FINAL PUBLISHABLE SUMMARY REPORT

Executive summary

Seasonal, or epidemic influenza, has a global incidence of 10-20%, is responsible for between 3 and 5 million cases of severe illness every year resulting in 500,000 deaths annually. Total direct and indirect costs of a severe flu epidemic are estimated at over $12 billion in the US alone. As a consequence, Seasonal Influenza remains one of today's biggest threats to the world's socio-economic health. Rapid, accurate diagnosis of influenza typically requires molecular biological techniques such as the Polymerase Chain reaction (PCR). Although accepted as the gold-standard diagnostic test, PCR remains constrained by its requirement for specialist laboratory staff and high capital equipment costs. As a consequence, PCR is traditionally unsuited to Point-of-Care (PoC) testing and is often beyond the reach of many developing nations which have a recognised need for decentralized disease diagnosis.

The RANGER programme successfully brought together a European consortium of clinical, scientific, engineering and regulatory expertise to develop the first of a new generation of molecular diagnostic systems - the Enigma®ML. From initial concept to a production-ready system in less than three years; the RANGER consortium has proven that with the focussed and collaborative effort of recognised experts, EU framework projects can deliver commercially viable diagnostic products into the global market.

The Enigma®ML is a revolutionary PCR-based diagnostic platform allowing fully-automated, rapid and accurate diagnosis of a wide spectrum of human pathogenic microorganisms in a range of clinical sample types. Under the RANGER programme, the system was developed specifically to provide a PoC molecular test for Seasonal Influenza viruses. Capable of providing laboratory standard Influenza results from a nasal sample in less than 60 minutes, the system requires no specialist operator knowledge, laboratory facilities or ancillary equipment. The system will also allow a more co-ordinated surveillance effort for future outbreaks enabling rapid deployment of resources to control disease spread.

During the three year programme, the consortium successfully delivered production-ready prototype Enigma®ML instruments and ambient-storage consumable cartridges containing all reagents required to perform a fully-automated RT-PCR diagnostic test for Seasonal Influenza A/B. Significant effort was invested in understanding the user requirements, the manufacturability of the system and expectations of the regulated diagnostics markets, the Enigma®ML system was designed from first-principles to meet these all these needs.

The Enigma®ML represents a paradigm shift in molecular diagnostics - for the first time, clinicians and global healthcare professionals will be directly empowered to make timely and informed diagnostic decisions critical to efficient medical intervention and effective patient management in influenza-like illnesses.

Summary description of project context and objectives

In addition to pandemic influenza, Seasonal influenza remains a major health concern globally and although vaccination may be effective in minimizing the health impacts of Seasonal Influenza in 'at-risk groups' such as the elderly, current global vaccination coverage is less than 5%. Continuous viral mutation requires constant review of vaccine efficacy. Consequently, the most effective means of controlling the spread of influenza remains early diagnosis, containment and where appropriate, antiviral therapy.

Currently available non-molecular diagnostic technologies lack standardization and often are unsuited for widespread use in both developed & developing countries. Consequently there is an immediate need for a rapid, robust, sensitive and cost effective point-of-care molecular diagnostic test for the identification of influenza which requires minimal expertise to operate & no specialist laboratory equipment. The RANGER programme was therefore established to develop a truly novel diagnostic platform to address this need. The RANGER programme unified European expertise in a range of disciplines including; influenza diagnosis and surveillance, molecular diagnostics, reagent stabilization, *in vitro* diagnostic instrument and software development and clinical regulatory affairs. The consortium was composed of the following members:

Beneficiary name	Beneficiary short name	Country
Enigma Diagnostics Limited	ENIGMA	UK
Health Protection Agency	HPA	UK
Bruhn Newtech Group	BRUHN	DK
QSNICH	QSNICH	THIA
Bioplastics BV	BIOPLAS	NL
Sagentia Limited	SAGE	UK
Pera Innovation Ltd	PERA	UK
Biopharma Technology Ltd	BTL	UK

The overall objective of the consortium was to develop a fully integrated, highly portable and multi-sample PCR diagnostic system providing affordable, rapid and reliable testing for influenza infections. The system needed to be completely at home in both laboratory and outreach environments at a cost and complexity not prohibitive to many third world settings where a PCR capability would otherwise not be available. The project intended to develop disruptive technology, making it possible to obtain reproducible, reference standard PCR capability (typically reserved for developed-world centralised laboratories) in any global care setting. In short, the RANGER consortium were to a develop a cost-effective diagnostic capability suitable for any global location, providing rapid laboratory standard results whilst operable by minimally-skilled users requiring no specialist knowledge in molecular biological techniques or data analysis. To achieve this, the specific technical objectives of the RANGER consortium were to:

- Develop a scalable, portable, fully automated PCR instrument for point of care environments & capable of being operated by clinical staff while the patient remains in the clinic, hospital, triage area, port of entry etc.

- Develop a consumable cartridge providing all the mechanical interfaces needed for automated sample preparation and PCR-based assays.

- Develop a multiplex RT-PCR assay capable of detection and differentiation of Human Seasonal Influenza type A and B viruses in nasal/ nasopharyngeal samples with the level of sensitivity & specificity needed for a high level of clinical utility:

- Develop a process by which the assay reagents could be stabilised for ambient storage conditions, eliminating the requirement for refrigerated storage.

- Integrate the instrument, consumable and assay into a single system capable of 'sample in, results out' in less than 60 minutes.

- Develop intelligent algorithms for the unambiguous interpretation of PCR data and automated diagnostic result 'calling' of clinically relevant information.

- Provide a secure internet based application that provides a central reference point for the clinical data produced during outbreaks coupled with global surveillance tools for the mapping and analysis of influenza transmission.

- Demonstrate the clinical utility of the system in influenza reference laboratories.

- Establish a manufacturing plan for commercial production of the system with Enigma®ML targets costs of <€25k (depending on number of modules) with a cost per test (consumable & reagents) <€15

The RANGER project deliverables were specified as part of the EU FP7 RANGER Description of Work (DoW) which centred around the delivery of pre-production prototype Enigma®ML instruments, cartridges and an assay suitable for eventual commercialisation and qualification as a human *in- vitro* diagnostic tool for influenza diagnosis and surveillance. To achieve these deliverables, the development programme was divided into 10 work programmes with responsibility for each divided between the consortium members according to their relevant expertise in each field. These work packages and associated deliverables can be summarised as:

Table 1. RANGER project Description of Work

WP	Title	Task #	Specified Task(s)	Parties Involved	Del #	Deliverable
1	**Sample preparation methodology**	1.1	Confirm the optimum sample type and sample preparation methods for the target strains of Influenza	EDL, HPA, QSNICH	D1.1	Report on the research, analysis and selected method of the clinical sample collection for influenza diagnosis
		1.2	Optimise the method of magnetic bead sample preparation, separation, purification and concentration for each influenza strain	EDL, HPA	D1.2	Protocol and validation report for the magnetic bead sample preparation
		1.3	Validation of the above magnetic bead sample preparation, separation, purification and concentration step	EDL, HPA		
2	**User friendly - diagnostic consumable**	2.1	Consumable research phase (pre-tooling phase)	EDL, SAGE, BIOPLAS	D2.1	Sample of 3 filled RANGER system consumables
		2.2	Consumable development (tooled consumable phase)	EDL, SAGE		
		2.3	Consumable filling process development	EDL, SAGE, BIOPLAS		
		2.4	End-user feedback and design evaluation	EDL, HPA, QSNICH	D2.2	RANGER consumable unit end user feedback report
3	**Instrumentation**	3.1	Design Definition	ALL	D3.1	Operational RANGER sample unit instrument & hub unit
		3.2	Sample processing unit development	EDL , SAGE		
		3.3	Hub unit development	EDL, BRUHN, SAGE		
		3.4	Packaging for shipment and transport	EDL, SAGE		
4	**Assay selection, optimisation & multiplexing**	4.1	Evaluation of candidate assays	EDL, HPA, QSNICH	D4.1	Selected RANGER assay shortlist to be developed & validated
		4.2	Preparatory assay suitability assessment	EDL, HPA	D4.2	Optimised candidate assays
		4.3	Assay Optimisation Process	EDL, HPA	D4.3	Performance characteristics of candidate assays in clinical samples
		4.4	Optimised assay performance characterisation with clinical samples	EDL, HPA		
5	**Lyophilisation**	5.1	Compatibility of the assays with excipients	EDL	D5.1	Lyophilisation protocol for the RANGER assays

		5.2	Lyophilisation protocol, process development & optimisation	BTL, EDL		
		5.3	Performance and stability analysis of lyophilised reagents	BTL, EDL		
6	**Information technology systems**	6.1	Ranger Instrumentation software development	SAGE, EDL	D6.1	Demonstration of the complete RANGER system software
		6.2	Demonstration of the Surveillance Management software	BRUHN, EDL	D6.2	Demonstration of the Surveillance Management software
7	**System integration**	7.1	Complete RANGER system preproduction prototype	EDL, SAGE	D7.1	Complete RANGER system preproduction prototype
		7.2	Electrical & environmental test results and report	EDL, SAGE	D7.2	Electrical & environmental test results and report
8	**Validation**	8.1	Ranger Instrument Validation	EDL	D8.1	Clinical validation results & report for both the developed & developing world trials
		8.2	Clinical Validation (developed world setting)	HPA, EDL		
		8.3	Clinical Validation (developing world setting)	QSNICH, EDL		
9	**Dissemination and Exploitation**	9.1	Dissemination & exploitation plans	ALL	D9.1	Dissemination & exploitation plan
		9.2	Technical Publication of Achieved S&T Objectives.	EDL, HPA, QSNICH	D9.2	Construction of project web-site
		9.3	Construction of project web-site	ALL		
10	**Consortium Management**	10.1	Six month progress reports, Mid Term Assessment Report and Final Report Submission of the costs	ALL	D10.1	Six month progress reports, Mid Term Assessment Report and Final Report Submission of the costs
		10.2	Formal administration responsibilities of consortium manager within the project	PERA	D10.2	Provision of audit certificates and bank guarantees
		10.3	Formal administration responsibilities consortium, technical & scientific management of the project	EDL		
		10.4	Intellectual property protection	EDL	D10.3	Report on gender, societal and ethical issues of exploitation
		10.5	Coordinate gender equality, society aspects and ethical issues within the project	PERA, EDL		

Description of the main Scientific and Technological results

The 3 year RANGER project involved the unification of multi-disciplinary teams within the core beneficiaries working on inter-related work packages to develop the core technologies behind the Enigma®ML system and Seasonal Influenza A/B test. The inter-dependencies of the project can be summarized in the following Pert diagram:

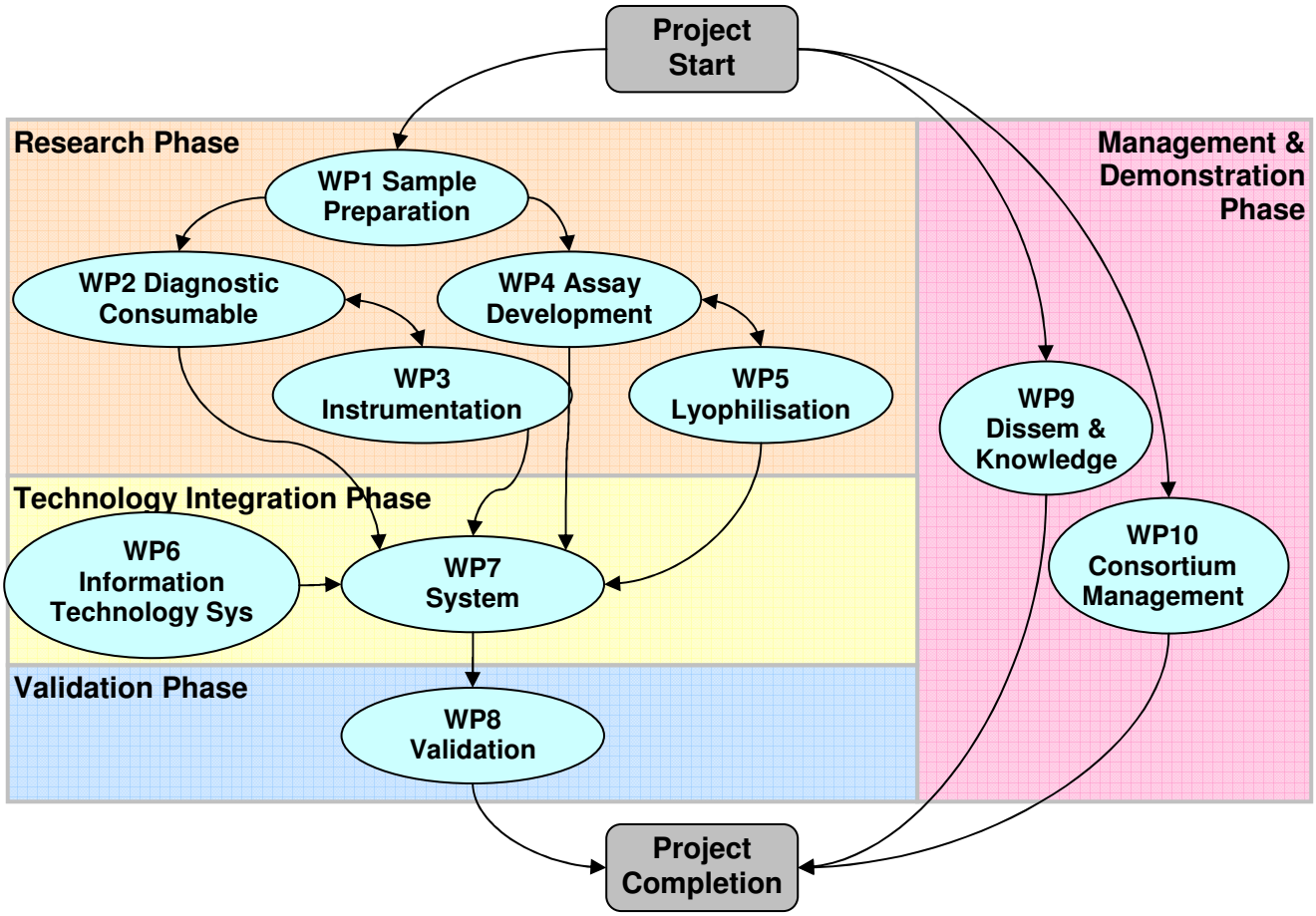

Fig 1. RANGER Work Plan Strategy.

