e-ECE 2020 Congress, 5–9 September 2020

Multicentre performance evaluation of the new, fully automated Elecsys ASD immunoassay and determination of reference ranges

Barbara Obermayer-Pietsch,[1] Roland Imdahl,[2] Marta de Ramon,[3] Claudia Reichmuth,[4] Garnet Bendig,[5] Stefan Hutzler,[5] Judith Taibon,[5] Christopher Rank,[5] Peter Findeisen[6]

[1]Medical University of Graz, Graz, Austria; [2]Labor Augsburg MVZ, Augsburg, Germany; [3]Laboratori de Referència de Catalunya, Barcelona, Spain; [4]Agent from TRIGA-S representing Roche Diagnostics GmbH, Penzberg, Germany; [5]Roche Diagnostics GmbH, Penzberg, Germany; [6]MVZ Labor Dr. Limbach & Kollegen, Heidelberg, Germany

AEP837

Introduction

- Androstenedione (ASD) levels are used to assess androgen production, adrenal gland and ovarian/testicular function.[1]
- Measurement of ASD is recommended for monitoring patients with hyperandrogenism from congenital adrenal hyperplasia, which is characterised by cortisol-related enzyme deficiencies.[2–6]
- According to international guidelines,[7] ASD can confirm biochemical hyperandrogenism in suspected polycystic ovary syndrome (PCOS) when total/free testosterone are not elevated.
- There are several methods available for ASD quantification:[8,9] isotope dilution liquid chromatography with tandem mass spectrometry (ID-LC-MS/MS) has been shown to deliver accurate and reliable results;[10] however, immunoassays are most suitable for large routine applications.[11]
- The Elecsys® ASD assay (Roche Diagnostics) is a competitive electrochemiluminescence immunoassay for in vitro quantitative determination of ASD in human serum/plasma.

Objectives

- **To evaluate the performance of the new, automated Elecsys ASD assay versus an ASD ID-LC-MS/MS-based reference measurement procedure (JCTLM database: C16RMP6)[10] and commercially available immunoassays.**
- **To determine reference ranges and expected values in different clinical cohorts.**

Methods

- Elecsys ASD assay performance (using cobas e 602/cobas e 801 analysers) was evaluated at three sites in Germany and Spain; method comparison was performed internally by Roche Diagnostics; reference ranges were determined internally by Roche Diagnostics.
- The cobas e 602 and cobas e 801 are fully automated analysers for processing of electrochemiluminescence-based immunoassays.
- Repeatability and intermediate precision were assessed according to Clinical and Laboratory Standards Institute (CLSI) EP05-A3 employing variance component analysis, using three control levels and five human serum pools (n=75 each) covering the assay measuring range (0.15–10.00 ng/mL); one run per day for 5 days. The results were compared with the target values and acceptance criteria defined.
- Method comparisons versus commercially available immunoassays (IMMULITE® ASD [Siemens] and LIAISON® ASD [DiaSorin]) and an ID-LC-MS/MS reference measurement procedure were conducted using 421 serum samples covering the Elecsys ASD assay measuring range; Passing-Bablok regression and Pearson's correlation coefficient were calculated.
- Reference ranges were determined in five clinical cohorts using samples from several sites/vendors; the central 90% and 95% regions with their respective 95% confidence intervals were estimated for each cohort using robust, non-parametric methods:
  - apparently healthy children (≤8 years [US vendor BioreclamationIVT])
  - apparently healthy women with proven fertile cycle (USA, 22–37 years [Trina Bioreactives AG, USA; Roche Wellness Center, USA]; EU/Rest of World, 18–37 years [UZ Brussel, Belgium; University Hospital Leipzig, Germany; Practice Dr Rohsmann, Germany])
  - apparently healthy men (≥18 years [Bavarian Red Cross, Germany])
  - apparently healthy post-menopausal women (55–70 years [NUVISAN GmbH, Germany])
  - women with PCOS according to Rotterdam criteria (18–45 years [Medical University of Graz, Austria]).

