bio-techne®

White Paper

Accelerating the Drug Development Journey

Bio-Techne's QuantideX® RT-qPCR CTA platform for ultra-sensitive nucleic acid detection and quantification

Contents

Executive Summary:

Bio-Techne offers a suite of precision medicine services spanning translational biomarker discovery through clinical trial assay (CTA) and downstream development, as well as commercialization of companion diagnostic products (FIGURE 1). As part of these services, Asuragen, a Bio-Techne brand, has developed a multiplex, reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) assay system (QuantideX) for ultra-sensitive RNA quantification, for which clinical and analytical performance has been demonstrated in multiple disease indications. The ultra-sensitivity of the QuantideX technology is extensible to multiple genetic targets and is portable between qPCR platforms. The QuantideX platform has a simple workflow that facilitates implementation across molecular laboratories and demonstrates excellent reproducibility to enable widespread decentralization of testing. This white paper showcases the effectiveness of utilizing QuantideX® as the underlying technology, emphasizing the importance of selecting appropriate molecular targets and developing high-performance clinical trial assays (CTAs) and companion diagnostics (CDx) that are easy to deploy. The paper also highlights how the implementation of QuantideX technology can significantly expedite patient stratification and enhance clinical trial enrollment for our pharmaceutical industry partners.

Key Findings:

- The QuantideX technology can be adapted to monitor disease burden in diseases requiring highly sensitive detection and/or quantitation across multiple nucleic acid targets. It can also be utilized for designing high performing, efficient, and simplified kits for genetic targets with unmet biomarker development and CTA needs

- The QuantideX platform has demonstrated broad applicability in multiplex nucleic acid testing. QuantideX technology has been incorporated into the first FDA-cleared diagnostic kit for use in Chronic Myeloid Leukemia (CML) management during monitoring of treatment with Tyrosine Kinase Inhibitor (TKI) therapy by ultra-sensitive detection and precise monitoring of BCR-ABL1 transcripts.

- The QuantideX technology has been clinically and analytically validated, and has demonstrated a robust level of sensitivity for the assessment of therapy response and measurement of minimal residual disease in select indications.

QuantideX Workflow

The QuantideX platform has a simple workflow (FIGURE 2) in which both RT and qPCR are performed on the same instrument.[1] Premixed reagents reduce the number of pipetting steps to minimize the risk of human error. The QuantideX platform allows a rapid time to result with minimal hands-on time (~1 h) due to streamlined reagent formulation, thereby improving the assay workflow and increasing the number of specimens that can be tested per run. This batch size flexibility makes for a testing solution which can support both small and large throughput testing demands. The QuantideX technology facilitates high ease of implementation to enable the flexibility of decentralized testing.