Over the 3 years of development, 10 work packages have been progressed to address the technical and project management elements of RANGER. Work packages 1-8 had tangible outputs feeding into all of the development, integration and validation activities associated with the Enigma®ML instrument, consumable cartridge and Seasonal Influenza A/B RT-PCR test assay components. Therefore, the main scientific and technological results of the RANGER programme will be described for each individual Workpackage and in the context of the whole Enigma®ML system.

WORK PACKAGE 1 - SAMPLE PREPARATION METHODOLOGY

The objectives for this work package were to confirm the optimum sample type and sample preparation methods required for clinical diagnosis of the target strains of Influenza. This involved selection of a sampling method/ process compatible with wider clinical practices and a sample preparation chemistry compatible with automation and capable of achieving the analytical and clinical diagnostic performance required.

Sample collection

The type and method of sample directly impacted the design of the consumable cartridge and the sample preparation protocol used processing. The sampling method also determines the ease-of-use of the system and it's compatibility for use in the point-of-care environment. Therefore it was important to establish the preferred method. Extensive Voice-of-Customer (VoC) work was conducted during the initial design phase of the Enigma®ML to fully understand the 'problem' and identify the most appropriate solutions for the identification of seasonal influenza in adult and paediatric patient groups.

The research was initiated via expert consultation with the clinicians involved in the RANGER consortium, led by Dr. Tawee Chotpitayasunondh at the Queen Sirikit Hospital (QSNICH) in Bangkok and Dr. Thomson from the UK Health Protection Agency (HPA). Subsequent to this a literature search was undertaken, notably including documents and recommendations published by the World Health Organisation. The understanding gained during these activities was utilised to design a user survey which was distributed widely to clinicians, laboratory professionals and surveillance experts specialising in seasonal and pandemic human influenza viruses. See https://www.surveymonkey.com/s.aspx?sm=0MEwjPfcdnbWi3S8X9GQLA_3d_3d. The survey contained 54 questions grouped into ten sections:

1. Patient management
2. Samples
3. Data requirements
4. Assay requirements
5. Current tests
6. Testing locations
7. System design
8. Waste disposal
9. Pricing
10. Surveillance

The results from this survey were then taken in person to a round-table of influenza experts at the World Health Organisation in Geneva for ratification. Using targeted feedback from clinicians, healthcare professionals, academic groups, key opinion leaders and diagnostics market experts, the following outputs were produced:

- Literature review to establish current clinical best practices
- Competitor analysis and market gap analysis
- Customer and Patient needs analysis
- Regulatory requirements

The research revealed that overall the most commonly taken samples were nasal swabs for seasonal influenza using off-the-shelf flocked swabs. These sample types were therefore selected for the primary system development activities within the Ranger programme and the specifications for a sample collection device were established. To minimise the technical and regulatory burden of developing a bespoke swab specimen collection device, existing CE IVDD swab and sample collection tubes were assessed for compatibility with the RANGER objectives test cartridge. The favoured design approach was to use a standard COPAN nasopharyngeal swab coupled with a modified sample collection tube lid featuring a piercible foil allowing automated sample release from a closed tube when inserted into the test cartridge (figure 2).

Fig 2. Selection of COPAN CE-marked nasal swab (top), standard COPAN UTM-RT sample tube (middle) modified COPAN UTM-RT with RANGER ML compatible lid modifications (bottom).

As part of the cartridge development programme, the swab and sample cartridge tube designs were fixed to allow design decisions to be made with respect to the physical dimensions of the consumable cartridge and interaction with the Enigma®ML instrument. The final COPAN-manufactured RANGER sample collection kit is pictured below (Figure 3).

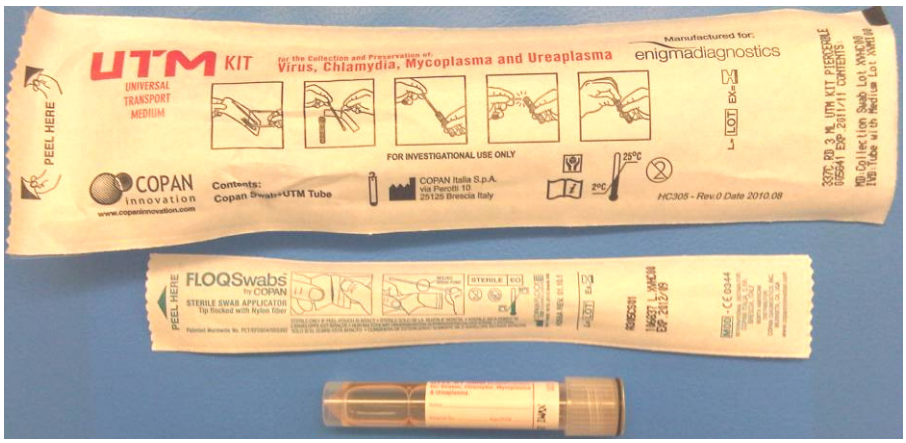

Fig 3. Final packaged sample collection kit manufactured by COPAN containing the COPAN FLOQSwab® and Enigma®ML-specific UTM-RT sample collection tube.

Sample preparation chemistry

Having defined the sample type and collection device, it was then possible to define the method of sample preparation required to purify the total nucleic acid from a patient sample. A variety of sample preparation methods were available depending upon the nature of the target agent, sample matrix type, sensitivity of downstream detection processes and the equipment available (automation *vs.* manual processing). The HPA with input from EDL conducted a review of the possible extraction methodologies for compatibility with the outline specification for the RANGER ML. Of the available methods, magnetic bead-based extraction chemistries and a number of commercially available chemistries were shortlisted based on presumptive functional/ analytical performance, long-term ambient storage stability and highest potential for automation. EDL, having developed magnetic bead-based nucleic acid extraction systems previously proposed the use of a commercially available chemistry - MagaZorb® (Promega).

Using an 'off-the-shelf' solution enabled the consortium to focus on instrument, consumable and PCR assay development activity whilst reducing the concomitant manufacturing QC and regulatory burden of developing an in-house chemistry. Proof-of-principle investigations using MagaZorb® on benchmark automated systems such as the KingFisher (Thermo) suggested that the chemistry was well suited to Influenza RNA recovery from clinical material and would readily translate onto the proposed RANGER instrument being designed in parallel. Fixing the chemistry early in the programme allowed further design decisions to be made wrt to the consumable cartridge configuration (material choice, number of reagent wells, volume, form-factor etc).

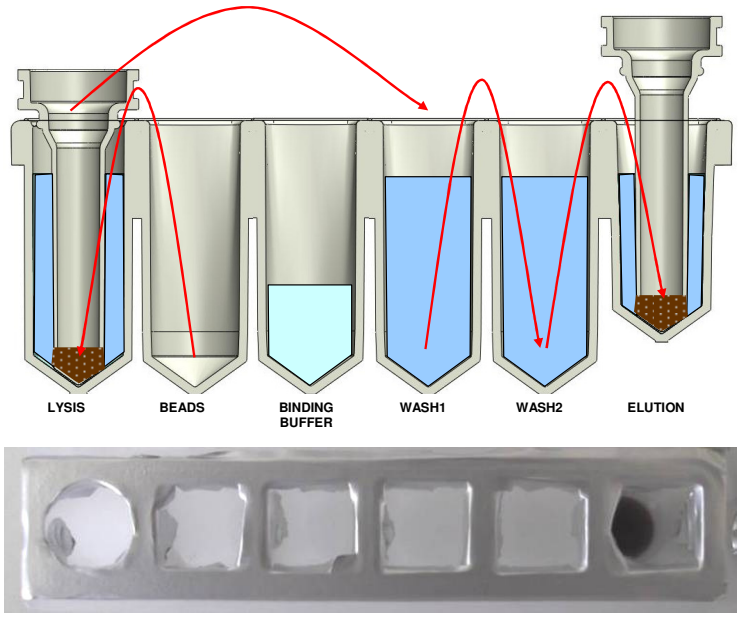

Fig 4. Process map of MagaZorb® magnetic-bead based sample preparation system (top) and integration with the Enigma®ML Sample Preparation Strip (SPS) consumable cartridge sub-component.

Optimisation and process validation activities were later conducted using the MagaZorb® chemistry once fully integrated with the test cartridge. This enable the Enigma®ML assay programme to be developed, performing the mixing and magnetic-bead transfer steps required for fully-automated sample extraction and influenza RNA purification from test samples. Influenza RNA recovery efficiency using the automated process was typically around 30% consistent with

most other manual and automated magnetic-bead systems. The chemistry showed a good level of performance in interfering substances removal when test samples were spiked with a range of common medicinal compounds. As a final suitability confirmation, investigations into SPS cartridge fill tolerances for each extraction reagent were conducted to assess overall cartridge manufacturability. These studies demonstrated that the RNA recovery efficiency of the MagaZorb® chemistry was unaffected by reagent fill variations of +/- 15% giving confidence that the chemistry was permissive to cost-effective bulk cartridge manufacture.

WORK PACKAGE 2 – USER FRIENDLY DIAGNOSTIC CONSUMABLE

This package of work focussed on the research & development of a user friendly, multi-component consumable cartridge enabling fully-automated sample preparation and PCR processes to be performed by the Enigma®ML instrument. As the consumable is an integral part of the system, efficient design and development was critical to the overall success of the RANGER programme. Due to the complexity and inter-dependencies of the cartridge with the RANGER Influenza assay and the Enigma®ML instrument, development of all three was a parallel and iterative process.

Having established the sample collection method and sample preparation chemistry for the RANGER system, significant project effort by EDL, SAGE and BIOPLAS was expended in the consumable research (pre-tooling) phase. Initially developed using CAD models and prototype SLA parts, early prototype cartridges were produced to support customer acceptance studies, technical feedback on the design and proof of principle experimentation.

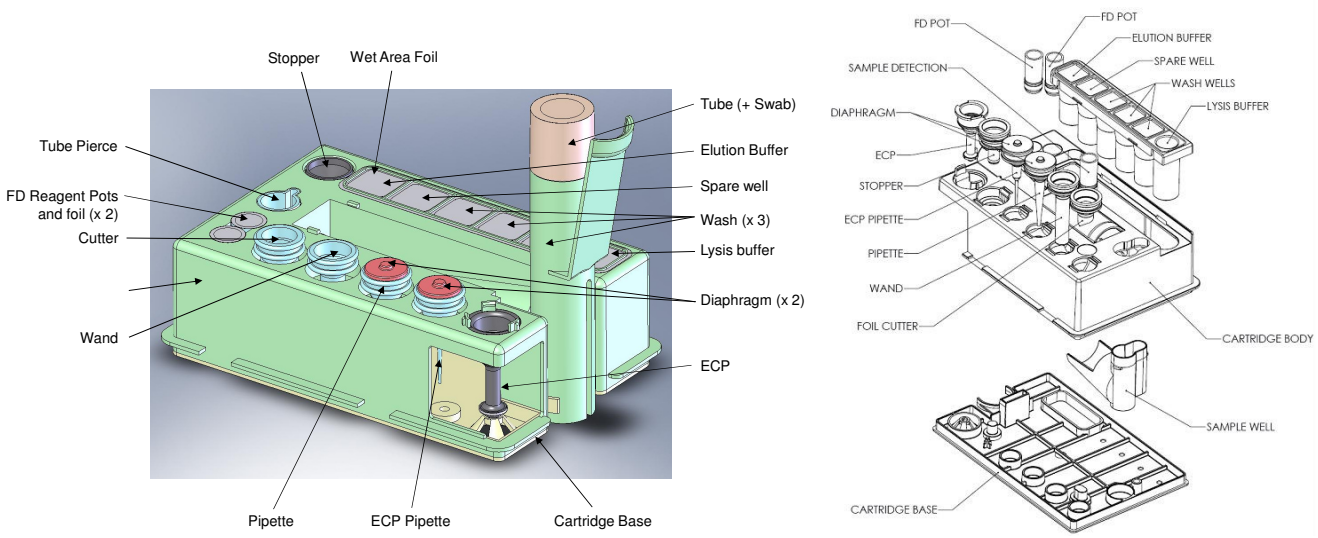

Fig 5. Original CAD design concept (left) for the consumable cartridge to final cartridge manufacturing specification (right).

