SOME STEPS IN THE DEVELOPMENT OF AN IMMUNOCHROMATOGRAPHIC TEST TO QUANTIFY CORTISOL IN HUMAN SALIVARY FLUID.

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Introduction

Cortisol is a corticosteroid hormone widely used as a marker of stress. Fenol A. et al. measured cortisol levels in conjunction with the presence of stress and periodontal disease in prison settings.[1] The authors found that the level of cortisol in the salivary fluid serves as a reliable marker of the stress state of the body and correlates with cases of periodontal disease. In his dissertation work, Brown C.W. studied the effect of cortisol on the state of anxiety mental disorder in adolescents, provides recommendations for the use of cortisol as a marker for the diagnosis of depression.[2] Bedini S. et al. conducted studies on the increase in salivary cortisol among ambulance dispatchers.[3] All of these studies have found a link between stress and increased levels of cortisol in the body. Due to its high speed and ease of analysis, immunochromatography has a potential advantage for detecting stress-dependent conditions, however, the development of a quantitative version of LFIA is a complex and complicated task.

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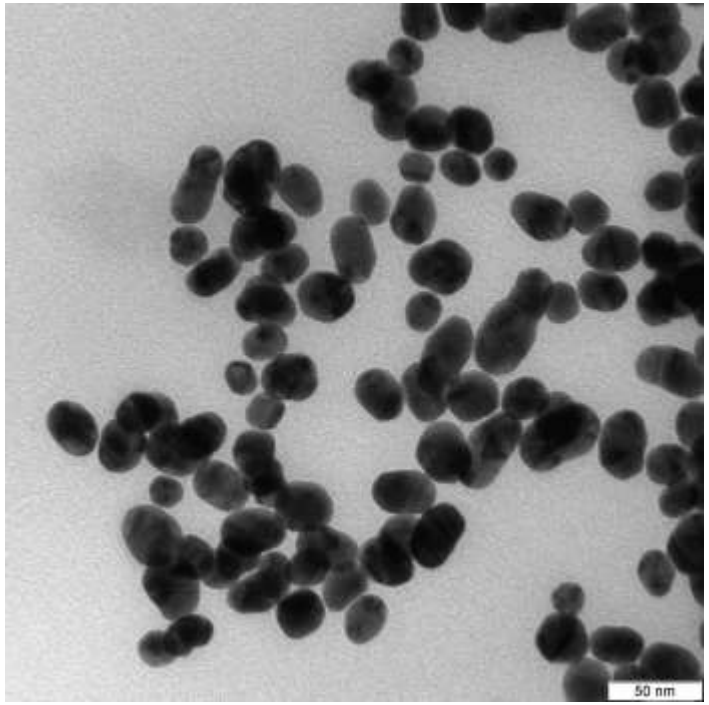
[1] Fenol A. et al. association of stress, salivary cortisol level, and periodontitis among the inmates of a central prison in Kerala // Dent. Res. J. 2017. № 3. V. 4. P. 288-292.

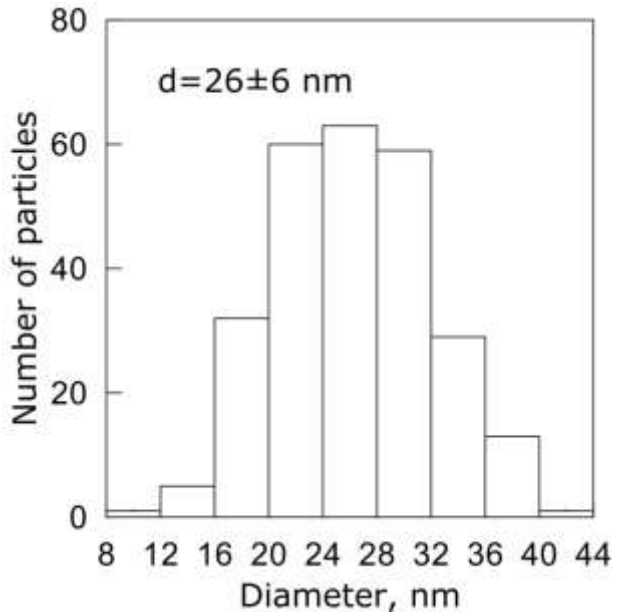
[2] Brown C.W. Salivary cortisol as a Measure of stress reactivity in adolescents with psychiatric disorders. 2016. Yale Medicine Thesis Digital Library. 2041. http://elischolar.library.yale.edu/ymtdl/2041

[3] Bedini S. Stress and salivatory cortisol in emergency medical dispatchers: a randomized shifts control trial // Plos One. 2017. doi.org/10.1371/journal.pone.0177094

How to make test-strips for cortisol detection?
Preliminary steps.

