Research Article

Journal of Molecular Recognition

Received: 6 July 2014, Revised: 1 September 2014, Accepted: 4 September 2014, Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jmr.2433

Pattern recognition of neuron specific enolase and carcinoembryonic antigen in whole blood samples

Raluca-Ioana Stefan-van Staden[a,b]*, Ionela Raluca Comnea-Stancu[a,b], Carmen Cristina Surdu-Bob[c] and Camelia Stanciu-Gavan[d]

New tools and methods for pattern recognition of neuron specific enolase (NSE) and carcinoembryonic antigen (CEA) were proposed for the screening of whole blood samples. The new tools were based on stochastic sensors designed using nanoporous gold microspheres, graphite, graphene, diamond paste as well as α-CDs, and 5,10,15,20-tetraphenyl-21*H*,23*H*-porphyrin. The best sensor for the assay of CEA was the one based on P/graphite (the limit of determination was 16 fg/ml and sensitivity was 2.32×10^7 s mg^{-1} ml), while for the assay of NSE the, best sensor was the one based on P/graphene (the limit of determination was 7.45 pg/ml and sensitivity was 2.49×10^8 s mg^{-1} ml). The sensor of choice for simultaneous detection of NSE and CEA is the one based on P/graphene because we need high sensitivity and low limit of determination for NSE. To our knowledge, this is the only one screening test for early detection of lung cancer, by identification of NSE and CEA in whole blood samples. **Copyright © 2015 John Wiley & Sons, Ltd.**

Keywords: carcinoembryonic antigen; neuron specific enolase; stochastic sensor; pattern recognition; nanostructured materials

INTRODUCTION

Lung cancer is the leading cause of cancer death due to its diagnostic at late stages when the chances of survival are very low (American Cancer Society, 2014). Early detection represents the most promising approach to grow the lung cancer survival, because the early treatment leads to better clinical outcomes (Pavlou and Diamandis, 2010). Unfortunately, to date, there is no screening test for specific biomarkers that can lead to early detection. The only way to detect it is simple X-ray radiography – which is usually performed for symptomatic patients when the illness is already in stage 3 or 4.

Predictive biomarkers as tools for lung cancer detection are useful for screening, early diagnosis, prognosis, treatment, and monitoring the disease progression and for therapeutic response. Neuron specific enolase (NSE) and CEA can be used for fast screening of whole blood and early detection of lung cancer (Schrohl *et al*., 2003; Maruvada *et al*., 2005; Vaidyanathan and Vasudevan, 2012).

Carcinoembryonic antigen is a highly glycosylated cell surface glycoprotein (180 kDa), belonging to a group of substances known as the tumor-associated antigens (Martin *et al*., 1976; Grunnet and Sorensen, 2012). CEA is the most widely investigated tumor biomarker for diagnostic as well as follow up of the treatment being over-expressed in various tumors, for example, colorectal, stomach, pancreas, liver, ovarian, breast, prostate, thyroid, bladder, kidney, and lung (Qu *et al*., 2013).

Neuron specific enolase (NSE) is a 78 kDa glycolitic enzyme that catalyzes the conversion of 2-phospho-D-glycerate to phosphoenolpyruvate. NSE is a sensitive, specific, and reliable diagnostic biomarker for small cell lung cancer, but its presence is also elevated in other malignancies such as melanoma, neuroblastoma, hormone-resistant prostate cancer, and semioma (Holmes *et al*., 2011; Han *et al*., 2012; Torsetnes *et al*., 2013); therefore, addition of CEA biomarker to the whole blood analysis will facilitate the correct diagnostic of patients.

Until now, CEA was measured by methods including enzyme linked immunosorbent assay (standard method) (Naghibalhossaini and Ebadi, 2006; He and Chen, 2009; Cedres *et al*., 2011), chemiluminiscence immunoassay (Tomida *et al*., 2009; Kaleta *et al*., 2013; Qu *et al*., 2013), immunohistochemistry (Jun *et al*., 2003; Quinones *et al*., 2013), time-resolved fluoroimmunoassay (Yan *et al*., 2005; Hou *et al*., 2012), piezoelectric immunoassay (Shen *et al*., 2005), radioimmunoassay (Wu *et al*., 2011), and various electrochemical methods, for example, difference pulse voltametry

