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2021

HANDS ON

Video Tutorial: Reboot the *AXINON® System*

INSIDE

Clinical diagnostics of CVD supported by lipoprotein particle analysis

NEWS

numares and *Oxford University* sign exclusive license agreement

PROLOG

Volker Pfahlert

Winton Gibbons

In this issue, we are also providing experts' views on lipoprotein particle analysis, important risk factors in cardiovascular diseases.

Welcome to *numares insider*

Together, *Oxford University* and *numares* recently reached and important milestone in the fight against Multiple Sclerosis (MS). Based on the preliminary work of Oxford, and having now signed an exclusive licensing agreement, *numares* will now be able to develop diagnostic biomarker constellations to help identify the transition from "relapsing remitting MS" to "secondary progressive MS" and urgent need. This partnership is also further extended to working together in Alzheimer's disease. Read more on this medical breakthrough on page 19.

In this issue we are also providing experts' views on lipoprotein particle analysis, important risk factors in cardiovascular diseases. We provide current information about ApoB-100, LDL-P and other important particles for lipoprotein profiling. Enjoy reading this exciting lead article on the following pages.

With the positive feedback of our customers, we are glad to introduce two further departments. Software Development & IT present themselves on page 9, and our head of US Service, Michael Grasse, gives insights on his daily routine on page 16.

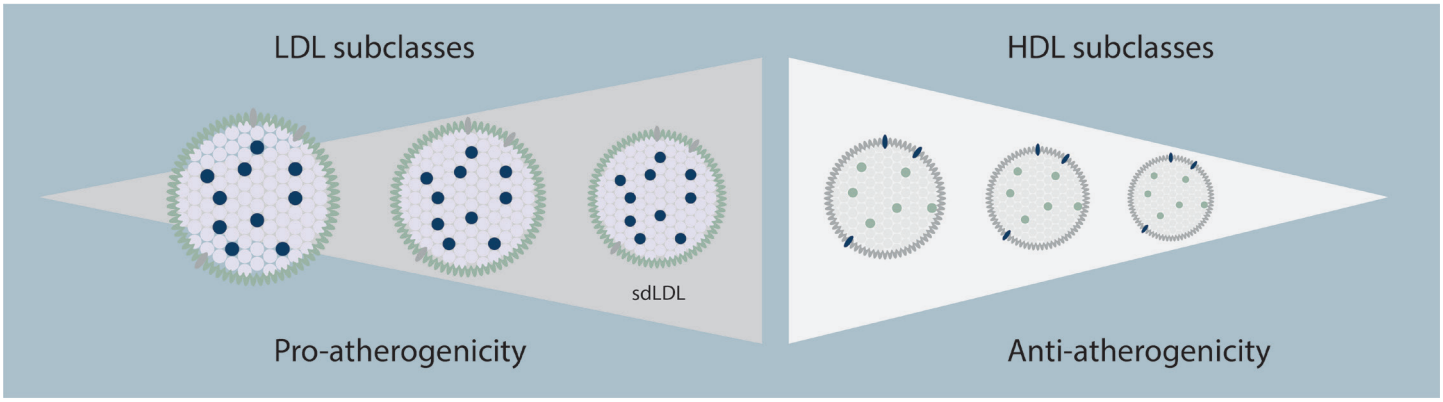
In our popular series "Hands On" page 12, we discuss our latest NMR topic, the magnet and vibration dampening system. Also, we recollect on how to deal with dry ice.

We wish you a healthy and happy rest of the year, and an enjoyable holiday season.

Volker Pfahlert, Chief Executive Officer
Winton Gibbons, President of numares GROUP Corp.

INSIDE

Clinical diagnostics of CVD supported by lipoprotein particle analysis



Schematic illustration of lipoprotein particles within LDL and HDL subclasses. When the particle diameter is reduced, the atherogenicity of LDL increases, while the anti-atherogenic effect of HDL decreases. (Illustration: numares AG)

Lipoproteins and Cardiovascular Disease

Elevated blood cholesterol is a well-known risk factor for cardiovascular disease (CVD), in particular coronary artery disease (CAD), myocardial infarction and other consequences of atherosclerosis such as stroke or peripheral artery disease. However, over the past decades, we have learned that CVD risk is characterized by a much more complex interplay of physiological events than just the elevation of cholesterol.

For transport in the blood, cholesterol and other lipids are packaged into lipoprotein particles. Originally, different lipoprotein fractions have been classified by density, because ultracentrifugation was an available method to separate them. The terms we still use for different lipoprotein types, today: VLDL (very low density lipoprotein), IDL (intermediate density lipoprotein), LDL (low density lipoprotein) and HDL (high density lipoprotein) are derived from the use of this technique.

By now, we know that there is much more to different lipoprotein classes: There are distinct pathways/life cycles for chylomicrons, LDL family and HDL family particles. Today we have the ability to characterize these particles much more precisely by their size, apoproteins, lipid composition and (patho-) physiological roles.

Half of the individuals who are hospitalized due to CAD have normal LDL cholesterol levels [1]. With respect to CVD risk assessment, the first historic improvement over total cholesterol determination was to consider LDL and HDL cholesterol and their ratio. This approach is still used in everyday CVD risk assessments in the clinic. Much momentum has been gained in further understanding of the cardiovascular events and it is now recognized that there is a continuous distribution of lipoprotein size and density that needs to be considered to assess atherogenic potential of a patient.

Why should lipoprotein subclasses be measured?

Certain lipoprotein subclasses are widely acknowledged as emerging risk factors for CVD [2-5]. The association between lipoprotein subclass cholesterol, particle concentration, particle size and CVD outcomes has been demonstrated by a large number of studies [6-27]. Within the LDL fraction, especially small dense LDL particles are positively associated with CVD risk [6, 10, 12-22]. High levels of large HDL particles seem to have a cardio protective effect [28]. Use of LDL/HDL ratio to assess CVD risk has been a common approach for many years but it needs to be scrutinized further due to the inconsistencies in findings between studies [19, 23-26].

