Transforming Routine Laboratory Developed Tests Using Hydrolysis Probe Chemistry into Cassette Based Molecular Assays for use on the Luminex ARIES® System

Sophie Jones[1], Angela Short[2], Laura Gifford[3] and Catherine Moore[3]

1. Public Health Wales Pathogen Genomics Unit. 2. Public Health Wales Microbiology Cardiff and 3. Wales Specialist Virology Centre. University Hospital of Wales, Cardiff, CF14 4XW.

Introduction

There is an increase in the number of rapid molecular diagnostic test systems available, however few can support laboratory developed tests (LDTs).

The Luminex ARIES® is a sample to answer real-time PCR system that can run up to 12 samples in less than two hours. The universal assay protocol allows different sample types and assays to be run simultaneously. The current Luminex ARIES® assays are based on MultiCode®-RTx technology, and although LDTs are supported, most laboratories use chemistry that requires a degree of assay redesign. Luminex have recently developed a new enzyme formulation ARIES® Exo+ Ready Mix that allows the transfer of hydrolysis probe assays to ARIES®.

An evaluation was undertaken to determine how well LDTs transfer, and to scope the utility of the system as a rapid option for a range of targets and sample types.

Methods

- A verification and validation was performed on the ARIES® System using a MERS-CoV in-vitro transcript (IVT) of the *upE* gene (obtained from the European Virus Archive). This was to prove concept prior to transfer of further assays.
- Serial dilutions of the IVT were performed in NucliSENS® lysis buffer (Biomérieux) and TE buffer (Sigma-Aldrich) from log 10^2 to log 10^5, equating to 5,000 to 5 copies of RNA per PCR reaction.
- The IVT dilutions in lysis buffer underwent extraction on the NucliSENS® easyMAG® (Biomérieux) and amplification using the ABI 7500 FAST Real-Time PCR System (Applied Biosystems) in triplicate using local protocols (1) .
- The ARIES® amplification default amplification profile is 50°C for 420s, 95°C for 120s and 45 cycles of 95°C for 5s and 58°C for 8s this is followed by an optional (for hydrolysis probe assays) melt step that will allow concurrent running of MultiCode®-RTx assays.
- The ARIES® assay process is outlined below

  - The ARIES® protocol is set-up using the User Defined Protocol (UDP) application within SYNCT™ (step 1). This is performed just once and the protocol saved for future use.
  - The primers and probe used in the routine assay are added to the lyophilised enzyme mix (final concentration per reaction 0.8µM primers and 0.4µM probe for targets and half concentration for the internal controls) (step 2)
  - The extraction cartridge is clicked into place (step 3)
  - Between 100-200µl of sample (dependant on type) is added to the cartridge (step 4)
  - The assay is run (step 5)
- The ARIES® System was further validated by testing a range of LDTs and sample types, including QCMD panels, which were compared to the corresponding routine assays following the local protocols.
- Assays were multiplexed (initially up to three targets) and compared to the routinely used multiplex assays
- Parallel testing of clinical samples was also performed and the results compared with the routinely used assays (Luminex NxTAG® RPP and LDT).

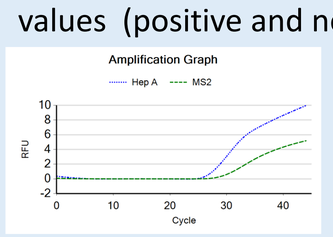
Target	Sample types	Input sample volume
Influenza A (LNA probe)	Respiratory	200µl
Influenza B	Respiratory	200µl
Adenovirus (LNA probe)	Respiratory, whole blood, stool	200µl (resp), 100µl blood and stool
Mycoplasma pneumoniae (LNA probe)	Respiratory	200µl
Hepatitis A	Serum and Stool	100µl
Measles	Respiratory and serum	200µl and 100µl
Enterovirus	Respiratory, whole blood, Stool	200µl (resp) and 100µl (blood and stool)
Norovirus	Stool	100µl
EV-D68	Respiratory and Stool	200µl (resp) and 100µl (stool)
Influenza A typing assays (H3, H1, H5, H7)	Respiratory	200µl
Internal controls (RNAseP and MS2)	All samples	Sample type dependant

Results

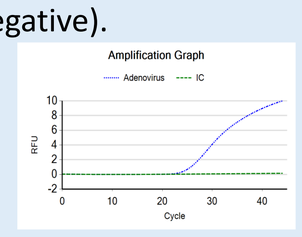
- The MERS-CoV verification showed excellent concordance with the routine process.
- The extraction efficiency was comparable between easyMAG and ARIES using an input volume of 200ul for both the MERS IVT dilution series and the MERS QCMD panel.
- The standard amplification profile defined by the ARIES UDP application proved sufficient to perform all of the LDT assays validated.
- No issues were observed with testing 'complex sample types' such as whole blood, serum and stool supernatant. All assays performing as well as the routine assays.
- Addition of multiple targets has so far proven not to affect the assay sensitivity although work is ongoing to increase the targets multiplexed.
- Parallel testing of clinical samples confirmed the routine assay results with equivalent Ct values (positive and negative).