* *Correspondence to: Raluca-Ioana Stefan-van Staden, Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, 202 Splaiul Independentei St., Bucharest 060021, Romania. E-mail: ralucavanstaden@gmail.com*

a *R.-I. Stefan-van Staden, I. R. Comnea-Stancu*
Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, 202 Splaiul Independentei St., Bucharest 060021, Romania

b *R.-I. Stefan-van Staden, I. R. Comnea-Stancu*
Faculty of Applied Chemistry and Materials Science, Politehnica University of Bucharest, 1-7 Polizu St., Bucharest 011061, Romania

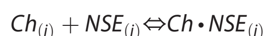
c *C. C. Surdu-Bob*
Low Temperature Plasma Laboratory, National Institute for Lasers, Plasma and Radiation Physics, Str. Atomistilor 409, Magurele 077125, Romania

d *C. Stanciu-Gavan*
Department of Surgery 4, University of Medicine and Pharmacy "Carol Davila", 8 Eroii Sanitari St., Bucharest 050474, Romania

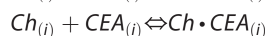
 Copyright © 2015 John Wiley & Sons, Ltd.

(Sun and Ma, 2012; Zhou *et al.*, 2012; Liu and Ma, 2013). The lowest limit of detection 0.015 fg/ml was obtained with an immunosensor based on poly(2-aminothiophenol) and Au nanoparticles (Liu and Ma, 2013). The most sensitive method for the assay of NSE reported to date is the one based on a portable and quantitative enzyme immunoassay, the limit of determination of NSE being 50 pg/ml (Fu *et al.*, 2014).

The purpose of this work was to develop a method and new tools for pattern recognition of NSE and CEA in whole blood samples. The new tools were based on stochastic sensors designed using nanoporous gold microspheres, graphite, graphene, diamond paste as well as α-cyclodextrins (CDs), and 5,10,15,20-tetraphenyl-21*H*,23*H*-porphyrin. Stochastic sensors are recognized as a class of sensors capable for single-molecule detection that are based up on the alteration of electrical current by distinct analytes that interact transiently or permanently with a functional recognition group located within a nanopore. The pore contains a binding site for an analyte, and each time the analyte binds to the pore, the current is modulated; the reaction occurring can be summarized as follows:



$$Ch_{(i)} + NSE_{(i)} \Leftrightarrow Ch \bullet NSE_{(i)}$$



$$Ch_{(i)} + CEA_{(i)} \Leftrightarrow Ch \bullet CEA_{(i)}$$

where *Ch* is the channel, and *i* is the interface.

The frequency of occurrence of the events reveals the concentration of the analyte (t_{on}), whereas the current signature (the mean duration and amplitude of the events) reveals its identity (t_{off}) (Bayley and Cremer, 2001; Movileanu, 2009). Therefore, the stochastic sensors represent a good alternative to the traditional methods because of their capability of determining in one run the quality and the quantity of different analytes in the sample. Their introduction as tools in biomedical analysis and nanomedicine can improve the limits of quantification of the substances of biological importance and accordingly can solve the problem of screening tests for pattern recognition and early detection of substances of clinical interest.

EXPERIMENTAL

Reagents and materials

Carcinoembryonic antigen, NSE, natural diamond powder having particle size of 1 μm (99.9%), graphite powder, graphene powder, 5,10,15,20-tetraphenyl-21*H*,23*H*-porphyrin (P), monosodium and disodium phosphate were purchased from Sigma Aldrich (Milwaukee, USA), paraffin oil and NaN_3 was purchased from Fluka (Buchs, Switzerland), α-CD was supplied by Wacher-Chemie GmbH (Germany). Engineered nanoporous 14 K gold microspheres were obtained from NILPRP. Monosodium phosphate and disodium phosphate were used for the preparation of phosphate buffer 0.1 mol/l, pH = 7,4. Deionized water obtained from a Millipore Direct-Q 3 System (Molsheim, France) was used for the preparation of all solutions. All standard solutions were prepared in buffer solution pH = 7.4, with NaN_3 0,1% in a ratio water : buffer solution 1:1 (v/v). Serial dilution technique was used for the preparation of solutions of different concentrations.

