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high-resolution respirometry

Mitochondrial Physiology Network

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Respirometry of Permeabilized Muscle Fibres: Towards Quality Assurance in the Diagnosis of Mitochondrial Function

2nd MiPNet Workshop 2009, Dec 9-10 (Med. Univ. Innsbruck, Austria)

Quality Assurance (QA) is too big a term [Note 1] for a small workshop on diagnosis of mitochondrial (mt) respiratory function, even when the focus is restricted to the model of permeabilized muscle fibres. There are several milestones in the quest ***towards QA***, including continuous quality control and incorporating 'Best Practice' in the laboratory. We are well aware of the fact that ***in practice*** we may aim at ***better*** standards, but will hardly ever establish any final ***best*** [Note 2]. QA in general may set impractically high demands on a laboratory, hence **the required level of QA depends on the specific application**. QA will be different in (i) an experimental study on mt-respiratory function of mouse skeletal muscle, (ii) a comparison of aged humans versus young controls using biopsies, or (iii) the diagnosis of a mt-myopathy in a single patient when an individual is evaluated (she/he really cares) versus a (matched – science has to care) control group.

Against this background, we can reach several unambiguous conclusions:

1. **Some level of QA is required** in all studies on mt-respiratory function – from basic science to clinical applications.
2. **Sharing the practical expertise** of different research groups in this rapidly expanding field provides an effective and economical approach towards higher standards of science by implementing QA.
3. **Multiple benefits** will result from the development and application of appropriate standards of QA - for the individual patient (very few laboratories are involved), the individual scientist (all of us, all of our collaborators), the laboratory, mt-respiratory physiology in terms of scientific reputation (including some companies with positive or negative impacts), the particular segment of our health care system.

The benefits and implications are potentially enormous, considering the **impact of mitochondrial medicine on the quality of life**: life style related to exercise and nutrition, obesity, degenerative diseases, metabolic syndrome, muscular and cognitive dysfunction, rare mt-diseases, healthy aging, cardiovascular diseases, reducing the risks for a range of cancers, immunological fitness, competitive and noncompetitive sports, hypoxia, ischemia-reperfusion, ...

It is far more exciting to discuss these challenging topics than to talk about quality control of the chemicals used as inhibitors or in mitochondrial respiration media, tissue wet weight or dry weight measurements, or about valid estimates of the proton leak in a respirometric protocol. A focus on QA, however, helps to eliminate unreliable data which are a source of confusion, lead to unnecessary controversies, or even overshadow the potential of accurate diagnosis of a disease. Explicit implementation of QA will provide a basis of **increased recognition and reputation of our field of research**, and may be considered as an expression of **corporate social responsibility within the mitochondrial physiology network**. This MiPNet QA-Workshop includes but is not restricted to instrumental performance, and presentations are invited to cover not only applications of the OROBOROS Oxygraph-2k but also extend the discussion to other instruments (e.g. see www.oroboros.at/index.php?hrr-versus-multiwell).

Aims

The topics and aims of the proposed workshop on 'Respirometry of permeabilized muscle fibres: towards quality assurance in the diagnosis of mitochondrial function' are:

1. **Presentation of presently available components of QA**: methodological details, exchange of laboratory protocols with updates [Notes 3.1 – 3.4].
2. **Science sessions**: protocols, traces, results, concepts, perspectives [Notes 3.4 – 3.7].
3. **Summary of practically important segments and perspectives of QA** [Note 4].

Is it worthwhile considering a joint project application? Can you come up with specific suggestions? Some of the workshop participants will attend the subsequent *Course on High-Resolution Respirometry* (IOC54; Schröcken, Austria), during which we might complete and extend the summary from the workshop in a small group.

Programme

Two morning sessions devoted to **QA topics** [Notes 3.1-3.4], keeping the first afternoon and evening free for **science sessions** with particular emphasis on protocols [Notes 3.4-3.7]. The programme developed according to the contributions and suggestions of the participants. A draft of a **summary** was prepared during the second afternoon/evening.

Wednesday, December 9

Morning: **Diagnosis of mitochondrial respiratory function: Methods and laboratory protocols , components of QA.**

Erich Gnaiger (AT) Aspects of quality assurance in respirometry of permeabilized muscle fibres: OXPHOS capacity in human skeletal muscle. (i) OXPHOS capacity at saturating concentrations of ADP and oxygen. (ii) Effect of substrate combinations. (iii) Indices of coupling.

Magnus Hansson, Saori Morota (SE) Aspects on multititration protocols in human permeabilized heart fibers and platelets.

Martin Mogensen (DK) Preliminary comparison of Oroboros and Hansatech respiratory measurements in isolated mitochondria.

Robert Boushel (DK) Effects of PG and NO inhibition on muscle oxygen consumption in-vivo and in permeabilized fibres: Experimental design and protocols.

Afternoon: **Mitochondrial respiratory function and capacity: body mass index – endurance and obesity**

Flemming Dela (DK) Mitochondrial respiration in human subcutaneous and visceral adipose tissue.

Erich Gnaiger (Innsbruck, AT) Fewer mitochondria - mitochondrial 'fever':– Quantitative relations between BMI, ergometric performance and OXPHOS capacity in human skeletal muscle.

Pablo M Garcia-Roves (SE) Constitutively active calcineurin in skeletal muscle increases endurance performance and mitochondrial respiratory capacity. Comparison of mitochondrial respiratory capacity in different muscle types.

Guy HEJ Vijgen (NL) Skeletal muscle fibre responses in morbidly obese subjects.

