IMI1 Final Project Report Public Summary

Project Acronym: RAPP-ID

Project Title: Development of RApid Point-of-Care test Platforms for Infectious Diseases

Grant Agreement: 115153
Project Duration: 01/04/2011 - 30/09/2016

Final[1] project report - Template

Development of Rapid Point-of)Care Test Platforms for infectious diseases

RAPP-ID

115153

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Last Period [January/2015] - [September/2016]

Reporting Period [5]

Duration of the project [April 1/2011] - [September 31/2016]

Description of work - version date 26/05/2016

November 29, 2016

[1] The Final project report also includes the periodic report of the last period (see Articles II.4 and II.4.2 of the IMI Model Grant Agreement).

(IMI Ref. IMI/INT/2014-02186)

Table of content

Declaration of the coordinator

I, the coordinator of this project, declare that,

The final report submitted is in line with the obligations as stated in Article II.2.3 of the Grant Agreement:

The attached report represents an accurate description of the work carried out in this project for the last reporting period as well as for the whole duration of the project;

For the last period, the project *(tick as appropriate):*

- ☐ has fully achieved its objectives and technical goals;
- ☐ has achieved most of its objectives and technical goals for the period with relatively minor deviations[2];
- ✓ ~~has failed to achieve critical objectives and/or~~ is not at all on schedule[3].

For the whole duration of the project, the project *(tick as appropriate):*

- ☐ has fully achieved its objectives and technical goals;
- ✓ has achieved most of its objectives and technical goals with relatively minor deviations[3];
- ☐ has failed to achieve critical objectives and/or is not at all on schedule[3].

The public project website www.rapp-id.eu is up to date.

To my best knowledge, the financial statements which are being submitted as part of this final report are in line with the actual work carried out and are consistent with the report on the resources used for the project (section 7) and if applicable with the certificate on financial statement.

All participants, in particular non-profit public bodies, secondary and higher education establishments, research organisations and SMEs, have declared to have verified their legal status. Any changes or deviations have been reported under section 6 (Project Management) in accordance with Article II.3.f of the Grant Agreement.

Name of the Coordinator: Jorge Villacian

Date: ...../ ...../ .....

Signature of the Coordinator: ……

[2] If either of these boxes is ticked, the report should reflect these and any remedial actions taken.

1. Executive summary

The executive summary will be made publically available, and therefore should not include information deemed as confidential by the consortium. It should be concise (preferably no more than 40 pages), comprehensive and should capture the updates for the last reporting period as well as the overall outputs of the project and its impact. It shall at least cover the following items:

1.1. Project rationale and overall objectives of the project

When RAPP-ID started in 2011 infectious disease diagnosis was generally culture-based and provided information on the identification of micro-organisms and antibiotic resistance within 2 to 3 days. As clinical practice in infectious diseases relies on the identification of microbial infections for adequate and targeted treatment, there was an urgent need for more rapid diagnostic solutions. The more rapid detection of pathogens was thought to improve treatment outcomes and health utilisation resources by guiding treatment decisions at the time and place where they were needed. In addition, there was an urgent need for more rapid diagnostics to guide patient enrolment during drug trials. The amount of patients to be enrolled can be larger than what would be economically feasible for any pharma company, especially in case of narrow spectrum antibiotics or for those aimed at developing drugs targeting resistant organisms. A diagnostic that rapidly identifies those patients that carry pathogens of interest can significantly reduce this patient population and thus enable drug development trials.

To come up with a clinically relevant rapid diagnostic test, several technological aspects need to come together. Foremost, the selection of appropriate targets for detection and identification of pathogens and their resistance characteristics is critical. Once selected, methodologies to extract antigens, genetic material or biomarkers need to be investigated as well as different techniques to label this material in order to detect it afterwards. All of these aspects need to come together in an easy to use, rapid and accurate test. Therefore the integration of the different elements in a cartridge or matrix as well as the development of instruments to read the results is key. The objective of RAPP-ID was to evaluate the clinical need and to address that need using state-of-the-art technologies, resulting in one or more diagnostic prototypes at the end of the project.

Despite massive technological advances in the diagnostic field, there is still a large unmet need for faster, sensitive diagnostics that can be used at the point of care.

1.2. Overall deliverables of the project

At the start of the project, the RAPP-ID consortium aimed to develop Point-of-Care Test platforms (POCT) for rapid (hospital <2h, primary care <30min) detection of bacteria, mycobacteria, fungi and viruses, including the determination of resistance to anti-microbial drugs. The platforms were supposed to be able to handle samples related to sepsis, Lower Respiratory Tract Infections (LRTI), including Community-Acquired Pneumonia (CAP) and Ventilator-Associated Pneumonia (VAP) and Tuberculosis (TB). As this focus was probably too broad for a project with the size and resources of RAPP-ID, during the mid-term review

of the project, the focus shifted to respiratory tract infections only. This focused approach enabled to shift time and other resources in order to develop a more limited amount of platforms, leaving some of the other developments for research tracks outside of RAPP-ID.

