# Evaluation Report

# Eurolyser ASO Test Kit (ST0250) on Cube and Smart Analysers

## Location

Location: Eurolyser Diagnostica GmbH
Operators: Michael Gruber; Simone Wieser
Date: 08.06.2015

## Specimens

The specimens used for analysis were taken from multiple sites and were frozen human serum and EDTA whole blood samples.

## Equipment

- Eurolyser SMART Analyser: Bc14261 Bc14262
- Eurolyser CUBE Analyser: Cb 12758 Cb 12759

- Test kit ASO ST0250: LOT 0215-1

Eurolyser Diagnostica GmbH
Bayernstraße 11a
5020 Salzburg, AUSTRIA

Tel: +43 662 432100
Fax: +43 662 432100 50

# 1. Introduction and scope

Eurolyser's ASO (anti-streptolysin-O) test is used to determine post streptococcal complications, including rheumatic fever and glomerulonephritis, as well as recent streptococcal infections.

The presence and level of ASO antibodies in human blood/serum directly reflects the extent and degree of infection. Elevated levels of ASO may also be present in other conditions including tonsillitis, acute rheumatoid arthritis, scarlet fever, and various other streptococcal infections as well as in health carriers.

Eurolyser's ASO assay can be analysed from a single drop of finger blood (5µl) or serum/plasma.

**Principle:**
Latex particles coated with streptolysin-O react with ASO in the patient sample, resulting in an increase in turbidity. The absorbance is measured at 700nm and is directly proportional to the concentration of ASO.

# 2. Comparison Studies

The comparison study is based on the correlation between the results of the Eurolyser ASO and the Beckman Coulter AU5822 (Item No: 469165).

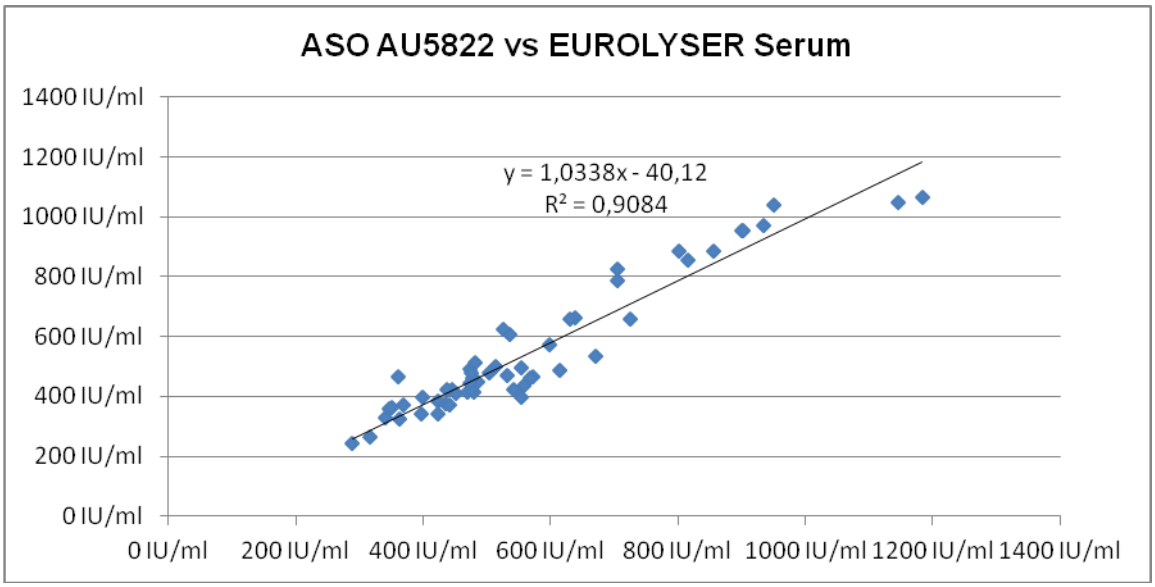
54 patient samples, both EDTA plasma and serum, have been tested.

The acceptance criterion for this comparison study is a coefficient of determination **$R^2 > 0.90$** obtained from linear regression between the Eurolyser and the AU5822 ASO.

