NOXYGEN Science Transfer & Diagnostics GmbH

With Passion for Innovation

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# EPR-APPLICATION TRAINING COURSE OVERVIEW (OPTIONS)

1. **Lectures**
   a. New updates in detection and imaging of ROS in vitro and in vivo using EPR spectroscopy.
   b. Endothelial vasomotion control and endothelial dysfunction: Detection of NO using colloid Fe(DETC)2, circulating NOHb concentration and EPR or in Real-Time using alternative chemiluminescence procedure
   c. The impact of hypoxia and hyperoxia on generation of ROS and NO with the consequences on the modulation of grow factors profile: Physiological oxygen level.
   d. Exploratory, diagnostic, differential-diagnostic relevance of "CMA" (cellular metabolic activity), "Extended CMA" and "Inflammatory Resistance" tests.

2. **Electron Spin Resonance Spectroscopy**
   a. Electron spin resonance spectroscopy – introduction, principles, technical background
   b. Introduction in the usage of EMXnano – new standard for Bench-Top EPR
      i. Acquisition, evaluation and simulation software (Xenon, SpinFit)
      ii. Sensitivity and stability
      iii. EPR settings (center field, sweep width, microwave power, modulation amplitude, time constant, conversion time, sweep time, number of scan, resolution)
      iv. Power saturation
      v. Overmodulation
      vi. Use of spin traps, spin probes, spin labels
      vii. Default protocols (frozen and non-frozen samples, short-term, long-term detection)
      viii. EMXnano accessories (liquid nitrogen finger dewar, temperature & oxygen/gas controller, UV irradiation unit, flat cell, aqua X cell, flow-through cell)
   c. Introduction in the usage of Noxygen System – exploratory & routine Bench-Top EPR system
      i. Acquisition, evaluation and simulation software
      ii. Sensitivity and stability
      iii. EPR settings (center field, sweep width, microwave power, modulation amplitude, time constant, conversion time, sweep time, number of scan, resolution)
      iv. Power saturation
      v. Overmodulation
      vi. Use of spin traps, spin probes, spin labels
      vii. Default protocols (frozen and non-frozen samples, short-term, long-term detection)
      viii. Accessories (liquid nitrogen finger dewar, temperature & oxygen/gas controller, UV irradiation unit, flat cell, aqua X cell, flow-through cell)

**3. Detection of Reactive Oxygen Species (ROS)**

a. Introduction in the usage of spin probes (cyclic hydroxylamine's) for detection of reactive oxygen species (ROS; O2●, OH-, ONOO-, H2O2)
b. Preparation of Krebs-HEPES buffer, spin probes, and other solutions
c. Calibration of EPR-signal for calculation of ROS generation/concentration
d. Value of time dependence ROS production in mitochondria, cultured cells, isolated tissue, isolated organs, in living mouse
e. Physiological and pathophysiological conditions:
    i. Temperature dependence (normothermia, hypothermia, hyperthermia)
    ii. Oxygen dependence (anoxia, normoxia, hypoxia, hyperoxia)
    iii. Pressure dependence (normotension, hypotension, hypertension)
    iv. Shear dependence (laminar, oscillatory, pulsatile shear stress)
f. Basal and stimulated ROS production/generation
g. Quantitative identification of intracellular and extracellular ROS generation
h. Preparation of samples for detection of ROS in frozen and non-frozen conditions
i. Problems, pitfalls, advantages of ROS- detection using EPR, interpretation of results