As a first deliverable, in a first stage of the project, the RAPP-ID consortium identified the clinical need related to sepsis, RTI, and TB and drafted user requirements specifications. This clinical need was then translated in a number of diagnostic platforms capable of addressing this need. In a second stage, the aim of RAPP-ID was to combine novel methods of sample preparation, novel specific probes and demonstrated ultra-high sensitive detection methods, delivering diagnostic prototypes at the end of the project. These diagnostics were conceived using four functional modules: 1) sample collection and interfacing, 2) up-concentration and extraction, 3) signal and/or sample amplification, and 4) detection. The aim was to integrate the minimum number of modules required for each disease/syndrome into a microfluidic cartridge, for which a breadboard reader with Graphical User Interface should provide the necessary optical/fluidic/electric/thermal interfaces. As a final deliverable, the aim was to test the integrated POCT using (spiked) reference samples and well characterised clinical samples and to compare these with reference standards and other standard available diagnostic tests.

Key objectives of the diagnostic prototypes were:

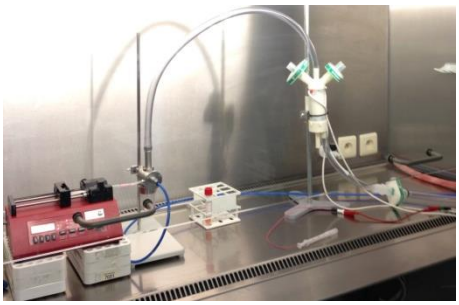
- The POCT can directly handle clinical samples and detect pathogens and resistance markers in these samples;
- The POCT provides a rapid result in close to 30 minutes and not longer than 2 hours;
- The POCT has higher sensitivity and specificity then than current Rapid Immunological Tests;
- The POCT can be integrated into a single system by assembling the associated modules into one cartridge and can provide a simple output.

1.3. Summary of progress versus plan since last period

During the first 4 years, the RAPP-ID consortium developed key technologies and iteratively integrated those, aimed toward the development of diagnostic prototypes for respiratory tract diseases. During this period, the consortium further iterated the integration of key technologies for the three remaining platforms:

Influenza platform: The aim is to develop a rapid qualitative test for detecting influenza virus within minutes (<30) after sampling and at improved sensitivity (<10^5/ml) compared to commercially available assays. This platform features a breath sampler, capable of collecting viral particles from exhaled breath into a tube containing a lysis buffer. This feature is thought to improve patient comfort over the use of nasopharyngeal swabs, which are the current standard of care. Upon capture, viral particles are lysed, releasing the nucleoproteins which are captured on a very sensitive anharmonic acoustic detection sensor (anharmonic detection technique: ADT).

During the current reporting period, the KTH team further optimised the design of the collector tube of the breath sampler and tested the overall concept in the biosafety level 3 facilities at JnJ. Capture of viral particles was shown. As literature related to the capture of virus from breath is scarce and contradictory, with only some groups reporting viral capture from exhaled breath, the team decided to test the breath sampler on actual patients. The study compared the capture of viral particles in exhaled breath using our collector tube with capture of breath trough a dissolvable filter. Both samples were processed using the standard protocol of the University Hospital Antwerp (UZA) consisting of DNA extraction using EasyMag, reverse transcription and qPCR. As for those studies demonstrating viral particles in exhaled breath, the amount of virus was low, nasopharyngeal swabs were also obtained from the patients and were analysed using both the standard UZA protocol and the newly developed Idylla Respiratory (IFV-RSV) Panel of Janssen diagnostics and Biocartis. After obtaining ethical approval, the study was initiated in several primary care facilities in the Antwerp region. For this, the setup was transported to Antwerp and KTH produced a number of breath sampling units, including disposable cage collectors and mouth pieces. The study was performed at the height of the influenza season. Patients first saw their normal primary care physician who evaluated their symptoms and, upon a putative diagnosis of flu, explained them the study. Patients who wanted to participate were then directed to the study team for further explanation, consent and sampling. A total of 20 patients were enrolled, 7 of which were shown to be positive for the nasopharyngeal swab sample as analysed using both the UZA protocol and the Idylla cartridge. However,



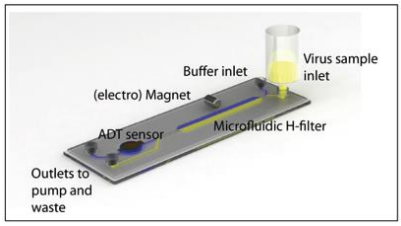
Experimental setup at JnJ: capture of influenza

Breath sample unit

none of the breath samples was positive demonstrating that the limit of detection of the UZA protocol was too low. As the normal detection protocol of the prototype developed in RAPP-ID captures nucleoproteins which are present in 100-1000 copies rather than single copy RNA, the team is now further evaluating the samples (stored in the UA biobank). On the one hand, the aim is to decrease the LoD of the UZA protocol. On the other hand, the team will use our in-house detection technologies to demonstrate the presence of viral nucleoprotein rather than RNA. In case the amount of viral particles in exhaled breath proves too low or unreliable, an alternative track, using nasopharyngeal swabs can be easily integrated on the platform. Although still more sensitive compared to current lateral flow assays, this would however not reduce the patient discomfort associated with nasopharyngeal swabs.

Experimental setup to test the breath sampler in a primary care facility in the Antwerp region

On the bioassay level, the team at KULeuven developed several aptamers which were evaluated for their binding capacity on the MyCartis Evalution platform. Together with the earlier generated antibodies, these will form the capture elements that will drive the sensitivity and specificity of our bioassay. Surface functionalisation protocols for these elements are in place. This bioassay will run on a disposable chip developed at KTH in which a magnet is used to extract the captured nucleoproteins that are bound to magnetic particles to a buffer, enabling their purification. This solution will then flow over the ADT sensor.