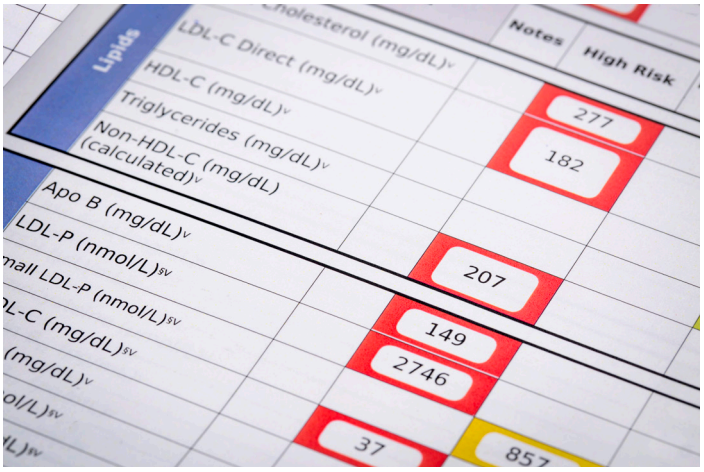
In a large-scale prospective study of more than 20,000 healthy women, NMR (nuclear magnetic resonance) determined lipoprotein particle concentrations and sizes predicted incident CVD events independently of classical risk factors [20]. Adjusted Hazard Ratios (HRs) of 2.51 (95% confidence interval (CI) 1.91 – 3.30) for LDL particle concentration (LDL-P), 0.91 (95% CI 0.75–1.12) for HDL particle concentration (HDL-P), 0.64 (95% CI 0.52–0.79) for LDL size and 0.65 (95% CI 0.51–0.81) for HDL size [20] were observed when ranking them among the major risk factors. The association between NMR measured LDL particle concentration and future CVD events was established in the large Multi-Ethnic Study of Atherosclerosis (MESA) with a HR of 1.32 (95% CI 1.19-1.47) [11].

Furthermore, the two nested case-control studies by Blake et al. [6] and El Harchaoui et al. [10] found that LDL-P was a strong predictor of cardiovascular risk. In addition, a matched case-control study within the Multiple Risk Factor Intervention Trial showed that higher levels of HDL-P were associated with a lowered risk of CVD death in men with metabolic syndrome (HR 0.50, fourth quartile compared to first quartile (95% CI 0.26-0.96)) [26]. Another nested case-control analysis by Kuller et al. showed significant differences between incident CVD cases and controls in LDL-P in women after bivariate analysis including LDL-C (odds ratio (OR) 1.11 per 100 nM (95% CI 1.03-1.09)) [7].

In addition, the concentrations of cholesterol in large HDL particles were significantly lower in women with myocardial infarction and angina (OR 0.73, fourth quartile compared to first quartile) compared to concentrations in women in controls [7]. In 2072 men from the Québec Cardiovascular study population, a strong and independent association between cholesterol in small dense LDL (sdLDL) particles and the risk of long-term ischemic heart disease in the first 7 years of follow-up was observed [27]. Taken together, a growing body of evidence indicates that lipoprotein subclass cholesterol, particle concentration and size are significantly and independently associated with CVD.

LDL-P, sdLDL & other independent CVD risk factors are captured by lipoprotein profiling

As it turns out, LDL cholesterol concentration only explains part of the atherogenic potential of LDL particles. The number of LDL particles (LDL-P) has been shown to more closely reflect the risk associated



Lipoprotein subclass cholesterol, particle concentration and size are significantly and independently associated with CVD.

with increased LDL, particularly if the concentrations of LDL-C and LDL-P are discordant [4-7, 11].

In addition, lipoproteins can be grouped into subclasses with different atherogenicity according to their size (see Figure 1). Within the LDL fraction, especially small, dense LDL particles (sdLDL) are strongly associated with CVD risk [8-20], while large HDL particles seem to have a protective effect [12, 21-27, 29-30]. In conclusion, it is evident that a detailed lipoprotein profile is necessary to capture the full picture of an individual patients' risk for CVD.

Equivalence of molar concentrations of ApoB-100 & of LDL, IDL, & VLDL particles

In the last years it has been established that the apolipoproteins A-I, B-100, and B-48 are specific for the different families of lipoproteins, apoA-I for HDL, apoB-100 for LDL, IDL, VLDL, and apoB-48 for chylomicrons and chylomicron remnants. It is generally assumed that these lipoprotein-specific proteins are bound in fixed stoichiometry to their particles. For the group of non-HDL-non-chylomicron lipoproteins an exact stoichiometry of one apoB-100 molecule per particle is confirmed by direct biochemical evidence as well as by the basis of their biogenesis [31, 32]. VLDL is produced by the liver and is successively metabolized to remnant particles, defined as IDL, and eventually to LDL. This means that the particles are converted into each other but the one structuring apolipoprotein remains. The molar concentration of apoB-100 thus equals the sum of molar concentrations of the LDL, IDL, and VLDL particles. The concentration ranges, however, vary significantly. While the concentration of LDL is typically around 1000-1500 nmol/l, IDL and VLDL only sum up to about 100 mmol/l [20, 33, 34]. As a result, the determination of the apoB-100 molar concentration equals the molar concentrations of LDL, IDL, and VLDL (LDL-IDL-VLDL-P, sometimes incorrectly called non-HDL). Independently, LDL correlates well with apoB-100.

LDL-p quantification is more complex since it is not a single molecule but an aggregation of lipids and proteins.

Methods comparison for the determination of the molar concentrations of apoB-100 / LDL-P

There are several methods available for absolute quantification of molar apoB-100 or LDL-P concentrations [31]. ApoB-100 can be determined using immunologic methods, often coupled with light scattering (immunonephelometry), or using trypsin digestion of the proteins and subsequent quantification of the specific peptides by Mass Spectroscopy, e.g. LC-MS/MS. Immunonephelometry is widely used because it is among the least expensive analytical options. However, there are indications that conformational changes of the binding epitope of apoB-100 which depend on the particle size or shape affect the efficiency of current immunoassays [35]. LDL-P quantification is more complex since it is not a single molecule but an aggregation of lipids and proteins. The absolute particle concentration can be determined using Ion Mobility methods, e.g. ES-DMA which couples an ES source with an Ion Mobility spectrometer, but also using combinations of techniques that take into account the particle composition.

With NMR spectroscopy all parts of the lipoprotein can be measured in one shot and also differentiates between the different classes and subclasses.

An example VAP test (vertical autoprofile) uses enzymatic cholesterol analysis after lipoprotein separation with ultracentrifugation. NMR spectroscopy on the other hand, offers an advanced technique which – once trained and standardized to retrieve the accurate information – directly measures all parts of the lipoprotein in one shot and also differentiates between the different lipoprotein classes and subclasses. This means that with only one measurement, a complete lipidomic analysis is accomplished that includes parameters that are required for a diagnosis. The data generated is fully quality-controlled and within the natural environment of the sample. Besides the classical lipid panel (total cholesterol, LDL-cholesterol, HDL-cholesterol, and total triglycerides), the cholesterol and triglyceride concentrations in all subclasses are obtainable. Moreover, the molar particle concentrations of lipoprotein classes and subclasses can be provided along with information on the size distributions in these classes. Because of the fixed stoichiometry of apoproteins/particles, the molar concentrations of apolipoproteins can be directly derived from the NMR data. The accuracy of the NMR analysis has been recently reviewed [36, 37].