**4. Detection of Nitric Oxide (NO)**

a. Introduction in the usage of a spin trap (Fe2+(DETC) for detection of NO
b. Introduction in the usage of NOHb, NOMg for detection of circulating NO, or bioavailable NO in muscle
c. Preparation of Krebs-HEPES buffer, spin trap, and other solutions
d. Calibration of EPR-signal for calculation of NO generation/concentration
e. Value of time dependence NO production in cultured cells, isolated tissue/organs, in vivo
f. Physiological and pathophysiological conditions:
    i. Temperature dependence (normothermia, hypothermia, hyperthermia)
    ii. Oxygen dependence (anoxia, normoxia, hypoxia, hyperoxia)
    iii. Pressure dependence (normotension, hypotension, hypertension)
    iv. Shear dependence (laminar, oscillatory, pulsatile shear stress)
g. Basal and stimulated ROS production/generation
h. Quantitative identification of NO-Fe(DETC), NO-Hb, NO-Mg
i. Preparation of samples for detection of NO in frozen conditions
j. Problems, pitfalls, advantages of NO detection using EPR, interpretation of results

**5. Diagnostics, differential diagnostics tests - clinical research**

a. CMA Score – metabolic, respiratory activity of human blood cells (HBC)
b. Physical performance – metabolic, respiratory activity of HBC before and after exercise challenge
c. Endothelial Dysfunction – correlation of circulating, bioavailable NOHb concentration in human venous blood to its physiological level
d. Cardiovascular Risk Assessment – correlation between "Vitality Score" and "Endothelial Dysfunction" tests

e. Extended CMA Score - Relation of total metabolic, respiratory HBC activity (total ROS generation) to:
   v. NADPH-oxidase dependent ROS generation in HBC
   vi. Mitochondria dependent ROS generation in HBC
   vii. Peroxidase dependent ROS generation in HBC
   viii. LDL dependent ROS generation in HBC
   ix. Glucose-dependent ROS generation in HBC
   x. Evaluation of bioactive ingredients of food supplement
   xi. Effects of environmental pollution (ozone, nanoparticles, UV-light, etc.)
   xii. Monitoring of treatment efficacy (pharmacokinetic, pharmacodynamic)
f. Inflammatory Resistance Assay - TNF-alpha-depended ROS generation in HBC
g. Platelet Aggregability Assay – Shear-stress-dependent metabolic, respiratory activity test
h. Blood conserve quality evaluation

6. **Functional related EPR applications – Basic and Clinical research**
   a. Intracellular/extracellular detection of ROS/$O_2^{\bullet-}$ (basal and stimulated ROS/$O_2^{\bullet-}$ production) in cultured cells, in isolated organs/tissue under physiological conditions and effected by:
      i. Hypo- or hyperthermia
      ii. Hypo- or hyperoxia
      iii. Hypo- or hypertension
   b. Intracellular/extracellular detection of ROS/$O_2^{\bullet-}$ in cultured or native cells exposed to laminar, oscillatory, pulsatile shear stress with or without combination of arrhythmia
   c. Monitoring of oxygen concentration and / or oxygen consumption in vitro and in vivo
   d. Mitochondria dependent generation of ROS/$O_2^{\bullet-}$ production in cultured cells, in isolated organs, in isolated tissue
   e. Simultaneous detection of ROS/$O_2^{\bullet-}$ and oxygen concentration/oxygen consumption in native or cultured cells
   f. Simultaneous detection of ROS/ $O_2^{\bullet-}$ and NO in cultured/native cells, in isolated tissue, in vivo
   g. Real-Time monitoring of ROS generation in a living mouse exposed to environmental pollution, vasoactive drugs, hyperglycemia, UV-light exposure
   h. Detection of SH-groups, Vit.-C, concentration in blood plasma, cell-/tissue lysates
   i. Detection of intra- and extracellular pH level
   j. Evaluation of membrane micro-viscosity changes

**7. EPR-Imaging**
   **a. System E540L (ELEXSYS L-Band spectrometer with 22 and 34mm probe head)**
      i. Monitoring of ROS generation in mouse (head, chest, abdomen, etc.), rat head
      ii. Monitoring of Oxygen concentration in mouse (head, chest, abdomen, etc.), rat head
      iii. Monitoring of pH-level in whole mouse (head, chest, abdomen, etc.), rat head
      iv. Monitoring of mitochondria-dependent ROS generation