Having established a viable cartridge design and initial bill of materials (BoM), first-off production moulded parts were made allowing characterisation and functional performance testing of all the cartridge subcomponents such as piercing tool, pipettors, magnetic wand sheath etc.

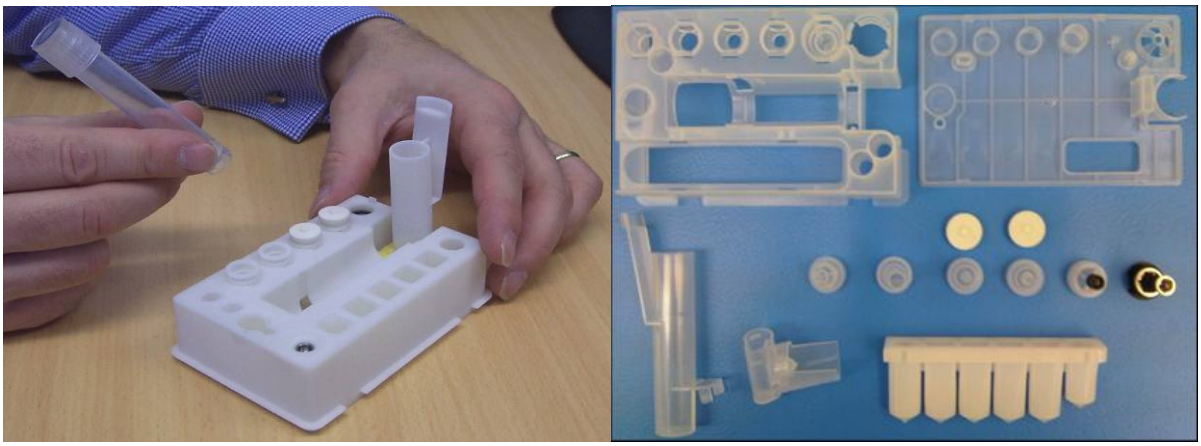

Fig 6. Early SLA cartridge prototypes (left) to realisation of production-ready prototype cartridge components (right).

After manufacturability studies and cost-down activities, the design of the Enigma®ML consumable cartridge was fixed and production transferred to a volume manufacturing organisation (Hi-P, Malaysia) for multi-cavity tooling, generating some 40k complete test cartridges to underpin further RANGER technical development and exploitation activities. Manufactured cartridge components were transferred to EDL for the development of pilot scale assay reagent filling and foil sealing processes and production line development. Final RANGER assay cartridges were successfully delivered concluding RANGER work packages 1 and 2.

Fig 7. Final RANGER test assay consumable, fully-assembled containing pre-packaged MagaZorb® sample extraction reagents, lyophilised Seasonal Influenza A/B RT-PCR reagents, lyophilised MS2 process control and ECP thermal reactor.

WORK PACKAGE 3 - INSTRUMENTATION

The instrumentation work package constituted the bulk of development activity undertaken by EDL and SAGE project teams to develop a robust, reliable Point of Care PCR clinical diagnostic instrument. During the initial deign consultations and VoC work conducted as part of Work Package 1, the desire to have a modular, scalable instrument architecture was identified such that multiple configurations of the Enigma®ML could be produced to suit differing test settings and throughput requirements. To achieve this, the Enigma®ML instrument was designed as two functional sub-units, the Control Module (CM) and Processing Module (PM).

Control Module (CM)

The CM was designed to act be the primary end-user interface with the Enigma®ML system, acting as a communications hub, data repository and power management centre for integrated system. Architected to control 1-6 Processing Modules (PMs), the CM was designed using a microcontroller-based PCB running QNX Neutrino embedded operating system (common to many IVD instruments) to coordinate system operations, drive the user interface and provide data reporting/ archiving functions.

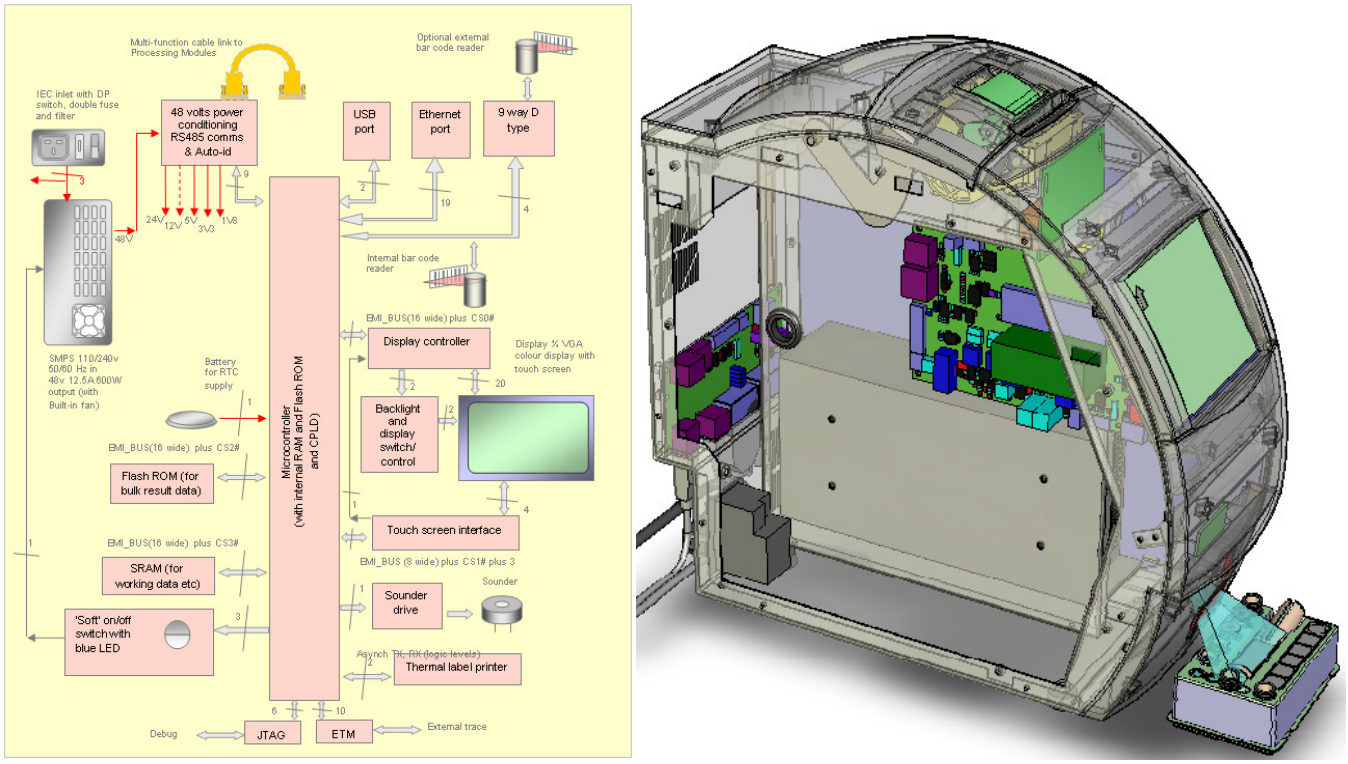

Fig 8. Original schematic of the Enigma®ML CM architecture (left), CAD industrial design (right).

Central to the success of the system was the development of the icon and virtual keyboard driven graphical user interface (see WP6 below), displayed via a 5.7" 256 colour ¼ VGA resolution resistive touchscreen, operable by clinicians with gloved hands. The CM has an embedded bar-code scanner providing automated data entry (operator ID, sample ID, cartridge type ID etc) for complete QC and result traceability. Also embedded within the CM is an RJ45 Ethernet port for

external connectivity to Information Management Systems (IMS), a USB programming port (for software and assay programme updates) and a 2” thermal printer to produce hard-copy test results.

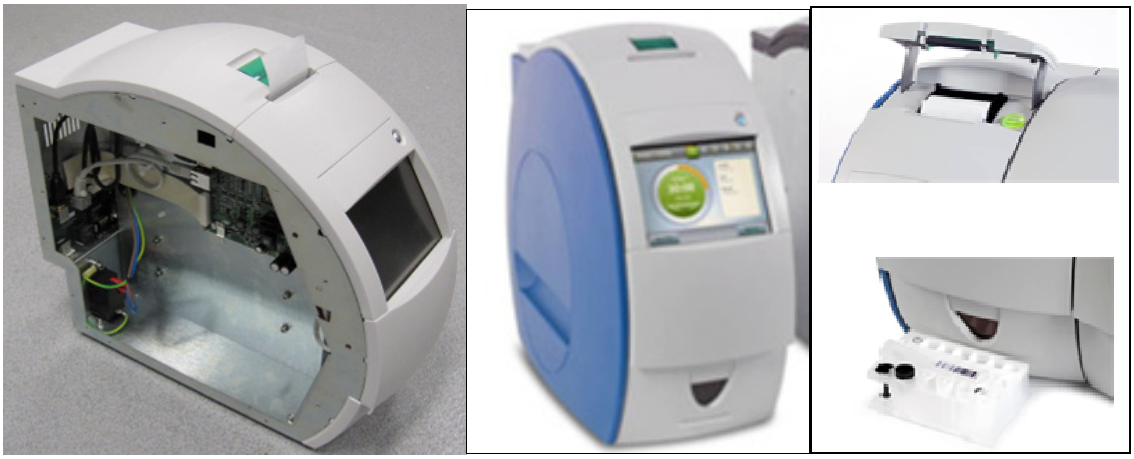

Fig 9. ‘A1’ early prototype CM (left), ‘A3’ production-ready prototype CM (centre), thermal printer (top right), external bar-code reader (bottom right).

Processing Module (PM)

The Processing Module (PM) is the Enigma®ML robotic subunit containing the mechatronic hardware required for automated sample preparation and the optoelectronic hardware for PCR thermocycling, fluorescence data acquisition, processing and result analysis. The PM has a microcontroller-based control PCB running software to communicate with the CM, run selected assay programmes and provide the low level mechatronic control functions.

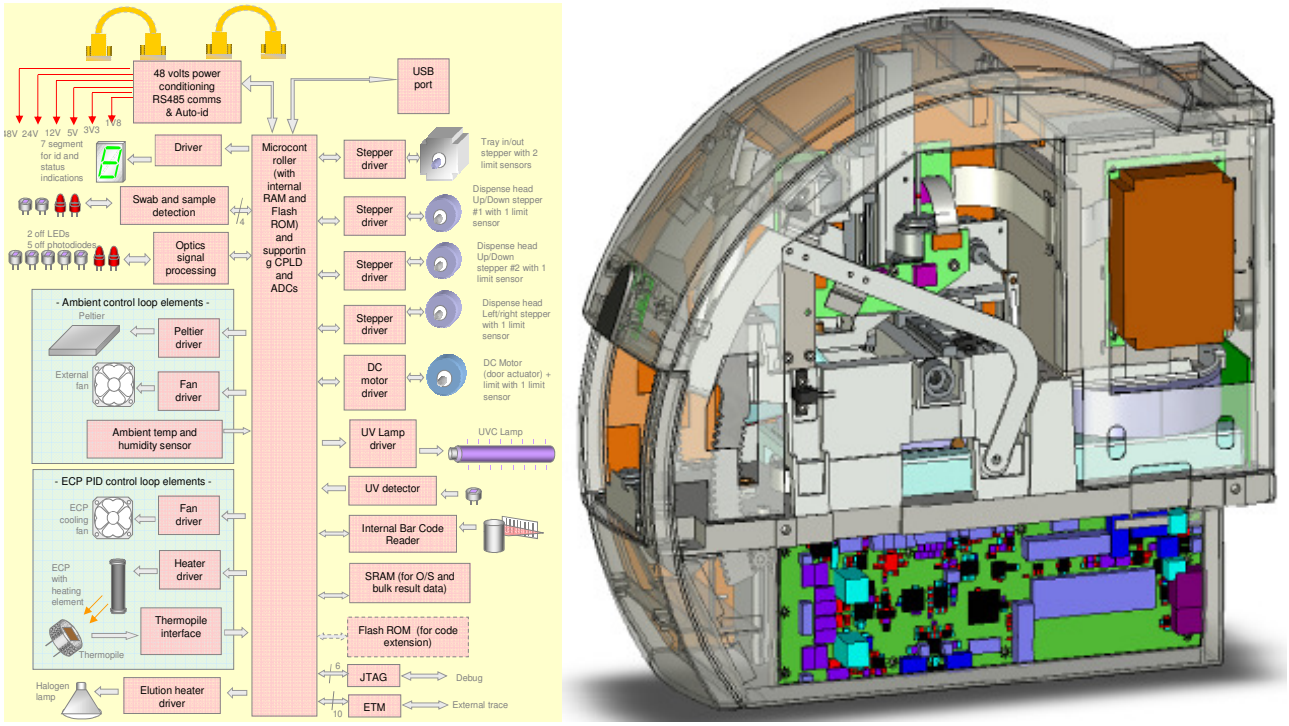

Fig 10. Original schematic of the Enigma®ML PM architecture (left), CAD industrial design (right).

