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Technical Performance and Diagnostic Utility of the New Elecsys® Neuron-Specific Enolase Enzyme Immunoassay

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This international multicenter study was designed to evaluate the technical performance of the new double-monoclonal, single-step Elecsys neuron-specific enolase (NSE) enzyme immunoassay (EIA) and to assess its utility as a sensitive and specific test for the diagnosis of small-cell lung cancer (SCLC). Intra- and inter-assay coefficients of variation, determined in five control or serum specimens in six laboratories, ranged from 0.7 to 5.3 (inter-laboratory median: 1.3%) and from 1.3 to 8.5 (inter-laboratory median: 3.4%), respectively. Laboratory-to-laboratory comparability was excellent with respect to recovery and inter-assay coefficients of variation. The test was linear between 0.0 and 320 ng/ml (highest measured concentration). There was a significant correlation between NSE concentrations measured using the Elecsys NSE and the established Cobas Core NSE EIA II in all subjects (n = 723) and in patients with lung cancer (n = 333). However, NSE concentrations were systematically lower (approximately 9%) with the Elecsys NSE than with the comparison test. Based on a specificity of 95% in comparison with the group suffering from benign lung diseases (n = 183), the cut-off value for the discrimination between malignant and benign conditions was set at 21.6 ng/ml. NSE was raised in 73.4% of SCLC patients (n = 188) and was significantly higher (p < 0.01) in extensive (87.8%) as opposed to limited disease (56.7%). NSE was also elevated in 16.0% of the cases with non-small cell lung cancer (NSCLC, n = 374). It is concluded that the Elecsys NSE EIA is a reliable and accurate diagnostic procedure for the measurement of NSE in serum samples. The special merits of this new assay are the wide measuring range (according to manufacturer's declaration up to 370 ng/ml) and a short incubation time of 18 min. Clin Chem Lab Med 2003; 41(1): 95–103

Key words: Neuron-specific enolase(NSE); Small-cell lung cancer (SCLC).

Abbreviations: AUC, area under the curve, ECL, electrochemiluminescence; ED, extensive disease; EGTM, European Group on Tumor Markers; EIA, enzyme immunoassay; LD, limited disease; NSCLC, non-small cell lung cancer; NSE, neuron-specific enolase; ROC, receiver-operating characteristic; SCLC, small-cell lung cancer.

Introduction

Neuron-specific enolase (NSE) comprising $\gamma\gamma$- and $\alpha\gamma$-isomers of the glycolytic enzyme enolase (2-phospho-D-glycerate hydrolase E.C. 4.2.1.11) is expressed preferentially in neuronal tissues and in amino precursor uptake and decarboxylation cells (1, 2). The enzyme was shown to be a valuable tumor marker for cancers of neuroendocrine type such as small-cell lung cancer (SCLC) (3–6), neuroblastoma (7), carcinoid tumors (8), melanoma (9), seminoma (10), Merkel cell carcinoma (11), medulloblastoma (12) or retinoblastoma (13). NSE was further shown to be released into cerebrospinal fluid and blood as a result of cerebral injury due to, for instance, cardiac arrest (14), open heart surgery (15), tonic-clonic seizures (16), *status epilepticus* (17), and Creutzfeldt-Jakob disease (18).

In SCLC, which accounts to 20–25% of lung carcinomas, pre-treatment NSE was increased in 70–80% of patients (2–6, 19, 20). In multivariate analyses, serum NSE proved to be a prominent prognostic factor together with well-established ones such as performance status and stage of the disease (20, 21). However, NSE measurement is of doubtful utility for the monitoring of chemo- and/or radiotherapy. It is true that NSE usually decreases to the normal range in SCLC patients who respond to chemotherapy but the decrease is irrespective of the extent of remission (6, 22), therefore, NSE cannot replace clinical response evaluations. Increasing NSE levels may indicate tumor progression or recurrent disease (23, 24) but the lack of curative second-line treatment limits the value of NSE measurement for this application. Some years ago, Roche (Basel, Switzerland) introduced the NSE enzyme immunoassay (EIA) on the Cobas Core system as a two-step assay employing a specific monoclonal antibody against NSE in conjunction with a polyclonal (rabbit) antibody. Clinical and methodological features of this EIA were evaluated in comparison with the NSE EIA developed by Boehringer

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Mannheim, Mannheim, Germany on the Elecsys ES700 analyzer and NSE radioimmunoassay of Pharmacia (Freiburg, Germany) (25). Further development of the Cobas Core NSE EIA by the manufacturer resulted in a one-step, solid- phase EIA employing two monoclonal antibodies to NSE. The analytical performance and the diagnostic utility of this Cobas Core NSE EIA II was evaluated in comparison with Cobas Core NSE EIA (26). The particular test configuration of the Cobas Core NSE EIA II has now been transferred to the Elecsys system by Roche Diagnostics. The Elecsys system benefits from chemiluminescence as the detection method that offers a wide measuring range and short incubation times. The aim of the present multicenter study was

1. to evaluate the technical performance of the new Elecsys NSE test by assessing intra- and inter-assay precision, dilution linearity and recovery;
2. to compare the results of NSE measurements using the Elecsys NSE test with the established Cobas Core NSE EIA II;
3. to establish clinical utility of the Elecsys NSE test as a diagnostic tool in a series of patients with lung cancer and benign lung diseases (reference group) and in healthy persons.