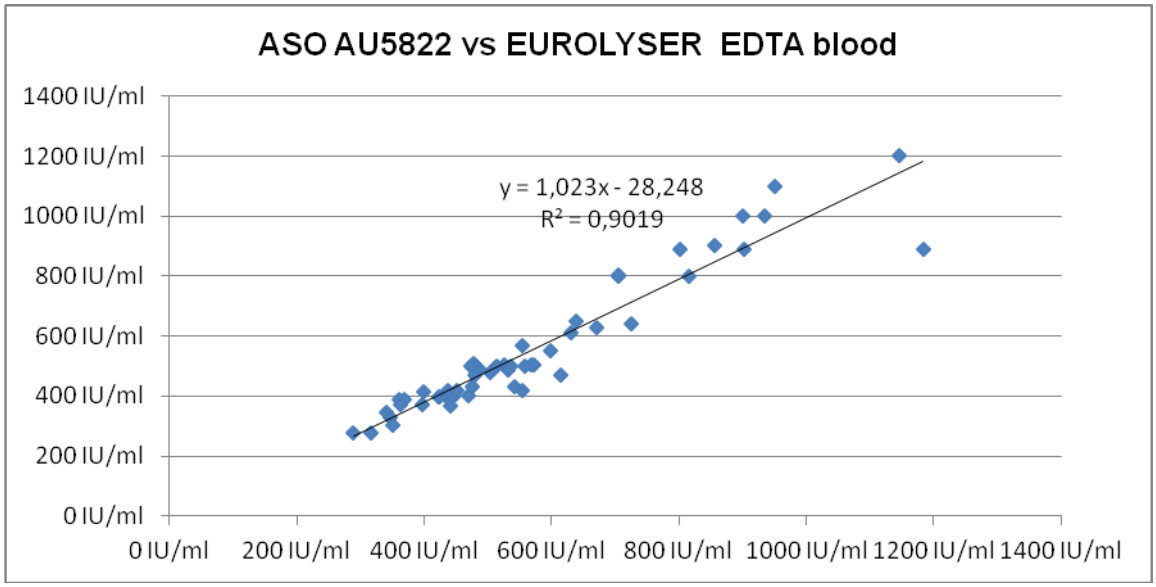
| ID | ASO Serum Beckman Coulter AU5822 (x) | ASO Eurolyser Serum (y) | ASO Eurolyser EDTA blood (y) |
|---|---|---|---|
| **1 (BC14261)** | 317 IU/ml | 264 IU/ml | 277 IU/ml |
| **2 (BC14262)** | 399 IU/ml | 397 IU/ml | 414 IU/ml |
| **3 (Cb12758)** | 289 IU/ml | 245 IU/ml | 277 IU/ml |
| **4 (Cb12759)** | 553 IU/ml | 398 IU/ml | 420 IU/ml |
| **5** | 423 IU/ml | 342 IU/ml | 399 IU/ml |
| **6** | 361 IU/ml | 465 IU/ml | 388 IU/ml |
| **7** | 422 IU/ml | 386 IU/ml | 399 IU/ml |
| **8** | 470 IU/ml | 413 IU/ml | 400 IU/ml |
| **9** | 481 IU/ml | 515 IU/ml | 500 IU/ml |
| **10** | 396 IU/ml | 340 IU/ml | 370 IU/ml |
| **11** | 435 IU/ml | 375 IU/ml | 399 IU/ml |
| **12** | 485 IU/ml | 449 IU/ml | 490 IU/ml |
| **13** | 368 IU/ml | 370 IU/ml | 390 IU/ml |
| **14** | 445 IU/ml | 422 IU/ml | 399 IU/ml |
| **15** | 473 IU/ml | 491 IU/ml | 501 IU/ml |
| **16** | 542 IU/ml | 424 IU/ml | 430 IU/ml |
| **17** | 340 IU/ml | 331 IU/ml | 344 IU/ml |
| **18** | 802 IU/ml | 884 IU/ml | 888 IU/ml |
| **19** | 638 IU/ml | 662 IU/ml | 650 IU/ml |
| **20** | 630 IU/ml | 659 IU/ml | 610 IU/ml |
| **21** | 451 IU/ml | 408 IU/ml | 420 IU/ml |
| **22** | 670 IU/ml | 533 IU/ml | 630 IU/ml |
| **23** | 553 IU/ml | 498 IU/ml | 570 IU/ml |
| **24** | 571 IU/ml | 466 IU/ml | 505 IU/ml |
| **25** | 504 IU/ml | 480 IU/ml | 477 IU/ml |
| **26** | 935 IU/ml | 969 IU/ml | 1000 IU/ml |
| **27** | 856 IU/ml | 885 IU/ml | 900 IU/ml |
| **28** | 704 IU/ml | 789 IU/ml | 801 IU/ml |
| **29** | 705 IU/ml | 826 IU/ml | 803 IU/ml |
| **30** | 614 IU/ml | 487 IU/ml | 470 IU/ml |
| **31** | 568 IU/ml | 462 IU/ml | 503 IU/ml |
| **32** | 558 IU/ml | 434 IU/ml | 499 IU/ml |
| **33** | 477 IU/ml | 460 IU/ml | 509 IU/ml |
| **34** | 725 IU/ml | 658 IU/ml | 640 IU/ml |
| **35** | 347 IU/ml | 361 IU/ml | 330 IU/ml |
| **36** | 351 IU/ml | 363 IU/ml | 302 IU/ml |
| **37** | 480 IU/ml | 414 IU/ml | 470 IU/ml |
| **38** | 536 IU/ml | 605 IU/ml | 500 IU/ml |
| **39** | 526 IU/ml | 626 IU/ml | 504 IU/ml |
| **40** | 951 IU/ml | 1040 IU/ml | 1100 IU/ml |