Instrumentation

For all chronoamperometric measurements, a PGSTAT 12 potentiostat/galvanostat connected to a three-electrode cell was used, and linked to a computer via an Eco Chemie (Utretch, The Netherlands) software version 4.9. The electrochemical cell was assembled with a conventional three-electrode cell: the working electrode, an Ag/AgCl (0.1 mol/1KCl) as reference electrode, and a Pt counter electrode. For the pH measurements, a Cyberscan PCD 6500 pH/mV-meter from Eutech Instruments was used.

Design of the sensors

Modified diamond (DP), graphene, and graphite pastes were prepared as follows: the powder of each material was mixed with paraffin oil to form a paste. 50 μl from the 10^{-3} mol/l solution of the electrochemical active compound (α-CD or 5,10,15,20-tetraphenyl-21H,23H-porphyrin) was added to each 100 mg of

Table 1. Response characteristics of the stochastic sensors used for the screening of carcinoembryonic antigen (CEA) and neuron specific enolase (NSE)

Stochastic senors based on	τ_{off} (s)	Linear concentration range (pg/ml)	Limit of determination (pg/ml)	Sensitivity (s mg^{-1} ml)	Equation of calibration*
CEA					
P/DP	2.7	6–160	1.6	1.76×10^5	$1/\tau_{on} = 0.07 + 1.76 \times 10^5 \times C$; r = 0.9998
α-CD/DP	2.1	0.16–160	0.16	3.19×10^5	$1/\tau_{on} = 0.08 + 3.19 \times 10^5 \times C$; r = 0.9968
Nanoporous Au microspheres	3	$160–1.6 \times 10^4$	160	2.92×10^3	$1/\tau_{on} = 0.02 + 2.92 \times 10^3 \times C$; r = 0.9995
P/graphite	1.5	0.016–1.6	0.016	2.32×10^7	$1/\tau_{on} = 0.03 + 2.32 \times 10^7 \times C$; r = 0.9957
P/graphene	2	$1.6–1.6 \times 10^3$	1.6	3.65×10^4	$1/\tau_{on} = 0.03 + 3.65 \times 10^4 \times C$; $r = 0.9934$
NSE					
P/DP	2.5	$4.77 \times 10^3–7.63 \times 10^3$	4.77×10^3	4.8×10^3	$1/\tau_{on} = 0.044 + 4.8 \times 10^3 \times C$; r = 0.9923
α-CD/DP	1.5	$7.63 \times 10^3–1.22 \times 10^5$	7.63×10^3	95.58	$1/\tau_{on} = 0.040 + 95.58 \times C$; $r = 0.9946$
Nanoporous Au microspheres	2	$1.22 \times 10^5–1.95 \times 10^6$	1.22×10^5	8.20	$1/\tau_{on} = 0.035 + 8.20 \times C$; $r = 0.9950$
P/graphite	2.7	$3.05 \times 10^4–1.95 \times 10^6$	3.05×10^4	35.14	$1/\tau_{on} = 0.039 + 35.14 \times C$; $r = 0.9991$
P/graphene	3	7.45–119	7.45	2.49×10^5	$1/\tau_{on} = 0.039 + 2.49 \times 10^5 \times C$; $r = 0.9921$

*$<C> =$ mg/ml; $<\tau_{on}> =$ s.

Copyright © 2015 John Wiley & Sons, Ltd.

