Chips 4 Life

Rapid Microbiologic Diagnostics

FINAL REPORT

Grant Agreement number: **278720**

Project acronym: ***CHIPS4LIFE***

Project title: ***Development of multi-analyte diagnostic Tests***

Funding Scheme: **Collaborative project**

Date of latest version of Annex I against which the assessment will be made: **28/11/2014**

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4.1 Final publishable summary report

4.1.1 Executive summary

Respiratory tract infections (RTIs) are a huge burden in terms of mortality and morbidity worldwide. They are very common and represent the main reason for seeking medical care. The disease burden for Acute Respiratory Infections (ARI) is estimated at 94 037 000 DALYs (Disability Adjusted Life Years and 4.2 million deaths per year. Acute respiratory infections are among the leading causes of death in children under 5 years, mainly because of Pneumoniae. When referring to RTIs, it is important to distinguish between diseases of the upper and lower respiratory tract (URT and LRT). Acute LRTIs are a persistent and pervasive public health problem. They cause a greater burden of diseases worldwide than HIV, malaria, cancer or heart attacks. As estimated by the WHO, lower respiratory tract infections (LRTI), mainly pneumonia, should be one of the 4 leading causes of death in adults in 2030. In addition, the issues of co-infections and resistance to antibiotics stress the needs for a rapid, selective and reliable diagnostic tool.

The overall objective of this 3.5 years project was to develop a panel of dedicated rapid diagnostic tests based on a one-tube multi-parameter molecular amplification of viral and bacterial respiratory pathogens as well as resistance markers using classical broadly available thermocyclers followed by direct analysis on one-step microfluidic chips on a dedicated reader. C4L took advantage of the powerful multiplexing capacity of the MLPA technology to target pathogens and antibiotic resistance markers coupled to the microfluidic technology for reaching speed, sensitivity, specificity and robustness.

The *Chips 4 Life* project developed two multiplex diagnostic tests to rapidly detect the etiological agent(s) responsible(s) for a respiratory pathology, including presence of genes encoding antibiotic resistance markers and virulence factors. The first test takes advantage of the MLPA technology and detects the respiratory parameters of interest with a classical PCR equipment. The second test detects a limited number of pathogens (6 viruses and 2 bacteria) without the possibility to identify the resistance markers. Nevertheless this second test is performed in a microfluidic chip. This test is run in a specific instrument allowing PCR and fluorescence detection of amplified products. Another chip for FRET detection was developed, but is not yet involved in a one-device model pairing the PCR chip and the FRET chip. Because of the problems faced during the chip development, it was decided to move to a “classical” fluorescence detection in place of the FRET detection initially considered. On the other hand, other problems appeared during the validations, and finally only a limited number samples were tested in clinical settings (Antwerp and Liege). Nevertheless, despite the problems faced during the course of this project, the consortium has delivered several achievements:

- two microfludic chips: one for FRET detection and one for continuous flow PCR with OC detection
- two readers: one for FRET detection and one for specific Cy5 detection
- 6 publications, 1 PhD thesis and 11 posters and presentations.
- several recommendations following the clinical validations performed in clinical settings
- adding more targets to the chip
- improving the capability to identify more pathogens on multi-infected samples,
- adding resistance markers
- involving the DNA extraction step in the chip.

4.1.2 Summary description of project context and objectives

RTIs and especially LRTIs are a leading cause of morbidity and mortality from infectious diseases, even in industrialized countries; they represent a major area where improvements are critically needed. The C4L project was conceived due to the need for accurate microbiologic diagnosis, which is essential for patient care, to optimize therapy, for infection control and for antimicrobial resistance containment.

The overall objective of this 42-month project was therefore **to develop a panel of dedicated rapid diagnostic tests to allow medical staff to link antibiotic prescription to evidence-based diagnosis.** These tests will be based on a one-tube multi-parameter molecular amplification of viral and bacterial respiratory pathogens as well as resistance markers using classical broadly available thermocyclers followed by direct analysis on one-step microfluidic chips on a dedicated reader.

Four specific objectives to achieve this overall aim were set out:

Specific objective 1: to identify and select the molecular targets to include in the test, representative of the more significant bacterial and viral causes of RTIs in Europe, but also of genes representative of significant resistance mechanisms among specific bacterial species or coding for important virulence markers.
Specific Objective 2: to design the probes for all identified targets, to validate these probes with reference to real-time PCR and to develop a rapid multiplex amplification protocol to be further implemented on a microfluidic chips analysis tool.
Specific Objective 3: to develop the C4L diagnostic test to detect analytes related to pathogen identification, resistance to antibiotics, and severity of the infection using a simple-to-use, accurate microfluidic chip. Part of this objective is also to provide a flexible, generic microfluidic chip technology that can easily be adapted for specific requirements of various tests (sample volume, number of tests per chip, etc.) and that can be efficiently manufactured.
Specific Objective 4: to validate in clinical setting the analytical and robustness characteristics of the new developed test and system by comparison to routine and/or gold standard diagnosis methods.

To meet reach these objectives, the project was organized into 6 WPs.

WP1: Management of the project: Leader: Coris

The main objective of this work package is to set up an effective management framework for the C4L consortium, which will ensure the correct progress of the project towards its planned objectives.
• To set up an effective management framework for the C4L consortium
• To act as the interface between the C4L consortium and the European Commission.
• To ensure that all actions are performed correctly and within the rules and regulations established by the European Commission and in the Consortium Agreement including financial and legal management and to ensure that the received funds are correctly distributed and accounted for.
• To ensure the work and tasks are performed on time, within budget and to the highest quality and create anearly warning and advisory system, also for IP-related issues.
• To keep each partner, including the Commission, fully informed about the project status, scientific issues, the work planning (adjustments) and all other issues which are important and relevant to partners in order to obtain maximum transparency and achieve synergy of the cooperation; to ensure that all partners are informed of all important and impacting information that can influence the outcome of the project.

WP2: Target Identification and multiplex molecular amplification: Leader: Pathofinder

The aims of WP2 were to:

- design the primers and probes for the multiplex assay to detect a-typical and typical pathogens and their antibiotic resistance patterns.
- validate the multiplex assay, in house
- incorporate of the assay into the final detection format on-chip

WP3: Microfluidic Chip design, development &Production: Leader: IBM

The objectives of WP3 were set out as follows:
• To develop low-cost, disposable microfluidic chips for the rapid detection of pathogens and analytes;
• To provide a flexible, generic microfluidic chip technology that can easily be adapted for specific requirements of various tests (sample volume, number of tests per chip, etc.);
• To align the microfluidic technology with realistic medical context/workflow and manufacture.

WP4: detection System: Leader: Coris

The objectives of WP4 were established as follows:
• To evaluate the best fluorophores to be used for having a high signal/noise ratio on the microfluidic chip and control optical signals;
• To adapt the currently developed reader to microfluidic chips developed in WP3 both in terms of hardware (chips carrier, thermal control) and software (results analysis);
• To validate the detection system.

WP5: Validation: Leader CHU Liège

WP5 aimed to:

• Validate the multiplex diagnostic test developed during the project: pre-analytical conditions, analytical characteristics, clinical relevance and accuracy, and robustness.
• Set up a web-central database available to all the involved members of the team participating at the validation studies of the project. Safety of data will be regulated according to the European Directive of 24 October 1995 (95/46/CE) regarding protection and circulation of personal data. Data management will be periodically reviewed in occasion of the semi-annual progress report.
• Perform the statistical analysis of all collected or produced data and to evaluate the clinical benefits of the new the multiplex developed diagnostic test by comparison to high quality routine tests used for diagnosis of respiratory tract infection.

WP6: Dissemination and Exploitation: Leader: IT

The objectives of WP6 were to:

• ensure maximum of international visibility of projects results towards the medical community, stakeholders and general public about the strains causing Respiratory Tract Infections and the multiplex test developed in C4L;
• conduct a market survey with benchmark of the available products and propose an optimized commercialisation strategy;
• ensure an optimal IP monitoring and IP-rights adequate protection.

4.1.3 A description of the main S&T results/foregrounds (not exceeding 25 pages)

The main results for each WP and individual tasks are described in this section.

WP1 Management of the project Work description
WP1 and its participants have achieved the following:

- maintenance of an effective management framework for the C4L consortium, ensuring the progress of the project towards its planned objectives and adequate reporting;
- organisation and compilation of the second periodic report for the Commission using partner-customized templates, timelines and simplified guidelines to help all partners to report their individual contribution and use of resources into the project's programme of work. A quality control check was also done for all partners' financial reports;
- maintenance of an efficient interface between the consortium and the European Commission (e.g. procedure for reinforced monitoring at Month 6 for partner 6 PF);
- support for the organisation of the kick-off general meeting in Namur (Belgium) in January 2012, 6 month meeting in Jena (Germany), first annual meeting in Liege (Belgium) in January 2013, 18 month meeting in Zurich (Switzerland) in June 2013, Maastricht in December 2014, Brussels (Belgium) in September 2014, Brussels (Belgium), January 2015 and Liège (Belgium) in June 2015;
- creation and update of the corporate C4L communication tools (poster, website); first results presented on the updated poster at ECCMID international conferences in London, United Kingdom (2012) and Berlin, Germany (2013).
- updating internal intranet regularly;
- all actions were performed correctly and within the FP7 rules and regulations established by the European Commission and in the Consortium Agreement including financial and legal management and correct distribution and accounting of received funds;
- each partner and the European Commission were continuously informed about the project status, scientific issues, the work planning (adjustments) and all other issues which were important and relevant to partners in order to obtain maximum transparency and achieve synergy of the cooperation;
- all partners were informed of all important and impacting information (regulatory, ethical, business issues) that could influence the outcome of the project, notably via meetings and minutes thereof. A special effort was also made for promoting, the gender equality within the consortium.

Task 1a - Contractual, financial and regulatory affairs management

The compliance by all Beneficiaries to the consortium agreement and FP7 financial and GA rules was continuously monitored by the coordinator CORIS and the project manager IT. Financial plans and human resources (change of personnel) were carefully monitored. In addition, good operating practices were proposed to all partners thanks to a "Management Handbook" prepared by partner 3 which includes guidelines on FP7 financial rules, costs justifications, time sheet models etc. This Handbook remains available 24/7 on the private section of the C4L website ('extranet').

Partner 3 IT acted as a permanent helpdesk for all partners to find adequate solutions to their administrative issues which led occasionally to the preparation of amendment.

The C4L consortium agreement was signed by all partners before the start of the research activities.

IT and Coris coordinated the request for Amendment no. 1 to the GA which was finalised and accepted in December 2014. Partners were kept up to date on the amendment process and were consulted on the main changes. Following the amendment, the new annexes were made available to the partners on the intranet.

The main changes in the tasks and timeline were highlighted during the Executive Council meeting in January 2015.

As part of the amendment process, IT requested to subcontract the D6.2 Market survey benchmarking the competing products and mapping the market. IT requested 4 offers from consulting firms. The Paris-based consulting company was selected.

Financial follow-up was carefully monitored. Updates on spending were requested from each partner for the consortium meetings and more frequently via email during the extension period. This was particularly important in analysing the possibility of an extension and during the extension period to monitor and prevent overspending.

Human resources (change of personnel) were monitored and solutions were found as quickly as possible where a change of staff occurred.

Task 1.b: Knowledge / Information management

Knowledge and information management was managed smoothly.

Each participant was adequately informed about all progress achieved to date by the others, as well as the activity plan for the next period and timing. Knowledge management activities included key meetings with archived minutes and the set-up of a web-based C4L management platform. Due to the tight schedule during the last 6 months of the project, monthly teleconferences were set up to ensure adequate flow of information and timely achievement of tasks.

During all project meetings, all the partners involved in the project were informed of the progress of each research task, delay in the work programme, or pitfalls when detected.

Due to the relatively small size of the consortium, all participants were invited to attend the physical 6 month meetings. The Management Team (Partners 1 and 3) informed the consortium of any decision taken by the Executive Committee via emailing and the upload on the extranet of the **minutes of any meeting** organised by the Consortium to assess the network progresses. Minutes were validated by the participants before being circulated.

Each participant, from the PhD student to the senior scientific had his/her own login and password to the extranet for sharing information and SOPs with all partners. The partners were active users of the intranet service. This private section of the C4L website is the repository for all C4L documents restricted to consortium members such as: i) contract related documents (Grant Agreement and the latest version of Annexes, Consortium Agreement), financial plans, reports; ii) updated address book with contacts for all partners (including scientific and administrative contacts for each partner); iii) information about activities conducted by each group; iv) scientific databases, protocols and internet-based data management tools; v) communication rules and guidelines with C4L and FP7 logos; vi) ethical and regulatory documents; vii) Intellectual property rights related documents.

Task 1.c: Work plan definition

The Coordinator (Partner 1) and the WP leaders (Partners 2, 3, 5, 6) further detailed the work programme and the budget on a sub/task partner level, notably during the general meetings of the consortium. The WP leaders informed the partners of the work to be conducted and discussed the work programme with them at

every consortium meeting: immediate action items, expected delivery dates and risk management/rescue plans adjustments.

Partner 3 IT explained the EC financial rules and presented the detail of financial plans and reporting at the occasion of the Consortium meeting in order to facilitate the reporting.

