



The Soma IgA / Cortisol Test With the SOMA Cube Reader

Lateral Flow Immunoassays for the rapid
quantitative measurement of salivary sample

English test instructions and information.



Tests for research or investigative purposes.
Not an in vitro diagnostic test.

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INTRODUCTION

Lateral-Flow assays represent a well established proven technology for a variety of point-of-care and field use applications. Although these simple diagnostic tests are established in many routine applications, this technology has not been widely applied when very sensitive, highly reproducible, quantitative results or electronic data documentation are required. The SOMA Cube Reader now makes this possible, by combining the major advantages of traditional lateral flow assay with modern technologies to fulfill the requirements for new quantitative tests.

Test specific data is transferred to the SOMA Cube wirelessly by radio frequency identification (RFID) before each measurement. The data is stored internally and can be transferred to a computer via USB using the Data Viewer Software.

It is essential to check before each measurement that the Lot No. on the RFID card matches that on the LFD foil pouch label. The component parts required for a test are a SOMA Cube Reader, a SOMA Oral Fluid Collector (OFC) swab, SOMA OFC Buffer and a SOMA LFD cassette, specific to the analyte under investigation.

Prior to first use, please read this manual carefully.

SYMBOLS USED IN THIS MANUAL AND ON THE CUBE

	Attention! Very important and safety relevant information
	Manufacturer's instructions
	Please follow the description
	<i>In-Vitro</i> -Diagnostics
 2004-06	Date of manufacture (year & month)
	Serial number
	Don't dispose in general trash. By disposal of device please refer to country specific rules and laws
REV	Part number
IP20	Protection class of electronic equipment
	CE-symbol

BEFORE USING THE SOMA CUBE



Prior to first use please read the instruction manual carefully. The SOMA Cube is designed exclusively for use on a straight and horizontal surface area. During the measurement it should not be moved and not be exposed to any bright light (e.g. sunlight). The device should never be opened (except for battery compartment) otherwise the warranty is invalidated.

BATTERY POWER IN THE SOMA CUBE



The SOMA Cube is powered by 3 lithium batteries (button cells), which are type CR2032. Replacement batteries can be purchased from a wide variety of retail stores. The SOMA Cube is supplied with 3 batteries in a separate blister pack and need be inserted to the SOMA Cube to enable operation.

When inserting the batteries take care to avoid grease from fingers coming into contact with the batteries. Such contamination can lead to a more rapid discharge of the batteries and reduce their life. Therefore gloves or plastic tweezers are recommended.

In case the device does not start after putting new batteries in please check the polarity of the batteries and clean the batteries by using a dry cloth.



After turning on the device for the first time or changing the batteries, date and time needs to be set. Please refer to “Setting date and time” below.

MATERIALS SUPPLIED, STORAGE AND STABILITY

Component	Cat. No.	Content	Storage at	Shelf Life
SOMA CUBE READER	SOMA LFD CUBE	1	4°C to 40°C	N/A
SOMA OFC SWAB	SOMA OFC	100	4°C to 37°C	24 months
SOMA OFC BUFFER	SOMA OFC	100	4°C to 37°C	18 months
SOMA LFD	SOMA LFD-	100	4°C to 37°C	12 months

SPECIMEN COLLECTION AND PREPARATION

The determination of quantitative levels in human oral fluid requires the collection of Oral Fluid using the SOMA OFC (Oral Fluid Collector) Swab and Buffer. The swab will collect 0.5 mL of oral fluid and is then placed in the SOMA Buffer. However the concentration values given on the reader will be the actual value of the target analyte in the saliva sample.

WARNINGS AND PRECAUTIONS

All reagents within SOMA test kits are strictly intended for *in vitro* use only. It is intended that the kits be used by staff who are informed and trained to carry out such tests. Please adhere strictly to the stated protocols in this document for safety and to ensure the gathering of effective information.

