# Methods for the Quantification of Salivary Cortisol and of α-amylase in Biosensors and Portable Devices


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*There is an increasing interest in the analysis of salivary biomarkers for medical practice. The objective of this article was to identify the specificity and sensitivity of quantification methods used in biosensors or portable devices for the determination of salivary cortisol and salivary α-amylase. There are no biosensors and portable devices for salivary amylase and cortisol that are used on a large scale in clinical studies. These devices would be useful in assessing more real-time psychological research in the future.*



*Keywords: biosensors; stress biomarkers; salivary cortisol; salivary α-amylase.*


Saliva sampling has the advantages of being non-invasive, stress free and allowing multiple sampling easily done by the patient. Analysis of saliva biomarkers is an increasing interest in medical practice. Rapid quantification of biomarkers in practical medicine is useful not only for rapid diagnosis, self-control of the disease and treatment management, but also for population studies [1-4].

Salivary cortisol is considered a non-invasive biomarker for the activity of the hypothalamus-pituitary-adrenal (HPA) axis, which is the main system involved in psychological and pathological stressors adaptation [5-7]. Currently, analysis of salivary cortisol is considered a standard method for the evaluation of the influence of stress [8].

Recently, salivary α-amylase has been indicated as a non-invasive surrogate biomarker for the quantification of the activity of the sympathetic system [9-11]. It was noticed that modifications of amylase activity in saliva precede modifications of salivary cortisol levels in case of psychological stress [12]. Salivary α-amylase is useful in quantifying psychological stress and to distinguish between eustress and distress [13].

According to the IUPAC definitions [14], biosensors are chemical devices that use specific biochemical reactions mediated by enzymes, immune system components (antibodies or antigens), tissues, organelles, whole cells, and even microorganisms or bacteria to detect chemical compounds usually through electrical, thermal or optical signals.

The objective of this article was to identify the specificity and sensitivity of quantification methods used in biosensors or portable devices for the determination of salivary cortisol and salivary α-amylase.

## Experimental part

*Material and methods*

Data sources were relevant studies from PubMed and SCOPUS databases, published in English from 1980 up to September 2017. Search terms used included *stress biomarkers; biosensor; salivary cortisol and salivary α-amylase*. We excluded the duplicates and studies which do not describe the methods. The articles with incomplete data, letters to the editors, reviews and commentaries were excluded as well. We recorded the method of detection, the range of analytes for detection, the sensibility and the specificity of the methods.

## Results and discussions

Most of the articles regarding the methods used in biosensors and portable devices for quantification of salivary cortisol and salivary α-amylase are based on optical or electrochemical transducers.

The most important step in the construction of an electrochemical based biosensor is the immobilization of the biological recognition material on the surface of an electrode. Biological elements for the detection of salivary cortisol are conjugate anti-cortisol antibodies. Electrodes used up to now for the detection of salivary cortisol are made of gold or graphene. Horseradish peroxidase (HRP)-labelled cortisol is used in optic methods for the detection of salivary cortisol.

Particularities of biosensors and detection methods for salivary cortisol analysis are presented in table 1.

Biological elements for the detection of salivary α-amylase are α-amylase specific antibodies. Enzymatic reaction time is used in optic methods for the detection of salivary α-amylase.

Particularities of biosensors and detection methods for salivary cortisol analysis are presented in table 2.

Salivary cortisol and amylase can be utilized as excellent indexes for stress evaluation. Salivary gland biomarkers respond quickly and sensitively to stress factors, similar to blood biomarkers. The levels of α-amylase in saliva are modified more rapidly than the levels of cortisol, therefore


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**Table 1**
SALIVARY CORTISOL: BIOSENSORS AND TRANSDUCTION SYSTEMS

| Biosensor transducer | Molecular recognition material | Detection method | Limit of detection | Sensitivity | Author |
|---|---|---|---|---|---|
| Electrochemical | conjugate anti-cortisol antibody | gold microelectrodes using 3,3'-dithiodipropionic acid di(N-hydroxysuccinimide ester (DTSP) as a self-assembled monolayer agent | 3 pg/mL-10 µg/mL | High | Khan et al., 2017 [16] |
| Electrochemical | anti-cortisol antibody | reduced graphene oxide electrode using a thermally denatured bovine serum albumin protein layer as an interlayer | 10 pM-100 nM | High | Kim et al., 2017 [17] |
| Optical | lossy mode resonance | nanocomposites having 20% zinc oxide (ZnO) in polypyrrole | $0-10^{-6}$ g/ml | High | Usha et al., 2016 [18] |
| Optical | peroxidase-cortisol conjugate | detected by adding the chemiluminescent substrate luminol/enhancer/hydrogen peroxide, with a smartphone camera as light detector | 0.3-60 ng/mL | Sensitive enough | Zangheri et al., 2015 [19] |
| Optical | Horseradish peroxidase (HRP)-labelled cortisol | colorimetric detection at 450nm, using a standard complementary metal-oxide-semiconductor (CMOS) silicon photodiode as the photodetector | 0.01-20 ng/mL | High | Pinto et al., 2017 [20] |
| Optical | Cortisol-specific monoclonal antibodies | surface plasmon resonance (SPR) detection | 1.5-10 ng/ml | High | Stevens et al., 2008 [21] |

