Molecular diagnostics

Overcome hurdles of MDx assay design and development using real-time PCR solutions

Key messages

- The growing demand for MDx assays warrants the need for fast and accurate workflows that reduce the cost and time to bring a new assay to market.
- Real-time PCR–based molecular diagnostics enable fast sample-to-result times and sensitive target detection and quantification.
- The QuantStudio Dx family of real-time PCR systems from Thermo Fisher Scientific provides an accurate, reliable, and IVD-ready platform for streamlining MDx assay development.

Introduction

Molecular diagnostics (MDx) refers to the class of diagnostic tests that assess a patient's health at the cellular level [1]. The development and use of tailored molecular-based assays have revolutionized the diagnostic sector, reducing the turnaround time for detecting and quantifying cancer, infectious diseases, and inherited conditions from patient samples [2-4]. The demand for MDx tests is growing due to the high prevalence of infectious diseases, emphasis on early disease identification, and increasing focus on biomarker discovery and transition to the clinical sector [5]. Given that it typically takes 3–7 years to bring these assays to the commercial market, efforts to meet the growing demand for MDx tests rely on the acceleration of the MDx development pipeline [6]. Critical to the success of MDx assay development are three factors:

- **Accurate results**—getting the right answer the first time
- **Reliability**—consistent performance and robust supply chains
- **IVD readiness**—compliance documentation and assay development support

Real-time PCR, also called quantitative PCR (qPCR), is a major technology in the field of MDx. qPCR assays offer both high sensitivity for the detection of low-level targets and high specificity for the precise identification of and differentiation between molecular targets [7].

Thermo Fisher Scientific offers the Applied Biosystems™ QuantStudio™ Dx family of real-time PCR systems as a solution for streamlining MDx assay design and development. The MDx-focused ecosystem includes the Applied Biosystems™ QuantStudio™ 5 Dx and 7 Pro Dx Real-Time PCR Systems integrated with Applied Biosystems™ Diomni™ Software, the portfolio of Applied Biosystems™ TaqPath™ master mixes, and Applied Biosystems™ TaqPath™ predesigned assays. Our global customer concierge services cover orientation, technical support, and digital and qualification services.

This white paper introduces our product and service ecosystem designed with the following in mind: high-quality and accurate results, consistent manufacturing standards and performance of reagents, and *in vitro* diagnostics (IVD) compliance and support.

QuantStudio Dx instruments: designed for streamlined MDx workflows

When developing an MDx assay, running experiments using a trusted, high-performance instrument designed with efficiency in mind can save time and resources. Thermo Fisher Scientific has been producing qPCR instruments for over 25 years and has over 10 years of clinical instrument manufacturing experience. With years of MDx support and experience, Thermo Fisher has developed reliable high-performance qPCR instruments for simplifying the path from development to commercialization. These instruments include the QuantStudio 5 Dx and QuantStudio 7 Pro Dx Real-Time PCR Systems.

The QuantStudio 5 Dx Real-Time PCR System is a compact, flexible, and affordable system designed to simplify MDx workflows. This instrument enables accurate and sensitive results by detecting differences in target quantity as small as 1.5-fold in singleplex reactions and providing 10 orders of magnitude of linear dynamic range. The system also supports multiplexing of up to six targets. Moreover, the short run times, minimal maintenance, compatibility with existing plastic consumables, and intuitive interface provide convenience for optimizing MDx assays with ease. These features help ensure accurate results that enable users to get the right answer the first time around, ultimately speeding up MDx assay development timelines.

The QuantStudio 7 Pro Dx Real-Time PCR System is a stand-alone system with interchangeable 96-well and 384-well block options designed for maximum efficiency. The system is equipped with a graphical user interface (GUI) that enables end-to-end IVD workflows to be carried out directly on the machine without the need for an accompanying computer.

Moreover, it offers smart features such as hands-free operation with voice commands as well as both smart support and smart help to report issues or request instrument services. This system also offers fully automated capabilities that use assay definition files (ADFs) to allow users to configure all the setup and analysis parameters required for a particular qPCR assay. Together, these features improve the efficiency of MDx assay development by offering automated, high-throughput workflows while reducing manual error.