Task 1.d: Periodic reporting

Partner 3 IT was responsible for periodic reports 1 and 2. Partner 3 IT presented the guidelines for periodic reporting at all the major meetings throughout the project. More detailed instructions were at the meetings in Brussels (September 2014, January 2015, Liège June 2015). In order to ensure a smooth process with all the partners, partner 3 IT launched the C4L activity and financial report by providing customised reporting templates to gather all necessary information. Continuous assistance and guidelines were provided to the scientific team for the activity report and to administrative officers in charge of compiling financial information, to facilitate the access to the FORCE platform (providing FORCE guidelines, answering to partners' questions). Partner 3 IT also performed a quality-check on the technical contributions, use of resources and Form Cs and Form Ds before assembling and editing the first periodic activity report. Partner 1 validated the reports before submission. Partner 3 IT made a quality-check for all the activity contribution and costs statements of all partners before assembling and editing the first periodic activity report.

Task 1.e: Intellectual Property management

The Consortium Agreement agreed upon by all C4L partners lays down the general rules for Intellectual Property (IP) protection and set frames for the discussion relating to IP sharing and exploitation. Partner 3 Patent attorney and DESCA specialist (Ms Denise Hirsch, Director of Intellectual Property Department) made a presentation on "Intellectual Property Issues in C4L (potential financial exploitation of samples or intellectual property)" during the first annual Meeting of the Consortium in January 2013 in Liege (Belgium). Partners were vigilant about requesting other partners' approval before inviting external individuals to meetings or publishing information. Relevant information was uploaded to the intranet. In the case of IBM's patent filed for the Orthogonal Flow Mixer, all partners were informed of IBM's intention in advance. IT ensured that MTI and ERDYN – subcontractors for the market study deliverable – signed confidentiality agreements before accessing project information. At the final meeting, the consortium discussed the importance of disseminating the results of the project. It was agreed that all partners' names will figure on future publications as long as they have contributed directly to the work which led to the publication.

WP2 Target Identification and multiplex molecular amplification

The main **S&T results of the WP2** are as follows:

1. Development of two multiparameter tests, based on the MultiFinder technology, consisting of (1) all relevant a-typical respiratory viruses and bacteria causing a respiratory tract infection and (2) all typical respiratory bacteria including the most relevant resistance patterns

Work performed and results obtained: oligonucleotides (primers and probes) were developed for (1) all relevant a-typical respiratory viruses and bacteria and (2) all typical respiratory bacterial and the most relevant resistance genes. The sequences of these oligonucleotides were described in Deliverables D2.1 and D2.2, respectively. These two sets of primers and probes were compiled in two multiplex assays (respectively assay (1) and (2)) which were described in Deliverable D2.4

2. Development of a fast amplification protocol

Work performed and results obtained: by extensive comparison of master mixes and research and optimization of reaction conditions, the total runtime of the multiparameter nucleic acid amplification was reduced from 5h30min to 3h30min.

3. Determining the analytical sensitivity of each target within the two multiparameter tests.

Work performed and results obtained: synthetic double-stranded DNAs were designed that represented native sequences of the resistance genes in the multiparameter assay (2). Dilution series of these synthetics DNAs, containing known copy numbers, were used to determine the analytical sensitivity for each target. For all resistance genes detected in assay (2), 100 copies per reaction were detected adequately.

4. Validating the specificity of the two multiparameter tests (for each probe)

Work performed and results obtained: for each of the targets detected in the multiparameter tests, a real-time PCR was developed of which the detection site coincided with that in the multiparameter tests. For the a-typical pathogens, a series of 700 samples were obtained from the University of Antwerp. These samples were screened with the developed assay for a-typical pathogens and by Real Time PCR as a reference. Overall, the sensitivity was 90% and the specificity was above 95%. For the typical pathogens, a well-characterized series of 10 samples was obtained from the University of Liège. The outcome of the multiparameter test matched with the description of the University of Liège.

WP3 Microfluidic Chip Design, Development & Production

WP3 deals with the development of the microfluidic chip based on inputs from WP2 and defines key characteristics that are needed for the development of the reader (WP4). During the first period, Tasks 3a-d were performed and led to four generations of designs of chips in silicon and polymer. The most advanced chips had a common inlet and outlet, 8 channels for reagents and analysis, and flow resistance structures for optimizing the flow distribution of liquid across the 8 channels. Preliminary work on the integration of reagents using inkjet technology has also been demonstrated.

By performing systematic studies on the filling of chips (at IBM and Coris in parallel) and dissolution and distribution of reagents in the channels, 2 major issues were identified:
- channels did not fill simultaneously and the amount of liquid passing through each channel varied, sometimes leading to the formation of air bubbles where channels merge toward the end outlet
- the dissolution of reagents was too fast and reagents accumulated in a very small volume of sample near the filling liquid front making their positioning in the reading areas of the chip virtually impossible.

These problems required major work under Task 3.e (Chip “troubleshooting”) for IBM and Microfluidic ChipShop, followed by extensive filling tests at IBM of chips produced at Microfluidic ChipShop (Task 3f “Production... and quality control”) and the introduction of an alternative assay implementation, which took advantage of oligochromatography (OC) tests from Coris. This later Task, 3g, was introduced by revising the workplan and was intensively worked out by Coris and Microfluidic ChipShop, who were able to combine PCR and OC functions in one polymeric chip.

Overall, 2 breakthroughs on novel microfluidic concepts (orthogonal flow mixers and synchronization junctions) were realized for dissolving homogeneously reagents in a sample and for filling channels reliably for both silicon and polymeric chips. The development of PCR + OC chips was successful as well and 200 polymer chips were supplied to support clinical testing in WP5.

Main S&T results are as follows:

WP3a – Design and fabrication of capillary systems for biological assays.

Due to problems encountered with filling and air bubbles and with the too strongly localized dissolution of DNA probes 3 new generations of chips (Gen 4, Gen 5, and Gen 5 revision 1) were designed with new functionalities. Gen 5 rev. 1 chips featured 2 new microfluidic functionalities: orthogonal flow mixers (OFMs) and synchronization junctions (see also Deliverables 3.3 and 3.4).

- OFMs solve a long-standing problem in microfluidics, specifically the dissolution of spotted reagents in channels without their strong local accumulation. This was done by filling OFMs with sample in an area not containing reagents followed by a brief, local flow of sample over spots of reagents. Using OFMs, the dissolution volume of reagents increased from ~100 nL to ~500 nL for each channel.

- Synchronization junctions used capillary valves and microfabricated rails to prevent liquids from parallel channels from exiting the channels prematurely and creating air bubbles. With these junctions, sample fills the 8 parallel channels one after each other but exit towards the outlet only when all channels had been filled.

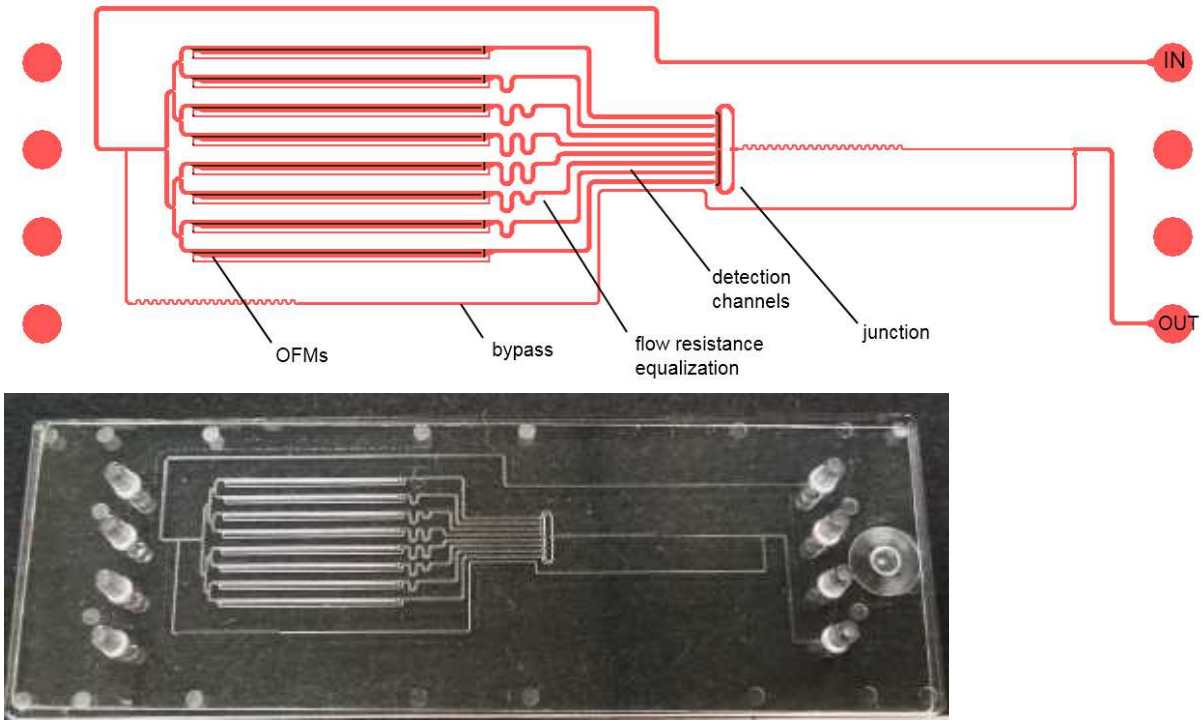


Figure 3.1. Layout of a Gen 5 chip and corresponding injection-molded polymeric chip. Such a chip has a footprint of 1 inch × 3 inch, needs as little as 11 microliters of sample and support a highly-multiplexed detection of DNA analytes owing to its 8 parallel channels that can hold different DNA probe combinations.

WP3b – Integration of assay components into microfluidic chips

The integration of DNA probes was performed using an inkjet spotter as reported during the first period report.

WP3c – Biofunctionalization of polymer surfaces

Active filling was selected and made surface functionalization non-critical. Chip lamination and sealing was done using the same method than during the first period.

WP3e – Chip troubleshooting based on test results

This task became essential in the second period due to the accumulation and too strong concentration of reagents during their dissolution in a sample and because many air bubbles were observed during testing of the chips and in WP4. The development of OFMs permitted a homogeneous dissolution of reagents in a micro-channel, see Figure 3.2. This was done iteratively using silicon and polymeric chips. Synchronization junctions were also developed for preventing liquid filling channels from creating air bubbles, Figure 3.3.

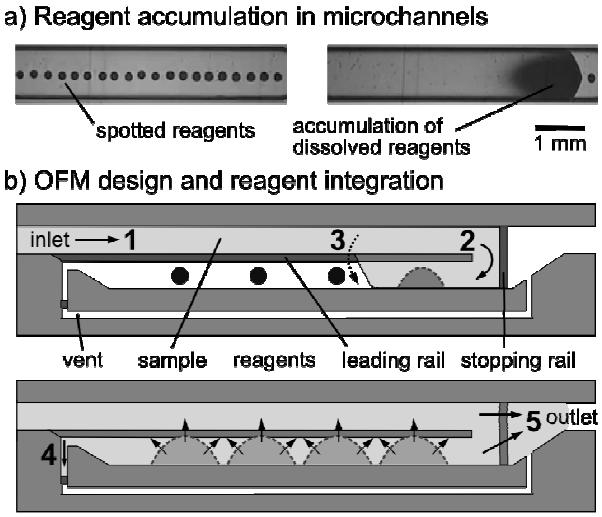


Figure 3.2. (a) The strong accumulation of reagents in a liquid filling a microfluidic device is solved using an OFM (b) wherein (1) incoming liquid is distributed parallel to spotted reagents using a leading rail, (2) liquid follows the stopping rail initiating an "orthogonal" flow (3) over the areas containing the reagents, (4) air leaves the OFM through a vent, and (5) liquid passes the stopping rail once the OFM is filled.

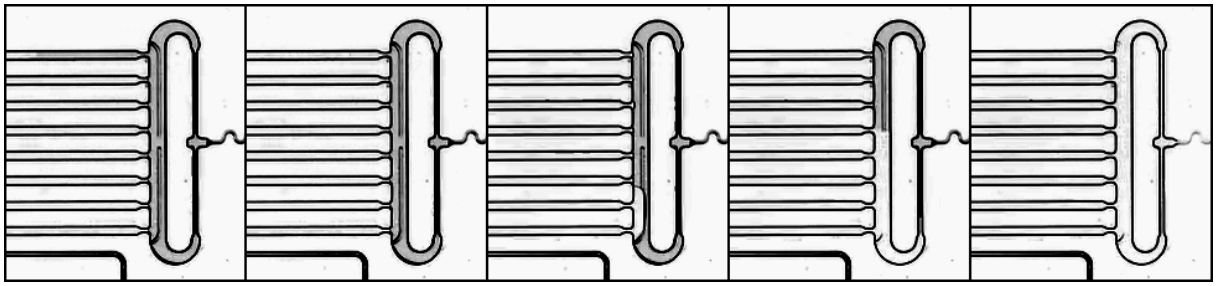


Figure 3.3. Time series images showing the filling of 8 parallel channels and the merging of the 8 streams of liquids at a synchronization junction. Areas filled with liquid appear lighter in the images.

WP3f – Production of microfluidic chips and quality control

Series of polymeric chips were produced using the various designs described above and the chips combining PCR with OC. Filling tests were conducted on chips using videos recorded with optical and fluorescence microscopes and with liquids having a similar chemical composition than samples for the diagnostic tests.

