COLLABORATIVE RESEARCH AGREEMENT

THIS RESEARCH CONTRACT is dated September 15, 2015 and is made

BETWEEN:

i. **KING ABDULAZIZ UNIVERSITY**, located in Jeddah, Saudi Arabia (hereinafter referred to as KAU),

Prof Abdelrahman Obeid Alyoubi, as Vice President For Educational Affairs of the KAU

And

ii. **UNIVERSITAT ROVIRA I VIRGILI**, located in Tarragona, Spain (hereinafter referred to as URV)

Prof Josep Anton Ferré Vidal, Rector of the URV by virtue of his appointment through Decree 72/2014, of 27 May (DOGC 6633, of 29 May 2014), represents this institution in accordance with the competencies established in article 66 of the statute of the URV, which was approved by Decree 202/2003, of 26 August (DOGC 3963, of 8 September 2003), and modified by the agreement GOV/23/2012, of 27 March (DOGC 6100, of 2 April 2012).

1. RECITALS

A. KAU and URV have expertise in the fields of Analytical Chemistry and Chemical Engineering and they have agreed in entering into research collaboration.

B. The objective of this contract is to set out the framework under which, the Parties will work together to conduct the Research Project and to set out the rights and obligations of each Party.

IT IS AGREED AS FOLLOWS:

1. DEFINITIONS

In this contract the following expressions have the meaning set opposite:

Academic Publication:	The publication of an abstract, article or paper in a journal or an electronic Repository, or its presentation at a conference or seminar; and in clauses 5 and 6 "to Publish" and "Publication" are to be construed as references to Academic Publication;
This contract:	this document, including its Schedules, as amended from time to time in accordance with clause 10.9;
Background Intellectual Property:	Intellectual information, techniques, Know-how, software and materials and other Intellectual Property (regardless of the form or medium in which they are disclosed or stored) that are provided by one Party to the other for use in the Project (whether before or after the date of this Agreement), except any Research Project Intellectual Property (or "Results") defined below
Confidential Information:	Each Party's confidential information is: any Background disclosed by that Party to the other for use in the Project and identified in writing as confidential before or at the time of disclosure; and any Research Project Intellectual Property;
The Effective Date:	The date of signature of this Agreement;
External Funding:	Any funding or assistance provided for the Project or to any Party for use in the Project by any third Party, including without limitation, any state or public body;
The Financial Contribution:	The financial contribution to be provided by KAU set out in Schedule 1;

The Good Data Management Practices:	The practices and procedures set out in Schedule 3;
Intellectual Property:	Patents, trademarks, service marks, registered designs, copyrights, database rights, design rights, confidential information, applications for any of the above, and any similar right recognised from time to time in any jurisdiction, together with all rights of action in relation to the infringement of any of the above;
The Key Personnel:	The Principal Investigators, liaison officer/project managers, and any other key personnel for KAU and URV identified in Schedule 2;
Know-How:	Unpatented technical information (including, without limitation, information relating to invention, discoveries, concepts, methodologies, models, research, development and testing procedures, the results of experiments, tests and trials, manufacturing process, techniques and specifications, quality control data, analyses reports and submissions) that is not in the public domain;
The Locations:	the locations, at which the Project will be carried out in KAU and URV as set out in Schedule 2;
The Parties:	with respect to this Research Collaboration contract shall mean KAU and URV;
The Project: "Research Project")	The programme of work described in Schedule 2, as amended from time (or to time in accordance with clause 10.9;
The Project Period:	the period described in clause 2.1;
Research Project Intellectual Property (or "Results"):	All information, Know-how, results, inventions, software and other Intellectual Property, which is identified, created, developed, discovered or first reduced to Practice or writing in the course of the Project.

2. THE PROJECT

2.1 The Project will begin on the Effective Date and will continue for a period of thirty six months or until any later date agreed in writing between the Parties, or until this contract is terminated in accordance with clause 8 or 9. If this contract is entered into after the Effective Date, it will apply retrospectively to work carried out in relation to the Project on or after the Effective Date.

2.2 Details of the contribution of URV to the collaboration are outlined in Schedule 2, and URV will provide the human resources, materials, facilities and equipment that are designated as its responsibility in Schedule 2. The Project will be carried under the direction and supervision of the Principal Investigators shown from the URV side and the Principal Investigators from the KAU side.

2.3 Each of the Parties will use all reasonable endeavours to obtain all regulatory and ethical licences, consents and approvals necessary to allow it to carry out the tasks allotted to it in Schedule 2.

2.4 Each of the Parties will ensure that its employees and students involved in the Project: observe the conditions attaching to any regulatory and ethical licences, consents and approvals; keep complete and accurate records of all research, development and other work carried out in connection with the Project and of all Research Project Intellectual Property and observations, signed by the people who obtained each result or made those observations, and countersigned by an employee of that Party who is not a member of the research team but who understands the work; and comply with the Good Data Management Practices.

2.5 Although each of the Parties will use reasonable endeavours to carry out the Project in accordance with Schedule 2, neither Party undertakes that any research will lead to any particular result, nor does it guarantee a successful outcome to the Project.

2.6 KAU and URV will communicate on a regular basis and will meet at a minimum of 6 month intervals throughout the Project Period with both Parties reporting on progress of the Project and providing copies of all Research Project Intellectual Property.

2.7 Each of the Parties warrants to the other that it has full power and authority under its constitution, and has taken all necessary actions and obtained all authorisations, licences, consents and approvals, to allow it to enter into this contract.

3. FINANCIAL CONTRIBUTION AND EXTERNAL FUNDING

3.1 URV will keep complete and accurate accounts of its expenditure on the Project. URV will invoice KAU in the amounts as set forth in Schedule 1. KAU will pay each invoice within 60 days after receipt thereof.

3.2 Because KAU is based outside of Spain, VAT is not applicable on payments made by KAU to URV.

3.3 KAU and URV will each own any equipment purchased for their respective contributions to the Project.

3.4 The budget of KAU team is independent of that assigned to URV team according to Schedule 1 (i.e. KAU has the right to decide upon its own budget for its contribution). URV shall not be responsible for any costs relating to the KAU contribution to the Project or the costs of the KAU equipment or research team.

4. USE AND EXPLOITATION OF INTELLECTUAL PROPERTY

4.1. Each Party agrees that it will not have any claim, ownership or interest in the other Party's Background Intellectual Property.

4.2. To the extent that they are free and able to do so, each Party grants the other Party a non-exclusive, royalty-free licence for the use of any Background Intellectual Property made available by the granting Party to the other for the purpose of carrying out the Research Project.

4.3.1 In consideration of the rights granted in this Agreement, the Parties agree that all rights title and interest to the Research Project Intellectual Property shall be equally owned by the two Parties.

