

numares
insider



2021

HANDS ON

Novelties for the
AXINON® System
preanalytical process

INSIDE

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A comparison...

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sign agreement

Welcome to *numares insider*



Volker Pfahlert



Winton Gibbons

In this issue, we highlight two milestones: The FDA submission of numares' AXINON® System and the partnership with NMR manufacturer Bruker to enable advanced metabolomics-based clinical diagnostics.

While the world still struggles to recover, there is anticipation of returning to the necessary routine care for patients. To help with this, we are working hard to provide solutions nearterm for current and unmet medical needs.

In this issue, the opening article discusses the advantages and differences of utilizing NMR and mass spec for metabolomics. The techniques serve different purposes and complement each other in their use for diagnostics. Moreover, in practice, organizations that develop assays often focus on different medical problems, resulting in unique tests.

Last month marked two milestones achieved by numares in establishing NMR diagnostics for routine clinical use. These included our collaboration with Bruker to enable advanced metabolomics-based clinical diagnostics, and our submission of an application to the FDA for the AXINON® System.

We also have in this issue our second article in the series about numares departments, "What it's like to work in the "Service" department at numares." Next, we focus on the components that come with kits for the preanalytical phase of the AXINON® System. There are some features that allow for the best automated and manual sample preparation. Finally, we conclude with technical instruction on the automated sample preparation process, derived from our online video tutorials. These videos support our customers in the required maintenance and qualification procedures of the AXINON® System.

As spring moves to summer, and the world moves back to normal, we hope everyone will remain healthy and thrive again.

Volker Pfahlert, Chief Executive Officer

Winton Gibbons, President of numares GROUP Corp.

NMR vs MS: Which metabolomics tool to use for clinical diagnostics? A comparison...



Mass Spectrometry (left) and Nuclear Magnetic Resonance Spectroscopy (right). Both analytical technologies are used in metabolomics research and biomarker commercialization. With standardization measures, both techniques entered the clinical diagnostics market, offering several benefits for various medical questions.

Biomolecules indicated in medical diagnosis typically fall under the broad categories of genomics, proteomics and metabolomics. Due to the developments in both the technology and access to technology in the field of proteomics and genomics in the last decades, the role of both genetic and protean biomarkers in diseases is much better understood than the role of metabolomics in human disease and progression. In this brief summary, we review and compare the most prominent analytical technologies currently used in metabolomics, namely mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy.

The Technologies

The two primary analytic technologies used in metabolomics research and biomarker commercialization include NMR and MS. Typical MS instrumentation include three basic components: 1. Ionization device to generate charged molecules for analysis; 2. An analyzer (or a sequence of analyzers) to select ionized molecules and to create diagnostic fragments (MS/MS) and separate ions and fragments into distinctive mass components; and 3. A detector to detect mass components according to their mass-to-charge ratio. Many different variations of MS exist making

standardization across labs a challenge, and the utility and performances of MS often vary from lab to lab.

An NMR system requires two major components: 1. NMR Magnet and 2. NMR probe. The NMR magnet generates a magnetic field identified by the MHz of the magnetic system. Although NMR systems up to 950 MHz exist, they are prohibitively expensive, whilst the additional sensitivity and resolution is often not required for the routine analysis of biological analytes and so more commonly a 600 MHz NMR system will suffice. The probe is the part of the NMR spectrometer that covers most of the work when it comes to excitation of the nuclear spins and detection of the NMR signal. There are several probes that, by design, respond to multiple frequencies and allow both excitation and discrimination of multiple nuclei* such as ^{13}C , ^{31}P , and ^1H , respectively, although these are not detected at the same time. ^1H probes are optimized to give the highest proton sensitivity (^1H) and therefore detect most of the organic molecules, which is a prerequisite for performing metabolomics.

Applications

Traditionally, NMR is used to identify the chemical composition of a complex solution. Additionally, it can be used to evaluate the molecular dynamics of a solution as well as for identification of molecules and their chemical properties. Over the last 10 years, NMR increasingly became of vital importance to metabolomics for clinical applications using liquid biosamples. Earlier NMR systems were limited by low sensitivity, but with more powerful magnets and increased development of probe technology, NMR systems evolved as quite capable tools for metabolomics biomarker discovery and identification in clinical samples.

MS is primarily used in structural characterization of biomolecules as well as in the quantification of targeted molecules within a sample. In MS, the biomolecule is isolated and broken down to its molecular or even atomic levels in order to analyze. Hence



The AXION® Platform: clinical diagnostics based on NMR and metabolomics: The highly standardized AXION® platform can be operated by any lab technician with one week of training.

unlike NMR, MS is not readily suitable for studying molecular dynamics. Also, MS instrumentation used can vary significantly according to the performance requirements, cost considerations and the variations in technological components employed in ionization, analysis and detection. Hence MS really exists as a family of instrumentation offering a wide array of choices for different targeted applications. This diversity in MS family makes the technique highly targeted to the specific utility of the lab and less amenable for standardization across labs. NMR on the other hand is much less variable in its technical performance and function across labs and is much more amenable to standardization across labs.