With Gen 5 rev. 1 chips, filling of chips and microfluidic structures reached nearly 100% success rate, see Table 3.1

Table 3.1: Characterization of the filling behavior of Gen. 5 Rev. 1 chips.

Feature	Number of trials	Number of successful fills
splitter group	7	7
OFM	56	55
synchronization junction	7	7
bypass	7	7
overall chip performance	7	7

The accuracy of producing the microfluidic structures during injection molding was assessed using scanning electron microscopy and cryogenic fracture of polymeric chips. Some chips were also prepared without lamination. These chips were coated with a few nanometer-thick layer of aluminium and inspected using optical interference microscopy, Figure 3.4. Dimensions and depths of structures in polymeric chips were almost exactly matching the designs.

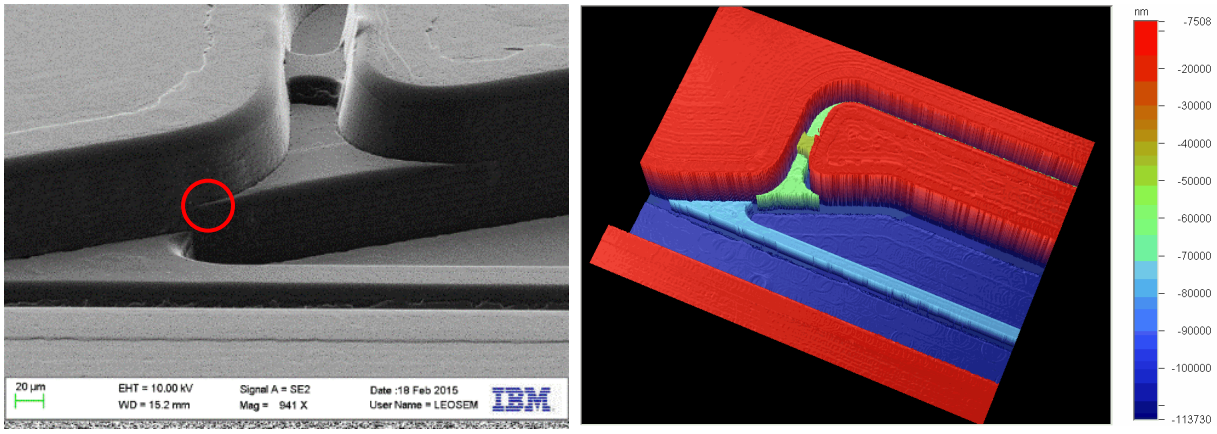
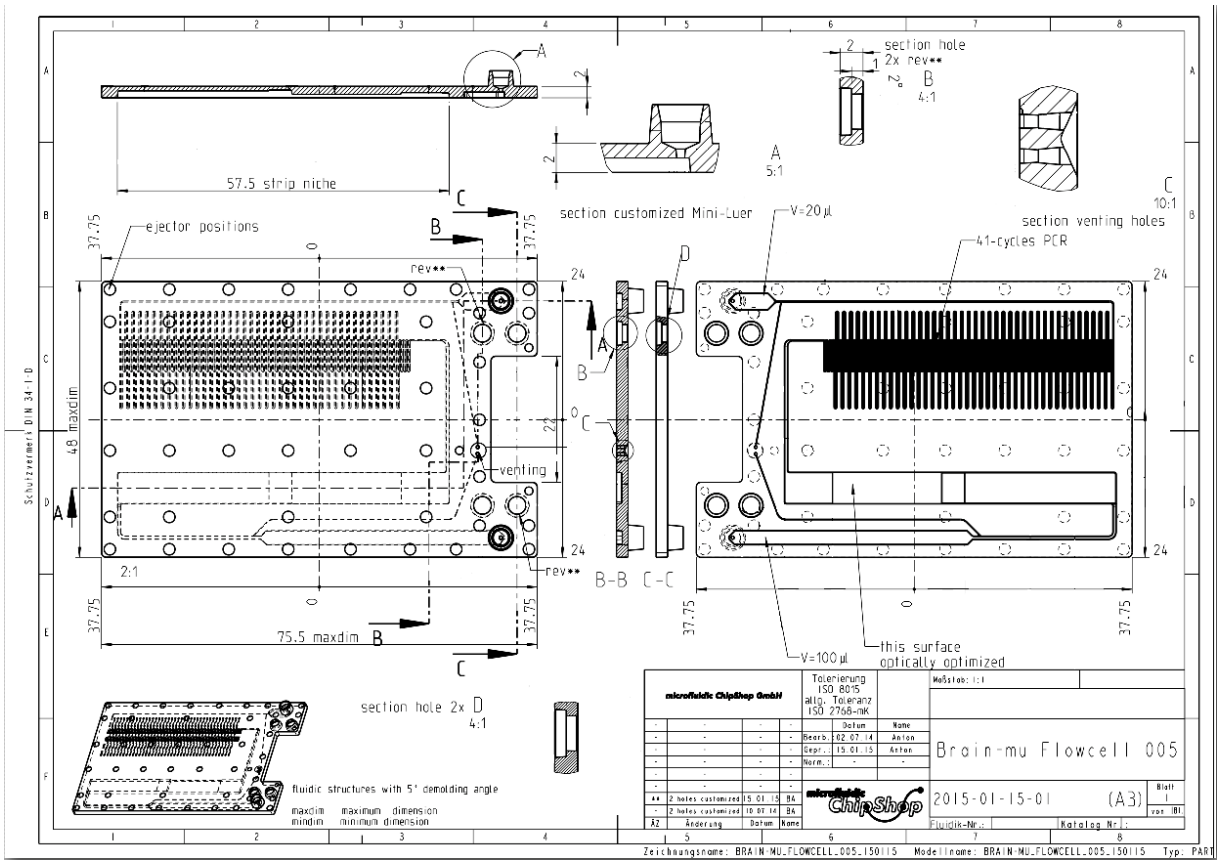


Figure 3.4. Metrology of polymeric chips using scanning electron microscopy (left) and optical interferometry (right) was invaluable in verifying the dimensions and depths of all structures reveals a protrusion at the vent entrance (red circle). The dimensions and heights of the structures in the OFM are otherwise perfectly implemented in the polymer chips.

WP3g – Production of microfluidic chips for PCR and OC on-chip

A chip combining PCR and OC was designed and produced in large series to support clinical validation (WP5). The PCR part of the chip comprises microfluidic loops for 41 PCR cycles and was combined with a plastic housing an OC strip test. The total footprint of this chip was made compatible with the reader and optical readout system developed in WP4. The layout of these chips and corresponding injection-molded polymeric chip are shown in Figure 3.5. A series of 200 chips was produced and two batches of 35 chips were sent to each clinical partner for validation.



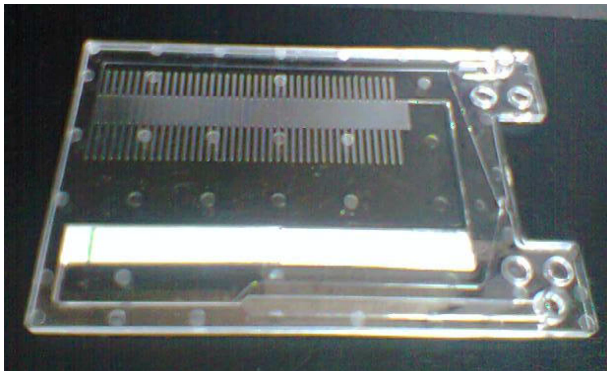


Figure 3.5. Layout of the PCR + OC chips for injection molding production and corresponding injection-molded polymeric chip.

The know-how developed is available to the research community and will evidently survived the C4L project because significant business opportunities have been realized for the production and commercialization of this technology.

WP4 Detection system

<u>Task 4a – Fluorophore evaluation and selection – Coris</u>

Plastic properties were studied especially for their autofluorescence and their tolerance to temperature from room temperature to 100°C.

All plastic chip tested were fine with low autofluorescence in the region of emission signals of the reporting dyes Cy5 and ROX after a FAM excitation. First generation of plastic chips was made with Cyclo olefin polymer (COP or Zeonor) based on their low autofluorescence properties and their high heat resistance.

Two fluorescence models were considered.

In the first model, a PCR product is labelled with FAM and probe is labelled with Cy5 or ROX as usually used by Pathofinder in their FRET experiment developments. The measured signal will be a decrease of fluorescence with de-annealing (relapse) of the probe.

The second model is adapted to the initial reader developed by Coris with Cy5 excitation and emission. In this case the PCR product would be labelled with Cy5 and the probe with a biotin. The measured signal will be an increase of fluorescence at the localisation of the probe spotted on the strip.

The first model was chosen for its best stability, precision in the quantification of a decrease of fluorescence rather than its appearance (second model).

Task 4b – Reader redesigning – Coris, CHULg

Work description and progress for the FRET reader (Prototype-V4)

For illumination, one white LED was used for focus and two different LED lights were tested for FAM excitation, a cyan LED and a blue LED (Figure 1).

(a) cyan LED (b) blue LED

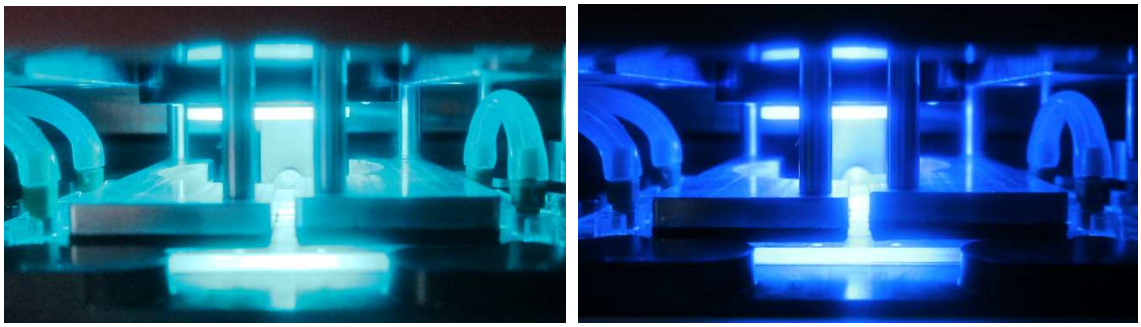


Figure 1: selection of two different LEDs for FAM fluorophore excitation, (a) cyan LED, (b) blue LED

Best fluorescent signal were obtained with the blue LED, as shown in Figure 2.

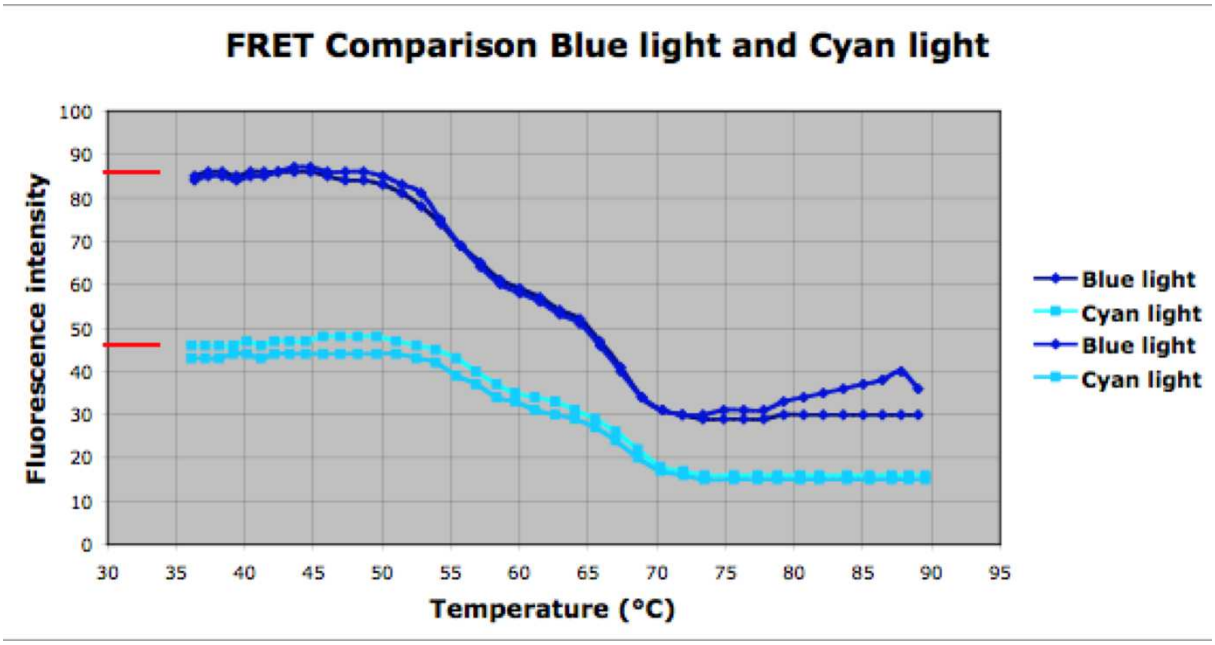


Figure 2: FRET intensity signal of FAM/ROX probes under blue or cyan illumination.

The intensity of FRET signal (ROX emission) was higher when the FAM fluorophore was excited by the blue light than by the cyan light. Although the background signal is a little bit higher with the blue light, the delta of fluorescence (signal-background) was the best with the blue light.