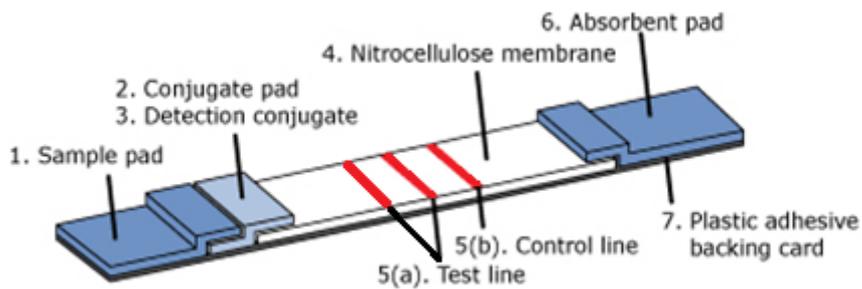
Timings are important and deviation from the stated protocol will increase the variability of the data gained.

Samples should be stored securely or disposed of responsibly upon test completion. It is usual to gain informed consent from humans before the commencement of testing procedures. Guidance on ethics and informed consent can be found on this WHO website:

http://www.who.int/rpc/research_ethics/informed_consent/en/

METHOD AND TEST PRINCIPLE

Most rapid diagnostic tests work by capturing analytes on a solid surface and then attaching molecules to them that allow detection by the naked eye. SOMA's test is based on the principle of Lateral flow, also called immunochromatographic strip (ICS) tests or simply strip-tests. The LFD consists of 7 components making the strip in addition to the housing plastic cassette. These are shown in the diagram below, the example of which is a dual analyte IgA / Cortisol test.



1. Sample Pad
2. Conjugate Pad- The conjugate pad contains the dried detection reagent (conjugate).
3. Detection Conjugate: Gold-labelled anti-IgA secretory chain antibody
4. Solid-phase Nitrocellulose Membrane.
5. Test lines: e.g. secretory IgA, cortisol and control reagent line.
6. Absorbent Pad.
7. Plastic-adhesive backing card

The test is carried out by adding the sample (buffer/saliva mixture) onto the sample pad. The liquid will move by capillary action through the conjugate pad hydrating the dried conjugate. The whole mixture continues its flow through the nitrocellulose membrane towards the wicking pad at the end of the strip. As the mixture flows across the membrane, the gold-labelled anti-secretory IgA (or other analyte) will be captured by the sIgA (or other analyte) test line resulting in the appearance of a red line. If secretory IgA is present in the sample, this will bind to the gold labelled anti-secretory IgA antibody, resulting in fewer gold particles being captured by the sIgA test line (similarly for the cortisol line). It follows that the test line intensity is inversely proportional to the sIgA and cortisol concentration in the sample. The SOMA LFD Reader measures the line intensity and converts these values into the corresponding sIgA and cortisol concentrations in the saliva sample, expressed in $\mu\text{g/ml}$ (IgA), ng/ml or nM (cortisol), on the basis of a specific programmed standard curve specific to each Lot of IgA/Cortisol test strips.

TEST PROCEDURE AND PROTOCOLS

The stages are:

1. Sample collection using SOMA OFC swab
2. Placement of completed Swab into SOMA OFC Buffer
3. Addition of Buffer / Sample mix to LFD and incubation
4. Measurement of the sample on the LFD with the SOMA Cube
5. Interpretation of data

SAMPLE COLLECTION WITH SOMA OFC

SOMA OFC SWAB:

The oral fluid collector consists of a specially formulated synthetic polymer-based swab material attached to a plastic tube containing a volume adequacy indicator. The collector is designed to collect 0.5mL oral fluid.

SOMA OFC BUFFER BOTTLE:

The SOMA OFC buffer contains sodium phosphate, salts, detergents and preservatives. It has a number of key properties to make it an effective tool for user-friendly oral fluid collection in the field. Not only does it contain extraction agents to draw the target analytes from the swab into the buffer, it also contains preservatives to prevent growth of microorganisms. Once the swab is in the buffer it is stable at 37°C for three weeks. It is recommended that continued storage (weeks) be in a refrigerator or (months) in a freezer, where IgA samples in the SOMA buffer are stable for at least 18 months.