Joris Hoeks (NL) Prolonged fasting-induced insulin resistance in humans is accompanied by a decreased mitochondrial capacity.

Pablo M Garcia-Roves (SE) Novel insights into mitochondrial function in a genetic model of obesity: the db/db mouse.

Dan Kane (Greenville, NC, USA) Hormonal considerations and skeletal muscle mitochondrial function in women: focus on estrogen and progesterone.

Dominique Marie Votion (BE) Muscle mitochondrial respiratory function measured in small samples from needle biopsies: applications in sport and myopathic horses.

Evening: Reception and discussion at the *MiPArt Gallery* – OROBOROS INSTRUMENTS.

Thursday, 10. Dec 2009

Morning: **Towards quality assurance in the diagnosis of mitochondrial function - Discussion**

David K Harrison (UK) Some general principles of quality assurance: From research to clinical investigations, with particular reference to diagnosis in peripheral arterial occlusive disease (PAOD). *General part of the presentation* [Note 5].

Erich Gnaiger (AT) Evidence-based instrumental resolution of oxygen flux.

Afternoon: **Instrumental demo: Oxygraph-2k and titration feedback control – MiR06 – Experimental perspectives**

Mario Fasching and Dominik Pesta (Innsbruck, AT) Automatic control of oxygen regimes for instrumental background - MiR06.

Dominik Pesta (Innsbruck, AT), Dan Kane (Greenville, NC, USA) Case report: Initiation of a project linking mitochondrial function with P-NMR in the context of a hypoxia-normoxia training study on human skeletal muscle.

Wenchich Laszlo (Prague , CZ) Problems of tissue sampling: Inadequate handling may lead to misinterpretation of the results.

Erich Gnaiger (Innsbruck, AT), Robert Boushel (DK) Functional criteria for elimination of outliers in a study on permeabilized fibres from human skeletal muscle – an optimization strategy.

Afternoon/Evening: **Workshop summary** [Note 6]

Friday, 11. Dec 2009: Departure / Arrival IOC54

Suggestions for presentations

1. Do you know of any QA guidelines that may be available for diagnosis of mitochondrial respiratory function?
2. Please send the preliminary title of your contribution(s) by return Email. Based on a return-circular of preliminary titles, you may adjust your title(s) later and add a short abstract for optimization of our workshop.
3. We encourage to present plenty of experimental traces that show the 'representative experiment' and explain the final protcol(s). In addition, show key experiments leading to the final protocol(s), and illustrate the rationale of how experimental problems have been resolved. Do not consider repetition of presenting protocols as a problem, but as a unique opportunity for in-depth discussions.
4. Input of material that may be circulated internally to all participants is very welcome.
5. If you cannot participate locally, but are interested in joining this informal MiPNet QA-project, please send us your suggestions, potential title(s) for a possible future meeting, and input of material. All participants (locally and non-locally registered) may contribute to and will receive the information collected and the workshop summary.

Notes

1. **Quality Assurance** (Wikipedia, Oct 2009): Quality assurance, or QA for short, refers to planned and systematic production processes that provide confidence in a product's suitability for its intended purpose. It is a set of activities intended to ensure that products (goods and/or services) satisfy customer requirements in a systematic, reliable fashion. Unfortunately, QA cannot provide and absolute guarantee of the production of quality products but makes this more likely.

   Two key principles characterize QA: "fit for purpose" (the product should be suitable for the intended purpose) and "right first time" (mistakes should be eliminated). QA includes regulation of the quality of raw materials, assemblies, products and components; services related to production; and management, production and inspection processes.

2. **From Best to Better Practice** (Wikipedia, Oct 2009): A 'Best practice' is a technique, method, process, activity, incentive or reward that is believed to be

more effective at delivering a particular outcome than any other technique, method, process, etc. The idea is that with proper processes, checks, and testing, a desired outcome can be delivered with fewer problems and unforeseen complications. Best practices can also be defined as the most efficient (least amount of effort) and effective (best results) way of accomplishing a task, based on repeatable procedures that have proven themselves over time for large numbers of people.

The term "best practices" has implications of finality, obedience, authority, and universality. The term 'best practices' implies that some source has the final answer to a matter in dispute or disarray. The matter is closed, decided, set and resolved. The term "**better practices**" seems to seek better ways, which may even lead to tweaking the suggested practice to make it even better. It **suggests that all of us together can come up with something better than any one of us can arrive at individually, and places authority in the community. The term may imply that the better practice is not universal, but depends on the specific situation.**

3. **Presently available components of Quality Assurance** – examples of the components of QA (E. Gnaiger):

3.1. General aspects of QA in the laboratory:
- Tools: pipettes, balances, …
- Chemicals: distilled/deionized water, substrates, uncouplers, inhibitors, components of media (MiR05, MiR06; BIOPS; …), drugs; storage, multiple freezing, …

3.2. Tissue preparation and quantification of tissue mass and mt-content
- Biopsy sampling
- Tissue storage
- Tissue preparation: mechanical separation, chemical permeabilization, short-term storage (ref.[1-3]; we want to improve and extend our protocols.
- Quantification of tissue: wet weight, dry weight
- Quantification of mt-content: marker enzymes (CS, aa_3), mt-DNA, mt-volume fraction

3.3. Instrumental performance of the respirometer (not restricted to the OROBOROS Oxygraph-2k; examples and references below relate to our expertise with high-resolution respirometry and touch on critical issues related to other approaches)
- Oxygen sensor calibration: Calibration standards (oxygen concentration in an air-saturated medium; zero oxygen calibration); signal stability and linearity (e.g. track records of calibration parameters; Fig. 4 in ref.[4]); drift and noise; time resolution (www.oroboros.at/index.php?hightimeresolution; Fig. 3 in ref.[5]).
- Instrumental performance of the respirometer; documentation of resolution of flux throughout the duration of a study: chamber leakiness and oxygen backdiffusion (e.g. testing and track record of instrumental background parameters and their application for the correction of oxygen flux; Fig. 5 and 6 in ref.[4]; Fig. 2 in ref.[5]).
- Stability of oxygen in mt-respiration medium, evidence against microbial contamination.