4.3.2 Neither Party shall do anything which would prejudice the rights of the other joint owner.

4.3.3 Each Party shall have a worldwide, non-exclusive, royalty-free right to use the Research Project Intellectual Property for its own non-commercial internal research, teaching and knowledge transfer purposes, subject to obligations of confidentiality towards the other Party.

4.3.4 If a Party intends to use the services of visiting fellows, students, contractors, consultants, agents or other third parties to undertake or participate in the Research Project it shall first ensure that they assign full ownership of any intellectual property which they may generate in the course of their work on the Research Project to that Party.

4.3.5 The Parties wish to ensure that Research Project Intellectual Property is commercially exploited in such a way as to achieve its maximum potential. They shall therefore collaborate to establish a co-ordinated strategy for the commercial exploitation of Research Project Intellectual Property which will deal with such matters as the identification of Research Project Intellectual Property, the management and protection of Research Project Intellectual Property, terms of any licences or rights for third parties to use Research Project Intellectual Property, the territories and commercial markets for such exploitation, the costs of commercial protection and the distribution of any net income arising from the commercial exploitation of Research Project Intellectual Property. To this end, the Parties shall enter into separate agreements which deal fully with these matters either when Research Project Intellectual Property is first identified as having the potential to be commercially exploitable, or if no Research Project Intellectual Property is so identified during the Project, no later than six months before the conclusion of the Project, although it is their intention to do so well before that date. In making such arrangements, the Parties shall ensure that the distribution of net revenue from the commercial exploitation of Research Project Intellectual Property between themselves fully and fairly reflects each Party's contribution to further research and development necessary to commercialise any Research Project Intellectual Property and the costs of commercial protection.

4.3.6 No Party shall licence or otherwise commercialise any Research Project Intellectual Property without the written permission neither of the other Party nor without an agreement to be negotiated in good faith by the Parties hereto providing for, *inter alia*, the sharing of income the commercial exploitation of Research Project Intellectual Property. To this end, the Parties shall determine amongst themselves which of them shall be responsible for the protection and management of the said Research Project Intellectual Property, and shall work together to ensure that it is protected effectively, and that the costs of protection are shared between

themselves in an agreed appropriate way. In determining such matters, the Parties shall take into account; *inter alia*, such factors as the willingness or ability of a Party to undertake the protection, prosecution and maintenance of the Research Project Intellectual Property.

4.3.7 Should a Party not wish to take out or maintain protection for any Research Project Intellectual Property, it shall promptly notify the other Party of its decision, and shall offer it the right to take out or maintain such protection at its own cost. If the other Party wishes to take over the protection of such Research Project Intellectual Property, the first Party shall make all the arrangements to facilitate this, including any necessary assignments or licences without undue delay.

4.3.8 All negotiations regarding the terms for the use, licensing, assignment or protection of Research Project Intellectual Property and/or Background IP shall be handled by the appropriate official in each Party's organisation who is responsible for technology transfer and/or the protection of intellectual property.

4.4 The intellectual property terms in this Agreement apply only to the Project and its approved Amendments by the Parties, and if any new work, which is not related to the project and its approved Amendments, is foreseen between the two Parties, then the Parties agree to re-negotiate the intellectual property terms for such envisaged further work.

4.5 If Key Personnel or other team members of either Party include students, both Parties agree that:

a nothing in this contract will inhibit the right of a student to have their thesis examined;

b All students shall own copyright in their thesis.

5 ACADEMIC PUBLICATION

5.1. Each Party will submit to the other Party, in writing, details of any results which could be considered to be Research Project Intellectual Property that any team member or student of either of the Parties intends to publish, at least 30 days before the date of the proposed submission for Publication. The Parties will agree whether the Research Project Intellectual Property will be protected through appropriate patent, trademark or other submission prior to publication. The Parties will have the right to delay publication by a further 60 days if necessary to reach a decision regarding Intellectual Property protection, through use of a Confidentiality Notice submitted to the individual requesting the right to Publish.

5.2. Any team member of either Party (whether or not directly involved in the Project) may, provided a Confidentiality Notice under clause 5.1 has not been given:

5.2.1. Discuss work undertaken as part of the Project in University seminars, tutorials and lectures; and

5.2.2. Publish non-Confidential results of the Project.

6 CONFIDENTIALITY

6.1. Subject to clause 5, neither Party will, either during the Project Period or for 3 years after the end of the Project Period, disclose to any third party, nor use for any purpose except carrying out the Project, any of the other Party's Confidential Information.

6.2. The obligations of confidentiality, non-disclosure and non-use shall not apply to the extent that information:

6.2.1. Is known to the receiving Party prior to receipt from the other Party, and not already subject to any obligation of confidentiality to the other Party as shown by written records;

6.2.2. Is or becomes publicly known without any breach of this Contract or any other undertaking to keep it confidential;

6.2.3. Is obtained by the receiving Party from a third Party in circumstances where the receiving Party has no reason to believe that there has been a breach of an obligation of confidentiality owed to the other Party;

6.2.4. Has been independently developed by the receiving Party ;

6.2.5. Is approved for release in writing by an authorised representative of the other Party.

6.2.6. Neither Party will be in breach of any obligation to keep any Research Project Intellectual Property, or other information, confidential and not to disclose them to any third party, by Publishing any of the same if that

Party has followed the procedure in clause 5.1 and has received no notice within the period stated in that clause.

7 LIMITATION OF LIABILITY

7.1. Neither of the Parties makes any representation or gives any warranty to the other that any advice or information given by it or any of its employees or participating students who work on the Project, or the content or use of any Research Project Intellectual Property, Background Intellectual Property or materials, works or information provided in connection with the Project, will not constitute or result in any infringement of third-party rights.

7.2. Except under the indemnity in clause 7.3, and subject to clause 7.6, neither Party shall have any liability or responsibility, in contract, negligence, misrepresentation or otherwise for any use which may be made by the other Party of any Research Project Intellectual Property, nor for any reliance which may be placed by that other Party on Research Project Intellectual Property, nor for advice or information given in connection with Research Project Intellectual Property.

