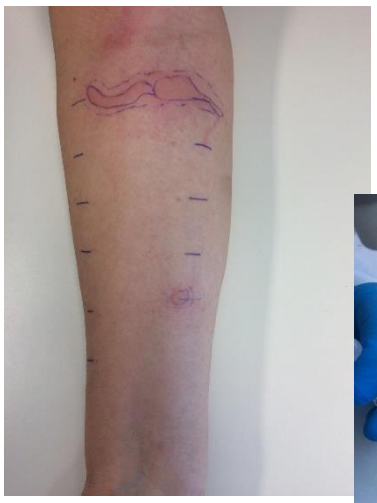
COBIOPHAD

COMPACT BIOPHOTONIC PLATFORM FOR DRUG ALLERGY DIAGNOSIS

From this…



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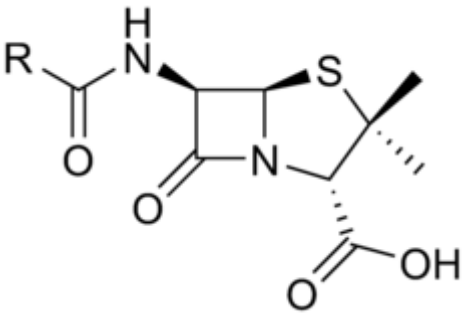

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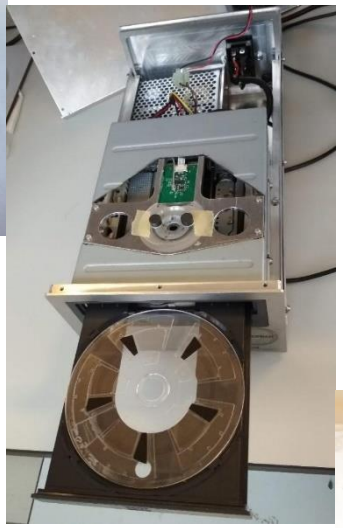


…to this…

COBIOPHAD

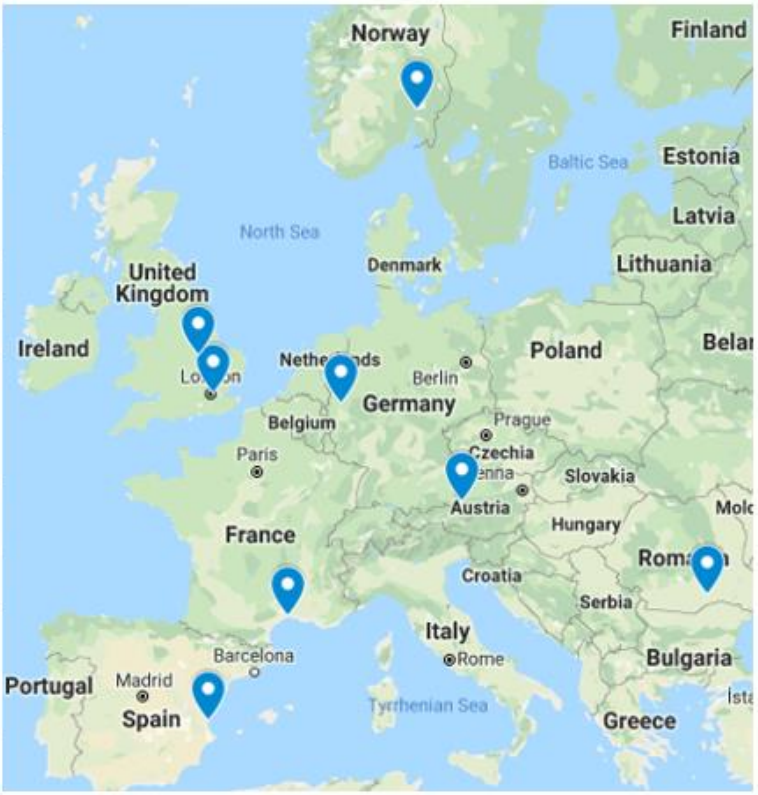
- Faster
  - More specific
    - More sensitive
      - Altogether SMARTER diagnosis of antibiotic allergies

THE FRAMEWORK PROGRAMME FOR RESEARCH AND INNOVATION
HORIZON 2020

COBOIOPHAD – The Partnership to Deliver the System

#	Partner Full Name	Partner Short Name	Country
1*	UNIVERSITAT POLITECNICA DE VALENCIA	UPVLC	ES
2	BIOTRONICS 3D LIMITED	B3D	UK
3	CENTRE HOSPITALIER UNIVERSITAIRE MONTPELLIER	CHRUM	FR
4	EUREXPLOIT LTD	EUX	UK
5	DR. FOOKE-ACHTERRATH LABORATORIEN GMBH	FOOKE	DE
6	FUNDACION PARA LA INVESTIGACION DEL HOSPITAL UNIVERSITARIO LA FE DE LA COMUNIDAD VALENCIANA	HULAFE	ES
7	OPTOELECTRONICA - 2001 SA	OPTOEL	RO
8	SINTEF AS	SINTEF	NO
9	STRATEC CONSUMABLES GMBH	STRATEC	AT
10	LUMENSIA SENSORS SL	LUM	ES
* Coordinator			

COBIOPHAD Partner Locations



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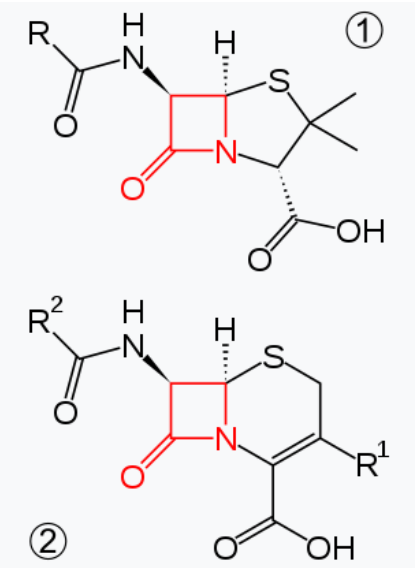
COBIOPHAD

…testing to save lives

…testing to better target drugs

Mis-diagnosis of allergy to penicillin and related antibiotics has the potential to lead to anaphylactic shock and even death in patients treated with these drugs. Conversely, mis-reporting means that many patients believing themselves to be allergic could be treated with them after all.

Penicillins are representatives of a huge range of β-lactam antibiotics that are so-called because they include a β-lactam ring in their molecular structures as shown in the diagram:



Core structure of penicillins (top) and cephalosporins (bottom). β-lactam ring in red.

These antibiotics can cause adverse drug reactions in sensitive patients. Such reactions can range from the relatively mild to full-blown anaphylactic shock which is life-threatening if not treated immediately. Usually, sensitivity is only detected when a patient experiences an adverse response following their first exposure to the drugs – often in childhood.

Problems relating to the administration of these drugs are compounded by the fact that the incidence of sensitivity is largely over-estimated. Recent clinical studies have found that as many as 10% of patients in Europe and across the USA self-report as being allergic to penicillin. However, these same studies have demonstrated that only 10-20% of those patients (1-2% of the total patient population) are truly at risk from further treatment with penicillin, amoxicillin and other β-lactam antibiotics.

Numerous explanations for this discrepancy have been proposed. Amongst them is that it has recently been discovered that allergy to penicillin can wear off with age, meaning that many of those who were diagnosed with it as kids may no longer be allergic as adults, but still think they are.

The downside to over-estimating allergy is that clinicians avoid the use of culprit antibiotics, choosing alternatives that may be less-efficient and will probably be more expensive than the first-choice β-lactams. Also, whilst these antibiotics may successfully treat the patient, there is a cost to them, including increased risk of developing infections and the potential for antibiotic resistance.

Ideally then, the clinician would like to have a specific, sensitive, rapid and cost-effective test for antibiotic allergy that could be deployed before first treatment and could identify patients that self-report as allergic but are not.

The aim of COBIOPHAD is to develop just such a test…

Adverse reactions to β-lactam antibiotics can be classified as **immediate** or **non-immediate** based on the timing of appearance of symptoms.

Immediate reactions have their onset in 1 to 6 hours (generally within 60 minutes) after exposure to a dose of an antibacterial. Patients show symptoms of an immunoglobulin E (IgE) mediated allergic reaction, ranging from urticaria or pruritus to angioedema and anaphylaxis.

