Whitepaper

Next generation multiplexing by LiquidArray®: streamlined diagnostic workflows in the clinical laboratory

Authors: Dr. Jennifer Dougan, Head of Business Unit Syndromic LiquidArrays,
Dr. Vera Allerheiligen, Head of Research and Development, Molecular Diagnostics

LiquidArray is a new microbiological detection technology from Bruker; combining innovative probe and thermocycling approaches with results-at-a-glance software visualization to realize the full potential of multiplexed polymerase chain reaction (PCR) assays. The comprehensive coverage offered by LiquidArray supports clinical decision-making by streamlining diagnostic workflows in the laboratory, providing faster results to the treating physicians. This whitepaper describes these technological advances and highlights how they can contribute to improved detection of mycobacterial, sexually-transmitted, and gastrointestinal infections. At a time when unprecedented demand for disease diagnostics is coming from every corner of the globe, the world's attention is turning to the potential of multiplexing. New molecular diagnostics approaches are faster and more sensitive than traditional culture-based methods, and play an important role in disease management.

Introduction

Molecular diagnostics - such as PCR - are widely used in clinical microbiology to detect and differentiate sources of infection and disease. Multiplex tests and syndromic panels can target multiple pathogens from a single patient sample in a single test - increasing laboratory efficiency and supporting clinical decision making.

LiquidArray is a next generation multiplexing technology from Bruker – delivering comprehensive results from a single patient sample on the high-performance thermocycler, the FluoroCycler® XT.

This whitepaper describes the innovative thermocycling, detection and analysis technologies that underpin LiquidArray and shows how the technology can be applied to the challenges of detecting specific species and resistances of mycobacteria, and offers syndromic panels for sexually-transmitted and gastrointestinal infections. LiquidArray brings next generation multiplexing to the clinical microbiology laboratory, making it more accessible in routine testing.

Next generation multiplexing with a familiar PCR-based workflow

Although high level multiplexing can be achieved by using bespoke cartridges or surface-based arrays, LiquidArray technology exploits the optical alignment and thermal precision of the FluoroCycler XT to deliver next generation multiplexing in a familiar, cost-effective PCR-based workflow. The combination of innovative thermocycling technology with expert probe design and automated results interpretation provides LiquidArray assays in an intuitive, easy to implement and accessible format for the routine laboratory.

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LiquidArray – next generation multiplexing underpinned by FluoroCycler XT

Three key features of the FluoroCycler XT that enable LiquidArray next generation multiplexing are optical alignment, thermal precision, and integrated analysis software:

Outstanding optical alignment

The FluoroCycler XT has outstanding optical alignment, based on Bruker-patented technology, enabling LiquidArray assays to make full use of five optical channels without cross-talk. This produces accurate results without the need for color compensation. As a result, assays can be designed for multiplex detection across all five optical channels simultaneously while maintaining the highest sensitivity.

Exceptional thermal precision

For many thermocyclers, a rapid temperature ramp causes substantial temperature overshoots. Whereas, the FluoroCycler XT exhibits exceptional thermal precision, adhering very closely to the programmed temperature throughout the entire cycle (+/- 0.2 °C) (Figure 1) with very low well-to-well variability. This allows precise temperatures to be reached in every well at any given point in time. The impact of this thermal control is that multiple probes can be used per optical channel, allowing the ability to multiplex within each channel. As a result, LiquidArray assays make full use of the thermal as well as the optical capabilities provided by the FluoroCycler XT.

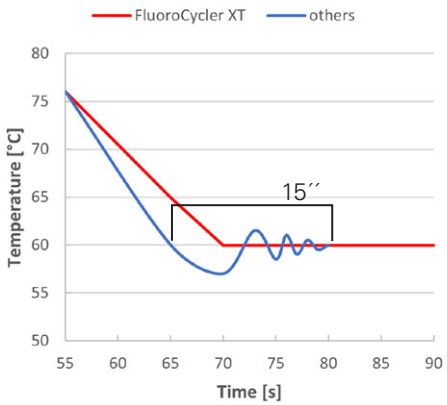

Figure 1
Comparison of the thermal precision achieved with FluoroCycler XT (red) and a thermocycler from another manufacturer (blue).