Materials and Methods

Subjects

Serum samples were taken before treatment from 562 consecutive patients with lung cancer admitted to Klinikum Großhadern, Munich (laboratory 1), Thoraxklinik-Heidelberg (laboratory 3) and Klinikum Gauting (laboratory 5) for diagnosis and therapy. Of these 562 patients, 188 suffered from SCLC (mean age: 62, range: 35–86 years) and 374 from non-small cell lung cancer (NSCLC) (mean age: 63, range: 34–86 years, including patients with squamous cell carcinoma, adenocarcinoma and with large cell carcinoma).

The reference group consisted of 183 patients (mean age: 56, range: 21–85 years) with benign lung diseases of different etiology including benign neoplasms. Diagnosis was performed using standard protocols including histopathological examination of biopsy material.

The control group of 258 apparently healthy volunteers was recruited exclusively from the employees of the clinics. The stage of malignant disease was determined by clinical staging after submitting the SCLC patients to the diagnostic protocol following the classification of the Veterans Administration Lung Cancer Group (VALG) as modified by Wolf and Havemann (27). Histological diagnosis was performed according to the guidelines of the World Health Organization (28) and was based on the predominant cell type.

Elecsys® NSE

The Elecsys® NSE is a one-step, solid-phase enzyme immunoassay of a sandwich type. The assay employs two monoclonal antibodies with different epitopes specific for γ-enolase (named 18E5 and DN84B10) raised in mice immunized with purified γ-enolase from human brain as described by Sterk and coworkers (29). No crossreactivity against the α-subunit has been found (29).

Patient specimens (sample volume: 20 µl), calibrators (Elecsys NSE CalSet) and controls (PreciControl Tumor Marker 1 and 2) are incubated with biotinylated monoclonal NSE-specific antibody (NSE 18E5) and monoclonal NSE-specific antibody (DN84B10) labeled with Tris(2,2′-bipyridyl)ruthenium(II)-complex to form a sandwich complex. After addition of streptavidin-labeled microparticles, the complex produced is bound to the solid-phase *via* biotin-streptavidin interaction. First and second incubation are performed at 37 °C. The reaction mixture is then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are removed with ProCell. The Elecsys NSE immunoassay makes use of the electrochemiluminescence (ECL)-technology. After application of voltage to the detection cell electrode, a peak of light emission occurs over a short time interval and can be detected as the resulting ECL-signal. A defined area under the curve (AUC) is measured around the intensity maximum. A lot-specific master calibration curve is established at Roche Diagnostics using lot-specific test kit reagents and master calibrators. The shape of the lot-specific master curve is characterized by a four-parameter Rodbard function. The data characterizing this curve are stored in the lot-specific reagent barcode. Lot-specific assigned values of the calibrator are read from the lot-specific master calibration curve and are encoded on the calibrator barcode card. At the customer site, the calibration results from two calibrators measured under routine conditions are mathematically combined with the encoded data from the barcode. From this combination, the instrument determines lot calibration from which the concentration of measured samples is reliably calculated. The Elecsys NSE immunoassay is standardized against the Enzymun NSE immunoassay (Boehringer Mannheim, Mannheim, Germany).

The NSE tests were performed according to the procedure specified by the manufacturer, Roche Diagnostics GmbH, Mannheim, Germany. Total assay time amounts to 18 min. The measuring range of the test is 0.05–370 ng/ml, defined by the lower detection limit and the maximum of the master calibration curve.

The potential influence of several serum components on NSE measurement was investigated by the manufacturer of the test. There were no interferences by bilirubin (up to 72 mg/dl (1236 µmol/l)), triglycerides (up to 2000 mg/dl (22.8 mmol/l)), biotin (up to 100 ng/ml (0.41 µmol/l)), rheumatoid factor (up to 1500 U/ml) and by frequently used therapeutic drugs. No high dose hook effect at NSE concentrations of up to 100000 ng/ml was observed. However, erroneous findings may be caused by the human anti-mouse antibodies (HAMAs) in patients who had been treated with monoclonal mouse antibodies or who had received them for diagnostic purposes. Elecsys NSE contains additives which minimize these effects. In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium may occur. The influence of hemolysis on NSE testing is well documented.

Comparison test: Cobas Core NSE EIA II

The test principle of the reference Cobas Core NSE EIA II has been previously described (26). Briefly, it is a one-step assay using a highly specific monoclonal antibody to NSE (18E5) immobilized on a polystyrene bead, in conjunction with a second monoclonal NSE antibody (DN84B10) covalently linked to horseradish peroxidase to form a sandwich with serum (or calibrator or control) NSE. The assay is run in a one-point calibration mode using NSE recalibrator 50 ng/ml and a predefined lot-specific master calibration curve.