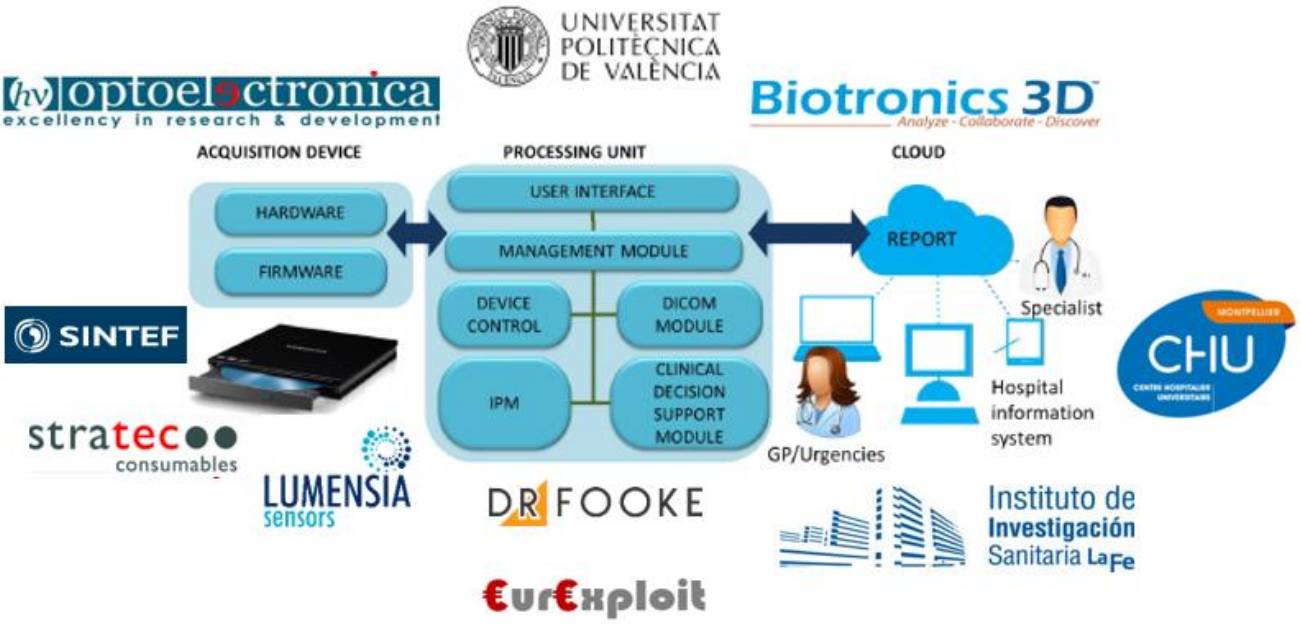
Non-immediate reactions, occurring more than 60 minutes (commonly several days) after exposure to penicillin, mainly result from the release of specific cytokines by activated T cell subsets. The most common non-immediate reactions are maculopapular or morbilliform and urticarial rashes.

The COBIOPHAD assay targets the IgE-mediated, immediate reaction

In comparison to other type of blood biomarkers, the sensitive and specific determination of IgE is by far the most important issue in drug allergy diagnosis. Certainly, there is no *in vitro* test on the market that can determine IgE concentrations below 0.35 kU/L. Besides that, the current allergy tests lack sensitivity, selectivity, and multiplex capability. Therefore, there is a clear need to overcome these limitations and COBIOPHAD addresses all these challenges by using an innovative approach:

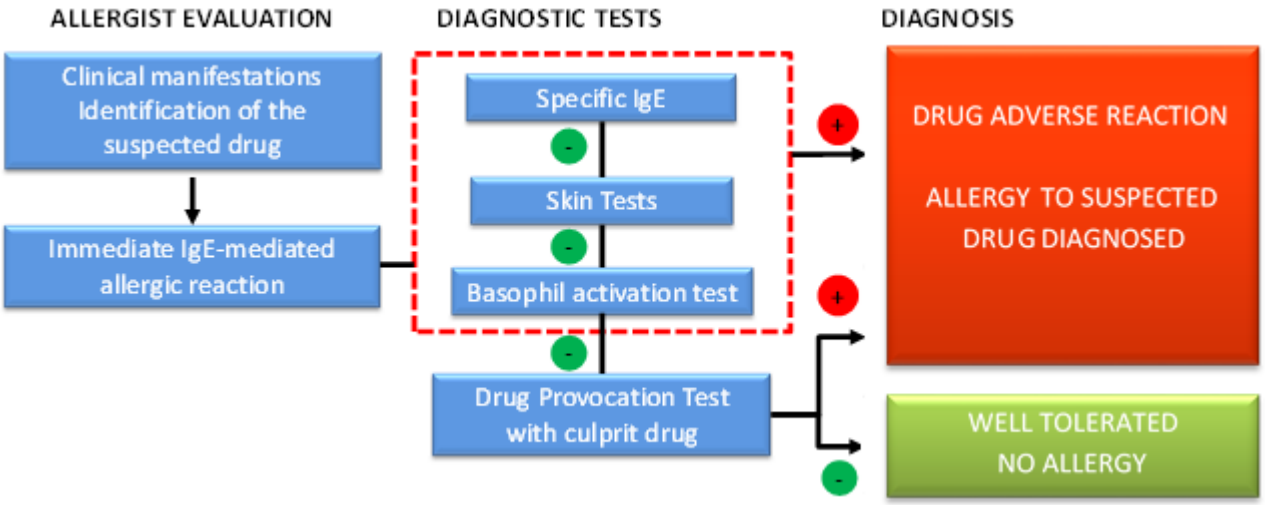
Objectives of the COBIOPHAD approach to specific IgE *in vitro* determination in patient sera

- ✓ Development of a fully compact functional readout device operating as a chemical analyser using electronics and photonics systems integrated on a compact disc drive.
- ✓ Design and fabrication of microfluidic centrifugal compact discs, combining well-established optical and production technologies with advanced material processing.
- ✓ Assay facilitating a highly sensitive, fast and selective IVD test for the ten most commonly prescribed BLC antibiotics, integrating biotechnological and chemical solutions.
- ✓ Synthesis and selection of new allergy determinants to detect a wider range of BLC effects.
- ✓ Development of telemedicine software for recording and analysis of test results and patient data management.

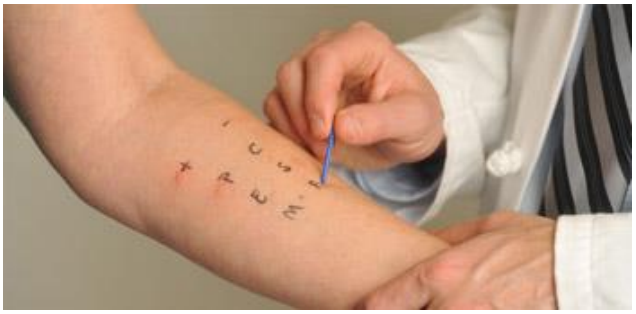


New Assays for Antibiotic Allergy Required

There are several methods to diagnose immediate drug allergy (hypersensitivity reactions Type I) including use of specialized methods looking for evidence, either by direct identification of specific IgEs or by indirect assays (skin tests, basophil activation test, and histamine release test). Rationalization of drug allergy evaluation has been issued as an International consensus on patient management[1] from which this diagram is derived:



There are multiple problems with existing tests for antibiotic allergy



***In vivo* tests –** Skin prick and intradermal tests (placing a drop of the allergen determinant solution) and provocation tests (exposure of the patient to the drug) must be performed by experienced allergy professionals and under strict controlled conditions to avoid the consequent risks.
The figure shows a skin prick test carried out on the inner forearm an allergic individual.
These tests can facilitate allergic sensitization or induce adverse reactions. In addition, they are not always available (as in emergencies, primary care centers) or reliable (due to the severity of the medical conditions or to the effects of concomitant medications). The sensitivity is not optimal, ranging from 60% to 70%.

***Ex vivo* tests –** Basophil activation and histamine release tests are based on the response of blood to addition of a suspected drug. These tests are suitable for testing several allergens. Currently, *ex vivo* tests show challenges such as unknown sensitivity and lack of standard doses for the majority of relevant drugs. Despite the fact that both tests have existed for decades, they are still under validation.

