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Multiplexing PCR technologies for biopharmaceutical research

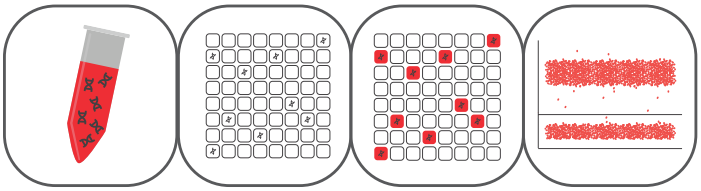
Multiplexed PCR: The next frontier for translational research

Quantification-based PCR technologies have long been widely used for nucleic acid measurement across research laboratories. As powerful tools for quantitative nucleic acid analysis, both quantitative PCR (qPCR) and digital PCR (dPCR) play a critical role in the research, development, and manufacture of biotherapeutics.

In drug discovery and development, these methods help researchers identify high-level targets, confirm and verify disease mechanisms, and assess genomic markers of therapeutic efficacy and safety.

dPCR

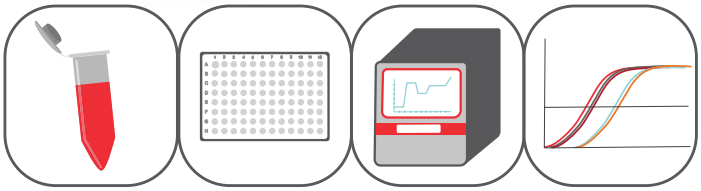
Preparation → Digitization → Amplification → Quantitation



- Absolute copy number quantification without the need for a calibration curve
- Technological advances enable high-sensitivity rare target detection, greater inhibitor tolerance, and efficient multiplexing

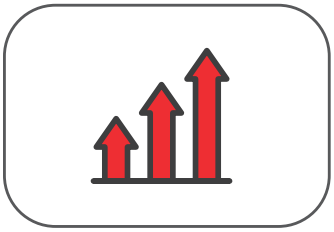
qPCR

Preparation → Plating → Amplification → Analysis

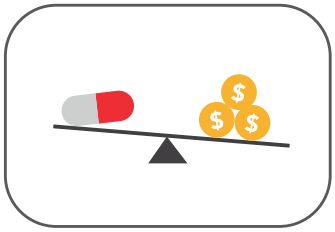


- Highly specific and robust, and provides exceptional target sensitivity
- Rapid and high-throughput, with 96- or 384-well runs in under 90 minutes

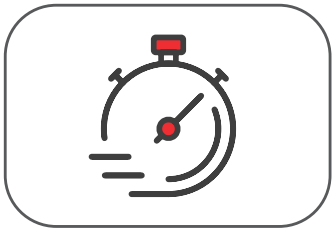
Multiplexed qPCR and dPCR enable you to measure multiple targets in a single reaction—reducing sample consumption, increasing throughput, and improving data precision. For biopharma researchers, this means faster target validation, more efficient screening, and accelerated discovery and development timelines.



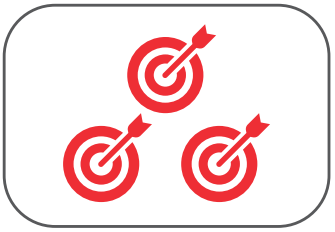
Increased throughput



Improved efficiency



Accelerated timelines



Consistent precision

Biopharma research

PCR-enabled drug discovery

From target identification to lead verification, researchers across biopharmaceutical institutions turn to robust methods and high-plex techniques that overcome common roadblocks in the drug discovery process. Quantitative genetic technologies with multiplexing capabilities, such as quantitative PCR (qPCR) and digital PCR (dPCR), are critical components of modern-day drug discovery workflows, allowing scientists to integrate molecular techniques for expansive discovery.

The drug discovery process

Drug discovery often begins with target identification and mechanistic studies that enable researchers to search for therapeutic candidates. During this pre-discovery stage, scientists identify targets using in silico methods, cellular assays, genomic studies, proteomic techniques, and many other strategies.[1] These initial investigations help researchers hypothesize if modifying a certain target or pathway will result in a potential therapeutic benefit, which gives way to the basic research stages of drug discovery that further confirm targets, screen and refine drug candidates, and verify lead compounds.[2]

Drug discovery pain points

Biopharmaceutical professionals face the primary challenge of balancing efficiency and innovation during drug discovery.[3] Pre-discovery and basic research methods are often laborious, requiring a large investment into effort and resources for assay verification and biomarker discovery.[4] Traditional discovery approaches such as immunoassays and standard PCR techniques are effective in certain situations but also include drawbacks that limit their general applicability in today's drug discovery and development landscape.[4]