7.3. Except as provided in clause 7.1, each Party (the "Indemnifying Party") will indemnify the other Party, its Principal Investigators and its other employees and students participating in the Project (the "Indemnified Parties"), and keep them fully and effectively indemnified, against each and every claim made against any of the Indemnified Parties as a result of the Indemnifying Party's use in the course of the Research Project of any of the Results or any materials, works or information received from the Indemnified Parties pursuant to the terms of this Contract and arising out of the Indemnifying Party's negligence or intentional misconduct or misrepresentation, provided that the Indemnified Party must:

7.3.1. Promptly notify the other Party of details of the claim;

7.3.2. Not make any admission in relation to the claim;

7.3.3. Allow the Indemnifying Party to have the conduct of the defence or settlement of the claim; and

7.3.4. Give the Indemnifying Party all reasonable assistance in dealing with the claim.

7.4 The indemnity in clause 7.3 will not apply to the extent that the claim arises as a result of the Indemnified Party's negligence, its breach of clause 6, its deliberate breach of this contract or its knowing infringement of any third party's Intellectual Property.

7.5 Subject to clause 7.6, and except under the indemnity in clause 7.3, the liability of either Party to the other for any breach of this Contract, any negligence or arising in any other way out of the subject matter of this Contract, the Project and the Research Project Intellectual Property, will not extend to any indirect or consequential damages or losses, or to any loss of actual or anticipated profits, savings or revenue, loss of data, loss of contracts or opportunity, whether direct or indirect, even if the Party bringing the claim has advised the other of the possibility of those losses, or if they were within the other Party's contemplation.

7.6 Subject to clause 7.5, and except under the indemnity in clause 7.3, the aggregate liability of each Party to the other for all and any breaches of this Contract, any negligence or arising in any other way out of the subject matter of this Agreement, the Project and the Research Project Intellectual Property, will not exceed in total the Financial Contribution.

7.7 Nothing in this Contract limits or excludes either Party's liability for:

7.7.1 Death or personal injury;

7.7.2 Any fraud or for any sort of liability that, by law, cannot be limited or excluded; or

7.7.3 Any loss or damage caused by a deliberate breach of this Contract or a breach of clause 6.

7.8 The express undertakings and warranties given by the Parties in this Contract are in lieu of all other warranties, conditions, terms, undertakings and obligations, whether express or implied by statute, common law, custom, trade usage, course of dealing or in any other way. All of these are excluded to the fullest extent permitted by law.

8 FORCE MAJEURE

If the performance by either Party of any of its obligations under this Contract is delayed or prevented by circumstances beyond its reasonable control, that Party will not be in breach of this Contract because of that delay in performance. However, if the delay in performance is more than 6 months, the other Party may terminate this Agreement with immediate effect by giving written notice.

9 TERMINATION

9.1 Either Party may terminate this Contract with immediate effect by giving notice to the other Party if:

9.1.1. the other Party is in breach of any provision of this Contract and (if it is capable of remedy) the breach has not been remedied within 60 days after receipt of written notice specifying the breach and requiring its remedy; or

9.1.2. the other Party becomes insolvent, or if an order is made or a resolution is passed for its winding up (except voluntarily for the purpose of solvent amalgamation or reconstruction), or if an administrator, administrative receiver or receiver is appointed over the whole or any part of the other Party's assets, or if the other Party makes any arrangement with its creditors.

9.2 URV will notify KAU promptly if at any time any of the Key Personnel appointed by URV is unable or unwilling to continue to be involved in the Project. Within one month after the date of that notice, URV will nominate a successor and KAU will not unreasonably refuse to accept the nominated successor. However, if a successor acceptable to KAU cannot be found within one month, then KAU may terminate this Contract by giving URV not less than 3 months' notice, and KAU will be entitled to a refund of any unused portion of the Financial Contribution in accordance with Section 9.4 below.

9.3 Clauses 1, 4, 5, 6, 7, 8, 9.2, 9.3, 9.4, and 10 will survive the expiry of the Project Period or the termination of this Contract for any reason and will continue indefinitely.

9.4 On the termination of this Contract, KAU will pay URV for all work done prior to termination. If KAU has paid any of the Financial Contribution in advance and the whole of that contribution has not, by the end of the Project Period or the termination of this Contract, been used by URV for the purposes for which that Financial Contribution was provided, URV will return to KAU the unused portion of that contribution.

10 GENERAL

10.1 **Notices:** Any notice to be given under this Contract must be in writing, may be delivered to the other Party or Parties by any of the methods set out in the left hand column below, and will be deemed to be received on the corresponding day set out in the right hand column:

Method of service	**Deemed day of receipt**
By hand or courier	The day of delivery
By pre-paid first class post	The second business day after posting
By recorded delivery post	The next business day after posting
By fax (provided the sender's fax machine Confirms complete and error-free transmission of that notice to the correct fax number)	The next business day after sending or, if sent before 16.00 (sender's local time) on the business day it was sent

The Parties' respective representatives for the receipt of notices are, until changed by notice given in accordance with this clause, as follows:

For KING ABDULAZIZ UNIVERSITY	For UNIVERSITAT ROVIRA I VIRGILI
Prof Abdelrahman Obeid Alyoubi	Prof Ciara Kathleen O'Sullivan
Vice President For Educational Affairs	ICREA Research Professor
Chemistry Department,	Nanobiotechnology & Bioanalysis Group
Faculty of Science,	Department of Chemical Engineering
King Abdulaziz University,	Universitat Rovira I Virgili
P.O. Box 80203, Jeddah 21589,	Avinguda Països Catalans, 26
Kingdom of Saudi Arabia	Tarragona 43007
	Spain
E mail: aalyoubi@kau.edu.sa	E mail: ciara.osullivan@urv.cat
Tel: 00966126965703	Tel: 0034977558740/0034977240312
Fax: 00966126952435	Fax: 0034977559667/0034977559721

10.2 **Headings:** The headings in this Contract are for ease of reference only; they do not affect its construction or interpretation.

10.3 **Assignment:** Neither Party may assign or transfer this Contract as a whole, or any of its rights or obligations under it, without first obtaining the written consent of the other Party. That consent may not be unreasonably withheld or delayed.

10.4 **Illegal/unenforceable provisions:** If the whole or any part of any provision of this Contract is void or unenforceable in any jurisdiction, the other provisions of this Contract, and the rest of the void or unenforceable provision, will continue in force in that jurisdiction, and the validity and enforceability of that provision in any other jurisdiction will not be affected.

10.5 **Waiver of rights:** If a Party fails to enforce, or delays in enforcing, an obligation of the other Party, or fails to exercise, or delays in exercising, a right under this Contract, that failure or delay will not affect its right to enforce that obligation or constitute a waiver of that right. Any waiver of any provision of this Contract will not, unless expressly stated to the contrary, constitute a waiver of that provision on a future occasion.

10.6 **No agency:** Nothing in this Contract creates, implies or evidences any partnership or joint venture between the Parties, or the relationship between them of principal and agent. Neither Party has any authority to make any representation or commitment, or to incur any liability, on behalf of the other.