3.4. Respirometric experiments and protocols:
- Documentation on instrumental settings (pdf file: DatLab [Crl+F3 window])
- Respirometric protocols: medium, temperature, substrate concentration, substrate combinations, sequence of substrate-uncoupler-inhibitor titrations (ref. [6])
- Stability of mt-function during respirometric incubation (stability of flux)
- Oxygen limitation of respiration (ref. [7,8])
- A *negative example* for QA is ref.[9] which will be discussed during the workshop; the respective authors are cordially invited to present their views during the workshop or by correspondence.

3.5. Presentation of data and publication of results:
- Tissue-specific OXPHOS capacity (per tissue mass; Table 2 in ref.[6])
- mt-specific OXPHOS capacity (per mt-marker)
- Flux control ratios (Table 1 in ref.[6])

- Units: SI (ref. [10,11])
- Terminology: e.g. State 2 – ambiguously used in the modern literature; State 3 – saturating or non-saturating ADP concentrations and P_i concentrations? (ref.[6,12])
- Presentation of methods and results: Are experimental conditions presented adequately (temperature, media, tissue preparation, experimental oxygen levels, ...)? Are units explained in sufficient detail, such that the data have quantitative meaning?
- References: Are references provided to original methods papers (other than Lowry et al 1951 for protein determination)?

3.6. Patient groups and controls
- Inclusion/exclusion criteria
- Subject information: signed informed consent form
- Number of patients and controls
- Gender, age, morphometric data of patients, clinical background: which data should always be published?
- Protocol compliance; adverse events and concomitant medication

3.7. Data and statistics
- Number of individuals per group, *N*
- Number of replicate assays per tissue sample, *n*
- Appropriate statistics (test for normal distribution, non-parametric tests)
- Detection of outliers and assessment of the validity of outlying data points – (*a*) statistical; (*b*) systematic tests, such as cytochrome *c* effect
- Data storage; data transfer from primary data acquisition to tables, figures and final reports (*an average error rate for keying text or numbers is known to be about 1 per 300 keystrokes*).

4. Perspectives of Quality Assurance (E. Gnaiger):
- What are the benefits of evidence-based management of the quality of data and of QA, as a system of procedures that helps ensure the quality of diagnosis of mitochondrial functional by respirometry and related methods?
- How can different modules of QA be defined to allow flexible systems of QA to be built in accordance with the requirements of a project or clinical programme?
- Maintaining accuracy and quality is a continual and dynamic process, since expectations and requirements may change during a study.
- Specific audits determine whether the activities evaluated were appropriately conducted and that the data were generated, recorded, analyzed, and accurately reported according to a selected system of QA procedures.
- Quality control (QC) includes periodic operational checks to verify that data are generated, collected, handled, analyzed, and reported according to a selected system of QA procedures.
- The system of QA procedures describes how the quality control and quality assurance processes are to be applied within a specific project or clinical programme.
- Which methods and modules are primarily important but not yet available to build an appropriate system of QA procedures? (For instance, a mitochondrial preparation that can be stored and shipped from a single source to all participants of an interlaboratory test; a mitochondrial preparation that can be stored for periodic monitoring of the reproducibility of intralaboratory results; such a project for preparation of a 'mitochondrial powder' is under way: Steve Hand, Baton Rouge, Louisiana, USA).

5. **Some general principles of quality assurance: From research to clinical investigations with particular reference to diagnosis in peripheral arterial occlusive disease** (by David Harrison, Hon. Senior Lecturer in Medical Physics, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK)
Some of the Elements Involved in QA: Best-Better Practice / Benchmarking / Cost-effectiveness / Risk Assessment-Management / Policies / Standard Operating Procedures / Documentation of Processes / Audit

5.1. **Best Practice** (BusinessDictionary.com): Methods and techniques that have consistently shown results superior than those achieved with other means, and which are used as benchmarks to strive for. There is, however, no practice that is best for everyone or in every situation, and no best practice remains best for very

long as people keep on finding better ways of doing things. / "Better Practice" may be more achievable. / See also "Evidence based practice".

5.2. **Benchmarking**: 'Benchmarking - finding and implementing best practice' (www.nhsbenchmarking.nhs.uk): 'A continuous, systematic process for evaluation of the products, services and work processes of organisations that are recognised as representing best practice for the purpose of organisation improvement' (Spendolini MJ. The Benchmarking Book 1992). / The primary reason for benchmarking is to learn from others and improve. A secondary use is for devising a rank order between organisations, to demonstrate some 'competitive advantage'. Being 'the best', however, is a goal only available to one organisation – assuming, of course, that the best can be measured. (Diffey et al. Benchmarking and Performance Indicators in Medical Engineering and Physics, 2004).

5.3. **Cost-effectiveness**: The degree to which a service or a medical treatment meets a specified goal at an acceptable cost and level of quality. (www.futurehealth.ucsf.edu/cnetwork/resources/glossary/gloC.html) i.e. "Best Practice" may not necessarily be cost-effective!

