Multiplexing
Solving for efficiency and efficacy

Abstract
Healthcare systems face growing pressure to deliver accurate diagnostics while minimising resource consumption and environmental impact. This paper examines how Roche's multiplexing technologies across various diagnostic platforms address these challenges by enabling simultaneous detection of multiple biomarkers or targets from single samples. From tissue diagnostics to molecular testing and point-of-care solutions, the implementation of multiplexing represents a significant advancement in sustainable healthcare practices, offering not only clinical benefit, but also environmental, social and economic value whilst maintaining diagnostic excellence.

Introduction
Conventional diagnostic methods often require multiple samples and separate testing procedures for individual analytes, resulting in increased resource consumption (multiple tests versus just one) and potentially delaying patient care decisions, if certain tests are done sequentially instead of simultaneously.

As healthcare facilities worldwide strive to reduce their environmental impact whilst improving patient outcomes, innovative diagnostic solutions that address both objectives have become increasingly important. Roche has developed multiplexing capabilities across its diagnostic portfolio to meet these dual demands of sustainability and clinical excellence.

Understanding multiplexing technology

Multiplexing is an advanced diagnostic approach that enables simultaneous detection of multiple targets from a single sample or reagent or test kit. While implementation varies across platforms, the fundamental principle remains consistent: maximising information yield while reducing sample requirements and resource utilisation. The result is more informed clinical decision-making, and less waste.

In tissue diagnostics, multiplexing allows sequential application of antibodies and detection systems on a single tissue section. This "holistic visualisation uncovers a world of interactions, enables co-localisation studies and allows for an overall greater understanding of cell populations and protein-protein relationships".[1] The process involves a carefully orchestrated sequence that maintains tissue integrity while extracting multiple data points.

In molecular diagnostics, multiplex PCR enables detection of multiple targets in a single reaction. Point-of-care multiplexing provides rapid results for multiple analytes from a single patient sample. Each application shares the common goal of extracting maximum diagnostic value without requiring additional sample, or more succinctly stated: More with less.

Multiplexing across Roche's research and diagnostic solutions

Tissue multiplexing research

The DISCOVERY ULTRA platform enables automated multiplexing of up to five biomarkers on a single tissue section.[2] This research approach provides comprehensive tissue characterisation while preserving valuable patient samples. The sequential staining process includes application of primary antibodies, binding of HRP-conjugated secondary antibodies and enzyme-mediated deposition of fluorophores, with heat deactivation steps between rounds to prevent cross-reactivity.[2] Unlike next-generation sequencing, flow cytometry and other protein expression technologies, IHC multiplexing provides simultaneous detection and analysis of multiple biomarkers from a single tissue section without the loss of tissue context.[1] The automation of DISCOVERY ULTRA ensures consistent results while minimising reagent waste and manual intervention.

Molecular diagnostic multiplexing

Roche's molecular diagnostic portfolio includes several multiplexed testing solutions that deliver significant sustainability benefits.

With a new technique that uses a standard PCR plate on existing **cobas®** 5800/6800/8800 systems, Roche scientists found a way to triple the number of targets (e.g. viruses or bacteria) that can be detected based on multiple thermo-activated dyes, temperature cycling and relatively simple data processing. Called TAGS – short for temperature-activated generation of signal – more information is available per single test, which can mean quicker results for patients and lower costs for healthcare professionals. And with no need for additional reagents and consumable parts, there is less waste for the landfill. Commercially launched with the name **cobas®** Respiratory flex, this system is designed for flexibility - because it allows users to test only what's needed, and to tailor how they use it, which may allow them to simplify their respiratory season logistics. Up to 12 results can be simultaneously detected and differentiated from from one test using the same kit. Results can be generated for the following viral targets: influenza A virus, influenza B virus, respiratory syncytial virus (RSV), SARS-CoV-2, adenovirus (species B, C and E), human metapneumovirus, human rhinovirus/enterovirus, common human coronaviruses (229E, HKU1, NL63, OC43), parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, and parainfluenza virus 4.[3] For more information, visit: https://diagnostics.roche.com/global/en/products/lab/cobas-respiratory-flex-pid00000771.html

