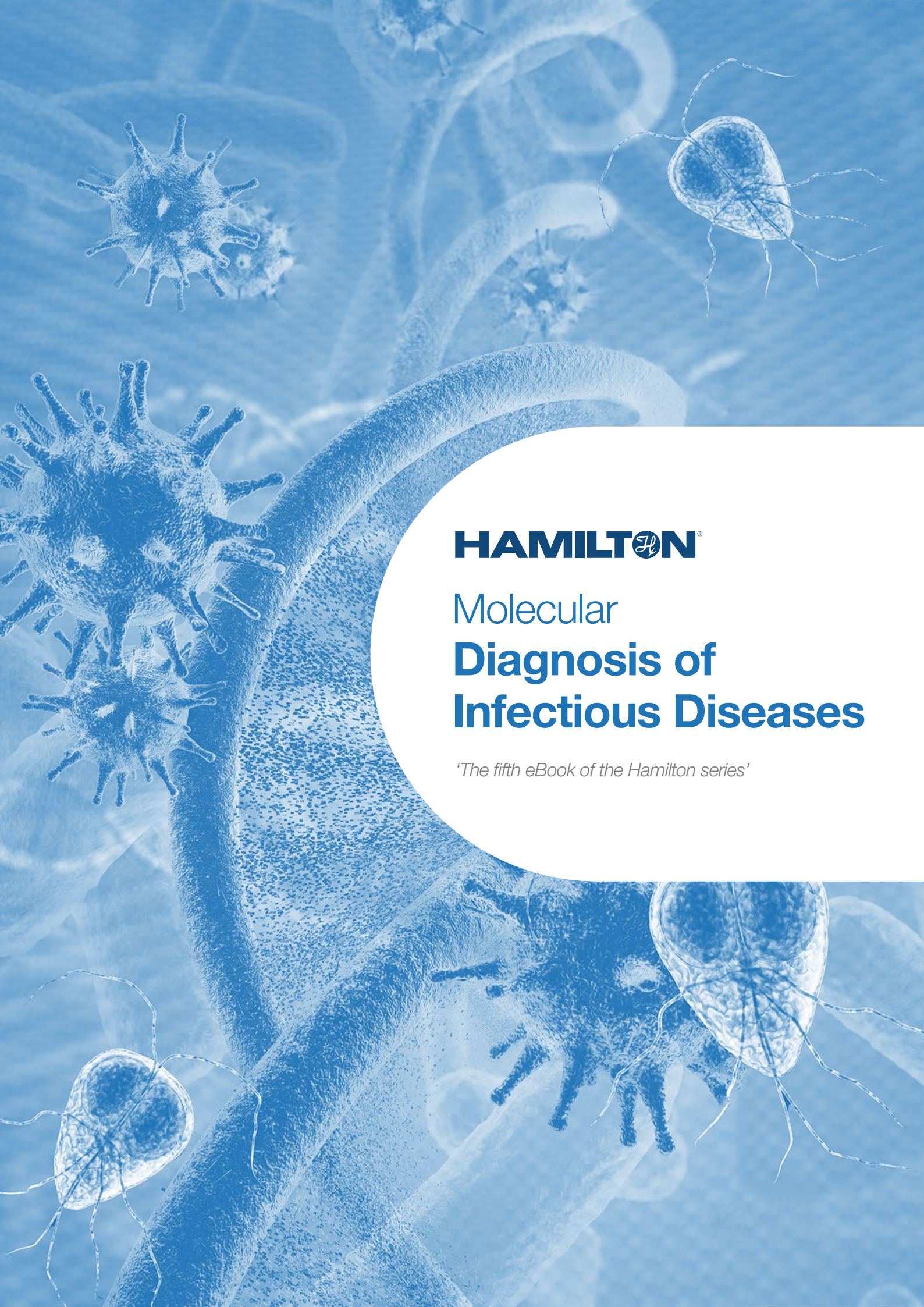

HAMILTON®

Molecular
Diagnosis of Infectious Diseases

'The fifth eBook of the Hamilton series'

Foreword

It is now widely accepted that a timely and accurate diagnosis is needed to ensure adequate patient treatment, effective infection control measures and attainable healthcare costs. This is particularly relevant when preventing antimicrobial resistance, as well as the spread of diseases during pandemics.

The remarkable developments of molecular technologies have allowed for faster, more accurate and more efficient diagnostic methods. Whereas traditional methods are based on the culture of pathogens, molecular methods are based on the identification of the genomic material unique to each pathogen. Current molecular diagnostic methods are based on various types of techniques including qPCR, Next-Generation Sequencing (NGS) and microarrays. The majority of the workflows developed for these techniques can be automated to improve variables such as standardization, sample traceability and throughput.

In this eBook, we discuss the impact of modern molecular diagnostic technologies on patient treatment and disease management, the drivers behind the need for automating the sample preparation workflows of molecular diagnostics methods and the solutions that Hamilton offers to customers working in this field.

This eBook is part of a dedicated campaign on the topic of molecular diagnosis of infectious diseases, where we aim to provide our readers with interesting educational resources and additional insights into the way our customers are using Hamilton solutions to accomplish their tasks. For readers particularly interested in the use of NGS in the fields of oncology and rare diseases, we highly recommend our previous eBooks, "NGS in the Field of Precision Medicine Oncology" and "NGS in Diagnosis of Rare Diseases".

I would like to thank our International Commercial Leader Diagnostics and Senior Market Segment Leader Genomics for their valuable contributions to this eBook. We hope you find the content beneficial.

Your kind feedback is always highly appreciated.

Yours sincerely,
Dr. Gabriela Boza-Moran
Scientific Content Manager
Hamilton Robotics Direct Sales
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For magnetic bead-based nucleic acid extraction

RT STARlet
Integrated solution from nucleic acid extraction to Real-Time PCR analysis

Clinical NGS STARlet
CE-IVD solution for NGS library preparation

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Diagnosis of Infectious Diseases: Exploring the Role of Modern Molecular Diagnostic Methods

Infectious diseases are caused by a wide range of pathogens comprising several species of viruses, bacteria, fungi, protozoa, and helminths.[1] The fast and accurate identification of these pathogens is a critical requirement for the adequate treatment of diseases and the control of their dissemination within the population.

There have been major advances in the technologies used for these purposes in the last few decades. Whilst traditional diagnostic methods are based on the culture of pathogens, modern methods use molecular technologies that target genomic material unique to each pathogen. As a result, the latter methods deliver quicker and – in many cases – more accurate results than conventional culture-based methods.[2] These two advantages are particularly important in the fight against Antimicrobial Resistance (AMR) and the control of pandemics. It is estimated that at least 700,000 people die every year from infections caused by antibiotic-resistant bacteria.[3] Furthermore, the total cost to society due to the burden of the health care system is estimated at US$ 35 billion a year.[4]

The main contributing factor to antibiotic resistance, and AMR in general, is the inappropriate use of antimicrobials (e.g. taking medication when it is not needed, not completing the full course prescribed, taking a lower dose than required, using the wrong medication).[4] One of the key drivers for the inappropriate use of antimicrobials is the fact that they are often prescribed without accurate knowledge of the disease-causing pathogen – presumably to save time and money in the short term.[3] Fortunately, rapid molecular assays are now offering healthcare professionals the opportunity to test for many different pathogens within a very short time. Furthermore, biomarkers for drug resistance can also be rapidly detected using these assays, resulting in the delivery of more appropriate treatments (e.g. a narrow-spectrum antibiotic instead of a broad-spectrum antibiotic). This approach not only limits antimicrobial resistance but also reduces the risk of sepsis.[5]

The management of pandemics also relies heavily on early, widespread and rapid diagnosis, especially in cases where the infection is frequently asymptomatic. Events occurring during the current coronavirus pandemic (COVID-19) clearly show that this is the case. During the first wave, the countries that implemented robust diagnostic testing managed to contain the spread of the disease early on.[6] Beyond COVID-19, a vast amount of studies investigating various infectious diseases show that an early and accurate diagnosis has a strong and positive effect on patient outcomes, infection control measures and healthcare costs.[5,7]

Several molecular technologies are currently being used in the clinical practice for the diagnosis and study of

infectious diseases, including Single-Target PCR, Multiplex PCR, quantitative PCR (qPCR), genotyping, sequencing, Fluorescent *In Situ* Hybridization (FISH), microarrays and Luminex-based assays.[8] Moreover, the adoption of droplet digital PCR[9] and Matrix-Assisted Laser Desorption Ionization–Time-of-Flight (MALDI-TOF) Mass Spectrometry[10] is expected to increase in the clinical practice.[11] Each technique has its advantages and disadvantages and they all provide slightly different types of information. An end-point PCR is a cheaper alternative to a qPCR; however, it only provides qualitative information and it cannot detect low copy numbers.[12] Likewise, a microarray can detect hundreds of targets simultaneously, yet it cannot distinguish genetic variants (single nucleotide variants and small insertions and deletions) among samples, as a sequencing method does.[13] The molecular diagnostic technology chosen, depends on the question being investigated and the final use of the data generated. In general, however, research laboratories tend to use more diversified approaches than diagnostic laboratories.