5.4. **Risk Assessment and Management**: Risk assessment is the determination of quantitative or qualitative value of risk related to a concrete situation and a recognized threat (also called hazard). Quantitative risk assessment requires calculations of two components of risk: *R*, the magnitude of the potential loss *L*, and the probability *p*, that the loss will occur. / Risk management is the identification, assessment, and prioritization of risks followed by coordinated and economical application of resources to minimize, monitor, and control the probability and/or impact of unfortunate events. / Policies: Policies and procedures are a set of documents that describe an organization's policies for operation and the procedures necessary to fulfil the policies. Departments and laboratories will also have local policies and procedures for their activities.

5.5. **Standard Operating Procedure**: An SOP is a step-by-step procedure that promotes uniformity in operations to help clarify and augment such operations. SOPs document the way activities are to be performed to facilitate consistent conformance to technical and quality system requirements and to support data quality. The use of SOPs is an integral part of a successful quality system (www.brownfieldstsc.org/glossary.cfm).

5.6. **Documentation of Processes**: This may be part of the SOP. It is the recording, by the operator, that specific steps in the process have been completed and that the appropriate subsequent quality and safety checks have been made. Such documents provide an audit trail for the outcome of the procedure.

5.7. **Clinical Audit**: A quality improvement process that seeks to improve patient care and outcomes through systematic review of care against explicit criteria and the implementation of change. (UK National Health Service) / The key component of clinical audit is that performance is reviewed (or audited) to ensure that what should be done is being done, and if not it provides a framework to enable improvements to be made.

6. Summary of QA Workshop (2009-12-10)

6.1. **The workshop group wants to continue this discussion and move towards a more formal QA-approach**, preferentially forming a working group within the MiP*society* (www.mitophysiology.org) and linking to other societies. A circular should be sent to all MiP*members*, with the summary of the QA workshop, future perspectives, and questions on feedback. A special evening session might be devoted to QA at MiP2010 (www.mitophysiology.org/index.php?mip2010; but will there be sufficient time?).

6.2. **QA working group**: The QA workshop and related correspondence showed that there is a critical mass for a working group; avoid forming a closed group of 'insiders', remain open but focused. The special topic of respirometry of permeabilized muscle fibres appears to be adequate and manageable as a first step, whereas the general correspondence suggest a broader perspective (including isolated mitochondria, intact and permeabilized cultured and primary cells) for future QA workshops.

6.3. **Publication**: Methods paper on the basis of a series of QA workshops; possible key words are:
- Methods of taking biopsies
- Sample handling and sample preservation
- Respirometric instruments
- Experimental conditions (temperature; media; controls incl. cyt c test; oxygen levels)
- Wet weight/dry weight; normalization of respirometric flux
- Mechanical separation of fibres
- Permeabilization of fibres (concentration of sponin, time, temperature, washes)
- Substrate concentrations (how to evaluate correct or optimal concentration; stock solution preparation)
- Miminal protocol (evaluation; minimal protocol for comparison and quality control)
- Pitfalls
- Exclusion criteria
- The methods paper should include a meta-analysis of published data

6.4. **QA website**: Place for protocols, study design, information for researchers on groups who are doing similar work.

6.5. **Towards QA is a theme of *Gentle Science*** –
www.oroboros.at/index.php?gentle-science

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Track record

1st /2nd draft: E Gnaiger (2009-10-29/2009-11-05); Printed: 2009-12-04;
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Disclosures

E Gnaiger is the founder and Managing Director of:
OROBOROS INSTRUMENTS Corp.
high-resolution respirometry
Schöpfstrasse 18
A-6020 Innsbruck, Austria

T +43 512 566796, F +43 512 566796 20
Email: erich.gnaiger@oroboros.at
http://www.oroboros.at - http://www.mipart.at
MiPNet - www.oroboros.at/index.php?mipnet

Correspondence address

Erich Gnaiger, A.Univ.-Prof., Ph.D.
Medical University of Innsbruck
Dept. General and Transplant Surgery

D. Swarovski Research Laboratory, Innrain 66/6
A-6020 Innsbruck, Austria
T +43 512 504 24623 (F 24625)
Email: erich.gnaiger@i-med.ac.at

List of participants

Last update: 2009-11-26

Bilet Lena, Department of Human Biology, Maastricht University, Maastricht, The Nederlands. - L.bilet@hb.unimaas.nl

Boushel Robert, Prof. Dr., Faculty of Health Sciences, Dept Biomedical Sciences, Copenhagen, Denmark. - rboushel@mfi.ku.dk

Burtscher Martin, Prof. Dr., Inst. Sports Science, Univ. Innsbruck, Austria. - Martin.Burtscher@uibk.ac.at

Dela Flemming, Prof. Dr., Faculty of Health Sciences, Dept Biomedical Sciences, Copenhagen, Denmark. - fdela@mfi.ku.dk

Fasching Mario, PhD, OROBOROS INSTRUMENTS, Innsbruck, Austria. - mario.fasching@oroboros.at

Garcia-Roves Pablo M, PhD, Senior researcher, Karolinska Institutet, Department of Physiology and Pharmacology, Integrative Physiology, Stockholm, Sweden. - pablo.garcia-roves@ki.se

Gnaiger Erich, A.Univ.-Prof., PhD, Dept. General and Transplant Surgery, Medical University of Innsbruck, D. Swarovski Research Laboratory Innsbruck, Austria; and OROBOROS INSTRUMENTS; Austria. - erich.gnaiger@oroboros.at