The time of exposure was also optimized to get the best fluorescence signal and the lower background and photobleaching of the dye. A time of 3000 ms was selected for the following experiments (Figure 3).

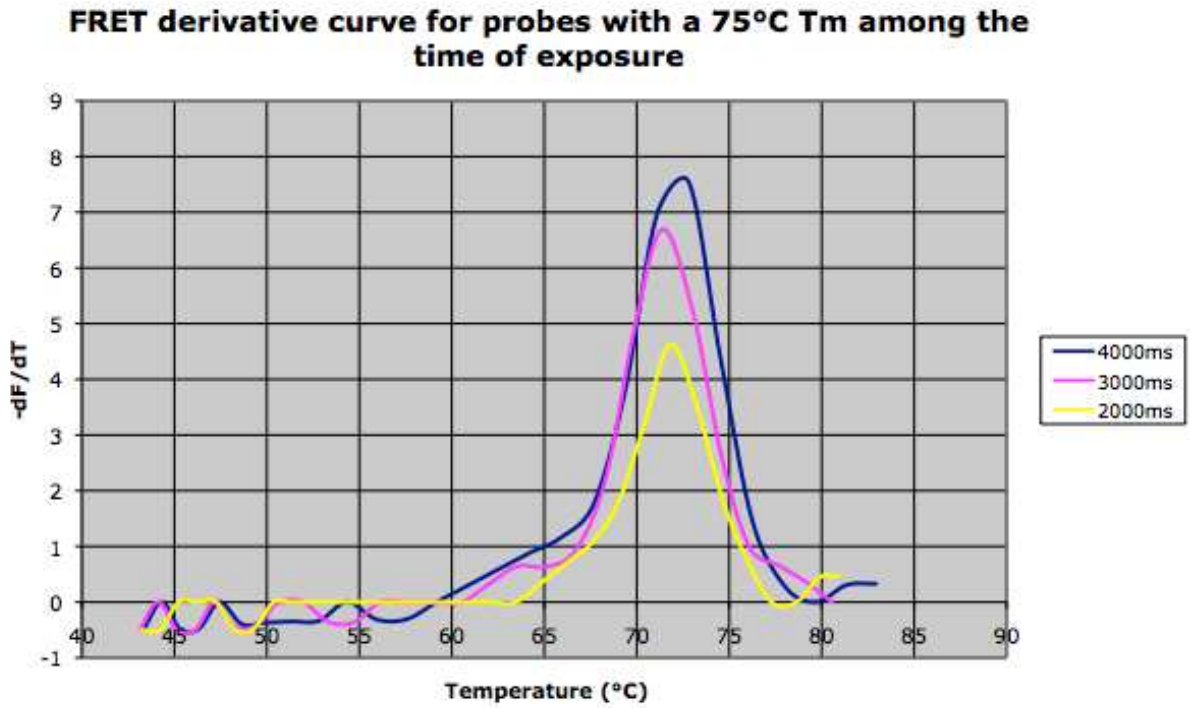


Figure 3: Comparison of the derivative FRET fluorescence curves at different time of exposure.

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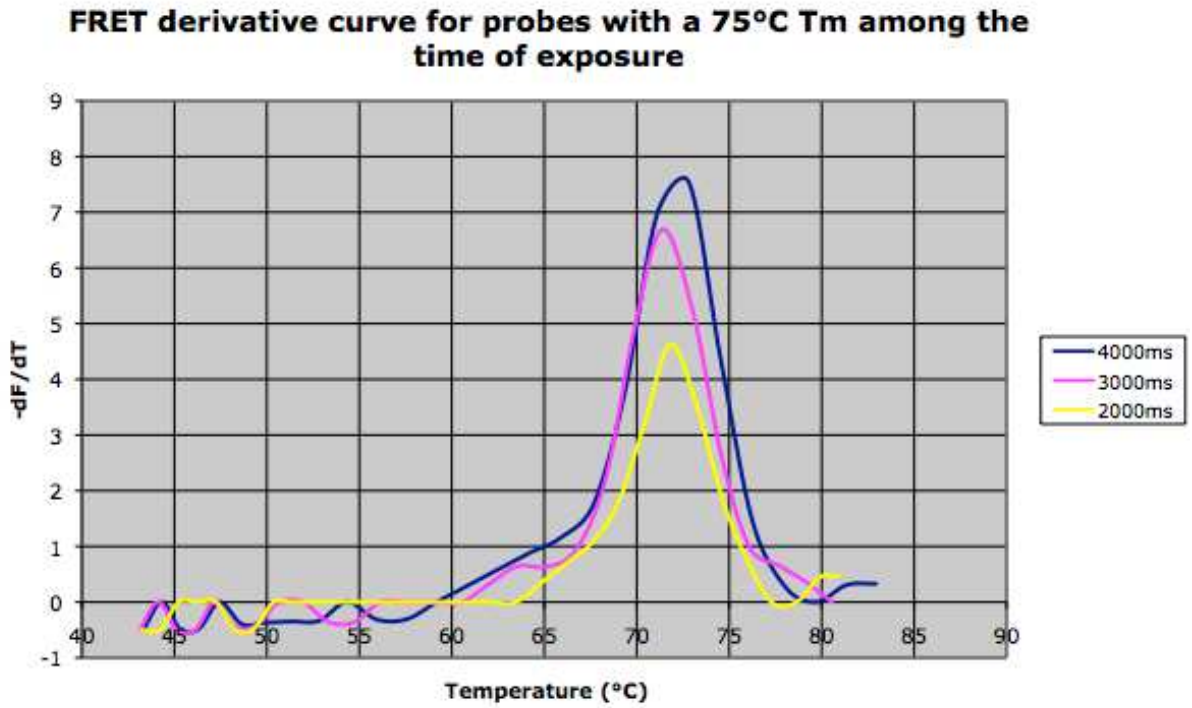


4 independent heating zones

Zone 1, 2 and 3 for PCR on chip
Zone 4: C4L detection chip

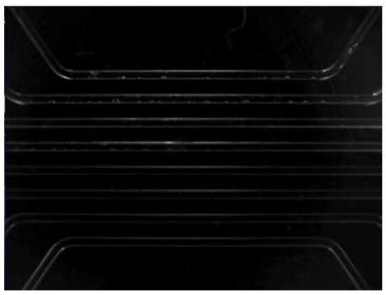
Figure 4: double slide size heating stage

The temperature ramping speed for the FRET melt curves was evaluated. The curves were perfectly superimposed whatever the ramping speed (5°C/min or 8°C/min or 10°C/min). (Data not shown). A ramping of 10°/min was chosen to reduce the total testing time.

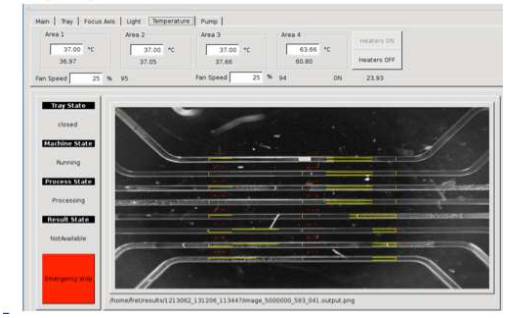
The software was adapted to quantify the fluorescence intensity in the 8 channels of the C4L FRET chip. A grid of quantification was applied on the detection zone of the chip.

Main steps for a FRET test:

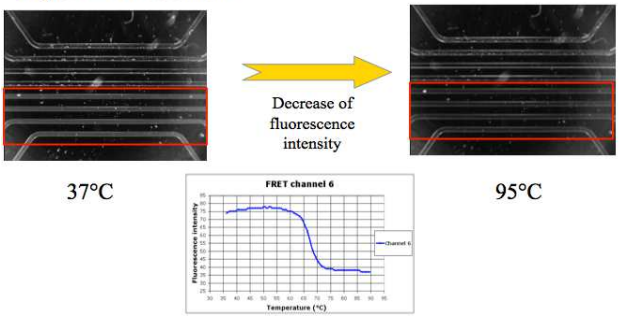
Step 1: Focus on detection zone 4 with white light



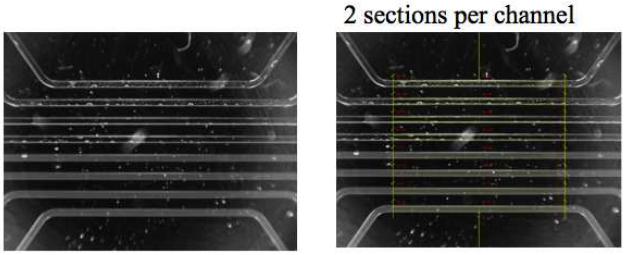
Step 2: Sequential programing of temperature and speed of the pump



Step 3: FRET detection



Step 4: Quantification of fluorescence



The best results were obtained with a mix of FAM/ROX probes with a 10°C melting temperature difference.

The analysis software was implemented in the FRET reader in order to smooth the raw data and to give the best interpretation of the test.

The result of the fitting analysis is presented in Figure 5.

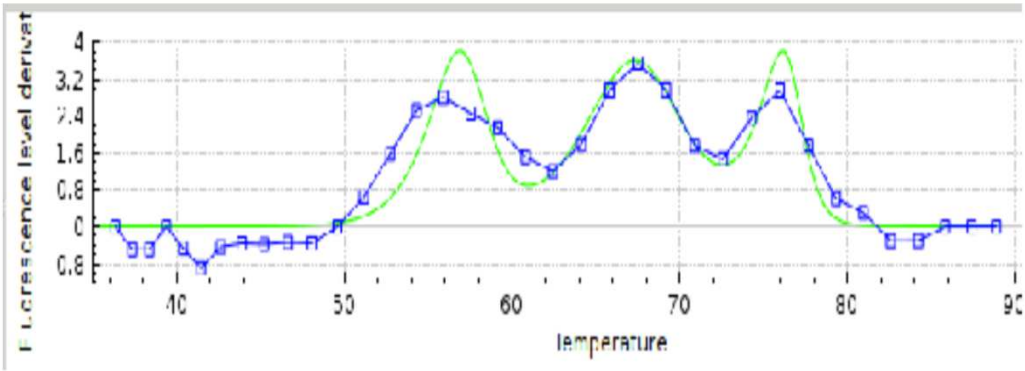


Fig 5: Fitting of the FRET melt curves of 3 FAM/ROX probes pairs (Tm 50, 60 and 70°C)

Blue line: raw data of the derivative of fluorescent melts curves

Green line: fitting curves of the derivative of fluorescent melts curves

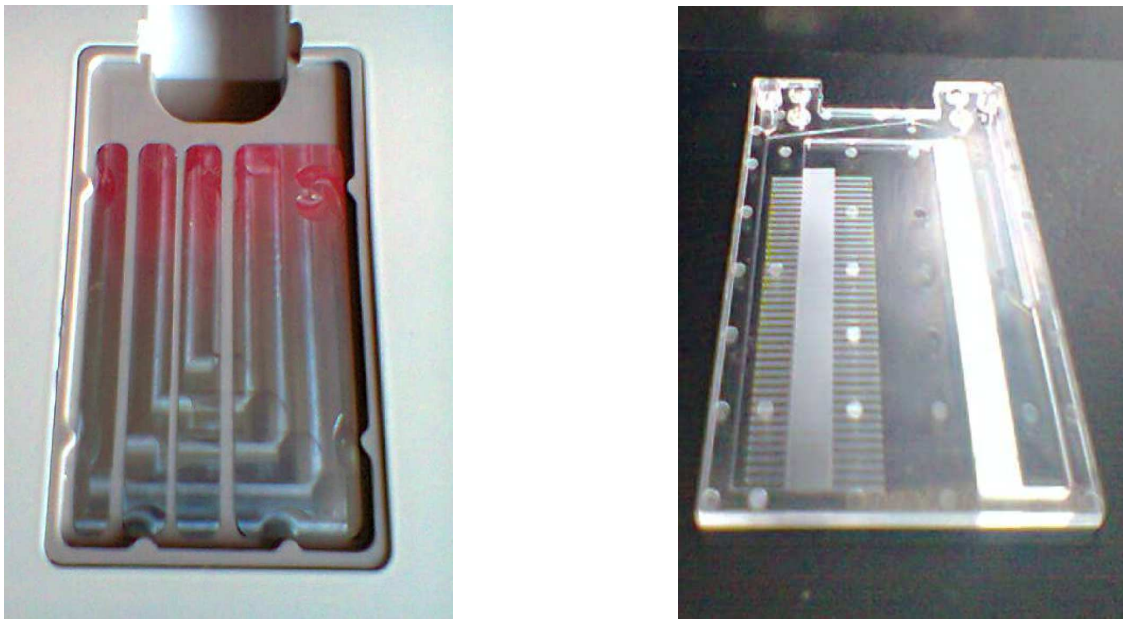
Work description and progress for Cy5 Reader (prototype-V5):

The Prototype-V5 (Cy5) reader was already under development at Coris (in collaboration with WoW technology) on the time the decision was taken to move to the continuous flow PCR/OC detection technology for finalizing tests to be performed during the validation study.

The source of the Cy5 light was not changed.

However, the size of the heating stage, in Figure 6, (4 heating zones, Z1, Z2 and Z3 for PCR on-chip, Z4 for OC detection) was adapted in the C4L project to the standard-plate combining the continuous flow PCR to the oligochromatography strip according to the specification of Microfluidic ChipShop [Deliverable D3.4] and to the new peristaltic pump system developed by Coris.