Integrated analysis software

The LiquidArray combination of multiplexing across 5 optical channels and within each optical channel can generate complex fluorescence signatures. The analysis of the assays is carried out using the FluoroCycler XT's integrated FluoroSoftware®. Avoiding the need for user interpretation, fluorescence signals are automatically interpreted, and an integrated machine learning algorithm provides results at a glance in a user-friendly report. A laboratory information management system (LIMS) connection ensures seamless and secure integration with existing laboratory processes.

Assay and probe design for next generation multiplexing

Exploiting the capabilities of the FluoroCycler XT, assays developed using linear after exponential PCR and novel probe design can deliver next generation multiplexing:

`Linear after exponential' PCR for high sensitivity

LiquidArray is a PCR-based technology that uses 'linear after exponential PCR' - with an excess, and a limiting primer. The reaction starts with both primers targeting a region of interest. After an exponential phase, in which double stranded DNA is produced, only the excess primer remains. Additional thermal cycles – in the linear phase – produce an abundance of single stranded PCR product (Figure 2). Since Taq polymerases are slightly inhibited by dsDNA, the 'linear after exponential' approach provides enhanced sensitivity.

Lights-ON/Lights-OFF probes to detect multiple targets

Polymerase chain reactions are frequently analyzed by fluorescence detection to determine whether a target is present. A common approach is to make use of probes containing a fluorophore and quencher, which are separated from one another in the presence of a target sequence, producing fluorescence, which is measured in a thermocycler.

In the case of LiquidArray, the single stranded DNA produced can be targeted by a combination of Lights-On and Lights-Off probes. Lights-On probes contain a fluorophore and a quencher; Lights-Off probes contain only a quencher. Lights-On probes only exhibit fluorescence when the fluorophore and quencher are spatially separated from one another by the probe binding to a target sequence. Depending on the application, assays can be designed so that Lights-Off probes sit adjacent to Lights-On probes on the target, selectively switching off fluorescence of the neighboring Lights-On probe (Figure 3). The binding and release of the probes is temperature-dependent, based on the sequence of the probe. Probes are therefore designed to bind and release from the target sequence, producing or quenching fluorescence at predictable temperatures. Thanks to the thermal precision of the FluoroCycler XT, multiple probes can be used with different melting temperatures per optical channel. Since the probes are released at specific temperatures in a predictable order, they generate unique fluorescence signatures.

Using multiple probes with distinct melting temperatures per optical channel greatly increases the multiplexing capacity. LiquidArray assays can be designed to generate multiplex results within each optical channel and across 5 optical channels - providing next generation multiplexing (Figure 3 and Figure 4).