The PM consists of a number of core components to provide the RANGER system with a means of mechanical interaction with the consumable cartridge to provide sample manipulation, sample extraction and RT-PCR. Briefly these include a motorised tray to move the consumable in and out of the module, motorised door to close entry and an actuated 'grabber arm' head to manipulate cartridge tools. A variety of internal 'error state' sensors detect whether the test cartridge correlates with the cartridge data entered during test start-up, optical sensors detect the presence of the sample swab and confirm correct release of sample into the test cartridge. Other features of the PM include an integrated UV lamp for decontamination and sample carry-over avoidance. PCR data acquisition is provided by the optics module 'block', solid-state a 'beam splitter' device using two LED excitation sources (blue and red) with six photodiodes (detectors) with front-end signal conditioning electronics and an interface to the main PCB. Algorithmic PCR and melt analysis data interpretation is performance within each PM, communicating the test result to the CM for display to the instrument operator.

Fig 11. 'A1' early prototype PM (left), 'A3' production-ready prototype PMs integrated with the CM subunit (right).

Having successfully delivered prototype CM and PM subunits, functional testing and reliability verification activities were undertaken on to confirm mechanical, electronic and optical performance prior to the system integration activities described in work package 7 (below).

WORK PACKAGE 4 - ASSAY SELECTION, OPTIMISATION & MULTIPLEXING

The clinical utility of the instrument is dependent on the availability of sensitive and specific RT-PCR assays for Seasonal Influenza A/B viruses. Work package 4 was designed to develop a multiplex Influenza RT-PCR assay, suitable for lyophilisation (work package 5) and ready for implementation on the Enigma®ML system as a clinical test assay. The work package was divided into several workstreams to evaluate existing assay capabilities, develop and optimise new candidate assays for RANGER implementation and determine their analytical performance against clinically-relevant samples. The HybProbe® (Roche) PCR assay signalling chemistry was chosen due to its wide acceptance in the molecular diagnostic sector, significant expertise within the EDL and HPA development teams and EDL having obtained intellectual property rights to commercialise this type of test. Significant effort was spent conducting a literature review of

existing Influenza PCR assays and conducting bioinformatics analysis of known human influenza strains to identify genetic targets suitable for specific detection and discrimination of circulating human seasonal type A and B influenza viruses. Based on this review, regions within the influenza Matrix Protein (MP) encoding gene region were selected for assay design and development.

After conducting several rounds of assay down-selection, formulation development and optimisation, initial influenza assay sensitivity was determined by using human nasal wash samples spiked with known concentrations of influenza A/Taiwan/1/86 (H1N1), A/Moscow/10/99 (H3N2) and B/Brisbane/60/08 (Victoria lineage). Quantified by plaque assay, these strains allowed the assay limit of detection (LoD) for the InfA and InfB constituents of the assay to be determined as; A/Taiwan/1/86 LoD = 4.7 pfu/reaction, A/Moscow/10/99 LoD = 8.5 pfu/reaction, B/Brisbane/60/08 LoD = 8.5 pfu/reaction.

For diagnostic use, the RANGER assay required a process control to protect against false-negative results possibly obtained during clinical sample testing. MS2 bacteriophage was chosen as it is an established surrogate organism for mimicking pathogenic RNA viruses (such as Influenza) and used in comparable molecular assays. MS2 would be introduced with the sample during processing, proving a control for both the nucleic acid extraction (chemical and mechanical processes), reverse transcription and PCR amplification reactions. To enable this, an MS2-specific detection assay was designed around the MS2 coat-protein and incorporated within the RANGER multiplex PCR assay. Re-optimisation of the candidate assay was then performed and the final formulation established in preparation for lyophilisation process development (work package 5).

To finalise the output of work package 4, the lyophilized assay was subjected to a comprehensive analytical performance evaluation to assess analytical sensitivity and specificity of the final assay. This involved assessment of cross-reactivity against a panel non-seasonal influenza organisms, assessment of pan-reactivity for multiple InfA and InfB strains and confirmatory testing against a panel of clinically representative samples.

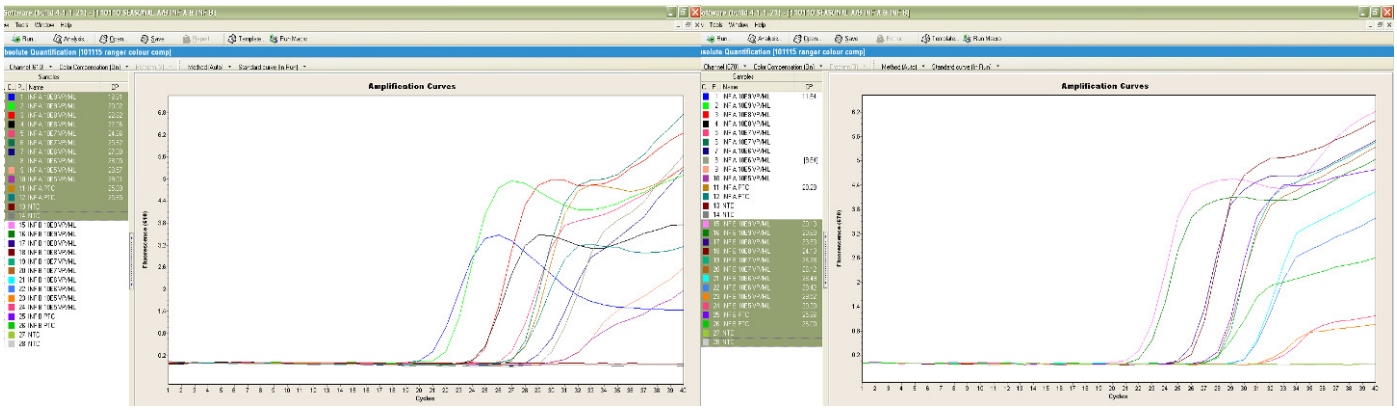

Fig 12. RT-PCR sensitivity determination of the Influenza A (left) and Influenza B (right) specific detection components of the RANGER assay a serial dilution series of Influenza A and B RNA templates.

Analytical specificity results for the RANGER assay were good, the practical experimental data supporting BLASTn *in-silico* sequence analysis. The assay demonstrated a high level of inclusivity (pan-reactivity) when tested against a panel of 62x InfA and 22x InfB strains influenza strain detection, no cross-reactivity shown against a panel of 53 non-influenza microorganisms. This data is indicative of efficient assay design and implementation by the HPA team. Overall sensitivity of the RANGER assay was also high, meeting the requirements for detecting Influenza A and B viruses within the clinically significant range for a nasal swab sample.

WORK PACKAGE 5 – LYOPHILISATION

To achieve compatibility with point-of-care and decentralised diagnostic test, eliminating the cold-storage requirements typically associated with molecular biological methods was critical. Work Package 5 objectives were to develop a lyophilisation process and reagent formulation suitable for freeze-drying the RT-PCR and MS2 process control components to achieve the 12-18 months stability with ambient storage desired for the RANGER assay consumable. This was conducted in parallel with cartridge development (WP3) since the PCR and MS2 control had a dependency on the design and integration of a bespoke reagent pot.

EDL had significant prior knowledge in the formulation of PCR reactions containing excipients needed for successful freeze-drying. Using the initial HPA PCR assay formulation, the RANGER multiplex assay was shown to be compatible with several excipient formulations which were transferred to BTL for development and refinement of the freeze-drying process needed to stabilize the assay and MS2 control as reagent 'cakes'. Several hurdles to this development programme were identified, specifically the ability to thermally probe and monitor cake formation during freeze-drying using standard equipment. The complex nature of a PCR reagent mix meant the inter-dependencies of the core reagent components (enzyme, buffers, salts etc) are greater than simple formulations. Also the low reagent mass/ volumes hampered determination of collapse temperature, glass transition temperature and residual moisture content. As such WP5 was also an analytical method development workstream, taking existing analytical technologies an applying them within the constraints of small volume reagents (<50µl) and prototype plastic wear.

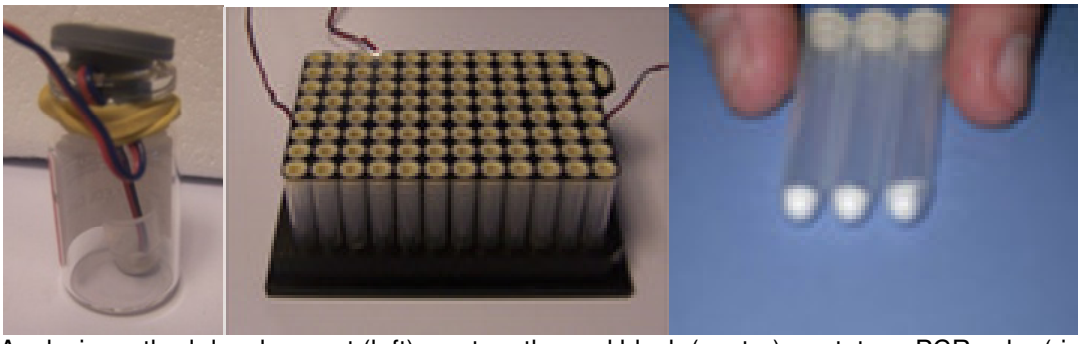

Fig 13. Analysis method development (left), custom thermal block (centre), prototype PCR cake (right).

Prototype freeze-drying protocols and excipient formulation were successfully developed by BTL, providing the ability to lyophilise the RANGER RT-PCR assay and MS2 process control in a 'cake' format without loss of assay/ control performance. Reagent cakes were compatible with the

consumable cartridge plasticwear and proof-of-principle ambient storage stability demonstrated within the prototype reagent pots. The BTL process and formulation were successfully transferred to a number of manufacturing organisations providing contract lyophilisation services confirming portability and scalability of the process for commercially viable product manufacture.

To enable the RANGER assay to be submitted for regulatory approval (CE marked IVD, FDA 510k etc), it was necessary to demonstrate pilot-scale manufacturing within a GMP facility. This was achieved by transferring the RANGER assay to Biolyph Inc, successfully integrating the Seasonal Influenza A/B RT-PCR and MS2 process control reagents into a proprietary Lyosphere® (bead) reagent format. This enabled significant improvement in manufacturing process scale-up and cost-reduction activities required to make the RANGER assay commercially viable and meet the <€15 customer cost per assay cartridge.

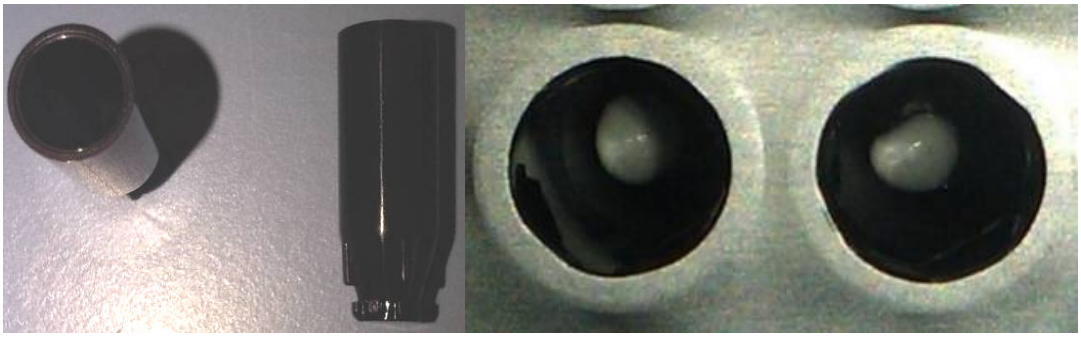

Fig 13. Production-ready freeze-dried reagent pot (left), RANGER RT-PCR reagent Lyosphere® beads.

The RANGER assay 'beads' were subjected to an analytical performance evaluation within EDL and HPA laboratories to establish the suitability of the final prototype assay prior to final validation activities (Work Package 8).