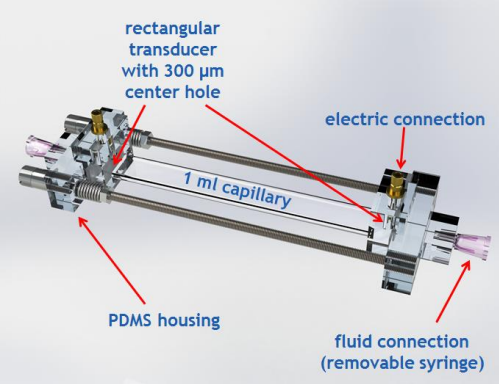
Overview of the cartridge

The 3 ADT devices that were earlier developed by UCAM and were distributed to UCAM, KTH and KUL were used to further test and optimise this technology. ADT cartridges were further optimised allowing to investigate the required flow rate, assay and drive parameters. The team showed a reliable detection up to 16 nM using our model IgE based system. Aptamers were shown to increase the specificity. Although the obtained results demonstrate results superior to quartz crystal microbalance, there are still technological hurdles that delay its integration in the overall prototype. For this reason, partner KULeuven allowed the consortium to make use of its proprietary digital ELISA platform, a sensitive detection technology which was developed outside RAPP-ID. Cartridges for this back-up technology are also available.

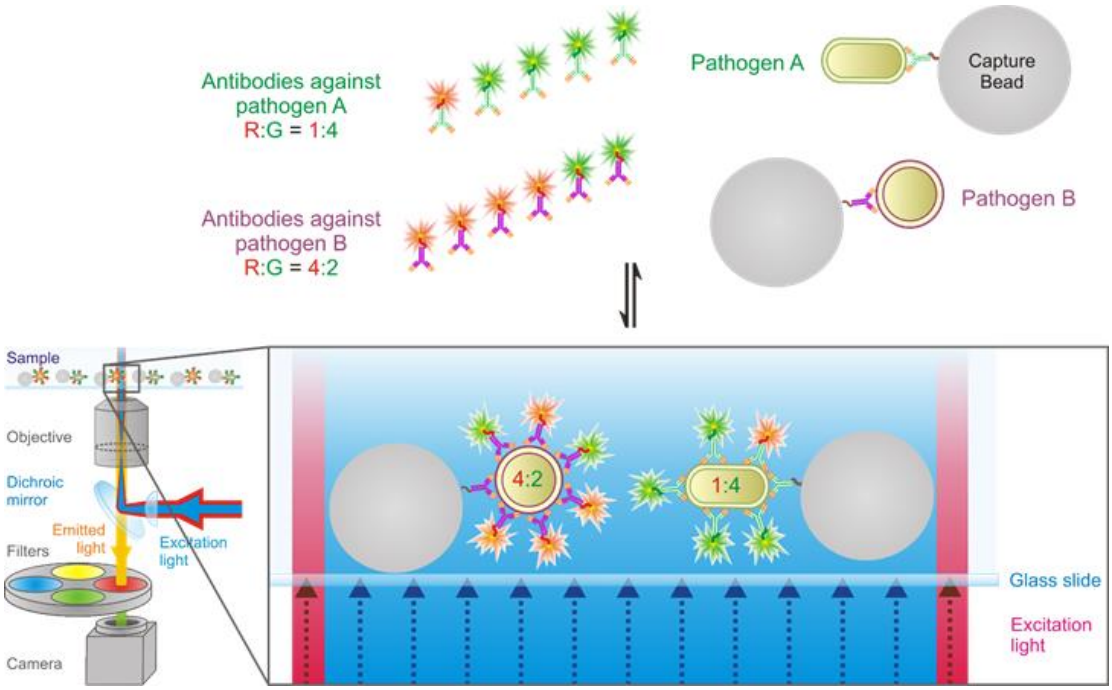
CA-LRTI platform: Aim is to develop a rapid qualitative test for differentiation of bacterial pathogens during a respiratory tract infection (<<30 minutes). Whole bacterial cells obtained from solubilised sputum are collected in a glass capillary where acoustic manipulation of the pathogens enables their rapid focusing over the detection area allowing fluorescence readout through an antibody-based labelling strategy.

During the current reporting period, the team further obtained antibodies to capture the 4 pathogens that form proof of concept of this technology: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenza* and *Bordatella pertussis*. Protocols for fluorescent labelling or attachment to silica beads are in place.

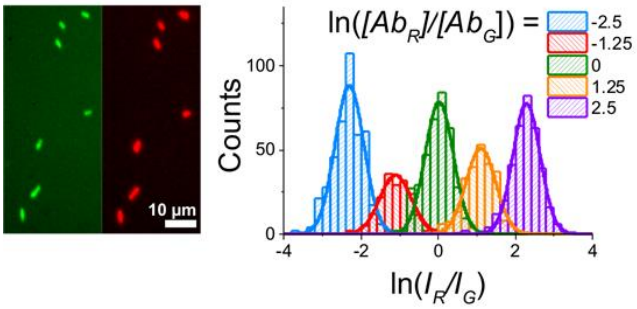
The platform now makes use of an upstream sputum solubilisation protocol which consists of bringing the sputum in a tube containing a chemical agent, a waiting time of 15 minutes and a vortexing step. The sample is then injected into the capillary where the target bacteria are captured and acoustically moved to the detection zone. This simultaneously ensures removal of waste products. The detection process itself makes use of antibodies that are labelled with multiple fluorophores in a certain ratio (PriSM coding). This dramatically increases the number of potential "codes" you can obtain using only a few colours. Indeed, using two colours in 5 ratios increases the coding potential of the two colours to 5. UCAM developed this technology and the image analysis algorithm. The latter makes use of the size of the bacteria to reduce false positives and measures the fluorescence in various channels.