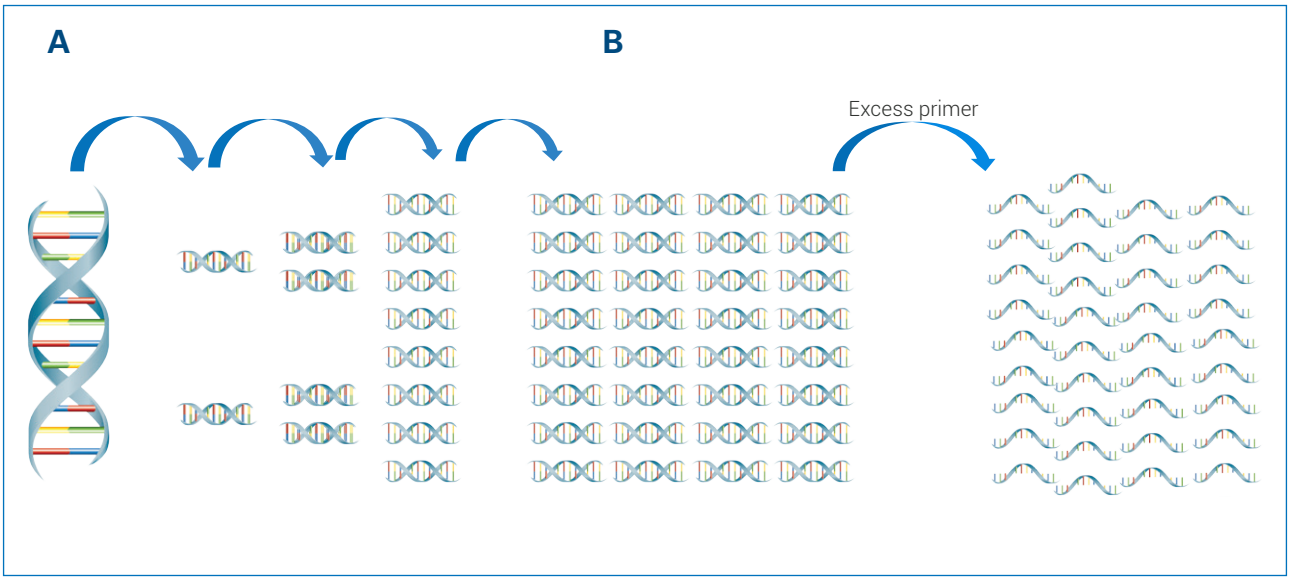

Figure 2
The 'linear after exponential' amplification approach used in LiquidArray: (A) Exponential phase; (B) Linear phase.

Figure 3
Operation of Lights-On/Lights-Off probes: The Lights-On probe contains a fluorescence dye (yellow) and a fluorescence quencher (blue); (A) When a Lights-On probe binds to DNA, fluorescence is emitted; (B) When a Lights-On probe is released from DNA, fluorescence is quenched; (C) The Lights-Off probe contains just a fluorescence quencher; (D) When a Lights-Off probe binds adjacent to a Lights-On probe, the fluorescence is quenched.