Journal of Molecular Recognition

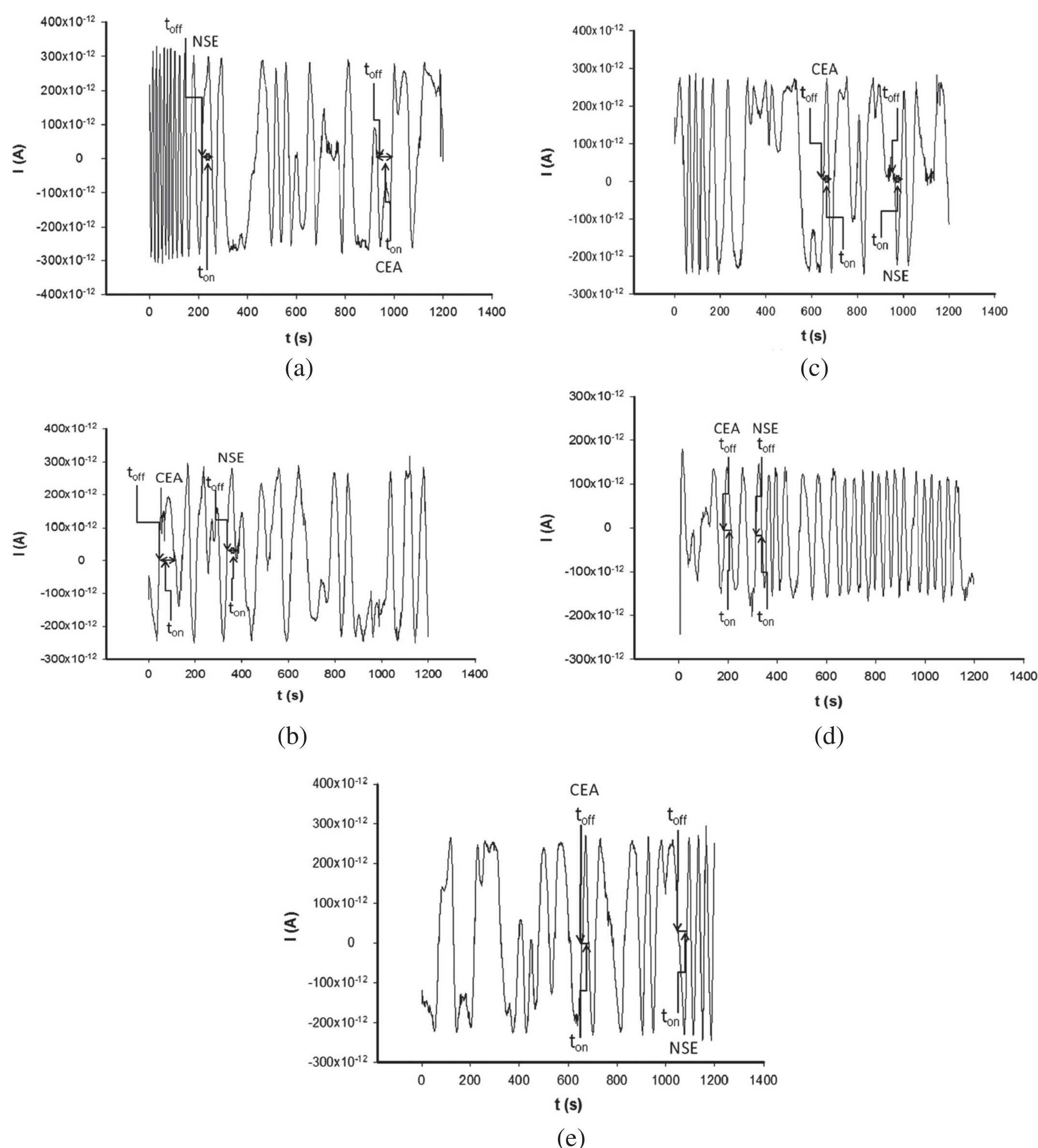


Figure 1. Diagrams obtained for the pattern recognition of carcinoembryonic antigen (CEA) and neuron specific enolase (NSE), resulted by screening tests of whole blood samples with the sensors based on (a) P/DP, (b) α-CD/DP, (c) nanoporous Au microspheres, (d) P/graphite, and (e) P/graphene.

paste. The modified paste was placed into a plastic tube with a diameter of 25 μm. Electric contact was obtained by inserting an Ag wire into the modified paste. The surface of the sensor was wetted with deionized water and polished with alumina paper (polishing strips 30144-001, Orion) before using. When not in use, the sensors were stored in a dry state at room temperature.

One nanoporous gold microsphere of a diameter of 300 μm was placed in a plastic tube so that half of the sphere was out and the other half was inside the tube. Electrical contact was carried out using a silver wire.

Stochastic method

For the stochastic sensing, a chronoamperometric technique was selected for the measurements of t_{on} and t_{off} at 125 mV. The electrodes were dipped into a cell containing solutions of analyte of different concentrations. Equations of calibration $1/t_{on} = f(\text{Conc.})$ are determined using statistics (linear concentration range – data obtained for t_{on} when solution containing different concentration of CEA and NSE are measured using the sensors considered, the values obtained for r will help in assessing the linear concentration range, and the pairs concentration (x) – $1/t_{on}$ (y) obtained on this range will be considered to calculate the parameters of the equation of calibration using statistic method based on linear regression equation). Unknown concentrations of NSE and CEA were determined using these equations.