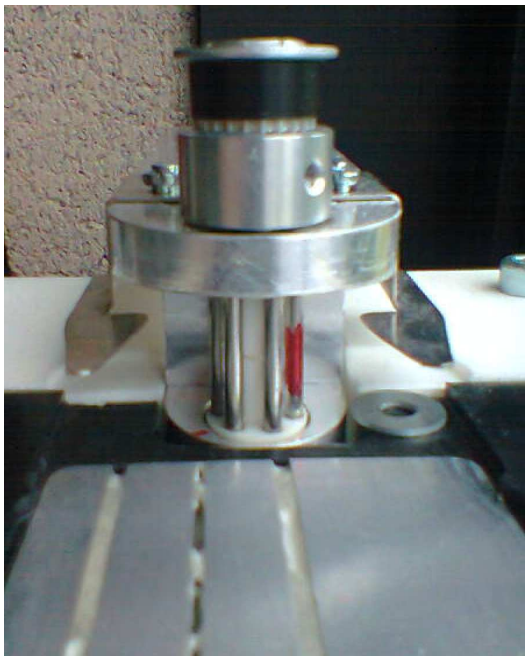
Connection to the peristaltic pump



(a) Heating stage **(b) PCR/OC chip**

Figure 6: reduced size of the heating stage (a) to the dimensions of the PCR/OC chip (b) and new design for a connection to the peristaltic pump

The model of the peristaltic pump was changed to reduce the length of the tubing between the chip and the pump by adding a pump head to the chip. In the same time, the number of rollers was increased from 4 rollers to 6 to allow a more constant flow speed. Figure 7 shows both versions of the peristaltic pump.



(a) **(b)**

Figure 7: (a) 4 rollers peristaltic pump link to the Gen 3 C4L chip, (b) 6 rollers peristaltic pump included in the Prototype-V5 Cy5 reader for clinical validation

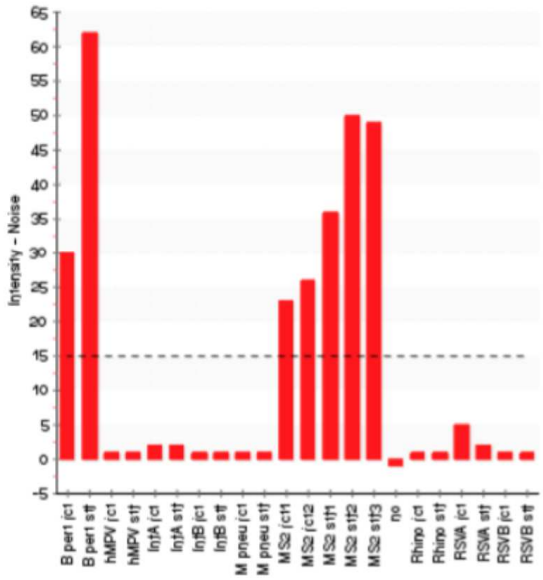
A specific program for the C4L application was created in the analysis software. The data were reported on two different reports, a general report, Figure 8 and a detailed report, Figure 9.

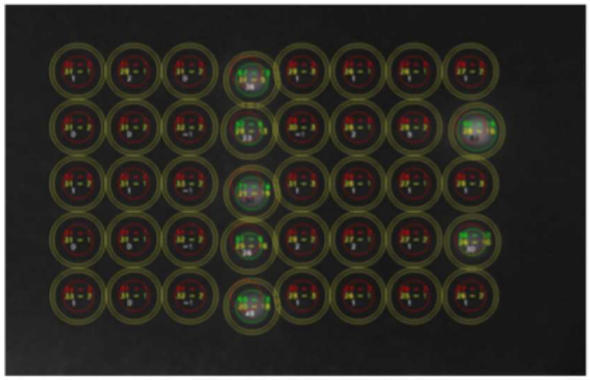
Operator	Chip #	Mix PCR #	Date	Sample Code
Root	201505272	201505272	2015-05-27 13:16:27	201505272

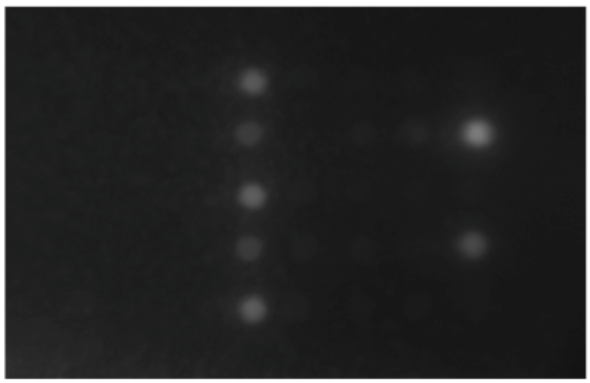
Name	Gene	Status	Validity
Control Amplification	MS2 stf1	Positive	Valid test
Control Amplification	MS2 stf2	Positive	Valid test
Control Amplification	MS2 stf3	Positive	Valid test
Bordetella pertussis	B pert jct	Positive	Valid test
Bordetella pertussis	B pert stf	Positive	Valid test
human Metapneumovirus	hMPV jct	Negative	Valid test
human Metapneumovirus	hMPV stf	Negative	Valid test
Influenza A virus	InfA jct	Negative	Valid test
Influenza A virus	InfA stf	Negative	Valid test
Influenza B virus	InfB jct	Negative	Valid test
Influenza B virus	InfB stf	Negative	Valid test
Mycoplasma pneumoniae	M pneu jct	Negative	Valid test
Mycoplasma pneumoniae	M pneu stf	Negative	Valid test
Control Amplification	MS2 jct1	Positive	Valid test
Control Amplification	MS2 jct2	Positive	Valid test
	no	Negative	Valid test
Rhinovirus	Rhino jct	Negative	Valid test
Rhinovirus	Rhino stf	Negative	Valid test
Rhinosyncitial A virus	RSVA jct	Negative	Valid test
Rhinosyncitial A virus	RSVA stf	Negative	Valid test
Rhinosyncitial B virus	RSVB jct	Negative	Valid test
Rhinosyncitial B virus	RSVB stf	Negative	Valid test

Figure 8: general report of the test; identification of the positive samples and the MS2 (amplification controls)

Operator	Chip #	Mix PCR #	Date	Sample Code
Root	201505272	201505272	2015-05-27 13:16:27	201505272







Position	Name	Int. Mean	Int. STD	Noise Mean	Noise STD
1-1	CP	32	2	31	1
1-2	no	31	1	29	1
1-3	no	31	2	31	2
1-4	MS2 stf1	67	13	31	5
1-5	Inf B jct	30	2	29	3
1-6	Rhino stf	27	2	26	1
1-7	RSVB jct	27	2	26	1
1-8	CP	28	2	27	2
2-1	no	31	1	31	2
2-2	no	31	1	31	2
2-3	no	31	2	32	2
2-4	MS2 jct1	52	6	29	13
2-5	no	29	1	30	3
2-6	Inf A jct	30	2	28	1
2-7	RSVA jct	34	3	29	2
2-8	B pert stf	90	19	28	16
3-1	no	32	2	31	2
3-2	no	32	2	31	1
3-3	no	32	2	33	2
3-4	MS2 stf2	71	14	21	19
3-5	hMPV jct	32	2	31	3
3-6	Rhino jct	29	1	28	1
3-7	RSVB stf	28	1	27	1
3-8	M pneu jct	30	1	29	3
4-1	no	31	1	31	1
4-2	no	31	1	31	1
4-3	no	31	2	32	2
4-4	MS2 jct2	51	6	25	16
4-5	Inf A stf	31	2	29	2
4-6	RSVA stf	29	2	27	1
4-7	CP	28	1	27	2
4-8	B pert jct	56	10	26	10
5-1	CP	34	2	33	2
5-2	no	31	1	31	1
5-3	no	31	1	32	2
5-4	MS2 stf3	69	13	20	18
5-5	hMPV stf	30	2	29	3
5-6	Inf B stf	27	2	26	1
5-7	M pneu stf	26	2	25	1
5-8	CP	27	1	26	2

Figure 9: detailed report of the test; histogram of the positive fluorescence peaks (MS2 controls and positive target detected), pictures of the fluorescent spots with or without the grid of quantification, detailed table of the fluorescence values (spots and background).

Task 4c – Validation of microfluidic chip reader

Validation of the FRET Reader (Prototype-V4):

The illumination (fluorescent light) and the temperature of the heating stage were evaluated for their homogeneity and stability.

The same FRET probes were filled in several channels of the FRET chip and fluorescence was quantified in the different channels for each segment of quantification (2 per channel). The homogeneity of the FRET signal is shown in Figure 10.

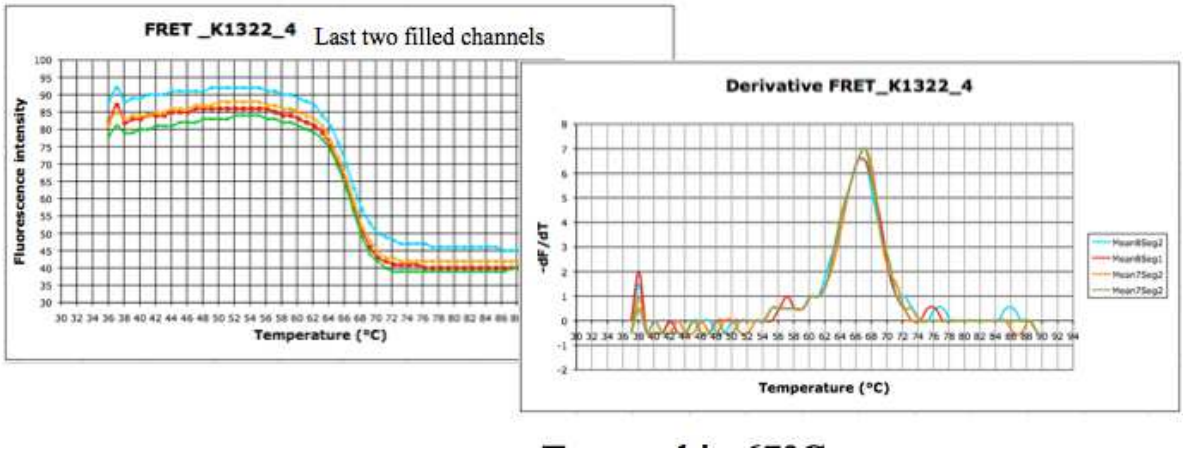


Figure 10: Homogeneity of the fluorescent signal/section channels; FRET melt curves and derivative melt curves for probes FAM/ROX with a Tm 60°C.

Task 4c – Validation of microfluidic chip reader

Validation of the FRET Reader (Prototype-V4):

The illumination (fluorescent light) and the temperature of the heating stage were evaluated for their homogeneity and stability.

The same FRET probes were filled in several channels of the FRET chip and fluorescence was quantified in the different channels for each segment of quantification (2 per channel). The homogeneity of the FRET signal is shown in Figure 10.

Temperature rises in the heating stage during the melt curve step is perfectly linear

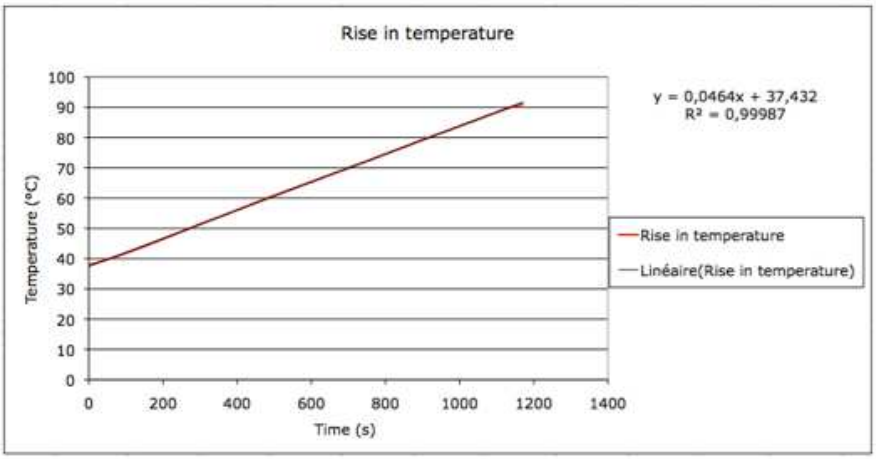


Figure 11: Temperatures measured during the ramping of the heating stage from 37°C to 90°C.

Validation of the Cy5 Reader (Prototype V-5):

The Prototype-V5 (cy5) reader was validated on low and high concentration of QCMD samples (DNA) for the 8 selected pathogens and on 10 negative samples. All the amplified PCR products were specifically detected by their appropriate probe on the OC strip. Sensitivity of the system has been partially evaluated in WP5 by the clinical partners.

Two Prototype-V5 (Cy5) readers were respectively placed at the CHU Liège and at the UZ Antwerpen for the clinical validation.

