General average (x) 1,2
 Sample average sd (s_x) 0,022
 Within-sample sd (s_w): 0,006
 Between-samples sd (ss): 0,022
 S_L in the Proficiency Test: 0,174
 S_R in the Proficiency Test: 0,178

Stability test Date: 15/5-25 DHBP

Sample	x(a)	x(b)
3	1,24	1,24
43	1,24	1,23
55	1,24	1,23

For stability

General average (y): 1,236667
 /x-y/ = 0,017917

Conclusions

ss = 0,02 0.3*sigma= 1,08	
/x-y/ = 0,017917	
Analytical quality	Is s _w < 0,15*sigma YES
Homogeneity:	Is ss < 0.3*sigma? YES
Stability:	/x-y/ < 0.3*sigma? YES

PT: SPIL-2 Parameter: pH Unit: Sigma: 3,6
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Responsible for tests: S7MS/DHBP
Approval of controltest FYE3

Homogeneity test Date: 2025-04-29

Sample	x(a)	x(b)	aver- age	sd	sd^2
1	7,2	7,2	7,2	0,000	0,000
10	7,2	7,15	7,2	0,021	0,000
11	7,1	7,17	7,2	0,028	0,001
16	7,1	7,17	7,2	0,021	0,000
26	7,2	7,2	7,2	0,007	0,000
32	7,2	7,18	7,2	0,014	0,000
33	7,1	7,13	7,1	0,007	0,000
42	7,1	7,15	7,1	0,021	0,000
43	7,1	7,15	7,1	0,014	0,000
51	7,1	7,14	7,1	0,007	0,000
55	7,2	7,16	7,2	0,007	0,000
65	7,1	7,15	7,1	0,007	0,000

For homogeneity

General average (x) 7,2
Sample average sd (s_x) 0,024
Within-sample sd (s_w): 0,015
Between-samples sd (ss): 0,021
 S_L in the Proficiency Test: 0,086
 S_R in the Proficiency Test: 0,092

Stability test Date: 15/5-25 DHBP

Sample	x(a)	x(b)
4	7,08	7,08
21	7,04	7,06
52	7,07	7,07

For stability

General average (y): 7,066667
 $|x-y| =$ 0,090833

Conclusions

ss = 0,02 0.3*sigma= 1,08 $ x-y = 0,090833$	
Analytical quality	Is $s_w < 0,15 \cdot \sigma$ YES
Homogeneity:	Is $ss < 0.3 \cdot \sigma$? YES
Stability:	$ x-y < 0.3 \cdot \sigma$? YES