In addition to speeding up assay development, the QuantStudio 5 Dx and QuantStudio 7 Pro Dx systems also help provide trustworthy, consistent performance and reliable, reproducible results. In a study testing the reproducibility of an assay with the same 20-sample panel, three different laboratories with 2 separate users each ran the assay over the course of 5 days. The groups found 97.9% and 98.7% concordance of results between sites using the QuantStudio 5 Dx and 7 Pro Dx instruments, respectively, demonstrating the reproducibility of results using these systems (Table 1).

In addition to highly accurate and reproducible results, each QuantStudio Dx instrument is equipped with specific IVD modes as well as a security, auditing, and e-signature (SAE) module, which helps provide a secure environment for running assays and assists in meeting regulatory compliance requirements. Overall, every instrument in the QuantStudio Dx family enables accurate results, establishes reliability, and supports IVD readiness, ultimately powering rapid and cost-effective development of MDx assays.

Table 1. Summary of site-to-site reproducibility results.

	Site-to-site call concordance	95% confidence interval	
		Lower limit	Upper limit
QuantStudio 5 Dx Real-Time PCR System	97.9%	97.1%	98.5%
QuantStudio 7 Pro Dx Real-Time PCR System	98.7%	98.0%	99.1%

Streamlined data analysis with Diomni Software

Diomni Software is an easy-to-use solution to boost the speed and efficiency of MDx assay development through simplified workflows and expanded connectivity. This web-based application seamlessly integrates into existing qPCR workflows using the QuantStudio Dx instruments and is built to assist assay developers at every stage of the product development cycle (Figure 1). This software enables users to create predefined protocols, automate QC and interpretation of results, connect to multiple instruments, run assays remotely, visualize and track samples, and integrate with your laboratory information system (LIMS or LIS).

Ultimately, the incorporation of Diomni Software can reduce the time to set up an assay, analyze results, and transfer data—accelerating qPCR-based MDx assay development.

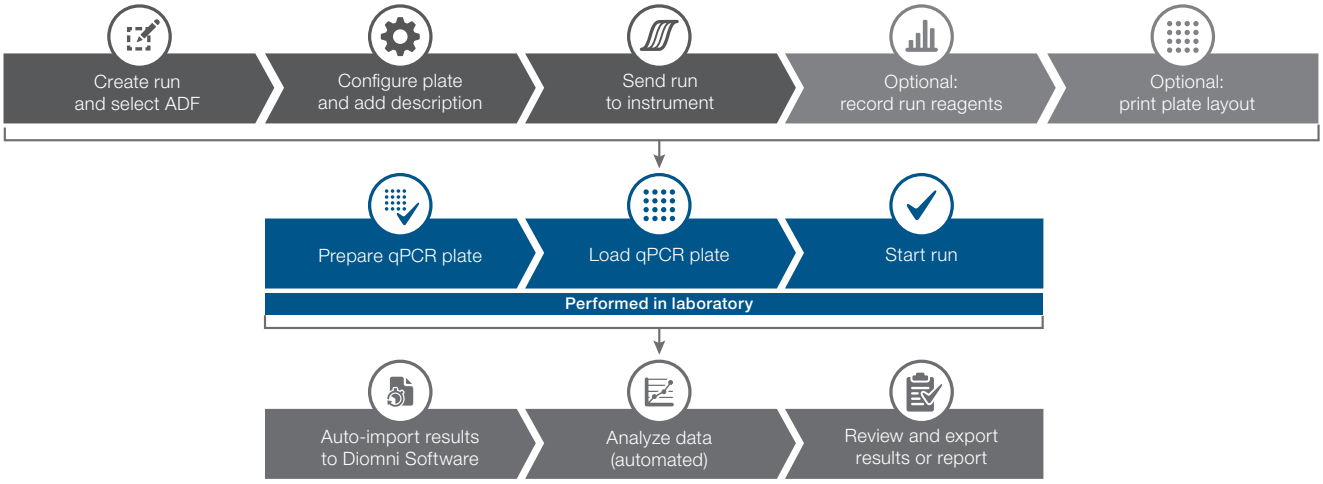

Figure 1. qPCR assay workflow with Diomni Software.

TaqPath master mixes: formulated with MDx assay developers in mind

A reliable assay can't be built without reliable reagents. Formulating an MDx assay with reagents that offer a limited detection range, unpredictable stability, manufacturing inconsistencies, uncertain supply, and lack of compliance documentation and support can all delay time-to-market as well as expand costs for development. When starting an MDx assay development pipeline, it's essential to ensure all reagents are well formulated, consistent, and able to be scaled to the IVD space to ensure prompt downstream regulatory approval and minimize time-to-market.