Haberberger Birgit, Dr., Institut für Humangenetik, Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt, Neuherberg, Germany. - birgit.haberberger@helmholtz-muenchen.de

Hansson Magnus, PhD, Laboratory for Experimental Brain Research, Wallenberg Neuroscience Center, Lund, Sweden. - magnus.hansson@med.lu.se

Harrison David K., PhD, Dr.Med.Habil., Hon. Senior Lecturer in Medical Physics, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK. - d.k.harrison@ncl.ac.uk

Hoeks Joris, PhD, Department of Human Biology, Maastricht University, Maastricht, The Nederlands. - j.hoeks@hb.unimaas.nl

Hüttenhofer Alexander, Prof. Dr., Innsbruck Biocenter, Innsbruck Medical University, Austria. - alexander.huettenhofer@i-med.ac.at

Iyer Shilpa, PhD, Univ of Virginia, Neurology Dept, Charlottesville VA, USA - si3s@cms.mail.virginia.edu

Janke Linda, Deutsches Diabetes-Zentrum, Leibniz-Zentrum für Diabetesforschung, Träger: Deutsche Diabetes Forschungsgesellschaft e.V., Düsseldorf, Germany. - linda.janke@uni-duesseldorf.de

Kane Dan, Ph.D., East Carolina University, Brody School of Medicine, Department of Exercise and Sport Science & Department of Physiology, East Carolina, Greenville, USA. - DAK1021@ecu.edu

Kraunsoe Regitze, Faculty of Health Sciences, Dept Biomedical Sciences, Copenhagen, Denmark. - regitzek@mfi.ku.dk

Kruse Shane, PhD, Dept. of Radiology, Univ. of Washington, Seattle, Washington, USA. - kruses@u.washington.edu

Mogensen Martin, Dr., Faculty of Health Sciences, Dept Biomedical Sciences, Copenhagen, Denmark. - mmogensen@mfi.ku.dk

Morota Saori, PhD, Lund University, Laboratory for Experimental Brain Research, Wallenberg Neuroscience Center, Lund, Sweden. - saori.morota@med.lu.se

Pak Oleg, PhD Student, Justus-Liebig-Universität Giessen, Medizinische Klinik II, Innere Medizin, Giessen, Germany. - oleg.pak@innere.med.uni-giessen.de

Pesta Dominik, Mag., D. Swarovski Research Laboratory, Dept. General Transplant Surgery, Medical University Innsbruck; and OROBOROS INSTRUMENTS, Austria. – dominik.pesta@i-med.ac.at

Renner-Sattler Kathrin, Mag. Dr., Regensburg, Germany. - kathrin.renner@mitophysiology.org

Schiller Evelyn, Experimentelle Genetik, Helmholtz Zentrum München - Deutsches Forschungszentrum für Gesundheit und Umwelt, Neuherberg, Germany. - evelyn.schiller@helmholtz.muenchen.de

Schmoll Dieter, Dr., Sanofi-Aventis Deutschland GmbH, R&D TD Metabolism FFM, Frankfurt am Main, Germany. - dieter.schmoll@sanofi-aventis.com

Sumbalová Zuzana, PhD, Comenius University, Medical Faculty, Pharmacobiochemical Laboratory, III. Medical Clinic, Bratislava, Slovakia. - zuzana.sumbalova@zoznam.sk

Timmers Silvie, Department of Human Biology, Maastricht University, Maastricht, The Nederlands. - s.timmers@hb.unimaas.nl

Tweedie Constance, PhD, East Carolina University, Brody School of Medicine, Department of Exercise and Sport Science & Department of Physiology, East Carolina, Greenville, USA. - tweediec@ecu.edu

Vijgen Guy, Department of Human Biology, Maastricht University, Maastricht, The Nederlands. - g.vijgen@mumc.nl

Vosselman Maarten, Department of Human Biology, Maastricht University, Maastricht, The Nederlands.

Votion Dominique-Marie, Equine Clinic, Faculty of Veterinary Medicine, University of Liège, Sart Tilman, Liège, Belgium. - dominique.votion@ulg.ac.be

Wenchich Laszlo, Dr., Laboratory for Study of Mitochondrial Disorders, Department of Pediatrics, First Faculty of Medicine, Charles University, Prague , Czech Republic. - wlaca@centrum.cz

Zeh Ramona, Experimentelle Genetik, Helmholtz Zentrum München - Deutsches Forschungszentrum für Gesundheit und Umwelt, Neuherberg, Germany. - ramona.zeh@helmholtz.muenchen.de

From the correspondence 1: The 2009 QA-Workshop

2009-11-30: We plan to discuss our preliminary findings and also plans for future experiments in an openly manner. I know you have unfortunately have had some bad experiences with your scientific openess. Is this something that should be discussed briefly in the beginning of the workshop so that everybody is comfortable with sharing new ideas even though several of us have projects that may be overlapping?

Hansson Magnus, PhD
Laboratory for Experimental Brain Research
Wallenberg Neuroscience Center
BMC A13, SE-221 84 Lund, Sweden
magnus.hansson@med.lu.se

A: Your suggestion is excellent - Quality Assurance requires reflections on the quality of science, the quality of progress, the quality of scientific communication, and the quality of fair references in publication. A short reflection might set the stage for a high quality of open communication in our workshop, for the advancement of the quality of our science. How can we meet the demands on competitive publication and at the same time be open for informal communication as an important strategy for optimization? We should spend some time on Thursday in our summary discussion: Quality Assurance requires an explicit consideration of open communication, sharing of protocols, in connection with fair citation. See also some points in Notes 3.4 and 3.5 (Erich Gnaiger).