Results

Precision analysis

- The 5-day CLSI EP05-A3 precision analysis experiment showed that the repeatability and intermediate precision coefficients of variation (CV) across all sites were 2.01–3.91% and 2.43–4.30%, respectively (mean ASD concentrations 2.23–9.92 ng/mL; Table 1).
- The pre-defined standard deviations (SDs) and CVs of the repeatability and intermediate precision experiment were fully met.

Table 1. 5-day CLSI EP05-A3, summary of complete data for all sites combined, outliers included

Complete data (outliers included)		Repeatability estimates		Intermediate precision estimates	
Sample (ASD target value [ng/mL]), n=75 each	Mean	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
HSP01 (0.34)	0.257	0.010	3.88	0.012	4.48
HSP02 (0.52)	0.474	0.013	2.72	0.016	3.45
HSP03 (2.44)	2.23	0.045	2.02	0.054	2.43
HSP04 (5.28)	5.47	0.118	2.16	0.169	3.09
HSP05 (9.66)	9.92	0.388	3.91	0.427	4.30
PC RH1 (0.6)	0.500	0.014	2.79	0.019	3.74
PC RH2 (3.0)	2.91	0.059	2.01	0.079	2.71
PC RH3 (7.0)	7.42	0.280	3.76	0.279	3.76
Acceptance criteria*		≤0.60 ng/mL: SD ≤0.04 ng/mL	>0.60 ng/mL: CV ≤6.0%	≤0.60 ng/mL: SD ≤0.06 ng/mL	>0.60 ng/mL: CV ≤8.0%

*SD/CV specification as defined in product specification document V7.0.
HSP, human serum pool; PC RH, PreciControl Reproductive Health.

Method comparison

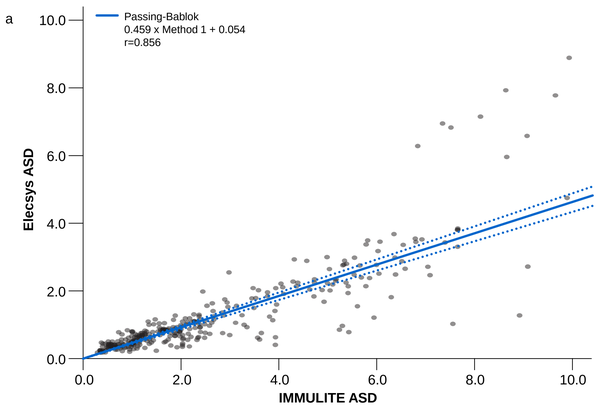
- Poor correlation was observed between Elecsys ASD assay and IMMULITE ASD (Table 2; Figure 1a), good correlation with LIAISON ASD (Figure 1b), and very good correlation with ID-LC-MS/MS (Figure 1c).

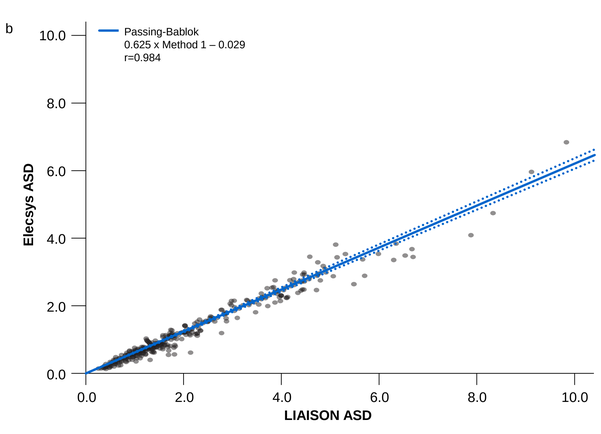
Table 2. Summary of method comparison for all sites

