Airway inflammation Diagnostic
EBC-Collection & analysis
The EBC-Collector is an innovative solution for non-invasive, fractionised collection of exhaled breath condensate (EBC), because of:
- a spirometers, which is built in, allows the measurement of volume and time controlled standardised collection,
- breath controlled collection into two separate chambers makes it possible to collect condensate from different depths of the bronchial system, so that the dead space condensate may be discarded,
- the material of the sampling bags is approved for the collection of exhaled biomarkers: hydrogen peroxide, L-lactate, proteins and lipids, like: prostaglandin and leukotrienes.
- \( \text{H}_2\text{O}_2 \) and L-Lactate can be analysed by EBC Analyzer:

**Advantages of the EBC Collector:**
- fractionised condensate collection
- transportable desktop device
- simple handling
- low noise level
- stable construction

**Diagnostic of inflammatory processes in the lung**

ECoCheck

ECoCheck is an innovative solution for measurements of \( \text{H}_2\text{O}_2 \) and L-lactate in exhaled breath condensate immediately after collection. The principle of the measurement is a highly specific reaction of the analyse substance with oxidase followed by amperometric detection.

The detection of these biochemical markers has become easier compared to chemiluminescence or fluorescence spectroscopy, because of:
- small amount of needed EBC volume (min. 300 ml),
- high sensitivity of the measurements: for \( \text{H}_2\text{O}_2 \) in linear range from 30 up to 3000 nmol/l and for Lactate in linear range from 5 up to 150 mmol/l,
- short measuring time (result within 5 min in \( \text{H}_2\text{O}_2 \) case),
- no calibration required (in \( \text{H}_2\text{O}_2 \) case),
- offline input of ventilation data,
- automatic recalculation of results according to collecting time and total exhaled volume.

The ECoCheck device gives opportunity to simultaneous measurements in EBC of pH and the temperature as well. The measurements are performed with an IsFet pH sensor (pH range from 0 to 14, temperature range from 0 up to 70°C).
EBC collection method with the EBC Collector F

The EBC Collector F allows the non-invasive collection of non-gaseous contents in exhaled breath air. This includes metabolic products from cells in the airways as well as from other organs, reaching the lung over the blood streams. Also, all the exhaled substances, which entered the body through the lungs, the alimentary canal or over the skin. The physiological features of exhaled substances use different ways into exhalation: as a volatile compounds, steam or aerosol. Therefore, the EBC analysis addresses important research needs by allowing non-invasive assessment of biochemical and inflammatory biomarkers in the airway. Measuring the biomarkers in the breath air is a very attractive approach to monitoring the short-term effect of interventions (i.e. medications) as well as the chronic diseases (i.e. asthma and COPD inflammation). The compounds which the most often identified in EBC are: hydrogen peroxide, isoprostanes, leukotrienes, nitrogen oxides, peptides and various ions. Additionally, the change of the pH level of the breath air is a assumed as a marker of the asthma and COPD.

For collection, these sensitive processes strongly rely on breathing pattern, depth and frequency of breathing. Additionally, for the standardisation of measurements, the time of sampling, exhaled volume and amount of condensate should be monitored during each collection as well. Relating measured substances in exhaled volume and/or collection time allows better intra-individual comparisons. The EBC collection has to characterise a way of sampling of the breath air which can be repeated frequently within only short breaks and with good reproducibility in EBC volume as well as in concentration for tested markers. These requirements fulfil the EBC-collector, which has a built-in spirometer to record breathing volumes and time for standardisation. It also controls the splitting of exhalation in up to four fractions. Control of the amylase activity and the viscosity - used to validate the method - show that no contamination of the sample by the saliva is observed. The measurements can be done with every patient or test person which is capable of spontaneous respiration. Only a constant rest respiration will be required with a duration of 5 to 15 minutes according to sample volume needed. Because of the short time and effortlessly to control the course of the measurements, this method can also be used in the routine analyses and in screening tests where the measurements should be repeatable as much as possible. No change due to the sampling analysis has been observed in the sample compared to the sampling of sputum. Immediate analysis of the collected condensate regarding the measurement of hydrogen peroxide (H2O2), L-lactate and pH may be done immediately after collection for example with the EBC analyser (ECOcheck) system.

Recommended publications of EBC collection and analysis:

- **Variability of exhaled breath condensate leukotriene B4 and 8-isoprostane in COPD patients**
  Int J Chron Obstruct Pulmon Dis, Volume 2, 2007, Pages 71-6
  Z.L. Borrell, R.C. Starkey, S.D. Singh

- **Gas chromatography/mass spectrometry analysis of exhaled leukotrienes in asthmatic patients**
  P. Cai, J. Chimade, F. Pehal, M. Mal, V. Petr, P.J. Barnes, P. Montuschi

- **Influence of conditioning equipment and temperature on exhaled breath condensate pH, total protein and leukotriene concentrations**
  Respiratory Medicine, Volume 102, Issue 5, 2008, Pages 720-725
  K. Creze, I. Barta, B. Antus, M. Valyony, I. Horvath, T. Kullmann

- **Quantitative Assessment and Repeatability of Chlorine in Exhaled Breath Condensate**
  Respiratory Medicine, Volume 72, No. 5, 2005, Pages 529-536
  A. Davidson, K. Naidsz Sjöström, L. Lundman, B. Schmeekel