Comparative Features

Sensitivity – While functional sensitivities can be matched between the two technologies for the analysis of a given biomolecule, MS often uses less sample volume than NMR.

Analyte Identification – Because NMR detection is not limited to a targeted range, the data generated by NMR is very useful for profiling where the NMR spectra generated represents all complex macromolecules within a sample that can be mined for targeted analysis prospectively. Analytical software can be employed post detection for targeted interrogation of metabolites across the profile. MS is useful for both targeted and profiling analysis but the capability to profile is limited by the specific technique used. For example, the mass range of analytes detected by mass spectrometry will depend on the ionization, analyzer and the pre-analytical conditions used. It is not possible to interrogate the complete range of analysis using a single mass spectrometry technique as versatile as NMR can do.

Sample Process – As mentioned in the above section, NMR allows for all metabolites within certain concentrations to be detected in a single measurement. In contrast, MS requires different instruments and ionization methods to identify metabolites in different classes.

Downstream Sample Processing – NMR is non-destructive and consequently samples can be stored after analysis for secondary or later analysis. Sample non-destruction allows for the sample to be archived as part of bio-repositories for further research and clinical analysis. MS has the advantage of using less sample volume but the exact sample aliquot cannot be used for subsequent analysis of the sample as it is destroyed during ionization process and cannot be retrieved for further analysis or storage is possible.

Repeatability/Reproducibility – Both techniques are highly precise and reproducible. As there are many more moving parts in MS workflow in a production setting, achieving consistent reproducibility in mass spectrometry requires highly trained technologists. NMR can be operated with relatively less training and complexity. This is especially true for the standardized AXINON® platform*, which can be operated by any lab technician with one week of training.

Sample Preparation – Performance of MS depends heavily on the extent of sample preparation prior to analysis because the separation and ionization process is highly competitive among chemical species and the purer the extract that is presented to MS for analysis, the more robust and consistent the performance would be. This can be a challenge in many scenarios and managing this would require an analyst that is highly trained in disciplines as varied as sample preparation, chromatography and mass analysis. NMR on the other hand has the advantage that little or no early sample processing is required and hence the threshold for skill and training is minimal compared to the operation of mass spectrometry platforms.

Metabolites Detected – A 600 MHz NMR instrument can detect about 400 metabolites from a single spectrum. The family of mass spectrometers combined can detect a larger variety of metabolites but an individual mass spectrometry platform has a much more limited coverage than the typical NMR platform. In order to fully exploit the coverage of MS, one will have to utilize many different types of MS platforms.

In summary, both NMR and MS are excellent technologies for biomarker analysis and are used routinely in clinical laboratories. Over the last few years, there has been significant growth in the metabolomics field driving greater adoption of both technologies in clinical applications. For targeted and single biomarker analysis, MS is the preferred technology for most laboratories and has a lower cost and a lower barrier for entry. In contrast, NMR is a more robust technology that is more optimized for the detection of multi-parametric biomarkers from a single sample and the data can be interrogated prospectively for additional data mining and analysis. In today's laboratory environment, both technologies are complementary in metabolomics research and commercialization. □

Maulik Shah, Medical Affairs

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NMR series:

Novelties for the *AXINON*[®] System preanalytical process

NMR diagnostics in the clinical laboratory is in itself a novelty, as Nuclear Magnetic Resonance (NMR) spectroscopy has been used as research tool in the medical and chemical research community for decades (see article this issue, page 3). Well established to clarify chemical structures or protein-protein interactions, NMR spectroscopy now entered the clinical laboratory market as a medical platform, capable of analyzing patient samples destructive-free and refined by sophisticated measures like *numares' Magnetic Group Signaling*[™] (MGS[®]) technology to ensure excellent inter-laboratory comparability, robustness, high throughput-capability and precision.

A potential bottleneck: The preanalytical phase

Those familiar with NMR analytics may see a bottleneck when it comes to the bulk preparation of samples for diagnostics with the, admittedly, fragile NMR tubes and humanly limited attention when it comes to preparing numerous samples manually. To ensure convenient handling and preparation of samples for NMR diagnostic evaluation with the *AXINON*[®] System, we have come up with several innovations to make the (pre-)analytical process as simple and error-resistant as possible - whether for manual or automated processing with liquid handling systems*.

1. Specimen-type specific, ready-to-use solutions**

With the *AXINON*[®] System come two different *AXINON*[®] kits for easy preparation, one for urine and one for blood (serum) samples, respectively. The kits are general purpose: they have been designed to be used universally for all *AXINON*[®] tests - just depending on whether the tests are serum or urine based. The consumables consist of additives solution, calibrator and control solutions. As all components are ready-to-use solutions, there is no need for time-consuming mixing, thawing or dissolving of different compounds, which makes their use in automated sample preparation in particular very convenient. The kits are available in different package sizes (for 20, 100, 500, 1000 tests) supporting a wide range of series lengths and throughput.