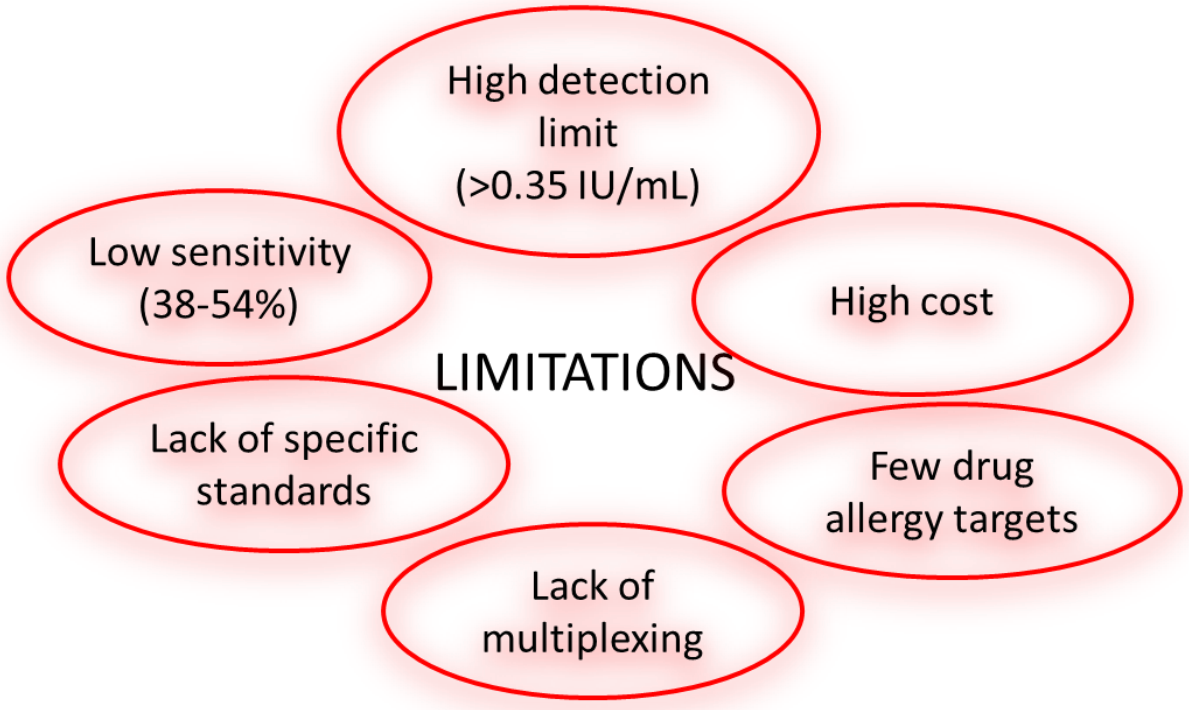
***In vitro* tests –** Due to the inconvenience and poor performance of both *in vivo* and *ex vivo* tests, safer, cheaper, and faster *in vitro* tests using physiological fluids are required. *In vitro* testing of drug allergies in blood is based on the immunoanalytical determination of the specific IgE concentration to a particular drug or derivatives. At present, most of the commercial kits determine three to five specific IgEs and are less sensitive than *in vivo* skin tests.

[1] Demoly P. et al. 'International Consensus on drug allergy' Allergy 2014 Apr 69 (4): 420-37

The main, commercial, currently used *in vitro* IVD tests are ImmunoCAP (PHADIA, Thermo-Fischer Scientific), 3gAllergy™ Universal Kit (IMMULITE, Siemens) and the assays commercialized by COBIOPHAD partner Dr. Fooke-Achterrath Laboratorien. All are devoted to detect only specific IgEs in serum to amoxicilloyl, ampicilloyl, cefaclor, penicilloyl G, and penicilloyl V, reaching different performances where PHADIA is the gold standard in the market. The detection of specific IgEs in allergy patients for other commonly prescribed BLCs is not commercially available. The three companies use different analytical strategies going from autoanalyzers for fully automated determination of specific IgEs and total IgEs to manual test, in time scales ranging from 60 to 180 min. The detection principles are absorptiometry, fluorescence and bioluminescence. The tests are always carried out in fully equipped laboratory settings. All these tests show a detection limit ≥ 0.35 kU/L. The price for instrumentation ranges from 15,000 € to more than 300,000 €, depending on the performance and working capacity.

Today, the clinical sensitivity of *in vitro* blood testing for drug allergy is only 25% to 30%, very poor compared to the skin test. The poor performance is the reason why these tests are not often included in regular clinical protocols. The drawbacks of current allergy *in vitro* tests are summarized in the following diagram:

Limitations of the current IVD drug allergy tests (L)



The main objective of the COBIOPHAD project has been to implement solutions to overcome these limitations

Patients and Sera

The development of New Assays referred to in the last section began with the isolation of specific IgEs (sIgEs) from serum of β-lactam allergic patients and non-allergic controls for further use as standards in the selection of the extended panel of determinants. This procedure was discontinued when ARTHUS were used as standards instead (p10).

For that purpose, serum samples were collected from β-lactam allergic patients with specific IgEs to β-lactam antibiotics and from controls with known tolerance to the exposure to these drugs. Both patients and controls were recruited by HULAFE and by CHRUM. Levels of β-lactam-specific IgE were measured using the ImmunoCAP assay. Allergic patients with specific IgE either for penicillin G, penicillin V, amoxicillin, ampicillin or cefaclor with a concentration above 0.5 KUA/L were selected for this study.

Serum samples were obtained by the standard procedure after blood was drawn. Sera were then stored at -80ºC pending further use.

Instituto de Investigación Sanitaria La Fe

HULAFE is a large tertiary-care academic hospital with extensive expertise in the management of drug-allergic patients including conventional *in vivo* and *in vitro* tests as well as the performance of *ex vivo* challenges by the basophil activation test.

In the COBIOPHAD project, they have provided support in identifying new allergen components, purifying human IgEs, and validating the final methodology for determining drug allergy in humans.

HULAFE's tasks have also included:

- identifying well-characterized BLC allergic patients
- obtaining serum samples from BLC allergic patients
- evaluating the performance of the final device in the diagnosis of the selected cases of IgE-mediated BLC allergy
- the evaluation of the final device in a pre-industrial trial.

Over the course of the project, up until the Eighth General Consortium Meeting in Neuss, Germany on 8th April 2019, sera from 173 patients and 150 controls were tested.

89 patients were recruited to HULAFE as part of a prospective study only whereas CHRUM analysed 30 sera from allergic patients that had already been stored as a retrospective study whilst also recruiting 54 new patients to their own prospective study. The results of the tests are recorded in the following table…

SERUM SPECIFICITY ⬇	CHRUM Patient sera		HULAFE Patient sera	TOTAL	%
	Retrospective n=30	Prospective n=64	Prospective n=97	CHRUM + HULAFE n=191	
	30	64	97	191	100.0
Aminopenicillin	18	36	45	99	51.8
Beta-lactam ring	8	13	16	37	19.4
Clavulanic acid	-	-	10	10	5.2
Cefazolin	1	10	2	13	6.8
Ceftriaxone	3	-	3	6	3.2
Cefuroxime	-	2	6	8	4.2
Ceftazidime	-	-	1	1	0.5
Cefotaxime	-	1	-	1	0.5
Tazocilline	-	1	3	4	2.1
Aminopenicillin + Clavulanic acid	-	-	1	1	0.5
Aminopenicillin + Tazocilline	-	1	-	1	0.5
Under investigation	-	-	10	10	5.2
CONTROLS	Retrospective controls n=10	Prospective controls n=75	Prospective controls n=76	Total controls n=161	

…which reveals that the majority of the patients studied (71.2%) were allergic to aminopenicillin or the core β-lactam ring.

It is noteworthy, however, that sera from 28.8% of patients showed other specificities, underlining the need for the development of an assay for an extended range of potential β-lactam allergens – as described in the following section – to define more fully the determinants that spark allergy in each individual patient.

We would like to take this opportunity to thank all the patients that allowed their sera to be used in these studies, particularly those that gave multiple blood samples, and all the hospital and clinic staff that collected and processed those samples.

CHRUM is a tertiary-care academic hospital with a research team specialized in the application of *in vivo* and *in vitro* tests for drug allergy patients and expertise in the development of diagnostic algorithms.

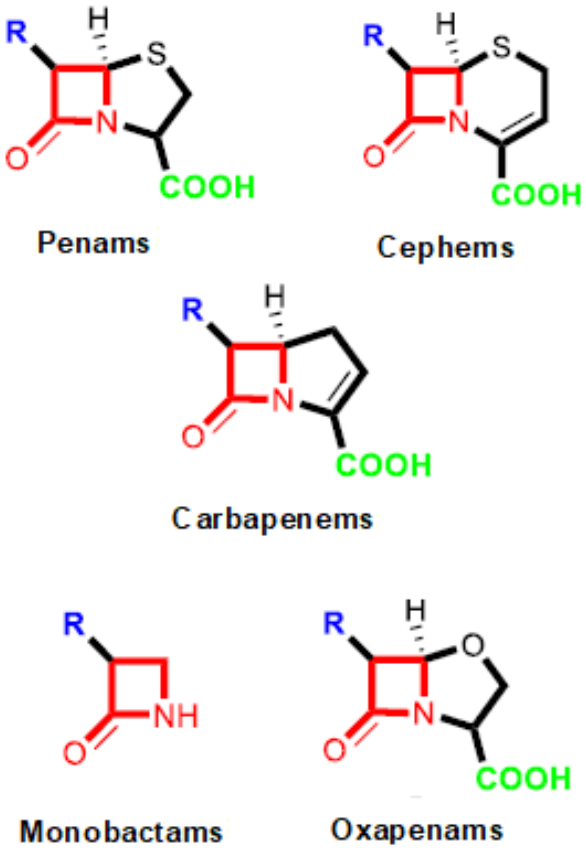
In the COBIOPHAD project they have been responsible for:

- identifying well-characterized BLC allergic patients
- obtaining serum samples from BLC allergic patients
- evaluating the performance of the final device in the diagnosis of the selected cases of IgE-mediated BLC allergy
- evaluating the different patterns of BLC IgE recognition depending of the type of allergic reaction
- contributing to optimizing assay methodology for use in diverse clinical settings

New Reagents for New Assays

Researchers at COBIOPHAD partner UPVLC have used novel approaches to developing new varieties of β-lactam determinants, and conjugates thereof.