10.7 **Entire agreement:** This Contract constitutes the entire Contract between the Parties relating to its subject matter. Each Party acknowledges that it has not entered into this Contract on the basis of any warranty, representation, statement, Contract or undertaking except those expressly set out in this Contract. Each Party waives any claim for breach of this Contract, or any right to rescind this Contract in respect of, any representation, which is not an express provision of this Contract. However, this clause does not exclude any liability which either Party may have to the other (or any right which either Party may have to rescind this Contract) in respect of any fraudulent misrepresentation or fraudulent concealment prior to the execution of this Contract.

10.8 **Formalities:** Each Party will take any action and execute any document reasonably required by the other Party to give effect to any of its rights under this Contract, or to enable their registration in any relevant territory provided the requesting Party pays the other Party's reasonable expenses.

10.9 **Amendments:** Both parties will work together to modify, amend, and fine tune the initial proposed research projects described in Schedule 2, within the first three months of the effective date of this Contract, and set out program deliverables and time table for delivery. However, no variation or amendment of the proposed research projects or this Contract will be effective unless it is made in writing and signed by each Party's representative.

10.10 **Third parties:** No one except a Party to this Contract has any right to prevent the amendment of this Contract or its termination, and no one except a Party to this Contract may enforce any benefit conferred by this Contract, unless this Contract expressly provides otherwise.

10.11 **Governing law:** This Contract is governed by, and is to be construed in accordance with Saudi Arabia law, insofar as it concerns activities to be undertaken in Saudi Arabia, and the laws of the Spain insofar as it concerns activities to be undertaken in Spain. The Saudi Arabian Courts will have exclusive jurisdiction to deal with any dispute which has arisen or may arise out of, or in connection with, this Contract or its subject-matter (including non-contractual claims), except that either Party may bring proceedings for an injunction in any jurisdiction.

10.12.1 **Escalation:** The Parties undertake to negotiate the terms of all agreements relating to the exploitation, use, development and protection of Research Project Intellectual Property in good faith and to endeavour to agree terms amicably between themselves. However, where such negotiations are solely between the Parties and where they are unable to agree terms amicably within a period of six months from the initiation of discussions, they will refer the matter to Prof A. O. Alyoubi on behalf of KAU, and to Prof C. K. O'Sullivan on behalf of URV in an attempt to resolve the issue within 14 working days after the referral. If the Parties are unable to reach agreement on any issue concerning Research Project Intellectual Property within this 14 working day period, the matter shall be escalated to senior management within each institution to be dealt with within a further 21 working day period. Either Party may bring proceedings in accordance with clause 10.11 if the matter has not been resolved within the cumulative 14 working day and 21 working day period and either Party may apply to the court for an injunction, whether or not any issue has been escalated under this clause.

10.12.2 If the Parties are unable to reach agreement on any issue concerning this Contract or the Project (other than any issue relating to Research Project Intellectual Property which shall be dealt with in accordance with the time frames as set out in clause 10.12.1 above) within 14 working days after one Party has notified the other of that issue, the matter shall be escalated to senior management within each institution to be dealt with within a further 21 working day period. Either Party may bring proceedings in accordance with clause 10.11 if the matter has not been resolved within the cumulative 14 working day and 21 working day period and either Party may apply to the court for an injunction, whether or not any issue has been escalated under this clause.

10.13 **Scope of This Contract**

This Contract states the entire contract between the parties as of the date of final signature below in respect to the subject matter of the service contract and supersedes any previous written or oral representations, statements, negotiations, or agreements. This service contract may be modified only by written amendment executed by the authorized representatives of both parties. DONE in Jeddah and Tarragona, in two originals, both are equally authentic.

FOR KING ABDULAZIZ UNIVERSITY,
KINGDOM OF SAUDI ARABIA
SIGNED for and on behalf of KAU
Name: Prof Abdelrahman Obeid Alyoub
Position: KAU Vice President for Educational Affairs,
Signature:

FOR UNIVERSITAT ROVIRA I VIRGILI,
SPAIN
SIGNED for and on behalf of URV
Name: Prof Josep Anton Ferré Vidal
Position: Rector of URV
Signature:

UNIVERSITAT
ROVIRA I VIRGILI
RECTORAT

SCHEDULE 1
The Financial Contribution

Total Cost of Project (in euros) = 429.032,50 €
Note no VAT is payable

Item	1st Year	2nd Year	3rd Year	Contract Total (EUROS)
Total	155.090,38 €	133.921,56 €	140.020,56 €	429.032,50 €
KAU FUNDS	129.033,52 €	107.864,70 €	113.963,70 €	350.861,92 €
URV FUNDS	26.056,86 €	26.056,86 €	26.056,86 €	78.170,58 €

Payments to be made for the attention of :
Name : Ciara O' Sullivan
(Reference: King Abdul-Aziz University)

All payments of the Financial Contribution will be made by bank transfer in Euro to:

BANCO SANTANDER
Rambla Nova, 33
TARRAGONA, Spain

CCC:	0049 1877 49 2910661321
IBAN (electronic):	ES64 0049 1877 4929 1066 1321
IBAN (paper):	ES64 0049 1877 4929 1066 1321
BIC/SWIFT :	BSCHESMM

The date payments will be:

Payments	Import (€)
payment 1 (31/10/2015)	64.516,76
payment 2 (30/04/2016)	64.516,76
payment 3 (31/10/2016)	53.932,35
payment 4 (30/04/2017)	53.932,35
payment 5 (31/10/2017)	56.981,85
payment 6 (30/04/2018)	56.981,85
Total	350.861,92

SCHEDULE 2

Project Description

Specific Aims and Objectives

Main Objective:

1. The proposed project has the potential to advance significantly beyond the SoA for detection of anabolic steroids (selected from a list of testosterone and a selection of testosterone analogues) in humans and animals (both in blood and urine) using aptamers. To date no aptamers against anabolic steroids have been reported and any outcoming publications would be in high impact factor journals.
2. Selection of family of aptamers for detection of anabolic steroids.
3. Use of aptamers in a variety of assays including enzyme-linked oligonucleotide assay (ELONA), aptasensors and apta-RPA for ultrasensitive detection.
4. Validation of selected aptamers using real human and animal samples and comparing performance with LC-MS.

Specific Aims:

The outcome will be a user-friendly rapid and inexpensive tests for the reagentless semi-quantitative and quantitative detection of a battery of steroids with the only required end-user intervention being sample addition (urine / blood),

Aim 1: Selection of aptamers against testosterone and analogues of testosterone (e.g. nandrolane, stanozolol, danazol, DNA aptamers have been demonstrated to be highly specific - the best example being an aptamer that shows 10,000 times better affinity for theophylline than caffeine, despite the small molecular size and the only differentiating group being a methyl group[1],

[1] G R Zimmermann, C L Wick, T P Shields, R D Jenison, and A Pardi, Molecular interactions and metal binding in the theophylline-binding core of an RNA aptamer., RNA. May 2000; 6(5): 659–667.