Roche also supports more focused multiplexed results in near patient care testing – for example SARS-CoV-2 (COVID-19), Influenza A/B and RSV – which may be more commonly used for the general population and when those viruses are known to be circulating. Multiplexed sexually transmitted infection (STI) panels that simultaneously detect multiple targets from a single patient sample are also now possible. This approach is highly useful as common STIs often present overlapping symptoms and can frequently be asymptomatic, making diagnosis challenging when relying solely on symptoms.[4] This consolidated approach also reduces plastic consumption, decreases biohazardous waste generation and reduces the need for repeat sample collection, which are important contributors to a more sustainable environment.

As a modular and scalable system, the **cobas® eplex** system uses specialized test cartridges to simultaneously detect and identify greater than 20 viral and bacterial targets from a single sample test for infectious diseases, specifically with test panels for respiratory infections and also bloodstream infections which can lead to sepsis.[5] Additional panels are in development for gastrointestinal infections, meningitis and encephalitis.

The **cobas® eplex** system is an easy to use sample-to-answer system that enables it to be used by any technician on any shift. With results in only about 90 minutes, this is especially useful for testing high risk or hospitalized patients that require a comprehensive result, rapidly. Using multiplex testing not only helps aids in clinical management for each affected patient, it also contributes to reducing antibiotic resistance and promoting antimicrobial stewardship efforts to help address the growing problem of antibiotic resistance.[6] By detecting multiple pathogens in a single test cartridge, comprehensive results can be obtained from a single sample that requires a small sample test volume and with reduced manual handling steps.

Point-of-care multiplexing

The **cobas® liat** system exemplifies sustainable point-of-care testing through multiplexed assays for respiratory panel testing to aid in the differential diagnosis, as well as other multiplexed panels targeted for STI pathogens. This compact system requires minimal sample volume yet delivers results for multiple pathogens in approximately 20 minutes.** The consolidated testing approach reduces plastic consumption and waste generation compared to single-target rapid tests, while enabling faster clinical decisions that benefit both patients and healthcare systems. Rapid molecular point-of-care testing assists in the clinical management of STIs in decentralised and community-based healthcare settings, enables more informed treatment strategies, and can help contain further transmission by providing timely diagnosis.

Sustainability impact

Environmental benefits

Multiplexing technology significantly reduces the environmental impact of diagnostic testing through several key mechanisms. The ability to analyse multiple targets from a single test kit or reagent cassette kit may dramatically decrease overall resource consumption in laboratory settings. This consolidation potentially leads to substantial reductions in plastic usage, reagent consumption and waste generation. For tissue diagnostics specifically, the technology results in fewer slides and less waste overall, including reduced consumption of glass, consumables and reagents. The consolidated processing steps across all platforms result in less computing cycles and resource time required. Additionally, reduced material requirements translate to decreased transport needs for samples and materials. Furthermore, digital pathology helps reduce courier costs as digital images of stained slides can be shared electronically, further lowering the environmental footprint of diagnostic processes.

Social impact

The social benefits of multiplexing extend beyond environmental considerations, directly impacting patient care and healthcare accessibility. By enabling multiple analyses from a single sample, precious patient specimens are preserved, that may be limited in quantity or difficult to obtain. Multiplexing helps use those sparse samples more effectively while avoiding situations in which clinicians put the patient through additional procedures. This preservation approach potentially reduces the need for a patient to endure additional or multiple sampling procedures.

The consolidated analysis process delivers faster time to multiple results, with the potential to also save patients from unnecessarily long wait or revisit time and enables earlier treatment decisions.Enabling earlier treatment can also help to reduce symptoms duration. This is particularly crucial in acute care settings where rapid differentiation between pathogens with similar clinical presentations can significantly impact treatment plans. Additionally, the efficiency of multiplexing enhances accessibility to comprehensive testing in resource-limited settings, democratising advanced diagnostic capabilities.