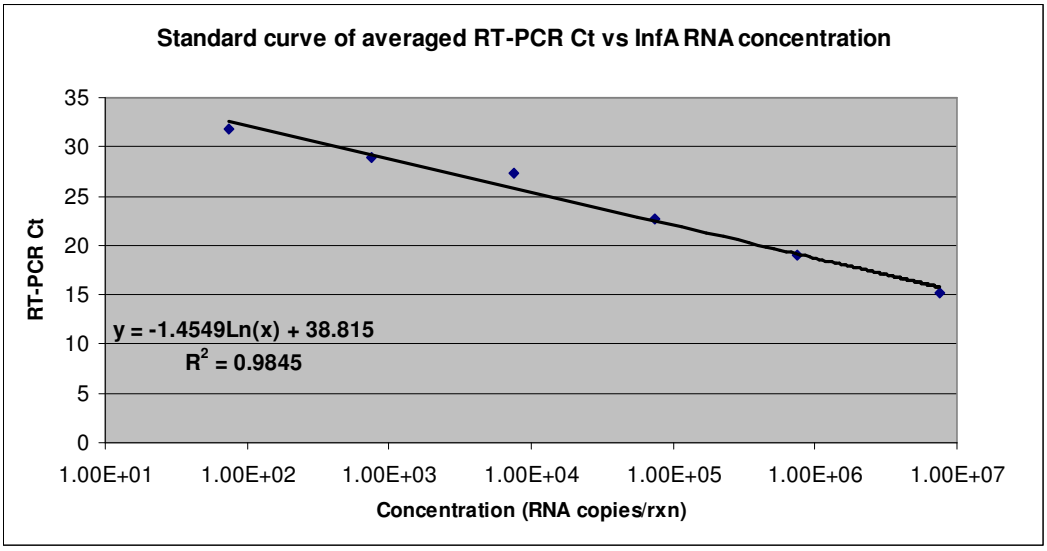

Fig 15. RANGER RT-PCR 'bead' analytical performance - determination of InfA PCR detection linearity

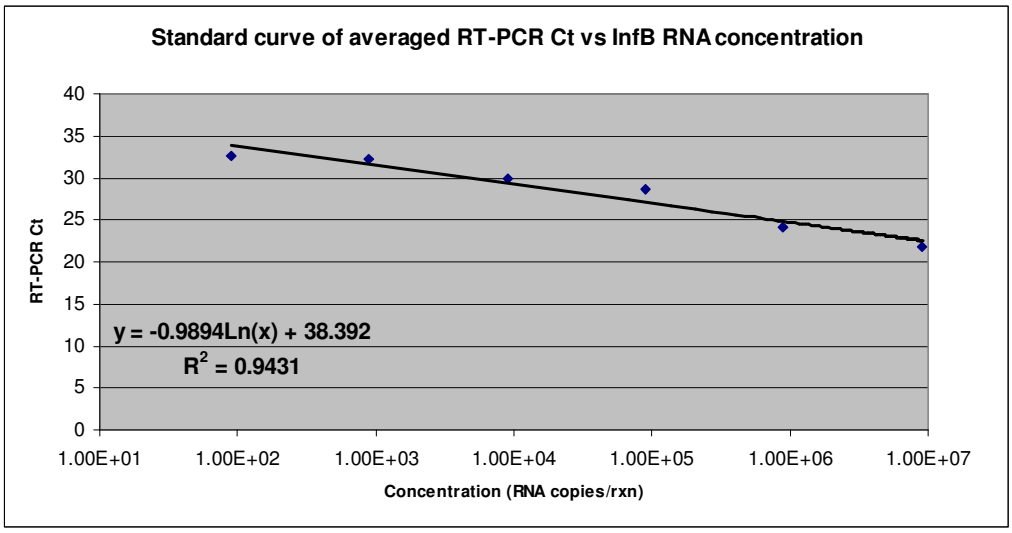

Fig 16. RANGER RT-PCR 'bead' analytical performance - determination of InfB PCR detection linearity.

Having established the analytical performance of the lyophilised RANGER assay, formal stability studies were conducted to verify that the requirement for >12 month ambient shelf-life could be successfully achieved by the product. These were conducted at 25°C and 40°C to assess real-time and accelerated stability as a function of maintaining RT-PCR performance over the storage period. Studies are ongoing, however >16 weeks stability at 40°C has been demonstrated equating to a predicted shelf-life in excess of 18 months. This gave significant confidence in the ability of the RANGER system to be deployed to remote locations where limited cold-storage facilities have previously been prohibitive to application of molecular diagnostic techniques. This has significant benefit for decentralized testing in the developing world where diagnosis and patient management are typically performed in mobile clinics and outreach centres.

WORK PACKAGE 6 – INFORMATION TECHNOLOGY SYSTEM

Work package 6 was structured to develop the information technology systems needed to control the Enigma®ML sub-systems, provide the graphical user interface (GUI) for system operation and develop the communication protocols needed to present data to a locally placed operator, and provide connectivity with Laboratory Information Management Systems (IMS). Transmission of data for wider surveillance and monitoring purposes was provided through the development of a bespoke Influenza Surveillance Management (SM) software package.

The software development process followed EN 62304: Medical Device Software – Software Life-cycle Process throughout the project. This standard maps out the development deliverables and activities required to create assured software components suitable for use in clinical/ medical equipment, critical for CE-marking as an *in-vitro* diagnostic device (IVDD). These guidelines were applied to all workstreams such as:

- Development of embedded firmware executing on the RANGER Control Module (CM)
- Development of embedded firmware on each RANGER Processing Module (PM)
- Development of the RANGER Graphical User Interface (GUI)
- Development of the spectral deconvolution and result calling algorithms

- Development of automated RANGER assay mechanical/ thermal/ optical programmes
- System integration, architecture verification and validation activities

To minimise development time and regulatory risk, the Enigma®ML instrument is driven by a QNX Neutrino embedded operating system (Standard Runtime with a Photon microGUI and a Mass Storage File System); an off-the-shelf operating system used in other CE-marked and FDA medical devices. This enabled the software development team to focus on the business and application layers of the Enigma®ML instrument, rather than bespoke coding for lower level software components. Conducted in parallel to the instrument hardware development, sequential system software release facilitated early engineering and assay related development activity required to fully characterise the mechanical, optical and thermal control requirements/ dependencies of the total system. This staggered release also allowed for refinement and enhanced functionality through continuous feedback, whilst allowing progressive debugging to accelerate other system integration activities (WP7)

Development of the RANGER Graphical User Interface (GUI)

A simple, intuitive, touch-screen GUI was essential to ensure that the Enigma®ML instrument was easy to use and could be operated without ancillary equipment (such as a PC) with minimal training burden and no specialist technical knowledge. As part of WP1 Voice-of-Customer (VOC) research, 20 detailed face-to-face interviews with potential end users (clinicians and health care professionals) were conducted to guide development of the graphical user interface. Using UML design techniques, design concepts generated under-subcontract by Areteworks Ltd were coded by MPC-Data Ltd software engineers - both groups experienced in medical device software development. The RANGER GUI was successfully coded onto the Enigma®ML Control Module as a fully functional touchscreen interface.

Fig 17. Areteworks RANGER GUI initial design concepts (left), fully functional GUI coded and embedded as a touchscreen interface on the Enigma®ML control module (right).

The GUI navigation logic ensures that the user is prompted through all test procedures involved in test set-up and execution before being presented with an unambiguous Influenza diagnostic result as both electronic (visual display and data file) and hardcopy (2" thermal print-out) formats. The system is fully operable from point of sample insertion to test result without any need for dexterous interactions or data interpretation such that, in future, the RANGER test could also be submitted against FDA CLIA Waiver regulations (in addition to the intended IVD CE marking activity).

To ensure the regulatory requirements on test traceability of the RANGER assay process are fulfilled, the GUI requires a number of successive information inputs (entered by barcode or virtual keyboard) such as Operator ID, Cartridge ID, Sample/ patient ID etc. The overall information input options are customisable depending on regional requirements. The system also provides hierarchical user access to ancillary functions including local/regional settings (language, DTG etc) performing QC tests, UV decontamination functions, recall of historical test results and data transfer options etc. In VoC studies, the user interface has proven simple and intuitive to use, highly responsive and very stable. The software has successfully met all specific requirements defined in the RANGER System Requirements Document (SRD).

Development of the Surveillance Management (SM) software package by Bruhn proceeded in parallel to the instrument development programme. Designed around Bruhn's existing SM capability in Chemical, Biological, Radiological, Nuclear and Explosive (CBRNE), the RANGER SM software if an off-board tool used to capture Influenza sample analysis data from the Enigma®ML instrument. The Influenza result data is then processed and presented in the RANGER SM programme using an embedded GIS (Geospatial Information System) to geographically monitor outbreak events.

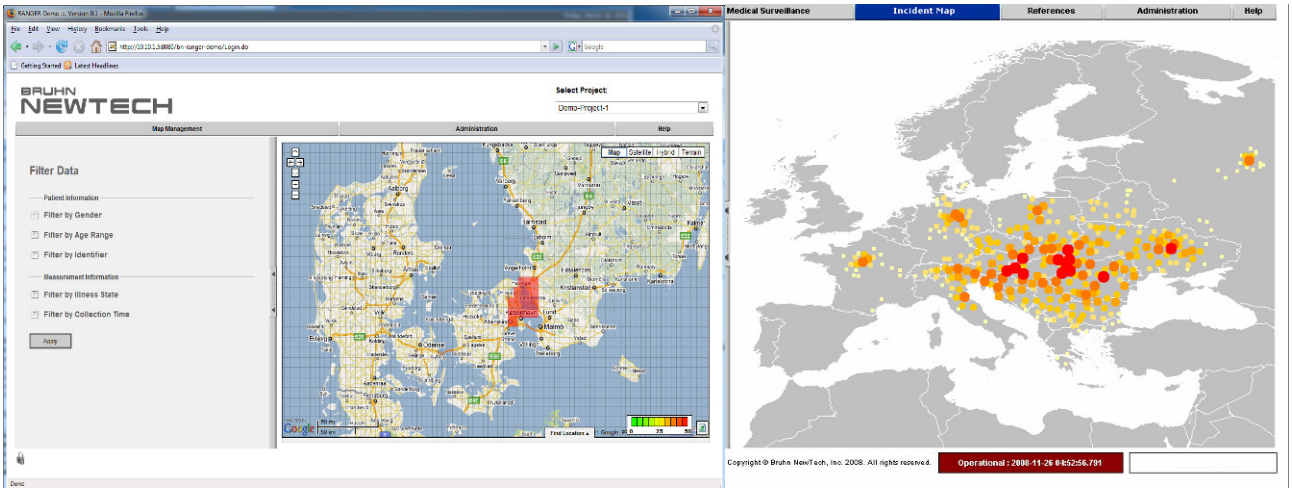

Fig 18. RANGER Surveillance Management software - GIS tracking of a simulated Influenza pandemic in Central and Eastern Europe.

The RANGER SM software will be a powerful epidemiological tool as different data filters can be applied based on certain criteria such as patient age, gender, symptoms and sample time. These

filters will allow Communicable Disease Control centres (CDCs) real-time access to surveillance data, rapidly identifying 'at risk' patient groups. A playback feature is also provided to monitor how disease spread evolves over time with significant benefits to tracking and management of future novel pandemic influenza viruses.

WORK PACKAGE 7 – SYSTEM INTEGRATION

The system integration work package was the concluding phase of all RANGER R&D activities. Specifically, WP7 drew together all the individual work packages into the prototype RANGER diagnostic system. This integration activity unified the following elements of the RANGER project:

- Final unification and testing of the Enigma®ML Control and Processing Modules
- Embedding of all higher and lower level software functions with the combined instrument
- Implementation, optimisation and testing of the RANGER assay test programme
- Confirmatory testing of total system functionality and pre-validation performance
- Colour compensation and result calling algorithm test harness data generation

During the development programme, integration of the Enigma®ML CM and PM subunits was a continuous activity as the CM provided power and communication management for multiple PM subunits (maximum of 6), as such the two could not be developed independently. This approach significantly de-risked the WP7 integration activities during the closing phases of the RANGER project allowing the focus on integrating the RANGER assay cartridge with the mechatronic and optoelectronic interfaces of the system to perform fully automated sample analysis.

Fig 19. Enigma®ML CM and PM system integration for a single-module system.

Cartridge integration was an iterative process as it was necessary to write programme scripts for the 4-axis robotic positional control needed for manipulation of the cartridge tools (foil piercer, pipettors etc) before defining the actual test process conditions needed (frequency & duration of wand mixing, magnetic bead acquisition period etc). These were established as a prototype test routine to allow further characterisation and optimisation of the integrated system.