Scientists may also struggle to meet precision and throughput demands during target identification and compound screening due to technical or resource limitations, such as sample scarcity and assay sensitivity.[4,5] Additionally, emerging high-throughput molecular techniques such as next-generation sequencing require extensive infrastructure and may be challenging to validate.[6]

Finally, refining a list of leads to determine optimal candidates involves evaluating large compound libraries, multiple target interactions, and a range of pharmacological effects. Lead refinement must include rigorous potency, selectivity, and safety analyses. Achieving reliability and reproducibility in such research workflows is challenging and further complicated by mechanistic heterogeneity among diseases, necessitating careful assessment of multiple pathways and potential off targets.[4]

Multiplexing with PCR technology

Highly specific, robust, and versatile, quantification-based PCR technologies have long been regarded as a gold standard for nucleic acid measurement across laboratory settings.[6,7] Multiplexed molecular assays such as qPCR and dPCR are useful methods for bioanalysis in bio-pharmaceutical research workflows. These methods are complimentary to one another. qPCR assays typically support a wide dynamic range, well suited for identifying large gene expression changes and differences between disparately expressed transcripts. dPCR helps detect

small fold changes and low-abundance transcripts with high-sensitivity precision. Integrating multiplexed strategies with both dPCR and qPCR helps accelerate discovery and verification of safe and effective targets and leads. For example, multiplexed qPCR may enable copy number variation or polymorphism genotyping to identify targets and investigate potential therapeutic mechanisms, while dPCR enables precision even amidst complex drug formulation buffers.

Multiplexing elevates the already dependable reliability and reproducibility of dPCR and qPCR assays, solving common drug discovery challenges such as throughput and efficiency.

As for any molecular method, scientists require the right reagents and instrumentation to perform multiplexed qPCR and dPCR research assays. For effective multiplexing, instruments must distinguish between separate fluorescent labels and accurately measure signals produced by each gene amplification. Researchers should also consider how combining primer pairs, probes, targets, and amplicons may influence the research for their specific assay.

Applied Biosystems™ real-time PCR instruments are equipped with multiple sets of filters, helping researchers multiplex numerous primers and probes in a single reaction. Developed to adhere to current good manufacturing practices (cGMP), Applied Biosystems™ TaqPath™ real-time PCR master mixes are specifically optimized for multiplexing, helping biopharmaceutical scientists avoid reagent competition and inhibition as more targets are assayed. Additionally, Applied Biosystems™ TaqMan™ Assays, compatible with both qPCR and dPCR, use this robust, highly specific, and flexible technology, allowing scientists to select probes with different dyes, and identify and screen more targets and candidates across a wide dynamic range.

The Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR System is a highly robust dPCR platform with multiplexing capability that is also compatible with TaqMan Assays.

Researchers use dPCR for higher plex gene expression analysis.[8] From discovery to confirming results obtained with qPCR or other methods, the QuantStudio Absolute Q dPCR system helps enable simple research workflows and multiplexing for multiple dyes.