| ID | ASO Serum Beckman Coulter | ASO Eurolyser Serum | ASO Eurolyser EDTA blood |
|---|---|---|---|
| **41** | 476 IU/ml | 421 IU/ml | 433 IU/ml |
| **42** | 437 IU/ml | 423 IU/ml | 420 IU/ml |
| **43** | 442 IU/ml | 372 IU/ml | 366 IU/ml |
| **44** | 362 IU/ml | 324 IU/ml | 370 IU/ml |
| **45** | 513 IU/ml | 500 IU/ml | 501 IU/ml |
| **46** | 475 IU/ml | 480 IU/ml | 499 IU/ml |
| **47** | 473 IU/ml | 444 IU/ml | 499 IU/ml |
| **48** | 532 IU/ml | 472 IU/ml | 488 IU/ml |
| **49** | 816 IU/ml | 853 IU/ml | 800 IU/ml |
| **50** | 598 IU/ml | 571 IU/ml | 550 IU/ml |
| **51** | 901 IU/ml | 952 IU/ml | 1001 IU/ml |
| **52** | 1183 IU/ml | 1066 IU/ml | 1133 IU/ml |
| **53** | 1146 IU/ml | 1050 IU/ml | 1200 IU/ml |
| **54** | 902 IU/ml | 954 IU/ml | 890 IU/ml |

Samples alternatingly measured with analyser serial No.: Bc14261, Bc14262, Cb 12758, Cb 12759;




Serum SAMPLE correlation:
The result for the correlation between AU5822 and Eurolyser is the linear regression function:
y (Eurolyser) = 1.0338 x(AU5822) – 40.12 and a **$R^2$ = 0.9084**.




Whole blood (EDTA) SAMPLE correlation:
The result for the correlation between AU5822 and Eurolyser is the linear regression function:
y (Eurolyser) = 1.023 x(AU5822) – 28.248 and a **$R^2$ = 0.9019**.

## 3. Imprecision “within-run”

*a. Precision / Reproducibility*

In-house precision:
The precision of the Eurolyser ASO test was evaluated according to Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline. In the study, two controls containing 329 IU/ml and 1000 IU/ml were tested.