The standard approach for diagnostic laboratories is to perform pre-defined test menus or panels (usually of a commercial nature) to screen for the most likely pathogens responsible for specific symptoms. For example, if a sexually transmitted disease is suspected, it is common to test for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, Human Papillomavirus (HPV) and Herpes Simplex Virus (HSV), among others.[7] Given the diversity of pathogens that can cause the same symptomatology – and the need to establish a rapid diagnosis – molecular tests have become the primary tool used for the diagnosis of infectious diseases. To ensure the adoption of these technologies in the places where they are needed the most, kit manufacturers collaborate with automation experts to develop and provide easier to use tools that can handle higher throughputs.

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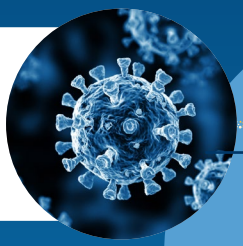
INFECTIOUS DISEASES

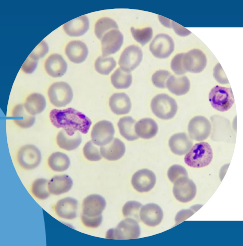
Infectious diseases are among the leading causes of death and disability across the globe. They pose a significant burden on public health and the global economy. Infectious disease impact is further deepened by the continued emergence of new pathogenic species and the resurgence of known diseases.[1]

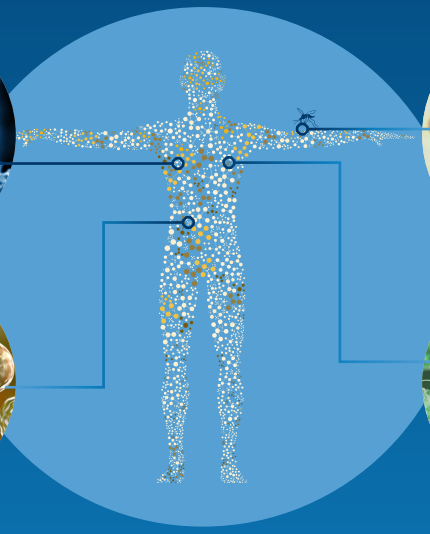
These diseases derive their importance from the type and extent of damage that they inflict on organs and/or systems when they gain entry into a host, and are generally caused by microorganisms, such as:

Viruses e.g. SARS-CoV-2, responsible for COVID-19

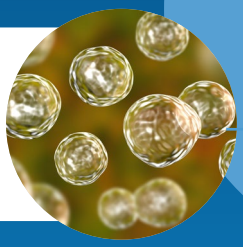


Parasites e.g. *Plasmodium vivax* responsible for Malaria

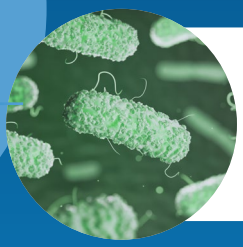




Fungi e.g. *Blastomyces dermatitidis* responsible for Blastomycosis



Bacteria e.g. *Mycobacterium tuberculosis* responsible for tuberculosis



ANTIMICROBIAL RESISTANCE

Antimicrobial Resistance (AMR) occurs when microorganisms evolve mechanisms that prevent the effects of antimicrobial compounds working against them.[2]

When there are no effective treatments against a microorganism due to resistance or limited research, an endemic, epidemic or pandemic may occur.

Endemic refers to a disease, or the level of a disease, that is present in a population or area all the time.

An **epidemic** refers to a rise in the number of cases of a particular disease, above the normal endemic level.

A **pandemic** is the worldwide spread of a new disease to which the majority of the population have no immunity. Movement restrictions, screening and vaccinations help to monitor spread. However, the rise of antibiotic resistance, the changes to our climate and the ever-increasing global movement of people and animals can make the prevention of pandemics all the more difficult.[3]

INFECTIOUS DISEASE TESTING

Several molecular technologies are currently being used in the clinical practice for the diagnosis and study of infectious diseases. In comparison with traditional culture-based methods, molecular diagnostic methods are more complex; however, their benefits include:

✓ Faster turnaround time | ✓ More accurate results | ✓ Easy scalability | ✓ Flexibility for multiplexing

References:

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Molecular Diagnostic Methods: Why Do We Need Automation?

Molecular methods are more complex and diverse than traditional culture-based diagnostic methods. To enable more widespread use of the latter, workflows must be standardized and scaled up. Automation allows for both, while catering for various levels of regulatory compliance and reducing the overall cost per sample.

Standardization and Compliance

Each type of molecular test has its own workflows, including those related to sample preparation. Several parameters determine the length, structure and complexity of these workflows; these include the type of clinical sample (e.g. swabs, bodily fluids, tissue biopsies), the type of genetic material to be analyzed (e.g. DNA, RNA), the species of the pathogen to be detected (e.g. bacteria, virus), the Nucleic Acid (NA) extraction technology (e.g. magnetic beads, silica-based membranes) and the specific detection method (e.g. qPCR, NGS, microarrays), among others.

Most molecular methods use complex workflows that are susceptible to manual processing errors. Furthermore, inter-operator variability is unavoidable even in the presence of experienced, qualified staff. Automation significantly reduces the risk of processing errors and standardizes the performance of each step in the workflow. The sample preparation steps for all molecular workflows can be automated, at least partially. Furthermore, several automated platforms offer integrated "sample-to-results" solutions that can perform sequential workflows in one deck without user intervention (e.g. NA extraction, reverse transcription and qPCR). Integrated solutions offer attractive benefits to the end-user by further reducing the hands-on time and ensuring complete traceability of the samples. These solutions, however, tend to offer less flexibility than the solutions based on fragmented workflows.

The need for higher quality control standards is one of the main reasons for transitioning from manual to automated workflows in the diagnostic setting. Molecular tests (including automated solutions) can be broadly classified into two groups in the US and Europe: Research Use Only (RUO) and *In Vitro* Diagnostic (IVD)-certified. In principle, RUO tests are not intended to be used for diagnostic purposes and are only required to comply with local quality control standards (e.g. CE marking in Europe). IVD tests, on the other hand, are specifically intended for diagnostic purposes and must follow extensive validation, in addition to local quality control standards.[1,2] In the US there is a third intermediate group called Laboratory Developed Tests (LDTs), which are a type of IVD test designed, manufactured and used within a single laboratory (a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory).[3] LDTs are known in Europe as "homebrew" or "in-house" tests, although the terms are not truly equivalent.[4] The regulatory pre-requisites for the use of each test in the diagnostic setting differs among countries.

In the US, only IVD tests or LDTs can be used for the diagnosis of infectious diseases. RUO tests can only be used in the diagnostic setting when they are granted an Emergency Use Authorization (EUA), as was the case for several tests during the COVID-19 pandemic. It should be highlighted that LDTs were also required to have a EUA in this particular case.[5,6]

In Europe, both IVD and RUO (with a CE mark) tests can be used for diagnostic purposes.[7] In 2022, however, a new EU regulation (EU 2017/746) will kick-in that changes the regulatory classification of IVD tests and introduces stricter compliance measures.[8] These new measures will be applicable for all aspects of the tests, including the kit, the instruments and the users. Automation will therefore play a critical role in all aspects of compliance, ensuring traceability, standardizing workflows, eliminating/minimizing human error, controlling user access and specifying how to deal with error handling. Reimbursement policies will likely be based on the use of IVD tests and their compliance with the new regulations.

Scaling Up and Cost per Test

The need for rapid scale-up is often observed in the field of infectious diseases. During the COVID-19 pandemic, for example, many automated solutions had to be rapidly implemented worldwide in order to reach the (theoretical) testing capacity needed to locally control the spread of the disease (10-50 people per 1,000 per day, as of the beginning of May 2020).[9,10] The UK alone increased its daily testing capacity from 26,000 virus tests per day in April 2020 to more than 500,000 in November of the same year (almost a 20X increase).[11] Luckily, not all infectious diseases become pandemics but many need to be continuously monitored and thus result in a high level of sample throughput. It would be virtually impossible to meet these demands without automation. Although automation requires a sizable initial investment (unless acquired through a reagent rental agreement), it generates significant savings over the long term. The cost savings provided by the automation of the workflows are the result of a reduction in labor and reagent use (due to the miniaturization of the assays), as well as from a reduction in mistakes and repetitions (due to significant improvements in sample safety and traceability).