Aim 2: DNA aptamers have been used in several lateral flow assays, including one for the detection of toxins, thus demonstrating the proof of concept[2]. The selected aptamers will be used for detection of steroid metabolites in urine in a semi-quantitative lateral flow assay.

Aim 3: Aptamers are much more flexible in their application than monoclonal antibodies and can be engineered into molecular aptamer beacons, exploiting either electrochemical of fluorescent detection[3], [4]. This format will allow for quantitative detection of the anabolic steroids, with the response being almost instantaneous. This format will be used for both blood (steroids) and urine (steroid metabolites) analysis. Blood analysis may need the integration of sample pre-treatment prior to detection, which can easily be achieved with a membrane.

Aim 4: For ultrasensitive detection of extremely low levels of anabolic steroids will also be explored where aptamer detection will be combined with nucleic acid amplification in a technique developed by URV, termed apta-RPA. This test can also be incorporated within a lateral flow type assay, which can be used on-site for both blood and urine analysis.

Aim 5: Analytical validation of the selected aptamers and different tests developed. The current gold standard for detection of steroids is GC-LC-MS, which requires considerable technical expertise to standardise assays and interpret results. A diagnostic test for rapid and cost-effective detection of steroids in humans and animals, for use on-site is currently not available. In order to demonstrate the accuracy and reliability of the selected aptamer(s) and developed tests, a number of human and animal urine and blood samples will be analysed both by GC-LC-MS and the developed tests and the degree of correlation established.

[2] Libing Wang, Wenwei Ma, Wei Chen, Liqiang Liu, Wei Ma, Yingyue Zhu, Liguang Xu, Hua Kuang, Chuanlai Xu, An aptamer-based chromatographic strip assay for sensitive toxin semi-quantitative detection, (2011), Biosesnors & Bioelectronics, 26, 3059-3062.

[3] Mairal, T., O'Sullivan, C.K., Rapid determination of total hardness in water using fluorescent molecular aptamer beacon. (2008) Analytica Chimica Acta, 610 (1), pp. 105-111.

[4] Radi, A.E., Sanchez, J.L.A., Baldrich, E., O'Sullivan, C.K., Reagentless, reusable, ultrasensitive electrochemical molecular beacon aptasensor. (2006) Journal of the American Chemical Society, 128 (1), pp. 117-124.

State of the art:

Currently, there are no conventional reagents available that will specifically detect steroids. The current gold standard for detection of nicotine metabolites is GC-LC-MS, which requires considerable technical expertise to standardize assays and interpret results. Thus, a diagnostic test for steroids suitable for use on-site is not feasible with existing technology, despite the fact there is a mature market need for such a device.

Nucleic acid aptamers offer the possibility of developing reagents that would specifically recognise the molecules. Significant research has been made in recent years in this field. Indeed, aptamers have been successfully developed for the recognition and detection molecules as diverse as thrombin[5], vitamin D[6], and the NMDA receptor[7]. These small molecules have the potential to bind any type of target molecule if correctly designed and engineered. So far, no aptamers have been developed for the specific binding of anabolic steroids, although aptamers have been described that can distinguish between molecules differing by only one methyl group which suggests that the task of recognising the different nicotine metabolites will be tractable. Moreover, an aptamer has been selected against 17-ß-estradiol, and exploited in a number of different sensors and assay formats, thus demonstrating the proof-of-concept that it is feasible and will indeed be possible to select aptamers against testosterone and testosterone analogues. The proposed project thus clearly has the potential to advance beyond SoA in the detection of steroids in humans and animals (both in blood and urine), developing new aptamers for expected large-scale utilization and impact.

Innovation:

The detection of the analyte will be performed using GC-LC-MS, which requires considerable technical expertise to standardize assays and interpret results, electrochemical techniques (electrochemical impedance spectroscopy (EIS), Stripping Voltammetry and Amperometry) at

5 Unfolding and Conformational Variations of Thrombin-Binding DNA Aptamers: Synthesis, Circular Dichroism and Molecular Dynamics Simulations.Sun L, Jin H, Zhao X, Liu Z, Guan Y, Yang Z, Zhang L, Zhang L.ChemMedChem. 2014.

6 Serum inverts and improves the fluorescence response of an aptamer beacon to various vitamin D analytes., Bruno JG, Carrillo MP, Phillips T, Edge A. Luminescence. 2012 Jan-Feb;27(1):51-8.

7 RNA based antagonist of NMDA receptors. Lee G, Maclean DM, Ulrich H, Zhao X, Aronowski J, Jayaraman V.ACS Chem Neurosci. 2014 Apr 7.

surface modified electrodes and fluorescence spectroscopy. Continuing this study, one propose of a collaborative of this project will be focused on improving the available methods to detect even lower levels of analye of interest and to overcome the interference in standard method caused by the complex matrices.

Work plan:

Aptamers are small, single stranded non-coding oligonucleotides (DNA or RNA), that fold into three-dimensional structures capable of binding to a range of target molecules, that can be exploited in a wide range of applications, including therapeutics, diagnostics, food quality control, environmental monitoring, drug development and drug delivery as well as in chemical biology. Most aptamers are generated by a process that combines combinatorial chemistry with in vitro selection, termed Systematic Evolution of Ligands by Exponential Enrichment (SELEX), from a complex library of randomized sequences of typically 10^{14} molecules. Targets of aptamers can be small, large, and simple or complex and aptamers are so widely applicable that new aptamer related reports are published every day.

Aptamers are significantly easier and cheaper to produce as compared to antibodies, and are neither immunogenic nor toxic. Aptamers are considerably more flexible that antibodies and can be engineered, and also show better specificity. Specifically, in this work package aptamers will be selected against various steroids. Each of the metabolites will be linked to a biotin tag using straightforward carbodiimide chemistry. Two modes of SELEX will be compared - solution phase and immobilized. For the immobilized, the biotinylated targets will be linked to streptavidin coated magnetic beads. A combinatorial DNA library containing between 10^{13} to 10^{15} diverse sequences will be used as the starting pool with these diverse sequences (30 bases in length) being flanked by two constant regions (each 25 bases in length for binding to primers for amplification following each SELEX cycle. Asymmetric PCR will be used to generate single stranded DNA. In both approaches the target will then be incubated with the DNA library for a defined amount of time at room temperature, in the presence of matrix elements that are present in urine and blood.