The initial target β-lactams are shown in this figure:



New determinants – These were synthesized to create new antigenic species/determinants that could represent those to which some patients are allergic but that had not previously been identified as the cause of allergies in those patients.

ARTHUS – As stated on p8, the selection and evaluation of novel β-lactam determinants began with the isolation of specific IgEs for them from patient sera.

However, the majority of the recruited patients (see previous Patients and Sera section pp8-9) were found to be allergic to the most commonly prescribed β-lactams (amoxicillin, penicillin G and clavulanic acid) and the number of patients found to be sensitized to the less frequently used β-lactams was low or null.

Also, the concentration of specific IgEs in serum is minimal, and the current analytical methodologies used to purify specific IgEs were not very efficient, resulting in low purification yields (20-35%), insufficient to develop the validation assay. These arguments drove the use of artificial human sera (ARTHUS) which can compensate the lack of patient sera with the specific IgEs required to perform the selection of the new determinants.

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Universitat Politècnica de València (Spain)

Project Coordinator

UPVLC is an academic institution. The research group involved in this project is specialized in the development of immunoreagents, surface functionalization and the development of biosensors and nanobiosensors, being a major European reference centre for compact disc technology analytical approaches.

Their main contributions to the project are:

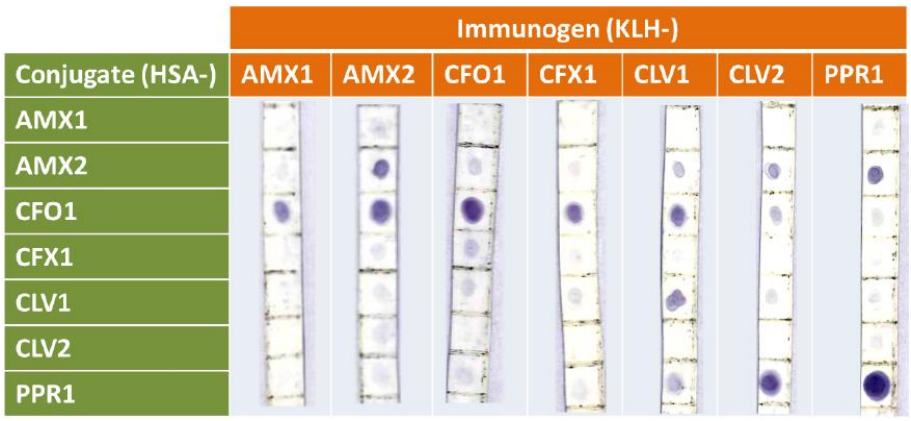
- the synthesis of structural determinants for target drugs/analogs;
- surface modification and probe immobilization strategies;
- assay development;
- testing of prototypes;
- the full integration of devices; and
- the evaluation of multiplexed allergen tests.

ARTHUS consists of allergen-specific rabbit IgG antibodies complexed with a chimeric adaptor molecule comprising the extracellular domains of the human FcγRI (CD64) molecule fused with human IgE Fc domains (CD64-IgE Fc).

New conjugates – Using novel β-lactam ring-opening and coupling technologies, a series of novel conjugates was sinthetized at UPVLC. These were designed to make the new determinants (haptens) antigenic so that they could be used to raise antisera in rabbits for the creation of ARTHUS at Fooke. For this purpose, the new determinants were conjugated to traditional 'carrier proteins' such as keyhole limpet haemocyanin (KLH) and human serum albumen (HSA), as well as to dendrimers specifically adapted to the purpose.

ARTHUS were expressed for a panel of β-lactam antibiotics that included penicillin G, aztreonam, meropenem, ceftriaxone, amoxicillin, cefotaxime, cefuroxime, clavulanic acid and piperacillin.

In particular, allergen-specific IgG antibodies were raised first in rabbits by immunization using -lloyl and -llanyl derivatives of the antibiotic haptens coupled to KLH. Dot-blot assays were then carried out to evaluate the selectivity of the sera by checkboard titration, using the same -lloyl and -llanyl derivatives coupled to human serum albumin (HSA). The results are shown in this figure:



Dot-blot analysis of sera produced against -lloyl (1) and -llanyl (2) β-lactam derivatives. AMX: amoxicillin, CFO: cefotaxime; CFX: cefuroxime; CLV: clavulanic acid; PPR: piperacillin.

As can be seen, most of the sera recognized the corresponding derivative. Also, some of the sera displayed cross-reactivity, for example, the sera produced against the -llanyl derivative of amoxicillin (AMX2) recognized the cefotaxime conjugate (CFO1).

DR FOOKE

FOOKE is an SME. For more than 24 years Dr. Fooke Laboratorien have been dedicated to developing, producing and distributing modern and innovative tests for *in-vitro* allergy and autoimmune diagnostics. Advanced *in-vitro* allergy and autoimmune test systems are the result of their developments leading to a product line that offers almost every user a custom solution exactly matching their needs.

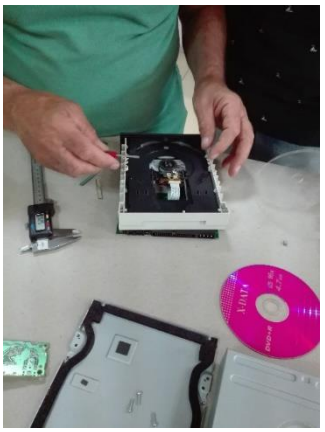
In the COBIOPHAD project, they have:

- produced Artificial Human Sera [ARTHUS]
- developed immuno-reagents for assay development and use
- lead the pre-industrial validation of the assay
- participated in the evaluation of results, and the analysis of the compliance with the defined requirements

Nevertheless, the combination of new determinants and conjugates, along with the development of corresponding ARTHUS has enabled the extension of the COBIOPHAD assay to a wide range of β-lactam determinants that are, or potentially are allergenic in humans.

COBIOPHAD Reader and Disc Hardware

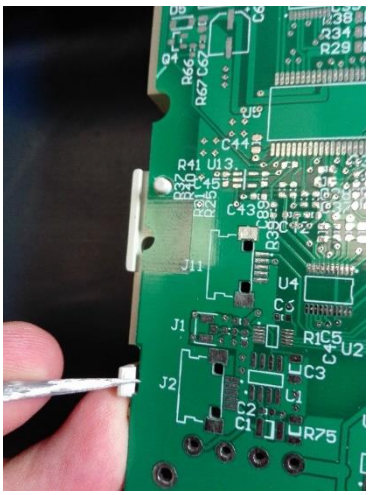
The hardware of the COBIOPHAD system is based on compact disc technology using a commercial optical disc drive (ODD) specifically designed to read the specially developed optical discs on which the assay takes place.



The engineers at OPTOEL collaborated with scientists and engineers from all the partners in the consortium to create a device that can evaluate the content of IgE specific for ten different β-lactams in six different patient serum samples on one optical disc.

To achieve this feat, they have had to overcome many significant challenges including:

- ✓ the choice of disc thickness, shape, size, etc.;
- ✓ how to clamp discs of the chosen thickness in place on the ODD;
- ✓ developing means to detect and control the speed of rotation of the disc;
- ✓ proposing and implementing means to detect and control the identification of the assay area on the disc;
- ✓ creating a laser sensor system capable of detecting and recording the range of intensities of the coloured spots on the disc that represent the assay results, using transmission spectroscopy;
- ✓ dealing with issues of disc flatness and the need or not for a central disc clamping flange.



[hv] optoelectronica
excellency in research & development

OPTOELECTRONICA is an SME specialized in technological developments in the optoelectronic domain.

The OPTOEL team have been responsible for the development of the optical drive disc [ODD] and development of the optical reading system's firmware and software.