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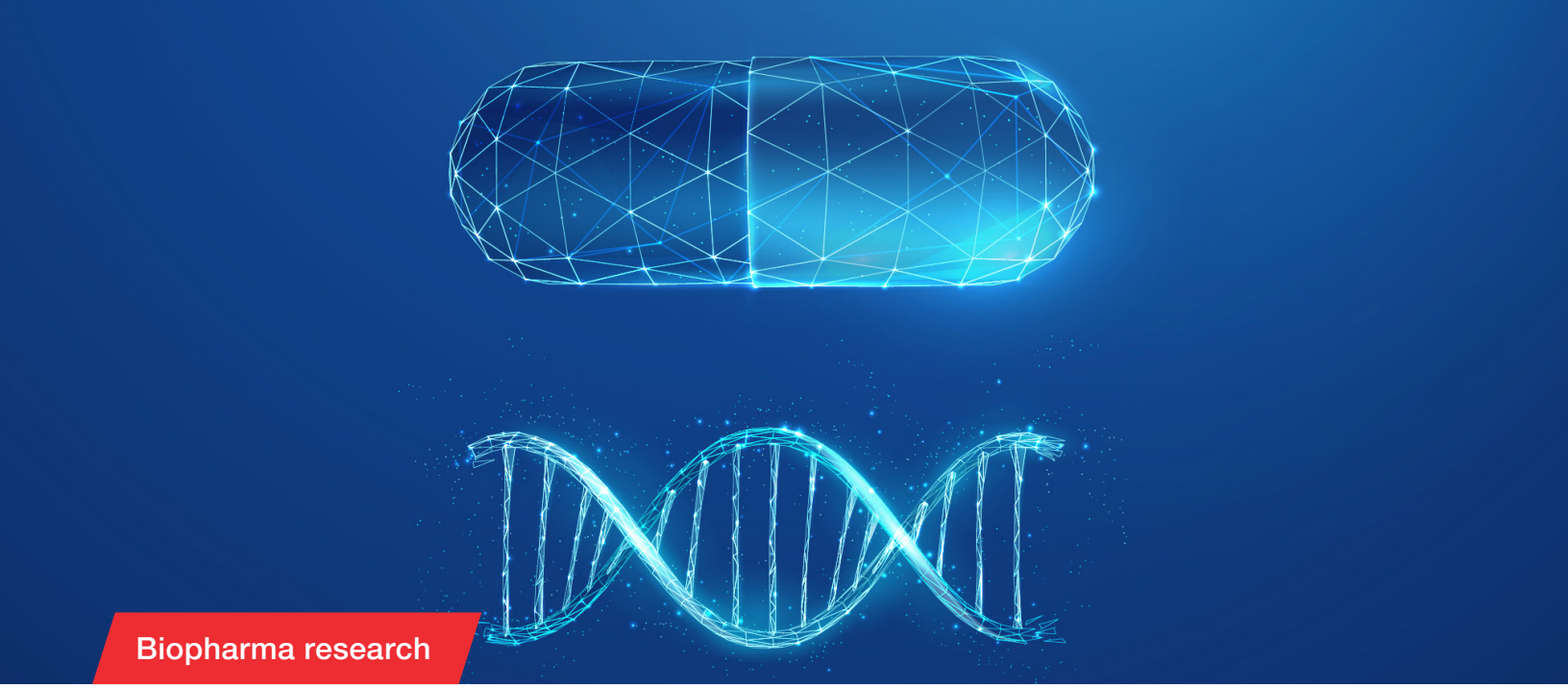

Biopharma research

The role of multiplex assays in drug development

Drug development is a multi-step journey that spans from target discovery to clinical implementation. Complex workflows and stringent compliance standards are common contributors to extensive development timelines.[1] In the dynamic biotechnology and biopharmaceutical research fields, dPCR and qPCR multiplexing has emerged as a crucial technique for analyzing multiple targets simultaneously, enabling accelerated and consistent workflows in bioanalytical research laboratories.

The drug discovery process

Therapeutic candidates must progress through three main stages to reach market: process development, preclinical and clinical studies, and manufacturing.[2] Process development relies heavily on early chemistry, manufacturing, and control (CMC), which encompasses the design, optimization, and validation of any methods and materials used to create new pharmaceutical products. CMC is an ongoing and iterative element of drug development. For example, adventitious agent tests such as microbial or nucleic acid contamination assays are major CMC considerations during both process development and later quality control (QC)-related manufacturing stages.[2,3]

A drug candidate may progress from process development into preclinical and clinical testing, which includes toxicology studies and clinical trials that help establish essential safety, dose, and efficacy data. Therapeutic candidates that succeed through preclinical and clinical studies must then go through rigorous manufacturing and QC steps, where consistency and compliance are key.[2]

Assays during drug development

Materials and methods chosen and validated during process development are transfered to preclinical, clinical studies, and QC testing. As researchers progress from working with small scale manufactured drug materials to commercial products, the assays they use to measure contamination or therapeutic endpoints must remain consistent and reliable while also being scalable.

Adventitious agents

All manufactured therapeutics products face the risk of adventitious agent contamination, but those produced in cell culture or requiring animal-based reagents and systems are at the highest risk. This includes many leading therapeutic modalities, such as biologics and cell and gene therapies.[4] Additionally, cell therapy materials are often collected from patients or donors, posing a potential source of contamination in a small and precious sample type that is difficult to purify. It is crucial to use sensitive, reliable, and rapid assays that can help ensure early detection and intervention during development.

Most adventitious agents, such as viruses, bacteria, and eukaryotic organisms, have nucleic acid components that can be used as readouts for contamination. PCR-based targeted nucleic acid detection is a preferred method because it is highly sensitive, inexpensive, and quick, with typical turnaround times in under a day.[4] Recent multiplexing advances have enabled even faster sample screening for multiple adventitious viruses in a single qPCR reaction.[4] Additionally, dPCR helps enable precise

and accurate absolute quantification of common contaminants, such as residual DNA and *Mycoplasma*.