## 8. Subject-specific /Research field-oriented applications

### a. Cardiology and Angiology

| | | |
|---|---|---|
| i. | Diagnostic tools | |
| ii. | Cell types | endothelial cells, smooth muscle cells, cardiomyocytes, blood, white and grey adipocytes, neutrophils, leucocytes |
| iii. | Isolated organs | aorta, coronary arteries, resistance vessels, heart |
| iv. | Isolated tissue | heart, muscle biopsies |
| v. | Animals | mouse, rat, dog, mini-pigs |

### b. Anesthesiology and Hematology

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|---|---|---|
| i. | Diagnostic tools | |
| ii. | Cell types | lung epithelial cells, endothelial cells, smooth muscle cells, cardiomyocytes, whole blood, white and grey adipocytes, neutrophils, leucocytes, erythrocytes, blood conserves |
| iii. | Isolated organs | aorta, coronary arteries, resistance vessels, heart, lung, kidney |
| iv. | Isolated tissue | liver, heart, lung, kidney, muscle biopsies, bone marrow |
| v. | Animals | mouse, rat, dog, mini-pigs |

### c. Sports and Rehabilitative Medicine

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|---|---|---|
| i. | Diagnostic tools | |
| ii. | Cell types | lung epithelial cells, endothelial cells, smooth muscle cells, cardiomyocytes, whole blood, white and grey adipocytes, neutrophils, leucocytes, erythrocytes |
| iii. | Isolated organs | resistance vessels, heart, lung, kidney, liver |
| iv. | Isolated tissue | liver, heart, lung, kidney, muscle biopsies |
| v. | Animals | mouse, rat, dog |

### d. Environmental Medicine

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|---|---|---|
| i. | Diagnostic tools | |
| ii. | Cell types | keratinocytes lung epithelial cells, endothelial cells, smooth muscle cells, whole blood, neutrophils, leucocytes, erythrocytes |
| iii. | Isolated organs | liver, heart, lung, kidney |
| iv. | Isolated tissue | skin, heart, lung, kidney, muscle biopsies |
| v. | Animals | mouse, rat, zebra-fish |

### e. Traumatology and Surgery

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|---|---|---|
| i. | Diagnostic tools | |
| ii. | Cell types | osteocytes, lung epithelial cells, endothelial cells, smooth muscle cells, whole blood, neutrophils, leucocytes, erythrocytes, neurocytes |
| iii. | Isolated organs | liver, heart, lung, kidney |
| iv. | Isolated tissue | brain tissue, skin, heart, lung, kidney, muscle biopsies, bone marrow |
| v. | Animals | mouse, rat, dog, mini-pigs |

**f. Toxicology and Pharmacology**

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|---|---|---|
| i. | Diagnostic tools | |
| ii. | Cell types | neurocytes, lung epithelial cells, endothelial cells, smooth muscle cells, whole blood, neutrophils, leucocytes, erythrocytes, fibrocytes, epithelial cells, |
| iii. | Isolated organs | liver, heart, lung, kidney, aorta, coronary arteries |
| iv. | Isolated tissue | brain tissue, skin, heart, lung, kidney, muscle biopsies, bone marrow |
| v. | Animals | mouse, rat, dog, mini-pigs, zebra-fish |

**g. Pulmonology and Internal Medicine**

| | | |
|---|---|---|
| i. | Diagnostic tools | |
| ii. | Cell types | lung epithelial cells, endothelial cells, neurocytes, smooth muscle cells, whole blood, neutrophils, leucocytes, erythrocytes |
| iii. | Isolated organs | liver, heart, lung, kidney, aorta, resistance vessels |
| iv. | Isolated tissue | brain tissue, skin, heart, lung, kidney, muscle biopsies |
| v. | Animals | mouse, rat, dog, mini-pigs |

**Do not hesitate to contact us for more detailed information about the requirements concerning the needed chemicals, accessories and devices.**

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