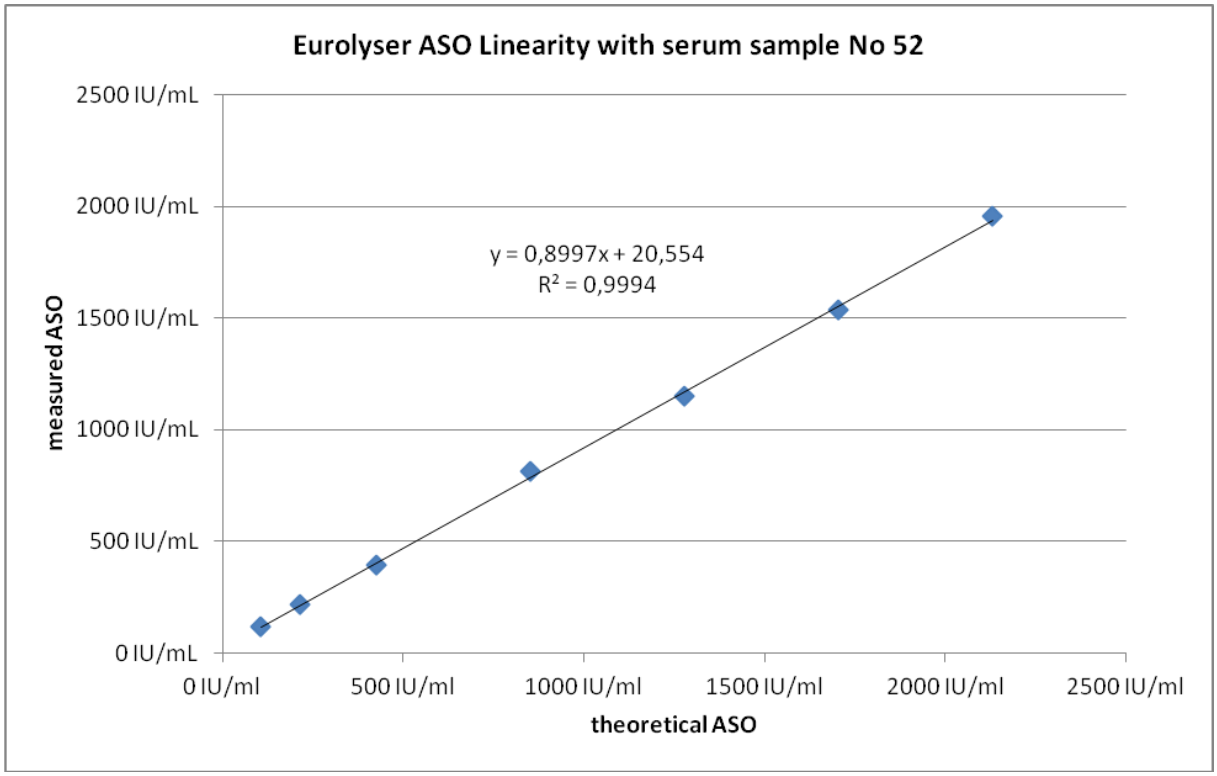
| **Day** | **Date** | **Run 1** | | **Run 2** | | **mean** | | |
|---|---|---|---|---|---|---|---|---|
| APTEC LOT S2097 | | Result 1 | Result 2 | Result 1 | Result 2 | run 1 | run 2 | daily |
| 1 | 04.05.2015 | 307 IU/ml | 333 IU/ml | 343 IU/ml | 349 IU/ml | 320 IU/ml | 346 IU/ml | 333 IU/ml |
| 2 | 05.05.2015 | 344 IU/ml | 339 IU/ml | 310 IU/ml | 356 IU/ml | 342 IU/ml | 333 IU/ml | 337 IU/ml |
| 3 | 06.05.2015 | 299 IU/ml | 298 IU/ml | 340 IU/ml | 290 IU/ml | 299 IU/ml | 315 IU/ml | 307 IU/ml |
| 4 | 07.05.2015 | 330 IU/ml | 339 IU/ml | 298 IU/ml | 299 IU/ml | 335 IU/ml | 299 IU/ml | 317 IU/ml |
| 5 | 08.05.2015 | 289 IU/ml | 299 IU/ml | 320 IU/ml | 299 IU/ml | 294 IU/ml | 310 IU/ml | 302 IU/ml |
| 6 | 11.05.2015 | 291 IU/ml | 289 IU/ml | 290 IU/ml | 339 IU/ml | 290 IU/ml | 315 IU/ml | 302 IU/ml |
| 7 | 12.05.2015 | 340 IU/ml | 343 IU/ml | 298 IU/ml | 349 IU/ml | 342 IU/ml | 324 IU/ml | 333 IU/ml |
| 8 | 13.05.2015 | 340 IU/ml | 349 IU/ml | 290 IU/ml | 360 IU/ml | 345 IU/ml | 325 IU/ml | 335 IU/ml |
| 9 | 14.05.2015 | 279 IU/ml | 278 IU/ml | 340 IU/ml | 288 IU/ml | 279 IU/ml | 314 IU/ml | 296 IU/ml |
| 10 | 15.05.2015 | 339 IU/ml | 301 IU/ml | 332 IU/ml | 344 IU/ml | 320 IU/ml | 338 IU/ml | 329 IU/ml |
| **Day** | **Date** | **Run 1** | | **Run 2** | | **mean** | | |
| Cal H Denka | | Result 1 | Result 2 | Result 1 | Result 2 | run 1 | run 2 | daily |