Elecsys immunoassay analyzer

Elecsys NSE is intended for use on the Roche Elecsys 1010/2010 and ModularAnalytics E 170 immunoassay analyz-

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ers. In all instruments, electrochemiluminescence is employed as a higly sensitive detection method, offering a wide measuring range and short incubation times. Elecsys 1010 is a sample-selective multibatch analyzer for small to medium-sized laboratories; Elecsys 2010 is a continuous analyzer for medium to large-sized laboratories. The recently introduced ModularAnalytics E 170 analyzer allows to combine the established Elecsys technology with various other analytical units.

Intra-assay and inter-assay precision

For the assessment of intra-assay and inter-assay precision the following specimens were used: PreciControl Tumormarker 1 (Lot 198815, mean concentration 12.4 ng/ml, range: 9.8–15 ng/ml, Roche Diagnostics), PreciControl Tumormarker 2 (Lot 198815, mean concentration 58.5 ng/ml, range: 46–71 ng/ml, Roche Diagnostics) and three human serum samples with low (range: 5.7–8.7 ng/ml), medium (range: 11.5–35.8 ng/ml) and high (range: 93.3–212 ng/ml) NSE concentrations. The latter specimens were from serum pools prepared at each site. For the assessment of intra-assay precision, the samples were measured 21 times within one run at each site. For determination of the inter-assay precision, the samples were measured in 21 independent runs at each site.

Laboratory-to-laboratory variability and recovery

Precision and recovery were assessed in a ring trial using three human serum pools distributed by Roche Diagnostics (target values: 5.6, 14.4 and 96 ng/ml). Median recovery was calculated from 10 independent runs at each site and compared to the target value.

Dilution linearity

Three human sera, containing NSE at a concentration 311.8 ng/ml (serum 1), 281.7 ng/ml (serum 2) and 317.8 ng/ml (serum 3) were each measured undiluted and as solutions of 0.9 (serum) + 0.1 (diluent or serum), 0.8+0.2, 0.7+0.3, 0.6+0.4, 0.5+0.5, 0.4+0.6, 0.3+0.7, 0.2+0.8 and 0.1+0.9. Dilution was performed using either the sample diluent from the Elecsys test or human serum with low NSE concentration (9.75 ng/ml). The measurements were done in duplicate in two independent series to exclude possible dilution errors. NSE value of 9.75 ng/ml was subtracted to correct for the NSE content of the diluent.

Comparison of the Elecsys NSE with Cobas Core NSE EIA II

To compare Elecsys NSE with established Cobas Core NSE EIA II, NSE concentrations were measured simultaneously with both methods at two sites (laboratory 1 and laboratory 3 in 723 serum samples drawn from a population consisting of 257 healthy individuals, 133 patients with benign lung diseases, 104 patients with SCLC, and 229 patients with NSCLC).

Statistical methods

The results for the different groups studied are given as 5%, 50% (median), and 95% percentiles. Group comparisons were analyzed descriptively using Wilcoxon's nonparametric rank-sum test (30). After careful inspection for possible outliers, parametric descriptive statistics (mean, CV) was used to estimate intra-assay and inter-assay precision. To protect against outlying (influential) observations in the method comparison analysis, the robust Passing and Bablok regression was used (31, 32). The details of the differences between the two analytical methods are shown in a normalized differences plot. Dif-

Table 1 Elecsys NSE test: intra- and inter-assay coefficients of variation.

	Intra-assay precision											
	Laboratory 1		Laboratory 2		Laboratory 3		Laboratory 4		Laboratory 5		Laboratory 6	
Material	Mean (ng/ml)	CV %	Mean (ng/ml)	CV %	Mean (ng/ml)	CV %	Mean (ng/ml)	CV %	Mean (ng/ml)	CV %	Mean (ng/ml)	CV %
PreciControl TM I	13.1	1.7	13.0	1.0	12.9	1.0	12.8	2.1	12.9	1.8	12.7	0.9
PreciControl TM II	62.6	2.2	60.5	0.7	61.9	1.0	61.5	1.8	60.6	2.0	60.3	1.2
Human Serum low	8.4	3.7	5.8	0.9	5.7	2.3	5.0	5.3	6.3	0.7	8.7	1.3
Human Serum medium	16.9	1.5	16.3	1.8	16.4	0.9	10.6	1.1	11.5	1.2	35.8	1.5
Human Serum high	165	1.0	149	1.3	116	4.1	118	1.3	93.8	1.2	212	1.1
	Inter-assay precision											
	Laboratory 1		Laboratory 2		Laboratory 3		Laboratory 4		Laboratory 5		Laboratory 6	
Material	Mean (ng/ml)	CV %	Mean (ng/ml)	CV %	Mean (ng/ml)	CV %	Mean (ng/ml)	CV %	Mean (ng/ml)	CV %	Mean (ng/ml)	CV %
PreciControl TM I	12.2	3.6	12.5	3.6	13.4	1.8	12.4	3.4	12.7	1.6	13.0	3.3
PreciControl TM II	56.6	3.8	58.2	4.3	63.2	2.1	59.9	4.0	58.0	2.2	61.7	2.8
Human Serum low	8.7	3.4	4.9	8.5	5.7	1.3	5.1	7.6	6.2	4.7	8.5	2.4
Human Serum medium	17.3	6.6	16.0	2.9	16.2	2.0	10.2	2.8	11.4	2.4	35.4	3.0
Human Serum high	168	4.9	145	3.6	121	2.2	119	4.4	94.4	3.6	210	3.5