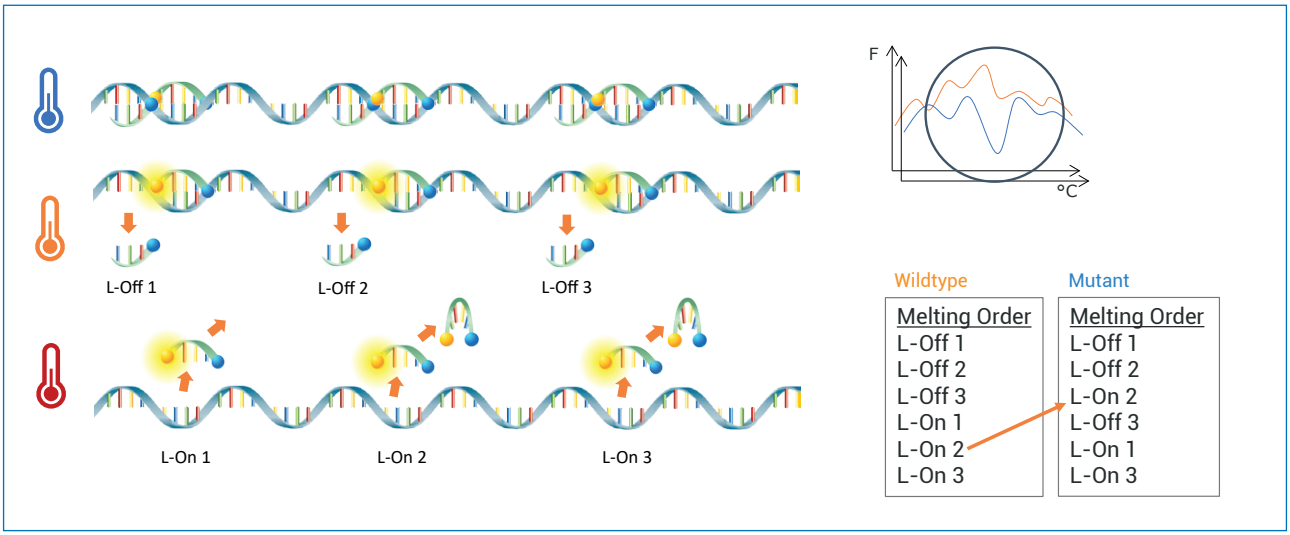

Figure 4
Example showing how the stepwise release of Lights-ON and Lights-OFF probes results in a complex fluorescence 'signature' in the melting profile. A mutation in the region probed by Lights-On 2 changes the melting order of probes and produces a different fluorescence signature, allowing the target sequence to be distinguished.

Application types

LiquidArray technology can be applied in two main ways (Figure 5):

- **LiquidArray applied to multiplex pathogen detection**
  A large number of pathogens can be targeted with multiple sequences probed simultaneously from a single sample using 'Lights-ON' probes only (Figure 5A). This approach can be applied to the development of LiquidArray syndromic panels.

- **LiquidArray applied to multiplex detection within a region/regions of a single target sequence**
  A target sequence can be multiply probed using 'Lights-ON' and 'Lights-OFF' probes, which allows LiquidArray to detect and differentiate a large number of mutations present within a single target sequence (Figure 5B). This approach allows rapid differentiation of treatment-resistant infections.

Application areas for LiquidArray

LiquidArray is currently available in three key application areas: mycobacteria species and resistance detection and syndromic panels for sexually transmitted, and gastrointestinal infections.

1. Mycobacteria species and resistance detection

FluoroType Mycobacteria VER 1.0 is powered by LiquidArray and allows differentiation of the *Mycobacterium tuberculosis (M. tuberculosis)* complex and 32 clinically relevant non-tuberculous mycobacteria including *M. avium, M. kansasii* and the different subspecies of the *M. abscessus* complex from culture in a single well. (Table1, Figure 6).

FluoroType MTBDR VER 2.0 is powered by LiquidArray and enables detection from patient sputum of more than 500 genotypes in the *M. tuberculosis* complex by the combined analysis of up to 45 different mutations (Figure 7). These allow detection of resistance to rifampicin and isoniazid from mutations of *rpoB*, *inhA* and *katG* genes. The assay is endorsed by the World Health Organization (WHO) [1].

Additional assays for the detection of tuberculosis and its resistant forms are also available from Bruker, ensuring efficient diagnostics across the *Mycobacterium* genus.