Examples: Complete NMR lipidomics analysis relative to isolated ApoB-100 determination

Risk assessment for the development of atherosclerosis and coronary heart disease (CHD).

Classically, the risk for atherosclerotic cardiovascular events has been derived from LDL-C concentration or, more recently, from non-HDL-C concentration calculated from the total cholesterol concentration (TC) minus HDL-C concentration. By many accounts, non-HDL particle concentration is a better predictor for the CHD risk which is highly correlated with ApoB-100 concentration [38]. After the establishment of high throughput NMR determination of lipoprotein subclasses it has become evident that the lipoprotein particle size distributions are even stronger predictors of the risk of CHD [39, 40, 41, 42]. It has been shown that the concentration of the small dense LDL particles is a parameter for the prediction of CHD that is independent of other parameters such as LDL-C or ApoB-100 concentration [41]. The mechanistic hypothesis is that the formation of atherosclerotic plaques is caused by the intrusion of the non-HDL particles LDL, IDL, and VLDL in the subendothelial space independent of their size. Only the number of these particles is assumed to be relevant [43]. If these plaques occur in the coronaries, CHD is to be expected. In contrast, a wealth of earlier studies suggests that the size distribution (e. g. the concentrations of small

dense LDL-particles) also matters in risk assessment and therapy. In line with this, the European Atherosclerosis Society consensus panel draws a more differentiate picture of the atherosclerotic CHD (ASCHD) where a more detailed lipoprotein profile is recommended including lipoprotein subclass analysis and the determination of the ApoA-1 concentration [44].

According to the current recommendations of the European and American societies of cardiology though, the risk should now be assessed using ApoB-100 concentrations [45, 46]. As the NMR based LDL particle analysis is only available in the US, the recommendations of the European societies do not currently benefit from the clinical utility of NMR for risk assessment or patient care.

Prediction of the development type-2 diabetes

In general, metabolic diseases are usually characterized by changes in lipid metabolism as well as lipoprotein sizes and concentrations. Typical changes in the lipoprotein subclass pattern are already observed for individuals with impaired glucose tolerance or impaired fasting glucose and get more significant for individuals with type-2 diabetes mellitus (T2D). Typically, small HDL concentrations are increased and those of large HDL particles are decreased. A similar pattern is observed for LDL with concentrations of small particles increased and those of large particles decreased. The most significant effect is observed for small VLDL particles with an increase of their concentrations [33, 47, 48, 49, 50]. Such an increase of concentration would not be visible in the ApoB-100 concentrations. In fact, it has been shown that the risk for developing T2D can be estimated from the lipoprotein patterns alone [51, 52].

Characterization of genetic and disease related dyslipidemia and dyslipoproteinemia

It is clear that a single parameter such as the ApoB-100 concentration is not sufficient to characterize, diagnose and treat the multiple types of dyslipidemias and dyslipoproteinemias that result from a multitude of different genetic disorders. Here, NMR lipoprotein profiling can provide a cost-effective initial test regime since it can provide not only the lipoprotein subclasses including the concentrations of the main apolipoproteins (ApoB-48, ApoB-100, and ApoA-1) but the lipid composition in all lipoprotein subclasses.

Typical changes in the lipoprotein subclass pattern get more significant for individuals with type-2 diabetes mellitus.

Conclusion Remarks

As described above, Apo-B can be used in lieu of non-HDL particle concentration and certainly represents an alternative method to help asses overall non-HDL related risk. However, high reagent costs currently prevent ApoB from being used in general risk screening although it appears better suited than the traditional approach using LDL-C. Several publications have demonstrated improved risk assessment based on these data especially for diseases such as CVD and type-2 diabetes mellitus (T2D). However, for patients with established risk factors, with already elevated lipid levels and/or are receiving lipid-lowering therapy, it is important to obtain a more comprehensive understanding of the patient's pathophysiology.

In addition to traditional markers such as total cholesterol, LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and total triglycerides, molar particle concentrations by lipoprotein (sub-)class and mean particle sizes by class can be highly informative.

NMR has the unique advantage of analyzing all critical parameters in a single standardized assay. Because of the fixed stoichiometry of apoproteins/particles, the molar concentrations of ApoA-1 as well as ApoB-48 can be directly derived from the NMR data.

For both risk assessment as well as for patients in therapy, NMR offers clinician a comprehensive and well standardized and operationally efficient advanced analytical method. □

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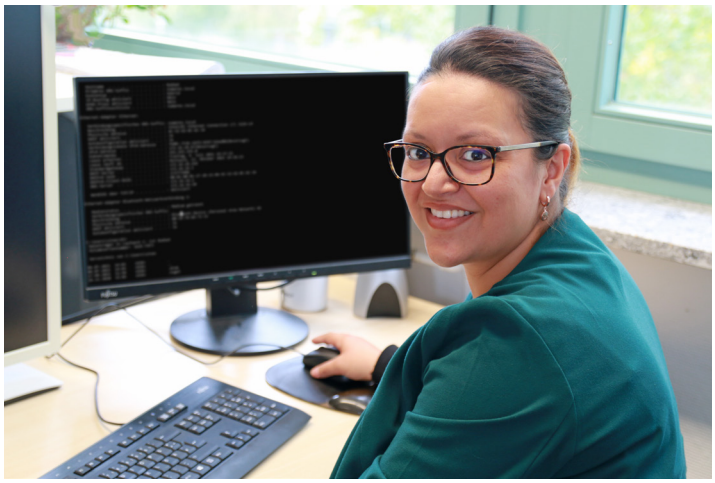
INSIDE

Introducing *numares* Departments

Software Development & IT

A special feature of *numares* is the development of diagnostic tests based on the evaluation of metabolic biomarker networks, so-called "metabolite constellations", by means of a convenient software. This is preceded by numerous algorithm developments and clinical validations. Ultimately, however, the sample analysis and its evaluation by the customer should be performed with as few clicks as possible. Both, pouring complex evaluations of human sample material into a software-based diagnostic test and making it controllable via a convenient software user interface is the task of numares' Software Development team.



ni: Sina, the *AXINON® Software* is the core of the diagnostic *AXINON® System*. The convenient user interface does not show the customer the immense computational processes running in the background. Can you give us an insight into the underlying structure?