Intra- and inter-assay coefficients of variation (CVs) obtained with the Elecsys NSE test in six laboratories. Specimens: PreciControl Tumormarker 1, PreciControl Tumormarker 2, and three human serum samples with low (range: 5.7–8.7 ng/ml), medium (range: 11.5–35.8 ng/ml) and high (range: 93.3–212 ng/ml) NSE concentration.

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Table 2 Ring trial: inter-assay CV and recovery of NSE in three human serum pools.

Material	Lab	Min	Max	Mean	Median	CV%
Pool 1	1	5.1	5.5	5.4	5.4	2.2
	2	5.3	5.8	5.5	5.6	3.0
Target:	3	5.8	6.0	5.9	5.9	1.1
5.6 ng/ml	4	5.4	6.0	5.6	5.5	4.1
	5	5.6	5.8	5.7	5.7	1.1
	6	4.9	5.3	5.1	5.1	2.6
Pool 2	1	13.7	14.5	14.0	13.9	2.0
	2	13.5	15.1	14.4	14.5	3.7
Target:	3	15.1	15.9	15.5	15.4	1.5
14.4 ng/ml	4	13.4	15.6	14.4	14.0	5.2
	5	14.6	15.3	14.9	14.9	1.4
	6	13.2	14.4	14.0	14.1	3.4
Pool 3	1	87.3	94.6	92.2	92.7	2.4
	2	88.1	103.3	94.6	95.6	4.9
Target:	3	100.6	107.7	103.3	103.7	2.3
96 ng/ml	4	87.2	105.8	93.0	90.5	6.9
	5	97.1	101.9	99.0	99.0	1.4
	6	88.5	97.9	93.8	95.5	4.0

Pools were measured 10 times each and were distributed by Roche Diagnostics (Ring trial). CV is based on median values of individual laboratories (Reagent lot No. 195768, Elecsys 2010 Instrument).

ferences between clinical groups are analyzed descriptively by means of the (outlier) box plot and to account for the non-normality of the data by means of the nonparametric Wilcoxon rank-sum test (30). Diagnostic performance is described in terms of sensitivity, specificity and AUC by receiver-operating characteristic (ROC) analysis (33). Nonparametric estimates have been used for the AUCs.

Results

The evaluation of precision and recovery

A summary of the results of intra-assay and inter-assay precision evaluation of the Elecsys NSE test is shown in Table 1. In the intra-assay precision study, CVs ranged from 0.7 to 5.3%. The inter-assay CVs ranged from 1.3 to 8.5%. Table 2 gives recovery and CV data determined at each site using serum pools distributed by Roche Diagnostics (ring trial). Median recoveries calculated from the data reported by the individual laboratories were 5.5, 14.5 and 96.2 ng/ml, respectively. These values are in excellent agreement with the target values assessed internally by Roche Diagnostics for pools 1–3: 5.6, 14.4 and 96 ng/ml, respectively. On the basis of mean recoveries determined at each site, the median values for between-laboratory CVs were calculated for serum pools 1–3 and yielded 5.7, 4.2 and 4.3%, respectively.

Dilution linearity

The results of dilution linearity experiments are presented in Figures 1 and 2. In the series using sample diluent, median recovery was 103.6% (mean: 106.6, range: 85.8–140.3%). When serum was used as a diluent, median recovery was 106.4% (mean: 109.6, range: 98.1–130.3%). Irrespective of the nature of the diluent, the deviation was highest in case of 1:10 dilution.