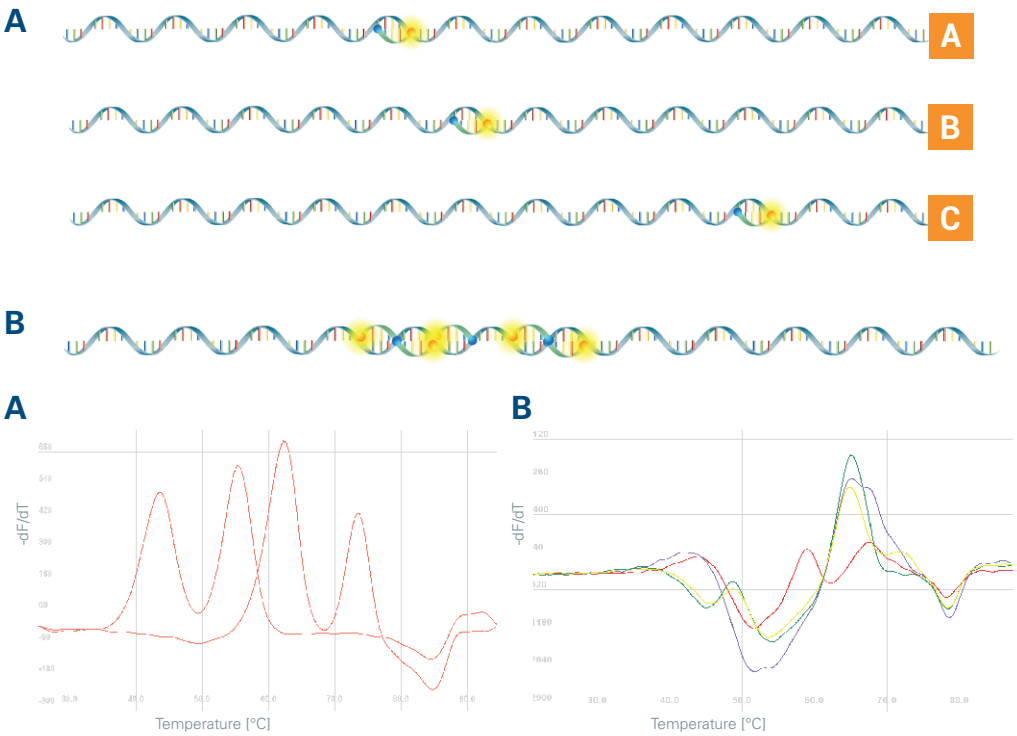

Figure 5
Use of LiquidArray for the two main categories of assays:
(A) Multiple pathogens – single sample
(B) Multiple mutations– single sample.

[1] Molecular WHO-recommended rapid diagnostic tests for detection of tuberculosis and drug-resistant tuberculosis, p3.
https://apps.who.int/iris/rest/bitstreams/1420014/retrieve
(accessed 17 August 2022)

M. tuberculosis complex	*M. genavense*	*M. marinum*
M. abscessus subsp. *Abscessus*	*M. goodii*	*M. mucogenicum*
M. abscessus subsp. *Bolletii*	*M. gordonae*	*M. peregrinum*
M. abscessus subsp. *Massiliense*	*M. haemophilum*	*M. phlei*
M. asiaticum	*M. heckeshornense / M. Xenopi*	*M. scrofulaceum*
M. avium	*M. intermedium*	*M. shimoidei*
M. celatum	*M. interjectum*	*M. simiae*
M. chelonae	*M. intracellulare*	*M. smegmatis*
M. chimaera	*M. kansasii*	*M. szulgai*
M. fortuitum	*M. lentiflavum*	*M. ulcerans*
M. gastri	*M. malmoense*	*M. xenopi*

Table 1
List of the mycobacteria species detected by FluoroType Mycobacteria VER 1.0 – *M. tuberculosis* complex and 32 non-tuberculous mycobacteria.

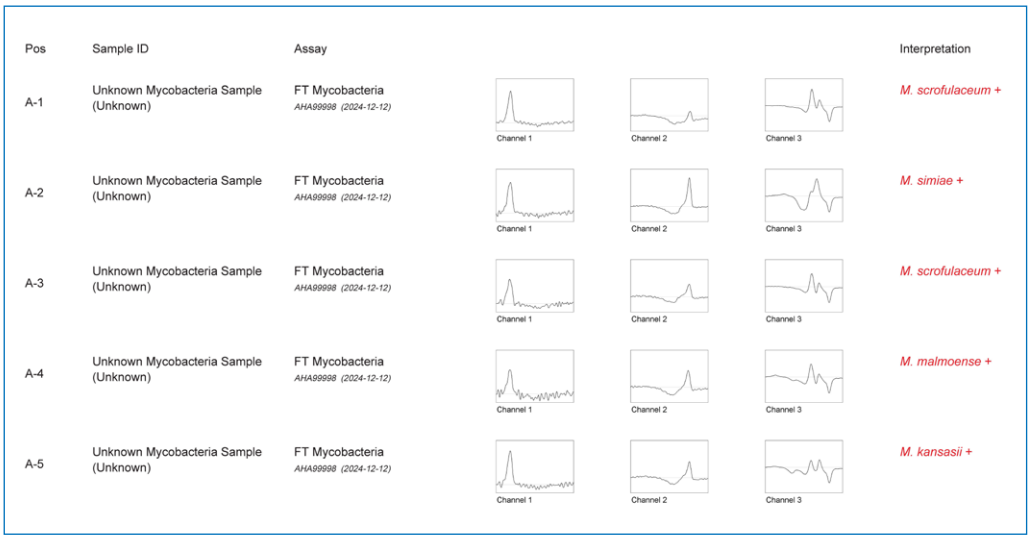

Figure 6
Example of a FluoroCycler XT report using FluoroType Mycobacteria VER 1.0 showing typical results interpretation of positive non-tuberculous mycobacteria samples.