Clinical research

Researchers and manufacturers must demonstrate that their manufacturing process consistently ensures final drug product identity, purity, and potency, but it can be challenging to determine clinically relevant readouts.[5] Newer therapeutic modalities often lack established assays that reliably indicate clinical activity in a patient for the intended indication and thus, biopharmaceutical scientists may rely on functional assays that reflect a drug candidate's mechanism of action.

Scientific and technological advances have enabled multiscale, longitudinal measurements of various biomolecules such as RNA, DNA, proteins, and metabolites, facilitating functional assays that reflect potential clinical potency.[6] Multiplexed qPCR can be used as a drug development tool to evaluate preclinical and clinical end points, including biodistribution, gene expression, and pharmacokinetics.[8]

Challenges and benefits of multiplexing

Whether performing safety or efficacy measurements, multiplexed dPCR and qPCR supports biopharmaceutical industry professionals with robust, scalable assays in early research workflows that carry through the entire development pipeline, from R&D process optimization to QC. For instance, either qPCR or dPCR can be used to test for adventitious agents at multiple points throughout a post-discovery research process, helping development stay on track while maintaining reliable readouts of lead potency and safety. However, multiplexing brings its own set of challenges that can affect accuracy and reliability. The chance of unwanted interactions between primers, probes, and amplicons increases when amplifying targets, and further elevates with each PCR cycle, limiting the potential of multiplexing while scaling up potency and safety research assays.

TaqPath qPCR and RT-qPCR master mixes help address nonspecific probe, target, and amplicon interactions by providing high sensitivity, specificity, and a wide dynamic range for optimal multiplexing. Along with real-time PCR instruments and highly specific custom Applied Biosystems™ TaqMan™ probes such as the TaqMan™ MGB Probe, these qPCR master mixes help ensure reproducibility and minimize experimental variability. Additionally, TaqPath qPCR and RT-qPCR master mixes are manufactured in an ISO 13485-certified facility that adheres to current good manufacturing practices (cGMP), with stringent production and process controls to help ensure lot-to-lot consistency, addressing reliability challenges in drug development research workflows.

The QuantStudio™ Absolute Q™ Digital PCR System employs microfluidic array plate (MAP) technology to deliver highly-accurate dPCR results, particularly for higher plex gene expression investigations, from measuring genes of interest to monitoring residual DNA. Along with the Applied Biosystems™ QuantStudio™ Absolute Q™ AutoRun Digital PCR Suite, multiplex dPCR assays help scientists scale precise nucleic acid quantification assays while keeping them simple.

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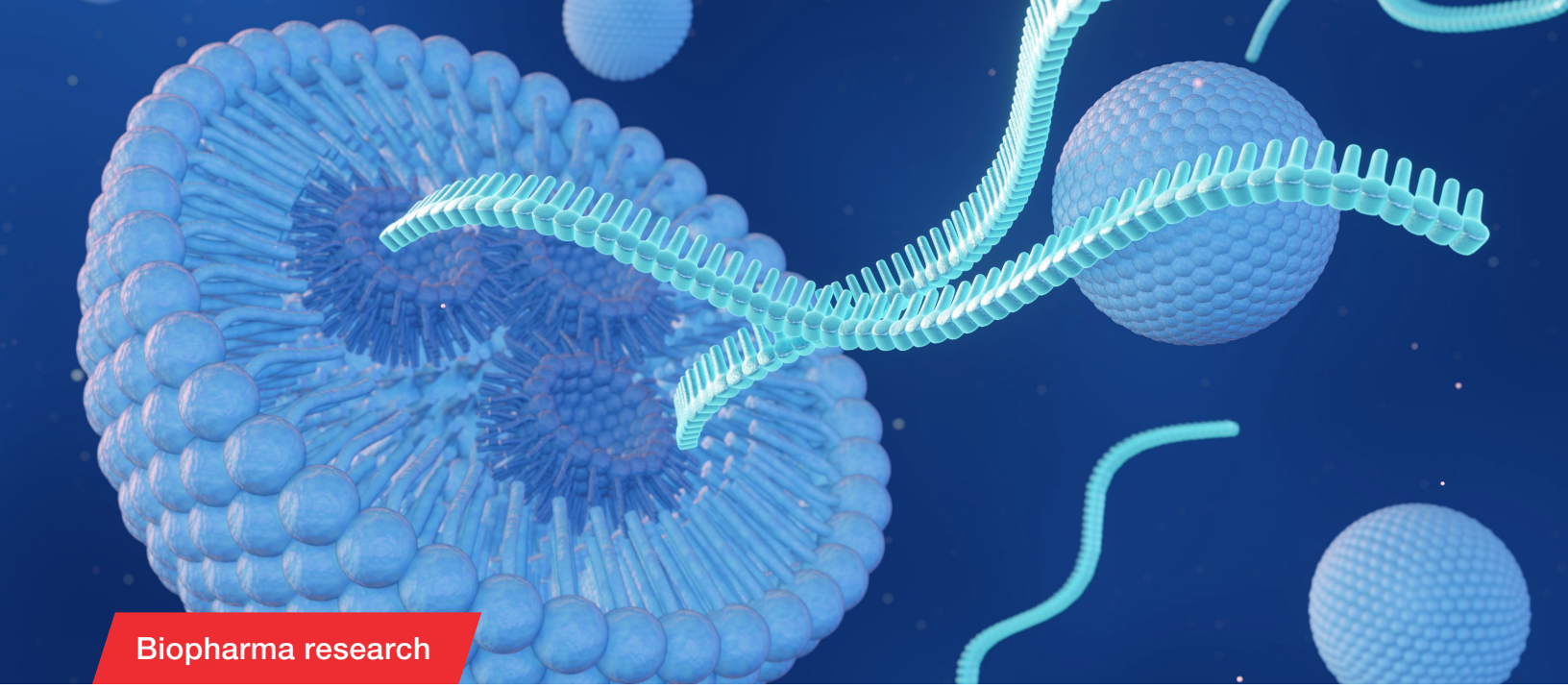