2009-12-02: It would be interesting to discuss the problems/dilemmas of the acquisition and manipulation with the tissue samples (the preanalytical phase). From my practice I know, that inadequate handling may lead to misinterpretation of the results.

Wenchich Laszlo, Dr.
Laboratory for Study of Mitochondrial Disorders
Department of Pediatrics
First Faculty of Medicine
Charles University
120 00 Prague 2, Czech Republic. - wlaca@centrum.cz

From the correspondence 2: Some steps

2009-10-29: *First circular QA-Workshop*: I just completed a draft for a proposed workshop (8-11 December) that could be a focus for further communication and perhaps a joint EU project application. The draft - or further evolved versions - will be circulated to a start-up group. Secondarily, we will circulate this proposal (very quickly) to our MiPNet Reference Laboratories (many are just starting now with human or mouse skeletal muscle fibers), for potential feedback. Please let me know your oppinion and all your suggestions and potential disagreement with the proposed details on the QA-Workshop.

Erich Gnaiger, A.Univ.-Prof., Ph.D.
Medical University of Innsbruck
Dept. General and Transplant Surgery
D. Swarovski Research Laboratory, Innrain 66/6
A-6020 Innsbruck, Austria
erich.gnaiger@i-med.ac.at
World-Wide MiPNet - www.oroboros.at/index.php?mipnet

2009-10-30: We highly appreciate the initiative to further standardize the assessment of mitochondrial function in (human) permabilized muscle fibers. We agree with you that a strong focus on protocols and possible pitfalls will be very valuable. We do think it would be appreciated to also relate to the protocols by presenting results. Separate sesssions for this seems fine, although it might also be good to more directly connect the results to the used protocols?

Schrauwen Patrick, Prof. Dr.
Department of Human Biology

Maastricht University, Maastricht, The Nederlands
p.schrauwen@hb.unimaas.nl

A: I fully agree that methodological aspects related to QA and protocols should be presented in connection with results, since one cannot be understood and discussed without the other. The suggested structure was meant to emphasize that results-only presentations should be the exception, if we agree to focus on the topic of Quality Assurance. - Erich Gnaiger, erich.gnaiger@i-med.ac.at

2009-11-02: The Neufer lab is delighted to see such an initiative, and agree that improving on these methodologies (e.g., optimization & standardization) will serve everyone's best interests. .. I can think of a dozen or more aspects of the permeabilized fiber approach that we have experienced problems/concerns with that deserve discussion, many of which are listed in the 4.2.2 QA Workshop document you sent. .. We could, for example, present aspect of the mechanical separation procedures, as we have a digital camera attached to our dissecting microscope. I can also try to bring with me some contraction inhibitors (blebbistatin and BTS) for experimental testing at the workshop.

Daniel A. Kane, PhD
6W54 Brody Medical Sciences Bldg.
East Carolina University, Greenville, NC, USA
DAK1021@ecu.edu

2009-11-13/16: To MITOCHONDRIA-L@LIST.NIH.GOV: Do you know of any Quality Assurance guidelines that may be available for diagnosis of mitochondrial respiratory function? I would be very grateful for receiving relevant information, which may be integrated with full references in our forthcoming QA-Workshop, which will be held on December 9-10 at the Medical University of Innsbruck, with support by OROBOROS INSTRUMENTS.

I thank all of you who are contributing to the discussion on QA in Mitochondrial Respirometry. Relevant sections of the correspondence are combined with previous and parallel correspondence on http://www.oroboros.at/index.php?qa2009-correspondence. Your feedback gives a high-quality momentum to our general objective. The announced QA-Workshop will thus provide a starting point for a QA-project, that promises to continue way beyond the direct workshop event. We will do our best to elaborate a workshop summary for distribution through the MIG list. I particularly agree that *in vivo* perspectives form an integral component of mitochondrial physiology, and I welcome a variety of high-quality approaches to be represented and discussed at our QA-Workshop and beyond. Thank you for your cooperation and feedback

Erich Gnaiger, A.Univ.-Prof., Ph.D.
Medical University of Innsbruck
Dept. General and Transplant Surgery
D. Swarovski Research Laboratory, Innrain 66/6
A-6020 Innsbruck, Austria
erich.gnaiger@i-med.ac.at
Mitochondrial Physiology Society: http://www.mitophysiology.org
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Innsbruck, Austria
erich.gnaiger@oroboros.at
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From the correspondence 3: Expressions of interest

2009-10-29: I'm in complete agreement about the need for such a program, because already there are small 'for-profit' industries doing mitochondrial function analysis in diagnosis of human pathologies using poor methodology. Such a QA program would assure that proper and uniform methodology is accounted for, at least with those individuals and groups willing and able to participate in such a program.

Towards that end, here is a study I completed that will be coming in the next issue of J Amer Coll Cardiol, where I compared mitochondrial function in the atrial tissue of the heart in type 2 diabetic patients with that in non-diabetic patients, both undergoing coronary bypass graft surgery. The methods I've used are a result of years of trial-and-error and careful practice, through the experience I've had with permeabilized fibers.

Ethan J. Anderson, PhD, Assistant Professor
Departments of Pharmacology & Toxicology & Cardiovascular Sciences
Brody School of Medicine, East Carolina Heart Institute
Greenville, NC 27834, USA. - andersonet@ecu.edu

2009-10-29: The exchange of ideas, protocols and best practices should be a constructive experience and I look forward to hearing feedback.

