COBIOPHAD
Compact biophotonic platform for drug allergy diagnosis
6th meeting
17th September 2018, Montpellier

Participants

Ángel Maquieira (UPVLC), Sergi Morais (UPVLC), Luís A. Tortajada (UPVLC), Teresa Molina (UPVLC), Estrella Fernández (UPVLC), Joao Correia (B3D), Eva Murauer (CHRUM), Pascal Demoly (CHRUM), Anca Chiriac (CHRUM), Audrey Boissin (CHRUM), Laetitia Ferrando (CHRUM), Ian A. McKay (EUX), Ethel Ibáñez (HULAFE), Dolores Hernández (HULAFE), Elizaveta Vereshchagina (SINTEF), Michal Mielnik (SINTEF), Werner Balika (STRATEC), Sergio Peransi (LUMENSIA), Alfredo Saez (LUMENSIA), Margrit Fooke (FOOKE), Nadine Offermann (FOOKE), Mr. Linte (OPTOEL)

Ian A. McKay (EUX) is delayed, and arrived at noon.
Werner Balika (STRATEC) will leave the meeting at 15:30.

Minutes

1. Welcome

Meeting started at 8:20.
CHRUM (Pascal Demoly and Anca Chiriac) welcomed the attendants and introduced the schedule of the day.

2. Status of the project

UPVLC (Ángel Maquieira) presented the status of the project and gave a general overview of the main achievements and challenges of the project.

<u>Project status</u>

- Extension of the project
  - The extension amendment is almost finished but still not submitted. Partners who did not provided the needed information are urged to send it as soon as possible to complete the process
- Reader (today demonstration)
- Disc design
  - Fluidics in development
  - Prototyping in process
- Disc fabrication
  - Design freezing 1 October
  - First batch 1 March

- Serum samples
  - Collection in progress
  - Pre-validation: not yet
  - Samples per validation: number reached (but low levels of IgE)
- Integration
  - Progressing but waiting for decisions, needs final outputs from WP1, WP2, WP3 to complete integration.
- Validation
  - Waiting for pre-validation results
- Diffusion
  - On time

Pending tasks and current challenges

To be done: Test the reader with prototyped discs to evaluate the analytical assay
To be done: Test the reader and the analytical assay with the final COBIOPHAD disc (could start in February, with first batch of moulded discs).

Currently: final design of the disc including fluidics is on-going, soon the discs should be prototyped and the analytical design tested on them.
There is a timing problem: the disc design should have been frozen on September 1st, now delayed to 1st October!
Ángel presented 3 different plans about how to proceed, including their risks. He stated that, today, it has to be decided which plan we should follow up. Most agreed that **plan 3** is the best: if fluidics design is frozen by the end of September, disc fabrication would start 1st October and validation would start on February using stamped discs.

Pascal Demoly asked if we have to show as results of the project, capabilities related to mass production.
SINTEF (Michel and Liza) replied that it is important to demonstrate the system using the proposed production methods. They also mentioned that each prototyped (milled) disc is unique, moulded (stamped) discs will be more regular, having repeatable behaviour. Moulded discs will provide better results in validation, compared to milled discs.

Samples.
147 positive & 150 controls already prepared. But still low representativity and low concentration of specific IgE for analytical assay pre-validation. We need 10 different representative samples with high marks and volumes of 50 ml.

New action in UPV concerning samples
Starting a campaign asking for volunteers allergic to b lactams among the personnel and students of the University. Also contacts with an Italian hospital which could provide samples and diagnostic information.

Internal reporting
Several partners do not report in time their contribution for the monthly report. Reminder for further collaboration.

3. Integration of the COBIOPHAD system. Part I

9:00
OPTOEL (Mr Linte) presented the work related to the integration of the COBIOPHAD system including a demonstration of the reader and its graphical interface.

As main conclusion, the reader is tested in all its performances
The focussing can be performed using both the reflective and the opaque trapezoids. The trapezoids should be as long as the spotted matrix.
To be tested: final prototypes of discs. Also, the effect of possible remains of liquid in the detection chamber when reading.
At the end of September one unit of the reader will be sent to UPV for validation.