Economic considerations

Multiplexing technology delivers compelling economic advantages that strengthen its value proposition for healthcare institutions. The consolidation of testing processes results in reduced per-test costs through more efficient use of reagents and materials. For instance, running a multiplexed panel potentially uses less resources than running each analyte test individually.

The reduction in physical waste translates to decreased waste management expenses, with fewer materials being a direct economic benefit. From a resource allocation perspective,

multiplexing also contributes to possibly less funds wasted on unnecessary or ineffective treatments. Without multiplexing, follow-up testing and/or treatment can require more funds and time, giving labs an opportunity for economic efficiency.
Laboratory efficiency and throughput may result in marked improvements through the consolidated testing approach. The reliability and comprehensive analysis capabilities of multiplexing may also reduce the need for repeat testing, as patients can leave with answers right away rather than requiring follow-up visits.

Implementation use cases
The following use cases illustrate the potential practical sustainability benefits of multiplexing technologies across different hypothetical healthcare settings. While specific metrics will vary by institution, these examples demonstrate the types of impact they could potentially expect when multiplexing is effectively implemented.

Regional medical centre respiratory testing
During high-volume respiratory seasons, a regional medical centre could implement multiplexed respiratory panels, allowing them to test for multiple pathogens simultaneously. A patient may come in for a COVID-19 test, but multiplexing would allow the clinic to test for influenza and RSV simultaneously, from the same sample. That way, a negative COVID-19 test wouldn't simply give the patient the "all clear" to continue living life normally, but instead may give them a "negative COVID-19, negative RSV, positive flu" result, saving both time and helping prevent disease spread.

University hospital laboratory transformation
A major university hospital that integrates tissue multiplexing technology may see a reduction in overall slide usage for complex cancer cases.

Public health STI testing
A public health laboratory may consider adopting multiplexed STI panels, allowing them to process more samples with the same staffing levels while reducing their plastic waste stream. The comprehensive testing approach may also improve treatment compliance by eliminating the need for patients to return for additional testing, thus achieving the goal of fewer patient visits that multiplexing can facilitate.

Future implications
As healthcare systems increasingly prioritise sustainability and efficiency, multiplexing technology represents a significant advancement in combining environmental responsibility with diagnostic excellence. Future developments may include:

- Enhanced automation capability
- Expanded target panels decreasing sample size requirements
- Increased integration with digital healthcare platforms
- Advanced data analytics improving testing efficiency and predictive insights
- Hybrid multiplexing approaches combining different methodologies for comprehensive results

Conclusion
Multiplexing technology across Roche's diagnostic portfolio demonstrates how innovation can address both clinical and environmental challenges in healthcare. By enabling more efficient use of resources while maintaining or improving diagnostic capabilities, this approach represents a significant step toward sustainable laboratory practices. The combination of environmental, social and economic benefits positions multiplexing as a key technology in the future of sustainable healthcare delivery.

*This document is for informational purposes only
**Turn-around times vary by assay

References:

1. Roche Discover your research potential brochure [Internet; cited 2025 Mar 31] Available from: https://diagnostics.roche.com/gb/en/article-listing/health-topics/immunohistochemistry/multiplexing-resources.html (MC--IE-02213)
2. Roche White paper, Bhaumik, Srabani. 2016 U DISCOVERY 5-Plex IF procedure: A fully automated immunofluorescence multiplex solution
3. cobas® Respiratory flex Instructions for use
4. World Health Organization, Guidelines for the management of symptomatic sexually transmitted infections, 15 July 2021 [Internet, cited 10April2025]. Available from: https://www.who.int/publications/i/item/9789240024168
5. Roche [Internet; cited 2025 Mar 31] Available from: https://diagnostics.roche.com/us/en/article-listing/innovations/esensor-technology.html
6. NIH. The Role of Antibiotic Stewardship in Promoting Appropriate Antibiotic Use. [Internet; cited 2025 April 1] Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6600622/

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