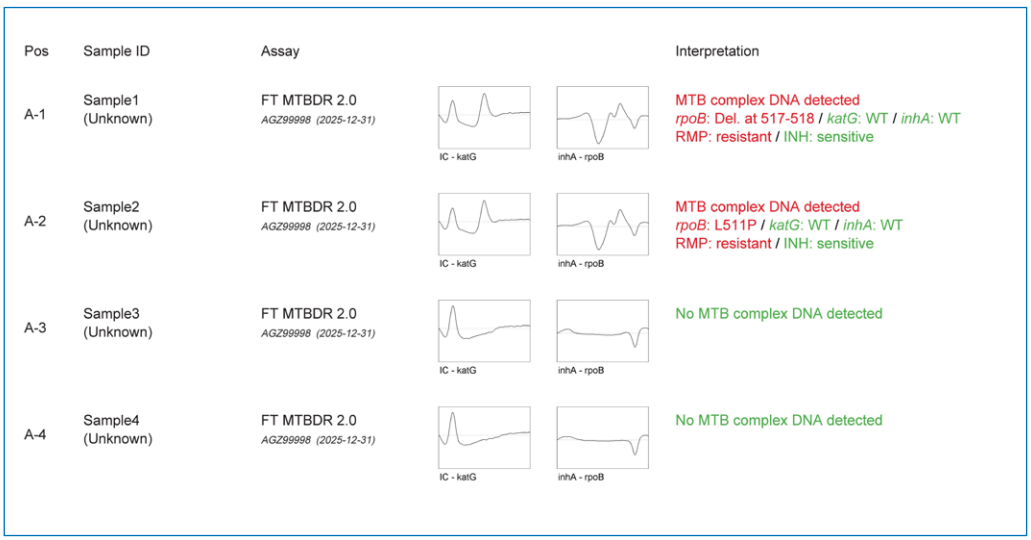

Figure 7
Example of a FluoroCycler XT report using FluoroType MTBDR VER 2.0 showing detection of multiple *M. tuberculosis* mutations and the corresponding drug resistance interpretation.

2. Syndromic panel for sexually transmitted infections (STI)

Fluorotype STI is powered by LiquidArray and detects 7 prevalent bacterial and parasitic STI pathogens in one well from urine and genital swabs.

For the leading two pathogenic bacterial species, dual-gene detection is used to ensure confident reporting:

- *Chlamydia trachomatis* (CT) is detected by a cryptic plasmid target and a genomic target (which covers 'drop out' strains).
- *Neisseria gonorrhoeae* (NG) is detected by both *porA* and *opa* targets. Since recombination between species in the *Neisseria* genus occurs frequently; requiring detection of both *porA* and *opa* genes to report an NG positive result increases the stringency of the test and means that a single test suffices for reliable positive detection.

Additionally, single-target detection is used for:

- *Trichomonas vaginalis* (TV)
- *Mycoplasma genitalium* (MG)
- *Mycoplasma hominis* (MH)
- *Ureaplasma parvum* (UP)
- *Ureaplasma urealyticum* (UU)

Results for all 7 species are reported from a single sample in a single well (Figure 8).

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LiquidArray®

Figure 8
Example of a FluoroCycler XT report using FluoroType STI showing detection of STI-causing species.