Mr Linte explained that possible errors fixing the disc were not only caused by disc but also by clamp quality and that OPTOEL has implemented an algorithm to know if the disc and the reading of the discs is correct or not.
He mentioned that the reading speed has been increased. Time of acquisition: 20-25 minutes for current chambers.
One bad news: when communication speed is configured at maximum: 5-10 % of data are wrong during acquisition, OPTOEL is still working on alternatives for speed up the reading process.

Mr Linte also gave an overview about some requirements for the disc and its specification and manufacturing.
The lateral sides of the trapezoids (reflective or opaque) should coincide with radial directions and the shortest width should be 3 mm.
The trapezoids must be darker than the spots to avoid problems with the focus algorithm.
The external ring is no longer needed, so there is more room for fluidics.
If the marker for the starting point of the reading process is properly located at the initial radius of the spotted matrix, this will reduce reading time. Current resolution: 5 micron (internal radius), 10 micron (external radius).
Speed range up to 5000 rpm. Minimum speed: 350 rpm (both rotating directions)

Discussion:
Estrella mentioned that it was also tested at lower speed with no problems in the communication process.

Liza asked why they do not focus on the surface. Linte replied that focus has to be done in the plane of the spots, on the edge of spots or edge of trapezoids.

9:45
LUMENSIA (Alfredo Saez) presented the work developed and their achievements on different spotting designs. Main focus was on general error corrections due, for instance, to the different size of the pixels at different reading radius, or also to loss of synchronization. He showed the results of real images (new spotting design).

Next steps: wait until a final spotting pattern is chosen in order to test everything with this design. Improvements of algorithm are expected after having the results of a certain number of tests, showing the real differences in different test results.

Discussion:
Werner asked for comparison of the reading process using black tape or aluminium reflective markers as optical references. Sergio answered that the work is more or less the same, since the markers are used for locating and referencing the spot matrix and background signal is the same. The difference between positive and background is important, but there is no big difference using any of the two possible markers.

Linte asked what happens if you find just 2 spots in one chamber. Luis replied that there will never be an image with only two spots as at least positive controls should be identified.
Linte stated that the maximum contrast should be between the background ("white signal") and the optical markers ("black signal"), the grey levels of the spots should be in between them.

10:05
B3D (Joao Correia) exposed the telemedicine feature of the software. Until now they were working on standardisation to DICOM and on user interface. The clinical analysis is in alpha. Final version expected in M35.

Presentation of the analysis module: the configuration of the assay can be easily done: 10x10 matrix of spots: easy to move the spots accordingly to the design of the matrix, values (and thresholds) can easily be changed to improve final results. In automatic mode everything is sent to cloud.

Joao further presented the Cloud platform and showed examples of presentation of allergy test reports. A user accounts for log in has to be set up. He showed a real demo.

10:25 Coffee break

4. Integration of the COBIOPHAD system. Part II

10:50
SINTEF (Elizaveta Vereshchagina) explained the status of the disc microfluidic design. So far there is no automated way for refilling and emptying chambers in a sequential delivery mode test. SINTEF will still work on it, however the time available will be too short.
Liza precised that the disc is fulfilling all needs of OPTOEL. Test will be performed on reference prototyped discs with updated clamping area (injection molded, Werner will present this later).

After the presentation, showing the current results and videos of the sequential delivery system, we discussed about the final design and the time line.

SINTEF presents different models for testing the prototypes and a model for manufacturing (which would be the frozen design) having 4 tests per disc: 2 manual delivery and 2 sequential deliveryof 25 µl load Freeze of design: expected in October.

Concerning the work to be done by SINTEF: it is needed to be considered with the available budget, there is only circa 600 hours remaining, ca 3.5 months of full work.

Teresa said that it was not clear to her how the final design will look like. Liza answered that the final design would have 5 loads expected (manual or sequential), the challenge is to keep the loads in the detection zone during the process (incubation, washing…), and after that empty completely the chamber prior to load it again, however this is not an easy task.

Werner said that, in the process of design and manufacturing of a complex platform, the task of finding the right design would usually would take 4-5 iterations and years to fulfil everything. In our project we should simplify and avoid problems with complex designs. If there is already one design that could be very close to a final design, we just have to decide if it is accepted.

Angel asks about 25 prototyped discs to work on the assay with a platform as close to the final disc as possible. This is to prevalidate the complete assay procedure.

STRATEC (Werner Balika) described the progress on disc manufacturing starting with the original and updated timeline. Werner then presented the current mold in preparation (to improve the clamping area). Current work on mold preparation is in progress (for the improvement of the clamping area): will be flat, no stack ring on the disc, clamping should be fine.