Biopharma research

Detecting distribution during mRNA therapeutic development

Biodistribution analyses detect the in vivo distribution, persistence, and clearance of a therapeutic product from the administration site to target and non-target tissues and fluids.[1] Although the European Medicines Agency (EMA) and United States Food and Drug Administration (FDA) recommend qPCR-based analyses for cell and gene biodistribution studies, the exact criteria for performing these studies are not yet well-defined, and until recently, this technique had not been reported in the context of capturing RNA product biodistribution.[1,2]

In the wake of successful mRNA-based vaccines reaching clinical research applications, there is increased interest in RNA-based therapeutic development among the biopharmaceutical and biotechnology sectors. Accurate and reliable mRNA quantification methods are essential for assessing biodistribution and pharmacokinetics for such products.[2] Scientists at a prominent pharmaceutical company initially chose banded DNA (bDNA) analysis for assessing biodistribution of mRNA vaccines but recently sought to validate a more adaptable qPCR-based approach for large-scale and multisite bio-distribution studies.[2-4]

Measuring mRNA pharmacokinetics

In work published in *Bioanalysis*, scientists compared the performance of bDNA and RT-qPCR-based assays to measure lipid nanoparticle (LNP)-encapsulated mRNA distribution across serum and other tissue samples.[3] The researchers administered 1 mg/kg intravenous doses of LNP-encapsulated luciferase mRNA to rat models and collected biological samples at different time points to measure mRNA concentrations using both assays. Various statistical analyses revealed that while both assays yielded consistent pharmacokinetic profiles across different tissue types, RT-qPCR demonstrated greater reproducibility across multiple contract research organization laboratories and a wider dynamic range and higher sensitivity compared to the bDNA assay.[3]

In a separate investigation, researchers also compared the sensitivity, specificity, correlation, and overall performance of each assay using a therapeutic mRNA candidate that they administered to non-clinical mouse models. The results, published in *The AAPS Journal*, revealed that inter-assay accuracy, precision, and total errors were comparable, reinforcing the use of RT-qPCR as a widely accessible alter-native and reliable platform for quantifying pharmacokinetics of subsequent mRNA-LNP therapeutics.[4]

Reliable biodistribution assays

Together, these studies provide valuable insights to help guide biopharmaceutical industry professionals in choosing the most suitable method for mRNA biodistribution quantification. While both bDNA and RT-qPCR are effective, qPCR's higher sensitivity, broader dynamic range, and greater reproducibility make it particularly advantageous for detecting low-abundance mRNA levels and large-scale studies. The researchers' findings are especially pertinent for the ongoing development and evaluation of RNA-based therapeutics, where accurate and reliable biodistribution measurements are critical for therapeutic safety

and efficacy. The demonstrated adaptability of qPCR across different biopharmaceutical laboratory settings also underscores its utility for large-scale and multisite studies, which are common in drug development processes.[3]