WP5 Clinical validation

WP5 was organised into 4 tasks:

The S&T results are described below for each task is described below

Task 5.a Collecting and testing samples

CHULg has collected and stored aliquots of 250 broncho-alveolar lavages (BAL), 250 naso-pharyngeal aspirations (NAPH), 150 tracheo-bronchial aspirates and 121 sputa. The distribution of bacterial pathogens among those samples is detailed in the tables below (5a-1: Typical bacteria). For virus, detection is not always requested by physician and for most of the tested specimens, they were mainly tested by antigen detection; a few samples were submitted to routine PCR methods. Among the tested samples, they were positive for influenza (N=4), RSV (N=79), parainfluenza (N=24) and human metapneumovirus (N=7). None was tested for rhinovirus. Pathofinders RespiFinder® 2SMART assay was performed on 28 samples that were routine positive for at least one target, on 232 routine negative or not routinely tested samples. Out of these, 175 virus and 7 atypical bacteria were detected by the RespiFinder 2SMART test. Out of these, a subset of 75 specimens was further tested with the C4L prototype. Results are presented in the table 5a-2.

Table 5a-1 Number of cultivated specimens (LBA, Tracheobronchial aspirate and sputum) positive for main typical bacteria (N = 521)

Task 5b Case report forms
The main results of this task were as follows:

The inventory of relevant data to collect for further analysis including demographic, clinical and biological data was discussed and approved by both partners CHULg and UA, involved in the clinical validation and collection of specimens and data. Partner UA has a specific expertise in this type of study related to respiratory tract infections. UA's contribution was invaluable. The content of the case report form is approved and its layout is in the process of being finalized. Shortly after beginning of period 2, the layout was finalized and available to fill in with demographic, clinical and biological data. Data for each collected specimen were recorded in these CRFs before capture in the database.

Task 5c - Database generation

The main results of this task were as follows:
The production version of a secured database was delivered by LambdaPlus in 2014. Shortly after starting the filling of the database, it was revised to improve the filling workflow. All demographic, clinical and biological data regarding all the samples collected in Liege were filled in from November 2014 until June 2015.

Task 5.d Statistical analysis and clinical relevance

The main results of this task were as follows:

In order to perform the statistical analysis, we had several meetings and discussion with the team of biostatisticians from CHULg. A statistical analysis plan (SAP) was set up. The SAP is structured in different chapters including the participants, a list of abbreviations, an introduction to present the project C4L, th primary and secondary objectives, the study design, the description of the patient's CRF and all parameters, the description of laboratory data, a description of the analysis subsets and the statistical methods of analysis (general principles, subject accountability, comparison of results, etc.).

By the end of June 2015, we have exported the data available from the C4L database and initial descriptive statistics have been performed. Further analysis will be dedicated to the comparison of C4L test with the gold standard and routine methods. As soon as all the data from Liege and Antwerp will be merged, further detailed analysis will be done in stratified groups to show in which groups of patients and/or type of specimens the C4L assay would be the more beneficial or in other words to identify the best clinical niches for the developed syndromic approach for diagnostics of respiratory tract infections.

Real-time graphs and statistics are available for the distribution of types of specimens and pathogens.

For the C4L prototype including 6 viral targets and 2 atypical bacterial targets, the calculated sensitivity of C4L compared with RespiFinder is 82% for specimens tested in Liege (BAL and NAPH collected from adults and children) and 84.5% for specimens tested in Antwerp (NAPH collected from children). The lowest sensitivity of C4L test is for rhinovirus and RSV B, and was observed mainly in case of co-infections.

Further statistical analysis per type of specimen within defined groups of patients will be more relevant. The statistical analysis is currently ongoing with the biostatistician's team of CHULg.

WP6 Dissemination and exploitation

Task 6.a Dissemination of Knowledge and raising awareness

The **main S&T results** of this task were as follows:

First communication rules and Standard Operating Procedures were agreed by all partners at the start of the project. They included for instance modus operandi for publishing quickly without hindering the chance of protecting Intellectual Property, or rules for acknowledging the EC grant support in publications resulted from the funded activities. Communication tools were developed for ensuring a better efficient and professional communication process: creation of the consortium's logo and graphical chart, a leaflet and presentation poster. The presentation poster was presented during the ECCMID conference in London in April 2012 and its updated version was presented during the ECCMID conference in Berlin (April 2013). These leaflets and poster remained available on the C4L website in their electronic version.

In addition, batches of the leaflet were distributed to every partner for their own local communication and networking on the project.

The C4L public website was regularly updated with new publications, participations to ECCMID conferences, announcement of C4L meetings. The private section of the website was also regularly updated with latest versions of Grant Agreement, Technical Annex, and any additional document useful to share between partners.

Finally, to ensure an optimal communication between partners, the consortium's address book was regularly updated and has remained available on the private section of the website 24/7.

The C4L public website was regularly updated with new project information and news on events. The private section of the website was also regularly updated with latest versions of Grant Agreement, Technical Annex, and any additional document that was useful to share between partners. Finally, to ensure an optimal communication between partners, the consortium's address book was regularly updated and has remained available on the private section of the website 24/7. All partners were active in disseminating knowledge about the project and raising awareness. The full details of such activities are included in the dissemination table online.

Task 6b Market study

A complete market study was produced. This study provided Coris with valuable information on the current market through a landscape of competitors and technology. Information was also gathered on clinician's expectations for such a test in 5 main target countries (Italy, Spain, France, UK, Germany), as well as the perspective of several distributors. Recommendations on the commercialization strategy and pricing were also put forward.

Task 6c Chips for Life workshop

The final workshop was held at the CHU Liège on the 29th of June 2015 and was co-organised by Inserm Transfert and CHU Liège. The aim of the C4L workshop was to present the results of the project to a vast

range of professionals. The consortium particularly sought to share their experience of the development of the test by explaining the methods used, challenges encountered and how they were overcome. The event aimed to reach out to professionals and students in the fields of microfluidics, molecular biology and microbiological diagnosis. 35 participants were enrolled in the workshop and 33 attended of which 17 from PMEs/companies, 6 from the academic sector and 9 from medical institutions. The EC was also represented by the C4L project officer, Patricia Paukovitz. The majority of participants were from Belgium, with the exception of the partners' representatives and one participant from the University of Auckland. Feedback from both the participants and the consortium was positive.

4.1.4 Potential impact and main dissemination activities and the exploitation of results

IMPACT

Due to its multidisciplinary nature and by bringing together companies, SMEs and end-users, the potential impact of the project can be summarized as follows.

Important impacts will directly result from this project:
- *Impact on public health* – The first impact will concern public health since effective treatment of bacterial infections is increasingly problematic due to the increase incidence of infections caused by pathogens resistant to antibiotics. Much evidence supports the fact that the total consumption of antibiotics is the critical factor in resistance selection. Microbiological tests are still often followed by bacterial identification tests and antibiotic susceptibility. The C4L test will enable users to distinguish between a viral and a bacterial infection within a few hours leading to more accurate treatments when necessary.

The appropriate decision regarding the cause of an infection will lower the antibiotic pressure and will give less chance to a resistance to emerge and also will have a significant impact on the social security/health expenditures since less antibiotic should be prescribed and stays in hospital won't be prolonged anymore. On the other hand, such a rapid identification of resistance will lower the long-term infected people living in the community and thus lead to less exposure of the general population to the risk of contracting a resistant strain of infection. An overall decrease in antibiotic consumption in the hospitals will also have a positive impact on reducing infections which are linked to antibiotic consumption such as *Clostridium difficile* infections.

The results of the C4L project have shown that the implementation of such a test should improve
- the Time-to-Result from collection to bedside and the detection of viral pathogens or a-typical bacteria.
- The management of patients suffering of LRTI, resulting in a decrease of morbidity and mortality and to reduce the inappropriate use of antibiotics aiming to prevent/control emerging resistances to antimicrobial agents, a true threat in the XXIst century.

- *Impact for diagnostic labs* – The final output of the project includes 2 multiplex assays for the detection of respiratory tract infections and their resistance markers. To our knowledge this will be the first (commercially available) assay that combines screening for both a-typical respiratory pathogens with typical pathogens and their resistance markers. The fast protocol, achieved in this project, allows comprehensive screening for all pathogens within the workflow of a normal working day, which enhances the clinical impact of the project. The generic technology developed in this project is also widely applicable as a screenings tool for infectious diseases.

For the clinicians involved in the project, even with the developed prototype limited to 8 of the expected targets, we have already demonstrated the advantages of performing this multiplexed assay when compared to routine methods performed on demand.

The C4L project provided test reader equipment to the clinical laboratories, which is capable of identifying the pathogens present in a respiratory specimen of a patient suffering from LRTI. Two Prototype-V5 readers were manufactured with all the functionalities needed for the C4L project (heating stage for continuous flow PCR and oligochromatography detection, high potential of multiplexing, low turn-around-time, high sensitivity and specificity). The Prototype-V5 readers perform the last PCR amplification of the 2SMARTFinder technology in a completely closed device, combining a continuous flow PCR to detection by oligochromatography, these two combined steps being performed within one hour. An important outcome is the potential of clinical applications as multiplexing and speed are two important parameters requested by the diagnostic laboratories.

The final result will therefore offer a new tool and technological approach to highly improve the turn around time (TAT) always critical in diagnostic. Such a reduction of TAT, skipping cultures to provide the first useful results, will improve management of ill infected patients. The panel of targets selected in this project will enlarge the usual panel of analysis routinely performed on respiratory tract specimens. As recommended in the market study, Coris BioConcept will also examine the possibility of offering different types of panel depending on the laboratory 1) reduced paned for low-throughput labs 2) large panel for high throughput labs.

The test offers to many labs the access to technologies and equipments currently reserved to research labs. The technologies and tools available at the end of the project will widen the diagnostic's horizon in general and will allow to go further to the expected dreamed theranostic approach. The validation process on an extensive sample of patients suffering of RTI will provide an opportunity to analyse invaluable data in a large epidemiological study including at the same time all the included targets of the project.

- *Impact on SME landscape* - The development of this multiplex test will also have an economic impact on the SME-landscape in Europe. Through this European Collaborative project, the 3 SMEs that are part of the consortium benefitted from EC support to help them bring a product to the market. The input of each partner will be taken into account in the Intellectual Property talks. European SMEs are the very heart of the economy of the European Union area; they contribute to more than 50 % of the total value-added created by business in the EU and employ 2 third of the employees of the private sector.1

Chips for Life will stimulate the dynamic activity of the field by bringing together several SMEs from different Member States and setting the appropriate frame for a successful collaboration. The consequences of the EC support to this project and to its SMEs will be multiplied by the economic activity of these SMEs.

The concrete economic benefit expected from each SME involved in the project can be summarized as follows:

Coris Bioconcept: Coris BioConcept will also take advantage of the project's deliverables: the present application with this new technological platform will allow CORIS to be positioned in a new growing diagnostic area. In addition, new products are foreseen using this new technological microfluidic platform. Several pathologies and/or physiological parameters need to be identified in a multiplex way to give the medical specialist more accurate answers and, consequently, improved and more specific patient treatments (theranostics or companion-diagnostics are two "new" applications of interest in the diagnostic field). The developed platform and the applications that will be developed on it will give CORIS a major advantage in this competitive market.

MFCS: As an example, the exploitation and the impact for MFCS will be realized on two levels. The first level is the direct transfer of manufacturing technologies and methods developed during the project to the range of MFCS's catalogue products, thus extending and improving the competitiveness of MFCS in the microfluidic component market. The second level of exploitation will take place in the product development phase after project termination where MFCS' plans to act as a polymer component supplier. CHIPSHOP has considerable expertise and capabilities for high volume polymer disposable manufacturing and this role is well aligned with MFCS's general business strategy to act as an OEM supplier of polymer microfluidic components for e.g. thediagnostic industry.

Pathofinder: the primer/probe design process has revealed the true diversity of resistance markers. This knowledge was used to improve the product design protocol at PathoFinder's laboratories. Further research is likely to be necessary to keep up with the continuous emerging of novel resistance genes/mutations. Commercial exploitation of R&D results: the primers and probes designed for this project can be used in diagnostic assays for the detection of atypical pathogens, and the detection of typical pathogens and their resistance markers. These assays are versatile in that they can be used in combination with many different nucleic acid extraction and detection platforms. This would allow implementation of the assays in different clinical microbiology laboratories. The assays would be based on PathoFinder's patented 2SMART method. To allow full development and commercialization, there is still need for design optimization, establishment of CE-IVD documentation, and internal and external clinical validation. Use of these assays would allow rapid and accurate diagnosis of respiratory infections, and would improve antibiotic treatment decision making.

- Impact related to *technological innovation* – Innovation is of obvious importance for SMEs and is also of great importance for a large company such as IBM because it creates new paths for its technologies and knowhow, new markets that are adjacent to its core business, and the necessary network of partners for co-developing, validating new technology and channeling it to the markets. In this context, the IBM microfluidic technology, which is developed in Zurich, made significant technical progress and was able to add an important reference point to its list of applications, which will generate licensing opportunities. New microfluidic functionalities were developed, which are state-of-the-art in research on microfluidics.

The project also contributed to attracting top talents – such as and employing them on technologies that are rapidly growing.

The parallel development of a chip for parallel detection of DNA analytes using continuous flow PCR and OC led to more options than initially thought for the deployment of diagnostics for the identification of pathogens related to respiratory tract infections. The inclusion of PCR with a detection part on one chip provides a simplification for the workflow of clinicians performing analysis while also reducing risks of contamination. Considering that one chip needs only 10 to 20 ?L for a highly multiplexed detection of analytes (a potential detection of 32 targets in the PCR/OC system) and using simple peristaltic pumping, our consortium is convinced that the work developed is broadly applicable to the detection of numerous infectious agents.