He further presented the disc specifications and limitations in terms of fabrication. Stratec proposes to outsource two processes (spotting and milling of 3rd notch, if it is finally needed) to manufacture the disc, in order to end up on-time and with a success in this project.

Werner asked how much time is needed for the prevalidation of the assay (prior to freezing the design). Stratec would like to fix the design by end of September, but up to 31st october is accepted, delaying the manufacturing of the different batches one month each. The number of discs they can deliver depends on the fabrication way (on how many manual processes will be), then, it has to be decided how many discs will be needed for validation. But it also will depend on the final design and the number of tests that could run in one disc.

Opaque markers are challenging. Thick markers are easy to manipulate but they would be a problem for bonding due to their thickness, if PSA bonding process is selected. Thin markers, should be less than 3 microns (big challenge), they are not a problem for bonding but very difficult to manipulate. The reflective markers are accepted as preferred option.

<u>Risk/limitations/decisions presented:</u>

- Unknown quantity of manual processes. Outsourcing not defined yet for spotting and milling (3rd notch)
- Aluminium sputtering will be good enough, this option is kept for producing the optical markers.
- Critical thing: flatness
- Preference for manufacturing process: solvent activated bonding. big chambers should have pillars to avoid problems with lack of flatness. The pillars should be about 300 microns in diameter. In the waste chamber, better if we use walls instead of pillars, as the chamber is quite big.
- Preference: aluminium coating for optical markers, instead of black tape
- Extra money spent to improve bonding equipment

- A reference check is needed (with Valencia) to define spotting process
- Goal: define the final design and freeze it to start manufacturing. In parallel other designs can be prototyped but possible improvements will not be included in the manufactured discs.

Questions raised during the discussion:

- Angel asks about the possibility to extend the design up to December, asks about implications in the delivery of manufactured discs.
- Werner: first batch could be in April 2019 it the freeze of the design is 1st october. He strongly suggest to avoid any delay in the freezing of the design. Freezing in December is a very high risk;
- Angel suggests to hurry up with the easiest way for the optimization of the design
- Sergi asks how many preliminary milled discs could be available pre prevalidation
- Michal: Sintef needs a number to organise prototyping, and confirm or not the possibility to produce them before freezing the design, and after that.
- Angel: 25 will be needed, not in one time: 2-5 to perform tests, then discuss the results, then receive 2-5 more units, …. until prevalidation of the design is complete)
- Michal explains that from their part it's a question of resources, there will be intensive dedication until freezing of the design, then dedication will slow down
- Liza proposes to f Freeze the manual design in order to have a reference disc, and to work after that 2 more months on other models of the disc that could be prototyped, even if they are not included in the frozen design.
- Werner does not want to delay the decision, proposes to : **simplify** in order to end up earlier
- Angel and Margrit defend that prior to freeze of the disc it should be tested at a prototype level.it is needed to know whether the reagents work on this device.
- Liza exposes that some tests with 100 µL were done on milled prototypes.
- Teresa: ask for some discs with similar design to the proposed one that will be frozen in order to test the assay
- Angel asks for discs to test fluidics and assay (in parallel to production, to fix the protocol for the assay process)
- Liza: Sintef will send as soon as possible 5 discs to UPVLC for testing. In November/December Sintef could deliver more discs where clamping area is ok.
- Werner: Moulding is better than milling considering the quality of the disc. Also it has to be considered that before preparation of mouldsthere is a time needed to work with the final design. During the period of mould preparation and while manufacturing is running, the milling is a solution for prototyping.
- After the discussion, Angel proposes to freeze the design as soon as possible to have first moulded prototypes (batch) in March
- Luis: Sintef should propose a design for prevalidation of the assay on disc, then UPV will perform some test, if results are ok, then that design could be frozen, then Stratec will start manufacturing. After design freeze: UPV will continue the work with integration activities using prototyped discs.
- Werner asks about how this process of prototyping/manufacturing will be explained at the next review meeting. This will be discussed later.