Benefits of Automation:

- Higher throughput
- Enhanced reproducibility
- Less manual processing errors (including inter-operator variability)
- Traceability
- Controlled user access
- Error management
- Cost-effectiveness (miniaturization + reduced labor)

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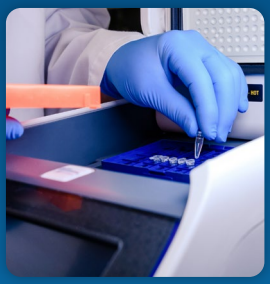
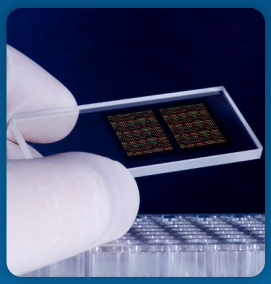
MOLECULAR DIAGNOSTIC WORKFLOW

Diagnostic testing is used to identify and manage diseases in infected patients. Accurate diagnostics can also monitor a patient's response to treatment with a particular therapeutic product, and therefore its safety.

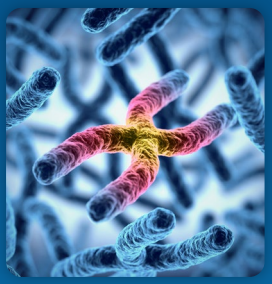
Several approaches for molecular diagnostics include[1]:



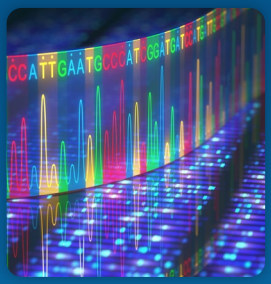
Single-Target PCR
Multiplex PCR
Droplet digital PCR
(q)RT PCR



Microarray Panels



Fluorescent *In Situ* Hybridization (FISH)



Genotyping

Next-Generation Sequencing

REGULATING MOLECULAR DIAGNOSTICS

The use of molecular tests in the diagnostic setting are tightly regulated. These regulations differ, depending on the geographic location.

USA

***In Vitro* Diagnostic (IVD) Tests**

IVD tests are intended for diagnostic purposes and must follow extensive validation, in addition to local quality control standards

Laboratory Developed Tests (LDTs)

IVD tests designed, manufactured and used within a single CLIA-certified laboratory. They are subject to quality control standards

Research Use Only (RUO)

Commercial RUO tests are not intended for diagnostic purposes – they are only required to comply with local quality control standards

Europe

Conformité Européenne *In Vitro* Diagnostic (CE-IVD) Tests

IVD tests are intended for diagnostic purposes and must follow extensive validation, in addition to local quality control standards

Home Brew or In-House Tests

Similar to USA LDTs, although not truly equivalent

Research Use Only (RUO)

Commercial RUO tests are not intended for diagnostic purposes – they are only required to comply with local quality control standards

Legend

- Used for diagnostic purposes
- Only used for diagnostic purposes until 2022 when the EU 2017/746 kicks in. See text for exceptions.
- Can only be used for diagnostic purposes if they are granted an Emergency Use Authorization (EUA)

References:

1. Messacar K, Parker S, Todd J, Dominguez S. Implementation of Rapid Molecular Infectious Disease Diagnostics: the Role of Diagnostic and Antimicrobial Stewardship. J Clin Microbiol. 2016;55(3):715-723. doi:10.1128/jcm.02264-16

What Can Hamilton Offer?

Hamilton is a market leader in precision liquid handling. Sample preparation for molecular diagnostic tests is our strongest focus area. To provide integrated solutions, we have developed automated platforms that can sequentially perform the various steps of the most popular molecular diagnostic workflows: Nucleic Acid (NA) extraction, quantitative PCR (qPCR), also called Real-Time PCR (RT-PCR), and Next-Generation Sequencing (NGS). We are aware of the fast-changing nature of this field and of the variety of commercial kits in the market, which is why we closely monitor new developments and keep in frequent communication with leading kit manufacturers. Our commitment to providing fast and targeted solutions to our customers can be seen in the rapid development of three new automated standard solutions ((Assay-Ready) Workstations) for COVID-19 testing, during the first weeks of the pandemic (i.e. MagEx STARlet, MagEx STAR and PCR Prep STARlet, see description of these systems in the following sections).

Hamilton strives to provide its customers with the broadest range of solutions possible – from customized to turnkey solutions, and from Research Use Only (RUO) to *In Vitro* Diagnostics (IVD)-compliant solutions.

From Customized to Turnkey Solutions

Our Assay-Ready Workstations (ARWs) provide ready-to-use solutions with fast implementation, which has been particularly advantageous during the COVID-19 pandemic. ARWs have standardized hardware and software packages with fixed deck-layouts, which allows us to develop and qualify specific workflows. These standardized solutions can rapidly implement updates from kit manufacturers and shorten the implementation time of newly qualified workflows. The deck-layout of our most popular ARWs for molecular diagnostics is seen in figures 1-2 and 5-6 and will be described in the following sections.

For customers seeking more flexibility, we can provide customized solutions based on existing workstations (e.g. PCR Prep STARlet and the RT STARlet) or fully customized deck layouts. Our platforms can be integrated with our various standard modules (e.g. modules for incubation, shaking, amplification and centrifugation), as well as with third-party devices. Furthermore, the RUO platforms can be upgraded at any time to add new pipetting channels, modules or accessories and we can provide complementary automation solutions for sample storage.

Due to our consultative sales approach and direct collaboration with kit manufacturers we can offer maximum flexibility and meet the demands of even the most customized projects.

From RUO to IVD-Compliant Solutions

Hamilton's software is designed to enable quality control measures, such as automatic loading, sample traceability, user access control and protocol set-up for error-handling. Furthermore, most of our platforms have an automatic barcode reader.

As previously mentioned, the regulatory standards for the new European IVD regulations (IVD-R) will require a higher level of compliance, which includes – but is not limited to – fixed software specifications and new documentation. In preparation for these changes, Hamilton has developed its first IVD-compliant ARW for molecular diagnostics: the Clinical NGS STARlet (see description below). Moreover, to better serve the needs of the diagnostic sector, Hamilton has formed an international clinical diagnostic team, who, along with our experts in regulatory affairs and application development,

can support our clinical customers and partners during the validation of the complete IVD-R workflow.

Our Standardized Solutions

1. Standardized Solutions for NA Extraction

Hamilton has developed three ARWs for NA extraction (DNA and RNA): the NIMBUS® Presto, the MagEX STARlet and the Genomic STARlet™ 2.0. The first two ARWs are designed for magnetic bead-based kits and the latter is designed for silica-based filter plate kits. A fourth workstation, the MagEx STAR, is also available. Several methods will be qualified in this workstation in the coming months. All of our ARWs process up to 96 samples simultaneously and they are all integrated with modules for incubation and shaking. The NIMBUS Presto, in particular, is equipped with ThermoFisher Scientific's KingFisher™ Presto Purification System.

We have worked with our application team and partners to develop automated workflows for the commercial solutions from various kit manufacturers.