Sample preparation

Whole blood samples were taken from the Universitary Hospital in Bucharest (Ethics committee approval no. 11/2013. Informed consent was obtained from all subjects), and they were used for the screening of NSE and CEA without any pretreatement. The apparatus cell was filled with whole blood sample, and the unknown concentration of NSE and CEA in whole blood samples were determined using the aforementioned stochastic method.

 Copyright © 2015 John Wiley & Sons, Ltd.

Table 2. Pattern recognition of carcinoembryonic antigen (CEA) and neuron specific enolase (NSE) in whole blood samples using stochastic sensors

Sample	Sensors based on					*t*-test
	P/DP	α-CD/DP	Nanoporous Au	P/graphite	P/graphene	
CEA (pg/ml)						
1	0.24	0.22	0.23	0.26	0.20	1.32
2	0.54	0.59	0.57	0.55	0.50	1.50
3	0.78	0.73	1.3	1.0	0.96	3.20
4	0.97	0.91	1.4	1.0	1.0	1.98
5	0.93	1.0	1.5	1.0	1.2	1.03
NSE (ng/ml)						
1	1.0	1.4	1.6	1.2	0.99	1.54
2	2.0	2.5	2.3	2.4	1.9	1.74
3	0.97	1.5	1.8	1.3	0.94	2.23
4	7.1	7.0	7.5	7.2	6.9	1.19
5	3.0	3.0	2.1	3.3	3.5	2.30

All values are the average of ten determination, RSD $< 1.0\%$.

RESULTS

Response characteristics of the stochastic sensors used for pattern recognition of neuron specific enolase and carcinoembryonic antigen

The diagrams obtained using stochastic sensors can be used for the qualitative as well as quantitative analysis. The qualitative assay of CEA is given by the values of τ_{off} (signature of the analyte), presented in Table 1, and the quantitative assay is given by the value of τ_{on}. The values of τ_{off} can be used for the identification of the biomarker in biological fluids (blood, serum, and saliva). Response characteristics of the stochastic sensors are shown in Table 1: quality is given by the values of τ_{off}, while using τ_{on}, one can determine the equation of calibration, sensitivity (slope of the electrode), and linear concentration range. All the sensors used for the screening of NSE and CEA showed very good response characteristics with high values of sensitivity, and low limits of quantification, of pg/ml magnitude order. As can be seen in Table 1, the best response characteristics in terms of sensitivity and limit of detection were recorded for the assay of CEA using the stochastic sensors based on P/graphite (the limit of determination of 16 pg/ml), while for the assay of NSE, the best results were obtained using the sensor based on P/graphene (the limit of determination of 7.45 pg/ml). Taking into account the sensitivities of the sensors based on P/graphite and P/graphene as well as their limits of determination for NSE and CEA, and the working concentration ranges, the best to use for simultaneous pattern recognition of NSE and CEA will be the sensor based on P/graphene. Compare with the results proposed previously for simultaneous assay of NSE and CEA using quantum dots based fluorescence and chemiluminescence immunoassay (Li *et al.*, 2010; Cao *et al.*, 2011; Li *et al.*, 2011), there were recorded lower limits of determination (in the range of pg/ml compared with ng/ml reported previously (Li *et al.*, 2010; Cao *et al.*, 2011; Li *et al.*, 2011)), and higher sensitivities. The stochastic method allows direct assay of the analytes in whole blood samples, while the previously reported methods (Li *et al.*, 2010; Cao *et al.*, 2011; Li *et al.*, 2011) required processed samples, namely serum samples for the simultaneous assay of the analytes.

The response characteristics were reproducible for more than 6 months of daily use, when relative standard deviation (RSD) values of the slopes were less than 1.00%.