As a summary of the discussion, everybody agrees on the need to use prototypes to allow testing prior to freeze the design, then, as soon as some successful tests could be performed (at least in the simple design: non sequential delivery) the process of manufacturing of inserts can start. About 2-4 weeks

will be needed for prototyping + testing, so the freeze of the design should happen not later than October 31st.
It is expected that the design will be similar to the "green model" exposed at slide 9 of SINTEF's presentation. It includes 2 chambers for sequential delivery (automatic assay) and 2 chambers for manual loading at each step of the test. At least, this simple way for performing the assay should be previously prevalidated by testing prototypes.
Sintef has to keep all partners in the loop of progress. Testing with real serum should be done at UPV or in Oslo

12:50 Lunch break

5. Assay and samples

14:00
HULAFE (Dolores Hernández) exposed the progress of serum sample collection and the main challenges. The main problem is that all cases have very low specific IgE for BLC (<1 UA/mL).
It is needed to detect these individuals. Dolores presented a contingency plan with 9 different ideas in order to obtain specific serum samples. In order to include volunteers it is suggested to check them via skin testing.

14:25
UPV (Sergi Morais) gave a short presentation on the assay development and showed that the COBIPHAD assay works well in batch on DVDs.
Important point: Create reference material.
Cobiophad assay has to be tested in the microfluidics COP disc to advance in the integration process.

Discussion:

Two solutions for pre-validation:
1. use samples with high IgE levels
2. use ARTHUS: however low representativity, might be used as a reference material for calibration.

The system has to move to a system where the results are either positive or negative (qualitative results).

Margrit: Tests can be performed with full blood if not clotted (heparin or EDTA). One possible solution: metabolize in vitro and isolate determinants.

Sergi appoints that when ARTHUS is included in the serum the sensitivity drops.

Margrit states that a very important point for the market: our results have to be comparable to the standard (CAP)

Pascal: even if with Cobiophad we are not increasing the sensitivity, good specificity is reached, and specificity is the most important.

Michal asks about the size of the array, why did the array shrink so much (now: 2.5 mm x 2.5 mm)?

Angel: The reader's resolution increased. It is also better for the analytical assay performances.

Sergi: Determinants: The idea is to have a chemical library of BLC

5. Validation

15:10
The validation will not be performed as planned, as the final result of the disc manufacturing process will not be a pre-commercial one. So that, there will be a testing programme defined to be run with the reader, the disc and the software developed to perform the evaluation of performances of the complete system.

6. Action plan to the end of the project

15:20
Luis: to perform tests with next prototypes 30-40 discs are needed (it is suggested to have 3 samples per disc and use bottom blank discs with black tape to have samples quicker).
Using prototyped discs, we should be able to perform complete tests (manual or sequential load) by the end of October – mid November.

Decision:
End of year: final version of reader, software, reagents, assay protocol.
Then: report: D4.2: performance evaluation.
Experiments, report D4.3 report on integration and assay testing.
See slide 10 of the presentation of WP4.

7. Dissemination and exploitation

15:40
Since 5th meeting:

3 abstracts and one presentation from IDM-UPVLC
Remark: Ian asks for details about conference journals for published abstracts and publications, links or references where publications could be find.

Each month is done a review of the patents published: found one patent application form STRATEC

Monthly reports: missing information because of missing monthly reports (up to six months). Asks for more cooperation.

16:00 Coffee-break

8. Management issues

16:00

UPVLC (Teresa Molina) presented management activities, deliverables; milestones and extension of the project.

The dates of pending deliverables and milestones are revised and confirmed by the partners.

The amendment for the extension of the project is still in progress, amendment not submitted since information of some partners is still missing. The process will be completed in the following days, the partners are urged to present as soon as possible the missing information.

There is a plan to prepare the information and presentations for the next review meeting. The schedule is shown. No discussion about proposed dates.

The next meeting will be 19/20 November 2018: Brussels (Review meeting).

Another general meeting is expected by the end February/ beginning of March 2019

The main contents for this meeting:

A review of the final results WP4 First results of WP5

Update of plan for final activities within WP5 (the testing programme defined)

WP6 exploitation; patents

The last meeting during the project execution period is expected for July 2019: final results of validation; results of COBIOPHAD; plan for documentation of results of the project and preparation of the final review meeting.

The final meeting of COBIOPHAD is expected in October 2019; at Brussels

Patent: all partners have money for this in the budget: money must be moved for patent if any is filed

Monthly teleconferences are proposed. Next one to be held on October 2018 to deal with the decisions taken concerning final design and manufacturing, as well as the preparation of the review meeting).