IMPORTANT: DO NOT INGEST THE BUFFER.

SAFETY NOTES:

- The oral fluid collector is designed for single use only.
- Do not chew or suck the oral fluid collection swab.
- Do not place the oral fluid collection swab in the mouth after it has been in the sample collection solution.

COLLECTION PROCEDURE:

1. Remove the SOMA OFC swab from the bag.
2. Place the swab in the mouth, on top of the tongue and close mouth, bringing pooled saliva to the swab (do not suck!). This method ensures reduced variability due to secretion of IgA at different rates from various saliva glands.

BEFORE



Volume indicator

3. Continue to collect until the volume adequacy indicator has turned royal BLUE in colour. This will typically take 20-50 seconds in most individuals, but can take several minutes if dehydrated, or flow rate is very low. In these rare instances be patient and await the colour change.

AFTER



Colour change to royal blue

PLACEMENT OF SWAB INTO SOMA OFC

Once the oral fluid has been collected, it should be placed in the Buffer bottle (unscrew the lid first), by holding the plastic tube and inserting the bud end of the swab into the Buffer, in the direction shown below. Take care not to spill any buffer from the bottle.



Replace the top of the Buffer bottle tightly.

The bottle should then be mixed *for a period of at least two minutes*. This should be done in a rhythmic up and down, or back and forth motion. The mixing is important to enable full extraction of the target analyte from the swab into the buffer. *Do not shake too vigorously*.

ADDITION OF SAMPLE TO LFD

Remove the SOMA IgA / Cortisol (or other analyte) LFD from the foil pouch by tearing at the notches on either side. The control line will appear as a green line, IgA blue and Cortisol an orange/red colour before the test has been run. All SOMA LFDs have coloured test lines corresponding to the LFD type; this is to more easily differentiate between LFDs when out of packaging.

As the LFD can be affected by humidity, it is important to check that there has been no colour change in the silica gel sachet that is also packed within each foil pouch with the LFD. If the silica gel has changed in colour (orange to green) then it is unlikely that the LFD is suitable for use and should be discarded.

Hold the buffer bottle perpendicular to the surface where the LFD is resting (for good repeatable performance, this should be a flat level

surface). Put two drops of the Sample / Buffer mix into the round test window of the SOMA IgA / Cortisol (or other) LFD. Within 30 seconds a reddish liquid will start to appear in the rectangular test window and run across the whole strip. You will notice that the original colour dyes will be washed away by the samples. In the unlikely event that red colour has not appeared within 90 seconds, add one more additional drop.

You should start to time the test from when the reddish colour first starts to appear in the rectangular test window. You will then scan your IgA / Cortisol (or other) LFD on exactly 15 minutes from when the reddish colour first appears in the test window. (Another option is to time the test from the application of the drops if testing many samples in one session). You will notice that within the test window there is the formation of three red lines, a (C) control and two (T) Test lines. Scanning the IgA / Cortisol LFD either before or after 15 minutes will add to the variability of your readings, so *it is important to be consistent and aim to read on 15 minutes on each occasion.*

MEASUREMENT WITH THE SOMA CUBE

The device offers the following options for the test measurements:

a) Immediate measurement

This type of measurement requires that the timing of the run or incubation time is done manually from when the test is started (drops added to LFD or the appearance of red liquid in the Test Window on the LFD. Deviation from the 10 minute incubation time will add to variability of your results.

This Method is best for when you are running a series of tests together

b) Timer measurement

This type of measurement follows a test specific configured incubation period, e.g. 10 minutes. At the end of the incubation period the measurement starts and displays the result. The measurement procedure can be stopped by pressing the control button.

Basic SOMA Cube Operation

1. When device is turned off, the display is empty.
2. To turn the device on, press the button briefly less than 1 sec.
3. After activation an audible alarm appears, the display shows 'ON'.
- 4a. Immediate Measurement (for Timer Measurement proceed to 4b)**

Press the button briefly, the display will show 'RFID'; proceed to 5.