Pattern recognition of neuron specific enolase and carcinoembryonic antigen in whole blood samples

Pattern recognition of NSE and CEA was carried out based on their signatures identified in the diagrams recorded using stochastic sensors (Figure 1). Table 2 shows the results for the assay of NSE and CEA in whole blood samples, based on τ_{off} values for the identification of CEA and NSE in the diagram (Figure 1), and on τ_{on} values from diagrams, and stochastic method described earlier for the assay of the concentrations of CEA and NSE. Based on the paired *t*-test performed for 99% confidence level, for which all values calculated for *t*-pair test at the 99.00% confidence level are less than the tabulated theoretical (*t*-value: 4.032), one can conclude that there is no statistically significant difference between the results obtained using the five stochastic microsensors, and they can be successfully used for the quantification of NSE and CEA in whole blood samples.

CONCLUSIONS

The proposed tools and method can be reliably used for pattern recognition of NSE and of CEA in whole blood samples, helping with fast and early detection of lung cancer. The proposed tools are stochastic sensors designed using nanoporous gold microspheres, graphite, graphene, diamond paste as well as α-CDs, and 5,10,15,20-tetraphenyl-21*H*,23*H*-porphyrin. The stochastic sensor of choice according with the limit of determination, sensitivity, and working concentration range is the one based on P/graphene. The proposed tools and method have great features in screening tests of whole blood for early detection of lung cancer. The test is carried out within minutes from unprocessed whole blood samples.

Acknowledgements

This work was supported by the PNII Program Capacity, 2012–2014, contract no. 3ERC-like/2012, by the Sectorial Operational Programme Human Resources Development 2007–2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/132395, and by PNII Ideas, project number PN-II-ID-PCE-2011-3-0953.

Copyright © 2015 John Wiley & Sons, Ltd.

REFERENCES

American Cancer Society. 2014. Cancer Facts & Figures. American Cancer Society: Atlanta.

Bayley H, Cremer P. 2001. Stochastic sensors inspired by biology. *Nature* **413**: 226–230.

Cao Z, Li H, Lau C, Zhang Y. 2011. Cross-talk-free simultaneous fluoroimmunoassay of two biomarkers based on dual-color quantum dots. *Anal. Chim. Acta* **698**: 44–50.

Cedres S, Nunez I, Longo M, Martinez P, Checa E, Torrejon D, Felip E. 2011. Serum tumor markers CEA, CYFRA21-1, and CA-125 are associated with worse prognosis in advanced non-small-cell lung cancer (NSCLC). *Clin. Lung Cancer* **12**: 172–179.

Fu X, Feng X, Xu K, Huang R. 2014. A portable and quantitative enzyme immunoassay of neuron-specific enolase with a glucometer readout. *Anal. Meth.* **6**: 2233–2238.

Grunnet M, Sorensen JB. 2012. Carcinoembryonic antigen (CEA) as tumor marker in lung cancer. *Lung Cancer* **76**: 138–143.

Han J, Zhuo Y, Chai YQ, Yuan YL, Yuan R. 2012. Novel electrochemical catalysis as signal amplified strategy for label-free detection of neuron-specific enolase. *Biosens. Bioletectron.* **31**: 399–405.

He H, Chen G, Zhou L, Liu Y. 2009. A joint detection of CEA and CA-50 levels in saliva and serum of patients wih tumors in oral region and salivary gland. *J. Cancer Res. Clin. Oncol.* **135**: 1315–1321.

Holmes JL, Davis F, Collyer SD, Higson SPJ. 2011. A new application of scanning electrochemical microscopy for the label interrogation of antibody-antigen interactions. *Anal. Chim. Acta* **689**: 206–211.

Hou JY, Liu TC, Lin GF, Li ZX, Zou LP, Li M, Wu YS. 2012. Development of an immunomagnetic bead-based time-resolved fluorescence immunoassay, for rapid determination of levels of carcinoembryonic antigen in human serum. *Anal. Chim. Acta* **734**: 93–98.

Jun Z, Jin G, Zhi Y, Zhen-Fu L, Ji-Chang Z, Li-Xin Z, Yi W, Guang-Wei X. 2003. Relationship among CEA expression in tumor, CEA serum level and radioimmunoguided surgery in colorectal cancer. *CJCR* **15**: 269–272.

Kaleta EJ, Tolan NV, Ness KA, Kane DO', Algeciras-Schimnich A. 2013. CEA, AFP and CA 19-9 analysis in peritoneal fluid to differentiate causes of ascites formation. *Clin. Biochem.* **46**: 814–818.