Well, our two main structural principles are modularity and simplicity. Modularity is essential, so we can easily add functionality, new tests or e.g., compatibility to different devices or reagents. This is particularly important as *AXINON® Software* is a multi-test platform, i.e., a variety of diagnostic and research tests can be used. Technologically, this means that our code is developed in small, generic units that can be reused in other settings. For example, an algorithm required for detecting a certain substance in human serum should be coded in a way that it is reusable for urine as well.

Simplicity is a concept that is crucial for our users, who typically work as lab technicians in a routine and high throughput settings. They require a simple user interface to easily access our rather complex measurement system. Here, the user only needs to decide on the samples and the test(s) of choice and our software guides through the entire process – from sample preparation to the results. Management of the NMR device and spectral analysis stays completely in the background and remains invisible for the user. Here, the challenge is hiding unnecessary complexity. Only that way the operator can focus on the lab processes and handling an NMR device becomes (almost) as simple as running a scanner.

ni: What must the *AXINON® Software* guarantee?

The *AXINON® Software* runs diagnostic tests. Consequently, we must comply with the various regulations for medical device software and in vitro diagnostics. In fact, this gives us a rather strict set of requirements on our software itself, but also on how we develop software. For example, we must follow a list of essential requirements and must maintain a trace for every single product requirement to its specification and to the various test cases.

Similar, but on a technical level, making NMR technology as simple as a scanner requires a lot of standardization on various levels from how we generate spectra, over the AI based algorithms to how we calculate scores. All this sums up to what we call *Magnetic Group Signaling™* (*MGS®*). Only with *MGS®* we can guarantee the degree of reproducibility and performance we need to use *AXINON® Software* as a multi-test platform.

ni: Software Development is closely involved in product development right from the beginning. What challenges do you encounter when developing a new product?

Interesting question. In fact, major parts of our products are software, what makes numares a diagnostics company and a software company at the same time. Consequently, product development almost always involves software development and a bug in one of our software components can have severe impact on the quality of the whole product. So, the challenge is to ensure that our software performs as intended. Of course, we have processes in place like risk management, design- and code- reviews that help us to avoid errors, but in the end, it requires extensive testing.

ni: What's the part of IT in this process and beyond?

We develop software that is installed on PCs. Consequently, the IT department of our customers, but also our own IT department require a product that can be managed in a compliant manner. Therefore, when we develop software, we must have IT operations in mind, which then give us some obvious requirements on e.g., hardware and software, but also on confidentiality, integrity and availability as most IT departments in the medical field must proof compliance to e.g., data privacy and security regulations. As an example, *AXINON® Software* may not contain any patient-related data like patient names, social security numbers or the date of birth. This has impact on how we design the graphical user interface, the user manual and even the database.

When it comes to security features, the relevance of IT for software development becomes even more obvious. No IT department would allow us to run our software on their systems if we didn't support e.g., the latest version of the operating system. Consequently, we are in a constant discussion and exchange with our own and our customers' IT.

ni: What implementations are planned for the future?

The software development team primarily focuses on test development and continuous improvement of the *AXINON® Software* and related applications. The pipeline for new tests is well filled and we constantly work on bringing new tests to the platform. Besides, we are currently preparing for the next generation of the *AXINON® Software*. This requires prototyping, technology evaluation, testing, etc.

However, maintenance and support of our existing tests and applications is equally important. On one hand, we must react on bugs or security issues in a timely manner, on the other hand customer feedback is an enriching resource for us to improve the *AXINON® Software* and to know what the next generation must support. □

Sina Schmitt, Software Development

Christiane Proll, Marketing

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HANDS ON

numares HEALTH

NMR series:

Why does the NMR Magnet need a Vibration Dampening System?

The appearance of an NMR magnet in a laboratory is quite impressive. Part of that comes from the big blue magnet stand which contains vibration damping systems in each of the three legs. There are many different types of vibrations damping systems available. They can range from rubber dampers, air spring dampers or, as used for the *AXINON® System**, air piston isolators. Its operation simply relies on a steady supply of compressed air. The magnet then floats on an air cushion. The air cushion prevents vibrations from the floor from being transmitted to the magnet. It is also needed to counteract movements from the Sample Jet.

Undamped, these vibrations would significantly reduce the signal-to-noise ratio and would result in distinctive signals which interfere with neighboring NMR signals. The reasons for such vibrations can be plenty, such as elevators, construction sites, doors moving and many more. [1]

For the *numares' AXINON® System*, consistent spectral quality is especially important. Vibrations lead to line broadening, phase shifting and peak asymmetry. Therefore, vibrations can lead to an elevated failure rate or even complete rack failures. The only time when the dampers should be switched off is during cryogen filling or maintenance work and should be switched on again right after. Otherwise, the next rack measured will fail as soon as the first sample is analyzed. In our maintenance procedure we check the presence of vibrations with the vibration test. In this test a specific sample is measured once, so possible vibrations cannot be averaged out and will be seen in