2. Additives solution

The additives solution is added to each of the analytical samples. It contains substances required for NMR measurement and quality control of the samples. The additives solution has to be mixed with the analytic sample in a defined ratio (see IFU**), and carefully transferred to the NMR tube. With automatic sample preparation, the additives solution can be used directly



There are two *AXINON*[®] kits - for urine and serum - providing ready-to-use consumables: control, calibrator and additives solution.

without preparatory handling. The liquid handling robot fills the 96-well plate positions A3 - H11 in one go. After adding and mixing the analytes, the mixed samples are transferred to the respective NMR tubes in the Automation NMR rack.

3. Calibrator solution

The *AXINON*[®] kits contain a ready-to-use calibration sample that ensures correct quantification of analytes in the analytical samples and that the results do not drift over time. Before each analytical run, a calibration measurement is automatically performed.

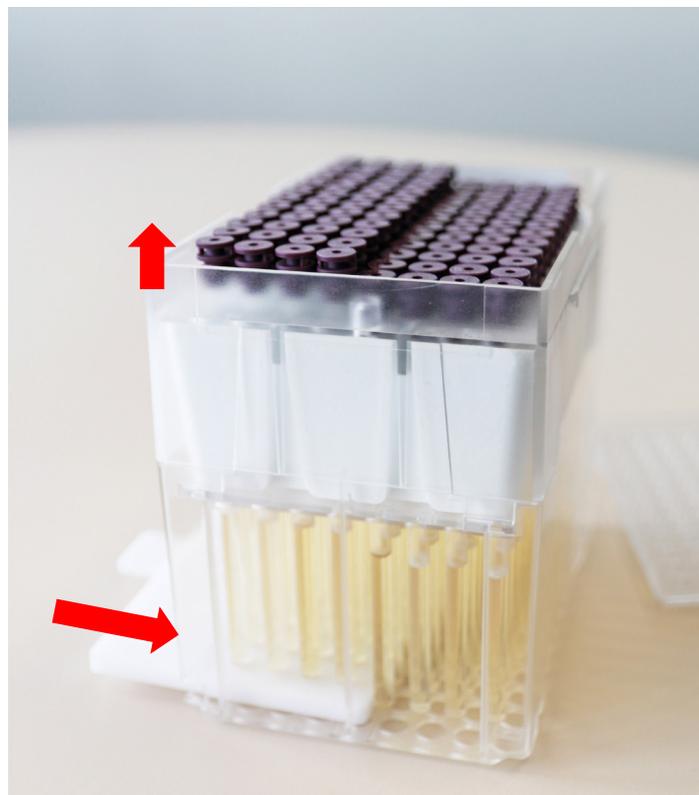
4. Control solution

The quality control samples contain several quality control substances that are used for controlling the quality of the measurement. In that way, the correct functioning of the *AXINON*[®] System, including the NMR spectrometer, is guaranteed. Before each analytical run, the internal quality control is performed using the first of two quality control samples. At the end this is repeated with the second one. This ensures valid results over the complete rack. Hence, the control solution is placed into the second and last position of the NMR rack.

Preparation of the samples conclude with the capping of the NMR tubes with barcode caps and subsequent scanning of the barcoded caps. To facilitate this final step, we developed an adapted „Automation NMR rack“.

5. Automation NMR rack

The Automation NMR rack was developed to facilitate capping and scanning of the prepared samples. Therefore we provide a refined NMR rack with a side slot along the entire length of the NMR rack, just above the bottom (see photo on the right). A supplied slider plate is slid into the slot below the NMR tubes. This raises the NMR tubes by approx. 4 millimeters, which enables a more convenient handling of the samples in the NMR rack during the capping process.



The "Automation NMR rack" has a side slot along the entire length of the NMR rack, just above the bottom. A supplied slider plate is slid into the slot below the NMR tubes. This raises the NMR tubes by approx. 4 millimeters, which facilitates capping.

With the Automation NMR rack, the capping time can be reduced by half. As a result, taking all these measures together, an automated sample preparation of 96 samples in parallel results in just approximately 25 min. processing time. A real improvement when several hundred samples are processed per day and NMR spectrometer e.g. just for lipoprotein profiling.

****For further details on the sample preparation process, please refer to the respective instructions for use of the *AXINON*[®] serum and urine kits. □**

*Christiane Proll, Marketing
Michele Salvi, Service*

Introducing *numares* Departments Service

Close to the customer and close to the technology. In this edition of the *numares insider* we continue asking *numares* employees to give an insight into their daily work. In this issue senior scientist Katja Barthelmes of *numares* Service provides insight into the challenges of a complete NMR (nuclear magnetic resonance) installation, the continuous improvements of a diagnostic system, and the most interesting support cases of customers.

ni: Katja, the Service department copes with the installation of *numares'* NMR-based diagnostic platform at the customer's site. How does one have to envision something like that?