4b. Timer Measurement

If you want to start the measurement after a certain incubation time has passed press the button longer than 1 sec. The display will show RFID. Each timer measurement can be stopped by pressing the button during the measurement.

5.
Put the lot specific RFID card onto the top side of the SOMA Cube reader **taking care that the Lot No. displayed on the RFID card is exactly the same as the Lot ID for the LFD cassettes you are measuring.**

6.
After successful wireless transmission of the data an audible signal starts. The display shows 'TEST'. The LFD that you wish to measure is now required.

- 7a.
The test has to be placed into the cavity on the bottom side of the device (in the case of Bulge cassette LFDs). Ensure the LFD and the bottom of of the SOMA Cube are flush and on a flat surface.

OR

- 7b.
Place the LFD into the LFD Housing, then place you SOMA Cube on top, ensuring that the fit is flush.

8.
Start the measurement by pressing the button shortly.

9.

The device measures and the display shows 'RUN'.

10.

After a few seconds an audible alarm starts and the result is displayed.

11.1 Storage of test results

(If no storage is required proceed with 12.1)

If the button is pressed longer than 1 sec. the measurement result(s) will be stored.

An audible alarm appears, the display shows 'SAVE'. The reader has an internal memory to store more than 100 readings. If the internal memory is full and a new result is to be saved, the reader overwrites the first saved result. Every new saved result will overwrite the saved results in a chronological order.

11.2

Press the button again for the next measurement to commence.

11.3

The display will show 'ON' again, as when the SOMA Cube was first turned on. Thereafter proceed with the above instructions from point 4.

12.1 No storage of test result

If no result storage is required, briefly press the button less than 1 sec. to not store the result.

12.2

'ON' appears in the display; continue with point 4 if another measurement is to be started .

13.

If the device is switched on and not activated for about 50 sec., the SOMA Cube automatically shuts down to save battery life. If a new measurement is to be started continue with point 2.

Please note: **There is no active function to shut off the SOMA Cube.**

It is important that the Lot ID displayed is the same as the Lot Number displayed on the Label of the LFD foil pouch; the specific calibration characteristics are programmed to the reader for each batch of strips manufactured.

CHANGING THE LOT ID

This is done via the RFID card supplied with your LFD test kits and the card must be used before each and every single measurement. If you are using LFDs from two batches in the same testing session, please be sure to use the correct RFID card for your LFDs.

ERROR MESSAGES ON THE SOMA CUBE

Display: 'ERR'

The device could not read the information from the RFID card.

SOLUTION

Press the button briefly (<1 sec.), the display will show 'ON' and continue with point 4a or 4b in the instructions above. If the error occurs for several times, please contact SOMA directly.

Display: 'DATE'

The expiry date of the LFD has past and performance could be impaired as real-time stability is only checked for 12 months from manufacture.

SOLUTION

The SOMA Cube checks the internally set date with the expiration date of the test.

Check the expiration date of the LFD batch (this will be displayed on the LFD foil pouch). If this has passed, select a new LFD from another shipment which has not expired. Press the button briefly, the display will show 'ON' and repeat point 4a or 4b in the instructions above. If the expiration date has not passed, check the internally set time and date of the device and adjust it if necessary.

Display: 'FAIL'

The device could not find a Control line or the signal of the Control line is very weak. The most common cause of this is insufficient sample applied to the LFD from the OFC or the test has not run properly. This can happen if there has been a large air bubble within one of the two drops, or only one drop was added to the LFD.

SOLUTION

Check that the LFD if it is placed correctly below the cube or in the Housing (section: measurement procedure, point 7a or 7b). Then press the button briefly (<1 sec.), the device shows 'ON' and repeat with 4a or 4b from the instructions above. If the error does not disappear, select a new test LFD and repeat the measurement procedure. Press the control button briefly (<1 sec.), the display will show 'ON' and continue with 5a or 5b.