| 1 | 04.05.2015 | 1000 IU/ml | 1103 IU/ml | 911 IU/ml | 890 IU/ml | 1052 IU/ml | 901 IU/ml | 976 IU/ml |
| 2 | 05.05.2015 | 910 IU/ml | 905 IU/ml | 1000 IU/ml | 912 IU/ml | 908 IU/ml | 956 IU/ml | 932 IU/ml |
| 3 | 06.05.2015 | 1005 IU/ml | 933 IU/ml | 1004 IU/ml | 936 IU/ml | 969 IU/ml | 970 IU/ml | 970 IU/ml |
| 4 | 07.05.2015 | 930 IU/ml | 1088 IU/ml | 1088 IU/ml | 929 IU/ml | 1009 IU/ml | 1009 IU/ml | 1009 IU/ml |
| 5 | 08.05.2015 | 1066 IU/ml | 965 IU/ml | 1099 IU/ml | 1001 IU/ml | 1016 IU/ml | 1050 IU/ml | 1033 IU/ml |
| 6 | 11.05.2015 | 1033 IU/ml | 956 IU/ml | 1100 IU/ml | 999 IU/ml | 995 IU/ml | 1050 IU/ml | 1022 IU/ml |
| 7 | 12.05.2015 | 994 IU/ml | 1002 IU/ml | 945 IU/ml | 938 IU/ml | 998 IU/ml | 942 IU/ml | 970 IU/ml |
| 8 | 13.05.2015 | 988 IU/ml | 930 IU/ml | 967 IU/ml | 923 IU/ml | 959 IU/ml | 945 IU/ml | 952 IU/ml |
| 9 | 14.05.2015 | 1001 IU/ml | 980 IU/ml | 988 IU/ml | 1099 IU/ml | 991 IU/ml | 1044 IU/ml | 1017 IU/ml |
| 10 | 15.05.2015 | 944 IU/ml | 1004 IU/ml | 1102 IU/ml | 1190 IU/ml | 974 IU/ml | 1146 IU/ml | 1060 IU/ml |
| | | **Control low APTEC** | **Calibrator high Denka** | | | | | |
| **Data points** | | 40 | 40 | | | | | |
| **mean** | | 319 IU/ml | 994 IU/ml | | | | | |
| **Standard dev (Day/day)** | | 16.026 | 40.193 | | | | | |
| **Cv (Day-to-day) (%)** | | 5.02% | 4.04% | | | | | |