Our department covers the whole customer support process for the installation of an *AXINON® System**. This includes all steps associated with the NMR device installation up to the final training of laboratory personnel.

The first interaction with our customers is usually during the site planning. Here we work closely together to define the optimal layout of the NMR laboratory. During installation, our NMR specialists coordinate the setup of the NMR spectrometer together with technicians from the NMR manufacturer Bruker. Our comprehensive qualification tests further ensure that the installed NMR device fulfills all requirements. After we installed *numares'* *AXINON® Software*, we start qualifying the complete system. Linearity, trueness and precision measurements for each installed NMR have to pass our strict protocols to proof the performance of the new instrument. Only when all requirements for the system have been met, it will be released for routine operation.



Besides installation, if requested, we can also help our clients to set up the connection to the Laboratory Information (& Management) System (LI(M)S) via the existing interface or provide guidance for the setup of internal standard operating procedures. The installation phase is completed with a hands-on training that enables our customer to get started quickly. In sum, the close collaboration with our clients on-site gives us the perfect opportunity to lay the foundations for a good and long-term customer relationship.

ni: What challenges do you have to overcome?

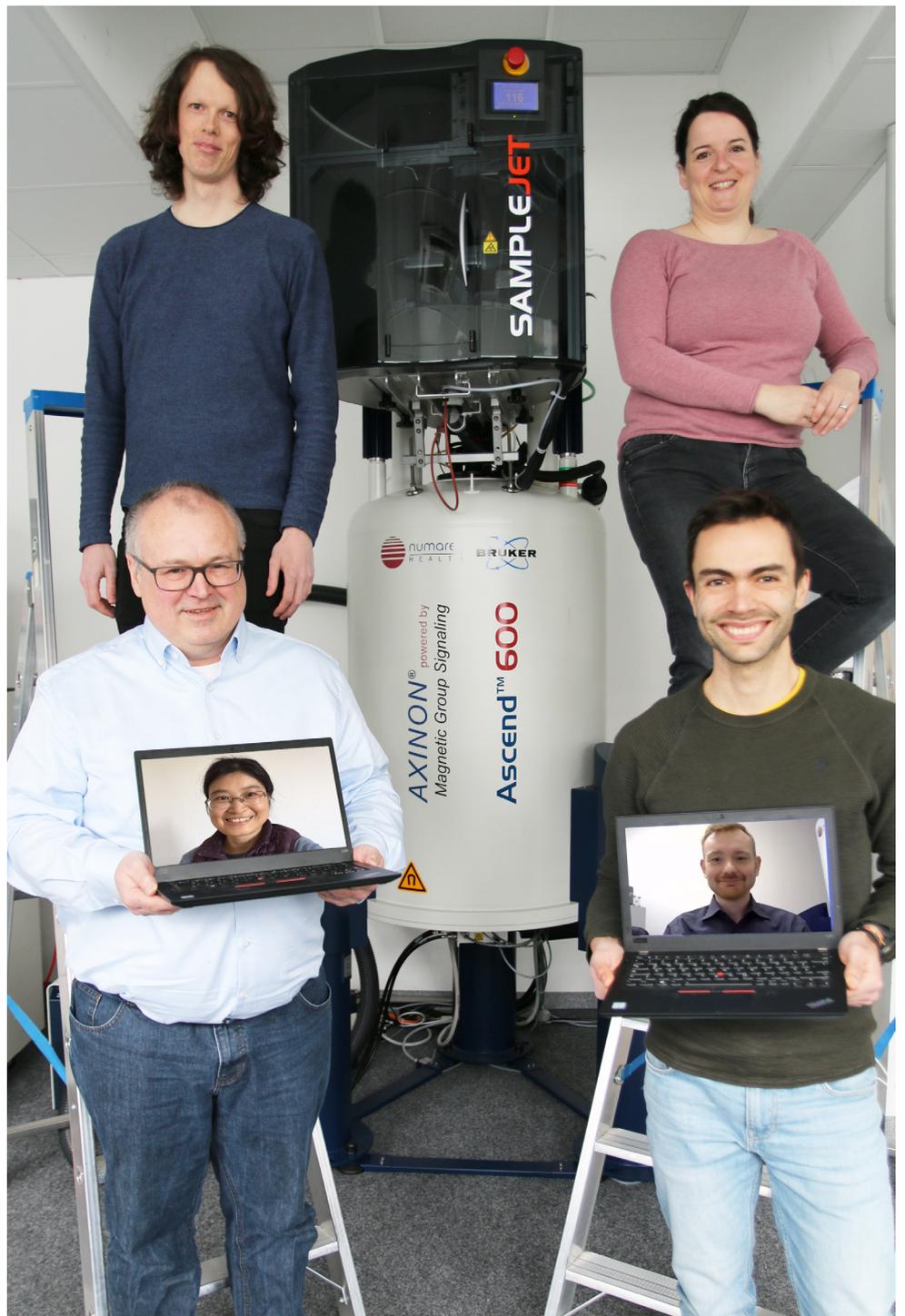
Once installed our *AXINON® System* is a very robust and easy-to-use platform. If at all, we face challenges during the installation phase, when we adopt the system to the special conditions of the customer laboratory. For example, finding the optimal settings of the air conditioning system (HVAC) can be tricky. They have to match the requirements given by the