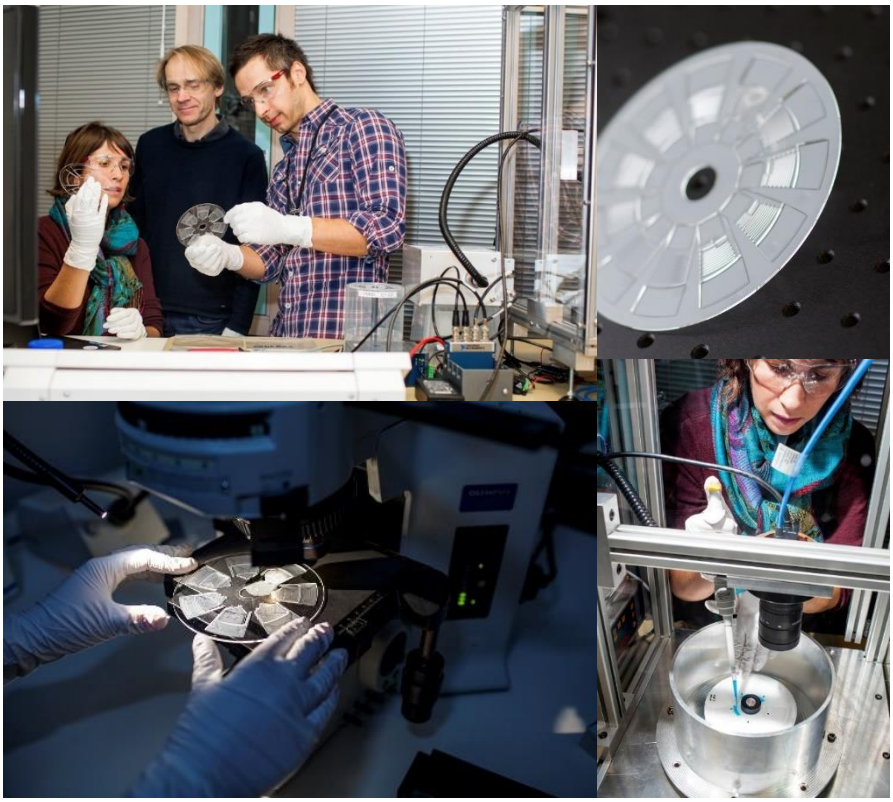
Their contribution has included:

- development of the sensor device from a commercial ODD
- integrating photo receiver blocks to detect and amplify light from the bioluminescent IgE assay
- providing the firmware to control the laser, the detector and the rotation of the ODD
- developing the user-friendly interface PC software for control of the sensor blocks
- carrying out optical simulation by ray tracing

In addition, they worked closely with partners LUMENSIA and B3D to develop the firmware and software, including a user-friendly GUI, for controlling the device, for recording and analysis of the assay results, and for transmitting those results to the operator and interested clinician(s), as well as to partner 3D for storage and potential meta-analysis.

Like OPTOEL, SINTEF had to work with numerous COBIOPHAD partners to specify and design the optical and micro-fluidic assay disc and to instantiate that design. They too had to overcome many significant challenges including:

- ✓ choice of polymer from which to manufacture the disc;
- ✓ design and composition of the top and bottom halves of the discs i.e. what parts of the assay and what parts of the control system go on which part of the disc;
- ✓ design and testing of the centrifugal system for mixing of patient sera with components of the assay and the required washing steps;
- ✓ issues relating to the manufacture and clarity of the microfluidics systems that underpin the assay system and are incorporated in the disc.



SINTEF is the largest independent research organisation in Scandinavia with multidisciplinary expertise in technology, medicine and the social sciences.

In the COBIOPHAD project, SINTEF has applied its expertise in microfluidics and polymer microfabrication, and has been responsible for the development of microfluidic design and prototyping of disc devices.

In particular they have:

- created the microfluidic design for on-disc implementation of the biological assay
- developed a novel combined 3D printing and micro-moulding approach
- been involved in prototype manufacturing and testing

Along the way, SINTEF has made several advances in the design and development of microfluidics systems based on the use of micro-milling and polishing of the chosen polymer to optimise surface quality for immobilization of bioreagents and optical transparency. In addition, SINTEF has developed novel combinations of micro-moulding with 3D polymer printing to create disc prototypes with fluidic channels and chambers with the required micro-tolerances.

Once the design sequence had been fixed and ‘final’ prototypes had been developed, it was the turn of partner STRATEC to develop the necessary procedures for manufacture of the discs to the highest quality with industrial equipment for mass production.

STRATEC's role in the project has been to work with the other partners to optimise the design of the consumable assay disc for use in the ODD reader – especially in optimising the necessary coating, spotting and bonding techniques – and then in developing a manufacturing strategy that would ensure maintenance of the tolerances and quality of the discs manufactured in bulk.

Working particularly closely with SINTEF, STRATEC too had to overcome many significant challenges in the development of manufacturing processes for the disc hardware including:

- ✓ optimisation of the disc clamping area and creation of samples for testing the modified area;
- ✓ creating samples of discs with either molded or milled micro-fluidics systems;
- ✓ testing molded vs milled systems;
- ✓ optimisation of coating and spotting technologies for final disc prototype design;
- ✓ developing bonding means to ensure correct union of bottom and top disc subunits;
- ✓ developing quality control systems for the handover of disc manufacture to their chosen supplier.

stratec consumables

STRATEC is a leading OEM supplier of smart polymer-based consumables to the *in vitro* diagnostics, life sciences and medical technology industries.

Their role in the project has been to supply their expertise in nano- and micro-structuring, coating technologies, polymer sciences and automated assembly in the application of centrifugal micro-fluidics to the fabrication of the consumable assay disc.

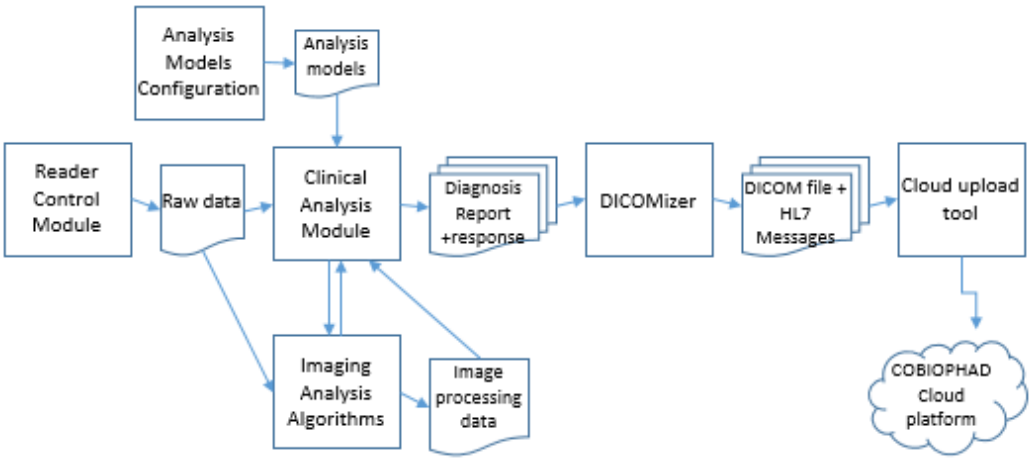
In addition, they have been responsible for:

- mastering, molding and bonding work-centres accompanied by operation of suitable QC
- development of surface chemistries to match the applications
- participate in all tasks related to disc fabrication.

Collaborations between OPTOEL, SINTEF and STRATEC along with significant imputs from UPVLC and the other partners, have resulted in the manufacture of sensitive, finely tuned, yet robust hardware for carrying out the COBIOPHAD assay.

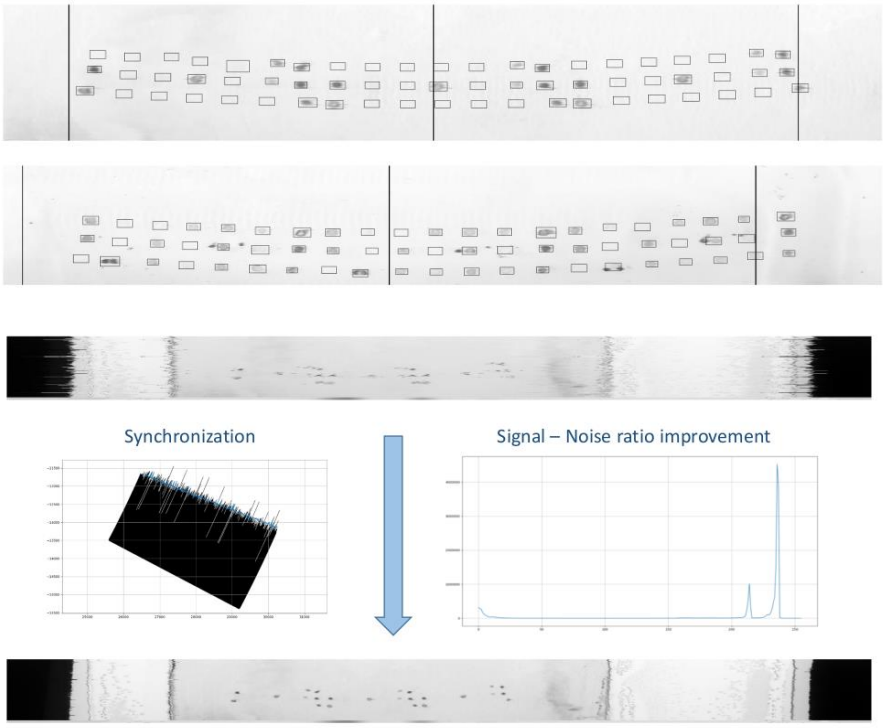
COBIOPHAD Firmware and Software

This figure shows all the firmware and software modules necessary to the implementation of the COBIOPHAD assay system that have been developed during the project:



Partners LUMENSIA, OPTOEL, UPVLC and B3D have worked closely together to specify, design and encode the software and firmware for the control of the ODD and on-disc assay system and for the capture and processing of assay data before its transmission for storage and analysis to the relevant end-users.

