Salivary alpha-amylase Test

Lateral Flow Immunoassay for the quantitative measurement of salivary salivary alpha-amylase in combination with IPRO LFD Reader.

English test instructions and information.

Test for research or investigative purposes.
Not an in vitro diagnostic test.
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1.0 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 IPRO Lateral Flow Device (LFD) 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. The component parts required for a test are an IPRO LFD Reader, an IPRO Oral Fluid Collector (OFC) swab, the standard IPRO OFC Buffer, an additional Dilution Buffer and an IPRO LFD cassette, in this case salivary alpha-amylase (sAA).

IPRO LFD Reader Safety Precautions:

• **Operating location:** The location of the reader should be preferably on a desk or stable surface with enough surrounding space in order to easily insert the cassettes or unplug the device. In case of emergency or under abnormal operating conditions the location should provide, at any time, enough space to allow the easy disconnection of the device.

• **Battery power:** The IPRO LFD Reader can be powered by batteries without external power supply. The batteries must periodically be recharged by connecting the external power supply for at least 4 hours (the complete charging time is 14 hours). It is important to charge the reader for 24 Hours before first use. **Remember: The reader must be switched on to enable charging.**

• **Ambient temperature:** The use of the IPRO LFD Reader in environments prone to large changes in temperature can cause measurement values to deviate from real values. Please take the environmental conditions into account when trouble shooting.

• **Ambient light:** The IPRO LFD Reader is a highly sensitive and precise optical device. The device has internal correction for normal levels of ambient light, but highly intense light falling into the test strip insertion port can cause serious interference with the measurement and must be avoided.

• **Vibration:** The IPRO LFD Reader is a highly sensitive and precise optical device. The result can be influenced by vibrations e.g. if the device is used close to vibrating machines. The device must be used on a stable and level surface.

• **Dirty environment:** If you plan to use the IPRO LFD Reader in a working environment prone to dirt build-up, you will need to clean the device regularly. For cleaning, use a damp cloth. For more persistent stains, it is also possible to clean the surface with a cloth dipped in pure alcohol (isopropanol or ethanol). Avoid the use of aggressive solvents such as acetone.
2.0 Materials supplied, Storage and Stability

Table One: Materials supplied, storage and stability

<table>
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<tr>
<th>Component</th>
<th>Cat. No.</th>
<th>Content</th>
<th>Storage at</th>
<th>Shelf Life</th>
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<td>IPRO LFD READER</td>
<td>IPRO LFD RDR</td>
<td>1</td>
<td>4°C to 40°C</td>
<td>N/A</td>
</tr>
<tr>
<td>IPRO OFC SWAB</td>
<td>IPRO OFC</td>
<td>100</td>
<td>4°C to 37°C</td>
<td>24 months</td>
</tr>
<tr>
<td>IPRO OFC BUFFER</td>
<td>IPRO OFC</td>
<td>100</td>
<td>4°C to 37°C</td>
<td>18 months</td>
</tr>
<tr>
<td>IPRO DILUTION BUFFER</td>
<td>IPRO-DB</td>
<td>100</td>
<td>4°C to 37°C</td>
<td>18 months</td>
</tr>
<tr>
<td>IPRO sAA LFD</td>
<td>IPRO LFD-AA</td>
<td>100</td>
<td>4°C to 37°C</td>
<td>12 months</td>
</tr>
</tbody>
</table>

3.0 Specimen Collection and Preparation

The determination of sAA levels in human oral fluid requires the collection of Oral Fluid using the standard IPRO OFC (Oral Fluid Collector) Swab and Buffer. The swab will collect 0.5 mL of oral fluid and is then placed in the IPRO Buffer. Two drops of this is placed in the Dilution Buffer and then two drops from here are placed onto the LFD. However the sAA concentration value given on the reader will be the actual value of sAA in the saliva sample.

4.0 Warnings and Precautions

All reagents within IPRO 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/
5.0 Methodology 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. IPRO'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 (shown in figure one) making the strip in addition to the housing plastic cassette.