Dilution Factor (MERS IVT)	EasyMAG extracts ABI 7500 (Mean C_t)	IVT RNA (TE) direct input ABI 7500 (Mean C_t)	ARIES® (Mean C_t)
Log 10^2	28.35	29.28	28.75
Log 10^3	31.81	32.59	32.00
Log 10^4	34.62	36.05	36.15
Log 10^5	37.25	39.44	38.90

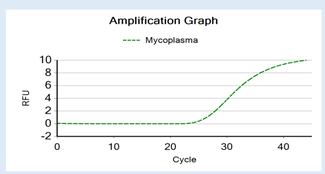
QCMD Sample	ARIES® (C_t)	ABI 7500 (C_t)
MERS 18S-01	20.8	20.63
MERS 18S-02	30.5	29.58
MERS 18S-03	23.8	23.08
MERS 18S-04	27.1	26.27
MERS 18S-05	33.6	32.86
MERS 18S-06	ND	ND
MERS 18S-07	ND	ND
MERS 18S-08	ND	ND



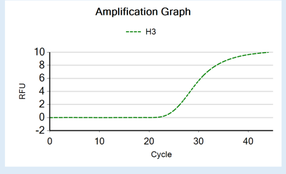
Hepatitis A in stool with IC



Adenovirus in whole blood



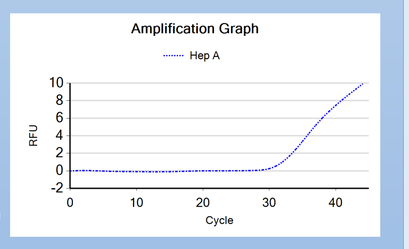
Mycoplasma in respiratory swab

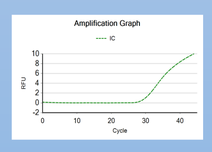


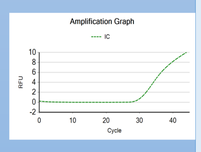
Flu A H3 typing assay multiplexed with H1N1pdm09 virus assay

Case Study – Hepatitis A Virus

- An 8 year old female recently returned from Pakistan presented to A&E with fever, jaundice and right upper quadrant pain.
- Serology showed reactive HAV IgG and IgM.
- 100µl of serum was tested by ARIES® assay on the same day to look for evidence of active infection. This was PCR positive (2)
- Her two siblings were asymptomatic at the time and immunised following the confirmation of the index case.
- Stool sample was collected to determine whether there was active infection in both prior to being allowed in school.
- Both ARIES® and LDT assays confirmed absence of infection within 48 hours of the index case being identified.







Conclusions

- We successfully transferred a wide variety of LDTs using hydrolysis probe chemistry to the Luminex ARIES® system
- The results obtained were equal to the results of our routine assays
- The ARIES® extraction cartridge gave good results for different samples types including respiratory swabs, whole blood, serum and stool
- Multiplexing the assays was a simple process with triplex assays showing no significant reduction in sensitivity when compared to single assays
- From sample to result the turnaround time was <2 hours
- Work is ongoing to further multiplex the assays and to introduce the ARIES® system as part of the laboratory repertoire for rapid molecular testing

1. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction V M Corman and I Eckerle and T Bleicker and A Zaki and O Landt and M Eschbach-Bludau and S van Boheemen and R Gopal and M Ballhause and T M Bestebroer and D Muth and M A Müller and J F Drexler and M Zambon and A D Osterhaus and R M Fouchier and C Drosten, Eurosurveillance, 17, 20285 (2012)
2. Multiplex Hydrolysis Probe Real-Time PCR for Simultaneous Detection of Hepatitis A Virus and Hepatitis E Virus Qiu, F., Cao, J., Su, Q., Yi, Y., and B, Shengli *Int. J. Mol. Sci.*, *15*, 9780-9788; (2014) doi:10.3390/ijms15069780

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