LUMENSIA has been specifically responsible for developing the algorithms for processing and analysis of images of the spots created by a positive result in the optical disc assay area. They have overcome major challenges in the areas of spot location and detection, synchronization, and improvement of signal to noise ratios:



In addition, LUMENSIA have collaborated closely with OPTOEL on the integration of the data and image processing software with the ODD control firmware, and with B3D and UPVLC on its integration with their data analysis and transmission software.

LUMENSIA sensors

LUMENSIA Sensors, S.L. is an SME devoted to the development of biosensing devices.

LUMENSIA's proprietary sensing technology is based on photonic structures manufactured on Photonic Integrated Circuits (PICs).

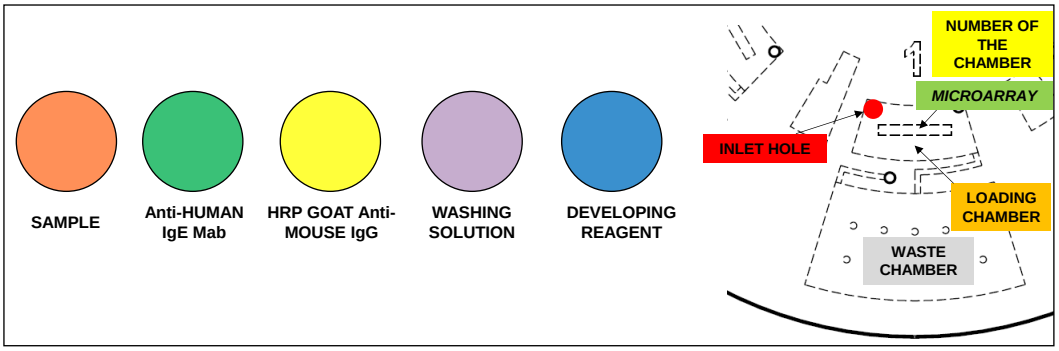
In the COBIOPHAD project they have been responsible for:

- design and optimization of the data and image processing algorithms
- assisting in prototype integration

COBIOPHAD Integrated Assay

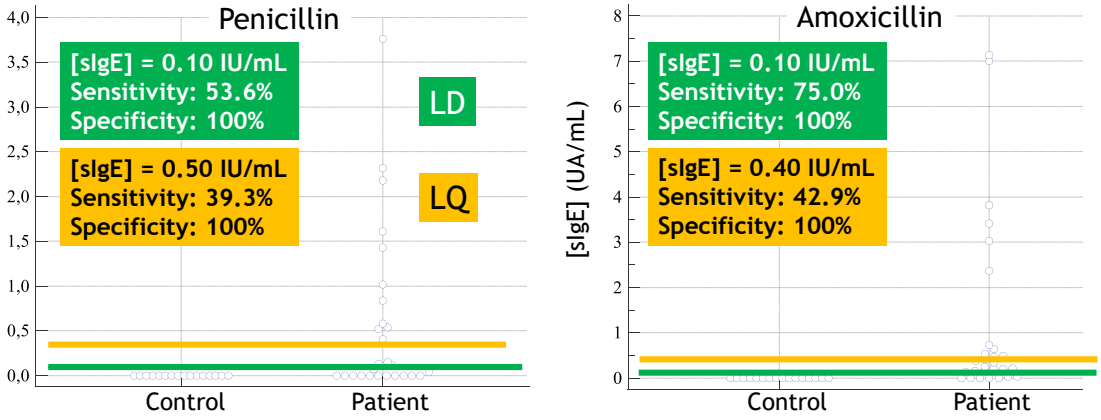
Assay principle – The COBIOPHAD test is an *in vitro* direct immunoassay in microarray format, developed in a five-step assay for the quantitative measurement of specific IgE for β-lactam antibiotics:

- ✓ Specific IgE from the patient sample is captured by the conjugate determinants coated to the microfluidic disc.
- ✓ Anti-human IgE monoclonal antibody solution is added and incubated.
- ✓ Peroxidase-conjugated goat anti-mouse IgG (Fc specific) antibody solution is added to the detection chamber and incubated.
- ✓ After a washing step, peroxidase-conjugate forming complexes are detected using 3,3',5,5'-tetra-methyl-benzidine (TMB) substrate solution, resulting in the development of a blue color precipitate.
- ✓ A centrifugal washing protocol removes surplus components a long the test from the assay assay chamber to the waste chamber.



Detection principle – The modified DVD drive is controlled by custom software, running on a personal computer and connected to it through a USB2.0 universal serial bus interface.

- ✓ The laser of the reader (λ = 650 nm) strikes the immunoreaction product which modifies the transmission properties of the disc surface
- ✓ The laser beam intensity is detected by a planar photodiode coupled to the reader.
- ✓ The optical density of the blue precipitate is related to the concentration of specific IgE for the panel of β-lactam antibiotics.
- ✓ The signals are digitalized and converted into images.
- ✓ A clinical assay algorithm provides the clinical results (concentration, error calculation and quality controls) for each array/chamber.



COBIOPHAD reader performance characteristics – These are shown in the following table:

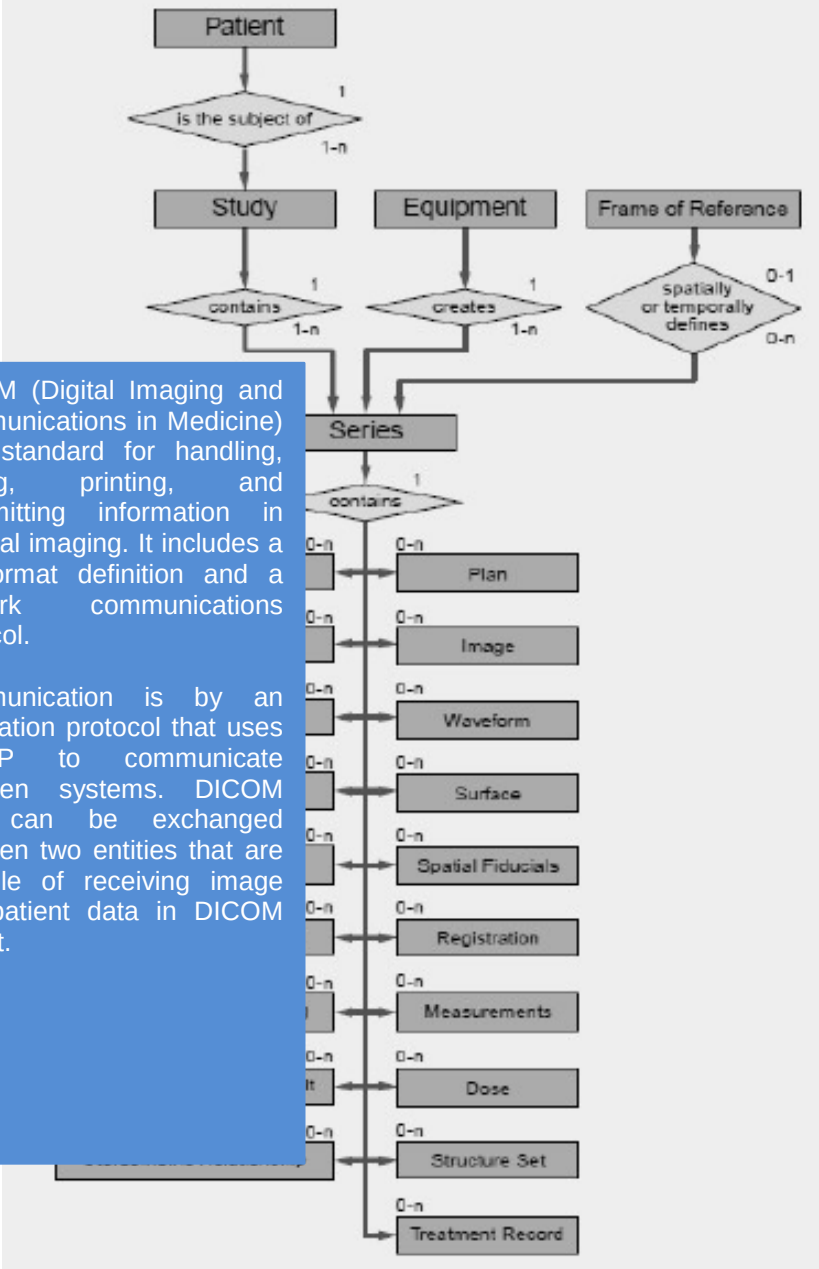
Parameter	Value
Programmable disc	Yes
Disc rotation	Clockwise: yes Counter-clockwise: yes
Min Speed (rpm)	Clockwise: 350 Counter-clockwise: 350
Max speed (rpm)	Clockwise: 6500 Counter-clock wise: 6500
Max Acceleration (rpm/s^2)	Clockwise: 1150 Counter-clockwise: 1150
Min Radial Position (mm)	24
Max Radial Position (mm)	56
Min Radial Step (microns)	10
Laser diode power control	CD diode: Yes (not implemented in FW/SW) DVD diode: Yes
Laser diode on/ off	780 nm diode laser: Yes 650 nm diode laser: Yes
Detector	Spectral response range 340 to 1100 nm Area 3 × 30 mm Photo sensitivity 0.66 A/W Dark current (max.) 10000 pA
Size	180 x 180 x 200 mm
Weight	1.7 Kg
Cost of optical reader	360 euro/pcs