Following incubation, partitioning of the small fraction of ssDNA that has bound to the target from unbound DNA will be achieved. In the case of solution phase SELEX, at this point the bound DNA-biotinylated target complex will be linked to streptavidin coated magnetic beads. For both approaches, the unbound DNA is removed via magnetic separation. The bound DNA is then eluted and amplified using asymmetric PCR for the next round of SELEX. Enrichment of binding DNA and evolution of the DNA pool towards potential aptamers will be monitored using microscale thermophoresis, electrophoresis and surface Plasmon resonance. Typically 10-15 rounds of SELEX will be carried out, and the DNA will then be cloned and sequenced using Ion Torrent next generation sequencing. The sequences obtained will be aligned and consensus motifs identified. The motifs with most hits will be synthesized and their affinity for the targets compared, and the aptamer with highest affinity will then be optimized. Optimization involves prediction of the structure of the aptamer, and based on this truncation will be carried out and the affinity of the truncated aptamers established. It is not unusual to achieve vastly shortened aptamers, just 15-25 bases in length, thus having a significant effect on the cost of the assay, which must be as inexpensive as possible to facilitate its' role as a companion diagnostic tool. These aptamers will be referred to as reporter aptamers. The SELEX process will be repeated with target-reporter aptamer complexes as the target, resulting in the selection of what will be referred to as a capture aptamer for use in lateral flow assay as demonstrated in Fig. 1.

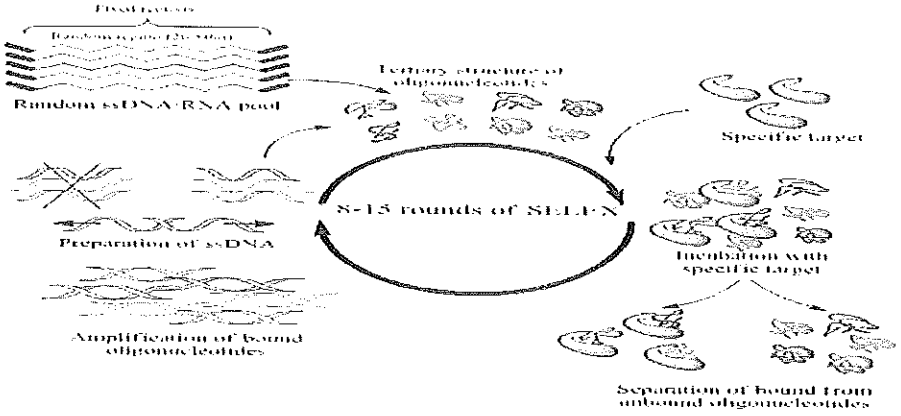


Fig. 1 Aptamer selection.

For a lateral flow assay, the concentrations of the capture and reporter aptamers will be optimised. The reporter aptamers will be linked to gold nanoparticles via thiol linkage. Once the urine/blood sample is introduced onto the chromatographic strip, it is wicked along the strip and

any analyte (i.e. steroids) present in the sample will interact with labelled reporter aptamer adsorbed on the membrane. This complex will continue to transverse the membrane until it reaches the detection zone where it will form a complex with the capture aptamer and the red colour due to the presence of the gold nanoparticles forms. Control will be DNA sequences complementary to each of the aptamer sequences. A colour bar will be used for semi-quantitative analysis as demonstrated in Fig. 2.

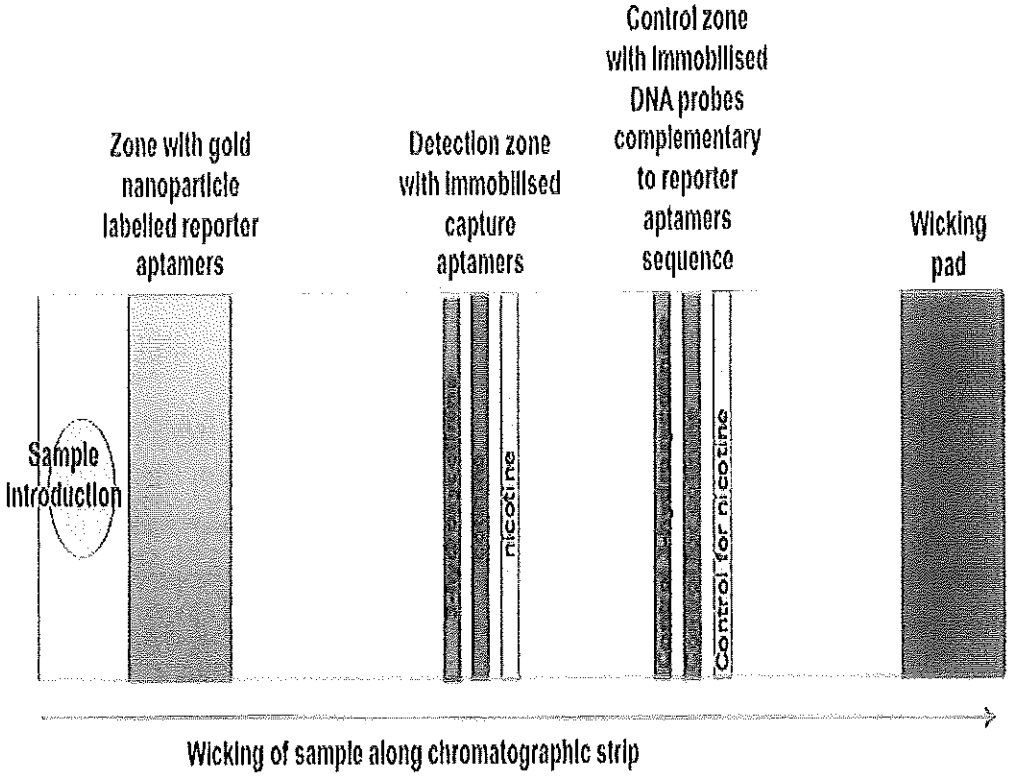


Fig. 2 Example of lateral flow assay based on aptamers.

In parallel, an array of electrochemical molecular beacon aptasensors will be developed. A design to allow for calibration and measurement of the three target analytes will be made for a total of 5 electrodes. Electrolytically gold plated printed circuit boards or screen - printed electrodes will be used (the cheapest solution will be used). Thiolated and redox molecule labelled aptamers will be chemisorbed onto these gold surfaces and a short chain alkanethiol used to backfill and space the aptamer beacons on the electrode surface to avoid any problems of

steric hindrance. In the absence of target the redox molecule (e.g. ferrocene) will be positioned far away from the electrode surface and those no signal observed. The aptamers will be engineered so that in the presence of target the aptamer will undergo a conformational change, bring the redox molecule close to the electrode surface and resulting in an increase in signal, with this signal being proportional to the amount of target present. This sensor architecture was pioneered by URV (reported in JACS, 2006 and cited >350 times) and the only required end-user intervention is sample addition, with the response being highly sensitive and achieved in less than 30 seconds. The various steroid targets will be quantitatively detected simultaneously and the cost of the sensor will be extremely low, with the PCBs being highly cost effective when produced on a large scale and the DNA aptamer cost being negligible. In addition, URV will develop sensors, sensor electronics and prototype reader technology of analyzing the ultra-trace concentration of the selected analytes.