Figure 1. Companion Diagnostics Capabilities

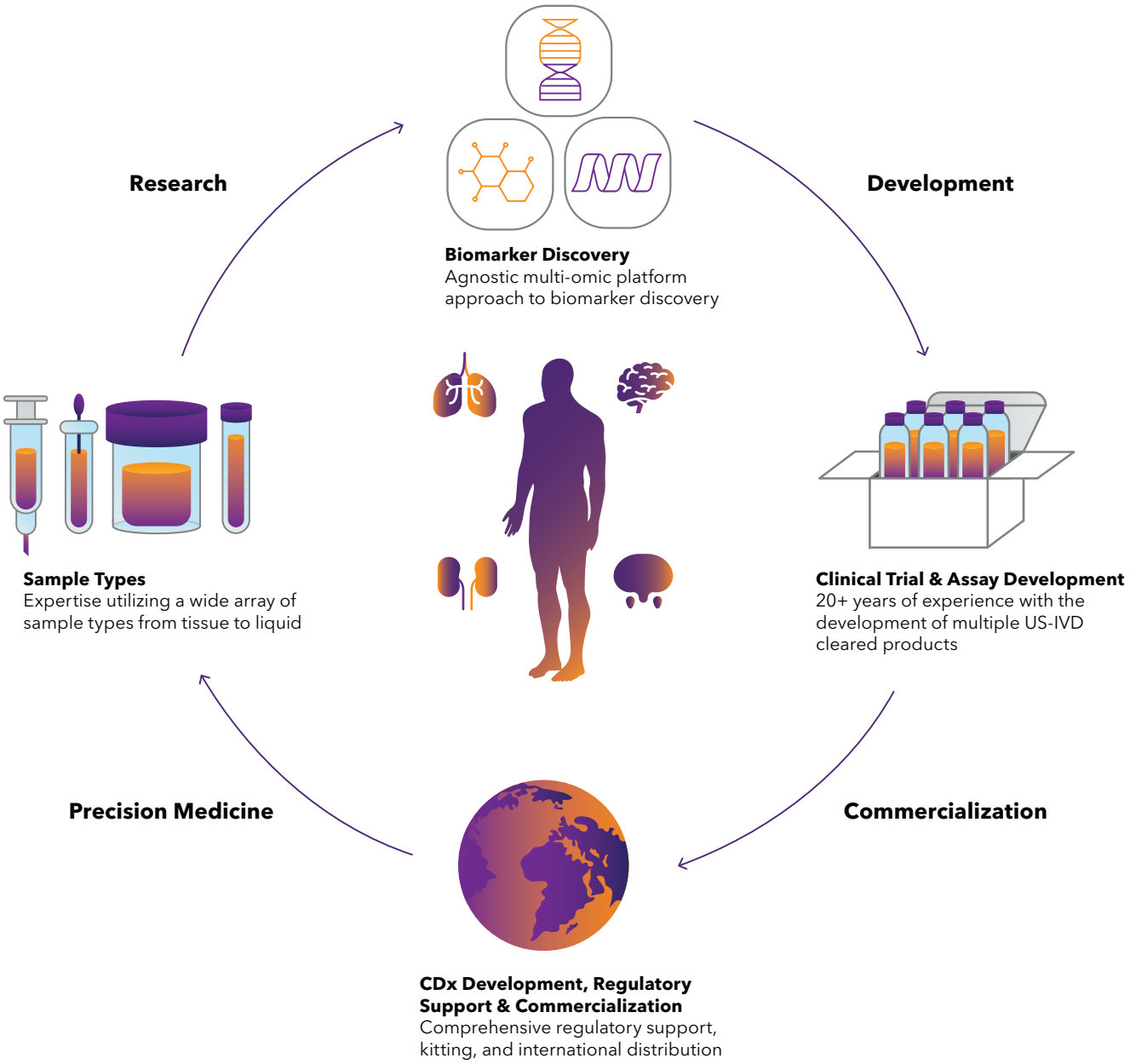


Figure 1. Highlights the broad range of companion diagnostic capabilities and services offered by Bio-Techne.

Figure 2. QuantideX Workflow

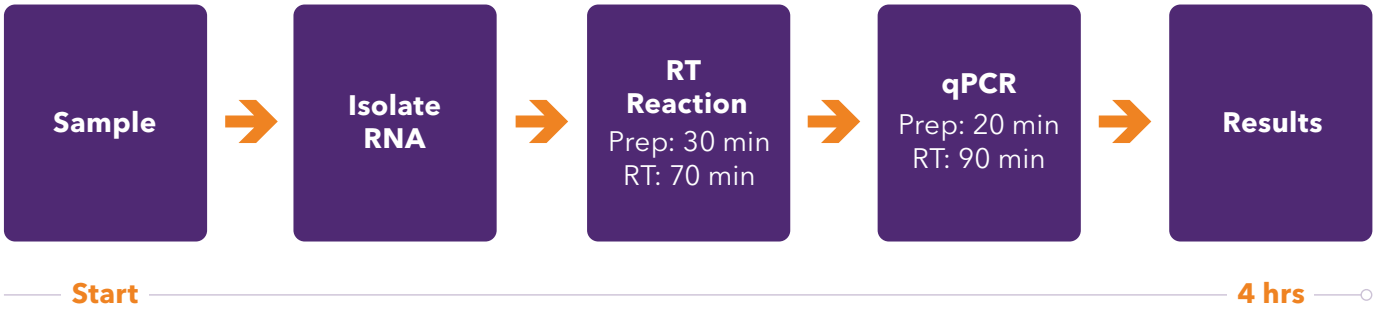


Figure 2. QuantideX Workflow. Approximately 4 hours from RNA to results.