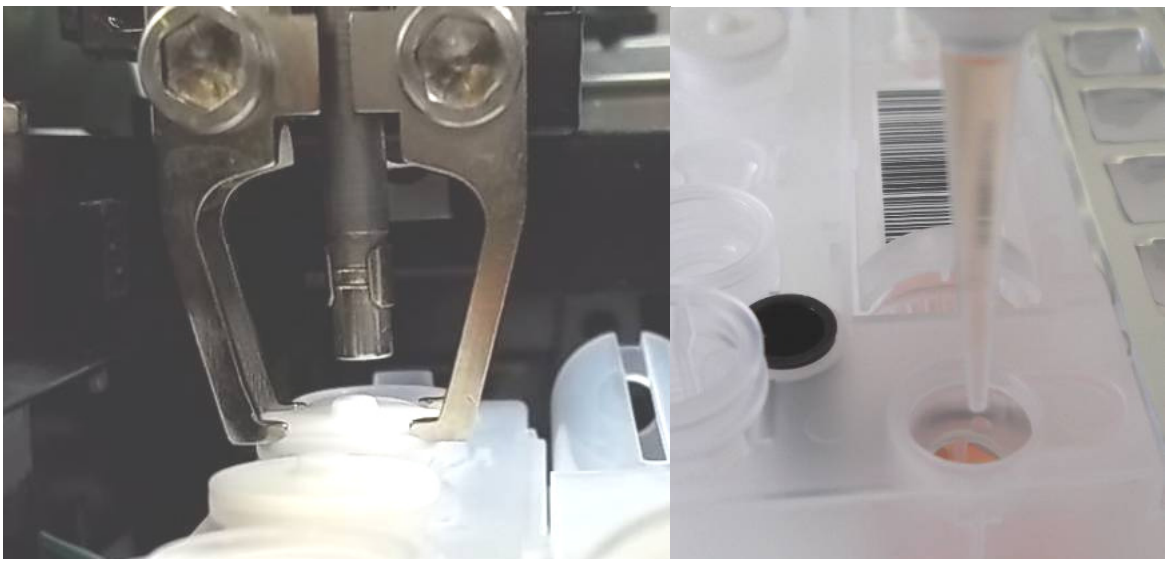

Fig 20. Positional optimisation of the Enigma®ML robotic grabber arm used for tool manipulation (left), example of automated pipette dispensing of 100µl of sample fluid into the MagaZorb® extraction (right).

In addition to the mechatronic elements of the system, it was necessary to extensively characterise the thermo-optical properties of the system needed for sensitive PCR and melt analysis. Significant benefit was gained using off-board orthogonal analysis tools to optimise and verify the optical and thermal precision of the Enigma®ML.

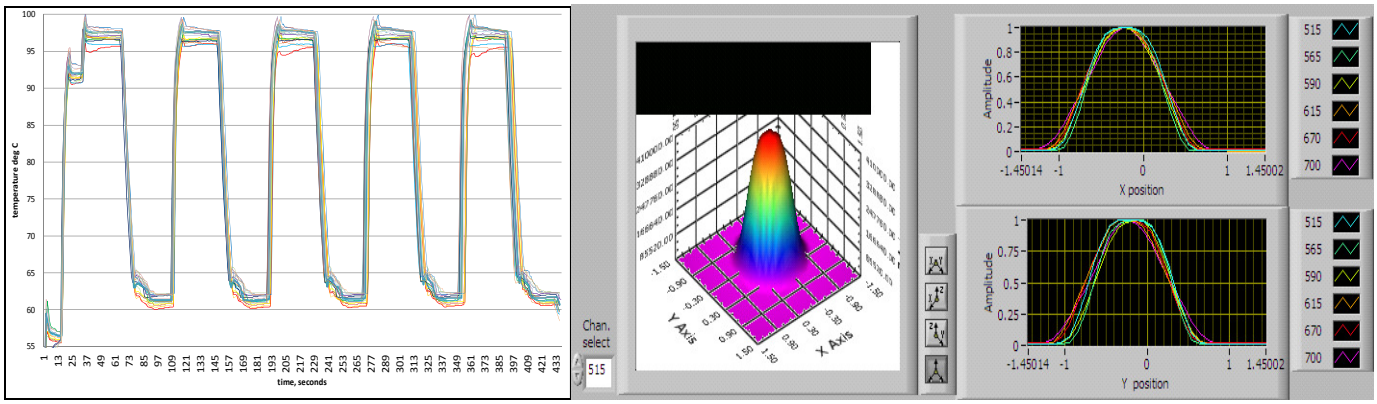

Fig 21. Characterisation of Enigma®ML PCR thermo-cycling accuracy & precision (left), ECP positional dependency of the Enigma®ML solid-state optics block (right).

Qualification of the sample extraction and RT-PCR functionality at the mechanical, thermal and optical performance level, system integration focussed on refinement of the automated test routine to replicate intended use. This included a comprehensive evaluation of sample extraction performance and RT-PCR performance. PCR data was then used to develop, train and finalize the colour compensation and PCR/ melt data analysis algorithms needed for automated diagnostic result 'calling'.

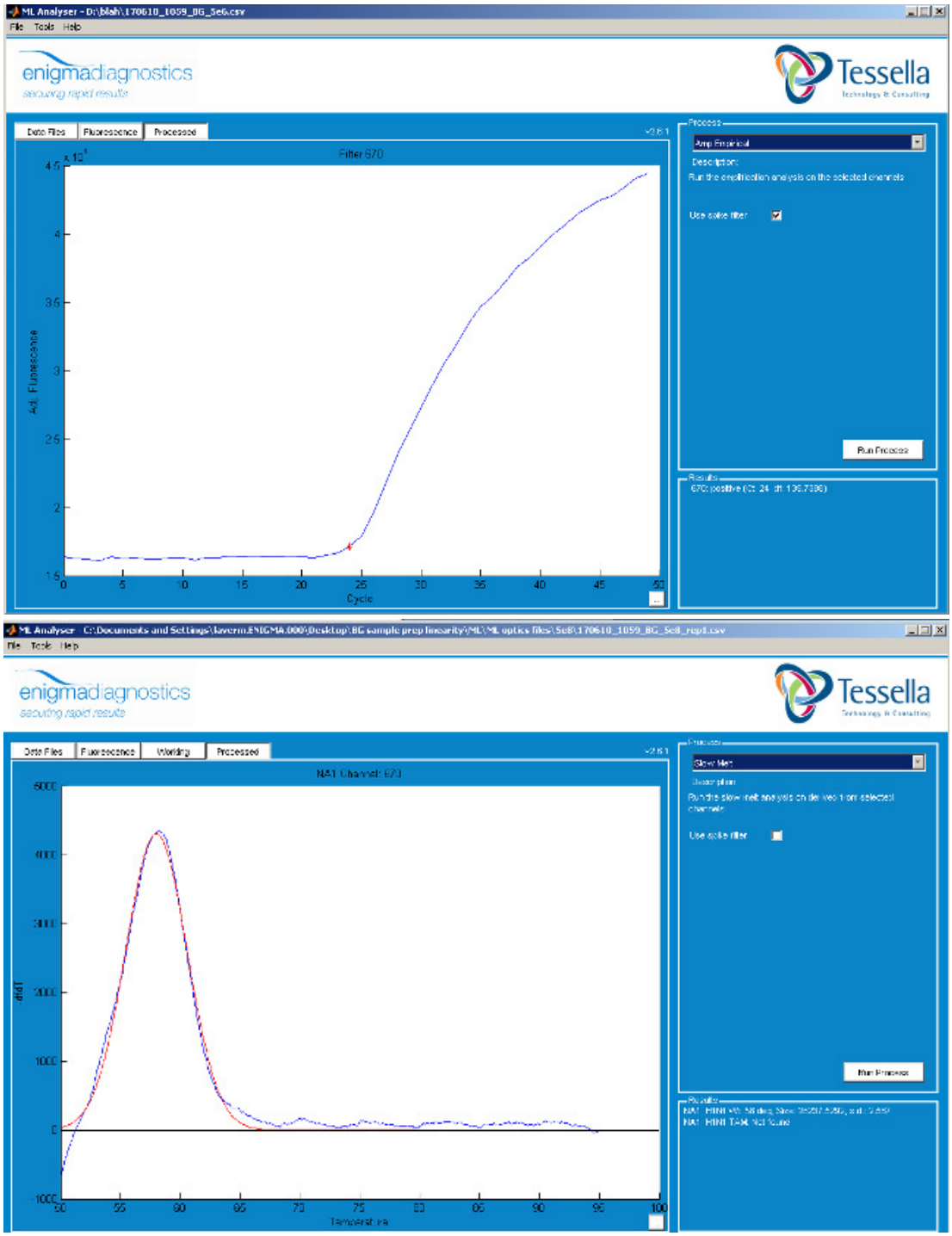

Fig 22. Training and verification of the Enigma®ML colour compensation and result calling algorithms using PCR amplification data (top) and melting peak data (bottom) required for automated interpretation and diagnostic result presentation to the operator.

The prototype result calling algorithm proved to be both sensitive and intuitive in RT-PCR and melt analysis data processing, demonstrating reliable result calling as part of the fully-automated RANGER test routine. To determine the level of diagnostic result variability across the prototype systems, a large testing programme was undertaken to characterise intra-module repeatability and inter-module reproducibility. Each module tested performed comparably in terms of sample preparation performance and RT-PCR sensitivity (example data shown in figure 23).

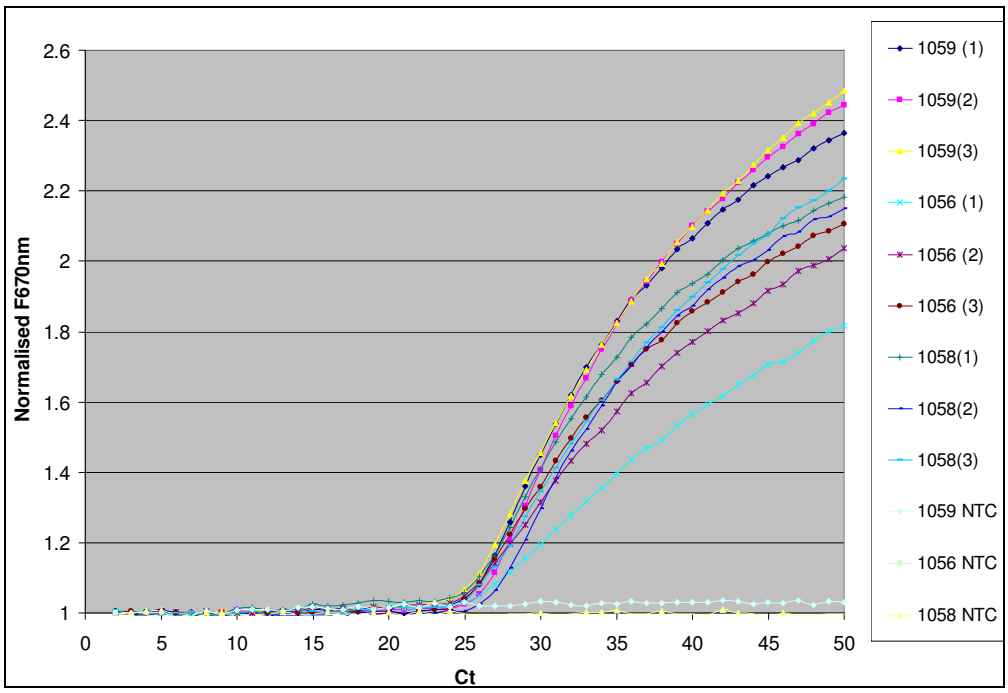

Fig 23. Inter- and intra-module PCR reproducibility testing on a three module Enigma®ML instrument to verify process reliability, functional performance and algorithmic result calling accuracy.

Once fully integrated and function performance of all core functions established, the RANGER assay protocol was optimised to enable the entire test routine to be conducted in less than 60mins to meet the desired 1 hour sampler to result end-user specification. This required rationalisation of all mechanical processing functions and PCR thermocycling conditions such that the assay could be run in the shortest period without impacting on analytical performance of the test. This was successfully achieved, the six module (higher throughout) instrument providing the capability to run 36-42 fully-automated diagnostic tests during a typical working day.

Fig 24. Enigma®ML CM and PM sub-system integration to a 6 module, higher-throughput diagnostic system.

The fully-automated RANGER diagnostic testing procedure from patient sampling to diagnostic result can be summarised schematically as follows:

Fig 25. RANGER Seasonal Influenza test procedure on the Enigma®ML instrument.

1. Clinical takes nasal/ nasopharyngeal swab sample from patient
2. Sample inserted into COPAN UTM sample collection tube and sealed
3. Sample collection tube inserted into Seasonal Influenza A/B assay cartridge
4. Cartridge presented to Enigma®ML bar-code reader for assay type and cartridge QC check
5. Operator ID and patient information entered via barcode reader or virtual keyboard through GUI
6. Test cartridge inserted into Enigma®ML for fully-automated sample preparation and RT-PCR
7. Real-time test status indicator with test information summary and predicted time to result
8. Algorithmic result calling and visual result presented to operator/ transmitted to LIMS system
9. Hard-copy 2” thermal print-out of test result for paper records

Having established the core system optima for the automated RANGER test procedure as part of the integration work package, significant effort was expended in functional reliability and in-service life testing. This involved rigorous testing of the core mechatronic components such as the instrument tray, grabber arm, heating probe etc to understand any possible failure-modes, wear rates and the predicted routine service/ maintenance schedules for the instrument. System stability and mechanical reliability testing indicated that the Enigma®ML would require only one scheduled annual service and that no user calibration or servicing was required (critical to Point-of-Care use and any future FDA CLIA waiver submission).