Monthly reports: they are posted in the docstore of the website. Some of them are missing. This work is to be continued in October (with the MR of September) with the participation of all partners.

Suggestion IAN: Put all the shared information into the minutes of the meeting to update the information (actually all the presentations will be available for the partners)

Liza: suggest to send the minutes of the teleconferences held with participation of some partners to IAN

Review meeting:

Structure similar to mid-term meeting

We have to prepare a report (general explanation of what has been done)

Document: there is a template (same as last time). It will be updated and send to the partners.

We have to present the distribution of the PM: for current period, for total project

Draft agenda will be sent to partners

Presentations organized by WP

PO need the digital version of the presentation 15 days before meeting (deadline 6 November) and in printed version the day of the meeting

9. Final remarks. Open discussion

17:15

Angel: asks Linte if reader will be finished and sent to UPV at the end of September: Linte agreed.

The compilation of the control software will be done at Valencia.

Mr Linte confirms that only 2 readers are available, it is better if one stays at OPTOEL.

Fooke would like to receive a reader and the discs to start testing processes.

Prototyped discs, how we proceed:

First units: 3 chambers, downscale to 25 microliters: Sintef will send some discs for integration tests, assay evaluation on disc and the performances of the design, the results will be used also to test the reader. Sintef will also prepare some units with the complex design, to be able to test them before freezing.

1st November at latest is the deadline for freezing the disc design.

SINTEF: exposes the problem of the low level of resources (budget) to perform the activities of prototyping. The costs of milling and prototyping were not initially expected only for SINTEF: cannot infinitely extend the tasks, therefore it will be discussed how many prototypes will be able to prepare after freezing, for continuing with the integration activities.

Luis states that the most important goal: to have a good sequential loading system. Michal does not agree: if we need to simplify we should assure that the manual delivery design works.

Samples: this task will be finished in December.50 ml blood of at least 10 patients of highly specific IgE levels (until December)

Anca asks if it make sense to continue collection until M 41 (max)? Answer: Yes, samples collected even in the last months could be very useful for validation.

Ian exposes that Stratec's contribution to INNODECs is low. Teresa answers that INNODECS are mainly containing presentations to conferences or articles, and STRATEC is not presenting this type of public communications. Nevertheless, this should be confirmed by STRATEC (not currently at the meeting as Werner had to leave about 15:30h).

End of meeting: 18:10

Annex: signature sheets

COMPACT BIOPHOTONIC PLATFORM FOR
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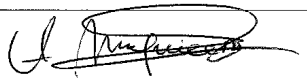
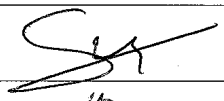
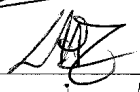
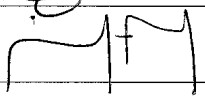
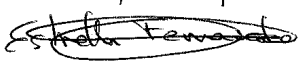
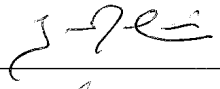
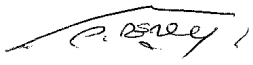
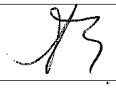
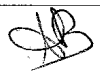
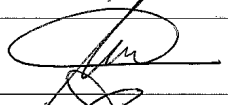
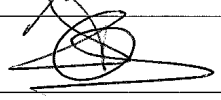
	Name & affiliation	Signature
1	Ángel Maquieira UPVLC	
2	Sergi Morais UPVLC	
3	Luís A. Tortajada UPVLC	
4	Teresa Molina UPVLC	
5	Estrella Fernández UPVLC	
6	Joao Correia B3D	
7	Eva Murauer CHRUM	
8	Pascal Demoly CHRUM	
9	Anca Chiriac CHRUM	
10	Sylvie Broussous CHRUM	
11	Audrey Boissin CHRUM	
12	Laetitia Ferrando CHRUM	
13	Ian A. McKay EUX	
14	Ethel Ibáñez HULAFE	
15	Dolores Hernández HULAFE	

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16	Elizaveta Vereshchagina SINTEF	
17	Michal Mielnik SINTEF	
18	Werner Balika STRATEC	
19	Sergio Peransí LUMENSIA	
20	Alfredo Saez LUMENSIA	
21	Margrit Fooke FOOKE	
22	Nadine Offermann FOOKE	
23	Linte OPTOEL	