Investigating the use of a Point of Care salivary Amylase test in the English Premier League Soccer Environment

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Introduction

The use of salivary diagnostics within the sporting community has gathered momentum in recent years; identifying hormone levels to assist in the optimisation of workloads, or antibodies such as sIgA to assess individual recovery status and potential immune suppression. Another analyte gaining popularity is salivary alpha-amylase (sAA) an acute stress biomarker. Immediate feedback for coaching and support staff via a Point of Care (POC) test would give a significant time advantage over standard laboratory techniques, which often reveal data to sporting squads only days later.

This paper assesses a new point of care product for the rapid determination of sAA in comparison to a novel antibody capture laboratory ELISA determination.

Methods

A total of 25 saliva samples were taken from a cohort of English Premier League soccer players (23.5 ±6.4 yrs) 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 analysed in this study were taken during routine monitoring: before training sessions, during a competitive season. The samples were analysed to determine sAA concentrations via laboratory ELISA and POC test using Lateral Flow Device (LFD) specific for sAA. This platform has previously been validated for sIgA in a similar manner (Dunbar et al., 2011; Coad et al., 2015). Both tests used a novel antibody capture, rather than enzymatic method.

Two drops of saliva/buffer mix from the original OFC were added to a second dilution buffer, giving a 90 + 1 dilution, then mixed for two minutes. Two drops of this dilution were then added to the sample window of the sAA LFD. The liquid runs the length of the test strip via lateral flow, creating a control and test line visible in the test window. Ten minutes after the sample is added, the test line intensity is measured in an IPRO LFD Reader. The test line intensity is inversely proportional to the sAA concentration in the sample giving a quantitative value.

The same samples were then taken to a laboratory for subsequent analysis, which was started within four hours of collection.

Measurement range for both ELISA and the POC sAA test is 31.25 - 4000 µg/ml.

The IPRO LFD showing both the Control and Test Line

Results

The sAA concentrations measured via ELISA ranged from 210 - 3295 µg/ml and with the POC test from 64 - 3679 µg/ml, with the POC test giving lower values in most cases. The relationship between the salivary sAA values obtained using the ELISA and LFD is shown in Figure 1 and was represented by the formula:

$y = 0.83x - 3.8109$, with R^2 0.8107. The Pearson correlation between the two test types was r=0.93 (95% CI, 0.84 - 0.97

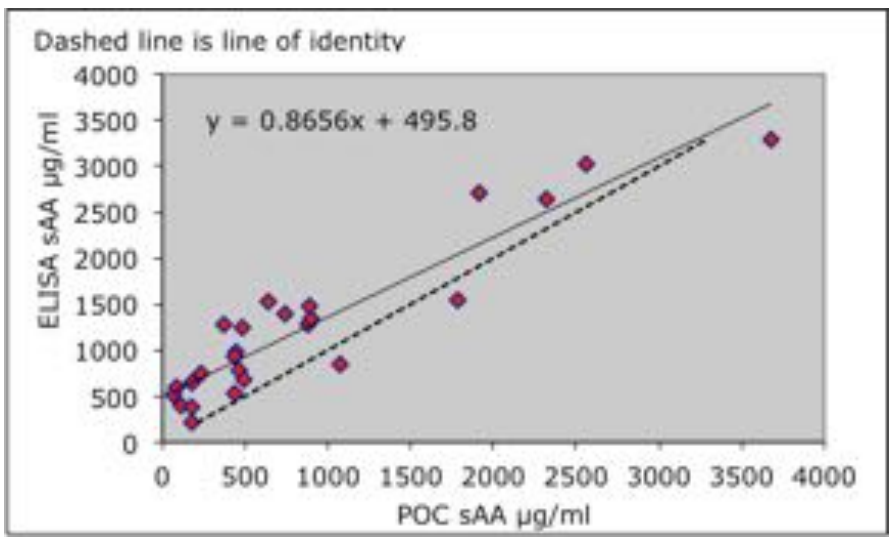

Figure 1: Relationship between sAA determination from ELISA & IPRO LFD

One of the most important aspects of such technology for the applied user is the repeatability of measurement. Athletes are more concerned about how their readings vary on a longitudinal basis, rather than how the LFD performs in comparison to the ELISA (which is far from a gold standard in its own right), so good repeatability is important.

Four of the samples were run as six replicates and the values, mean, S.D. and Coefficient of Variation (CV) of these replicates (expressed as a percentage) are displayed in Table 1.

The mean CV for the 6 replicates in four samples was 7.8%.

Table 1: Repeatability of the sAA LFD test with four of the samples run as six replicates

	sAA value in (µg/mL)			
Run No	GH	YK	HP	YO
1	202	191	645	1358
2	187	220	583	1487
3	189	198	608	1211
4	196	230	622	1140
5	204	213	629	1151
6	171	238	560	1162
Mean	191	215	608	1251
S.D.	12.1	18.2	31.5	140.6
% CV	6.31	8.45	5.19	11.24

Conclusion / Practical Implications

The point of care test shows good agreement with the ELISA method for the determination of salivary Alpha Amylase. 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 sAA concentrations can now be performed on site, in the training environment alongside other markers such as sIgA and cortisol 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 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.

Dunbar J, Jehanli A & Skelhorn S (2011) Investigating the use of a point of care sIgA test in English Premier League Soccer players. *Proceedings 10th Symposium of International Society of Exercise Immunology.*

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 IPRO OFC, LFD and LFD Reader

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