STEP 1. Synthesize 25 nm Au colloid.

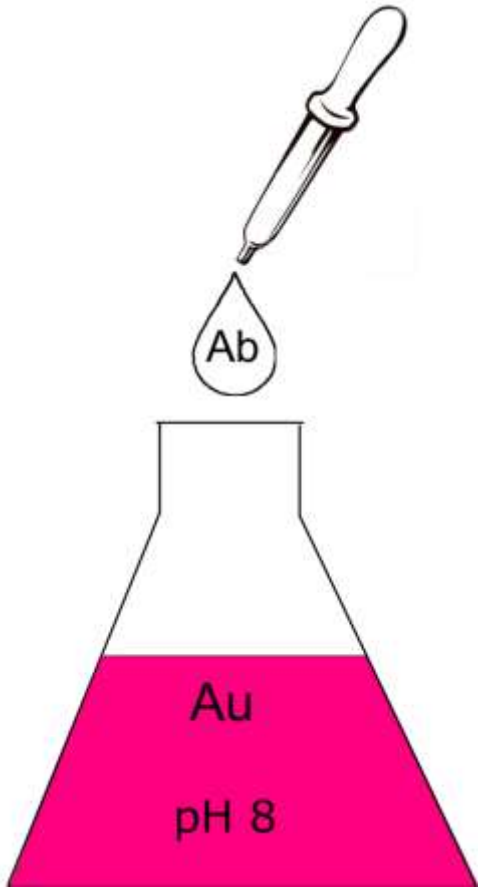




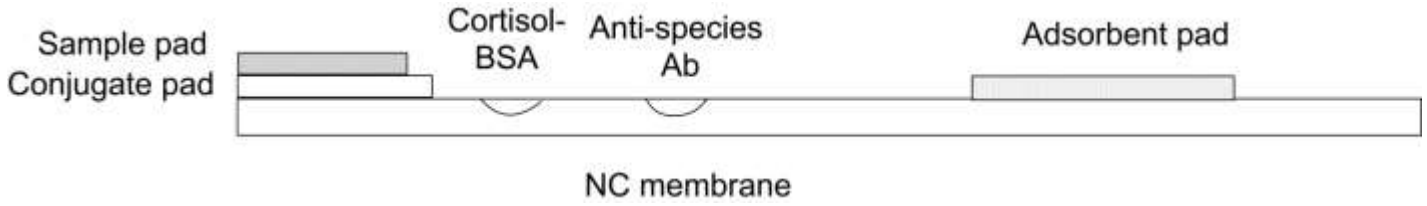
STEP 2. Make conjugate.

Protocol:

The colloidal gold suspension with a diameter of 25 nm was adjusted to pH 8 using 200 mM potash. Then a-cortisol antibody was added to the Au colloid, with a final concentration of 30 μg/ml. The conjugate was left for 1 hour. Then a BSA solution was added to a concentration of 0.1%, and 10 minutes later the suspension was centrifuged at 6000 rpm for 15 min. Re-dissolved in 10 mM phosphate buffer.



STEP 3. Collect the test strip.



To make test-strips, we use NC membranes type CNPF-SN-12-L2-H50, sample pad type GFB-R7L, adsorbent pad type AP 045, conjugate pad type PT-R7. All from mdi Advanced Microdevices Pvt. Ltd, India.

STEP 4. Determine the content of cortisol in cortisol-BSA applied to the membrane.