Li H, Cao Z, Zhang Y, Lau C, Lu J. 2010. Combination of quantum dot fluorescence with enzyme chemiluminescence for multiplexed detection of lung cancer biomarkers. *Anal. Meth.* **2**: 1236–1242.

Li H, Cao Z, Zhang Y, Lau C, Lu J. 2011. Simultaneous detection of two lung cancer biomarkers using dual-color fluorescence quantum dots. *Analyst* **136**:1399–1405.

Liu Z, Ma Z. 2013. Fabrication of an ultrasensitive electrochemical immunosensor for CEA based on conducting long-chain polythiols. *Biosens. Bioelectron.* **46**: 1–7.

Martin EW Jr., Kibbey WE, Divecchia L, Anderson G, Catalano P, Minton JP. 1976. Carcinoembryonic antigen: clinical and historical aspects. *Cancer* **37**: 62–81.

Maruvada P, Wang W, Wagner PD, Srivastava S. 2005. Biomarkers in molecular medicine: cancer detection and diagnosis, *Biotechniques* **38**: 9–15.

Movileanu L. 2009. Interrogating single proteins through nanopores: challenges and opportunities. *Trends Biotechnol.* **27**: 333–341.

Naghibalhossaini F, Ebadi P. 2006. Evidence for CEA release from human colon cancer cells by an endogenous GPI-PLD enzyme. *Cancer Lett.* **234**: 158–167.

Pavlou M, Diamandis EP. 2010. The cancer cell secretome: a good source for discovering biomarkers? *J. Proteomics* **73**: 1896–1906.

Qu S, Liu J, Luo J, Huang Y, Shi W, Wang B, Cai X. 2013. A rapid and highly sensitive portable chemiluminiscent immunosensor of carcinoembryonic antigen based on immunomagneti separation in human serum. *Anal. Chim. Acta* **766**: 94–99.

Quinones W, Ziober A, Yao Y, Bing Z. 2013. Immunohistochemical markers for the differential diagnosis of nephrogenic adenomas. *Ann. Diagn. Path.* **17**: 41–44.

Schrohl AS, Holten-Andersen M, Sweep F, Schmitt M, Harbeck N, Foekens J, Brunner N. 2003. Tumor markers from laboratory to clinical utility. *Mol. Cell. Proteomics* **2**: 378–386.

Shen GY, Wang H, Deng T, Shen GL, Yu RQ. 2005. A novel piezoelectric immunosensor for detection of carcinoembryonic antigen. *Talanta* **67**: 217–220.

Sun X, Ma Z. 2012. Highly stable electrochemical immunosensor for carcinoembryonic antigen. *Biosens. Bioelectron.* **35**: 470–474.

Tomida M, Mikami I, Takeuchi S, Nishimura H, Akiyama H. 2009. Serum levels of nicotinamide N-methyltransferase in patients with lung cancer. *J. Cancer Res. Clin. Oncol.* **135**: 1223–1229.

Torsetnes SB, Lovbak SG, Claus C, Lund H, Nordlund MS, Paus E, Halvorsen TG, Reubsaet L. 2013. Immunocapture and LC-MS/MS for selective quantification and differentiation of the izoenzymes of the biomarker neuron specific enolase in serum. *J. Chromatogr. B* **929**: 125–132.

Vaidyanathan K, Vasudevan DM. 2012. Organ specific tumor markers: What's new? *Ind. J. Clin. Biochem.* **27**: 110–120.

Wu Y, Wu Y, Wang J, Yan Z, Qu L, Xiang B, Zhang Y. 2011. An optimal tumor marker group-coupled artificial network for diagnosis of lung cancer. *Expert Syst. Appl.* **38**: 11329–11334.

Yan F, Zhou J, Lin J, Ju H, Hu X. 2005. Flow injection immunoassay for carcinoembryonic antigen combined with time-resolved fluorimetric detection. *J. Immunol. Methods* **305**: 120–127.

Zhou J, Zhuang J, Miro M, Gao Z, Chen G, Tang D. 2012. Carbon nanospheres-promoted electrochemical immunoassay coupled with hollow platinum nanolabels for sensitivity enhancement. *Biosens. Bioelectron.* **35**: 394–400.

Copyright © 2015 John Wiley & Sons, Ltd.