Elecsys NSE vs. Cobas Core NSE EIA II

The Passing-Bablok graphs for the comparison of Elecsys NSE with Cobas Core NSE EIA II are shown in Figure 3A (all samples) and Figure 4A (cancer patients). The Passing-Bablok equation which includes all samples measured shows that the Elecsys NSE results were on average 9.1% lower than these obtained using

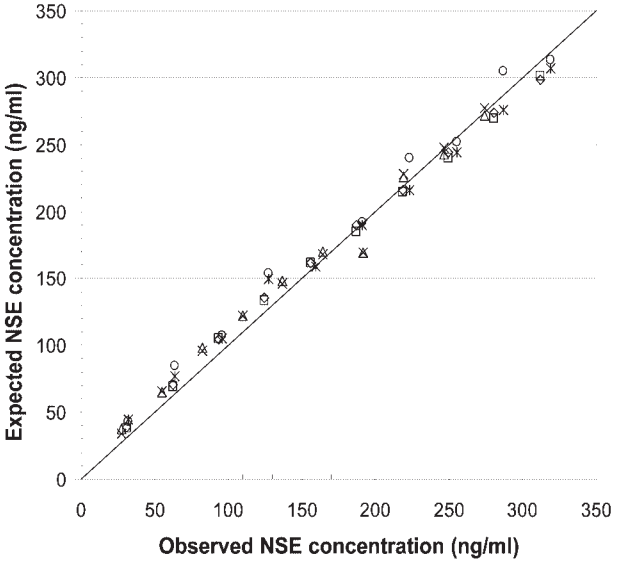

Figure 1 Test linearity assessed with three human serum samples assayed in duplicate. Dilution was performed with sample diluent. Each symbol represents an individual dilution series.

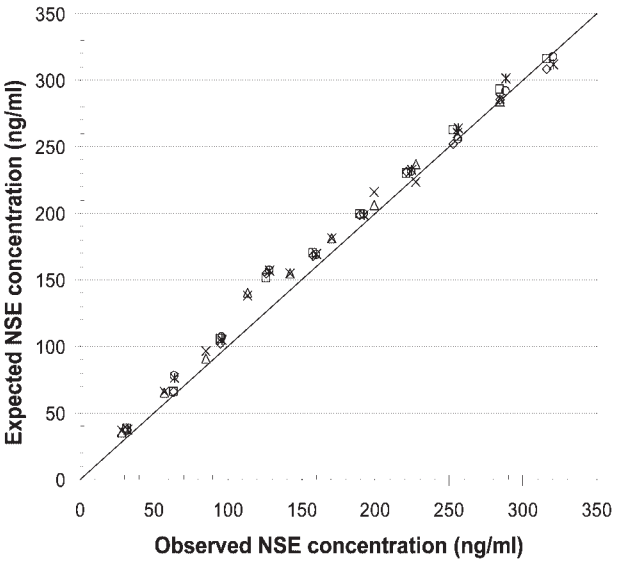

Figure 2 Linearity assessed by measurements of three human serum samples assayed in duplicate. Dilution was performed with serum with low NSE concentration (9.75 ng/ml). This value was then subtracted to correct for the individual values. Each symbol represents an individual dilution series.

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Cobas Core NSE EIA II. A similar result was obtained when comparing only the samples from the cancer patients; in this case the values obtained with the Elecsys NSE test were on average 8.4% lower. The two assays correlated well with nonparametric Spearman's rank correlation coefficient 0.977 for both the total population and the cancer patients. The normalized differences between both methods did not vary significantly as a function of NSE concentration (Figure 3B, Figure 4B)

The very high degree of agreement between both methods is also demonstrated by the ROC curves in Figure 5.

Clinical evaluation

Distribution of the NSE concentrations measured with the Elecsys NSE test in patients with untreated SCLC, NSCLC, benign lung diseases and in healthy persons is illustrated as Whisker plots in Figure 6. Listed in Table 3 are 5%, 50% and 95% percentiles and maximum values of NSE concentrations. For SCLC, the values are further stratified into Limited Disease (TNM I – IIIa) and Extensive Disease (TNM IIIb/IV). The highest NSE concentrations were obtained in patients with SCLC. The NSE concentrations of the SCLC group differed significantly from all the other groups ($p < 0.001$, Wilcoxon test). There was also a significant difference in NSE concentrations between limited and extensive disease ($p < 0.001$, Wilcoxon test).

Table 3 NSE concentration in patient groups.

		NSE concentration (ng/ml)				
	N	Min	5%	50%	95%	Max
Apparently healthy	258	2.5	6.2	9.7	16.6	32.3
Benign	183	4.1	5.9	11.6	21.6	60.7
SCLC	188	4.3	9.4	43.5	357.3	1053.5
LD*	60	4.8	8.8	25.3	217.1	748.0
ED*	90	4.3	12.0	74.9	370.0	770.0
NSCLC	374	2.1	5.4	13.1	38.6	355.0

Shown are the 5%, 50% (median), 95% percentiles and maximum values of NSE concentrations assessed by the Elecsys NSE test in patients with SCLC, NSCLC, benign lung diseases and in healthy persons. SCLC patients are further stratified into limited and extensive disease (*staging not available in 38 cases).