Reader software functions – The different SW elements of the reader and their functions are summarised in this table:

Reader software functions	
Set-up:	Create/save/load the disc image
Manual:	Display all reader actions: rotation, laser on/off, data acquisition progress, magnet on/off and current value
Acquisition:	Set acquisition parameters: start/stop, step and spindle speed, perform manual acquisition
Script:	Create/edit/save / load whole sequence
Result Image:	Display acquired image
Graph:	Display dynamic signals: acquisition data, focus error - not available for ordinary user
Log:	Display progress: save log, show possible errors

COBIOPHAD Data Management and Analysis

B3D have been responsible for converting the data to a medical industry standard for image analysis and presentation – DICOM.



DICOM (Digital Imaging and Communications in Medicine) is a standard for handling, storing, printing, and transmitting information in medical imaging. It includes a file format definition and a network communications protocol.

Communication is by an application protocol that uses TCP/IP to communicate between systems. DICOM files can be exchanged between two entities that are capable of receiving image and patient data in DICOM format.

Biotronics 3D
Analyze - Collaborate - Discover

BIOTRONICS3D (B3D) is the SME in charge of the cloud-enabled features for remote access, automated processing, analysis and reporting of the generated data and the Clinical Decision Support Software.

As an internet-based solution, images and results can be reviewed via secured login through any browser both by referring physicians and by specialists in and outside the clinical environment.

In addition, they have been responsible for:

- DICOM standardization
- development of the user interface
- protocols for the integration and conformance to 3DNet
- QMS of the online sample analysis SW and quality control SW.

In addition to development of the data analysis and transmission software, and the DICOMISATION of patient data, B3D, along with other partners, has been responsible for the development and integration of a complete Clinical Analysis Software suite, the 'COBIOPHAD Protocols and Gateway Software' and conformity with their own proprietary cloud platform: 3DNet Medical.

In sum, proprietary software has been developed to enable gathering of data from the COBIOPHAD assay and its processing and transmission to a cloud platform for viewing by authorised parties in standard DICOM format.

Exploitation and Dissemination of COBIOPHAD Results

EUREXPLOIT LTD (EUX) is responsible for the Exploitation and Dissemination of the COBIOPHAD project results, through exploitation activities that have included the management of all Foreground IP generated during the course of the project, and dissemination through multiple media outlets including the project website: www.cobiophad.eu, social media, scientific journals and this magazine.

For the purpose of these tasks, and in respect of its coordination in Spain, each result of the project, of whatever type, has been designated as an INNODEC: INNOvación DE Cobiophad.

Up until month 41 of the project, EUX has recorded in excess of 70 INNODECs with contributions from each partner. These include such as:

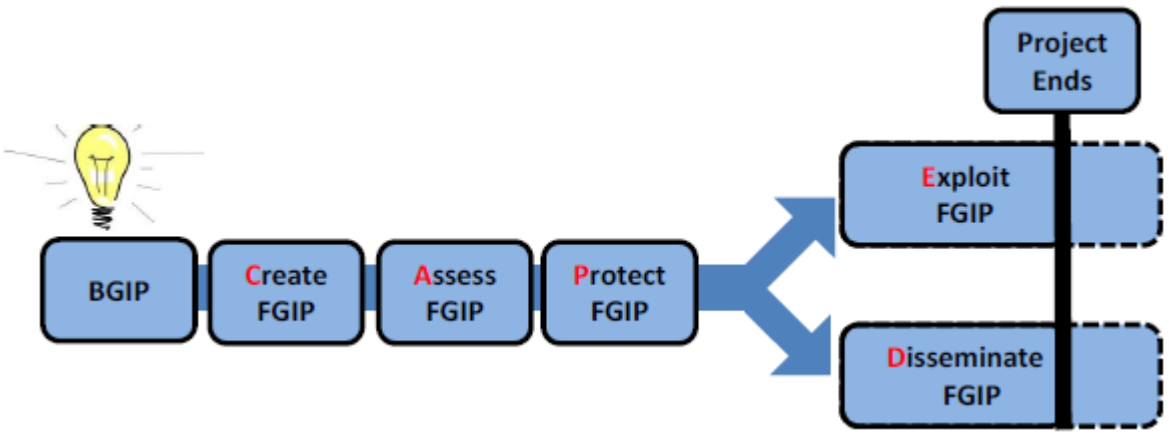
- ✓ each item of novel hardware, firmware and software;
- ✓ novel β-lactam determinants and conjugates;
- ✓ retrospective and prospective databases of β-lactam sensitive patients and tolerant controls;
- ✓ novel ARTHUS reagents;
- ✓ publications in scientific and medical journals;
- ✓ articles in medical trade papers and magazines;
- ✓ databases of assay development results;
- ✓ project videos;
- ✓ databases of images collected from the assays;
- ✓ deliverable reports e.g. on analysis of patenting in the disc-based *in-vitro* diagnostic device sector.

EUREXPLOIT LTD (EUX) is an SME dedicated to supporting other SMEs in managing national and European R&D & Innovation projects and in valorising their project results.

EUX provides consultancy in: proposal writing, dissemination and exploitation activities, intellectual property (IP) management, IP evaluation, technology sector mapping and patent analytics

EUX is responsible for the Exploitation and Dissemination of the COBIOPHAD project results

Following its **C**reation and record, each INNODEC has been subjected by EUX to the application of the APED algorithm as illustrated here:



The main intention of this application was to ensure that each project result was properly **A**ssesed for its value to the COBIOPHAD consortium members, and, if necessary, **P**rotected e.g. by patenting, BEFORE its **E**xploitation and/or **D**issemination.

Plan for the Exploitation & Dissemination of the (Project) Results (PEDR)

Four versions of this document have so far been prepared with a final version to be created for the end of the project (31st July 2019). This version will include plans for the exploitation of the INNODECs through sale and/or licensing and the plans for post-project dissemination of those plans and any further INNODECs recorded and assessed before the project's end.

The COBIOPHAD project website – This was established early in the project at www.cobiophad.eu:

It includes both public-facing elements, such as the PROJECT NEWS posts and list of PUBLICATIONS, both available on the Home page, as illustrated above, and elements such as the Docstore that are password-protected so that they can only be accessed by the COBIOPHAD consortium members. It will play a major role in the post-project dissemination activities proposed in the final PEDR.

Other dissemination activities that have been undertaken during the project have included, and will further include, the targeting of different potential stakeholders in the exploitation of the INNODECs such as :

- ✓ end-users of the assays – including clinicians and pathology services providers;
- ✓ hospital and clinic purchasing authorities;
- ✓ potential investors and/or manufacturing licensees;
- ✓ actual and potential β-lactam allergy sufferers;
- ✓ the general public e.g. for educational purposes.

These stakeholders have been and will be reached using a wide range of approaches including through conferences, seminars, workshops, trade fairs, scientific and trade publications, the website and this magazine.