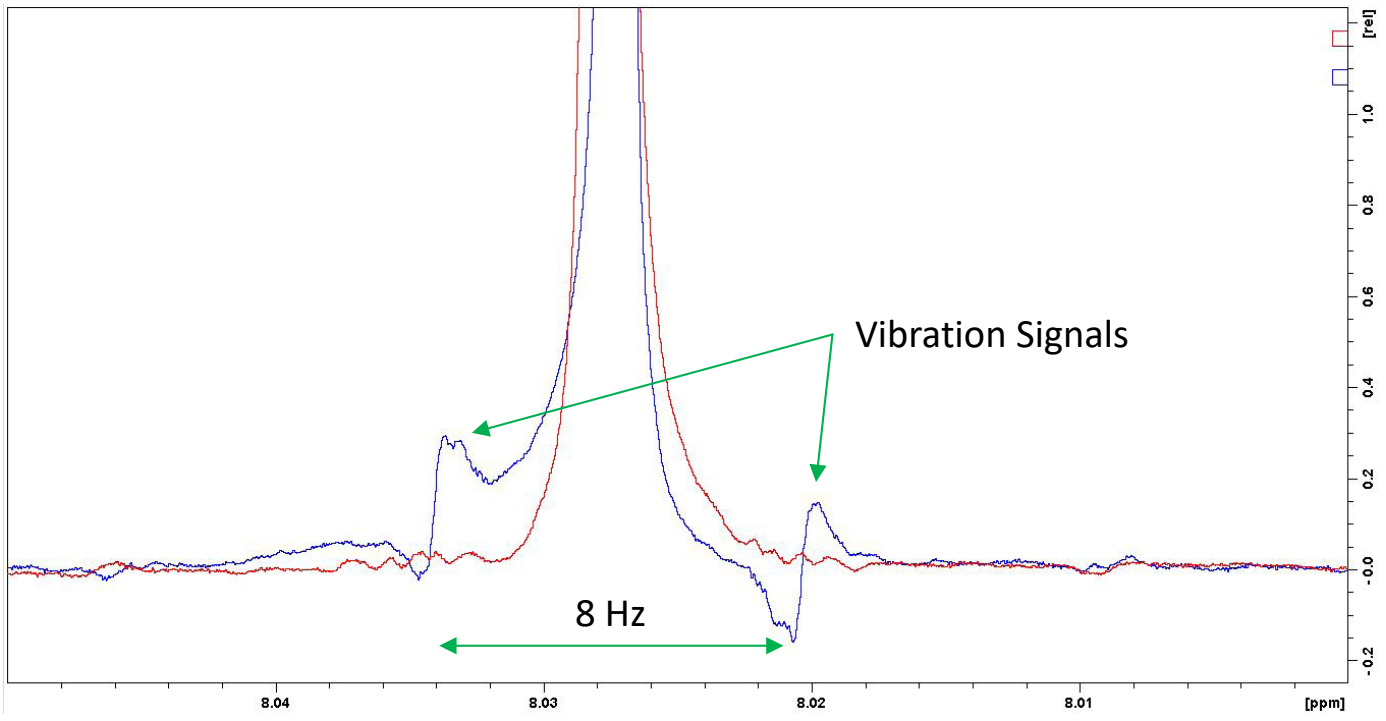

Figure 1: Overlay of a maintenance spectrum with vibrations (blue) and without (red) vibrations. The vibrations can be seen as peaks at +/- 4 Hz around the main signal.

the resulting spectrum (see Figure 1).

The damping system needs compressed gas which can be either nitrogen or dry air. The working pressure is 5-8 bar (70-115 psi). A metal piston is surrounded by aircushions in all three room directions. The vertical or z-axis, which is parallel to the magnet field is the most important. The vibrations are then damped by these aircushions.

The system doubles as levelling system. Controlled by a lever the magnet can be adjusted to be perfectly upright. The lever, which is adjusted by a screw, should always be horizontal. If the piston is moving, the lever is pushed in the same direction. Thus, the system regulates the gas pressure accordingly so that the piston returns to the starting position (see Figure 2). The system is very sensitive, a 600 MHz ASCEND™ NMR magnet weighs around 800 kg but the whole Magnet can be easily moved with the tip of a finger.

Usually, the vibration damping system is covered by a metal hood and is often overlooked because of its passive, virtually maintenance-free operation. For high-resolution NMR spectra, this is as important as a well-shimmed magnet. In the feet lies not only the reason why you need a ladder to load your samples, but also an important yet unremarkable part why your results of the *AXINON® System* can be delivered to the patients.

Disclaimer: Please be very careful when working on the magnet dampening system and follow the safety instructions by the manufacturer strictly. □

Andreas Döring, Service

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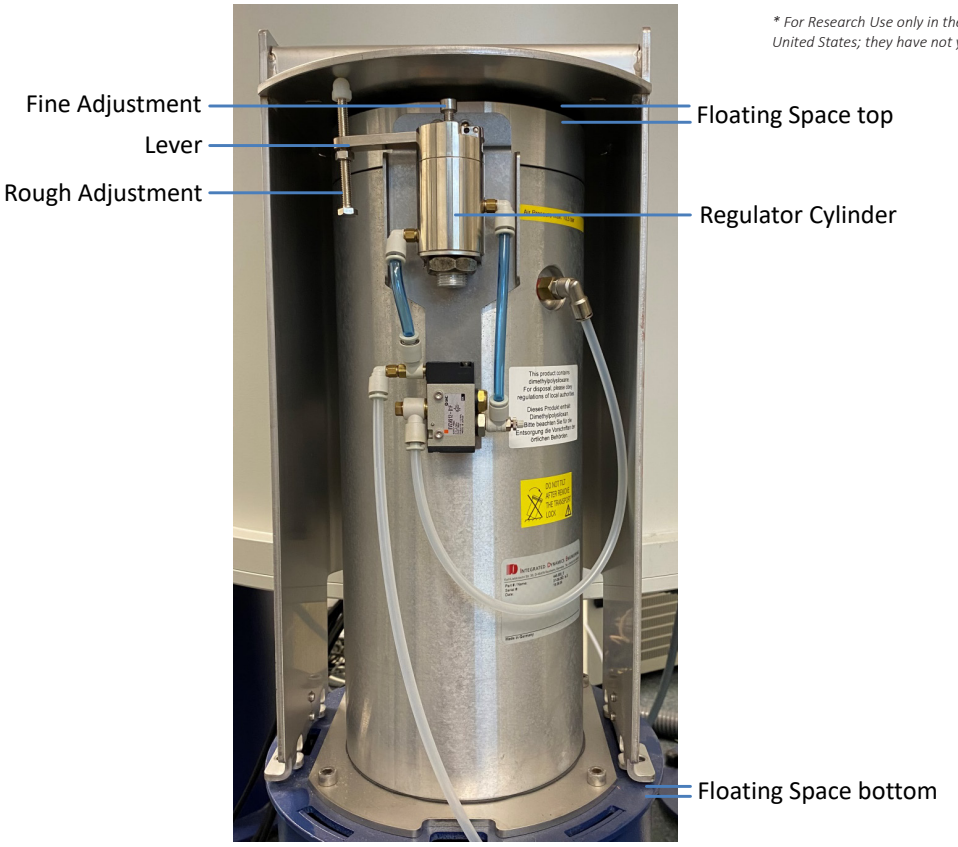
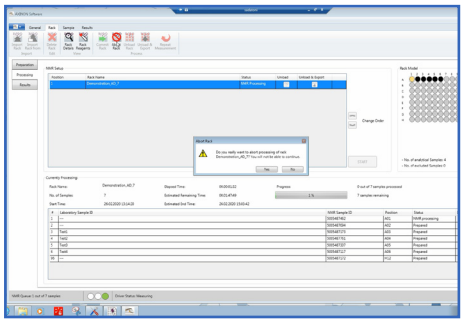


Figure 2: A look behind the cover of the magnet stand: The piston should be float freely above the blue magnet stand at the bottom and above the inner cylinder at the top. Right: Schematic drawing of a damper.

numares' Video Tutorial Series:

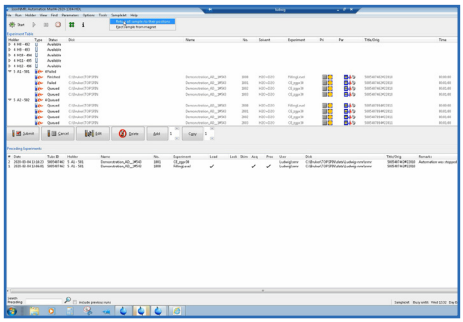
Reboot the *AXINON® System**

**Only for trained personnel. Also refer to numares' Maintenance Manual.*



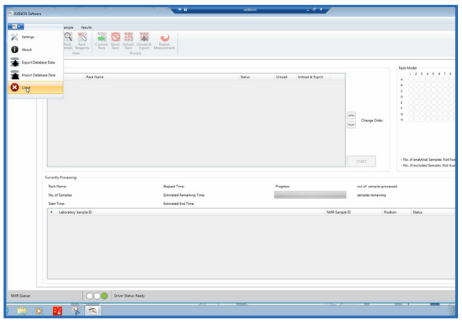
AXINON® PC:

To reboot the AXINON® System, stop all running tasks within AXINON® Software.



BRUKER PC:

Stop the Bruker ICON NMR run and click „Return all samples to their positions“.

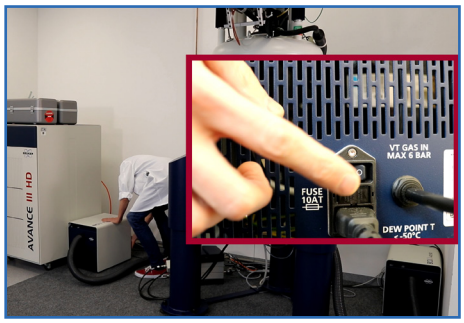


AXINON® PC:

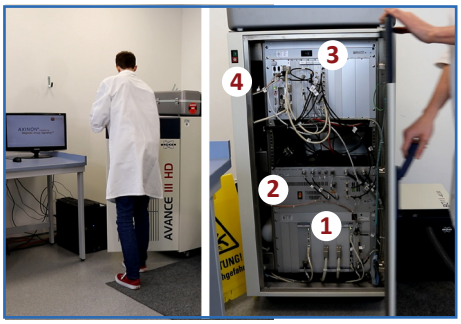
Close AXINON® Software...

BRUKER PC:

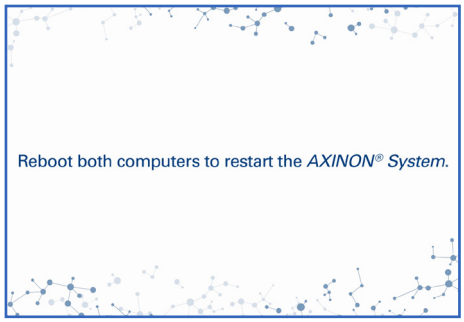
...and TopSpin Software. Turn off the SampleJet by clicking “Shutdown system” in the web interface of SampleJet.



Power off all BCUs.



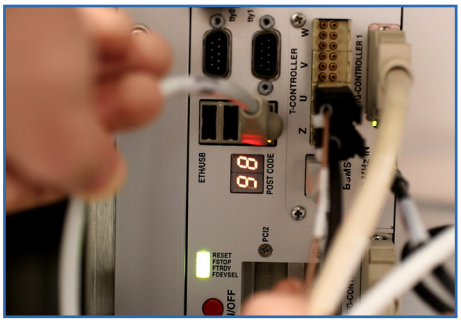
Open the NMR console. Switch off all units from bottom to top (1-3). Give each unit time to shut down completely to avoid any system errors. Then turn off the console power (4).



To restart the AXINON® System, reboot all computers.

Turn on the power console and wait at least for 30 sec.

Note: This will automatically reactivate the SampleJet.



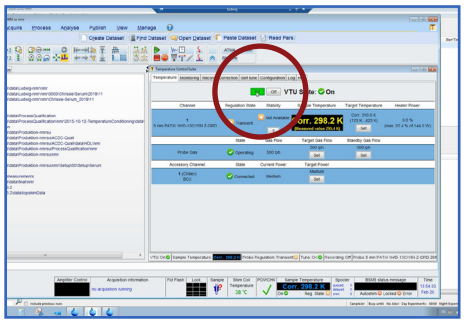
Switch on the top unit. Give it time to display green control lights and the permanent status code („98“ on IPSO for AVANCEIII console) before continuing with next unit.

Continue with other units from top to bottom. Each unit should display green control lights before proceeding to the next unit.

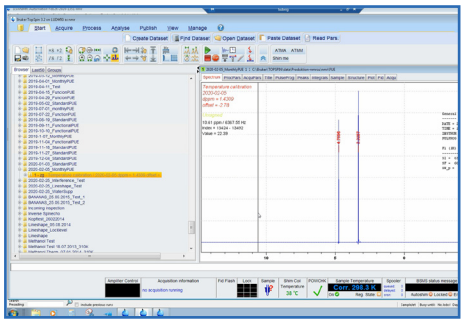
Turn on all BCUs.

Check that the controller is set to "remote" and the remote control light is green. Wait at least for 30 minutes.

Perform an Error Recovery. Follow the instructions at the SampleJet display.

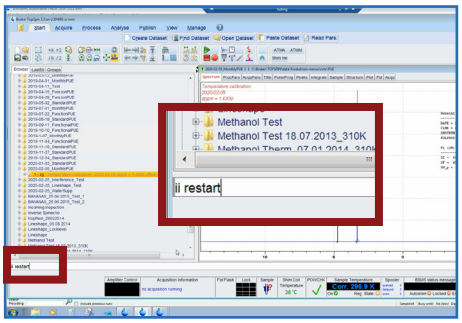


Open the TopSpin Software and switch on the vtu temperature control. Wait until temperature display is green.



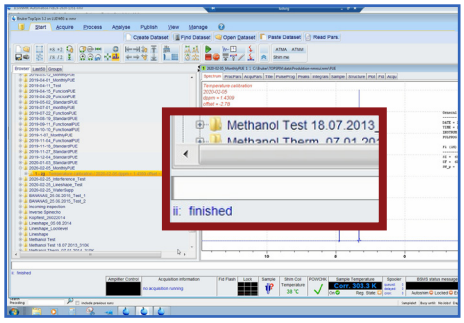
BRUKER PC:

Open an active data set in TopSpin...