The developed chips are generic and concepts are scalable. We have advanced the state-of-the-art for designing microfluidic chips and producing polymeric chips with high precision. Some of this work has been published or will be submitted for publication, a patent was filed, and many chips were produced.

DISSEMINATION ACTIVITIES AND THE EXPLOITATION OF RESULTS

To achieve effective communication and dissemination as well as exploitation of the knowledge generated within the Chips For Life consortium, the Project Management Team set up a relevant communication Framework to ensure the information flow within the Consortium, as well as to raise awareness and dissemination activities outside the consortium.

Communication outside the Consortium.

Careful IP assessments were made before any dissemination activities, as per The Chips For Life Consortium Agreement. The Project Management team provided the partners with a set of coherent communication tools to ensure homogeneity (logo, graphical chart, presentation leaflet, poster).

A public website www.chips4life.eu was created to present the Consortium and list our activities and will be available online until 9 months after the project end. The website was updated with information on C4L meetings and other news.

All the Publications stemming from Chips For Life's activities acknowledged the EC-Support and refer to the 7th Framework Programme with the addition of the following sentence : "The research leading to these results has received funding from the European Community's Seventh Framework Programme FP7/2007-2013 under grant agreement n°278720". 6 publications were done by IBM during the project, 3 of which provide open access.

The partners participated in congresses, events and symposia of the field (Clinical Microbiology, Respiratory diseases, Intensive Care Medicine) in order to **raise awareness** around the strains causing infection issues and the need to tackle them in clinical environment and in the community:

- 8 presentations were made at scientific events
- 2 posters were presented
- 5 lectures were given
- Representation at various international conferences on behalf of C4L

In addition 1 masters' thesis was supervised by Coris.

Partner 4 (CHIPSHOP) chairs every year a year the SPIE symposium of Microfluidics, BioMEMS and Medical Microsystems, which was be a very convenient platform to communicate around our activities as well. Furthermore, CHIPSHOP will conduct training courses on microfluidics, both for academic and commercial audiences, where the technologies utilized in this project will be disseminated.

Chips For Life Partners linked with relevant learned societies such as ESCMID (European Society of Clinical Microbiology and Infectious Diseases), ESPID (European Society for Paediatric Infectious Diseases), ESICM (European Society of Intensive Care Medicine) and ERS (European Respiratory Society) among others in order to raise awareness on our activities and benefit from their insights and exposure. The patient's associations too will be a selected target of our communication since they will be very much concerned by our activities, and can have a strong impact on decisions made within hospitals and clinics' direction. The partners participated in ECCMID 2015 (Coris, IT, Pathofinder). IT distributed flyers to promote the final workshop.

At the end of the project a Chips For Life Workshop to communicate on our final results was held in Liège on the 29th of June. It brought together 40 professions (academics, industry, SMEs, clinicians, EC project officer) specializing on the disciplines of the project. It provided an opportunity for the consortium to conclude the project and share lessons learnt and challenges met with colleagues.

Exploitation of results

In order to ensure that the product answers end-users' needs, a market study was conducted. Competitors and technologies were benchmarked and an analysis was conducted to see where to position the product on the market. Coris has been provided with clear information on the next steps and strategy that could be adopted to commercialise the product.

The results of the project will be exploited as follows:

- Microfluidic design for highly multiplexed FRET-based detection of analytes in small volumes of samples. All aspects of the chip design were documented, reported and made available to WP3's partners. This know-how, potentially subject to a license from the other partners if combined with background IP, is therefore potentially exploitable by Pathofinder, Coris, MFCS and IBM. Background IP might be 2SMART technology (Pathofinder), PCR/OC (Coris) and flow mixers/bypass channels (IBM).

This foreground in principle allows any of the partners to design its own microfluidic chip for multiplexed assays, which are based on the optical detection of ligand-receptors interactions in solution. In terms of IP, no specific patent application to be filed was identified because the chips use known principles from the field of microfluidics and know-how on making them working appropriately was seen as more important.

Potential exploitation might be MFCS producing and selling polymer chips, Pathofinder and Coris assembling diagnostic kits using such chips for in vitro diagnostic applications, IBM potentially licensing IP on flow mixers/bypass channels, and/or Coris potentially commercializing an integrated diagnostic solution (reagents, chips, reader).

The foreground would likely be exploited at the end of the project, after clinical testing. It was decided in the meeting at month 18 not to publish all details of this foreground to prevent competition (large *in vitro* diagnostic companies, in particular) from copying our technology and "racing" to be the first on the markets.

Microfluidic designs for highly multiplexed resuspension of chemicals in parallel flow paths with controlled and optimized filling. Synchronization of parallel filling liquids, designs, proof-of-concepts (silicon), mathematical modeling were documented using the intranet, reported and explained to partners. IBM has filed a patent application. This IP/know-how is available for licensing to partners and 3rd parties. Discussions on access to this foreground have started and will be pursued beyond C4L. This foreground is likely to be exploited after C4L.

The combination of PCR on a chip and oligochromatography was successfully realized. This incorporated polymer chips with PCR areas from MFCS and oligochromatography tests from Coris. This will likely lead to products and Coris and MFCS will evaluate how to proceed with the opportunity arising from this synergy.

Exploitation of this know-how might occur via selling polymeric chips and/or diagnostic chips.

Further research and publications

At the final meeting, all partners agreed that it is necessary to work on publications on the project results. All partners also agreed that only individuals who directly worked on the results being published would be listed as a co-author.

4.1.5 Address of project public website and relevant contact details

A full list of the beneficiaries and main contacts is presented below:

Institution	Name	Email address
Coris BioConcept	Thierry Leclipteux (TC)	th.leclipteux@corisbio.com
IBM	Emmanuel Delamarche (ED)	emd@zurich.ibm.com
Inserm Transfert	Sinéad Quigley (SQ)	sinead.quigley@inserm-transfert.fr
Microfluidic ChipShop	Holger Becker (HB)	hb@microfluidic-chipshop.com
CHU Liege	Pierrette Melin (PM)	pierrette.melin@chu.ulg.ac.be
CHU Antwerp	Greet Ieven (GI)	Greet.Ieven@uza.be
PathoFinder	Guus Simons (GS)	Guus.Simons@pathofinder.com

www.chips4life.eu

4.2 Use and dissemination of foreground

According to the **original Plan for Dissemination of the foreground** and use of results, the following activities were conducted during the project.

PLAN FOR THE USE AND DISSEMINATION OF FOREGROUND

Section A (public)

In continuation of the plan for use and dissemination of the foreground, the information provided in tables A1 and A2 are publically available information.

In addition, the consortium has planned to exploit the foreground in the following ways in the post-project period:

Table A1 – list of scientific publications

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES											
NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[1] (if available)	Is/Will open access[2] provided to this publication	WP
1	Reagents in Microfluidics: an 'in' and 'out' Challenge	Hitzbleck, M. (IBM)	Chem. Soc. Rev.	2013 42	RSC	UK	2013	8494-8516		no	WP3
2	Advanced Capillary Soft Valves for Flow Control in Self-Driven Microfluidics	Hitzbleck, M. (IBM)	Micromachines	4		--	2013	1-8		yes	WP3
3	A microfluidic architecture for efficient reagent integration,	Eker, B. (IBM)	microTAS	2013		--	2013	1150-1152		no	WP3

	reagent release, and analyte detection in limited sample volume										
4	Heterogeneous integration of gels into microfluidics using a mesh carrier	Eker, B. (IBM)	Biomed. Microdev.	16		Netherlands	2014	829-835		yes	WP3
5	Lab-on-a-chip devices: How to close and plug the lab?	Temiz, Y. (IBM)	Microelectronic Engineering	2015		Netherlands	132	156-175		yes	WP3
6	Highly-controlled dissolution of reagents integrated to microfluidics using passive orthogonal flow mixers	Goekce, O. (IBM)	microTAS	2015				submitted		no	WP3
7	PhD Thesis Martina Hitzbleck	IBM	Advanced capillary-driven microfluidic chips for diagnostic applications	07/06/2013	ETH Zurich (Switzerland)		50				WP3/ WP6

8	Master degree's	Coris	Développement d'un test de diagnostic moléculaire multiplex sur plateforme microfluidique	01/06/2015	HELHa Mons, Belgium	Scientific community	15	Belgium		no	WP2 WP3 WP4

		TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES							
NO.	**Type of activities**[1]	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience**[2]	**Size of audience**	**Countries addressed**	**Link to WP**
1	Presentation	PathoFinder	The development of a multiplex PCR assay for the detection of antibiotic resistance markers in respiratory pathogens	12/06/2013	Mosa International Students Research Conference on Health	Scientific community	100	Europe	2
2	Poster	PathoFinder	The development of a multiplex PCR assay for the detection of antibiotic resistance markers in respiratory pathogens	12/05/2014	ECCMID, Barcelona	Scientific community	Total no. of participants in meeting is 10000	Europe	2
3	Presentation	PathoFinder	Performance Characteristics	27/04/2015	ECCMID, Copenhage	Scientific	Total no. of	Europe	2

			of RespiFinder® 2SMART, a novel 22-plex molecular diagnostic assay for respiratory infections		n	community	participants in meeting is 10000	2	
4	Presentation	CHU Lg	Apport et stratégie de bonne utilisation du laboratoire de microbiologie et des ressources disponibles	17/12/2014	Liege, Belgium	Scientific community (specialists in oto-rhino-laryngology)	50-60	Belgium	5
5	Presentation	CHU Lg	idem	16/10/2014	Brussels	Scientific community (Infectious disease specialists, clinical microbiologists and paeditricians)	50	Belgium	5
6	Lecture	CHU Lg	idem	18/02/2014	University of Liege	Scientific community (MD	250 280	Belgium	5

						Students)			
7	Lecture	CHU Lg	idem	22/02/2015	University of Liege	Scientific community (MD Students)	280	Belgium	5
8	Lecture	CHU Lg	Du bon usage des antibiotiques	25/04/2014	University of Liege	Scientific community (senior MD Students)	120 135	Belgium	idem
9	Lecture	CHU Lg	Du bon usage des antibiotiques	26/03/2015	University of Liege	Scientific community (senior MD Students)	135	Belgium	idem
10	Lecture	CHU Lg	Introduction à la biologie clinique	17/03/2015	University of Liege	Scientific community (Bioclinical sciences Master Students)	24	Belgium	idem
11	Conferences	CORIS	ECCMID Conferences	April 25-28 2015	Coppenhagen	Scientific community	5000	Europe	WP2 –WP5
12	Conference (1st International Conference on	IBM	Advanced Capillary Soft Valves for Flow Control in Self-Driven	10/10/2012	Enschede (NL)	Scientific community & Industry	<100	Europe	WP3/WP6

	Microfluidic Handling Systems)		Microfluidics						
13	Invited presentation WAM-NANO 2012	IBM	Miniaturized assays using capillary-driven microfluidics	11/06/2012	Barcelona (S)	Scientific community	100	South Europe	WP3/WP6
14	Invited presentation EIC 2012	IBM	Miniaturized assays using self-powered microfluidics	5/07/2012	Montpellier (F)	Scientific community, policy makers, industry	150	South Europe	WP3/WP6
15	Invited presentation Nanobiotech Montreux 2012	IBM	Pumps, valves, bead traps and other functional microfluidic elements for capillary-driven diagnostic chips	12/11/2012	Montreux (CH)	Scientific community	120	Europe, America and Asia	WP3/WP6
16	invited presentation CHEMSEM Annual Seminar	IBM	Microfluidics	12/12/2012	Turku (Finland)	Scientific community	60	Scandinavia	WP3/WP6
17	Frontiers in nanotechnology	IBM	Micro/Nanofluidics for Research and Diagnostics	april-june 2013	ETH Zurich (CH)	Scientific community	30	CH	WP6

	(teaching + student projects evaluation)		in the Life Sciences						
18	Conference	Delamarche, E. (IBM)	Capillary-driven microfluidics for point-of-care diagnostics	Oct. 2013	Freiburg, DE	Scientific Community	800	International	WP3
19	Conference	Eker, B (IBM, Coris, Pathofinder, Microfluidic Chipshop)	A microfluidic chip for highly multiplexed detection of DNA for the diagnosis of respiratory tract infections	Nov. 2013	Montreux, CH	Scientific Community	100	Europe	WP3
20	Short Course	MFCS	Micro- and Nanofluidics in Diagnostics and Life Sciences: Technologies, Applications and Markets	16.4.2012	Irvine, USA	End users, Industry	30	Global	WP3
21	Presentation	MFCS	Integrated on-chip sample	25.4.2012	Boston, USA	Industry	50	Global	WP3

			preparation for nucleic-acid based diagnostics and bioanalysis						
22	Exhibition	MFCS	BioMeDevices2012	25.-26.4.2012	Boston, USA	Industry	100	Global	WP3
23	Presentation	MFCS	The Microfluidic Toolbox: Lowering Entry Barriers in Difficult Markets by Off-the-Shelf Components	26.6.2012	Tonsberg, Norway	Industry	50	Global	WP3
24	Short Course	MFCS	Micro- and Nanofluidics in Diagnostics and Life Sciences: Technologies, Applications and Markets	20.8.2012	Washington D.C.	Industry	25	Global	WP3
	Short Course	MFCS	Microfluidics from the Concept to a Product: Fabrication	5.6.2013	Hamburg	Industry	25	Global	WP3

			Technologies and Commercialisation Strategies						
26	Exhibition	MFCS	Clinical Lab Expo	29.7-1.8.13	Houston	Industry	5000	Global	WP3
27	Presentation	MFCS	Microfluidics from an industrial perspective: Hype, hope and the real world	22.7.2013	Bozeman	Scientific Com.	40	US	WP3
28	Workshop	MFCS	Conceiving and manufacturing polymeric chips	29.6.2015	Liege	Scientific	35	Europe	WP3

[1] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

[2] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias, Other ('multiple choices' is possible).