NMR specifications. But so far we always found the perfect solution – even for several NMR devices in one go.

ni: You also support the customer in his daily work. How does that look like, especially now in the times of Covid-19?

In general the situation with Covid-19 did not affect our usual day-to-day business very much. Our service agreements include support for our customers via email, phone calls, video calls or remote access. As we have service offices in the U.S. and Germany, our team is always reachable via video calls with short response times. So overall it was no problem to transfer our regular working routines to home office. Our U.S. team covers the few urgent cases where an NMR expert is needed on-site. Thanks to the robustness of the system this happens extremely rarely.

In the last months we have even taken advantage of the current situation. Updates of already installed and qualified *numares' AXINON® Systems* to new versions are now easily done remotely by our specialists. That



enables us to react more flexible to customer inquiries. Additionally, we significantly improved the *AXINON*[®] Maintenance Manual (former NMR Maintenance Guide, editor's note) to support our customers with guidance on routine maintenance tasks or operational qualification checks in order to keep their instruments in good condition. Nonetheless, the complete team looks forward to meeting our customers in person again as soon as circumstances permit. It is important for us to be a partner for our clients even beyond the daily challenges. We always enjoy the technical discussions and mutual exchange.

ni: What have been your most interesting/outstanding support cases? Can you give us an example?

I can remember one extraordinary case that turned out to be easily fixed but caused some amusement in retrospect. We saw a phenomenon, which we called the "dancing magnet": the magnet (the NMR spectrometer, editor's note) seemed to be slightly tumbling between its three legs, which looked like dancing. Recalibration of the air stream that goes into the dampening legs could fix the issue pretty quickly.

ni: The service department is also taking up feedback from the customer, which often results in system or service improvements. How does that look like?

Since we are in continuous contact with our customers, we can take up wishes and requirements, e.g. for an

adaptation of an application, which can be incorporated into the system requirements. This is especially true when the system has to be integrated into existing workflows. Then, our NMR specialists provide their expertise for improvements implemented by the experts of our development department.

In addition, we have noticed that customers like to consult with us before doing their maintenance tasks or operational qualification checks. This is understandable, as some tasks only occur once a year or only after an even longer period of time. In order to be able to provide optimal assistance and as a sort of memory aid, we already started creating video tutorials in which the individual steps of such tasks are shown in detail. The video tutorials are accessible – together with printable photo instructions – on the *numares* website (www.numares.com) and on the *numares* YouTube channel (<https://www.youtube.com/channel/UCKG5kLOYo0I6q1aMzJPNH3Q>). This is a great success! We received very positive feedback from our customers and are planning to continue with this series. Both, customer feedback and satisfaction are always an immense motivation for our team to give more than our best! □

Katja Barthelmes, Service

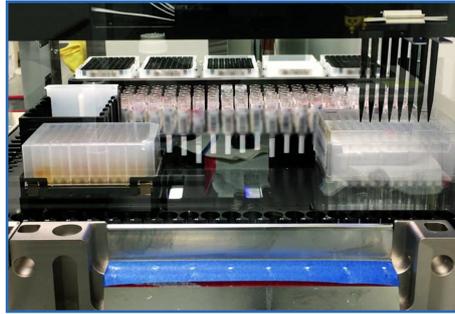
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numares' Video Tutorial Series: AXINON[®] System - Automated high-throughput sample preparation*

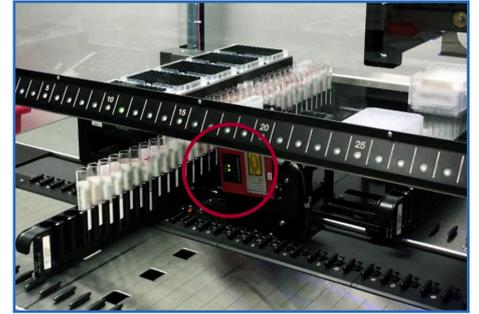
**For sample preparation refer to respective IFUs of numares' AXINON[®] serum and urine kits. .*



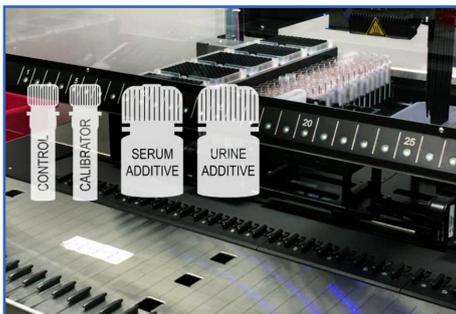
For high sample volumes, we recommend sample preparation with liquid handling robots (e.g. Tecan[®], Hamilton[®]).