The Applied Biosystems™ TaqPath™ qPCR master mixes, which are compatible with QuantStudio Dx instruments and Diomni Software, were designed to reduce the cost of MDx development and advance the timeline to market. The TaqPath master mixes (Table 2) were all formulated to help ensure the right answer the first time, consistent performance and reliability, and IVD readiness.

TaqPath master mixes: getting the right answer the first time with specific target detection and inhibitor tolerance

- When developing an MDx assay, it is essential to use trustworthy, stable reagents capable of reproducible, sensitive detection of targets. With reliable reagents, optimization times and troubleshooting steps can be reduced to ultimately drive high-speed and low-cost assay development.
- For MDx assay developers, using a reliable master mix with proven performance in accurately detecting molecular targets is key to success. TaqPath master mixes enable specific target detection and quantification with wide dynamic ranges and low background to facilitate reliable detection during assay development.
- The TaqPath BactoPure Microbial Detection Master Mix has been optimized for highly reproducible, low-level detection from a wide variety of samples. To that end, the proprietary manufacturing process of the TaqPath BactoPure Microbial Detection Master Mix minimizes the amount of spurious DNA in the background signal that could interfere with low-level target detection. In a study comparing the TaqPath BactoPure master mix to master mixes from three other suppliers, the TaqPath BactoPure master mix did not detect background signals, which can lead to ambiguous results, while master mixes from other suppliers did (Figure 2).

Table 2. TaqPath master mixes: their nucleic acid targets and MDx applications.

Product	TaqPath™ 1-Step RT-qPCR Master Mix, GC	TaqPath™ BactoPure™ Microbial Detection Master Mix	TaqPath™ ProAmp™ Master Mix
Target	RNA	DNA	Genomic DNA
Applications	Pathogen detection, gene expression	Microbial detection and quantitation	Genotyping, copy number variation

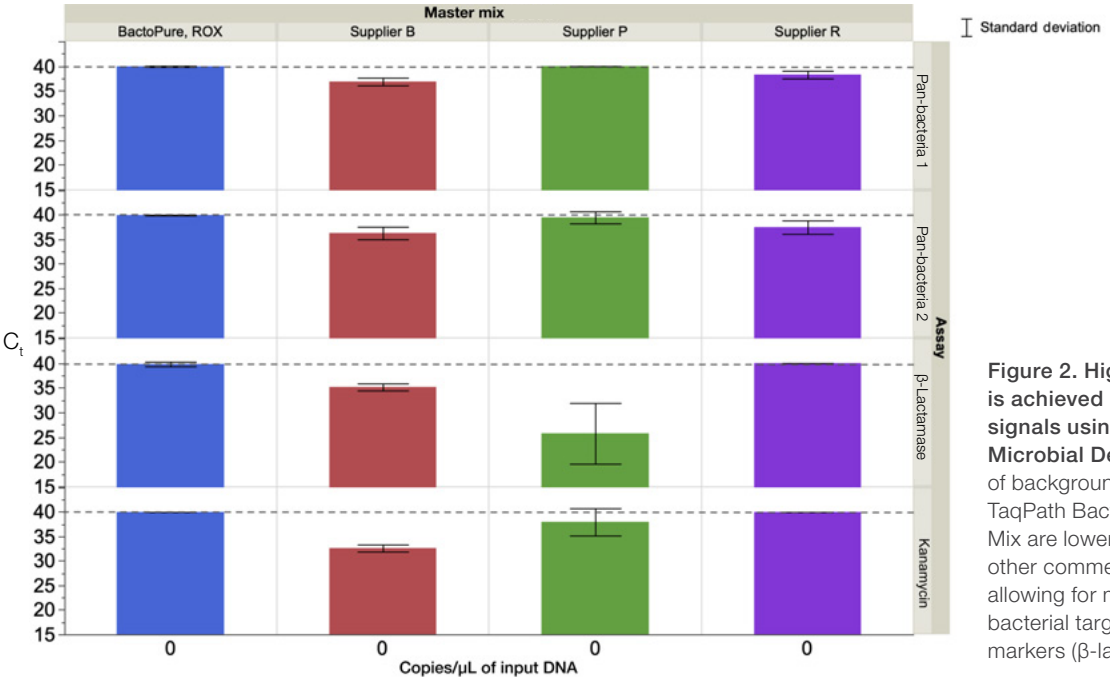

Figure 2. High specificity for MDx targets is achieved by limiting the background signals using TaqPath BactoPure Microbial Detection Master Mix. The levels of background nucleic acid detected using TaqPath BactoPure Microbial Detection Master Mix are lower than levels detected by three other commercially available master mixes, allowing for more sensitive detection of pan-bacterial targets and antibiotic resistance markers (β-lactamase and kanamycin).