Christopher G.R. Perry, PhD, Post-Doctoral Scholar
The Metabolic Institute
Departments of Exercise and Sport Science & Physiology
The Brody School of Medicine, East Carolina University
Greenville, NC 27834, USA. - perrych@ecu.edu

2009-11-06: I might be interested in a meeting at a future time, and would like to be copied on discussions in this area. We would also be consider attending a general workshop in this area.

Kristal Bruce S., Prof. Dr.
Department of Neurosurgery, Brigham and Women's Hospital
Boston, MA, USA. - bruce.kristal@gmail.com

2009-11-09: I would be keen to attend a session where the Oroboros respirometry is discussed along with other complimentary methodologies. My immediate interest would be to seek some discussion and feedback regarding possible future application of our fluoroscent method for following ADP:ATP ratio in permeabilized fibres (see attached PDF). Presently, we use the method to evaluate the mechanisms that govern strain dependent mechanoenergetics in skeletal muscle fibres and cardiac trabeculae. We use the method to evaluate ADP generation. But in principle, with the right cocktail of ATPase inhibitors etc, the method could also be used to monitor aspects of mitochondrial ATP production kinetics in permeabilized fibres. The method has high time resolution, sensitivity and probes the cytosolic domain of permeabilized fibres. My plan would be to modify our OROBORUS Oxygraph so that I could have an intact muscle preparation (eg rat trabeculae or papillary muscle) in which I could monitor mechanics and O2 kinetics.

Tim West, PhD
Imperial College London, NHLI (Molecular Medicine Section)
London, UK. - t.west@imperial.ac.uk

2009-11-09: I think it is a great idea to bring people who works on fibers together to discuss. I would be happy to participate in discussion and correspondence.

Lemieux Hélène, Dr.
Case Western Reserve University School of Medicine
Department of Pharmacology, Center for Mitochondrial Diseases
Cleveland, OH, USA. - helene.lemieux@case.edu

2009-11-09: For newcomers to the field of mitochondrial physiology, I was wondering if you could add to the workshop a short discussion on other new technologies in the field, for example, oxygen sensitive dyes and the use of fluorescent-based optical sensors that do not use Clark electrodes. Are they less sensitive? Advantages and Disavantages? Do the technologies complement one another?

Dworetzky Steven, Ph.D
Knopp Neurosciences
Pittsburgh, PA, USA. - dworetzky@knoppneurosciences.com

2009-11-09: I am interested in future workshops, but would prefer a more general workshop, since we work also with isolated mitochondria and permeabilised cultered cells.

Bekkenkamp-Grovenstein Melissa
RIKILT - Institute of Food Safety
Wageningen University, Wageningen, The Netherlands. - Melissa.Bekkenkamp@wur.nl

2009-11-09: We would certainly like to participate in events like this and we would also like to share our results.

Slinde Erik, Prof. Dr. philos., Principal scientist
Institute of Marine Research
Bergen, Norway. - erik.slinde@imr.no

2009-11-09: I'm very interested in working on respirometry of permeabilized muscle fibers. In fact, I'm doing my first experiments in mice skeletal muscle, but I'm not sure if I permeabilize the fibers well or if I perform all the protocol correctly. I will be very glad if you could check my preliminary results and give me some advices (I don't know if I am in the correct range of values of oxygen consumption, and I have some problems with the stability of the measurement).

Segalés Jessica
Dr. Antonio Zorzano's lab
Institute for Research in Biomedicine
c/ Baldiri Reixac 10
08028 BARCELONA. - jessica.segales@irbbarcelona.org

2009-11-10: Recently we did some measurements on heart and skeletal muscle, with so many samples that we had to try homogenates. I can say that homogenates worked wery well. Mitochondria were well coupled (RCI with glutamate about 10) also inhibitors worked well and all substrates respired. May be you could extent your next seminar also to measurements in homogenates.

Drahota Zdenek, Dr.
Dept. of Bioenergetics, Institute of Physiology
Academy of Sciences of the Czech Rep.
PRAGUE, Czech Republic. - drahota@biomed.cas.cz

2009-11-11: I would be very keen to attend any follow up workshop if I can. I am happy to contribute information if it will help but would be especially keen on receiving, or downloading the results of the workshop. In the short time that we have been working on permeabilised fibres we have noted that some protocols are better than others and we have had some problems that may relate to overenthusiastic teasing of muscle bundles.

Boyle John P., Dr.
Senior University Cardiovascular Research Fellow
Division of Cardiovascular & Neuronal Remodelling, LIGHT
University of Leeds, UK. - medjpb@leeds.ac.uk

2009-11-12: I'm very interested on future follow-up workshops on respirometry on fibers, but also on isolated mitochondria and permeabilized cells.

De Palma Clara, Dr.

Dip Scienze Precliniche, LITA-Vialba, Lab Farmacologia Cellulare
Università degli studi di Milano, Milano, Italy. - clara.depalma@guest.unimi.it

2009-11-12: I am potentially interested in a future follow-up QA-Workshop on respirometry of permeabilized muscle fibres. I think that QA-Workshop should be focused both to a more general topics and to a less general topics, for example respirometry of adipose tissue.

Hejzlarova Katerina, MSc
Department of Bioenergetics
Institute of Physiology AS CR
142 20 Prague Czech Republic. - katerina.hejzlarova@biomed.cas.cz

2009-11-13: I can not participate this workshop but am still interested in that. I would like to contribute to your discussions and presentations through correspondence. I also appreciate that in the future the focus can be extended to a more general QA workshop on respirometry of other cells and mitochondria.