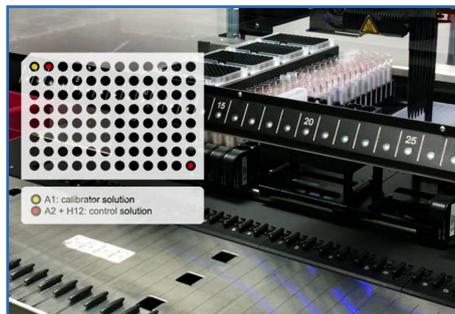
The automated sample preparation of 96 samples in parallel takes approximately 20 minutes. numares provides ready-to-use reagents for serum & urine samples.



Sample IDs are automatically scanned by a barcode reader.



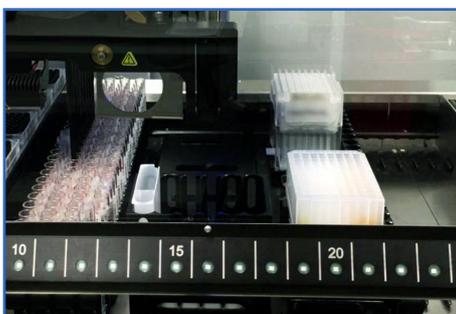
The AXINON[®] kits consist of 3 ready-to-use components: control, calibrator and additives solution.



In a 96 well plate, position A1 is filled with calibrator solution, position A2 and H12 with control solution.



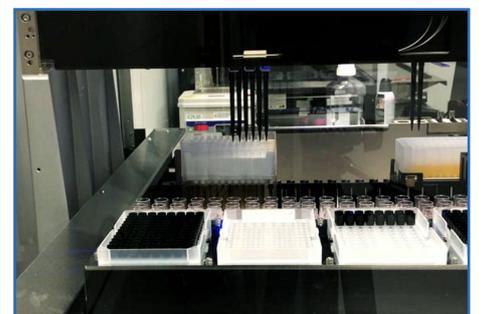
The other 93 wells are prefilled with the additives solution.



The analytes are transferred to the 96 well plate containing the additives solution.



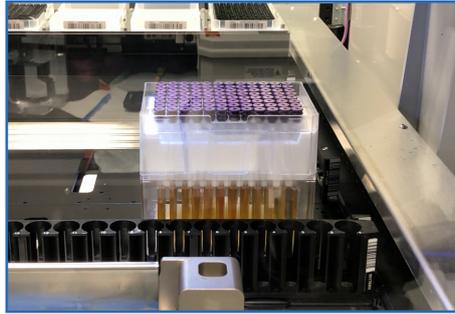
The analytes are mixed carefully by the automatic multi-pipette of the robot.



600µL of the prepared samples have to be transferred to the NMR automation rack holding the NMR tubes.



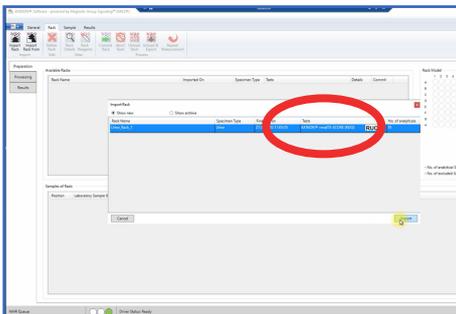
Alternatively, thin metal tips can be used for filling and air inclusion-free mixing.



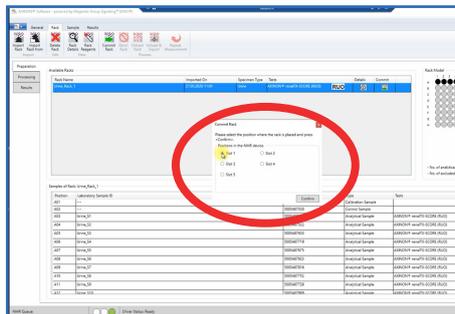
NMR tubes are closed with barcode caps and scanned. The cap barcodes are automatically assigned to the sample ID. The data is captured by the LI(M)S.



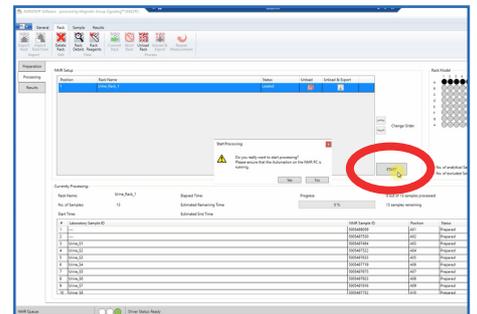
After the automatic pipetting process, the rack is transferred to a free slot in the SampleJet®. Keep slot number in mind.