Overview of platform



PriSM coding principle: different ratios of fluorescently labelled probes increase the amount of bacteria that can be differentiated using only a few colours.



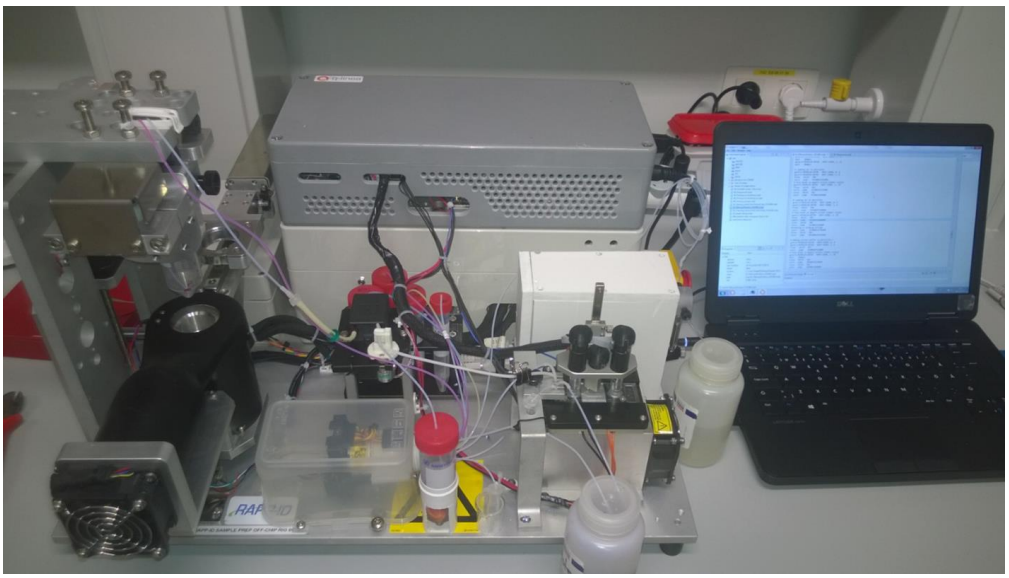
PriSM coding using 2 colours and and 5 ratios

Using fluorescent bacteria (genetically altered to code for a fluorescent protein), the technology was shown to be able to discriminate up to 5 codes using 2 colours. Aim in the current reporting period was to integrate the antibody-based labelling with the differentiating power of PriSM coding and to test this on patient sputum. The lower immunofluorescence for these antibody-labelled bacteria compared to fluorescent bacteria increased the contribution from autofluorescence which resulted in "compressed" PriSM codes. In addition, shifts in the ratios were observed which were attributed to cross-reactions between the antibodies. Considerable time was spend to resolve these issues. On the one hand, calibration curves were made for each of the antibodies. On the other hand, optimised reaction conditions positively affected the cross-reactivity problem. As the PriSM coding principle builds on the positions of the Gauss curves, the observed shifts in those positions make the detection principle less reliable and perhaps unsuitable for the current application. Obviously, the acoustic manipulation, the antibodies and the PriSM coding separately have their value and should be readily applicable in other concepts.

VAP-NAAT: Aim is to develop a molecular platform capable of detecting the most important organisms and resistance markers related to Ventilator Associated Pneumonia (VAP). The prototype should take the clinical sample as is and within two hours should generate the results without further hands-on time.

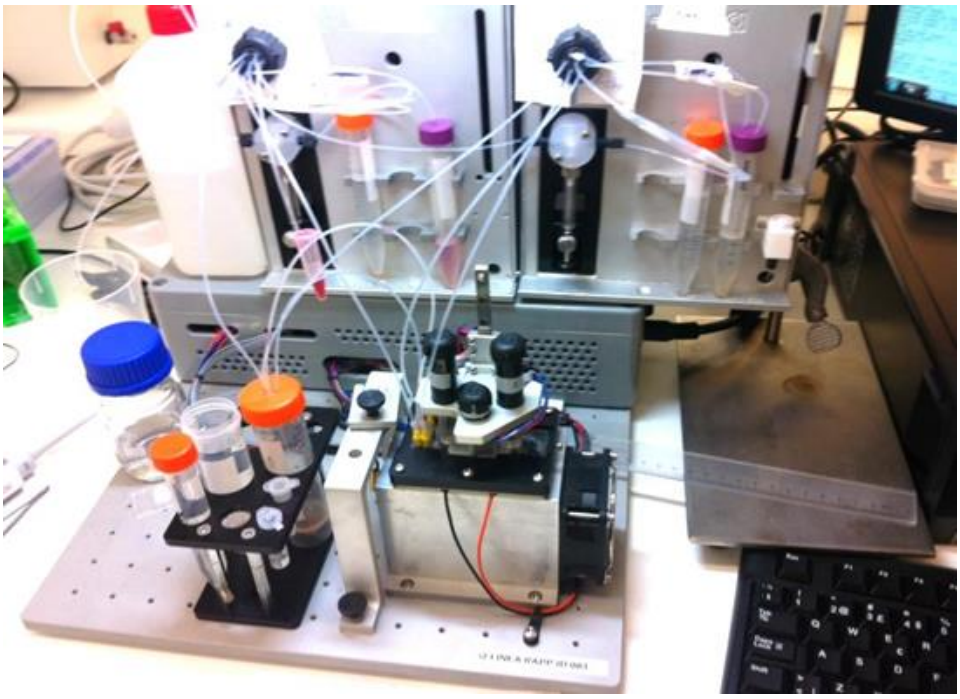
Previously, the team succeeded in developing two modules:

- The sample prep module is capable of taking up to two ml of sputum or endotracheal aspirate. This sample is then solubilised using a combination of acoustics and chemical lysis. This simultaneously ensures extraction of the DNA and fragmentation to a size suitable for the downstream amplification process. This DNA is then captured and transferred to the amplification and detection module.