Zheng Huaien, Dr.
Research Associate
Dept. Anesthesia, McGill University
Montreal, Quebec, Canada H3G 1V6. - huaien.zheng@mcgill.ca

2009-11-14: QC is a very important issue. We participate in the ERNDIM QC scheme for other inborn errors of metabolism. However, to my knowledge a scheme is not available for mitochondrial diseases. I recall the French made an effort to draw up some guidelines. You may ask Anne Lombes anne.lombes@upmc.fr. Please update this forum < MITOCHONDRIA-L@LIST.NIH.GOV > of your progress in this matter.

Ann Saada (Reisch) PhD
Department of Human Genetics and Metabolic Diseases
Hadassah-Hebrew University Medical Center
Jerusalem 91120 Israel. - annsr@hadassah.org.il

2009-11-15: The Diagnostic Standards Committee of the Mitochondrial Medicine Society (MMS) has published clinical guidelines for diagnosis to supplement the Modified Walker and Nijmegen Criteria used in the diagnosis of children with mitochondrial disease. These papers address the larger issues of clinical diagnosis. There is also an excellent review about interlab variation of respiratory chain enzymology results by Frank Gellerich which appeared in the 2004 Mitochondrial Medicine issue of the journal Mitochondrion. I have attached these papers.

However, to my knowledge, there has not been a comprehensive review of interlab variation in polarographic analysis of either patient samples, or a standardized non-human tissue source. New technologies, and new technology platforms make such an effort very timely. The MMS strongly supports these QA efforts, which serve both our patients and the broader mitochondrial research community.

Naviaux Robert K., MD, PhD
Departments of Medicine, Pediatrics, and Pathology
President, Mitochondrial Medicine Society, Professor and Co-director
The Mitochondrial and Metabolic Disease Center, Biochemical Genetics and Metabolism
UCSD School of Medicine
214 Dickinson St., Room C-103,
San Diego, CA 92103-8467. - naviaux@ucsd.edu
MMDC Website: biochemgen.ucsd.edu; Mitochondrial Medicine Society Website: www.mitosoc.org

2009-11-15: I believe David Thorburn has lead the way on this regarding oxphos enzymology, I remember him workshopping it at a UMDF meeting years ago, not sure if published but worth a search. I'm not aware of similar attempts re respirometry.

Trounce Ian, Prof.Dr.
St Vincent's Hospital,
The University of Melbourne, Australia. - ianat@MEDSTV.UNIMELB.EDU.AU

2009-11-15: In 1955 Chance and Williams defined the metabolic state of mitochondria under In Vitro conditions. This concept led to the development of In Vivo Monitoring of NADH redox state using surface fluorometry. The introduction of UV transmitting optical fibers enabled us to apply the technology many organs in the body exposed to various pathophysiological conditions. Two years ago an invited review was published in Am. J .Physiology. During the years, new light sources appeared in the market enabling us to built a stable device that was cleared by the FDA few years ago. A new model of NADH fluorometer was developed by Prizmatix.com and is available for experimental animals studies. I am sure that In vivo monitoring of mitochondrial function is a critical factor in advancing the knowledge of organ energy metabolism in real-time mode.

Mayevsky Avraham, Prof.Dr.
Faculty of Life Sciences
Bar Ilan University
Ramat Gan, Israel. - mayevsa@gmail.com

A: It seems a disclaimer re: COI/financial interest is in order here, unless i'm missing something.

Rhead, William J. MD, PhD
Children's Hospital of Wisconsin
Professor of Pediatrics and Pathology
Medical College of Wisconsin, USA. - wrhead@MCW.EDU

A2: I apologise for the problem created by not sending the conflict of interest statement on time. I have tried to download the conflict of interest document but I was unable to get it. I did not want to hide any information. I am a consultant to Prizmztix although we do not have any formal agreement yet ,I believe that I will get payment for my consultation. Due to my wide experience in this field I am the only one that could

support the optimal R&D of the new product, the Mito-Viewer. Just to clarify, I also have another product developed by CritiSense (www.critisense.com) for multiparametric patients monitoring and I am the owner of most of the shares of this company. This company is not active at the moment due to the lack of funding. Sincerely, Avraham, mayevsa@gmail.com

2009-11-17: I can´t participate the QA-workshop on Dec 8-11 in Innsbruck, but I´m interested in a summary of the discussions and presentation from this workshop. It´s a good idea to extend the focus in the future also on respirometry of isolated mitochondria.

Haindl Susanne, biomed. Analytikerin
Ludwig Boltzmann Institut für experimentelle und klinische Traumatologie
Wien, Austria. - susanne.haindl@lbitrauma.org

2009-11-19: QA is an extremely important aspect of for all laboratory procedures and especially so for laboratories offering diagnostic services, such as the lab that I work in. I would love to attend a meeting on QA for this particular area of interest, but it is too short notice for me. A QA workshop should cover all materials. I am always happy to contribute to the improvement of quality and quality assurance. It maybe be good to know that we have an organisation in Europe, called ERNDIM (European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism). I am a representative for this organisation. We offer EQA schemes in several forms and we are now also including EQA schemes for enzyme diagnostics. At the moment I cannot see how mitochondrial enzyme could easily be implemented in an EQA, but it may be something to consider. Moreover, in collaboration with SSIEM, ERNDIM also organises training courses.

Bierau Jörgen, Dr. PhD
Laboratory of Biochemical Genetics
Dept. Of Clinical Genetics, Maastricht University Hospital
Maastricht, The Netherlands. - jorgen.bierau@gen.unimaas.nl
ERNDIM: http://www.erndim.unibas.ch/ .