White paper

qPCR diagnostics

Quantitative PCR–based assay development made simple by Thermo Fisher Scientific

Abstract

Many clinically focused laboratories have resorted to the development, optimization, and eventual validation of assays developed in-house. These tests are developed and used by a single laboratory to provide diagnostic information that is classified differently from that provided by an FDA-approved diagnostic test. Because of the sensitivity and specificity inherent to quantitative PCR (qPCR), this approach has become a popular methodology for clinically focused laboratories. Combined with superb service and support, Thermo Fisher Scientific teams can support end-to-end qPCR assay development, validation, scale-up, and implementation. With the qPCR product solutions, support, and workflows provided by Thermo Fisher, your assays can enhance diagnostic information and potentially improve treatment plans for patients.

Assays developed in-house are central to enhancing patient care

Biotechnology has spurred countless innovations in medicine and helped improve patient outcomes through enhanced understanding of mechanisms of pathogenicity, better diagnostic assays, and more effective therapeutic interventions. These improvements are continually shaping the way modern medicine is practiced and constantly forcing medical scientists and practitioners alike to develop new diagnostic assays and treatment regimens to reflect the knowledge gained through biomedical research. Many clinically focused laboratories have implemented strategies to address current deficiencies in diagnostic testing through development optimization, and eventual validation and diagnostic use of laboratory-based diagnostic tests. These assays incorporate varied methodologies including molecular diagnostics, immunoassays, cell culture, and mass spectrometry [1].

Quantitative PCR is a central methodology in novel assay development

Multiple assay methodologies have proven to be suitable for use in LDT-based applications, including immunoassays, flow cytometry, mass spectrometry, and PCR. Compared to other methodologies, PCR offers a higher level of sensitivity and specificity [2]. For example, rapid antigen detection tests (RADTs), dependent on immunochromatographic, enzyme immunoassay, and optical immunoassay formats, offer significant advantages when it comes to time-to-results and ease of use. However, these assay formats have significant setbacks when it comes to sensitivity for novel pathogen strains and thus have a high rate of false negatives [3]. In one study, RADT sensitivity was only 41% in the diagnosis of respiratory syncytial virus (RSV) [3]. As such, PCR-based methods have gained significant adoption in the context of LDTs (Figure 1).

qPCR offers significant advantages over conventional endpoint PCR. The foundational basis of qPCR is in the ability to monitor DNA amplification by fluorescence during the exponential phase of a qPCR [4]. This inherent capability enables enhanced sensitivity compared to traditional endpoint methodologies and provides a wider dynamic range of experimental signal [5]. Further, when the fluorescence is measured after each cycle of amplification and combined with a reference material standard curve, qPCR can be quantitative [4]. Finally, amplification of multiple target sequences can be done simultaneously. This multiplexing feature is central in diagnostic qPCR, since internal amplification controls are required [3].

Collectively, this methodology offers the opportunity for high-throughput detection of multiple target nucleic acid sequences in variable matrices with less amplification time and elevated sensitivity [4,5]. In the context of diagnostics, qPCR has been used in several different areas including infectious disease [6-8], cancer [9-11], and rare inherited disease [12-14], as well as in disease target discovery [15]. However, regardless of the precision, sensitivity, and specificity of the methodology, users developing in-house assays can still face technical challenges in implementing qPCR if not properly equipped or supported during the development and optimization processes. Users can have significant setbacks in the laboratory if using qPCR instruments with nonideal or overly complicated user interfaces. Further, analysis software supplied with instruments may be inadequate and may skew obtained results, causing misinterpretation and, as a result, loss of additional time and resources. In addition, lack of quality standards for consumables used in the assay can further complicate assay optimization and subsequent validation through the inability to obtain consistent results. These issues may further be exacerbated by the inability to access customer support from the manufacturer. Thus, partnering with vendors with adequate experience, high quality standards, and strong qPCR products are helpful for successfully implementing qPCR for diagnostic assay development.