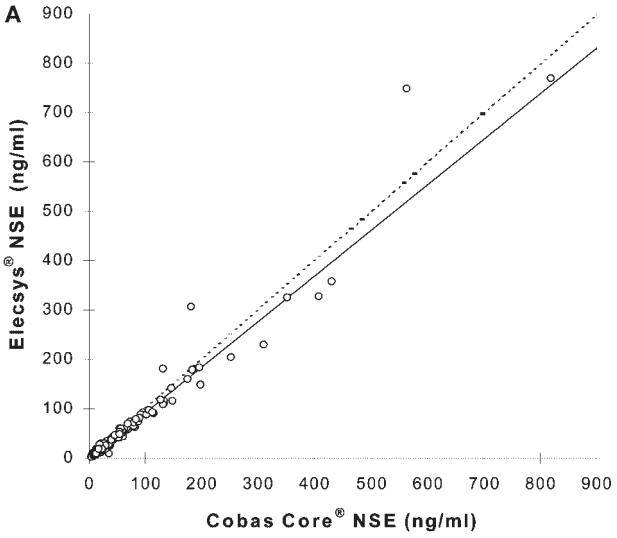

Figure 3A Correlation between NSE concentration measured by Elecsys NSE and Cobas Core NSE EIA II (Passing-Bablok) in all studied subjects. Spearman's rank correlation coefficient r_s: 0.977 ($p < 0.001$) (regression equation: $Y = 0.924X - 0.558$; $N = 723$).

Figure 7 presents the positivity rates of the Elecsys NSE tests with respect to SCLC, the SCLC subgroups, NSCLC and the group with benign lung diseases and healthy persons. The positivity rates are based on a cut-off value which corresponds to the 95% specificity of the NSE test compared with the group with benign lung diseases, following the criteria of the European Working Group of Quality Control and Standardization of Tumor Marker Assays (34). Based upon this specificity, the cut-off value was found to be 21.6 ng/ml. The sensitivity of the Elecsys NSE test was 73.4% for total SCLC and 56.7% for the Limited Disease subgroup and 87.8% for the Extensive Disease subgroup.

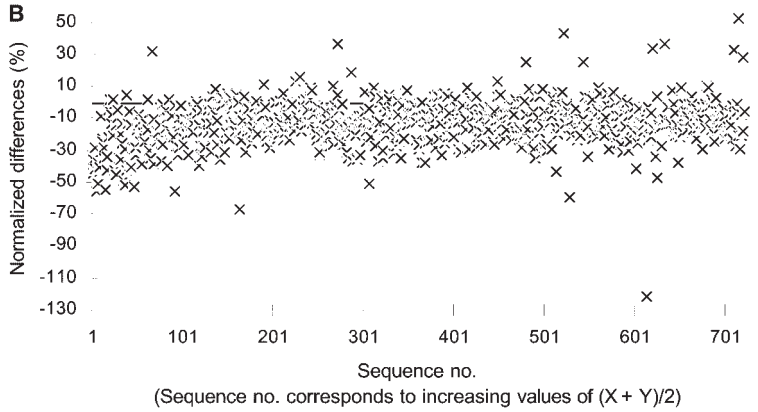

Figure 3B Normalized difference plot showing distribution of differences between Elecsys NSE and Cobas Core NSE EIA II methods as a function of NSE concentration in all subjects. (Method X = Cobas Core NSE EIA, Method Y = Elecsys NSE EIA).

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Discussion

The results of the precision and dilution linearity assessment indicate that the new Elecsys NSE test is a reliable and accurate diagnostic procedure for the measurement of NSE concentrations in serum. Intra- and inter-assay CVs were in the range 0.7–5.3% (median CV: 1.3%) and 1.3–8.5% (median CV: 3.4%), respectively. In addition, it was shown that laboratory-to-laboratory comparability was excellent with respect to recovery and CVs calculated from the median recoveries obtained in the individual laboratories. Intra- and inter-assay CVs for the Elecsys NSE test are in the same order of magnitude as those of reference Cobas Core NSE EIA II where intra- and inter-assay CVs range from 2.1 to 4.6% and from 3.0 to 7.0%, respectively (26). They were clearly better than those observed during the evaluation of Cobas Core NSE EIA (25), where reported intra-assay CV was 11.7% and inter-assay CV was 14.7%. It can be assumed that the use of two monoclonal antibodies to NSE improves the precision of the test. The lower inter-assay CVs of the Elecsys NSE may be particularly important for disease monitoring in SCLC because in the assessment of response to therapy inter-assay CVs are used for the calcu-

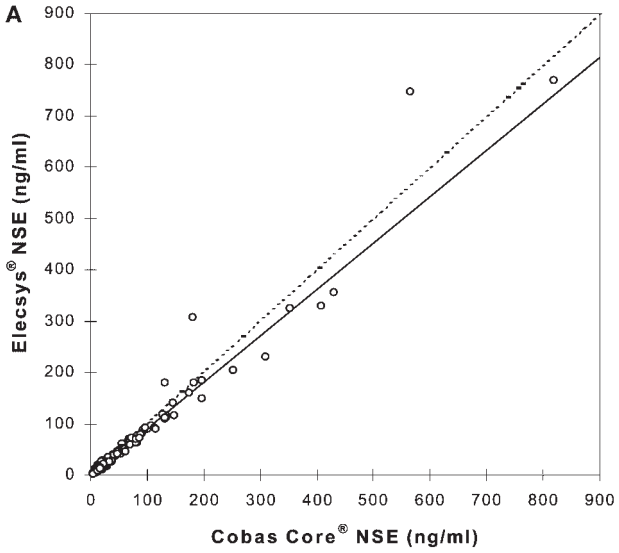

Figure 4A Correlation between NSE concentrations measured by Elecsys NSE and Cobas Core NSE EIA II in the cancer patients (Passing-Bablok). Spearman's rank correlation coefficient r_s: 0.977 ($p < 0.001$) (regression equation : Y = 0.906X –0.725; N = 333).