Table 1: *Hamilton Assay-Ready Workstations for NA extraction*

	NIMBUS® Presto	MagEx STARlet	Genomic STARlet™ 2.0
Hamilton's platform	Microlab NIMBUS® HD	Microlab® STARlet	Microlab® STARlet
Throughput*	96 samples in 1.3-1.7 hours	96 samples in 1.7-2.0 hours	96 samples in 2.0 hours
NA extraction technology	Magnetic bead-based	Magnetic bead-based	Silica-based filter plate
Development partner	ThermoFisher Scientific	-	MACHEREY-NAGEL
Kit providers from which there are available or qualified methods applicable to the field of infectious diseases	MACHEREY-NAGEL, ThermoFisher Scientific, Omega Bio-Tek	MACHEREY-NAGEL, ThermoFisher Scientific, Omega Biotek, Promega, Zymo Research, Molg3n	**MACHEREY-NAGEL

**Depending on the kit chosen and starting point of the workflow*

***Method to be requalified due to transition from Genomic STARlet to Genomic STARlet 2.0 (deck layout not yet available)*

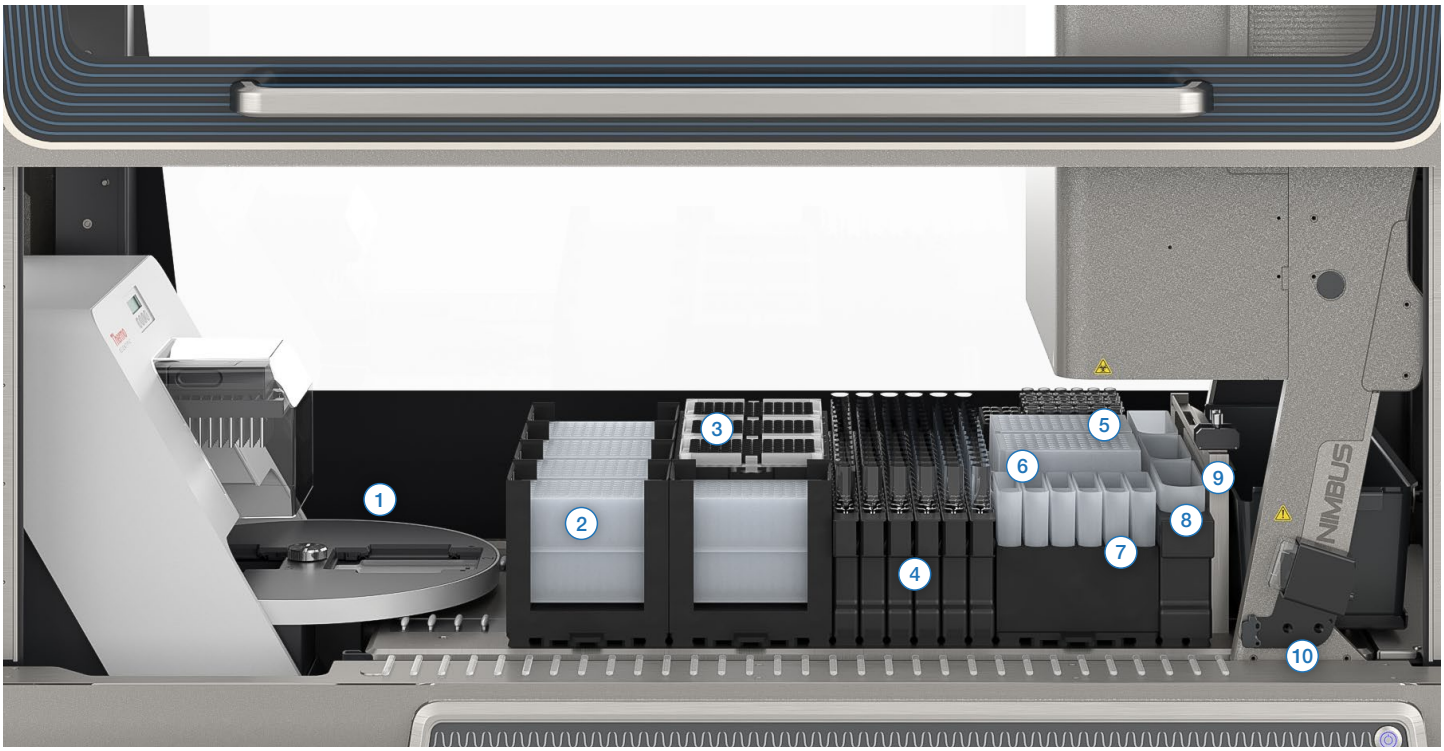


Figure 1: NIMBUS® Presto, Deck Layout. *(1) KingFisher™ Presto and Turntable, (2) 5X Deep Well Plates Stacks (storage), (3) 3X Tip Modules, (4) Sample Loading Area, (5) Carrier with Multitube Adapter Tube, (6) 2X Deep Well Plate Modules (working area), (7) 6X 60 ml Trough Modules, (8) 3X 200 ml Trough Modules, (9) CO-RE® Gripper, (10) Barcode Scanner. The system includes 4X CO-RE® 1 ml channels that are not visible in this figure.*

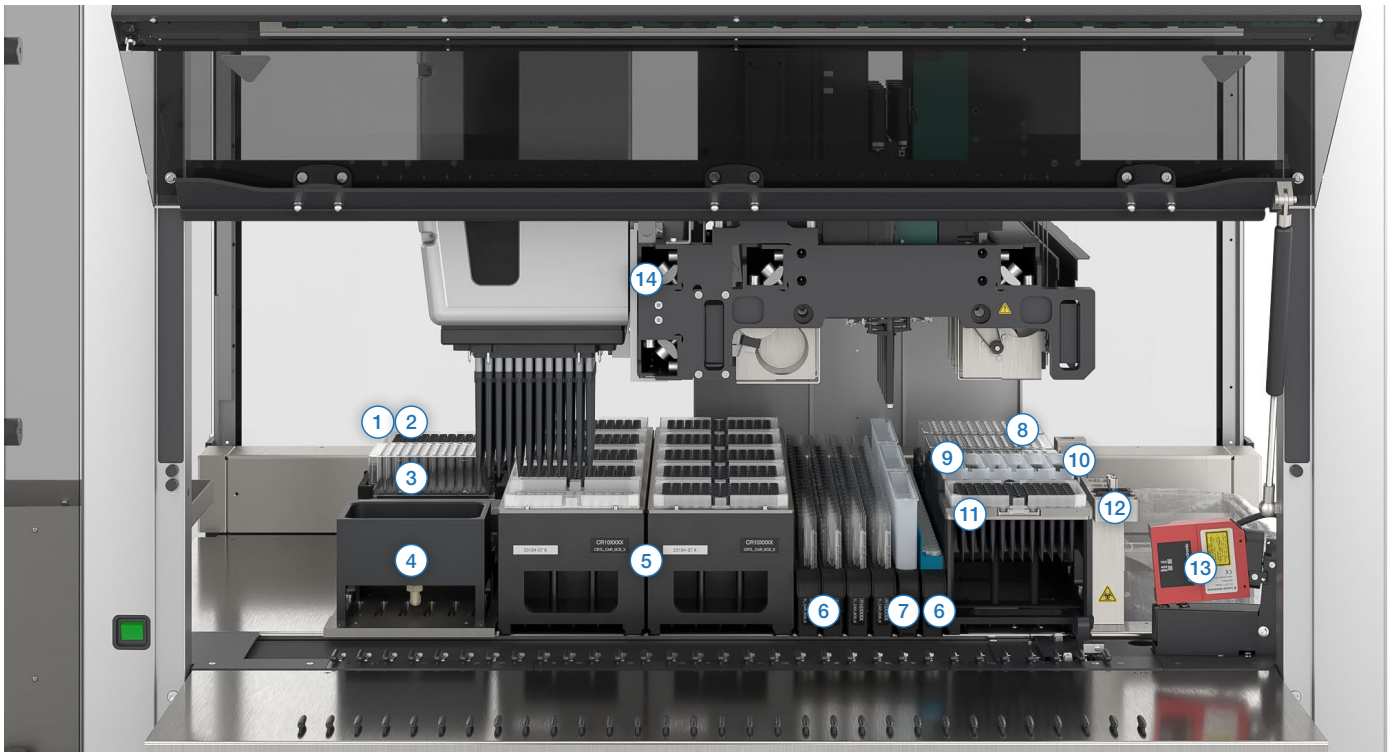


Figure 2: MagEx STARlet, Deck Layout. *(1) Shifted Tip Pickup Adapter, (2) Hamilton Heater Shaker™, (3) Magnetic Stand, (4) Gravity Liquid Waste for CO-RE® 96 Multi-Probe Head (MPH), (5) 2x Tip Carriers, (6) Sample Carriers, (7) 3X 120 ml Trough Modules, (8) 2X Deep Well Plate Modules, (9) Microtiter Plate Module, (10) 6X 60 ml Trough Modules, (11) Tip Module, (12) CO-RE® Gripper, (13) Barcode Reader, (14) CO-RE® 96 MPH + 8X CO-RE® 1 ml channels.*

2. Standardized Solutions for (q)PCR

Hamilton has developed two workstations focused on qPCR solutions: the PCR Prep STARlet and the RT STARlet. The PCR Prep STARlet automates the pre-PCR sample set-up and the RT STARlet provides a fully integrated solution from NA extraction to qPCR/RT-PCR run, including reverse transcription for RNA pathogens. The RT STARlet is integrated with a magnetic stand (for magnetic bead-based NA extraction) and up to two Bio Molecular Systems' Magnetic Induction Cyclers for qPCR/RT-PCR, specially adapted for Hamilton STARline integration (MIC4Hamilton).