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Looking toward multiplexed qPCR biodistribution assays

Biodistribution assays benefit from multiplexing, which accelerates research workflows by helping scientists detect more than one target in a single test.[1,2] In the future, biodistribution assays that implement multiplexed qPCR may further support RNA therapeutic development. By decreasing the amount of sample required and increasing the number of targets analyzed simultaneously, multiplexing in qPCR assays helps scientists scale up while helping to reduce sample analysis costs.[1]

Applied Biosystems™ qPCR and RT-qPCR master mixes are optimized for multiplexing in a variety of qPCR applications and formulated to maximize target amplification. In addition to the qPCR and RT-qPCR master mixes, TaqMan Multiplex solutions include TaqMan™ QSY and QSY2 Probes for high-level multiplexing, and pre-designed TaqMan assays for endogenous controls such as GAPDH and RNase P genes. From single step preparation to sensitive high-throughput analysis, these solutions provide application-specific formulations for multiplexing, enabling assay flexibility so that researchers and manufacturers can choose the most suitable approach for their drug development needs.

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References

Article 1: PCR-enabled drug discovery

1. Singh N, et al. Drug discovery and development: Introduction to the general public and patient groups. *Front Drug Discov.* 2023;3:1201419.
2. Hughes JP, et al. Principles of early drug discovery. *Br J Pharmacol.* 2011;162(6):1239-1249.
3. Gu M, et al. Forward or backward: Lessons learned from small molecule drugs approved by FDA from 2012 to 2022. *Molecules.* 2023;28(24):7941.
4. Vo D, Trinh KTL. Polymerase chain reaction chips for biomarker discovery and validation in drug development. *Micromachines (Basel).* 2025;16(3):243.
5. Goodsaid F. Quantitative real time polymerase chain reaction in drug development. *Drug Dev Res.* 2004:62(2):151-158.
6. Hays A, et al. Best practices in qPCR and dPCR validation in regulated bioanalytical laboratories. *AAPS J.* 2022;24(2):36.
7. Hays A, et al. Bioanalytical assay strategies and considerations for measuring cellular kinetics. *Int J Mol Sci.* 2022;24(1):695.
8. Measuring changes in gene expression with the QuantStudio Absolute Q Digital PCR System. *Thermo Fisher Scientific.* 2025.

Article 2: The role of multiplex assays in drug development

1. Shareef U, et al. A comprehensive review of discovery and development of drugs discovered from 2020–2022. *Saudi Pharm J.* 2024;32(1):101913.
2. Buckley LA, et al. Drug development 101: A primer. *Int J Toxicol.* 2020;39(5):379-396.
3. Gombold J, et al. Systematic evaluation of in vitro and in vivo adventitious virus assays for the detection of viral contamination of cell banks and biological products. *Vaccine.* 2014;32(24):2916-26.
4. Valiant WG, et al. A history of adventitious agent contamination and the current methods to detect and remove them from pharmaceutical products. *Biologicals.* 2022:80:6-17.
5. Gutierrez L, et al. The confluence of innovation in therapeutics and regulation: Recent CMC considerationsv. *J Pharm Sci.* 2020;109(12):3524-3534.
6. Seyhan AA. Lost in translation: the valley of death across preclinical and clinical divide – identification of problems and overcoming obstacles. *Transl Med Commun.* 2019;4:18.
7. Hays A, et al. Best practices in qPCR and dPCR validation in regulated bioanalytical laboratories. *AAPS J.* 2022;24(2):36.

Article 3: Detecting distribution during mRNA therapeutic development

1. Ma H, et al. qPCR and qRT-PCR analysis: Regulatory points to consider when conducting biodistribution and vector shedding studies. *Mol Ther Methods Clin Dev.* 2020;20:152-168.
2. Vervaeke P, et al. Regulatory guidelines and preclinical tools to study the biodistribution of RNA therapeutics. *Adv Drug Deliv Rev.* 2022;184:114236.
3. Ali S, et al. A comparison of branched DNA and reverse transcriptase quantitative polymerase chain reaction methodologies for quantitation of lipid nanoparticle encapsulated mRNA. *Bioanalysis.* 2024;16(21-22):1141-1154.
4. Ortiz J, et al. Comparison of RT-qPCR with branched dna to quantify a lipid nanoparticle–encapsulated mRNA therapeutic in serum and liver tissue samples from nonclinical PK studies. *AAPS J.* 2025;27(1):27.

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