No function

Despite of pressing the button no information is visible on the screen.

Cause: The batteries maybe discharged.

SOLUTION

Open the battery compartment and replace the 3 batteries with new ones as described in section "Battery Power in the SOMA Cube" near the start of this manual.

Should the device still not react after changing the batteries, please contact the SOMA.

Setting date and time

Bring the SOMA Cube to position 'ON' according to step 1. Press the button twice short (<1 sec.).

Year, time and date will appear on the display

Press the button for about 1 sec., a flashing display appears with the first time specification, year. By repeated short (<1 sec.) pressing of the button, the displayed value can be changed. When the desired value as reached (e.g. year) press the button longer (>1 sec), the required year will be stored and the next time information will be presented. Repeat these steps to successively move to year, month, day, hour and minute. After setting the date and time information minutes accordingly the device will display

‘OK’. Press the button one more time, the reader will show ‘ON’ and is now ready for use. Repeat this process with every battery change.

Data transfer

The SOMA Cube has the capability of data transfer to a PC or laptop. Therefore the unique USB cable and the Cube Data Reader software are required.

Returning the product

In case of any defect, it may be necessary to send back the device to the manufacturer. As the device might have been contaminated by residue substances, the surface has to be disinfected before submitting. Therefore please use a suitable disinfectant cleaning. The disinfectant should be approved and not attack the material of the device (e.g. *Mikrozid® AF Liquid* or similar products). The disinfection has to be documented.



Attention: Please note that a returned device will not be accepted without any proof of disinfection.

Maintenance and cleaning of the window

The SOMA Cube is maintenance free. Before each measurement the glass on the bottom should be checked for impurities, liquids or residues. For cleaning we recommend a commercial cloth together with a commercial cleaning fluid, e.g. for glasses products.

Disposal of the device



As the device can be contaminated by any material it should be disinfected with appropriate safety equipment.

Dispose of the used device after removing the batteries in accordance with the applicable regulations.

IgA / Cortisol Dual LFD product specifications

SOMA Product Code:

SOMA LFD - sIgA/Cort

Test Kit Contents:

100 OFC (Swab & Buffer)

100 Dual analyte LFDs

For the measurement of sIgA and salivary cortisol.

Applications:

For the analysis of saliva samples for the quantitative determination of sIgA & cortisol when read in the SOMA LFD Reader or Cube Reader. For use in Sport, Exercise, Corporate, Healthcare and Research.

Incubation Time:

15 minutes from the addition of sample.

Sample Volume:

Two drops of saliva / buffer mix from dilution buffer.

Shelf-Life:

Typically 12 months

Storage:

4°C to 37°C

Specificity:

Specific to secretory IgA and salivary cortisol.

L.O.D.

sIgA 18.1 µg/mL Cortisol 0.17nM

L.O.Q.

sIgA 24.3 µg/mL Cortisol 0.58nM

Calibration Range

sIgA:

25 - 800 µg/mL

Cortisol:

1.25 - 40 nM

Evaluation of a new Point of Care quantitative Cube reader for salivary analysis in Premier League soccer clubs

Dunbar J, Hazell G, & Jehanli A. IPRO Interactive, Wallingford, UK.

Introduction

The use of salivary biomarker responses has gathered momentum in recent years in sports, exercise and behavioural sciences. A Point of Care (POC) platform using Lateral Flow Device (LFD) technology, which takes just over 10 minutes to measure salivary IgA, has previously been validated (Coad et al., 2015).

Technological advancement has seen the introduction of a new reader device, the Ipro Cube Reader, that is smaller (2 inch cube), quicker (4 seconds scan time as opposed to 22 seconds) and considerably cheaper than the previous model. The ability to multiplex sampling with such a small and quick reader would save processing time and speed up delivery of data in the applied setting when the assessment of more than one biomarker is often required and such a POC test would certainly give a significant time advantage over standard laboratory techniques, which often reveal data to sporting squads only days later. This paper assesses a new Cube LFD reader in comparison to the previously validated LFD Reader.