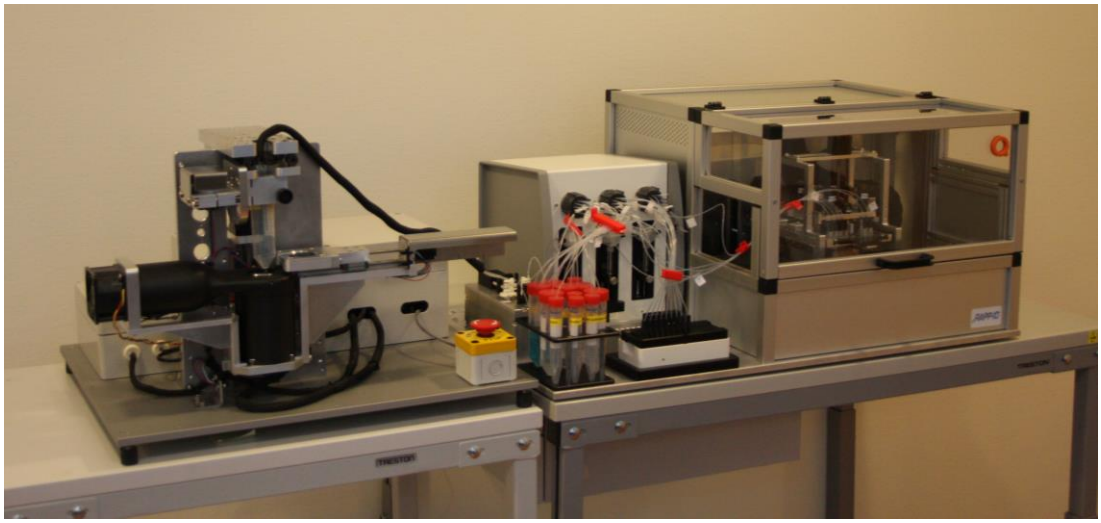
Sample prep module

- The amplification and detection module receives the fragmented DNA from the sample prep module. This simultaneously bridges the macroscale sample prep to the microscale downstream processes. These on-chip processes start with a further DNA purification, leading to amplification using an isothermal rolling circle amplification (RCA) process. As a proof of concept, a panel of over 20 targets are included, consisting of 11 species and 8 resistance markers (some covered by multiple targets to improve specificity). This demonstrates the multiplexing capacity of the method which is robust to the addition of further targets. Amplified products are then further transferred to an array where the multiplex panel is captured and visualised using a fluorescence readout.



Amplification and detection module

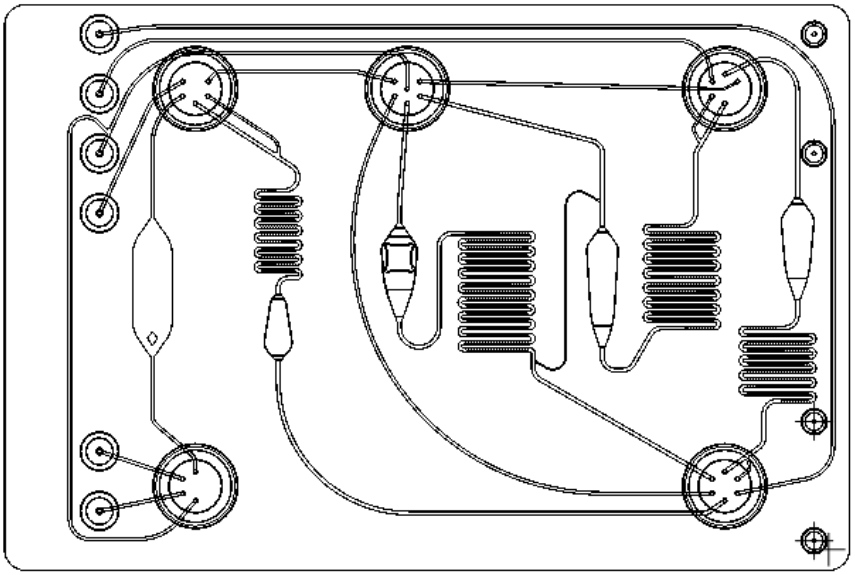
During the current reporting period, both modules were further refined, for example shortening the overall fluidics and improving the mixing strategy during the DNA purification process. The sample prep module was tested on endotracheal aspirates obtained from the University Hospital Antwerp and was shown to be able to solubilise all of those samples. The padlock probes used during RCA were scrutinised for specificity and multiplexing capacity at Stockholm University and some probes were further improved. In addition, both modules were connected, leading to a single prototype platform that integrates a very rapid endotracheal aspirate solubilisation protocol, bacterial lysis and DNA release, a highly multiplexed DNA amplification strategy and detection into a single automated device.