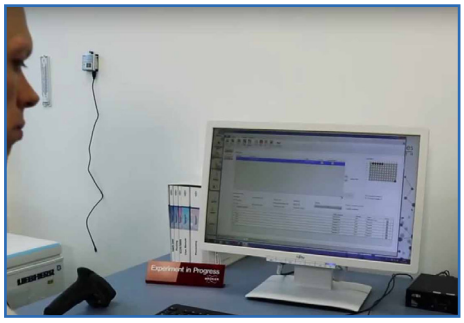
BRUKER PC:

...and enter „ii restart“ in the command line.



When „ii finished“ appears below the command line, TopSpin is ready for use.

If „ii aborted” appears, try “ii”/”ii restart” again 2-3 times. If the error persists, contact our technical service.



Start pre-tests (Operational Qualification) before using the system.*

**Please refer to numares‘ Maintenance Manual.*

Andreas Döring, Service

numares employee portrait

Dr. Michael Grasse, US Service

Close to the customer and close to the technology. In this edition of the numares insider we ask numares employees to give an insight into their daily work. In this issue Michael Grasse, Head of *numares'* US Service provides insight into the close interaction with the customer, the challenges in implementing new software releases during clinical lab routine, and the most interesting support cases.

ni: Michael, for five years now you've been heading the US Service department of numares. What does a typical working day look like for you?

A typical day starts with our daily stand-up meeting with the whole service team. Together we are working on incoming service tickets. Customer support is often time-sensitive and an immediate reaction to current problems is required. Once assigned to me, I start digging into troubleshooting: why does the instrument not work properly, what are the reasons of results not being within specifications etc. I also assist in improving the laboratory workflow or help during the validation of new tests. Besides solving problems, planning and performing installations of new instruments/software releases is a big part of my work. No day is like the other. The variety of my daily work is what makes my job exciting. Different installations on site, providing assistance over the phone, email or via remote access and understanding our customer's needs. Being at our customer's site is most interesting part for me. I love to be there and help them. I have to travel on call very spontaneously to accompany customers on urgent issues. New installations on the other hand are planned in advance but require a longer stay. This gives me the opportunity to build better relationships with the persons who actually use our platform. I also get to explore new cities on the weekend, which is a nice perk.

ni: Michael, the US Service department copes not only with the installation of numares' NMR-based diagnostic system on the customer's site, but also with implementation of new software releases. What work does this entail?

While installation of our diagnostic platform means site planning, qualifying the system and training for laboratory personnel, etc., new software releases are a different challenge because they must not interfere with the daily routine work in the laboratories. That means we have to plan around the client's daily workload and established routines in the laboratory. In close exchange with the laboratory personnel we align the required measurements, shift to available measurement time or vice versa. This can also be very late at night.In preparation for a new software release, be it of the *AXINON® Software**, which is the platform for controlling the measurements, or of the software-based test applications integrated into the *AXINON® Software*, we always have to check the compatibility of all components: all software versions have to fit together, match the operating system. Although the effort for a test application is less than for a new installation of our diagnostic platform, the device must work as good as before - this is the purpose of all qualification measurements we perform. Qualification in nearly all cases takes much more time as the actual installation.

ni: As you are obviously often on site and have built a very close relationship with the clients: How does your skills and experience support your work with customers?

As a chemist and scientist, I love technical challenges and to optimize things. When I'm at a customer's site, I appreciate the collegial exchange at eye level and the willingness to discuss/implement new ideas. I like to reduce fears and inhibitions about NMR technology. High-tech can be very intimidating and especially the NMR device with its impressive size and not insignificant value creates inhibitions in the user to work with the device. But that is unnecessary, as our system is quite robust and easy to operate via the *AXINON® Software* – as long as maintenance routines and qualification tasks are strictly followed. There is hardly anything that the user can break. I try to provide help, tips and best practices whenever I notice uncertainty or fear. On the whole, in person meetings are very important, because they create save spaces where people are open to communicate that something has gone wrong or someone feels insecure about techniques or precedires. It also unique to assess our customers' requirements. Furthermore, it is a source of suggestions and improvements for *numares'* products. Whenever I can I pass ideas and needs to my colleagues in the R&D or product management department.

ni: Can you give us a recent example?

It is important for our customers to be quickly informed about errors or problems. Therefore, we have introduced automatic notification emails, which immediately inform the user about a deviation and possible reasons. To be as helpful as possible, we have integrated initial tips into the email on how the customer should proceed. Another improvement was the re-measurement of samples if they have an error due to internal quality checks in the measurement process. In previous software versions, the abort of a complete rack was the consequence. Now, the measurement of the previously failed sample is repeated at the end when the complete rack has been measured successfully. This is a big plus as it saves time, and the patients get their results faster. The core objective of the *numares* Customer Support is to help our clients in achieving their goals as easy and effective as possibleThis is the core objective of the numares Customer Support – assist the clients and satisfy their needs. □

Michael Grasse, US Service & Christiane Proll, Marketing

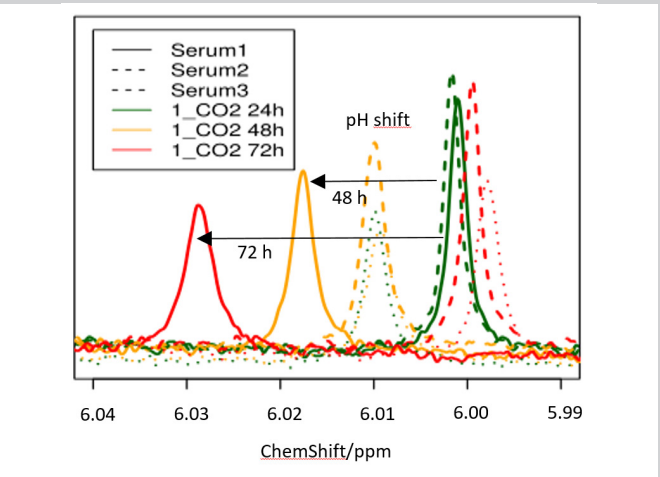
Things to remember: Avoid Dry Ice

Diagnostic samples are commonly shipped from the doctor's office to the diagnostic lab on dry ice in a frozen state.

Usage of dry ice may interfere with the quality checks performed by the *AXINON® Software,* thus impeding or invalidation the analysis of specimen samples.

Please avoid usage of dry ice for sample storage, sample shipping and *AXINON® kits* storage.

If you want to know more about this topic, contact us at technicalsupport@numares.com or refer to *numares insider 2* on our website. □



CO_2 diffuses into the samples causing a decrease in pH over time. NMR signal will experience a shift to higher ppm values & loss of spectral quality.