3. Syndromic panel for gastrointestinal infections

LiquidArray Gastrointestinal detects up to 26 gastroenteritis-causing pathogens from stool samples. The test is performed in a familiar PCR workflow across two wells. Bacteria, bacterial toxins, viruses, and parasites are all covered in a single protocol (Figure 9 and Figure 10).

Stool samples are first prepared in Stool Buffer, which stabilizes samples at room temperature until processing begins at a time that is convenient for the laboratory.

Reducing hands-on time, a single user can easily run the entire workflow and laboratories can replace multiple tests with one. Automated extraction, PCR setup and results interpretation combined with the comprehensive coverage of the assay and remarkably low invalid rates provides efficient gastrointestinal testing. This streamlines gastrointestinal testing in the routine laboratory.

Well 1

Bacteria and toxins:
Campylobacter spp.
C. difficile toxin A
C. difficile toxin B
E. coli O157
Enterohemorrhagic *E. coli* (EHEC)
Enteroinvasive *E. coli* (EIEC)/*Shigella* spp.
Enteropathogenic *E. coli* (EPEC)
Salmonella spp.
Shiga toxin-producing *E. coli* (STEC)
Yersinia enterocolitica

Viruses:
Norovirus GI
Norovirus GII
Rotavirus

Parasites:
Cryptosporidium spp.
Entamoeba histolytica
Giardia lamblia

Well 2

Bacteria:
Enteroaggregative *E. coli* (EAEC)
Enterotoxigenic *E. coli* (ETEC)
Plesiomonas shigelloides
Vibrio cholerae
Vibrio spp.

Viruses:
Adenovirus 40/41
Astrovirus
Sapovirus

Parasites:
Ascaris spp.
Cyclospora cayetanensis

Figure 9
26 gastroenteritis-causing pathogens covered by LiquidArray Gastrointestinal

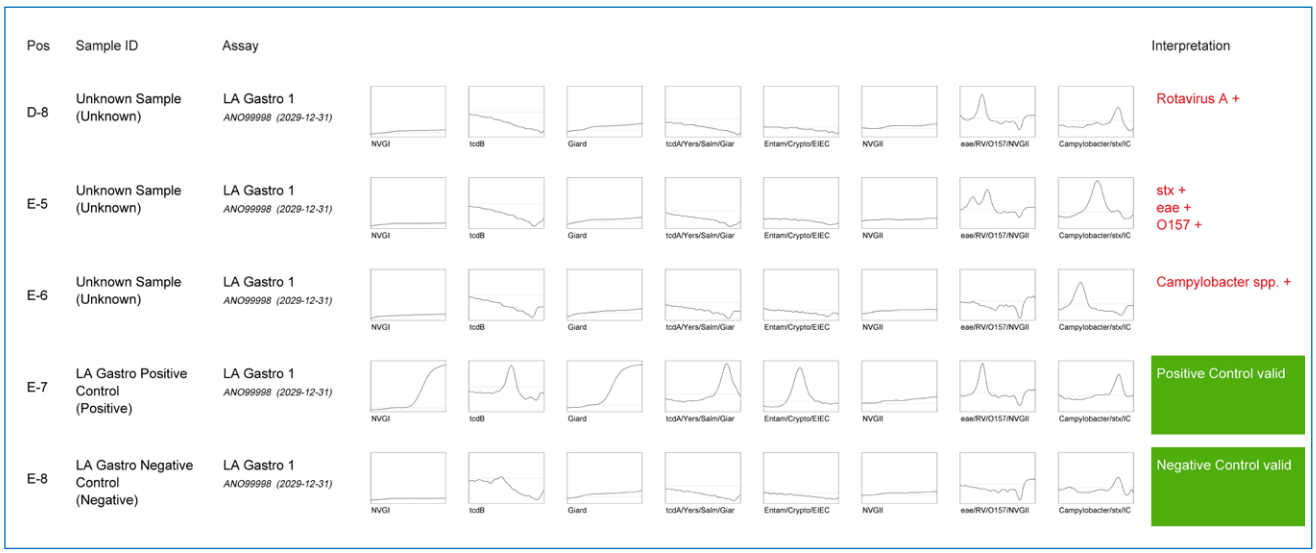

Figure 10
Example of a FluoroCycler XT report using LiquidArray Gastrointestinal showing detection of gastroenteritis-causing pathogens.