The high sensitivity of the QuantideX technology, its extensibility to multiple genetic targets, and portability between qPCR platforms is demonstrated below:

Case Study 1: QuantideX qPCR BCR-ABL IS Kit Detects and Quantifies Tumor Burden in CML Patients on TKI therapy

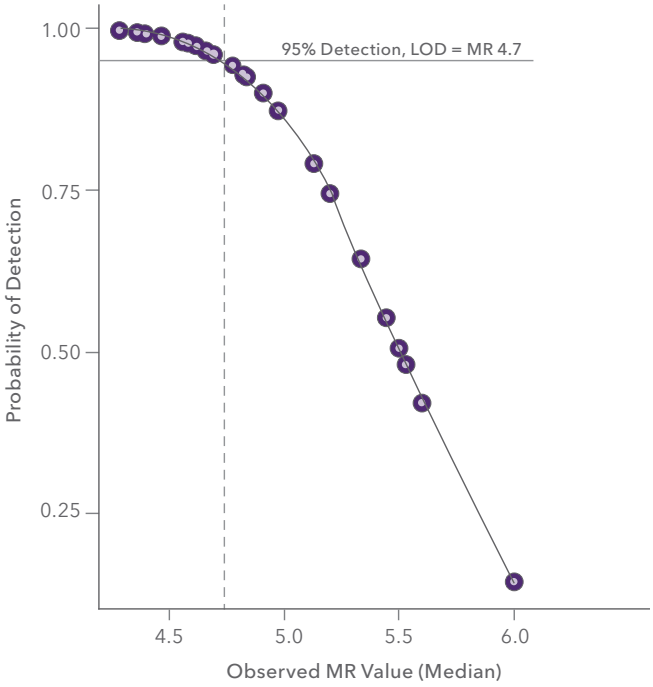
The QuantideX technology is the backbone of the QuantideX qPCR BCR-ABL IS kit, used to quantify BCR-ABL1 Major fusion transcripts resulting from translocation t(9;22) in CML for monitoring of patients on TKI therapy. The BCR-ABL1 translocation is a hallmark of CML.[2,3] TKIs act by targeting the BCR-ABL1 fusion protein, and their treatment efficacy is monitored by measuring the level of BCR-ABL1 transcript. Serial quantitation of BCR-ABL1 e13a2 and e14a2 (Major breakpoints) transcripts with RT-qPCR is now an established standard of care for assessing deep molecular responses in patients on TKI therapy. Previously, multiple laboratory-developed tests for BCR-ABL1 monitoring were developed as CTAs in seminal clinical trials. These tests generated different values from one another, leading to the effort to standardize results reporting on the International Scale for BCR-ABL1. The combination of TKIs and harmonized monitoring led to a revolution in CML treatment, making it a manageable disease. The QuantideX qPCR BCR-ABL kit was the first FDA-cleared diagnostic kit for use in CML management, rendering it the predicate for later tests.

Proven Clinical Utility and Clinical Validity of QuantideX Technology in Monitoring Treatment Efficacy in CML Patients

The QuantideX qPCR BCR-ABL IS Kit assay uses Armored RNA Quant® (ARQ) technology to deliver a highly reproducible, nuclease-resistant standard curve containing multiplexed mixtures of BCR-ABL1 and ABL1 transcripts, thereby reducing variability and removing the need for the costly, complex sample exchange previously required for reporting on the International Scale (%IS). The push-button QuantideX® Reporter software objectively and automatically reports results on the IS via a four-point calibrator system directly traceable to the values published for a reference material set World Health Organization (WHO) that was provided to select commercial entities. Together the complete system obviates the need for validating and re-validating conversion factors from burdensome sample exchange efforts, and eliminates the time and risk of errors from manual calculations.