Michele Salvi, Service

** For Research Use only in the United States. numares' products are not yet available for sale within the United States; they have not yet been approved or cleared by the U.S. Food and Drug Administration.*

NEWS

#*numares*@Social Media

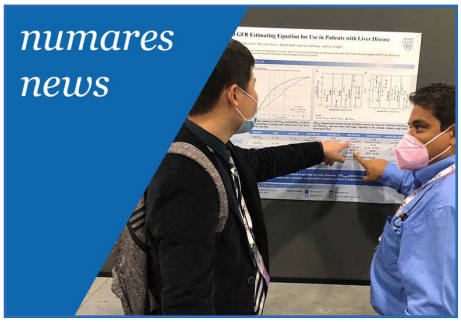
Welcome to the social media world of *numares*! By following the LinkedIn (https://www.linkedin.com/company/numares-ag/) profile of *numares*, you will get a continuous flow of news around our products, developments and the company itself. Here is an excerpt from the latest news:

We were happy to share this article in Lab Manager on "Expanding Diagnostic Applications of NMR Spectroscopy":

https://lnkd.in/e-7tJy6

This article summarizes the huge potential of NMR utilizing biomarker constellation and XAI for clinical diagnostic tests to address several types of disease, including kidney disease, liver cancer, and cardiovascular disease (CVD).

We are proud to be named as "... one organization leading the way [...] bringing advanced NMR diagnostic techniques to the frontline health care industry [...] for the swift, cost-efficient diagnosis of a broad spectrum of diseases."



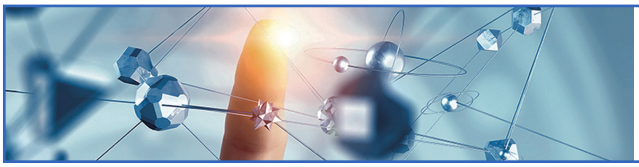
numares was at this year's 2021 AACC Annual Scientific Meeting & Clinical Lab Expo in Atlanta, Georgia! At our booth we showed how to transform data deriving from MGS processed NMR spectra with machine learning into multi-marker based diagnostic tests to support clinical decisions, e.g. in kidney function and CVD assessment, kidney transplant surveillance and neurological disorders.

Our Chief Medical Officer Maulik Shah presented a poster and informed about latest developments in kidney function diagnostics using "biomarker constellations" – a combination of metabolite and demographic biomarkers.

Introducing children and young adults to STEM subjects - that is the mission of Regensburg's MINT-Labs!

Since the end of June, the *MINT-Labs Regensburg* have made great use of the low Corona numbers and welcomed many children, teenagers, adults of all ages: the youngest were just four years old, the oldest well into student age.

In this project week, each day was dedicated to a STEM topic. All courses to dive deep into mathematics, computer science and natural sciences and technology were completely booked up. We are happy to be part of this initiative and congratulate the *MINT-Labs Regensburg* for this great progress. Learn more: https://lnkd.in/e9sW2C6U



@AgNumares
@numares NMR diagnostics
@numares AG
@numaresAG

Julia Hertlein, Social Media Marketing

NEWS

numares and *Oxford University* sign exclusive license agreement for novel multi-marker diagnostics in multiple sclerosis

Regensburg, Germany, and Oxford, UK – October 28, 2021. Leading NMR diagnostics company *numares AG* today announced that the company signed an exclusive licence agreement with *Oxford University Innovation.* This allows numares to translate and exploit preliminary work of *Oxford University* on Multiple Sclerosis (MS) biomarkers for the development of an urgently needed in-vitro diagnostics (IVD) test able to detect disease progression earlier to improve patient management and outcome. The exclusive licence enables numares to further develop the biomarkers into a multi-marker based diagnostic test and commercialization thereof.

In about 85% of patients with MS, the disease initially takes a relapsing-remitting course. Most patients with "relapsing remitting multiple sclerosis (RRMS)" will eventually transition to "secondary progressive multiple sclerosis (SPMS)" with continuous worsening of symptoms. This transition occurs subtly and is difficult to define clinically, requiring retrospective evaluation of the disease course over the past 12 months. This delay in diagnosis prevents timely adoption of treatment regimens for effective patient management and improved long-term clinical outcomes.

The collaboration between *Oxford University* and numares began in 2017 with the common goal to validate a set of biomarkers, which had been previously identified by Oxford researchers, for detecting transition from RRMS to SPMS. This used clinical data acquired from a multi-centre cohort collected by Oxford's partners.

numares provided its proprietary *AXINON® IVD System* that delivers standardized *Magnetic Group Signaling (MGS®)* measures of metabolite levels in patient samples from Nuclear Magnetic Resonance spectroscopy (NMR). Based on such NMR data, the company develops multi-marker algorithms for several diagnostic tests, by combining relevant biomarkers into "biomarker constellations" and applying machine learning and other modelling approaches.

"We are enthusiastic to enter the next phase of the collaboration and utilize Oxford's excellent preliminary scientific work and *numares'* expertise for the development of an MS IVD test based on the multi-marker approach," comments Volker Pfahlert, CEO of *numares*. "Our mission is to improve patient care by providing better diagnostic tools to help physicians better manage their patients. This fruitful collaboration with Oxford researchers gets us closer to our mutual goal to bring first class research to the bedside of MS patients."

Professor Daniel Anthony, Head of Experimental Neuropathology Laboratory, Department of Pharmacology at the *University of Oxford* and lead scientist on the project, adds: "We are very pleased to have such an experienced industrial partner in numares to commercialize our research findings as a breakthrough test, which enables early detection of the transition from RRMS stage to SPMS stage for the first time. This will have a significant impact on the care of individuals living with MS. The test opens up the possibility to monitor the condition more closely and thus improve therapeutic decision making."

The IVD development will start in 2022. In early 2021, both parties also agreed to extend their partnership for another two years and expand the approach of multi-marker use in diagnostics to the field of Alzheimer's disease.

Christiane Proll, Public Relations

numares is going to participate at the upcoming meetings & conferences

Due to the threat of the ongoing coronavirus pandemic, many congresses and trade fairs 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!

National Kidney Foundation (NKF) Spring Meeting 2022

April 6-10, Boston, MA
https://www.kidney.org/professionals/news/meetings

2022 American Transplant Congress (ATC)

June 4 - 8, Boston, MA
https://atcmeeting.org/

American Society for Clinical Laboratory Science 2022 Annual Meeting

July 24-28, Chicago, IL
https://www.aacc.org/meetings-and-events/annual-meeting-dates-and-locations

ASN Kidney Week 2022

November 1-6, Orlando, FL
https://www.asn-online.org/education/kidneyweek/archives/future.aspx

For appointments, please contact: marketing@numares.com □

Julia Hertlein, Marketing

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