COBIOPHAD – List of Publications

Authors	Title	Publication
Nadine Offermann et al.	Establishment of artificial human sera (ARTHUS) based on Chimeras of fcgammari and Human Immunoglobulin Domains	Abstracts from the European Academy of Allergy and Clinical Immunology Congress, 6–10 June 2015, Barcelona, Spain Allergy 2015; 70, Issue Supplement S101
Nadine Offermann et al.	Human serum substitution by artificial sera of scalable allergen reactivity based on polyclonal antibodies and chimeras of human FcγRI and IgE domains	Allergy 2016; 71: 1794–1799.
Teresa Molina	Compact biophotonic platform for drug allergy diagnosis	Brief presentation of the COBIOPHAD project in Photonics Public Private Partnership Annual Meeting 2016. Parallel session Working Group 3: Life, Science and Health.
Mª José Juárez, et al.	Diagnóstico in vitro de alergias a antibióticos β-lactámicos	Book of articles (ISBN: 978-84-617-5330-7) of the X International Workshop on Sensors and Molecular Recognition. Valencia 7-8 July 2016, Chapter 21, p117-121
Fernández E, et al.	Compact biophotonic platform for drug allergy diagnosis (COBIOPHAD)	Book of abstracts of the X International Workshop on Sensors and Molecular Recognition. Valencia 7-8 July 2016, 110
Sergi Morais	The analytical side of compact disc technology	Lecture Adlershofer Kolloquium Analytik (AKA) Berlin, 2nd May 2017
Nadine Offermann et al.	Artificial Human Sera (ARTHUS) as a tool for validation and standardization of bee and wasp specific in-vitro diagnostic systems	European Academy of Allergy and Clinical Immunology (EAACI) Helsinki, June 2017
Sergi Morais	Chemical strategies for designing structural determinants for β-lactam allergy	Abstract presented for a poster presentation at the XXXVI Biennial Meeting celebrated by the Spanish Royal Society of Chemistry (RSEQ). Sitges Barcleona 25-29 June 2017.
Estrella Fernández et al.	Compact biophotonic platform for drug allergy diagnosis (COBIOPHAD)	XI International Workshop on Sensors and Molecular Recognition 2017. Valencia 6-7 July 2017. Poster exhibited at the Workshop.
Mª José Juárez, Sergi Morais, Ángel Maquieira	Abstract: Estrategias químicas de anclaje covalente de alérgenos a superficies de policarbonato (*Chemical strategies of covalent anchorage of allergens to polycarbonate surfaces)*	XI International Workshop on Sensors and Molecular Recognition 2017. Valencia 6-7 July 2017. Poster exhibited at the Workshop.

Authors	Title	Publication
Salvador Mas, Sergi Morais, Ángel Maquieira	Abstract: Biosensor óptico para la determinación de IgE totales (*Optical biosensor for the determination of total IgE*)	XI International Workshop on Sensors and Molecular Recognition 2017. Valencia 6-7 July 2017. Poster exhibited at the Workshop.
David Ruzafa et al.	Abstract: Desarrollo de inmunoensayos quimioluminiscentes para el diagnóstico de alergias a antibióticos (*Development of chemiluminescent immunoassays for the diagnosis of antibiotic allergies*)	XI International Workshop on Sensors and Molecular Recognition 2017. Valencia 6-7 July 2017. Poster exhibited at the Workshop.
Elizaveta Vereshchagina et al.	Synergy of 3D printing and injection molding: a new prototyping method for rapid design optimization and manufacturing of microfluidic devices	Abstract: The 21st International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2017) October 22-26, 2017
Elizaveta Vereshchagina et al.	Rapid prototyping of polymer microfluidic devices for optical detection	Abstract: The 21st International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2017) October 22-26, 2017
Elizaveta Vereshchagina et al.	Synergy of 3D printing and injection molding: a new prototyping method for rapid design optimization and manufacturing of microfluidic devices	Full paper: The 21st International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2017) October 22-26, 2017
Elizaveta Vereshchagina et al.	Simultaneous improvement of surface finish and bonding of centrifugal microfluidic devices in cyclo-olefin polymers	Abstract submittted to MEMS2018 conference.
Elizaveta Vereshchagina et al.	Simultaneous improvement of surface finish and bonding of centrifugal microfluidic devices in cyclo-olefin polymers	Full paper submittted to MEMS2018 conference.
Elizaveta Vereshchagina et al.	Local deposition and characterization of antifouling coatings in cyclo-olefin polymer microfluidic devices	Abstract submitted to Biosensors 2018 conference. Miami, FL, USA. 12-15 June 2018.
Sergi Morais, Luis A. Tortajada-Genaro and Ángel Maquieira	Abstract: The COBIOPHAD approach. Towards in vitro antibiotic allergy diagnosis.	Abstract submitted to Biosensors 2018 conference. Miami, FL, USA. 12-15 June 2018.
Sergi Morais et al.	Abstract: Covalent anchoring of b-lactam determinants on digital surfaces for developing in vitro tests for the diagnosis of allergy	Abstract submitted to DHM 2018, Drug Hypersensitivity Meeting. Netherlands, 19-21 April 2018.

Authors	Title	Publication
Luis A. Tortajada-Genaro et al.	Abstract: COBIOPHAD: progresses towards in vitro diagnosis of drug allergies by a point-of-care device	Abstract submitted to DHM 2018, Drug Hypersensitivity Meeting. Netherlands, 19-21 April 2018.
Sergi Morais, Angel Maquieira and Luis A Tortajada-Genaro	Oral: The COBIOPHAD approach. Towards in vitro antibiotic allergy diagnosis	Oral presentation: Biosensors 2018 conference. Miami, FL, USA. 12-15 June 2018.
Estrella Fernández et al.	COBIOPHAD project: Progress towards in vitro diagnosis of drug allergies by a point-of-care device	Abstract submitted at the XII International Workshop on Sensors and Molecular Recognition. Valencia 5-6 July 2018. Poster exhibited at the Workshop.
E. Ibáñez Echevarría et al.	Diagnosis of betalactam immediate reactions	Abstract: XXXI Congreso de la Sociedad Española de Alergología e Inmunología Clínica (SEAIC2018) . Valencia. 24-27 October, 2018.
Julia Oto Martínez et al.	Serum IgE isolation & purification from betalactam allergic patients	Oral presentation: XXXI Congreso de la Sociedad Española de Alergología e Inmunología Clínica (SEAIC2018). Valencia. 24-27 October, 2018.
Julia Oto Martínez et al.	Serum IgG isolation & purification from betalactam allergic patients	Abstract: XXXI Congreso de la Sociedad Española de Alergología e Inmunología Clínica (SEAIC2018) . Valencia. 24-27 October, 2018.
Estrella Fernández and all partners	COBIOPHAD: An innovative *in-vitro* device for immediate drug allergy diagnosis	Poster: Lab-on-a-Chip and Microfluidics Europe 2019. Rotterdam. 18-19 June, 2019.
Estrella Fernández et al.	COBIOPHAD: An innovative *in-vitro* device for immediate drug allergy diagnosis	Poster: International Workshop on Sensors and Molecular Recognition (IWOSMOR) XIII UPVLC. Valencia. 4-5 July, 2019.
María José Juárez, et al.	Microimunoensayos para la determinación multiplex de IgE específica de antibióticos ß-lactámicos en suero humano	Oral communiucation: International Workshop on Sensors and Molecular Recognition (IWOSMOR) XIII UPVLC. Valencia. 4-5 July, 2019.
María José Juárez, et al.	Diagnóstico “in vitro” de reactividad cruzada en la alergia a antibióticos ß-lactámicos	Poster: International Workshop on Sensors and Molecular Recognition (IWOSMOR) XIII UPVLC. Valencia. 4-5 July, 2019.
Pedro Quintero-Campos, et al.	Detección in vitro de alergia a amoxicilina mediante inmunoensayo luminiscente	Poster: International Workshop on Sensors and Molecular Recognition (IWOSMOR) XIII UPVLC. Valencia. 4-5 July, 2019.
Elizaveta Vereshchagina et al.	A novel diagnostic device for rapid testing of antibiotic allergies: focus on fluidic design and manufacturing of disposable discs	Poster: MicroTAS2019. Basel. October 27-31, 2019.

COBIOPHAD Consortium Members

UPVLC (re COBIOPHAD)
Coordinator: Ángel Maquieira
Email: info@cobiophad.eu
URL: www.cobiophad.eu

OPTOEL
Brindus Comanescu
Email: brindus@optoel.ro
URL: www.optoel.ro

SINTEF
Fabrice Lapique
Email: fabrice.lapique@sintef.no
URL: www.sintef.no

LUMENSIA
Santiago Simón
Email: ssimon@lumensia.com
URL: www.lumensia.com

HULAFE
Dolores Hernández
Email: hernandez_dol@gva.es
URL: www.hospital-lafe.com

CHRUM
Anca Chiriac
Email: ancamirelachiriac@gmail.com
URL: www.chu-montpellier.fr

FOOKE
Margrit Fooke
Email: mfooke@fooke-labs.de
URL: www.fooke-labs.de

B3D
Harry Hatzakis
Email: hhatzakis@biotronics3d.com
URL: www.biotronics3d.com

STRATEC
Werner Balika
Email: w.balika@stratec.com
URL: www.stratec.com

EUREXPLOIT
Ian McKay
Email: ian.mckay@eurexploit.org
URL: -

PHOTONICS PUBLIC PRIVATE PARTNERSHIP

The COBIOPHAD Project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 688448. It is an initiative of the Photonics Public Private Partnership (www.photonics21.org)