Prototype VAP NAAT

Obviously, some of the components, especially the pumps, of this prototype are still relatively large. This is however to allow fine-tuning of the overall process and many of these components can be replaced by smaller versions in downstream versions of this prototype. It is believed, the size of the final version of the device would be in line with other molecular platforms currently on the market.

Simultaneously, the chip was further improved, resulting in a more optimised design, including all of the fluidic channels, the valves and chambers as well as the array for readout. To demonstrate the potential multiplexing capacity of the technology, the current bioassay enables to detect the most important VAP-associated pathogens (*S. Aureus*, *P. aeruginosa*, *A. baumannii*, *S. maltophilia*, *S. marcescens*, *E. coli*, *E. cloacae*, *E. aerogenes*, *K. pneumonia*, *K. oxytoca* and *P. mirabilis*) and resistance markers (SHV, TEM, CTX-M, VIM, KPC, NDM, OXA, *mecA*).



Chip design with 5 turning valves and chambers for sample purification (left), amplification (bottom two) and detection (middle).

The team is currently optimising the fluidics of the overall chip-device combination and the software-hardware integration. Following this, the device will be tested on (spiked) clinical samples.

1.4. Significant achievements since last report

The RAPP-ID team further integrated the technologies and further tested (parts of) those devices on actual patient samples. The influenza breath sampler was tested in primary care facilities and was shown to be more comfortable compared to nasopharyngeal swabs. Although the breath sampler was earlier shown to be able to collect influenza from air in an artificial setup, no virus could be recovered from exhaled breath using the standard

molecular methods present at the University hospital Antwerp, indicating either no or only very low amounts of viral particles are present in exhaled breath as was indicated in the scarce literature around this topic. The samples were stored and will be analysed with our more sensitive detection technology. The VAP NAAT, became a fully integrated platform where up to 2 ml of endotracheal aspirate can be directly inserted into the device. Within two hours, the prototype fully extracts, fragments, purifies and amplifies this DNA allowing the detection of the 20 most clinically relevant targets (*S. Aureus*, *P. aeruginosa*, *A. baumannii*, *S. maltophilia*, *S. marcescens*, *E. coli*, *E. cloacae*, *E. aerogenes*, *K. pneumonia*, *K. oxytoca* and *P. mirabilis*) and resistance markers (SHV, TEM, CTX-M, VIM, KPC, NDM, OXA, *mecA*). Although RAPP-ID ended, the team will continue to develop this device and will initiate testing of the full prototype on clinical samples.

1.5. Scientific and technical results/foregrounds of the project

Main output of the project are the prototypes as described above:

- Influenza test
- VAP NAAT

In addition, the RAPP-ID consortium developed a number of technologies. Although not all of those developments were finally integrated into the prototypes, those are valuable for future research. These include:

- Acoustic manipulation of bacteria
    - Very fast manipulation of free bacteria or bead-captured molecules allowing for the targeted depositing of those in a detection or manipulation area.
- PriSM coding principle and software
    - Technology that helps to increase the number of potential targets that can be distinguished using a fixed amount of wavelengths.
- Antibodies for various CA-LRTI associated pathogens
    - Antibodies and cell lines for *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenza* and *Bordatella pertussis*
- Antibodies and aptamers for influenza
    - Including an assessment of performance compared to commercial antibodies
- Breath sampler technology
    - Technology that allows to capture all kinds of (bio)molecules from air, be it exhaled breath or from the environment.
- ADT technology
    - Very sensitive detection platform based on Quartz Crystal Microbalance technology.
- Antibodies for LAM (TB biomarker)
    - Currently taken forward by Lionex in rapid diagnostics (ELISA, Lateral flow test)

- Padlock probes for VAP and TB associated pathogens
    - Expanding set of target probes for various infectious diseases. Outside of RAPP-ID the SU team developed probes for other targets (oncology) as well.
- Sample preparation module for BSI
    - Isolation of bacteria from whole blood, followed by DNA extraction.
- Sample preparation module for endotracheal aspirates and sputum
    - Combination of acoustic and chemical lysis allows to solubilize larger volumes (up to 2 ml) of those relatively complex samples. In one go, the DNA can be isolated, purified and delivered on chip. The technology was successfully tested on dozens of clinical samples
- On chip rolling circle amplification module
    - This rolling circle technology forms a more robust, isothermal alternative compared to PCR technology.
- Sample preparation strategy for TB sputum
    - Lionex developed TB sputum solubilisation solutions. The Sample preparation module for VAP samples can probably also process TB sputum samples, but this remains to be validated
- Lateral flow test for TB samples detecting LAM
    - Currently under development by Lionex

Furthermore, the project generated:

- A regulatory white paper
    - This document was generated in a collaborative effort between regulatory experts from big pharma as well as Medical Device Company. This document aimed at sharing the knowledge among all partners within the consortium.
- User requirement specifications for TB, BSI and LRTI
    - A set of documents outlining the clinical need for TB, BSI and LRTI and how this need can be addressed from a technological point of view.