Profile:

Universitat Rovira i Virgili (URV):

Description of the legal entity Universitat Rovira i Virgili is a public university located in Tarragona, Spain. Interfibio is a "consolidated research group" recognized for its excellence by the ministry of Education of the autonomous government of Catalonia and comprises 4 permanent staff members, 1 tenure track, 6 postdoctoral researchers, 14 PhD students, 6 masters' students and 2 administrative assistants. Interfibio is a research group formed in 2004 to create a critical mass of researchers of the Department of Chemical Engineering of the URV working at the bio/material interface. The team combines long experience in bioelectrochemistry and physical chemistry, molecular biology and nanotechnology/materials science. The most productive and active research of the group is centered in the exploitation of breakthroughs at the confluence of bio-, micro- and nano- technologies to create low-cost non-invasive intelligent diagnosis systems that can be applied to clinical, environmental and food analysis. Research activities also focus on the development of new sensors and relevant amplification systems for applications in microarrays and the selection and exploitation of aptamers.

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work is outlined. At present, the group works in 4 laboratories of 200 m^2

total research space, each specialised in infrastructure in a matrix-like organisation. The facilities include a dedicated synthesis/materials laboratory, microbiology (class 2) and a cell biology lab, an analytical lab, a molecular biology lab and a measurements (electrochemistry) laboratory. Equipment include potenciostates/galvanostats with impedance and coulometry modules, an electrochemical SPR, a BIACORE, a spectrofluorimeter with a Peltier module, a zeta-sizer, screen printer and surface tension measurement apparatus. The group has co-financed and has direct access to characterization equipment such as AFM, SEM, TEM, DNA sequencer, real-time PCR, NMR, MS, MALDI-TOF-MS. The group also has direct access to a fully equipped microfabrication clean room containing sputtering and laser equipment as well as a DRIE. Additionally the group has access to a milling machine as well as a rapid prototypes and 3-D printer for microsystem manufacture.

King Abdulaziz University (KAU):

King Abdulaziz University is a public university located in Jeddah, Kingdom of Saudi Arabia. KAU is a well-known university, recognized for its excellence by the ministry of Education of the autonomous government of Saudi Arabia. The research team combines long experience in Quantum Chemistry, Environmental Chemistry, Electroanalytical and spectroscopic techniques, bioelectrochemistry, physical chemistry and nanotechnology/materials science. The most productive and active research of the group is centered in the computational chemistry, electroanalytical, spectroscopic and separation techniques of small and large and nanotechnologies to create low-cost non-invasive intelligent diagnosis systems that can be applied to clinical, environmental and food analysis. In the present time, the research activities focus on the development of new optical and electrochemical nano sensors and relevant amplification systems for applications in analysis of different species e.g. inorganic and organic pollutants, drug residues in complex matrices.

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work is outlined. It is worthwhile to mention that in addition to the data archiving, the software used for data acquisition on the used instruments e.g. An Autolab PGSTAT302N potentiostat/galvanostat (Metrohm, Netherlands) connected to 663 VA Stand and operated with General Purpose Electrochemical System software (GPES v 4.9007) and

Frequency Resonance Analysis software (FRA v 4.9007); a Metrohm 757 VA trace analyzer and 747 VA stand (Basel, Switzerland); a scanning electron microscopy (SEM) (JEOL-JSM6301-F) (Peabody, MA, USA); An Agilent HPLC Technologies 1200 Series, USA, G1361A pump, G1315B DAD UV detector, analytical reversed phase symmetry C18 waters column (4.6×150.0 mm, 5 µm), A Milli-Q Plus system (Millipore, Bedford, MA, USA), a Perkin-Elmer LS 55 spectrofluorimeter (UK), equipped with a xenon lamp and a 10 mm quartz cell. This spectrofluorimeter has the data acquisition software compliant with the 21 CFR Part 11 regulations. The band passes were at 5 nm and 10 nm for excitation and emission monochromators, respectively. An agilent UV–visible spectrophotometer (190–1100 nm) (model 8453, Germany), A Perkin Elmer FTIR (200–4000 cm^{-1}) spectrometer 100 series (Beacoonsfield, Bucks, and UK), A Perkin Elmer inductively coupled plasma optical emission spectroscopy (ICP-OES) (model Optima 8000, UK) and ICP-MS spectrometers and A centrifuge (Clay Adams Safety Head, Becton-Dickinson and Company, USA), an ultrasonic bath cleaner (Aquawave 9377, Barnstead/Lab-Line, Germany), a water bath shaker (GFL 1083, Germany), a micropipette (Proline 20-200 µL and 100-1000 µL, Isolab, Germany), and a Milli-Q Plus system (Millipore, USA) were available.

Key Personnel:

The present project is a collaborative effort between Department of Cemistry at King Abdulaziz University (KAU), Kingdom of Saudi Arabia whom have strong expertise in quantum chemistry and Analytical Chemistry and Department of Chemical Engineering at Universitat Rovira i Virgili (URV), Spain whom have strong background in the area of the proposed project. The researchers involved in the proposed project are as follow:

KAU	URV
Prof. Abdulrahman O. Alyoubi	Prof. Ciara K. O' Sullivan
Prof. Mohammad S. El-Shahawi	Prof. Ioannis Katakis
Dr. Abdulaziz S. Bashammakh	

Expected Outcomes

- At least one PhD student graduating from each of King Abdulaziz University and Universitat Rovira i Virgili.
- At least 8 peer-reviewed research articles related to aptamer selection, assay development, sensor development, validation with real samples from animals (horses, camels) and humans. Target academic journals include:
    - Journal of American Chemical Society (Impact Factor = 12.113);
    - Analytical Chemistry (Impact Factor = 5.636);
    - Biosensors & Bioelectronics (Impact Factor = 6.045);
    - Angewandte Chemie (Impact Factor = 11.261);
    - Chemistry: A European Journal (Impact Factor = 5,731);
    - Methods (Impact Factor = 3,685)
- At least 10 Oral/Poster Presentations in International Conferences related to aptamers (e.g. Aptamers April 2016, May 2017), nucleic acids (e.g. ICNA 2017: 19th International Conference on Nucleic Acids, January, 2017) biosensors (Biosensors & Bioelectronics May 2016), American Chemical Society (ACS Spring/Fall meeting 2016, 2017), Veterinary Diagnostics (NAVC, January 2016, World Association of Veterinary Laboratory Diagnosticians, June 2017).
- Co-owned patent regarding assay/sensors exploiting aptamers for detection of anabolic steroids in humans and animals.
- Prototype devices for exhibiting at International Trade fairs (e.g. Medica; Analytica)

SCHEDULE 3

Data Management and Practice

No conventional reagents currently available that will specifically detect steroids. The current gold standard for detection of nicotine metabolites is GC-MS, LC-MS. These technique require considerable technical expertise to standardize assays and interpret results. So a diagnostic test for steroids suitable for use on-site is not feasible with existing technology. On the other hand, nucleic acid aptamers (NAA) offer the possibility of developing reagents that would recognize the molecules specifically.