Advancements in research investigating TKI treatment efficacy and therapy discontinuation resulted in the establishment of new guidelines for BCR-ABL1 assays, recommending analytical sensitivity sufficient to detect molecular responses (MR values) of ≥4.5 logs below the standardized IS baseline.[4,5] The QuantideX qPCR BCR-ABL IS Kit can detect residual amounts of disease down to 0.002%IS (MR4.7 limit of detection [LOD]) and has a linearity ranging from MR0.3 (50%IS) to MR4.7 (0.002%IS). The kit has been validated using clinically representative samples (FIGURE 3), not the more common RNA derived from cell lines, which can display bias.[6]

Figure 3. Proven Sensitivity Based on Rigorous Testing



The limit of detection (LOD) of the QuantideX quantitative polymerase chain reaction (qPCR) BCR-ABL IS Kit as determined by CLSI EP17-A2 guidelines by testing human RNA blends ranging from MR4.4 to MR6.0, with 60 replicates at each dilution for a total of 1680 possible data points. Note that high detection probabilities exist below the LOD, despite the stringent application of α=0.05 (95% detection).

Similarly, precision testing of the kit used 5 different MR levels composed of 5 unique positive specimens per level in the context of singleton testing (TABLE 1).[2,6] This approach lessens the burden of RNA isolation from clinical samples, the total hands-on time, and the risk of transposing samples in comparison to other methods that rely on replicate testing.

Table 1. Minimal Variability Across the Entire Dynamic Range of MR Values Demonstrates the Robustness of the Assay

Target MR	Mean MR	Std Dev
1	0.697	0.08
2	1.634	0.08
3	2.658	0.08
3.5	3.185	0.10
4	3.675	0.13

MR, molecular responses; Std Dev, standard deviation; qPCR, quantitative polymerase chain reaction. Testing spanned 3 lots, 3 operators, 20 runs, and 3 qPCR instruments

QuantideX Technology is Portable to Other Quantitative Real-time Instruments

The QuantideX technology is capable of portability to other quantitative real-time instruments as demonstrated by the validation of the QuantideX qPCR BCR-ABL IS Kit on the **cobas**® z 480 Analyzer** (Roche). Deep characterization of the assay on the **cobas**® z 480 using human RNA produced equivalent performance as compared to that on 7500 Fast Dx instrument, with an LOD/LOQ = MR4.70 (0.0020%IS).[1] Performance was similar for the QuantideX qPCR BCR-ABL minor Kit as well. Further, expert fluorescent dye and quencher selection by Asuragen can optimize any test to the desired platforms.

Case Study 2: Pharma Partnership with Immunexpress to Develop CTA for Advancing Clinical Trial for Sepsis

Asuragen has partnered with numerous pharmaceutical companies to meet their needs for robust, efficient CTAs to support their clinical trials. The QuantideX technology can be tailored to support a wide range of qPCR projects. This section highlights a case study where QuantideX RT-qPCR technology was successfully developed for CTA use in infectious disease clinical trials.