We are continuously working on the qualification of new workflows/kits on these two workstations.

Table 2: *Hamilton Workstations for qPCR solutions*

	PCR Prep STARlet	RT STARlet
Hamilton's platform	Microlab® STARlet	Microlab® STARlet
Workflow automated	pre-PCR sample set-up	NA extraction, reverse transcription, pre-PCR sample set-up, qPCR/RT-PCR run, amplicon detection
Throughput	96 samples	48 samples
Kit providers for which there are available or qualified methods applicable to the field of infectious diseases	ThermoFisher Scientific	MACHERY-NAGEL (for NA extraction) and Fast Track Diagnostics (for qPCR set up)

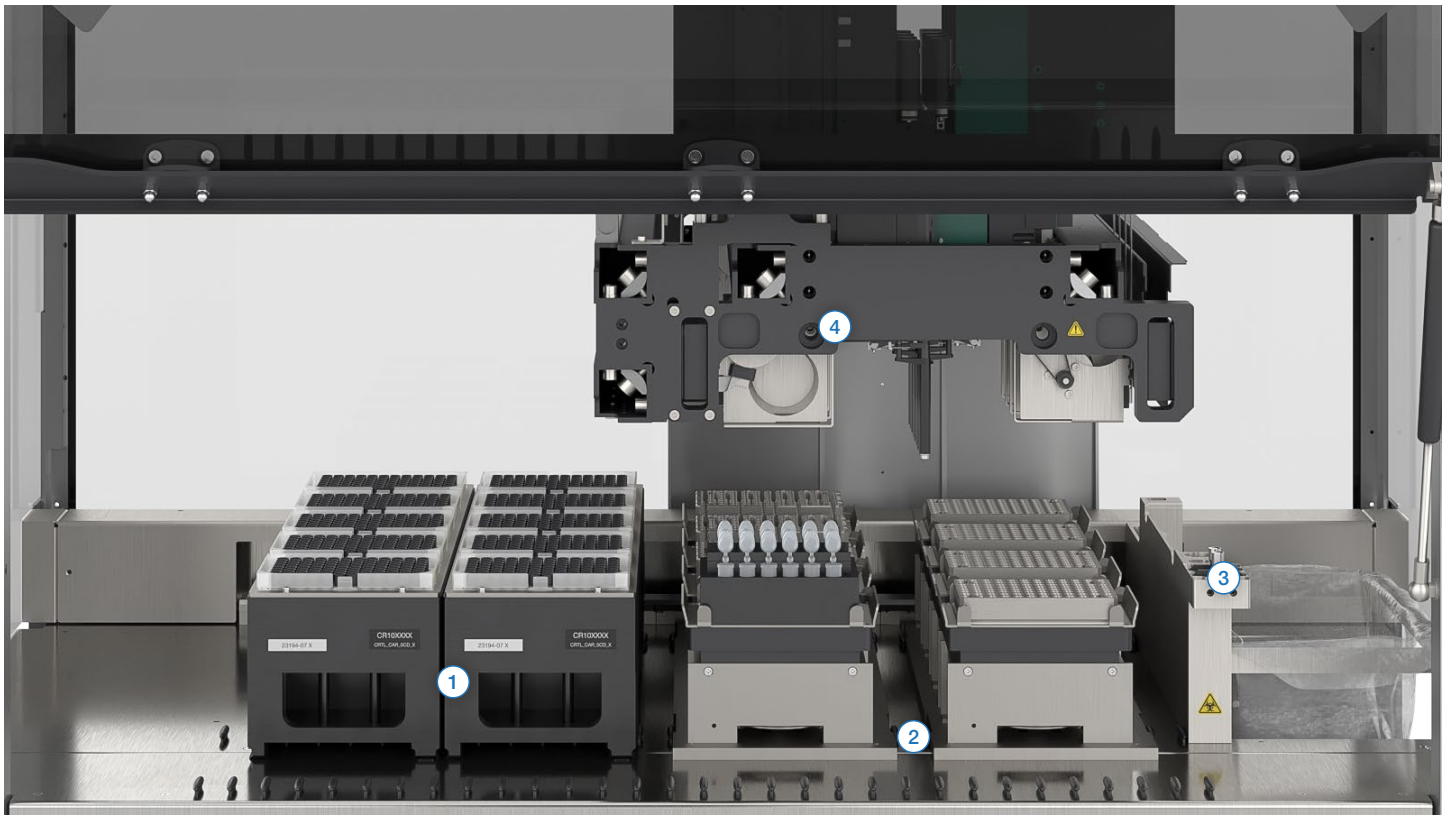


Figure 3: PCR Prep STARlet, Deck Layout. *(1) 2X Tip Carriers (2) 8X Inheco CPAC with respective adapters (3) CO-RE® Gripper (4) 8X CO-RE® 1 ml channels.*

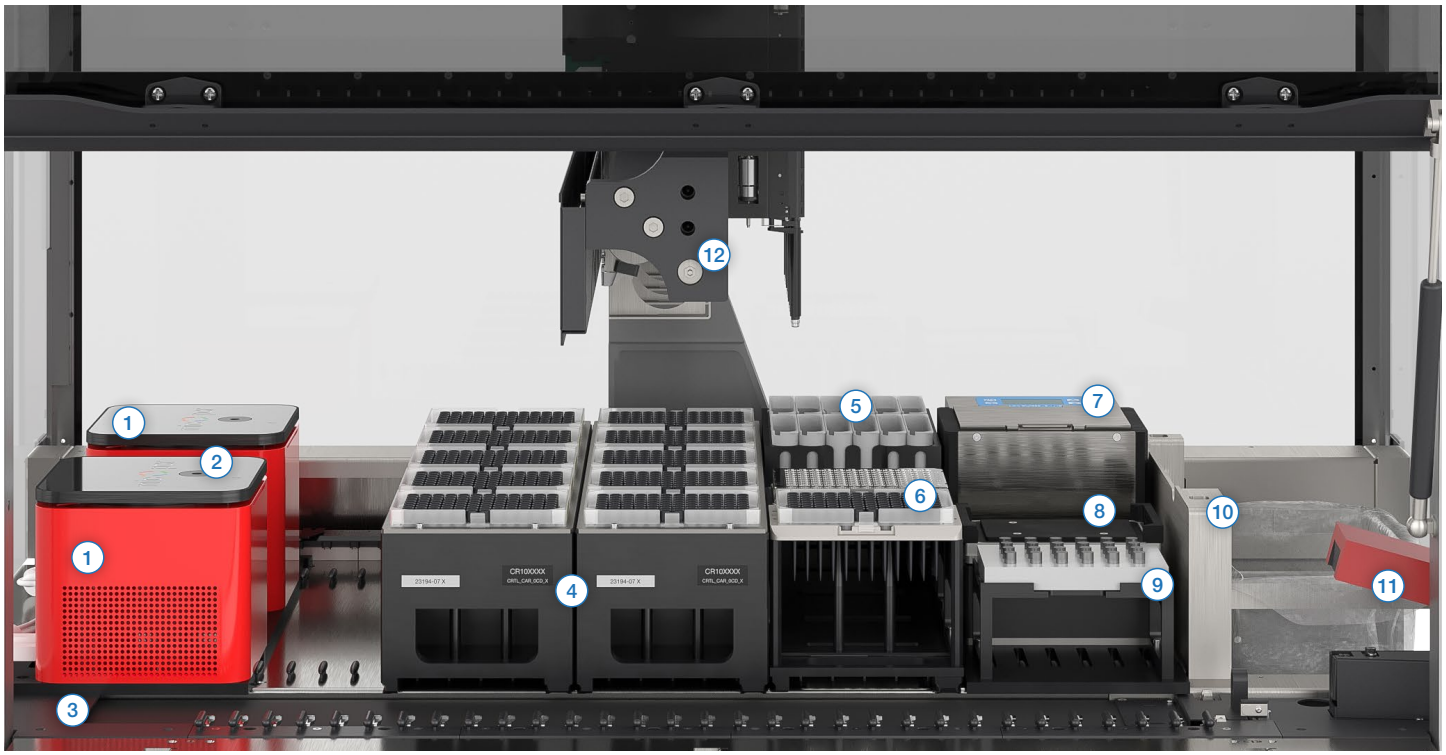


Figure 4: RT STARlet, Deck Layout. *(1) 2X Bio Molecular Systems' MIC4Hamilton (2) Teaching Adapter (3) Autoload (4) 2x Tip Carriers (5) 12X 60 ml Trough Modules (6) Deep Well Plate Module (7) Cooling-Heating Module (8) Hamilton Heater Shaker™ (HHS) (9) Magnetic Stand (10) CO-RE® Gripper (11) Barcode Reader, (12) 4X CO-RE® 1 ml channels.*

3. Standardized Solutions for NGS

We currently offer two ARWs specifically designed for NGS library prep workflows: the NGS STAR and the Clinical NGS STARlet. These platforms differ with regard to maximum throughput and the level of regulatory compliance. The NGS STAR is a general laboratory instrument that can process up to 96 sample libraries at once, while the Clinical NGS STARlet is an *in vitro* diagnostics device optimized to process up to 24 sample libraries. A third ARW based on our Microlab VANTAGE line will soon be available as well.