Recent years have seen an upsurge of interest in directing significant research in this field to develop reagents specific for detecting steroids. Indeed, aptamers have been successfully developed for the recognition and detection molecules as diverse as thrombin,[1] vitamin D[2], and the NMDA receptor.[3] These small molecules have the potential to bind any type of target molecule if correctly designed and engineered. So far, no aptamers have been developed for the specific binding of anabolic steroids, although aptamers have been described for 17ß-estradiol, as well as aptamers that can distinguish between molecules differing by only one methyl group which suggests that the task of recognizing the different nicotine metabolites will be tractable. The proposed project thus clearly has the potential to advance beyond SoA in detection of steroids in humans and animals (both in blood and urine), developing new aptamers for large-scale utilization and impact.

The dissemination and sharing of the data will be provided through the publication in peer-reviewed international journals in the fields of analytical chemistry, surface chemistry, veterinary and human diagnostics and spectroscopic research. The intellectual property is shared to the scientific community through the scientific publication of our data in peer-reviewed journals and

[1] Unfolding and Conformational Variations of Thrombin-Binding DNA Aptamers: Synthesis, Circular Dichroism and Molecular Dynamics Simulations.Sun L, Jin H, Zhao X, Liu Z, Guan Y, Yang Z, Zhang L, Zhang L.ChemMedChem. 2014

[2] Serum inverts and improves the fluorescence response of an aptamer beacon to various vitamin D analytes. Bruno JG, Carrillo MP, Phillips T, Edge A. Luminescence. 2012 Jan-Feb;27(1):51-8.

[3] RNA based antagonist of NMDA receptors. Lee G, Maclean DM, Ulrich H, Zhao X, Aronowski J, Jayaraman V.ACS Chem Neurosci. 2014 Apr 7

presentations (oral or poster) at conferences where physicochemical properties of bio macromolecules are the main focus.

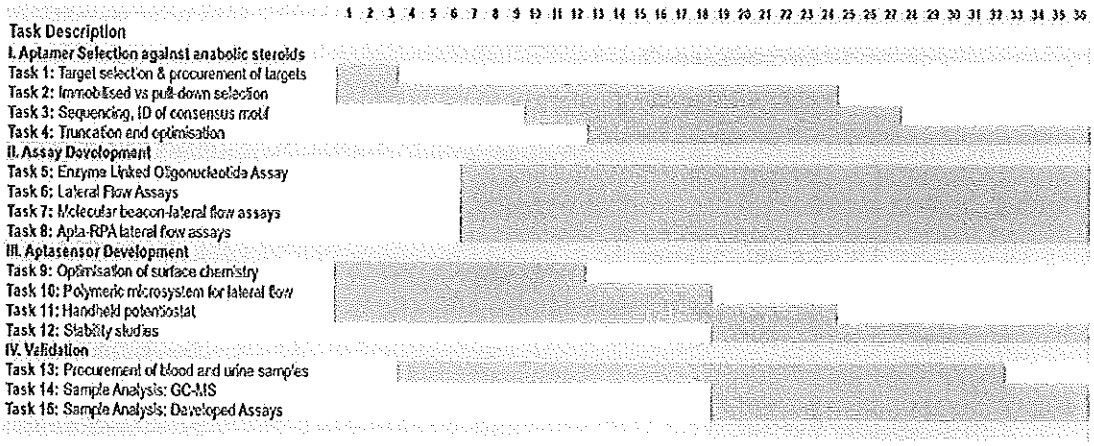
In this project, the analytical and surface chemistry approaches and the surface characterization methods employed in the project will be made available to our colleagues through a dedicated web page, after the data is published in peer-reviewed journals. Data archiving is also performed in the PI's laboratory. All original data are kept in the computers that are used for the data acquisition for a time period of 5 to 10 years, depending on the relevance of the data.

The PIs can assure not only the archiving of the data but also that the data is original and was not altered or manufactured. Each step in the data processing can be traced from the original raw data to the published results.

The PIs from both universities are confident that the overall proposed objectives and aims can be achieved successfully within the period of grant. This research project will provide the following:

i. Several tests that can be used on-site for the rapid semi-quantitative/quantitative detection of testosterone and/or testosterone analogues, where the only required end-user intervention will be sample addition (blood/urine).
ii. The tests to be developed will be highly specific and cost-effective, due to the inherent reduced cost of nucleic acids as compared to, for example, monoclonal antibodies, as well as the proposed experimental design of the detection scheme, where there is no need for a signaling antibody.
iii. Different types of tests will be explored capable of semi-quantitative analysis (lateral-flow), quantitative analysis (molecular beacon lateral flow), which will both aim to achieve detection limits of < 0.01 ng/mL, as well as an ultrasensitive test capable of detecting < 0.01 pg/mL (apta-RPA lateral flow).
iv. A handheld potentiostat will also be developed within the project by the partners at URV, where a portable instrument akin to a glucose-meter will be used for use with the lateral flow assays.

Milestones of the Project:



It should be noted that several targets will be addressed - starting with testosterone, and then moving on to various testosterone analogues. It is for this reason that Tasks 1-4, and in association, Tasks 5-8, run for almost the full duration of the project - however, it is expected that the first aptamers be selected in the first half of the project and the second set in the second half. From Day 1 of the project, the established 17ß-estradiol aptamers will be used as a model to allow immediate initiation of Tasks 9-11. A biobank will be established to store blood and urine samples obtained from humans and animals. For human samples, ethical permissions will be obtained. Following the development of the first set of assays and sensors using the first set of aptamers, validation studies correlating the performance of the developed molecular tools with the golden standard assay of GC-MS, will be established. The major objective of the project will be to produce a battery of very high-level publications with impact factor >5. The minimum expected number of articles is 8, but KAU and URV will aim to produce at least double that number.