1.6. Potential impact and main dissemination activities and exploitation of results

Please explain how the project scientific/technical outputs contribute to the overall IMI objectives:

- to provide socio-economic benefits for European citizens,

  When fully developed and validated, diagnostic tests in general are a tool that the clinician can use to guide patient treatment. For infectious disease diagnostics, this mainly means antibiotic stewardship, the evidence-based use of antibiotics only in those cases where they are needed. This helps the patient in many ways:

  - Diagnostics help to identify potentially resistant pathogens, including those not covered by empiric treatment. As the latter would result in treatment failure, this significantly impacts on the patient's morbidity and mortality.
  - A similar association can be made with the overuse of antibiotics. Empiric treatment often means inclusion of antibiotics that not directly target the pathogen of interest. This negatively impacts on the patient's health status. First of all, they destroy the beneficial microbiome which in turn can lead to superinfections and even long-term side effects. In addition, as many antibiotics are toxic, they by themselves negatively impact on the patient's health status. Both in turn can again lead to increased morbidity and mortality rates.

  These increased morbidity and mortality rates mainly translate into a more extensive treatment and a longer length of stay. Apart from the impact on the patient's health, this leads to increased health care costs and thus impacts on national health care systems.

  Within RAPP-ID, the consortium developed a prototype VAP NAAT. As VAP is still relatively common in intensive care units and since many patients under ventilation are critically ill, they would significantly benefit from a more targeted use of antibiotics and the avoidance of antibiotic-associated side-effects. As this test allows the identification of the most common pathogens for VAP and many potential resistance markers, this test perfectly fits into an antibiotic stewardship program. Since the costs of treatment at intensive care are high, a reduced morbidity and length of stay can easily save thousands of euros per patient.

- to contribute to the health of European citizens,

  Diagnostics have the potential to guide the use of antibiotics. This impacts on the health of European citizens in a number of ways:

  - The association between the use of antibiotics and increased levels of resistance are meanwhile well established. Diagnostics help the physician to decide whether to give antibiotics or not. In primary care, the majority of patients presenting with acute cough are suffering from a viral infection. However, without proper diagnostics, the physician cannot base the decision to give antibiotics or not on hard evidence. Especially for certain risk groups, improper treatment might lead to a rapid worsening of their situation and hospitalisation. Physicians will thus use the cautionary principle and will rather prescribe antibiotics than to withhold them and

wait. This in turn leads to a massive overuse of antibiotics and thus pressure toward antibiotic resistance.

In RAPP-ID, the consortium developed a rapid breath-based influenza test. This test, which is comfortable for the patient, helps the physician to make an evidence-based diagnosis and will thus impact on his/her antibiotic prescription behaviour.

- In addition to the decision on whether to give antibiotics or not, diagnostics also help to decide which antibiotics to give. Clinicians rely on epidemiological data to decide on their empiric treatment. With rising antibiotic resistance levels, this empiric treatment has to include more and more last resort antibiotics this in spite of the fact that the majority of the population would still benefit from those antibiotics to which only a minority of organisms already developed resistance. Again, because of a lack of an evidence-based diagnosis, the clinician decides for a more stringent empiric treatment. Diagnostics that allow to identify those patients that would still benefit from those earlier generation antibiotics would help safeguard later-generation antibiotics and would slow resistance development against these antibiotics.

  In RAPP-ID, the VAP NAAT includes resistance markers associated with many antibiotics and has the potential to further expand this panel, the latter because of the high multiplexing capacity of the RCA technology. This high multiplexing capacity helps to assure the clinician that a more narrow spectrum treatment and/or an earlier generation antibiotic will suffice.

- Many antibiotics are toxic. Examples include the nephrotoxicity associated with certain cephalosporin's and colistin. Knowledge about resistant organisms can help to avoid the use of those antibiotics which might be especially critical for patients in intensive care units.

  The RAPP-ID VAP NAAT provides ample information regarding the presence of antibiotic resistance genes. This will help guide treatment toward the best overall health status for that patient.

- to increase the competitiveness of Europe and help to establish Europe as the most attractive place for biopharmaceutical research and development.

There is an urgent need to develop rapid point of care diagnostics. Although it remains to be seen to what extent RAPP-ID will result in the commercialisation of any of its technologies, the development process as such has been beneficial for Europe in a number of ways.

- It allowed the further development of certain technologies which will be taken forward in future projects/developments.
- The groups involved in RAPP-ID had a widely varying background ranging from more clinically oriented partners over technological partners to partners with expertise in regulatory and validation aspects. The combined effort delivered in RAPP-ID helped those groups to bridge those fields which will be of significant value in future projects as a better understanding of the complete picture associated with dx development will allow for more focused future developments.
- Many of the young researchers (PhD students) within the project have been confronted with the wide scope of disciplines that surround diagnostic test

development. Through RAPP-ID they have gained an understanding of the clinical need as well as the biological and technological aspects related to dx development. Moreover, they have been confronted with regulatory and validation aspects. Because of this wide range of encountered expertise, these young researchers have gained the background to become future leads in diagnostic development programs.

Please outline how the project outputs have/will have the potential to be rapidly and broadly spread and taken up within the scientific/industrial community and healthcare professionals.

Within RAPP-ID, the consortium developed a number of technologies. Some of those were integrated in the diagnostic solutions developed within RAPP-ID, others were not integrated, but were often progressed further outside RAPP-ID. There are a number of ways by which those developments might be taken forward toward commercialisation or uptake within the scientific/industrial community and healthcare professionals.