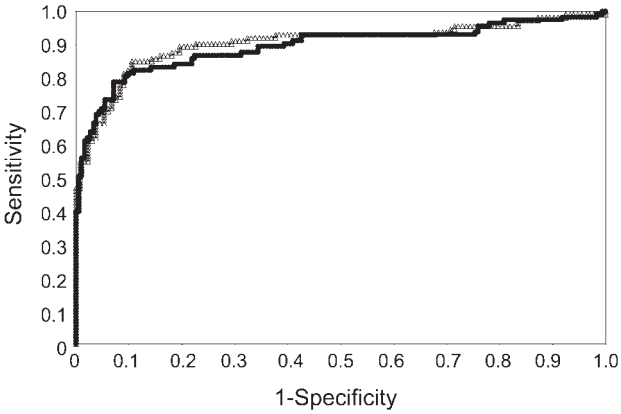

Figure 5 Receiver-operating characteristic curves of Elecsys NSE and Cobas Core NSE EIA II methods for SCLC (sensitivity) in relation to the reference group with benign lung diseases (specificity). AUC Elecsys: 0.89; AUC Cobas Core: 0.91). Elecsys: —●— Cobas Core: —△—.

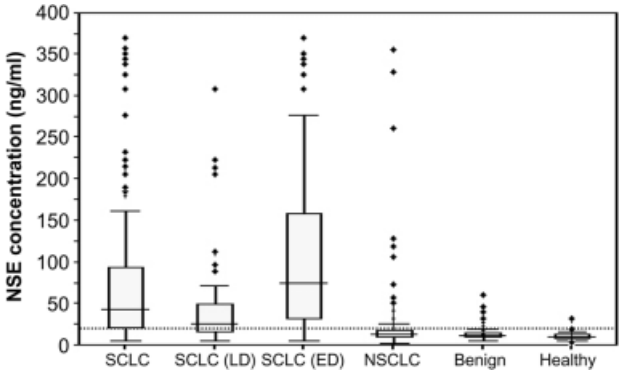

Figure 6 Distribution of NSE concentrations assessed by Elecsys NSE in the various study groups. Data are presented as multiple box and whisker plots showing median value (horizontal line), mean value (x), upper and lower quartile and range. Extreme values are plotted separately. Values greater than 400 ng/ml are not shown. LD: limited disease, ED: extensive disease.

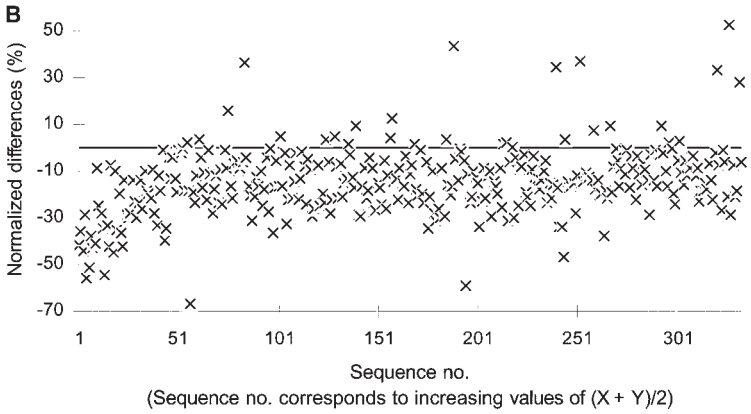

Figure 4B Normalized difference plot showing distribution of differences between Elecsys NSE and Cobas Core NSE EIA II methods as a function of NSE concentration in cancer patients. (Method X = Cobas Core NSE EIA, Method Y = Elecsys NSE EIA).