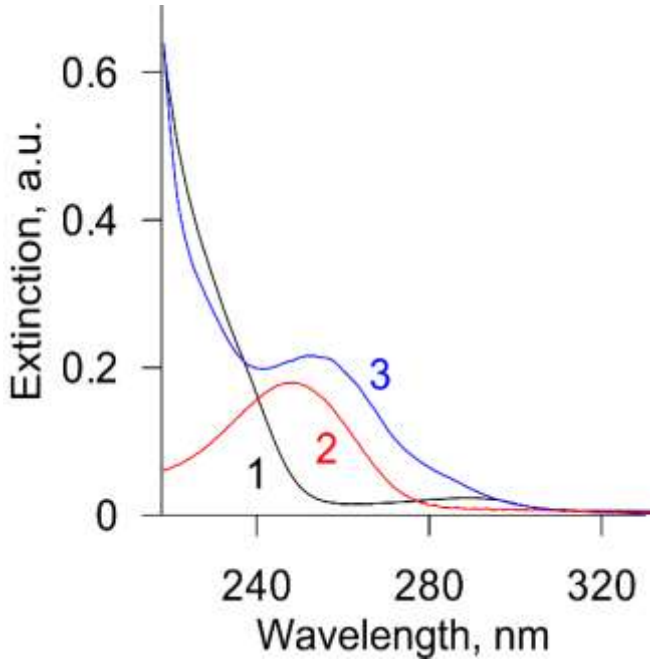


Figure. 1. Extinction spectra of BSA (1), cortisol (2), cortisol-BSA complex (3). The shift in the absorption maximum from 248 nm to 251 nm is associated with the chemical modification of cortisol, with its presence in a bound state.

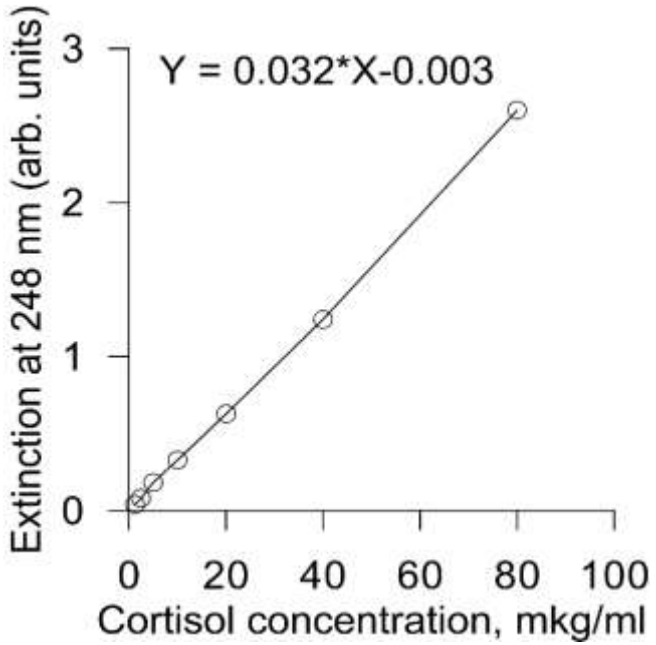
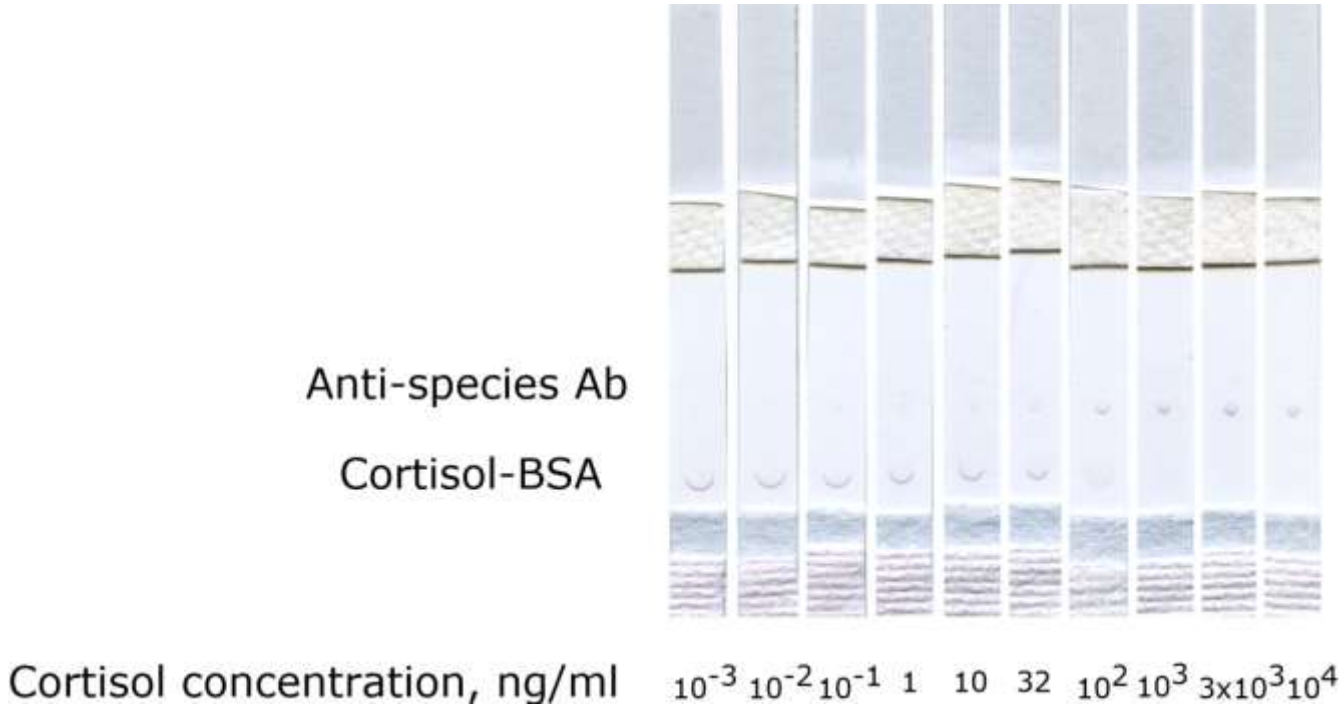