Figure One: Components within the IPRO LFD

1. Sample Pad
2. Conjugate Pad: The conjugate pad contains the dried detection reagent (conjugate).
3. Detection Conjugate: Gold-labelled anti-alpha-amylase antibody
4. Solid-phase Nitrocellulose Membrane.
5. Test line: sAA 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/absorbent pad at the end of the strip. As the mixture flows across the membrane, the gold-labelled anti-alpha-amylase will be captured by the alpha-amylase test line resulting in the appearance of a red line. If alpha-amylase is present in the sample, this will bind to the gold labelled anti-alpha-amylase antibody, resulting in fewer gold particles being captured by the alpha-amylase test line. It follows that the test line intensity is inversely proportional to the sAA concentration in the sample. The IPRO LFD Reader measures the line intensity and converts this into the corresponding sAA concentration in the saliva sample, expressed in ng/ml or nM, on the basis of a specific programmed standard curve specific to each Lot of sAA test strips.
5.1 Test Procedure and Protocols

The stages are:

1. Sample collection using IPRO OFC swab
2. Placement of completed Swab into the standard IPRO OFC Buffer
3. Addition of two drops from OFC Buffer to the Dilution Buffer
4. Addition of Dilution Buffer to LFD and incubation
5. Measurement of sAA sample in the LFD
6. Interpretation of data

5.2 Sample Collection with IPRO OFC

5.21 IPRO 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.

5.22 IPRO OFC BUFFER BOTTLE:
The IPRO 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 sAA samples in the IPRO buffer are stable for at least 18 months.

IMPORTANT: DO NOT INGEST THE BUFFER.

5.23 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.
5.24 COLLECTION PROCEDURE:

1. Remove the IPRO OFC swab from the bag, by tearing the perforation.

2. Place the swab in the mouth, either on top of the tongue and close mouth, or actively swab the cheeks and gums. It does not matter which method is used, as long as users are consistent on each occasion, because oral fluid can come from different saliva glands and the location of the swab is thus important.

Figure Two: OFC Collection Before

![Before Image]

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.

Figure Three: OFC Collection After

![After Image]

Colour change to royal blue
5.3 Placement of Swab into IPRO OFC Buffer

Once the oral fluid has been collected, it should be placed in the Buffer bottle, by holding the plastic tube and inserting the bud end of the swab into the Buffer, in the direction shown below.

Figure Four: Placement of Swab into the Buffer 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.

5.4 Dilution of Sample

Because the IPRO sAA assay is highly sensitive, it requires a further dilution than that for all of the other IPRO tests. The advantage of this process is that if you are running other assays on the same saliva sample, these can all be done from the standard OFC Buffer and then the dilution is done afterwards for your sAA test.

Flip the cap on the standard OFC buffer / saliva mix after mixing and then add two drops of this mix to the Dilution Buffer Bottle. Mix in a similar manner to how the mixing was done after adding the OFC Swab to the standard IPRO OFC Buffer i.e. mixing by gentle inversion for 1-2 minutes.
5.5 Addition of sample to LFD

Remove the IPRO sAA LFD from the foil pouch by tearing at the notches on either side. The sAA LFD will have a green test line and a green control line in the test window before running. All IPRO LFDs have coloured test lines corresponding to the LFD type; this is to more easily differentiate between LFDs when out of their 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 Dilution 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 Dilution Buffer mix into the round test window of the IPRO sAA LFD.

Within 30 seconds a reddish liquid will start to appear in the rectangular test window and run across the whole strip. In the unlikely event that this has not happened 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 sAA LFD on exactly 10 minutes from when the reddish colour first appears in the test window.

You will notice that within the test window there is the formation of two red lines, a (C) control and a (T) Test line.

Scanning the sAA LFD either before or after 10 minutes will add to the variability of your readings, so it is important to be consistent and aim to read on 10 minutes on each occasion.
5.6 Measurement in the IPRO LFD Reader

Figure Four: The IPRO LFD Reader

1. Display Window
2. ENTER and ON / OFF Button
3. BACK Button
4. FORWARD Button
5. UP Button
6. DOWN Button
7. Draw for IPRO sAA LFD
8. Battery Compartment

5.6.1 Basic Reader Operation

Full details of the operation and maintenance of the IPRO LFD Reader can be found in the separate manual documentation supplied on the memory stick with your IPRO LFD Reader (IPRO LFD RDR Manual). Basic instructions for general operation follow:

Press and hold the ON / OFF (2) button for two to three seconds. The IPRO LFD Reader will then start to boot up, while it displays a WELCOME message and go through a series of checks, which takes about two minutes.

Pull out the draw (7) and insert the sAA LFD cassette into the housing compartment, before closing the draw again. The reader will bleep if the cassette holder is not fully closed.
The default display on the reader will be to show the assay METHOD (in this case sAA) and the Lot ID, it also gives you the opportunity to enter a Sample ID.

*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.