Several of the whole genome/transcriptome sequencing methods already qualified in our NGS STAR are commonly used in the diagnosis and study of pathogens causing infectious diseases: Illumina's Nextera XT DNA Library Preparation Kit, Illumina DNA Prep (formerly known as Illumina Nextera DNA Flex Library Prep), Oxford Nanopore Technologies' Genomic DNA by Ligation Kit, Qiagen's QIAseq FX DNA Library Kit, Roche's KAPA HyperPrep kits, New England Biolabs' NEBNext® Ultra™ II DNA Library Prep, Illumina's TruSeq Stranded Total RNA Library Prep and New England Biolabs' NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina®.

The final decision about which instrument/ARW to use will depend on the specific needs of the customer. However, independent of the selection, all our solutions ensure:

- **Precision**, accurate pipetting achieved through our various proprietary technologies: (1) Compressed O-Ring Expansion (CO-RE®) for tip attachment and positioning; (2) Liquid Level Detection (LLD) to detect the exact level of liquids in tubes of plates and; (3) Anti-Droplet Control™ (ADC) for correctly pipetting volatile organic solvents.

- **Reproducibility**, consistency ensured through repeated workflow testing.

- **Traceability**, automated barcode verification of samples, reagents, plates and tips as well as dynamic tracking of each aspiration and dispensation step using our Monitored Air Displacement (MAD) and Total Aspiration and Dispensing Monitoring™ (TADM).

Table 3: *Hamilton solutions for NGS workflows*

	NGS STAR	Clinical NGS STARlet
Hamilton's platform	Microlab® STAR™	Microlab® STARlet
Classification	RUO	IVD
Throughput	96 samples	24 samples
Kit providers for which there are available or qualified methods applicable to the field of infectious diseases	Illumina, Qiagen, Roche, New England Biolabs, Oxford Nanopore Technologies	Illumina

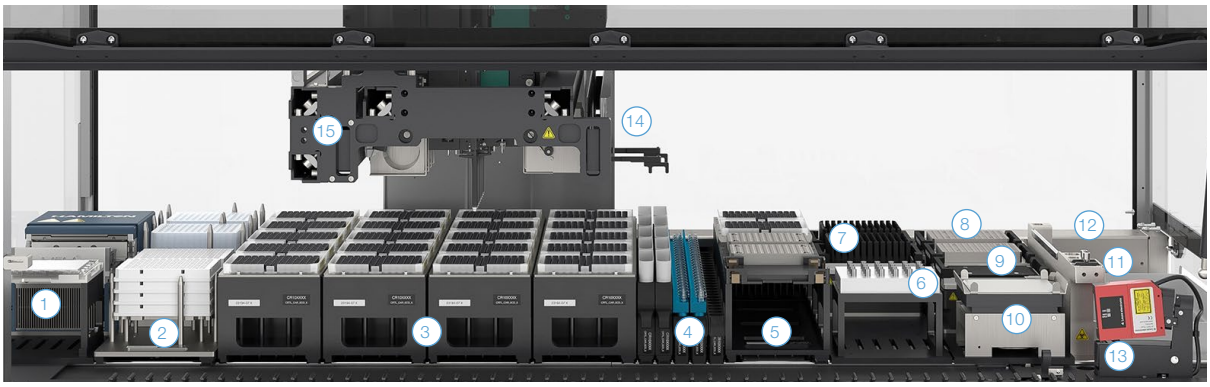


Figure 5: NGS STAR™, Deck Layout. *(1) Hamilton On-Deck Thermal Cycler (ODTC) with lid parking position (optional), (2) Plate stacker (HSP and MIDI plates), (3) Tip carriers (50, 300 & 1000 µl filter tips), (4) Reagent carrier (Troughs, Tubes, and Vials), (5) Plates and Tip carrier (HSP and MIDI Plates), (6) Magnetic stand (Thermo Fisher-Ambion), (7) Hamilton Heater Shaker™ (HHS) with adapter for MIDI Plates, (8) Hamilton Heater Shaker™ (HHS) with adapter for PCR Plates, (9) Hamilton Heater Shaker™ (HHS) with flat bottom, (10) INHECO Cold Plate Air Cooled™ (CPAC, optional cooling adapter for microtubes), (11) CO-RE® Gripper, (12) Solid/liquid waste, (13) Autoloader with Barcode reader, (14) iSWAP, (15) 8X CO-RE® 1 ml channels + optional CO-RE® 96 Multi-Probe Head (MPH).*

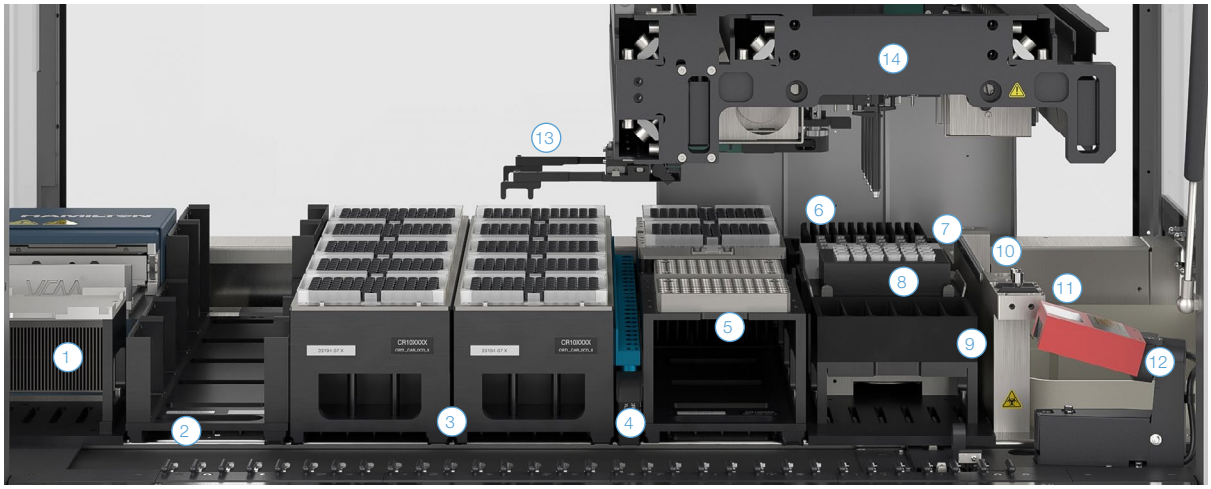
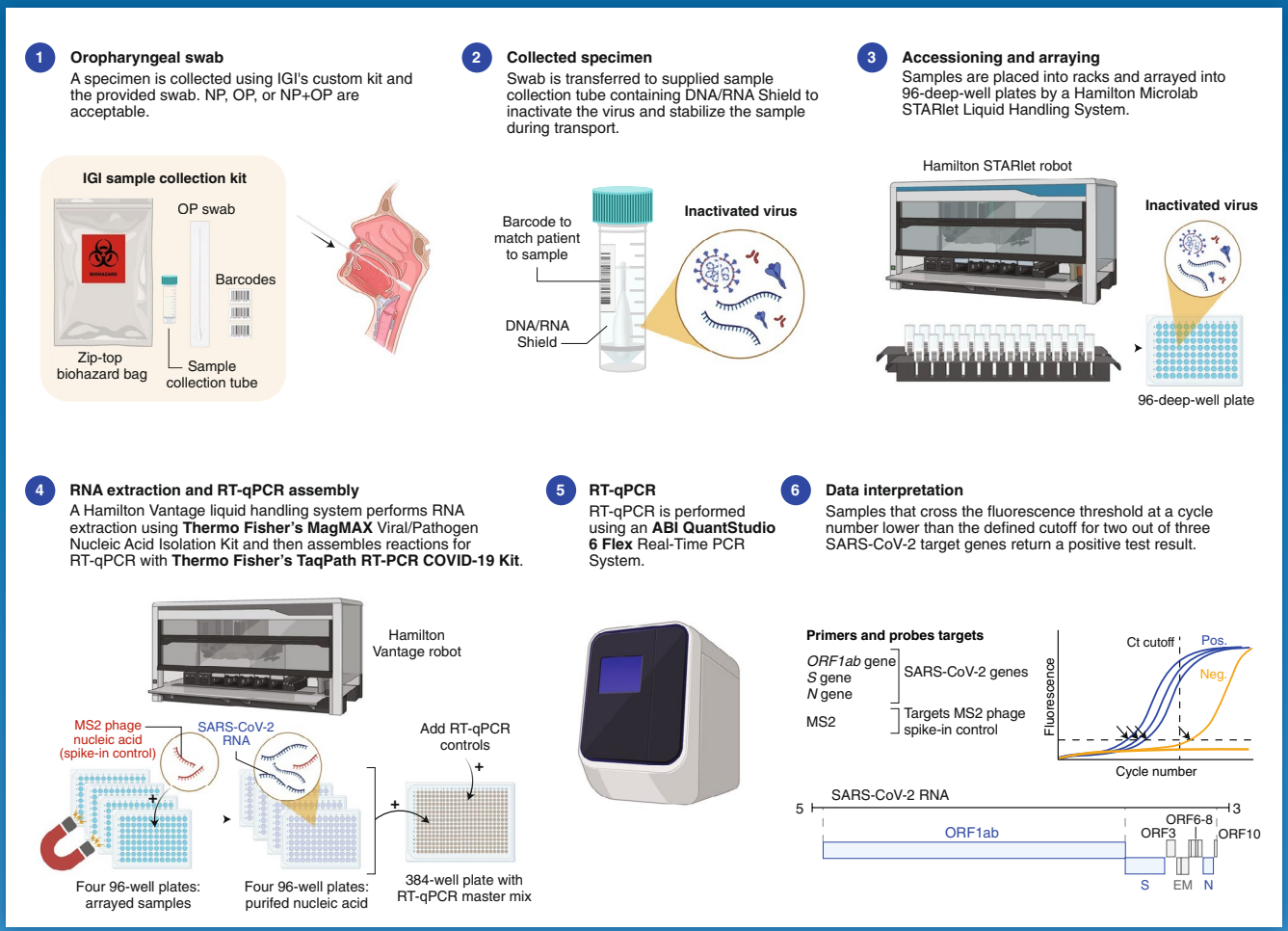