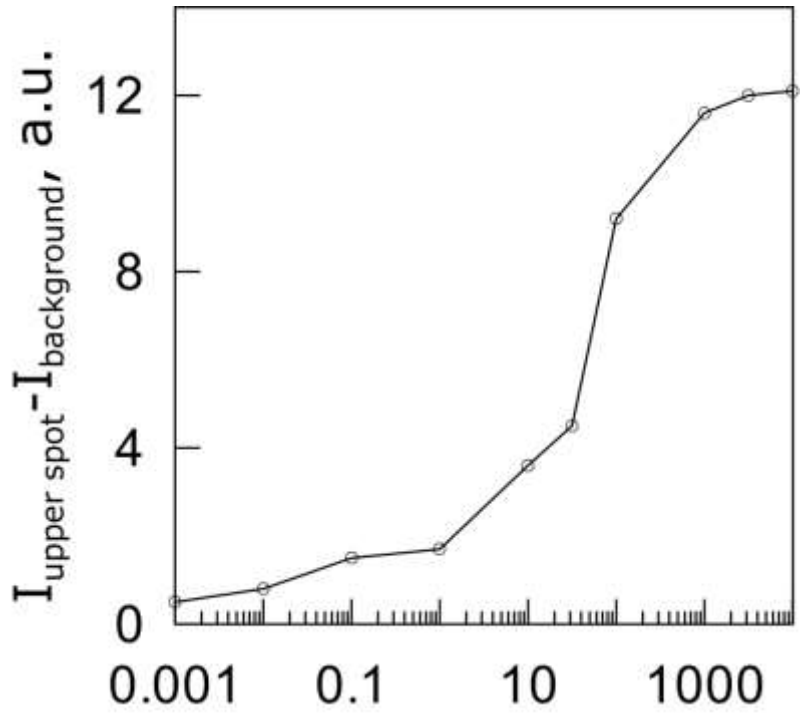
Figure. 2. A calibration graph of the dependence of the absorption of solutions of cortisol with a concentration of 1.25 to 80 μg / ml from concentration at 248 nm. An approximating line equation: Y = 0.032 * X-0.003.

STEP 5. Prepare calibration solutions

We prepare 10 calibration solutions with cortisol concentration from 0,001 ng/ml to 10 mg/ml and make the test.



STEP 6. Digitize test-strips and make a calibration curve.



Now you are ready to make test using saliva samples

Conclusion

In this work, we carried out preliminary stages in the development of an immunochromatographic test for the quantitative determination of cortisol in biological samples. As a result we obtained a calibration curve for the dependence of the intensity of staining on an immunochromatographic test strip on the concentration of cortisol in the analyzed liquid. Solutions of the cortisol-BSA complex with a cortisol content of 0.001, 0.01, 0.1, 1, 10, 31.62, 100 ng / ml and 1, 3.162, 10 mg / ml were used as calibration solutions. The content of cortisol in the complex was determined spectrophotometrically. A solution of the cortisol-BSA complex at a concentration of 1.05 mg / ml and secondary polyclonal antibodies at a concentration of 200 μg/ml were applied to a nitrocellulose membrane. After assembling all test components 60 μL of the calibration solution was preincubated with 10 μL of a solution of colloidal gold conjugate with (d = 25 nm) with antibodies to cortisol, then applied to the sample pad of the test. Within 15 minutes, staining of one or two (depending on the concentration of cortisol in the analyzed fluid) spots on the membrane occurred.

The membranes were dried and scanned. The stained spots were digitized using the Image J. program. Based on the data obtained, a calibration curve was constructed to determine the concentration of cortisol in the analyzed liquid by the intensity of staining the spot on the test strip.

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