Section B1 - confidential

List of patents

Type of IP Rights Choose between: - Patents, - Trademarks - Registered designs, - Utility models, etc	Application reference(s) (e.g. EP123456)	Intellectual property organisation (code)	Subject or title of application	Confidential [1](Yes / No)	Foreseen embargo date (dd/mm/yyyy)	Applicant(s) (as on the application)	URL of application	Status	Actions	Link to which WP?
Patent application	US 146711097	US	Microfluidic device with longitudinal and transversal liquid barriers for transversal flow mixing	No	none	Goekce, O. and Delamarche, E. (IBM)		filed March 27, 2015		WP3

[1] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

Patent application	WO2014/005969A1		PCR/OC chips and reader	YES		Coris		In progress	In progress	WP3-WP4

Table B2 – Overview table with exploitable foreground

In addition to the table, please provide a text to explain the exploitable foreground (= General advancement of knowledge, commercial exploitation of R&D results, exploitation of R&D results via standards, exploitation of results through EU policies or exploitation of results through (social) innovation), in particular:

- Its purpose
- How the foreground might be exploited, when and by whom
- IPR exploitable measures taken or intended
- Further research necessary, if any
- Potential/expected impact (quantify where possible)

Exploitable Foreground 1 :	IBM - Microfluidic design for highly multiplexed FRET-based detection of analytes in small volumes of samples. All aspects of the chip design were documented, reported and made available to WP3's partners. This know-how, potentially subject to a license from the other partners if combined with background IP, is therefore potentially exploitable by Pathofinder, Coris, MFCS and IBM. Background IP might be MLPA technology (Pathofinder) and reagent integrators/bypass channels (IBM). This foreground in principle allows any of the partners to design its own microfluidic chip for multiplexed assays, which are based on the optical detection of ligand-receptors interactions in solution. In terms of IP, no specific patent application to be filed was identified because the chips use known principles from the field of microfluidics and know-how on making them working appropriately was seen as more important. Potential exploitation might be MFCS producing and selling polymer chips, Pathofinder and Coris assembling diagnostic kits using such chips for in vitro diagnostic applications, IBM potentially licensing IP on reagent integrators/bypass channels, and/or Coris potentially commercializing an integrated diagnostic solution (reagents, chips, reader). The foreground would likely be exploited at the end of the project, after clinical testing. It was decided in the meeting at month 18 not to publish all details of this foreground to prevent competition (large in vitro diagnostic companies, in particular) from copying our technology and "racing" to be the first on the markets.

Exploitable Foreground 2 :	IBM The integration of DNA probes to microfluidic chips is strongly linked to the possibility of sealing the chip after the integration. This is done at MFCS using specific know-how. It is also related to the chemical composition of the buffer used for spotting the reagents. Since this foreground might not be detectable in a final product, it would probably not be advisable to seek patent protection for it. Exploitation of this know-how might occur via selling polymeric chips.
Exploitable Foreground 3 :	IBM Microfluidic designs for highly multiplexed resuspension of chemicals in parallel flow paths with controlled and optimized filling. Synchronization of parallel filling liquids. Designs, proof-of-concepts (silicon), mathematical modeling were documented using the intranet, reported and explained to partners. IBM has filed a patent application. This IP/know-how is available for licensing to partners and 3rd parties. Discussions on access to this foreground have started and will be pursued beyond C4L. This foreground is likely to be exploited after C4L.
Exploitable Foreground 4 :	IBM The combination of PCR on a chip and oligochromatography was successfully realized. This incorporated polymer chips with PCR areas from MFCS and oligochromatography tests from Coris. This will likely lead to products and Coris and MFCS will evaluate how to proceed with the opportunity arising from this synergy. Exploitation of this know-how might occur via selling polymeric chips and/or diagnostic chips.
Exploitable Foreground 5:	Pathofinder General advancement of knowledge: the primer/probe design process has revealed the true diversity of resistance markers. This knowledge was used to improve the product design protocol at PathoFinder's laboratories. Further

	research is likely to be necessary to keep up with the continuous emerging of novel resistance genes/mutations.
Exploitable Foreground 6:	Pathofinder Commercial exploitation of R&D results: the primers and probes designed for this project can be used in diagnostic assays for the detection of atypical pathogens, and the detection of typical pathogens and their resistance markers. These assays are versatile in that they can be used in combination with many different nucleic acid extraction and detection platforms. This would allow implementation of the assays in different clinical microbiology laboratories. The assays would be based on PathoFinder's patented 2SMART method. To allow full development and commercialization, there is still need for design optimization, establishment of CE-IVD documentation, and internal and external clinical validation. Use of these assays would allow rapid and accurate diagnosis of respiratory infections, and would improve antibiotic treatment decision making.
Exploitable Foreground 7:	CHU Liège: Publications by the consortium after further statistical analysis to demonstrate the benefits of using the technique developed and the use of a syndromic approach
Exploitable Foreground 8:	CHU Liège : Promotion of the approach at a regional Belgian symposium in 2016 (CHU Liège)
Exploitable Foreground 9:	- Processes and technologies for making higher aspect ratio microstructures, especially pillar structures The technology developed in the project will allow MFCS to use higher aspect ratio microstructures in the design and manufacture of own as well as OEM products. This is especially of importance in the field of cell-based and immuno-diagnostics, as it allows an increase in surface area. As it is company policy, process parameters will be treated as company secret and not disclosed (or filed in a patent). The company will continue to optimize these technologies and processes. As these elements will be part of a variety of microfluidic elements, it is difficult to quantify the impact. The availability of the technologies however will in any case strengthen MFCS's market position and therefore help secure jobs.

Exploitable Foreground 10:	- Processes and technologies for making higher aspect ratio microstructures, especially pillar structures The technology developed in the project will allow MFCS to use higher aspect ratio microstructures in the design and manufacture of own as well as OEM products. This is especially of importance in the field of cell-based and immuno-diagnostics, as it allows an increase in surface area. As it is company policy, process parameters will be treated as company secret and not disclosed (or filed in a patent). The company will continue to optimize these technologies and processes. As these elements will be part of a variety of microfluidic elements, it is difficult to quantify the impact. The availability of the technologies however will in any case strengthen MFCS's market position and therefore help secure jobs.
Exploitable Foreground 11:	General advancement of knowledge: The initial goal of this project was to develop a multiplex test for detecting respiratory pathogens, including viruses and bacteria and antibiotic resistance markers. The technology to be used was the MLPA developed by PathoFinder followed by a FRET detection. This detection step had to be housed in a specifc microfluidic chip. Because of the numerous technical problems linked to the design of the FRET chip, it was decided to focuse on the Cy5 detection by using the OC detection, already patented by Coris. In parallel, the FRET chip has been improved and could be now integrated within the microfluidic PCR chip.
Exploitable Foreground 12:	Commercial exploitation of R&D results: A first microfluidic chip was developed. It allows the detection of 6 viral pathogens and 2 bacteria. This chip involves a microfluidic PCR, followed by a detection with the patented OC technology. This chip has been clinically validated in two hospital settings. According to the data recorded, some more development would be needed to improve this first version, mainly multiple pathogens are involved in the infection.
Exploitable Foreground 13:	Commercial exploitation of R&D results: A specific reader for analysing the FRET fluorescence was developed. Nevertheless, a lot of technical problems appear during this development and finally, it was decided to go ahead by using a chip involving a microfluidic PCR and a Cy5 OC detection. A specific reader was developed accordingly. This reader is now fully functional and ready to be used with the chips: i.e. temperature control (PCR), fluorescence readings and data

	analysis. Implementation of the final version (v6) is now ongoing.

Section B (Confidential[2] or public: confidential information to be marked clearly)
Part B1

Type of IP Rights **Choose between:** - Patents, - Trademarks - Registered designs, - Utility models, etc	**Application reference(s) (e.g. EP123456)**	**Intellectual property organisation (code)**	**Subject or title of application**	**Confidential [3](Yes / No)**	**Foreseen embargo date** (dd/mm/yyyy)	**Applicant(s) (as on the application)**	**URL of application**	**Status**	**Actions**	**Link to which WP?**
Patent application	US 146711097		Microfluidic device with longitudinal and transversal liquid barriers for transversal	No	none	Goekce, O. and Delamarche, E. (IBM)		filed March 27, 2015		WP3

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Not to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

[3] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

			flow mixing							
Patent application	Patent WO 2014/005969 A1		PCR/OC chips and reader	YES		Coris Bioconcept		Filed	In progress	WP3 – WP4

Type of exploitable foreground. **Choose between :** - General advancement of knowledge, - Commercial exploitation of R&D results, - Exploitation of R&D results via standards, - exploitation of results through EU policies, - exploitation of results through (social) innovation.	**Exploitable Foreground (description)**	**Confidential YES/NO**	**Foreseen embargo date** (dd/mm/yyyy)	**Exploitable product(s) or measure(s)**	**Sector(s) of application**	**Timetable for commercial use or any other use**	**Patents or other IPR exploitation (licenses)**	**Owner & Other Beneficiary(s) involved**	**Status**	**Actions**	**Link to which WP?**
General advancement	Microfluidic design for highly multiplexed	no	Details on MLPA potentially to be delayed	Diagnostic chips	In vitro diagnostics	At and after the end of the project (1-3 years	Know-how, with good potential with	All WP3 partners: CORIS, IBM,	DNA reagents details	Publish & disseminate	WP3

	FRET-based detection		until Sept 2014			after C4L project)	background IP from partners	MFCS, PF	on hold until clinical tests roll out	work on microfluidics but no chip demo in commercial booths yet	
General advancement	DNA probe integration to microfluidics and sealing chips		Details on MLPA potentially to be delayed until Sept 2014	Functionalized diagnostic chips that are sealed	In vitro diagnostics	At and after the end of the project (1-3 years after C4L project)	Know-how, with good potential with background IP from partners	All WP3 partners: CORIS, IBM, MFCS, PF	DNA reagents details on hold until clinical tests roll out	Publish & disseminate work on microfluidics but no chip demo in commercial booths yet	WP3
General advancement	New microfluidic concepts (mixers, junctions,	no	Until end of 2015 for complying with conferences	Microfluidic functions can be integrated into	In vitro diagnostics, life sciences, analytical	Know-how can be licensed now and then patent	Patent application filed, licensing discussions	IBM	License available	Negotiations with potential	WP3

	etc.)		and journals embargo policies	microfluidic products	sciences	if granted	started, discussions on next projects started			licensees	
General advancement	Potentially new foreground on microfluidic concepts (PCR + OC) chip	no	Potentially until end of clinical validation and/or regulatory approval	Chip production, diagnostics	In vitro diagnostics, life sciences, analytical sciences	Available after validation and regulatory approvals	Know-how potentially combined with background IP from Coris and/or MFCS	Design and manufacturing of OC tests owned by Coris. PCR part of chips and manufacturing of polymer chip owned by MFCS		To be discussed beyond C4L	WP3
Commercial exploitation of R&D results	Processes and technologies for making higher aspect ratio microstructures, especially pillar structures	Yes	31.12.2025	Process technologies for mold insert manufacturing and molding	Microfabrication, Life Sciences	Directly applicable for own products and OEM products	Company internal process know-how	MFCS	available		WP3
General advancement of	Know-how on capillary-	No		Design methodologi	Microfabrication, Life	Directly applicable	Company internal	MFCS, IBM	available		WP3

knowledge	driven microfluidic structures and their manufacture			es and manufacturing processes	Sciences	for own products and OEM products	process know-how				
General advancement of knowledge	Chips design adapted for Cy5 fluorescence via an OC detection and FRET detection.	NO	NA	One chip with both PCR and Cy5 OC detection fully integrated.	IVD Labs	Starting 2017	Patent submitted by Coris	Coris BioConcept	Ongoing	Not applicable	2
Commercial exploitation of R&D results	A first assay has been developed for identifying 8 respiratory pathogens. Preliminary clinical validations have been performed	YES	December 2016	Diagnostic test for detection of viral and bacterial respiratory pathogens	IVD Labs	Diagnostic test for detection of viral and bacterial respiratory pathogens	The 2SMART method already patented by Pathofinder	Coris BIoConcept	Stand-by	Further developments needed and clinical validations to be performed	2,4,5
Commercial exploitation of	Two reading protocols	YES	October 2015	Instrument for managing	IVD Labs	Starting 2017	NA	Coris BioConcept	Ongoing	Final version	4

R&D results	have been integrated in an ins-trument for reading at different wavelengths for Cy5 detection as well as FRET detection			the diagnostic chips developed. A first chip integrates the Cy5 fluorescence detection with the dedicated reader						to be implem ented	