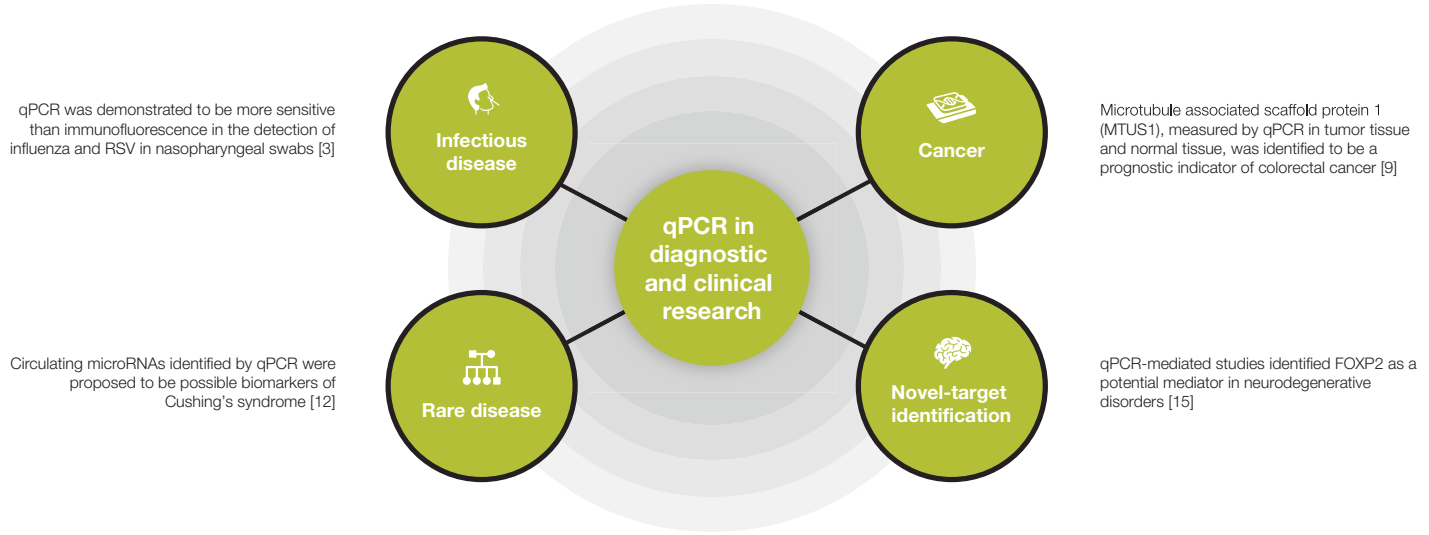

Figure 1. Utility of qPCR in multiple clinical disciplines.

The Thermo Fisher quantitative PCR portfolio enables in-house assay development capabilities

Thermo Fisher, a leader in qPCR-based diagnostics, has more than 25 years of qPCR experience. We provide a comprehensive portfolio of high-quality instruments, consumables, software, and service and support in the field of qPCR. Collectively, our all-inclusive qPCR product and service offering can help facilitate in-house assay development by providing you with up-to-date instruments and high-quality reagents.

Sample extraction and amplification hardware

Thermo Fisher is a leader in nucleic acid extraction and qPCR technologies. Our intelligent designs produce instruments with customer-centric features and benefits, such as:

- Touchscreens that allow for the editing of experimental conditions even after the run is complete, to prevent or reduce errors.
- Alerts users on maintenance due date to keep the instrument in sufficient working order.
- Remote access to determine progression of a qPCR run via a mobile app.
- Easy and concise views of all run parameters and plots to allow better result interpretation and detection of potential cross-contamination events or signal outliers.

- Robustness and durability; users have noted more than 50,000 nucleic acid extractions and PCR tests without any reported instrument malfunctions.

High-quality master mixes and consumables

Thermo Fisher offers high-quality master mixes that are among the best on the market. A key feature of these master mixes is enhanced stability. This feature gives users additional flexibility and consistency in both high-throughput and low-throughput applications. Manufactured in an ISO 13485–Wregistered facility, each product is functionally tested to help ensure lot-to-lot consistency.

Figure 2. Multiple ways Thermo Fisher can facilitate your assay development.

World-class customer support for end-to-end assay development

When you partner with Thermo Fisher for your qPCR-based assay development work, you gain access to excellent service and support throughout the in-house assay development, optimization, and validation processes. These services help support the successful application of the Thermo Fisher qPCR product line in all phases of assay development. For early implementation of our instruments and reagents, setup, maintenance, and technical support services include:

- **SmartStart Orientation**
  - Training for real-time PCR principles and Applied Biosystems™ QuantStudio™ instrumentation and software features*
- **AB Platinum Service Plan**
  - Efficient support/repair plan and personalized technical support*
- **Qualification services**
  - Instrument installation, operation, and performance to support compliance with regulatory standards
- **Digital services**
  - Customer access to Thermo Fisher support, troubleshooting, and scheduling network
- **Rich content library**
  - We provide you with rich technical literature on varied topics from quick start guides and basic instructional assets to detailed, novel data analysis methodologies
- **On-site support**
  - We support you in multiple ways, including coming on-site to assist in experimental setup, troubleshooting, and tutorials*

Further, Thermo Fisher offers an array of custom services and specialty manufacturing services that support qPCR development and applications that include:

- **Custom dye offerings**
- **Specialized formulation and volume requirements**
- **Specific assay plating arrangements**
- **Custom probes and GMP oligos**

For customers who are focused on late-stage qPCR assay development and validation of their in-house assay, Thermo Fisher provides additional support services. Specifically, we can provide training for and assistance with performing the assay validation, provide the standard operating procedures (SOPs) during the validation process, and guide the developer on required documentation for assay approval.

These services have been described by our current customers as essential. First, for developers who lack specific experience in assay validation and eventual diagnostic use, common errors and mistakes that will cause delays and deplete precious resources can be avoided. Second, Thermo Fisher's support services may reduce the need to utilize external consultants to navigate some critical steps in assay validation. Altogether, Thermo Fisher can provide crucial assay validation support that may help reduce development time and costs.

* Separate purchase required for service plans. See your sales representatives for purchase options in your region.

Putting it all together

With more than 25 years of qPCR experience, including 10 years of experience specifically in diagnostics, our extensive product portfolio and support service offerings facilitate every development step for our qPCR customers. Our robust instrument line can provide reproducible nucleic acid extractions and subsequent amplifications for tens of thousands of reactions with consistency and sensitivity when paired with high-quality Thermo Fisher consumables. The Applied Biosystems™ QuantStudio™ Dx Real-Time PCR Systems have been shown to detect as little as a 1.5-fold difference in single-plex amplification assays with 10 orders of magnitude of linear dynamic range (data on file). This enhanced sensitivity often means fewer amplification cycles are required, allowing increased efficiency.

Applied Biosystems™ Diomni™ Software is simple and user-friendly, yet powerful. Efficiency can be maximized by using a browser-based application that is connected to multiple instruments and users. Software users quickly learn the software, which allows data analysis and interpretation to be done with confidence. In the case of atypical results, further analysis can be performed to verify the obtained results and inform secondary clinical tests.

Thermo Fisher can also offer end-to-end customer support throughout the process. From support early in the development process like instrument installation, to custom manufacturing services and late-stage validation support, Thermo Fisher has your assay development efforts covered at every step.

Collectively, Thermo Fisher can help ultimately reduce time and resources required to develop assays for clinical research.

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