To verify product safety of the Enigma®ML prior to design freeze and finalization of the manufacturing datapack, early Electromagnetic Compatibility (EMC) testing by TÜV was conducted on the prototype system in preparation for formal CE safety certification of the final production system. Minor design changes were required for total EMC compliance and were successfully implemented in the final production/ commercial system design. The Enigma®ML should therefore attain CE marking (EMC) and CE marking (IVD) in Q1 2012 once the safety and regulatory compliances have been formally demonstrated.

WORK PACKAGE 8 – VALIDATION

Work Package 7 integration activities defined the RANGER test programme and key performance metrics needed to validate the Enigma®ML instrument for clinical use as a PoC medical device. To validate the system (WP8), it was necessary to confirm the essential CE marking requirements from the IVDD (98/79/EC) had been met (in principal) for future regulatory submission of the productionized system (post-RANGER project). This meant the system had to be assessed against the original RANGER User Requirements Specification (URS), Functional Requirements Specification (FRS), Detailed Design Specification (DDS) and Intended Use claims. In addition, it was necessary to complete the ongoing risk management and hazard analysis process used in defining the system architecture and system requirements.

To validate the diagnostic system, a multi-centred approach was adopted with sites encompassing clinical settings in both developed and developing world countries. The validation programme had 3 distinct workstreams:

- Instrument validation - formal engineering/ scientific validation of the system (EDL)
- Proof-of-principle clinical testing - developed world (HPA)
- Proof-of-principle clinical testing - developing world (QSNICH)

Instrument validation

Undertaken within EDL's laboratories, the Enigma®ML was subjected to a focussed and rigorous Master Validation Plan (MVP) to cover all functional and performance metrics associated with the system and the RANGER Seasonal Influenza A/B test cartridge. Briefly, this involved validation of all mechatronic, optical and thermal sub-system functionalities against defined metrics (specified in the SRD). System validation began with qualification of all sensory components of the system used in failure-mode prevention and human error-proofing of the system.

Bar-coding

Confirmation that the system could reliably interpret cartridge, sample ID, user ID bar-codes presented to the external bar-code reader as part of the test set-up procedure. This process checks the correct cartridge type is being presented to the system for the intended test type (i.e. a Seasonal Influenza cartridge for Seasonal Influenza testing) and checks the cartridge expiry

date has not lapsed as part of a QC procedure. The instrument then 'dials-up' the correct test protocol required for that cartridge/ sample type. A secondary check using an internal barcode reader is performed to confirm that the cartridge indentified to the system during test set-up, is identical to the one placed into the instrument for testing. This negates potential human error associated with confusing samples/ cartridges during the test routine. Validation of the bar-code functions successfully demonstrated that test set-up and error proofing was 100% reliable (fig 26).

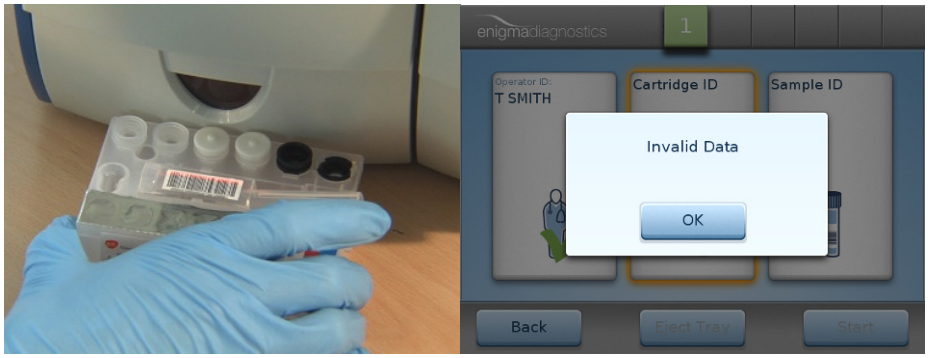

Fig 26. Barcode identification of a RANGER assay cartridge (left), detection of an incorrect cartridge (right).

Swab and sample detection

To protect against an operator failing to insert a test swab into the cartridge during RANGER assay set-up, the Enigma®ML swab detect function automatically verifies the presence of a swab within the sample tube prior to test execution. In addition, a secondary sensor verifies that during processing, sample was successfully released from the UTM sample tube. Both sensors provide protection against false-negative (no sample) results and proved to be 100% reliable during the validation package.

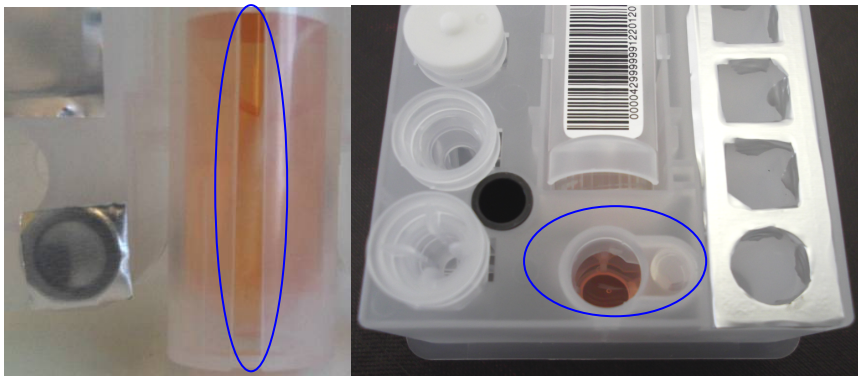

Fig 27. Swab detection within the UTM sample collection tube (left), detection of test sample correctly released into the cartridge sample well (right).

Where processes (such as pipetting, lysis heating, PCR thermocycling etc) had a tangible volume/ temperature accuracy and precision metric, these were measured orthogonally to qualify system performance and validate against the original system specification. All critical performance metrics were successfully achieved.

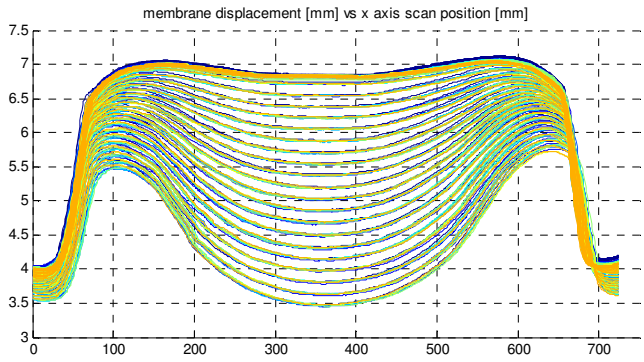

Fig 28. Pipette diaphragm elasticity and displacement during volumetric dispensing validation.

Validation of sample preparation

The MagaZorb® sample preparation chemistry within the RANGER assay cartridge was evaluated for its ability to remove potential interfering substances from a test sample. This was validated by spiking UTM samples with enumerated Influenza virus in the presence of a range of prescription and off-the-shelf medicinal compounds typically used during a symptomatic influenza infection. Samples were tested on the Enigma®ML, the automated sample preparation process successfully removing interfering substances without any significant impact on the performance of the RANGER test.

Validation of RT-PCR analytical sensitivity and specificity

Pre-clinical analytical performance was validated to confirm sensitivity and dynamic range of the Seasonal Influenza A/B test against the LightCycler® 2.0 (Roche) development platform using Influenza A/PR/8/34 (fig 29) and Influenza B/Lee/40 reference strains.

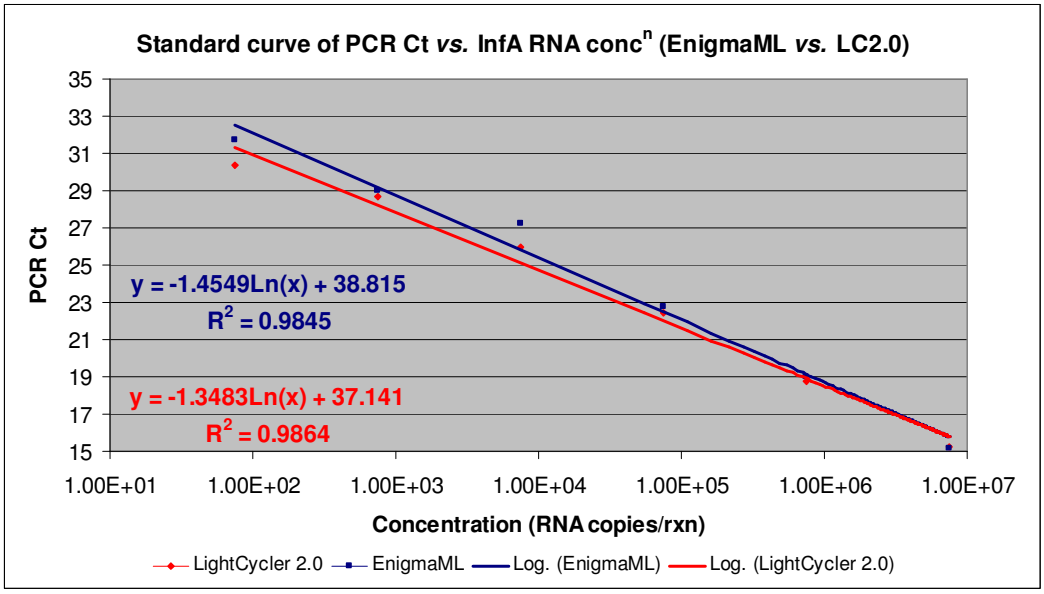

Fig 29. Validation of Enigma®ML PCR sensitivity and dynamic range of detection for Influenza A virus.

Having achieved a comparable performance to a 'gold-standard' real-time PCR thermocycler, the data confirmed that the RANGER product was likely to match or improve upon the performance benchmark of the Prodesse ProFlu+ predicate test during future formal clinical trials. To confirm analytical specificity as a function of pan-reactivity and absence of cross-reactivity, the RANGER system was evaluated by the HPA.

A pan-reactivity panel of 62x Influenza A and 22x Influenza B strains was selected for analytical testing, encompassing a number of recommended strains identified from competitor FDA 510k submission documents for comparable influenza diagnostic assays (such as Prodesse ProFlu). In addition, a much wider spectrum of influenza strains was tested to gain additional confidence in the global applicability of the assay to detect commonly circulating variants of type A and B influenza strains. All influenza A and B strains tested were tested positive by the RANGER assay confirming its pan-A and pan-B intended use. Analytical specificity (cross-reactivity) was validated using a panel of 53 non-influenza organisms, successfully confirming the specific nature of the assay and absence of false-positive results in the presence of high background levels of other micro-organisms.

Clinical evaluation (developed world)

A proof-of-principle clinical evaluation was conducted in the UK (HPA Porton Down) to evaluate the clinical performance of the prototype Enigma®ML system with the RANGER assay. This was conducted using 298 clinical samples obtained from King's Hospital, London. Original diagnostic test data was available, 58 of the sample originally reported as influenza A positive, 5 influenza B positive and 9 others being identified as other respiratory viruses or 'influenza negative' samples. Repeat testing of these samples on the Enigma®ML successfully identified 40 as influenza A positives, 31 of these results were consistent with the original diagnostic test. The ML was able to detect 2/5 expected influenza B samples. Data from this study will be used to refine/ improve algorithmic result calling prior to formal clinical testing for regulatory submission planned in 2012.

Clinical evaluation (developing world)

It was not possible to conclude clinical evaluation of the Enigma®ML system at the developing world trial site within the RANGER project timeframe. However, during the project 300 children from Queen Sirikit National Instutitue of Child Health and 300 adults from Rajavithi Hospital (RH) were recruited with suspected influenza infection. Patient samples were stored at -80°C for future testing (outside of the RANGER programme) to conclude the developing world clinical site activities, data which may be used to support future regulatory application.

Overall, validation data raised confidence in the performance of the system to meet future regulatory submission activities and that the Enigma®ML system would fully satisfy an existing market for fully-automated, Point-of-Care diagnostic test equipment capable of generating laboratory standard results.

The potential impact and the main dissemination activities and exploitation of results

The RANGER project work packages have successfully generated the Enigma®ML instrument and RANGER Seasonal Influenza A/B assay cartridge as specified as the overarching project deliverables. The first system of its kind, the Enigma®ML was designed for Point-of-Care and decentralised testing environments by fully-automating the procedures of complex molecular tests, making them operable by minimally skilled users in non-laboratory settings. The major impacts of the project are:

- The Enigma®ML will ensure the EU is better prepared technologically, to manage emerging infectious disease pandemics/ epidemics such as the 2009 H1N1 swine-lineage influenza global outbreak.