Inhibitor tolerance

An important consideration when developing an MDx assay is ensuring the performance of your master mix even in the presence of PCR inhibitors commonly found in clinical samples, such as heparin, hematin, humic acid, and IgG. The unique formulations of TaqPath master mixes offer higher tolerance and stability of the reagents. For example, C_t values for specific targets amplified in the presence of these inhibitors using TaqPath 1-Step RT-qPCR Master Mix, CG, as well as other suppliers' products demonstrated the TaqPath master mix's tolerance of these common PCR inhibitors (Figure 3).

Consistent performance provided by TaqPath master mixes

Development of MDx assays relies on consistent performance of reagents to help ensure reproducible results. To meet consistency parameters, the TaqPath reagents offer lot-to-lot consistency and help secure availability with a stable supply chain you can count on.

Lot-to-lot consistency

Uncertain consistency in reagents used to develop MDx assays can delay time-to-market and increase costs needed for optimization and troubleshooting. TaqPath master mixes have been assessed for amplification across multiple manufacturing lots and show proven consistency in detection. For example, three distinct lots of TaqPath ProAmp Master Mix produced the same target amplification levels, regardless of the sample input (Figure 4).

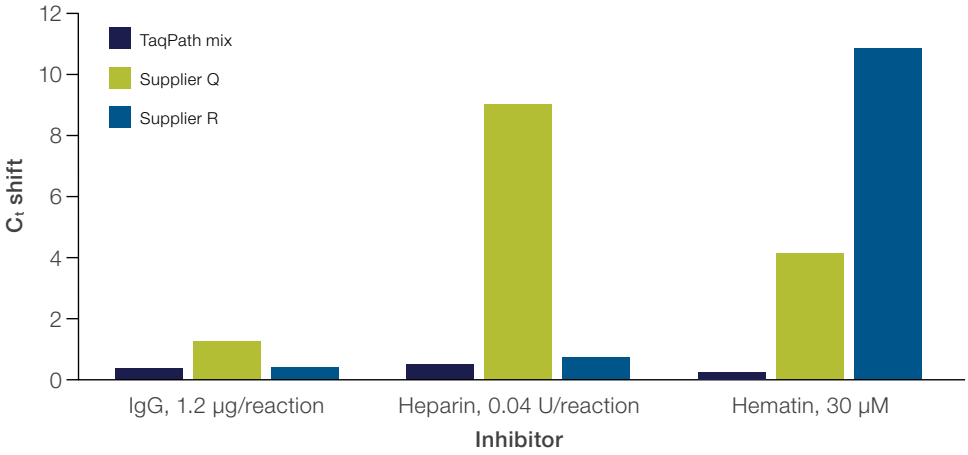

Figure 3. TaqPath master mixes are formulated to ensure performance even in the presence of PCR inhibitors. The shifts in C_t values determined by RT-PCR using TaqPath 1-Step-RT-qPCR Master Mix, CG, and two other suppliers' products in the presence of PCR inhibitors IgG, heparin, or hematin reveal the high stability and inhibitor tolerance of this TaqPath master mix.