**Table 2**
SALIVARY α-AMYLASE: BIOSENSORS AND TRANSDUCTION SYSTEMS

| Biosensor transducer | Molecular recognition material | Detection method | Limit of detection | Sensitivity | Author |
|---|---|---|---|---|---|
| Optical | enzymatic reaction time | maltose as a competitive inhibitor to a substrate Ga1-G2-CNP | 0–200 kU/1 | High | Yamaguchi et al., 2004 [13] |
| Electrochemical | α-amylase specific antibody | detected by electrochemical impedance spectroscopy in a polyaniline layer | 0.025-1000IU/L | High | Teixeira et al., 2016 [4] |

the use of both biomarkers can be useful to quantify the modifications induced by stress.

Salivary α-amylase activity depends on the saliva volume, but a recent study demonstrated that a new method for the analysis of a very small sample volume (5 µL) does not need to determine the salivary volume quantitatively [13].

Numerous analytical methods (including high-performance liquid chromatography, mass spectrometry, ultraviolet spectroscopy etc) have been successfully used for the detection of salivary cortisol and salivary α-amylase, but their major disadvantages are the need of expensive equipment, highly skilled personnel and long analysis duration [21]. Detection of cortisol and of α-amylase in a complex matrix such as saliva has been simplified by the development of new and very sensitive methods that allows the measurement of very low quantities of analytes.

Classical methods for the quantification of salivary cortisol and salivary α-amylase are largely used and recognized for studies on stress influence in different diseases [22-24].

Socioeconomic status and psychological factors are currently evaluated in different pathological states [25], therefore there is a need for simple and precise methods of detection.

New methods in biosensors and portable devices for the detection of salivary biomarkers are compared with clasical methods clinical studies, to evaluate the limits of detection of the analyte and the sensitivity of the methods [26-28]. The development of rapid, sensitive, selective and low cost biosensors is needed for the rapid detection and monitoring of salivary cortisol and of α-amylase in clinical studies on stress.

## Conclusions

The most used methods for the detection of salivary cortisol and for salivary α-amylase detections are optical. No portable device is currently used for the concomitant detection of these biomarkers in saliva.

There are no biosensors and portable devices for salivary amylase and for cortisol used in large scale in clinical studies. Biosensors and portable devices for salivary amylase and for cortisol biomarkers would be useful to assess more real-time psychological research.