- The consortium will continue to work on the two main prototypes developed within RAPP-ID: the VAP NAAT and the influenza breath sampler. The teams will continue the evaluation of these platforms and will aim for follow up funding with smaller, more targeted consortia, potentially including new partners.
  Key advantages of the VAP-NAAT include

Asset	**Benefit**
Clever sample collection tube	Minimal impact on current clinical practice as the tubing and size of the collector are nearly identical to those currently in use + easy insertion of sample into device. ETAs are heterogeneous in nature and difficult to divide into pieces (more viscous samples might require a scalpel).
Capable of handling sample volumes up to 2 ml	Because of the heterogeneous nature, larger volumes are thought to yield a less variable result while reducing the possibility that the "wrong" location of the ETA is sampled.
Isothermal amplification method	Reduces the technical complexity of the device (no need for temperature ramping)
High multiplexing capacity	RCA allows for hundreds of simultaneous reactions in a single reaction chamber. This is important for infectious disease diagnostics in which the amount of potential pathogens can be significant.
Robust to the addition of	Contrary to PCR, adding a target hardly influences the existing bioassay. This is especially

targets	important for the resistance markers as novel genes and variants, especially for the gram negatives, are continuously discovered.

Key advantages of the influenza POC include

Asset	**Benefit**
Patient friendly sampling method	Nasopharyngeal swabs can cause discomfort for the patient, especially while being sick. Merely blowing into a tube was shown to be more comfortable.
POC friendly bioassay	The sample can be collected directly in the lysis solution present in the collection tube which by itself is directly connected to the chip/cartridge.
Sensitive detection technology	Although dependent on the efficiency of the antibodies or aptamers used, the technology should in theory be able to detect very low amounts of viruses (even further decreased by targeting high copy nucleoproteins)

- The consortium will continue to develop core technologies. Although RAPP-ID as such has come to an end, many of the partners still collaborate in other projects and strive to obtain more joint funding in the future. Examples include the ND4ID project in which the RCA technology will be taken forward and the Pocket project in which the ring resonators form the core detection technology.
- The RAPP-ID team previously discussed core technologies and platforms with industry and will continue to do so.
- Developments, once beyond initial verification will be further validated using the IMI COMBACTE framework. The COMBACTE consortium is building a clinical trial network throughout Europe aimed at bringing new antimicrobials and diagnostics to the market.

1.7. Lessons learned and further opportunities for research

Please indicate how the collaboration in a public private partnership (PPP) has been an added value to achieve the objectives of the project.

Apart from the scientific input from the EFPIA companies, they provided valuable information regarding the regulatory landscape. At the start of the project, a regulatory white paper was developed and presented to the consortium. In addition, EFPIA brought expertise related to the validation of diagnostics.

From your experience, please propose any recommendations/ solutions which could be useful for a PPP.

RAPP-ID started as a very broad project, aimed toward the development of diagnostics for BSI, RTI, TB and including antimicrobial resistance. It was the need for diagnostics to guide patient selection in clinical trials that drove this call. Now, after 5 years, that need is still there and, despite massive advancements in diagnostics, pharmaceutical companies are still struggling to find suitable tests. A PPP can be very useful here, but would need to start from a more focused call. In advance of any call launched within the ND4BB program, IMI could launch a diagnostic development call to identify, modify and validate potential platforms that could aid patient selection in those ND4BB trials. As RAPP-ID demonstrated, diagnostic development is more time, labour and cost intensive than anticipated with a fully-fledged platform easily taking a decade and several hundreds of millions to develop. Hence to enable the use of any platform in these trials, the PPP would need to start with very clear target product profile specifications and platforms/technologies starting from TRLs of 5 and above. Only under those circumstances can suitable diagnostics be developed and validated within a timeframe of 2-3 years and with a reasonable budget.

In view of your project achievements, please provide your views on potential new research to further advance the field.

One of the main achievements of RAPP-ID were the user requirement specifications and target product profiles that were developed at the start of the project. These definitely benefitted the technological advancements made within the RAPP-ID project and are still valid. As both academia and industry have lots of technological developments in the pipeline, these might benefit significantly from such user requirement specifications and target product profiles. Once the problem and need are thoroughly defined from a clinical perspective and are made publically available, technologically oriented institutions/groups can take up the challenge. There are many advanced technologies out there that might be perfectly fit for integration into diagnostics. However, lack of knowledge of the clinical need limits their uptake or integration.

Developing diagnostics requires a lot of time and resources. Compared to oncology diagnostics, infectious disease diagnostics have the significant drawback that treatment (antibiotics) is cheap. The same is true for current culture-based diagnostics although everybody acknowledges these take too much time to really impact on antibiotic stewardship, especially in primary care settings. Modern solutions like cartridge-based molecular diagnostics are fast, but relatively costly compared to treatment and culture-based diagnostics. This really limits their uptake and reduces their impact on antibiotic use, antibiotic stewardship and the development of antibiotic resistance. Improvement of the situation probably on the one hand requires an assessment of the economics associated with development, production and validation and on the other hand requires an assessment of the impact of inclusion of diagnostics on health care systems, improved antibiotic use and reduced resistance and the impact of the latter on society as a whole.

As for the developments made within the framework of RAPP-ID, both the VAP and influenza platforms will be taken forward by the respective teams, be it in their entirety or specific parts which will then be integrated in further enhanced concepts. Related to the influenza test, this can be the coupling of the breath sampler to a sensitive digital elisa platform, while for the VAP NAAT, apart from the VAP developments, the RCA technology will be taken forward within the framework of the ND4ID project, where it will be coupled to alternative sample preparation and detection technologies aiming to develop a rapid diagnostic test for BSI. Ultimately, bringing any of those diagnostic concepts to the market will be challenging for academia, so the teams will aim to team up with industry partners after having successfully demonstrated proof of concept, including preclinical validation on clinical samples. Follow-up national or European funding will be required.