Sepsis is systemic inflammation triggered by an underlying infection and is a life-threatening medical emergency. Without timely treatment, sepsis can lead to rapid tissue damage, organ failure and death. Each year, according to the Centers for Disease Control and Prevention (CDC), at least 1.7 million adults in the US develop sepsis, and nearly 270,000 die as a result.[7]

There are multiple challenges associated with the diagnosis of sepsis. Patients in the early stages of sepsis are often quite difficult to distinguish from patients with infection-negative systemic inflammation. Making an incorrect distinction between these two clinical presentations has significant clinical and economic implications. Incorrect diagnosis can lead to inappropriate patient management, over-prescription of antibiotics, and, in worst-case scenarios, patient death or long-term debilitation.[8,9] Asuragen was selected to partner with Immunexpress to assist in the design of a clinical grade assay that would (1) determine which patients with systemic inflammation had sepsis, (2) be robust across independent patient cohorts, (3) be insensitive to disease severity, and (4) provide diagnostic utility.[10]

The gold standard for diagnosis of most bacterial infections, including sepsis, is the use of culture-based methods. However, culture-based methods are associated with multiple limitations, including inadequate sensitivity, specificity and predictive value for diagnosing sepsis; in addition, a rapid diagnosis is essential for sepsis, yet culture takes days to perform. Sepsis can also be the result of fungal and viral infections, further complicating assessment. Analysis of the host immune response provides an alternative approach to diagnosing sepsis[11], and the most studied host response biomarker is procalcitonin. However, a single biomarker with sufficient accuracy for diagnosing sepsis may not exist.[12] Recognizing this difficulty, the researchers at Immunexpress turned to investigating panels of biomarkers for interrogation of the host response.[13-17]

Asuragen performed the feasibility work using samples from trials conducted by Immunexpress to aid in the selection of the appropriate molecular targets. Asuragen then migrated the assay from an array-based signature to RT-qPCR utilizing the QuantideX platform. Asuragen was able to significantly reduce the time required to perform the test so that it could be completed in less than a day, and developed the underlying software to automate the reporting of results. The analytical and clinical validation of the test was performed by Asuragen, who also assisted with preparation of 510(k) submission to the FDA.

The SeptiCyte Lab test was validated on five independent, heterogenous cohorts of patients (n=345) from two medical centers in the Netherlands[10] and considered standard metrics of disease severity. The diagnostic utility of SeptiCyte Lab was evaluated by comparison to various clinical and laboratory parameters available to a clinician within 24hrs of ICU admission.

**cobas® is a registered trademark of Roche Diagnostics Operations, Inc.

SeptiCyte Lab was found to be significantly better at differentiating cases from controls than all tested parameters, both singly and in various logistical combinations, and more than halved the diagnostic error rate compared to procalcitonin in all tested cohorts and cohort combinations.[10] The work performed by Asuragen that aided in the development of the SeptiCyte Lab test enabled the development of a rapid molecular assay that was demonstrated to have appropriate sensitivity and accuracy, and sufficiently robust to be implemented into a rapid turnaround time.

QuantideX Platform Can Accelerate Your Drug Development Journey

The QuantideX platform demonstrates broad applicability in multiplex nucleic acid testing and is transferable to a broad range of genetic targets of interest. For applications in which serial quantitation is under investigation, frequent monitoring of genetic variants in patients will result in superior outcomes, as demonstrated by superior survival outcomes and lower healthcare costs in CML patients on TKI therapy, compared with those with less frequent monitoring.[19] As demonstrated by Case Study 1 above, the QuantideX platform is also extensible to other qPCR systems, with a potential to reduce capital expenditures by enabling increased utility of existing equipment. The QuantideX qPCR system's unprecedented level of sensitivity coupled with simple-to-run testing facilitates high-performance CTA development for novel analytes. When necessary, the technology can be applied as a multiplex design that amplifies and detects both test and control genes in the same reaction. Furthermore, a rapid time-to-result with minimal hands-on time (~1hr), due to streamlined reagent formulation, improves the assay workflow and increases the number of specimens that can be tested per run, important for trials with large cohorts to assess.

In addition to monitoring deep molecular responses to therapy, the QuantideX technology also has the potential of qualitatively detecting biomarkers with a high sensitivity. The well-established QuantideX platform can be easily adapted to monitor disease burden in diseases requiring highly sensitive detection and/or quantitation of a wide range of nucleic acid targets, and in designing high-performing, efficient, and simplified kits for genetic targets with unmet CTA needs.

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