## References


1. HASSANI, S., MOMTAZ, S., VAKHSHITEH, F., et al. Biosensors and their applications in detection of organophosphorus pesticides in the environment. Arch. Toxicol. 91, nr. 1, 2017, p. 109.
2. CAPRANICA, L., CONDELLO, G., TORNELLO, F., et al. Salivary alpha-amylase, salivary cortisol, and anxiety during a youth taekwondo championship: An observational study. Medicine (Baltimore). 96, nr. 28, 2017, p. e7272.
3. RUTHERFURD-MARKWICK, K., STARCK, C., DULSON, D.,K., ALI, A. Salivary diagnostic markers in males and females during rest and exercise. J. Int. Soc. Sports. Nutr. 14, 2017, p. 27.
4. TEIXEIRA, SR., LLOYD, C., YAO, S., ANDREA SALVATORE GAZZE, et al. Polyaniline-graphene based α-amylase biosensor with a linear dynamic range in excess of 6 orders of magnitude. Biosens. Bioelectron. 85, 2016, p. 395.
5. OUANES, S., CASTELAO, E., GEBREAB, S., et al. Life events, salivary cortisol, and cognitive performance in nondemented subjects: a population-based study. Neurobiol. Aging. 51, 2017, p. 1.
6. COZMA, S., DIMA-COZMA, L.,C., GHICIUC, CM., et al. Salivary cortisol and α-amylase: subclinical indicators of stress as cardiometabolic risk. Braz. J. Med. Biol. Res. 50, nr. 2, 2017, p. 5577.
7. GHICIUC, C.,M., DIMA-COZMA, L.,C., BERCEA, R.,M., et al. Imbalance in the diurnal salivary testosterone/cortisol ratio in men with severe obstructive sleep apnea: an observational study. Braz. J. Otorhinolaryngol. 82, nr. 5, 2016, p. 529.
8. STALDER, T., KIRSCHBAUM, C., KUDIELKA, B.,M., et al. Assessment of the cortisol awakening response: Expert consensus guidelines. Psychoneuroendocrinol. 63, 2016, p. 414.
9. ORTU, E., PIETROPAOLI, D., MAZZEI, G., et al. TENS effects on salivary stress markers: A pilot study. Int. J. Immunopathol. Pharmacol. 28, 2015, nr. 1, p. 114.
10. DIMA-COZMA, C., PATACCHIOLI, F.R., GHICIUC, C.,M., et al. Current perspectives in stress research and cardiometabolic risk. Rev. Cercet. Interv. So. 45, 2014, p. 181.
11. PATACCHIOLI, F.,R., TABARRINI, A., GHICIUC, C.,M., et al. Salivary biomarkers of obstructive sleep apnea syndrome in children. Pediatr. Pulmonol 2014, 49, p. 11, p. 1145.
12. SCHUMACHER, S., KIRSCHBAUM, C., FYDRICH, T., STROHLE, A. Is salivary alpha-amylase an indicator of autonomic nervous system dysregulations in mental disorders?-a review of preliminary findings and the interactions with cortisol. Psychoneuroendocrinology. 38, nr. 6, 2013, p. 729.
13. YAMAGUCHI, M., KANEMORI, T., KANEMARU, M., et al. Performance evaluation of salivary amylase activity monitor. Biosens Bioelectron. 20, nr. 3, 2004, p. 491.
14. *** IUPAC. Compendium of Chemical Terminology, 2nd ed. (the Gold Book). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). XML on-line corrected version: http://goldbook.iupac.org (2006-) created by M. Nic, J. Jirat, B. Kosata; updates compiled by A. Jenkins. ISBN 0-9678550-9-8. https://doi.org/10.1351/goldbook. Last update: 2014-02-24; version: 2.3.3.
15. KHAN, M.,S., MISRA, S.,K., WANG, Z., et al. Paper-Based Analytical Biosensor Chip Designed from Graphene-Nanoplatelet-Amphiphilic-diblock-co-Polymer Composite for Cortisol Detection in Human Saliva. Anal. Chem. 89, nr. 3, 2017, p. 2107.
16. KIM, Y.,H., LEE, K., JUNG, H., et al. Direct immune-detection of cortisol by chemiresistor graphene oxide sensor. Biosens Bioelectron. 98, 2017, p. 473.
17. USHA, S.,P., SHRIVASTAV, A.,M., GUPTA, B.,D. A contemporary approach for design and characterization of fiber-optic-cortisol sensor tailoring LMR and ZnO/PPY molecularly imprinted film. Biosens. Bioelectron. 87, 2017, p. 178.
18. ZANGHERI, M., DI NARDO, F., MIRASOLI, M., et al. Chemiluminescence lateral flow immunoassay cartridge with integrated amorphous silicon photosensors array for human serum albumin detection in urine samples. Anal. Bioanal. Chem. 408, nr. 30, 2016, p. 8869.
19. PINTO, V., SOUSA, P., CATARINO, S.,O., et al. Microfluidic immunosensor for rapid and highly-sensitive salivary cortisol quantification. Biosens. Bioelectron. 90, 2017, p. 308.
20. STEVENS RC, SOELBERG SD, NEAR S, FURLONG CE. Detection of cortisol in saliva with a flow-filtered, portable surface plasmon resonance biosensorsystem. Anal Chem. 80, nr. 17, 2008, p. 6747.
21. SONGA, E.,A., OKONKWO, J.,O. Recent approaches to improving selectivity and sensitivity of enzyme-based biosensors for organophosphorus pesticides: A review. Talanta. 155, 2016, p. 289-304.
22. BERCEA, R.,M., PATACCHIOLI, F.,R., GHICIUC, C.,M., et al. Serum testosterone and depressive symptoms in severe OSA patients. Andrologia. 45, nr. 5, 2013, 345.
23. PIPPI, R., PATINI, R., GHICIUC, C.,M., et al. Diurnal trajectories of salivary cortisol, salivary α-amylase and psychological profiles in oral lichen planus patients. J. Biol. Regul. Homeost. Agents. 28, nr. 1, 2014, p. 152.
24. GEORGESCU, S.R., ENE, C.D., NICOLAE, I., MITRAN, M., MUSETESCU, A., MATEI, C., RUSU, L.C., TAMPA, M., Rev. Chim. (Bucharest), **68**, no. 5, 2017, p. 1103
25. DIMA-COZMA, C., MITU, F., SZALONTAY, A., COJOCARU, D.,C. Socioeconomic status and psychological factors in patients with essential hypertension. Rev. Cercet. Interv. So. 44, 2014, p. 147.
26. USHIKI, K., SATO, Y., ARAI, K., et al., Evaluation of mental stress tests among medical students based on salivary sample collected just before the national license examination. Rinsho. Byori. 59, nr. 2, 2011, p. 138.
27. DELLA VENTURA, B., SAKAC, N., FUNARI, R., VELOTTA, R. Flexible immunosensor for the detection of salivary α-amylase in body fluids. Talanta. 174, 2017, p. 52.
28. KAUSHIK, A., VASUDEV, A., ARYA, S.,K., et al. Recent advances in cortisol sensing technologies for point-of-care application. Biosens. Bioelectron. 53, 2014, p. 499.


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