- The Enigma®ML will permit rapid disease identification and surveillance by removing the necessity for specialist laboratory facilities and skilled operators. In time-critical scenarios, this obviates the need to courier samples to centralised laboratories which may be some distance from the point-of-test.

- The Enigma®ML was designed for global application in both developed and developing countries. With a low cost-of-goods, the RANGER project will improve accessibility to advanced diagnostic technology and assist in global method standardisation.

- Development of lyophilisation technology could be applied to other molecular diagnostic reagents that could be routinely stabilized for ambient storage. This will reduce the carbon-footprint and environmental impact associated with refrigerated transport and storage chains.

- Involvement of partners from East-Asia helped to strengthen international collaboration in infectious disease research which will be beneficial to future collaborative effort and co-ordinated responses.

- The high proportion of EU-based SMEs involved in the RANGER project helped to maintain and improve the competitiveness of the European pharmaceutical and biotech industry in the disease surveillance field.

Beyond Seasonal Influenza surveillance, the Enigma®ML and consumable cartridge have sufficient flexibility for other PCR assay 'content' to be added as part of broader commercialisation of the system. Currently, 25% of worldwide deaths each year may be directly attributable to infectious disease according to World Health Organization (WHO) data. Discussions with clinical partners and key opinion leaders indicate that the technology developed during the RANGER project is directly applicable to a number of secondary markets:

- Food borne diseases (Salmonella, Campylobacter, Listeria)
- Water borne disease (gastroenteric viruses, cholera, typhoid)
- Hepatitis viruses
- Tuberculosis
- Sexual health (Chlamydia, Gonorrhoea)
- SARS (severe acute respiratory syndrome) corona virus
- Hospital-acquired infections (MRSA, *C.difficule*)
- Bacterial sepsis

All the above areas of human infectious disease have a comparable need to that of Influenza diagnostics, namely; rapid, sensitive laboratory standard results at the point-of-care. As such, significant commercial effort at EDL has been undertaken to understand the market-needs for future content beyond the RANGER project. The point-of-need compatibility of the system has also alerted tertiary (non-human diagnostic) markets which have expressed interest in its potential application in industrial monitoring, veterinary pandemic diseases etc:

- Avian influenza
- Foot & mouth disease
- Newcastle disease
- Swine Fever
- Biothreat threat agents in defence & homeland security applications
- Detection of microbial contamination in pharmaceutical products and foodstuffs

Overall, the technology developed during the RANGER project provides for the first time (i) a robust and reliable point-of-care diagnostic solution, accessible to both the developed and developing worlds (ii) surveillance tools for tracking and information management during an influenza epidemic to aid disease control and patient management. Both these technologies will allow public health authorities to be better prepared for detecting emerging disease threats and make better informed and timely mitigation decisions critical to disease control.

Dissemination activities

The primary target populations of RANGER dissemination activities are the predicted customers and end-users, namely international and national health care authorities and primary healthcare hospitals within the developed world. Once established as a commercial product and clinical market uptake has been successfully demonstrated, activities will then focus on dissemination to developing world nations. The technical/ engineering knowledge developed during the project was disseminated to consortium members, interested parties and end users of the Enigma®ML through links already fostered with influenza diagnostics and community focussed organisations. Beyond influenza, dissemination and exploitation will also ensure optimal use of the RANGER project results for alternative applications in secondary and tertiary markets. These will include point-of-care diagnostics for other viral and microbial infection diseases (human and veterinary applications) as well as analysis of microbial contamination in foodstuffs and pharmaceutical processes.

In all instances, multiple dissemination media formats were used to optimally communicate with the wider industry:

Project website (see below) - this was the initial hub for consortium progress communication and advertisement of the project to non-consortium members.

Pre-market stimulation activities - During the RANGER project, the prototype Enigma®ML was showcased through 'live' technology demonstrations to government officials, industry representatives, academic opinion leaders and healthcare professionals:

- Abu Dhabi Medical Congress (17 – 19 Oct 2009)
- Medica (Nov 2009, Dusseldorf)
- Arab Health (Jan 2010, Dubai)
- Molecular Diagnostics (Nov 2009, Beijing)
- American Association of Clinical Chemists, Laboratory Expo (25 – 29 July 2010, Anaheim)
- InnovHealth Summit 2010 (April 2010, Abu Dhabi)
- Reducing HAIs Conference (June 2011, London)
- American Association of Clinical Chemists, Laboratory Expo (26 - 28 July 2011, Atlanta)

Press releases on the project to trade journals -

- Article written by EDL published in Genetic Engineering & Biotechnology News, April 2009.
- Enigma announces successful completion of EU Framework 7 RANGER project
- Enigma announces the start of US Clinical Trials for Influenza A/B detection assay (11/4/11)
- Enigma Diagnostics to Feature the Enigma ML at the AACC Expo in Anaheim (21/7/10)
- Enigma Showcases the Enigma ML at InnovHealth Summit 2010 in Abu Dhabi (22/4/10)
- Tecan & Enigma sign global manufacturing & supply agreement for ML instrument (5/2/10)
- Enigma to Demonstrate Rapid Detection of Swine H1N1 Influenza at Arab Health 2010 (15/1/10)
- Enigma Diagnostics Showcases Enigma ML (Mini Laboratory) at Medica 2009 (18/11/09)
- GSK & Enigma Announce Commercial Partnership to Develop Point-of-Care Influenza Diagnostics (21/7/09)
- Press releases on initiation, cited by online press such as Genome Web News
- Partner Press Releases on award/initiation of the project

Preparation of product and project brochures, posters - detailing the diagnostic system, its benefits and the format of use for potential collaborators and end users.

- HPA presented abstract at HPA Annual Conference, Sept 2009
- Poster presented at XI International Symposium on Respiratory Viral Infections February 19 - 22, 2009 Bangkok, Thailand

- QSNICH presented poster abstract to 7th World Congress of the World Society for Pediatric Infectious Diseases, November 2011

Promotion through profession bodies, networks and trade associations

- World Health Organisation (WHO)
- European Diagnostic Manufacturers Association (http://www.edma-ivd.be/)
- Association of Medical Diagnostic Manufacturers (http://www.amdm.org/default.cfm?id=87)
- British In Vitro Diagnostics Association (http://www.bivda.co.uk/)
- European Society for Clinical Virology (http://www.escv.org/)
- Medicines and Healthcare products Regulatory Agency (MHRA)

Due to the requirement to protect the Intellectual Property underpinning the Enigma®ML system, consumable cartridge and test assay, it was not possible within the project timescales to conduct technical authorship of papers for peer-reviewed journals until patent applications covering sensitive information were been granted. However, once clinical data is available, further promotion the clinical results of the system will be undertaken in learned journals.

Exploitation of results

The global market for *in vitro* diagnostics (IVDs) is estimated to be worth more $40bn, however the market is heavily segmented market with diagnostic products ranging from highly automated centralized laboratories to simple tests that can be performed in the home (figure 29).

IVD Market Segments	
Market Segment	**Description**
Clinical chemistry tests:	Used to test blood, urine and other bodily fluid samples for the condition of a patient's liver, kidney, heart or thyroid;
Immunochemistry:	To analyze proteins in order to diagnose a patient's immune system. Used for cancer, fertility, thyroid, anaemia and cardiac testing;
Haemostasis/Haematology	For blood cell analysis, such as measuring a patient's ability to form and dissolve blood clots before and during surgical procedures, or for monitoring coagulation disorders such as haemophilia
Microbiology	To identify infection-causing bacteria and detect emerging antimicrobial resistance;
Infectious Immunology	Used to screen for infections such as hepatitis, HIV and sexually transmitted diseases;
Point-of-care Testing	Tests used at the patient's bedside in hospitals, in doctors' offices and by the patient at home. Commonly-used home tests include blood glucose monitors and fertility/pregnancy tests. Near-patient tests used by physicians and healthcare providers include tests for blood gas/electrolytes and cardiac assays;
Diabetes Monitoring	Tests used by patients and healthcare professionals to monitor blood glucose are commonly treated as a discrete product segment within the point-of-care market;
Molecular Diagnostics	Gene-based tests that allow earlier detection of disease, selection of appropriate therapies and monitoring of disease progression. Currently, molecular diagnostics are most commonly used to detect infectious diseases, such as HIV and sexually transmitted infections.

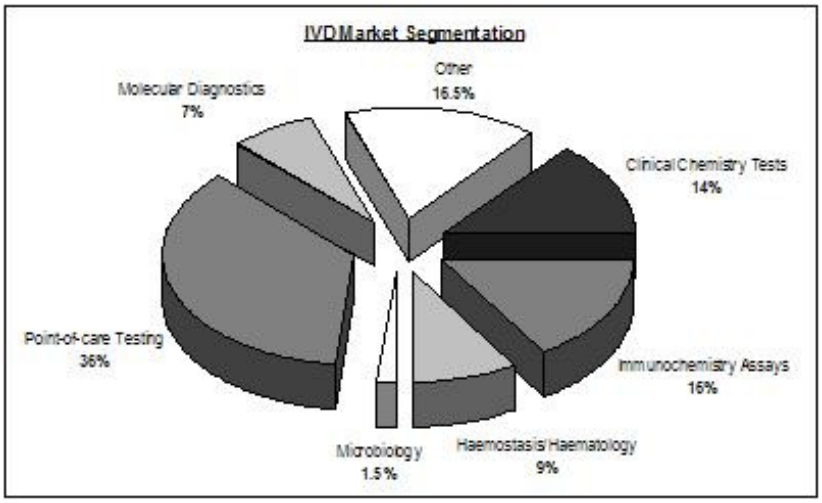

Fig 30. IVD market sector breakdown

Marchant J. **2006**. Innovations in Diagnostics: Next generation molecular and point-of-care diagnostics driving personalized healthcare.

Currently the largest market segment is point-of-care diagnostics, which includes markets such as glucose monitoring for diabetes, with 36% of the market and recorded sales of approximately $12bn in 2005. Point-of-care diagnostics is also one of the fastest growing market segments with an estimated compound annual growth rate (CAGR) of 7.8% to 2010. However, molecular diagnostics, of which infectious disease diagnostics accounts for 75%, is currently the fastest growing IVD sector with a CAGR of 14% to 2010. This growth has been primarily driven by advances emanating from the human genome project and despite only having 7% of the IVD market, in 2005 sales of molecular diagnostics were recorded at $2.6bn, with sales reaching > $5bn in 2010. Therefore, significant commercial exploitation of the RANGER project technology exists within the EU and the wider global diagnostic markets.

The Enigma®ML system will penetrate into all four major markets, Europe, North America, and Asia Pacific and the Middle East & Africa (MEA) during 2012/13. Designed to comply with the requirements of ISO13485 (the global medical device industry quality standard), the Enigma®ML has a lower regulatory risk than other laboratory systems adapted for clinical use. As such, attaining regulatory approval eg: IVDD or FDA CLIA waiver on a worldwide basis is likely to be less challenging.

The 'reagent rental' business model is standard in the diagnostics industry where the capital cost of the Enigma®ML instrument is amortised across the number of tests sold over a two or three year period. This eliminates the requirement for a capital budget and stimulates uptake of the platform in the developed world whilst providing accessibility to developing countries. If the instruments were to be sold stand-alone, the target cost for the instrument is <€25,000 for a six-module Enigma®ML instrument. Cost per test for the Seasonal Influenza A/B assay is currently <€15 and with enhanced volume production, <€10 is also commercially viable.

It is envisaged that the following exploitation route will be explored:

Group	Exploitation routes	Time to market (Yrs)
1	Core applications and markets serviced by the proposers 1. Human influenza diagnostics market in EU 2. Human influenza diagnostics market in developing countries 3. Human influenza diagnostics market in US	 3 4 3
2	Secondary applications to be realised by the proposers 1. Human *in vitro* diagnostics for other infectious disease testing 2. Veterinary diagnostics for influenza (including H5 N1) & other pandemic diseases	 5 3
3†	Non-core applications for specific project results for exploitation by third parties. • Laboratory research (academic and the biotech/pharmaceutical industries) • Food and drink microbial contamination testing in EU, US, MEA & PAC	 5 5
† New knowledge, technological capabilities and know-how will be made available through licensing agreements.		

Enigma Diagnostics have generated a considerable amount of background IPR prior to engaging in the RANGER project, including some 43 patents, relating to the core technology underpinning the RANGER project in the areas of PCR reagent stabilization, ECP thermalcycling and plastic consumable development. In addition the innovative and technically demanding foreground intellectual property developed in the form of patents, designs (registered and unregistered), copyright, software and know-how was protected throughout as part of the exploitation strategy. Technology licensing and sub-licensing activities may be explored to contribute to wider technology dissemination throughout the industry in future. In particular, it will be necessary to engage in licensing with manufacturing and distribution partners to provide the market access required to achieve the forecasted market penetration.

Project portal

The RANGER project portal can be found at: www.rangerfp7.com