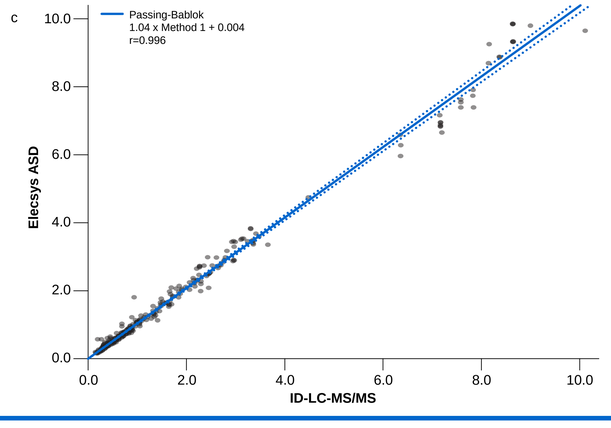
Elecsys ASD versus	N[1]/N[2]	Regression formula y = m x + a	Pearson's r	p-value	Kendall's tau	p-value
IMMULITE ASD	320/421	y = 0.459 x + 0.054	0.856	<0.001	0.721	<0.001
LIAISON ASD	327/421	y = 0.625 x – 0.029	0.984	<0.001	0.872	<0.001
ID-LC-MS/MS	332/421	y = 1.04 x + 0.004	0.996	<0.001	0.922	<0.001

N[1], number of evaluable samples; N[2], total number of samples measured.

Figure 1. Method comparison at all three study sites a) Elecsys ASD versus IMMULITE ASD, b) Elecsys ASD versus LIAISON ASD, c) Elecsys ASD versus ID-LC-MS/MS







Reference ranges

- Within the healthy children cohort, there were equal numbers of male and female children and a mean age of 4.4 years (range: 1.0–8.0 years).
- Age ranges for the healthy women and healthy men were 18.0–37.0 years and 18.0–70.0 years, respectively; mean ages were not known due to the anonymisation process.
- Mean ages (range) for the post-menopausal women and PCOS cohorts were, 59.6 years (55.0–70.0 years) and 27.7 years (18.0–43.0 years), respectively.
- More than the required 120 evaluable samples were available to determine the central 95% interval in apparently healthy children, apparently healthy men, post-menopausal women, and women with PCOS.
- Only 84 evaluable samples were available for the apparently healthy women cohort, allowing determination of the central 90% interval.
- The ASD expected values and reference ranges (5th–95th percentiles) were established in the five clinical cohorts (Table 3).

Table 3. Summary of ASD reference ranges and expected values of five clinical cohorts; the central 95% and central 90% intervals are shown

Cohort	Evaluable samples	Quantile*	Range (ng/mL)
Apparently healthy children	140	2.5% to 97.5% 5% to 95%	<0.150 to 0.519 <0.150 to 0.382
Apparently healthy women with proven fertile cycle†‡	84	5% to 95%	0.49 to 1.31
Apparently healthy men	138	2.5% to 97.5% 5% to 95%	0.280 to 1.52 0.355 to 1.26
Apparently healthy post-menopausal women	140	2.5% to 97.5% 5% to 95%	0.187 to 1.07 0.208 to 0.990
Women with PCOS	125	2.5% to 97.5% 5% to 95%	0.645 to 3.47 0.756 to 3.03

*2.5% to 97.5%: Central 95% interval. ≥120 samples are needed for the establishment of a statistically significant reference interval. 5% to 95%: Central 90% interval.
†As the sample size was <120 in the apparently healthy women cohort, the statistical confidence of the estimated central 95% region, i.e. 2.5% to 97.5% quantile, did not meet the requirements for the estimation of reference ranges. Therefore, these values are not shown in this table.
‡The first sample drawn in the follicular phase was selected for measurements.

Conclusions

- **The Elecsys ASD assay demonstrated excellent precision and very good correlation with an ID-LC-MS/MS-based reference measurement procedure.**
- **Reference ranges were established to support the interpretation of results from this assay in routine clinical practice.**

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Acknowledgements

The authors would like to thank Martin Hund, Gabriella Bobba, Denis Strugala, Michaela Fink, Sabine Mathis, Christine Chowanetz-Ernst, Simone Hertlein, Silke Trackner, Maria Schoedl, Mireia Canal and Sabine Hopfmann. Third-party medical writing support, under the direction of the authors, was provided by Rebecca Benatan, BSc (Gardiner-Caldwell Communications, Macclesfield, UK), and was funded by Roche Diagnostics International Ltd, Rotkreuz, Switzerland. COBAS, COBAS E, and ELECSYS are trademarks of Roche.