Open AXINON® Software: Import data from the LI(M)S, which will provide the chosen test application.

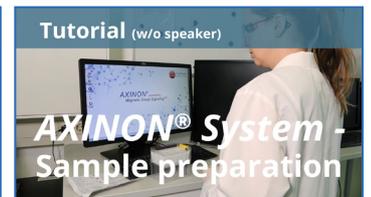


Assign to the respective slot...



...and press „Start“ to start measurements.

More from the numares' Video Tutorial Series:



Find more helpful video tutorials on numares' YouTube channel... [Subscribe](#)

#numares@Social Media

Welcome to the social media world of *numares*! By following the LinkedIn (<https://www.linkedin.com/company/numares-ag/>) profile of *numares*, you will get a continuous flow of news around our products, developments and the company itself. Here is an excerpt from the latest news:



numares NEWS: *numares* announces FDA 510(k) Submission of the **AXINON[®] System**, an NMR platform for metabolomics-based, AI-driven diagnostics.

"This U.S. regulatory filing for its diagnostics platform is an important milestone for *numares*," says Winton Gibbons, President - US and co-CEO of *numares*. "After entering into collaborations on joint diagnostics development with analytical instrumentation and solution provider *Bruker Corporation* in January 2021, and with *Mayo Clinic Laboratories* in 2019, this FDA submission is the first step in our common endeavor to bring NMR diagnostics into routine clinical use, and make metabolomics-based diagnostics accessible to the patients."

On International Women's Day we say thank you to all the female colleagues at *numares*, whose deep expertise, vision and leadership is fundamental to our success in developing advanced NMR diagnostics. Be it heads of, seniors or juniors, over all departments.

Without you we wouldn't be this far.

Thank you!

numares NEWS: *numares* recently published data showing that its novel, multi-marker approach to assess kidney function allowed for a more precise estimation of glomerular filtration rate (GFR).

In a proof of concept-study, the combination of 4 biomarkers incl. creatinine in a simple serum test surpassed common, recommended eGFR equations.

This holds the potential for an improved assessment of kidney function and at the same time deeper insights into kidney pathophysiology associated with CKD (chronic kidney disease).



@AgNumares
 @numares AG

@numares NMR diagnostics
 @numaresAG

numares and *Bruker* sign agreement to enable advanced metabolomics-based clinical diagnostics

numares and analytical instrumentation and solution provider *Bruker Corporation* have signed an agreement to collaborate to bring new diagnostics tests utilizing NMR to the laboratory market for unmet medical needs. The tests are based on the automated measurement of metabolites using a strong magnet field, and artificial intelligence. The platform is marketed under the *numares* brand **AXINON®**.

Based on a longstanding collaboration between the two companies, the agreement creates a combination of *numares*' expertise in leveraging metabolomics for software-based diagnostics with world-leading nuclear magnetic resonance (NMR) technology from *Bruker*. This leads to more rapid, accurate and personalized information about the severity and progress of diseases.