Conclusion: For two levels of ASO controls reproducibility data showed that the within-run imprecision ranged from 4.04% (994 IU/ml) to 5.02% (319 IU/ml).

## 4. Linearity Study

A linearity set was prepared by diluting (and double concentrating) a sample containing 1066 IU/ml ASO with NaCL.

| **Target 90-110% above 28 U/L** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5µl x 2=10µl | **Sample 52** | **Smart** | **Cube** | **Smart** | | | | |
| **Dilution NaCL 0.9%** | **Theoretical** | **meas 1** | **meas 2** | **meas 3** | **mean** | **stabwn** | **cv** | **Recovery** |
| 100 | 2132 IU/ml | 1980 | 2001 | 1890 | 1957 IU/ml | 58.97 | 3.01% | 92% |
| 80 | 1706 IU/ml | 1470 | 1660 | 1480 | 1537 IU/ml | 106.93 | 6.96% | 90% |
| 60 | 1279 IU/ml | 1077 | 1190 | 1189 | 1152 IU/ml | 64.95 | 5.64% | 90% |
| 40 | 853 IU/ml | 773 | 833 | 830 | 812 IU/ml | 33.81 | 4.16% | 95% |
| 20 | 426 IU/ml | 414 | 387 | 379 | 393 IU/ml | 18.34 | 4.66% | 92% |
| 10 | 213 IU/ml | 239 | 193 | 220 | 217 IU/ml | 23.12 | 10.64% | 102% |
| 5 | 107 IU/ml | 104 | 123 | 126 | 118 IU/ml | 11.93 | 10.14% | 110% |




Conclusion: The bias between expected and recovered ASO is less than 10% for the linearity set, ranging from 107 µmol/l to 2132 IU/ml ASO. The assay is linear from 107-2132 IU/ml with a correlation coefficient of **$R^2$ = 0.9994**.

## 5. Interference Study

The interference study was not repeated, as the ASO latex reagent used in the Eurolyser ASO test kit is manufactured by a leading supplier for ASO assays and an interference study was already conducted by said manufacturer (available upon request).

## 6. Limit of Quantitation (LOQ) of the ASO Assay

To calculate the LOQ of the test kit, one serum sample from a commercial source was diluted with NaCL to targeted concentrations and tested with the test kit reagent on 4 different analysers.

**LOQ (limit of quantitation) is determined as the lowest sample run that displayed CV < 20%**

| Reproducibility | 245 U/L sample 3 | Sample 3 diluted to 50% with NACL | Sample 3 diluted to 33.3% | |
|---|---|---|---|---|
| 1 | 245 U/L | 122 U/L | 81 U/L | Bc 14261 |
| 2 | 239 U/L | 109 U/L | 60 U/L | Bc 14262 |
| 3 | 251 U/L | 111 U/L | 61 U/L | Cb 12758 |
| 4 | 223 U/L | 99 U/L | 101 U/L | Cb 12759 |
| **Reproducibility** | **245 U/L sample 3** | **Sample 3 diluted to 50% with NACL** | **Sample 3 diluted to 33.3%** | |
| 5 | 244 U/L | 79 U/L | 90 U/L | Bc 14261 |
| 6 | 229 U/L | 139 U/L | 80 U/L | Bc 14262 |
| 7 | 211 U/L | 100 U/L | 73 U/L | Cb 12758 |
| 8 | 229 U/L | 110 U/L | 82 U/L | Cb 12759 |
| 9 | 227 U/L | 103 U/L | 105 U/L | Bc 14261 |
| 10 | 233 U/L | 111 U/L | 99 U/L | Bc 14262 |
| 11 | 244 U/L | 99 U/L | 109 U/L | Cb 12758 |
| 12 | 255 U/L | 88 U/L | 84 U/L | Cb 12759 |
| 13 | 267 U/L | 109 U/L | 88 U/L | Bc 14261 |
| 14 | 239 U/L | 114 U/L | 106 U/L | Bc 14262 |
| 15 | 222 U/L | 120 U/L | 80 U/L | Cb 12758 |
| 16 | 239 U/L | 100 U/L | 59 U/L | Cb 12759 |
| 17 | 221 U/L | 103 U/L | 129 U/L | Bc 14261 |
| 18 | 229 U/L | 107 U/L | 99 U/L | Bc 14262 |
| 19 | 244 U/L | 83 U/L | 84 U/L | Cb 12758 |
| 20 | 270 U/L | 101 U/L | 95 U/L | Cb 12759 |
| **mean** | **238 U/L** | **105 U/L** | **88 U/L** | |
| **stabwn** | **15.17** | **13.53** | **17.80** | |
| **cv** | **6.37%** | **12.84%** | **20.17%** | |

Estimated LOQ is set as 100 U/l