When ready to scan the sAA LFD (10 minutes after the reddish colour has appeared in the LFD test window), scroll down to MEASURE on the reader display using the DOWN (6) button (press 3 times with short presses). Finally, press the ENTER button for half a second to commence the Scan.

The Scan takes about 20 seconds and you will then see the sAA result displayed.

In the top left hand corner of the display NT (Next Test) is highlighted, press the ENTER button to bring back the default menu, enabling the immediate performance of another scan.

5.62 CHANGING THE LOT ID

This is done by connecting the IPRO LFD READER to a PC via the serial USB link cable supplied. The process requires the supplied IPRO software and installation of drivers supplied on the memory stick that comes with the LFD Reader. The appropriate calibration method is usually emailed by IPRO to the client when a new Lot ID is dispatched.
5.63 OTHER READER BASICS

To turn the Reader off, press and hold the ON / OFF button for three seconds. You will hear three beeps and the reader will shut down.

The reader will shut down after a period of inactivity, in order to preserve battery power. The length of time for this shut down can be adjusted within the SETUP menu.

**Charging the rechargeable batteries:**
To do this, the reader must be switched ON. The reader will not charge the batteries when it is turned OFF, *even if it is plugged into mains power.*

The Reader will record and store up to 100 readings in its internal memory. When it has 100 readings, it will start to overwrite previous readings, starting with the oldest.

Do not move the reader when it is scanning. Movement whilst scanning can increase the risk of damage to the reader!!
Only valid results are shown on the IPRO LFD Reader. If the test result states INVALID, then it is advised that the test be repeated. A likely cause of this is that the sample has not been mixed sufficiently in the buffer before adding to the LFD. It is also possible that insufficient sample has been added to the sample window; this can be the case if large air bubbles are present in the drops. Try to hold the bottle perpendicular to the surface on which the Reader is placed to obtain consistent drop size; holding the bottle at an angle can whilst dispensing the sample / buffer mix can add to variability in your readings. Another possible cause of INVALID readings is that the test result is outside of the effective measurement range. If it is suspected that the value is very high, it is possible to titrate the sample and test again. Alternatively the sample can be sent to the IPRO Laboratory for confirmation.

The sAA LFD shows good agreement with the IPRO sAA laboratory ELISA assay. Given that this latter process takes a number of hours to complete, the data in Figure Six shows a comparison of the sAA LFD results, run 4 hours after collection, with the laboratory ELISA assay. Although the agreement is good, it should be remembered that the ELISA itself is a laboratory test, it is no gold standard in its own right.

**Figure Six: sAA values run on the LFD and ELISA**

![Graph showing the correlation between LFD and ELISA values]
The measurement of the sAA LFD test result can only be done in combination with the IPRO LFD Reader. This is a fully quantitative result. Monitoring of sAA in saliva is a useful way of measuring the stress response of an individual. It is worth remembering that this antibody capture method for determining sAA values in saliva is different to the many enzymatic methods available on ELISA. The range expected can be from 0-7500 µg/mL with little difference between males and females. The correlation between the amount of sAA in saliva and activity (U/ml) has been shown to be r=0.61. Mandel AL, Peyrot des Gachons C, Plank KL, Alarcon S, Breslin PAS (2010) Individual Differences in AMY1 Gene Copy Number, Salivary a-Amylase Levels, and the Perception of Oral Starch. PLoS ONE 5(10): e13352. doi:10.1371/journal.pone.0013352

8.0 ASSAY CHARACTERISTICS

8.1 sAA LFD CHARACTERISTICS

Sample Material: 0.5 mL of oral fluid, collected with IPRO OFC
Dilution: 2 drops from OFC Buffer into Dilution Buffer
Incubation Time: 10 minutes from first appearance in test window
Sensitivity: 31.25 µg/mL
Dynamic Range: 34.4 µg/mL to 8000 µg/mL
Specificity: Specific to salivary alpha-amylase not pancreatic amylase.
### 9.0 Technical Specifications of IPRO LFD Reader

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<th>Batteries</th>
<th>3 x 1.2V&lt;sub&gt;DC&lt;/sub&gt; AA Ni-MH rechargeable batteries 2700mAh</th>
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<tr>
<td>Power Port</td>
<td>DC 12 V, 1.25 A</td>
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<tr>
<td>Storage Conditions</td>
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<tr>
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<tr>
<td>Relative humidity (non condensing)</td>
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<tr>
<td>I/O Output</td>
<td>5 V logic</td>
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</table>

**Storage of IPRO LFD:**
Temperature: +5°C to +30°C

**Operation of IPRO LFD:**
Temperature: +15°C to +30°C