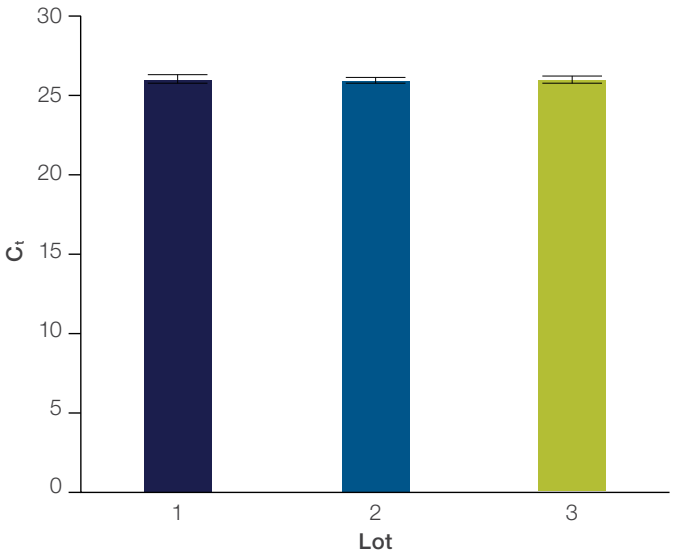

Figure 4. TaqPath master mixes maintain lot-to-lot consistency. Amplification of genomic DNA using three distinct lots of TaqPath ProAmp Master Mix show consistent C_t values for target detection.

Robust supply chain

During development of an MDx assay, unforeseen global crises can lead to shortages and manufacturing delays that extend a product's time-to-market. Thermo Fisher's worldwide manufacturing networks help ensure our products are available with short lead times to avoid potential delays in MDx assay development. Trust our secure supply chain to power your MDx development at every step.

Seamless integration with regulatory requirements using IVD-ready TaqPath reagents

Developing MDx assays using instruments and reagents specifically designed to meet IVD regulatory requirements can save developers time during the regulatory approval steps of the product development cycle. Our TaqPath master mixes are supplied with compliance documentation to help meet regulatory requirements. Moreover, using Thermo Fisher products means partnering with our assay development support team of experienced professionals to get your MDx product to market.

Compliance documentation

TaqPath master mixes are general-purpose reagents labeled for laboratory use and are manufactured in ISO 13485–certified facilities that adhere to cGMP principles. Our reagent manufacturing facilities provide quality management with rigorous analytical and functional QC standards. Our exceptional QC standards and optimized TaqPath formulations help ensure excellent product consistency. We also provide comprehensive documentation, including stability statements, supplier assessments, and change notifications, to simplify IVD assay submission and provide peace of mind.

Assay development support from the original equipment manufacturer (OEM) and commercial supply team

At Thermo Fisher, we help our partners commercialize MDx assays by offering full support, services, and insight throughout the entire MDx development workflow, from bench to commercialization. Our unique OEM and Commercial Supply team provides global connections, personalized support, industry and technology perspectives, customization of reagents, analytical validation consulting services, and more.

Global customer concierge services

In addition to qPCR instruments and accessories, Thermo Fisher offers orientation and training resources to our global customers as part of our concierge services to help ensure seamless implementation of our systems in your laboratory. These services include:

- SmartStart™ Orientation to help your team gain proficiency in real-time PCR principles, QuantStudio instrumentation, and related software features
- Applied Biosystems™ AB Platinum Service Plan to help ensure personalized technical support, optimized instrument performance, and efficient support and repairs
- Qualification services to help meet regulatory standards for instrument installation, operation, and performance in the clinical market
- Digital services to access our network of over 2,000 trained professionals for support, troubleshooting, and scheduling

With these services, the timeline for training, receiving technical support, and installing instrumentation can be reduced, ultimately reducing the cost and time involved in bringing a new assay to market

Pivoting your program: powered by our qPCR ecosystem

Once you have Thermo Fisher instrumentation installed in your lab, you can use our qPCR product portfolios to power your transition between disease areas. The time and costs saved by using the Thermo Fisher qPCR ecosystem of products can free up the ability to pivot your MDx program into other therapeutic areas such as infectious disease genetic testing, cancer screening, cancer staging diagnosis, and disease staging.

Summary

Partnering with Thermo Fisher can shorten the time and lower the costs associated with MDx assay development. Our QuantStudio Dx instruments are designed to meet the needs of MDx developers. These systems come with features such as automation capabilities and integrated Diomni Software that streamline qPCR workflows, enabling faster time-to-results. TaqPath master mixes can reduce time delays and resource consumption needed for troubleshooting and optimization by enabling consistent performance and the right answer the first time. Furthermore, TaqPath reagents are formulated to meet regulatory requirements, advancing the downstream regulatory approval steps in the product development pipeline. Beyond our product portfolio, our world-renowned support can streamline your MDx assay development pipeline by putting you in touch with experienced professionals at each step of the way. In summary, by implementing the Thermo Fisher qPCR product portfolio in your lab, you can save costs and time, paving the way to development of MDx assays that can potentially address new, highly relevant therapeutic areas.

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