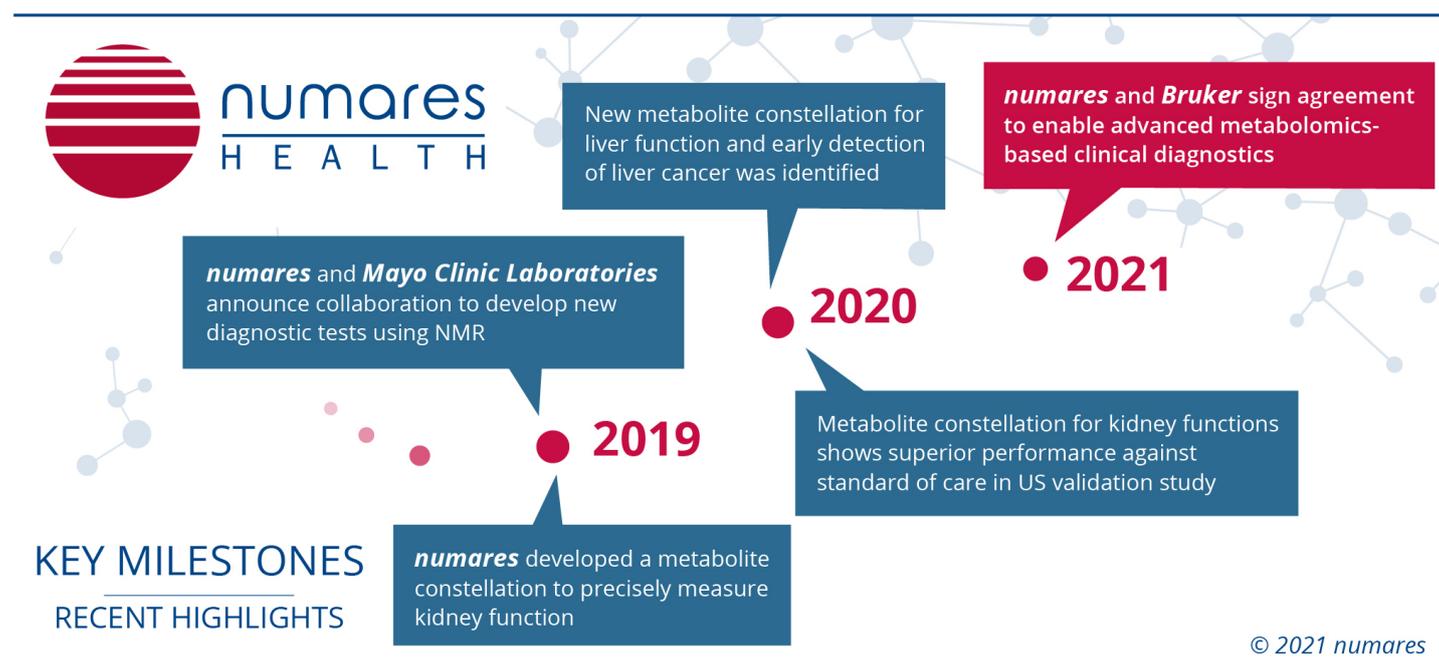
This collaboration will support the ongoing transition of **AXINON®** platform from research into routine clinical use, and makes it available to all patients as an affordable, day-to-day diagnostic standard. Two years ago, *numares* started a collaboration with US-based *Mayo Clinic* for joint diagnostic test development using metabolomics and NMR. The collaboration of *numares* and *Bruker* now marks a further milestone in the *numares* vision to bring these technologies into routine diagnostics.

The **AXINON®** platform based on NMR spectroscopy is a non-invasive technique that is uncovering new potential for the prevention and treatment of



disease, including conditions in the kidney, heart, and liver, as well as cancer. The *numares* system measures various metabolic patterns and applies artificial intelligence (AI) to cluster and translate the results into clinical diagnosis. This rich source of information gives healthcare professionals new insight into disease management, and gives patients fast access to the right treatment. Likewise, the **AXINON®** platform also supports disease risk prediction, paving the way to new developments in precision medicine and companion diagnostics.

Dr. Volker Pfahlert, Director of the Board of *numares* says: "Our agreement will help to expand the exciting potential of NMR technology, long established in academic and clinical research, to the clinical diagnostics industry where *numares* already has a strong customer base. It paves the way for market entry of NMR diagnostics under FDA regulations, a prerequisite to make it accessible to all patients as



day-to-day applications in routine clinical diagnostics. It will support *numares* to better utilize its test development in several indications such as kidney disease, liver disease or neurological disorders.”

Dr. Iris Mangelschots, President of *Bruker BioSpin's* Applied, Clinical and Industrial division comments: “The pandemic of 2020 has brought unimaginable challenges on a global scale. With the world’s spotlight sharply focused on the healthcare industry, our business has accelerated to develop the technology and diagnostic solutions that is desperately needed. The innovations born out of this demand have paved the way for future, highly advanced new product developments. Partnering with *numares* will bring our technology to the frontline healthcare industry, where it is most needed. NMR technology delivers the

robust and reproducible results needed to support the advanced AI modelling techniques deployed by *numares*. Together, our complementary technologies offer a powerful arm in the fight against disease of the future.”

Co-CEO and President of *numares Group Corp.* Winton Gibbons concludes: “This agreement is a further important step in the commercialization of our products in the US. With GFR_{NMR} we will soon launch a novel blood test to help identify and quantify impaired kidney function in patients with this more accurate, easy-to-access, and affordable diagnostic standard.”

□

Christiane Proll, Public Relations

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numares is going to participate at the upcoming meetings & conferences

Due to the threat of the ongoing coronavirus pandemic, many conferences and trade shows have switched to new dates and digital platforms. We will be part of it and invite you to get in contact with us on the dates below.

If you want to contact us independently from any event, please get in contact with our President - US Winton Gibbons (winton.gibbons@numares.com) to arrange an individual appointment via Zoom or in person. Thank you for your comprehension and stay healthy!



American Transplant Congress

June 4-9, Virtual Event

<https://atcmeeting.org/>

POSTER PRESENTATION



ASCLS Annual Meeting: 2021 Joint Annual Meeting

June 27 - July 1, Louisville, Kentucky

<https://ascls.org/annual-meeting/>

POSTER PRESENTATION



2021 AACC ANNUAL SCIENTIFIC MEETING & CLINICAL LAB EXPO

September 26-30, Atlanta, Georgia

<https://meeting.aacc.org/>



ASN Kidney Week 2021

November 2-7, San Diego

<https://www.asn-online.org/education/kidneyweek/>

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