Methods

A total of 48 saliva samples were taken during routine monitoring of a cohort English Premier League soccer players on two occasions in the same week, using IPRO Oral Fluid Collection (OFC) kits. The OFC kits collect 0.5mL of oral fluid and contain a colour changing volume adequacy indicator within the swab, giving collection times typically in the range of 20-50 seconds (Jehanli et al., 2011).

The samples were analysed for cortisol concentration using Ipro Cortisol LFDs on both the standard LFD reader and the new Cube Reader.

Similarly, the next week 50 samples were taken and assessed for IgA concentration using Ipro IgA LFDs and the two types of LFD Reader.

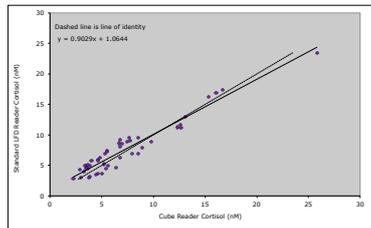
After sample collection and mixing (2 minutes), two drops of saliva/buffer mix from the OFC were added to the sample window of the cortisol or IgA LFFD. The liquid runs the length of the test strip via lateral flow, creating control and test lines visible in the test window. The test line intensity is inversely proportional to the cortisol or sIgA concentration in the sample giving a quantitative value on the reader.

Measurement range for cortisol is 1.5 - 40 nM on both readers and 18.5-900 µg/ml for sIgA, thus similar to typical ranges seen in laboratory ELISA analysis.

Results

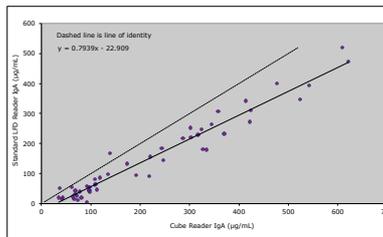
The cortisol values ranged from 2.24 - 25.8 nM on the Cube reader and 2.73 - 23.5 nM on the standard LFD Reader. Agreement between the two readers was good with Pearson correlation $r = 0.96$ (95% CI 0.93 - 0.98) with typical error of estimate 1.18nM (95% CI 0.98-1.148) and no significant difference between the two readers (Cube mean 7.1 ± 4.6 nM and LFD Reader 7.5 ± 4.3 nM).

Figure 1: Cortisol values on both LFD readers



The IgA values ranged from 34.7 - 621.1 µg/ml on the Cube reader and 20.0 - 518.8 µg/ml on the standard LFD Reader. The agreement between both readers was good, with the Pearson correlation $r = 0.98$ (95% CI, 0.96 - 0.999) and with typical error of estimate 29.91 (95% CI 24.94 - 37.36) µg/ml, with slightly higher values on the Cube (mean 215.6 ± 163.8) than the LFD Reader (148.3 ± 142.1).

Figure 2: IgA values on both LFD



Conclusion / Practical Implications

The new Cube LFD Reader Point of Care device show suitable validity for use in the sporting environment. Given the quick data turnaround and efficiency in terms of cost, it represents a suitable alternative method for use in sports teams.

Given the fact that both IgA and cortisol concentrations can now be performed on site, in the training environment, alongside other markers such as alpha-amylase on the same device; this test represents a true paradigm shift in the way athletes can be monitored, in that results are gained within twelve minutes from sample collection and subsequent intervention strategies can be applied immediately where appropriate.

References

Coad S, Mclellan C, Whitehouse T & Gray B (2015) Validity and reliability of a novel salivary immunoassay for Individual Profiling in Applied Sports Science. *Research in Sports Medicine* 23 (2): 140-150.

Jehanli A, Dunbar J & Skelhorn S (2011) Development and validation of an oral fluid collection device and its use in the immunoassay of salivary steroids and immunoglobulins in sports persons. *Proceedings 10th Symposium of the International Society of Exercise Immunology*.

The whole IPRO POC system in use



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