Figure 6: Clinical NGS STARlet, Deck Layout. *(1) Hamilton On-Deck Thermal Cycler (ODTC) with lid parking position, (2) Plate stacker (HSP and MIDI plates), (3) Tip carriers (50 & 300 µl filter tips), (4) Reagent carrier (tubes), (5) Plates and tip carrier (1000µl filter tips, HSP and MIDI plates), (6) Hamilton Heater Shaker™ (HHS) with adapter for MIDI plate, (7) Magnetic stand (Thermo Fisher-Ambion), (8) INHECO Cold Plate Air Cooled™ (CPAC) with cooling adapter for microtubes, (9) Reagent module (troughs), (10) Solid/liquid waste, (11) CO-RE® Gripper, (12) Autoloader with Barcode reader, (13) iSWAP, (14) 4X CO-RE® 1 ml channels.*

Overview of the IGI SARS-CoV-2 Testing Consortium Assay on Hamilton Automated Platforms

The figure below is extracted from the article "Blueprint for a pop-up SARS-CoV-2 testing lab".[1] The figure shows how Hamilton automated platforms are employed in the workflow implemented by the Innovative Genomics Institute (IGI) at UC Berkeley for the diagnosis of SARS-CoV-2.



Reference 1. IGI Testing Consortium., Amen, A.M., Barry, K.W. et al. Blueprint for a pop-up SARS-CoV-2 testing lab. *Nat Biotechnol*. 2020;38(7):791-797. doi:10.1038/s41587-020-0583-3

Access the full free article here

HAMILTON®

Automated purification of viral RNA/ DNA and microbial DNA from clinical samples on the Hamilton NIMBUS® Presto Assay-ready Workstation

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[2] Hamilton Bonaduz AG, Via Crusch 8, 7402 Bonaduz, Switzerland

Introduction

Isolation of pathogen nucleic acids (e.g. viral RNA and DNA, bacterial DNA) from clinical samples is the basis for a large variety of molecular tests that have become standard methodology in research and diagnostic laboratories.

Due to the diversity of clinical sample material – swabs, blood, plasma, body fluids, tissue biopsies, etc. – the isolation procedure itself often poses challenges to laboratory staff and workflows. The purification process needs to be suitable for a wide variety of sample materials. In addition, the molecular diagnostic market demands extraction methods that are adaptable on automation platforms and reliable in terms of pathogen DNA detection.

To meet these requirements MACHEREY-NAGEL developed the NucleoMag® Pathogen kit allowing the automated isolation of nucleic acids from various starting materials using magnetic bead technology.

Together with Hamilton, MACHEREY-NAGEL has established its NucleoMag® technology on the NIMBUS Presto. In this application note we demonstrate the utility and advantages of combining these technologies to fully automate your high throughput nucleic acid extractions for pathogen detection workflows.

- **Proven NucleoMag® lysis and purification procedure suitable for diverse clinical samples**
- **Automated plate prefilling and plate handling by the Hamilton NIMBUS Presto**
- **High speed nucleic acid purification by the integrated KingFisher™ Presto instrument**

Method Description

The NucleoMag® Pathogen kit is designed for common clinical sample material, such as whole blood, swabs, serum or plasma, feces, and tissue. Up to 200 µL of liquid or homogenized sample material (e.g. swab wash solution) is mixed with Proteinase K, Carrier RNA (optional) and Lysis Buffer NPL1 prior to lysis incubation. The subsequent isolation is based on reversible adsorption of nucleic acids to paramagnetic beads (NucleoMag® B-Beads). Nucleic acid binding is enabled by addition of Binding buffer NPB2. After magnetic separation and removal of the supernatant, contaminants and salts are removed by three subsequent washing steps. The NucleoMag® B-Beads are air dried before highly pure nucleic acids are finally eluted under low ionic strength conditions in Elution Buffer NPE5.

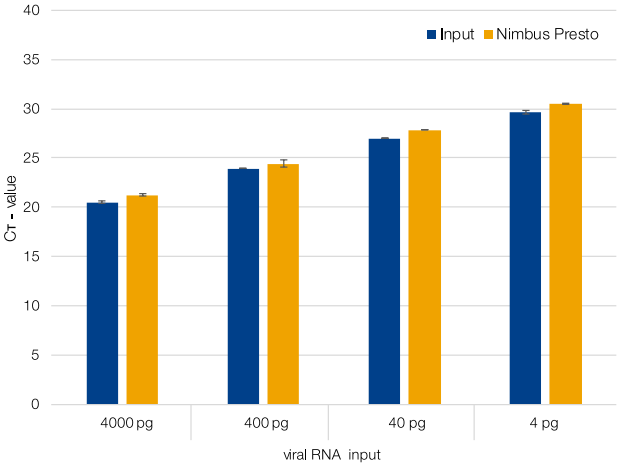
Figure 1: The Hamilton NIMBUS® Presto

We demonstrate this automated purification workflow for spiked viral RNA and DNA exemplarily. The tailored protocol allows flexible processing of up to 96 samples per run.

Application data

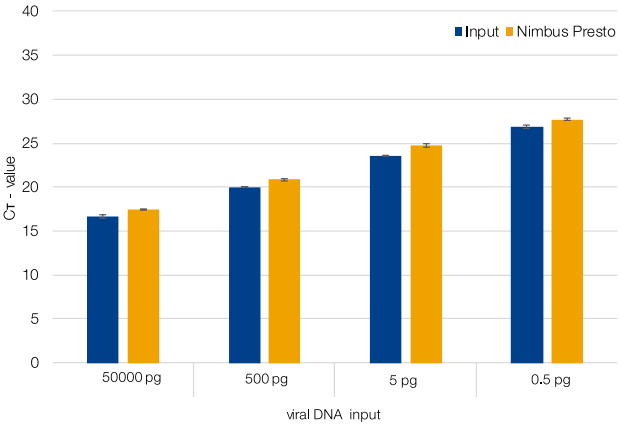
High sensitivity detection of viral RNA recovered from liquid samples

RNA was isolated from liquid samples (200 µL; n = 3 for each dilution) using the NucleoMag® Pathogen kit on the NIMBUS Presto workstation. MS2 bacteriophage RNA was spiked into a sample solution in a dilution series. The recovery rate was determined by measuring the input value in comparison to the Ct value after RNA extraction. The analysis was performed with a Taqman® PCR probe for MS2 RNA using the SensiFast™ Probe One-Step Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System.