Sample-to-result solutions

LiquidArray® is performed in a familiar PCR-based workflow, which is easy to implement in the routine laboratory. Bruker provides a range of sample-to-result solutions for LiquidArray assays, depending on the application. Each assay has been developed in combination with compatible nucleic acid extraction chemistry to ensure high quality processing alongside validated workflows designed to suit the throughput needs of routine laboratories – from low to high throughput. For minimal hands-on time, the GenoXtract® fleXT is Bruker's automated extraction system that also offers automated PCR preparation for up to 96 samples in parallel. Intuitive software guides users through a simple setup process and, with seamless data transfer to the FluoroCycler XT, provides data integrity and confident reporting. With results automatically interpreted and provided in a user-friendly report, labs can optimise workflow efficiencies and report accurate results with confidence.

The LiquidArray syndromic panels, LiquidArray Gastrointestinal and FluoroType STI are processed using the GenoXtract fleXT system for sample extraction and PCR setup (coming soon for FluoroType MTBDR 2.0) followed by LiquidArray processing on the FluoroCycler XT, and automated analysis of the fluorescence signatures using FluoroSoftware (Figure 11).

Looking ahead

LiquidArray technology brings next generation multiplexing to the routine clinical microbiology laboratory. By realizing the full potential of multiplex diagnostic solutions, LiquidArray supports laboratories by providing comprehensive results to solve clinical challenges with accuracy.

Click here to get in touch to discuss how LiquidArray next generation multiplex technology can support you.

Next Generation Multiplexing
Powered by LiquidArray®

Replace multiple tests with one

Streamlined diagnostic workflows

Results at a glance

Powered by
LiquidArray®

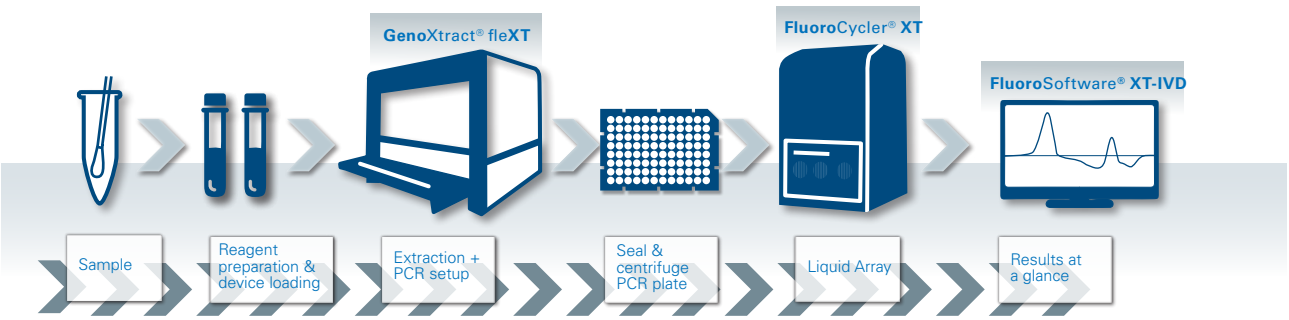

Figure 11
Streamlined worfklow from sample to results at a glance for LiquidArray Gastrointestinal and FluoroType STI. Coming soon for FluoroType MTBDR 2.0. Other validated extraction workflows are available.

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GenoXtract® fleXT Hamilton Bonaduz AG, Via Crusch 8, CH-7402 Bonaduz, Switzerland
FluoroCycler® XT Hain Lifescience, Hardwiesenstrasse 1, 72147 Nehren, Germany

Hain Lifescience GmbH

Nehren · Germany
+49 (0) 7473-9451-0

info.mdx.de@bruker.com

Online information
bruker.com/microbiology