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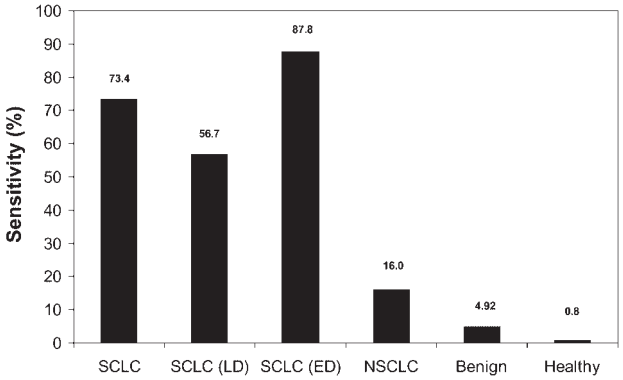

Figure 7 Positivity rates (sensitivity) of the measurement of NSE concentration with Elecsys NSE in the various study groups. Cut-off value set at 21.6 ng/ml corresponds to the 95% specificity of NSE measurement in patients with benign lung diseases (34).

lation of the critical difference between two consecutive marker levels (35). In our hands, the Elecsys NSE was linear up to a concentration of 320 ng/ml. The manufacturer claims that the analytical range of the Elecsys NSE extends to 370 ng/ml. There was a significant correlation between NSE concentrations measured using the Elecsys NSE and the established Cobas Core NSE EIA II, both in all subjects and in patients with lung cancer. However, the NSE concentrations measured by the new assay were lower compared to those obtained by the Cobas Core version of the assay. This was true for all investigated groups.

NSE was raised in 73.4% of SCLC patients and was significantly higher ($p < 0.01$) in extensive (87.8%) as opposed to limited disease (56.7%). This detection rate is in accordance with the overwhelming majority of data reported in the literature (3–6, 19, 20). The sensitivity of 73.4% is based on a cut-off level of 21.6 ng/ml, which corresponds to the 95% specificity calculated from NSE concentrations measured in the group with benign lung diseases. The cut-off level of 21.6 ng/ml is considerably higher than values observed in previous studies, which ranged from 11.9 to 16.4 ng/ml (19, 25, 26). Although the results of NSE measurement may differ with different test versions, these discrepancies are mainly caused by the differences in populations of individuals with benign lung disorders, who are used for the calculation of the cut-off point. The rather large number of 183 patients in this study allows a realistic view of the quality of the NSE assay to discriminate between SCLC patients and those with benign lung diseases. We observed rather high NSE concentrations in patients suffering from (pleural) fibrosis (60.7 ng/ml), benign paraganglioma (45.3 ng/ml), benign fibrous mesothelioma (39.8 ng/ml) or tuberculosis (28.9 ng/ml, 32.2 ng/ml). In all these cases, hemolysis as a potential explanation for elevated NSE levels was excluded. It is well-known in clinical practice that patients with tuberculosis (or HIV infection) often have increased NSE concentration. In general, patients with alveolar infiltrates or an interstitial pattern on chest X-ray had higher NSE levels than those with normal radiographs (36). Direct damage to the neural or neuroendocrine lung cells or a degree of local hypoxia are likely to play a role in the increase in NSE in these patients. Interestingly, NSE elevation was reported as a frequent event in patients with terminal hypoxia in the course of benign lung diseases (37). In case of benign paraganglioma, elevated NSE may not be uncommon since resected tumors reveal vascular mass with nests of epithelial cells and the presence of neurosecretory granules (38).

Further potential application of NSE measurements includes the differential diagnosis of malignant tumors in the thoracic space. As was found in other studies, elevated NSE levels were observed in the various subgroups of NSCLC patients (16.0%) probably due to the presence of small subpopulations differentiated as small cells. It has been reported that 30–50% of bronchogenic carcinomas consist of heterogeneous cell populations (39) but their histology is, as a rule, classified according to the dominating cell type (28). However, it would be interesting to know whether patients with non-resectable NSCLC with elevated NSE would benefit from chemotherapy. To our knowledge, such a study has not been performed to date. Also, NSE is not able to differentiate unequivocally between SCLC and mediastinal tumors such as thymoma, teratoma and Hodgkin's and non-Hodgkin's lymphomas. Elevated NSE was also reported in 13.2% of the latter tumors (40). This is of particular importance because both malignancies express similar roentgenographic features, presenting with a hilar mass and mediastinal widening. This means that the definite diagnosis can only be established by biopsy.

Despite these limitations, NSE assays may be helpful in the primary diagnosis of SCLC as was claimed by the European Group on Tumor Markers (EGTM; 41). They stated that NSE levels >25 ng/ml are suggestive of lung cancer and NSE levels > 100 ng/ml are suggestive of SCLC. In the present study it was found that 169 out of the 562 patients with lung cancer (30.1%) had NSE levels above 25 ng/ml. Of the 50 lung cancer patients with NSE levels above 100 ng/ml, 44 suffered from SCLC (23.4% of all SCLC patients) and six from NSCLC (1.6% of all NSCLC patients). These figures are in good agreement with the EGTM recommendations (40). However, these findings also show that the definite diagnosis in an individual patient cannot be made on the basis of NSE level.

Conclusions

The new Elecsys NSE is a reliable test for the measurement of serum NSE concentration showing high precision and good recovery and linearity. The test is distinguished by fast throughput, with the incubation time of 18 min. Its sensitivity with regard to SCLC is comparable to the established Cobas Core NSE EIA II. NSE measurement is a helpful diagnostic tool in patients in whom invasive techniques cannot be used and in those where diagnosis could not be made using standard procedures.

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