High sensitivity detection of viral DNA recovered from liquid samples

DNA was isolated from liquid samples (200 µL; n = 3 for each dilution) using the NucleoMag® Pathogen kit on the NIMBUS Presto workstation. T7 bacteriophage DNA was spiked into a sample solution in a dilution series. The recovery rate was determined by measuring the input value in comparison to the Ct value after DNA extraction. The analysis was performed with a Taqman® PCR probe for T7 DNA using the SensiFast™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System.



Absence of cross contamination

Positive (T7 bacteriophage DNA) and negative (no DNA) control samples (200 µL each) were arranged in a checkerboard pattern on a 96-well deepwell plate and subjected to the NucleoMag® Pathogen kit procedure on the NIMBUS Presto. Presence of DNA in the eluates was examined by qPCR with a Taqman® PCR probe for T7 DNA using the SensiFast™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System. Absence of qPCR signal (Ct undetermined) in the negative control samples indicates a cross contamination free workflow.

	1	2	3	4	5	6	7	8	9	10	11	12
A	20,46	*	20,47	*	20,51	*	20,45	*	21,10	*	20,70	*
B	*	20,67	*	20,38	*	20,31	*	20,45	*	20,45	*	20,42
C	20,40	*	20,43	*	20,61	*	20,55	*	20,47	*	20,46	*
D	*	20,55	*	20,32	*	20,48	*	20,42	*	20,34	*	20,33
E	20,54	*	20,29	*	20,46	*	20,58	*	20,55	*	20,34	*
F	*	20,45	*	20,32	*	20,44	*	20,36	*	20,33	*	20,38
G	20,43	*	20,38	*	20,30	*	20,59	*	20,32	*	20,12	*
H	*	20,45	*	20,20	*	20,51	*	20,45	*	20,13	*	20,03

Sample (C_T; positive control) No sample (*C_T undetermined; negative control)

A rapid, fully automated solution for pathogen nucleic acid extraction from clinical samples

MACHEREY-NAGEL and Hamilton deliver a tailored solution for your high throughput viral RNA, viral DNA, and microbial DNA extraction needs from various clinical sample materials. We adapted the NucleoMag® Pathogen procedure on the NIMBUS Presto workstation to meet the expectations of the molecular diagnostic market.

Hamilton and MACHEREY-NAGEL have demonstrated that the Assay-ready Workstation NIMBUS Presto is a powerful solution to automate NucleoMag® Kits.

NIMBUS Presto Assay-Ready Workstation Information

For Research Use Only.

Pipetting Volumes Specifications	
Tipsize 50 µl	1 µl volume ([R] 5%; CV 4%)
Tipsize 300 µl	50 µl volume ([R] 2%; CV 0.75%)
Tipsize 1000 µl	100 µl volume ([R]2%; CV 0.75%)

Pipetting Channels Specifications
CO-RE technology
Independent channels
Capacitive liquid level detection (cLLD)
Pressure liquid level detection (pLLD)
Tip Type detection

System Specifications
15 - 35°C
Humidity: 10%-90% with no condensation
Altitude: 0-2000 m above sea level

Power	
Input Power	100-240 VAC, 50-60 Hz, 5 A
Output Power	42 VDC, ± 5%; 600 W maximum

Ordering Information

Ordering Configurations
NIMBUS Presto Base L tube + Bar code reader + CO-RE Paddles
NIMBUS Presto Base S tube + Bar code reader + CO-RE Paddles
Laptop Win10 NIMBUS

Accessories
Large Tube Carrier
Small Tube Carrier

Additionally Required:

Labware; Manufacturer: Hamilton Bonaduz AG
Hamilton 50 µl filtered tips
Hamilton 300 µl filtered tips
Hamilton 1000 µl filtered tips

Additionally Required:

KingFisher Options; Manufacturer: Thermo Fisher Scientific
KingFisher Presto 96 Well Head
KingFisher Presto 96 + 24 Well Head
KingFisher Presto 24 Well Head (optional)

NucleoMag® Pathogen Kit. Section

Technology	Magnetic beads
Sample material	≤ 200 µl whole blood, serum, plasma ≤ 200 µl swab wash solution ≤ 25 mg tissue ≤ 200 µl feces
Elution volume	50- 200 µl
Fragment size	300 bp - approx. 50 kbp
Preparation time	Approx. 70 min (excl. Lysis) /96 samples

KIT Information	Manufacturer
NucleoMag® Pathogen 1x 96 preps	Macherey Nagel
NucleoMag® Pathogen 4x 96 preps	Macherey Nagel

NIMBUS Presto Assay-Ready Workstation: Specifications

Physical Dimensions	
Length	135.9 cm
Width	70.9 cm
Height with closed door	83.1 cm
Height with open door	124.0 cm
Weight	136 kg approx.

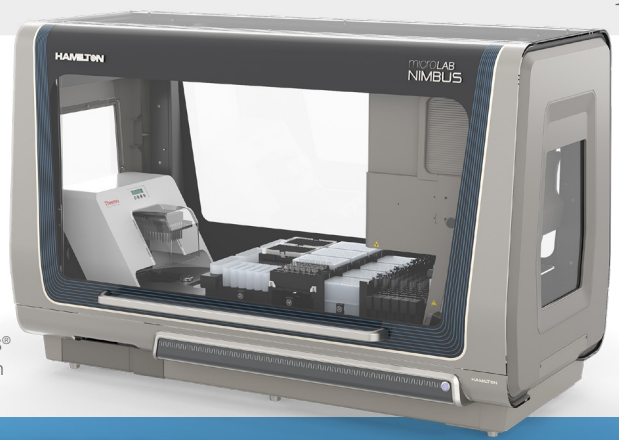


Figure 2: The Hamilton NIMBUS® Presto Assay-ready Workstation

Remarks

NucleoMag is a registered trademark of MACHEREY-NAGEL; Hamilton and NIMBUS are registered trademarks of HAMILTON; KingFisher is a trademark of Thermo Fisher Scientific; SensiFast is a trademark of Bioline Reagents; Taqman is a registered trademark of Roche Diagnostics.

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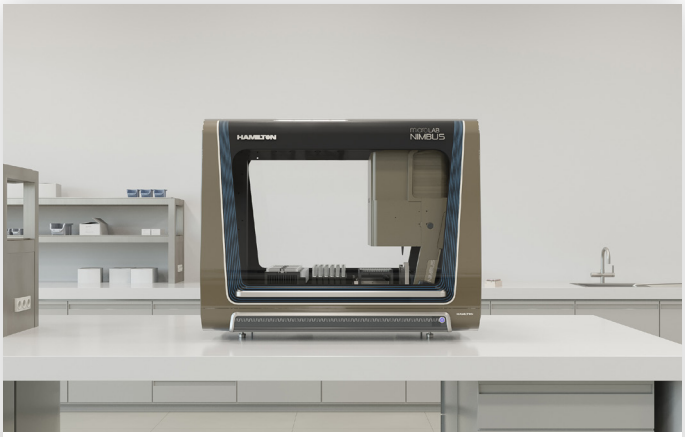
The next generation of liquid handling automation

Microlab® STAR line
The classic Hamilton automated liquid handler



Microlab® NIMBUS line
A compact, multi-channel automated liquid handler

Microlab® Prep™
Our smallest footprint liquid handler

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HAMILTON

(Assay-Ready)

WORKSTATIONS for Molecular Diagnostics

OUR PORTFOLIO OF FULLY AUTOMATED SOLUTIONS

- **MagEx STARlet**
  For magnetic bead-based nucleic acid extraction
- **NIMBUS® Presto**
  Integrated with ThermoFisher Scientific's KingFisher Presto for magnetic bead-based nucleic acid extraction
- **PCR Prep STARlet**
  For pre-PCR sample set-up
- **RT STARlet**
  Integrated solution from nucleic acid extraction to Real-Time/quantitative PCR analysis. Platform equipped with two dedicated qPCR machines (Magnetic Induction Cyclers)
- **NGS STAR™**
  For NGS library preparation
- **Clinical NGS STARlet**
  (CE-IVD) For NGS library preparation

Qualified methods from leading, third-party kit manufacturers are available in our (Assay-Ready) Workstations:

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