- **Factors influencing breath condensate volume**
  Pneumologie, Volume 55, 2001, Pages 414-419
  C. Gesnzer, H. Kuhn, M. J. Seyfarth, H. Pankau, I. Winkler, J. Schauer, H. Wirtz

- **Exhaled breath condensate: An evolving tool for noninvasive evaluation of lung disease**
  Journal of Allergy and Clinical Immunology, Volume 110, Issue 1, 2002, Pages 28-34 John Hunt

- **Comparison of Biomarkers in Exhaled Breath Condensate and Bronchoalveolar Lavage**
  American Journal of Respiratory and Critical Care Medicine, Volume 175, 2007, Pages 222-227
  A.S. Jackson, A. Sandri, K. Campbell, S. Choe, P. Thomas, D.H. Yates

- **Effects of breathing pattern and inspired air conditions on breath condensate volume, pH, nitrite, and protein concentrations**
  Thorax, Volume 59, 2004, Pages 694-698
  J.B. McCafferty, T.A. Bradshaw, S. Tate, A.P. Greening, J.A. Innes

- **Differences in exhaled breath condensate pH measurements between samples obtained with two commercial devices**
  Respiratory Medicine, Volume 101, Issue 8, 2008, Pages 1715-1720
  L. Prieto, A. Ferre, J. Palop, J. Domenech, R. Lluis, R. Rosas

- **Method optimization and validation for the simultaneous determination of arachidonic acid metabolites in exhaled breath condensate by liquid chromatography-electrospray ionization tandem mass spectrometry**

- **Biomarker reproducibility in exhaled breath condensate collected with different condensers**
  Eur Respir J, Volume 31, 2008, Pages 934-942

**Breath condenser coatings affect measurement of biomarkers in exhaled breath condensate**

- **Multiplex analysis of cytokines in exhaled breath condensate**

- **Comparison of two methods for exhaled breath condensate collection**
  Allergy, Volume 61, Issue 8, 2006, Page 1016-1018

- **Validation of a new method to measure hydrogen peroxide in exhaled breath condensate**
  Respiratory Medicine, Volume 99, Issue 9, 2005, Pages 1132-1137
  W.B. Gerritsen, P. Zanen, A.A. Bauwens, J.M. van den Bosch, F.J. Haas

- **Evaluation of H2O2 and pH in exhaled breath condensate samples: methodological and physiological aspects**
  Biomarkers, Volume 13(3), 2008, Pages 319-41
  H. Knobloch, G. Becker, M. Decker, P. Reinhold

- **Exhaled breath condensate in patients with asthma: implications for application in clinical practice**
  Clinical & Experimental Allergy, Volume 38 Issue 4, 2008, Pages 557-565
  K. Kostikas A. Koutsokera S. Papiris K.I.ourgoulionaris S. Loukides

- **H2O2**

- **Markers of inflammation and oxidative stress in exacerbated chronic obstructive pulmonary disease patients**
  Respiratory Medicine, Volume 99, Issue 1, January 2005, Pages 84-90
  W.B. Gerritsen, I. Aisik, P. Zanen, J.M. M van den Bosch, F.J.L. Haas

- **Exhaled breath condensate: methodological recommendations and unresolved questions**
  Eur Respir J, Volume 26, 2005, Pages 523–548
  I. Horvath, J. Hunt, P.J. Barnes

- **Exhaled Markers of Pulmonary Disease**
  Am J Respir Crit Care Med, Volume 163, 2001, Pages 1699–1722
  S.A. Kharitonov and P.J. Barnes

Collection of exhaled breath condensate and analysis of hydrogen peroxide as a potential marker of lower airway inflammation in cats

The Veterinary Journal, Volume 169, Issue 3, 2005, Pages 385-396

- **N. Kirschvink, D. Marlin, F. Delvaux, J. Leemans, C. Clercx, A. Sparkes, P. Reinhold**

**Clinical tests proved that the concentration of the marker H₂O₂ is 2–3 times higher in the central respiratory tract than from the alveolar periphery.**

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**Procedure of fractional EBC collection**

The spirometer controlled collection of EBC can be defined by the following parameters in the setting:

Vges = Total volume of the whole collection of exhalations, SampleVol per exhalation = Setting for the acoustic signal to control patients breathing. VVol1 for wasting a defined volume, if more peripheral breathing gas is targeted in sample volume 1 – SV1, VVol2 for wasting a defined volume, if more peripheral breathing gas is targeted in sample volume 2 – SV2.

The diagrams demonstrate the two modes:

**Example 1 – Accurate sample volume**

The collection happens exactly according SV1 + SV2, the rest volume until end expiration is wasted. The total volume Vges is exactly the addition of (SV1 + SV2) times exhalation numbers. SampleVol per exhalation SolV has to be higher than the sum of SV1 + SV2.

**Example 2 – Sample volume until end expiration**

The collection is the accumulated out of SV1 + SV2 until end expiration. SV2 has to be higher than SolV. That means that the sample volume 2 is dependent from the difference of the patient exhalation volume and contains the breathing gas until the end of expiration. The total volume Vges is different from the calculated SV1 + SV2 times exhalation numbers.

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**Example 1 – Accurate sample volume**

Vges: 1000; SolV: 1100ml; SV1: 100ml; SV1: 500 ml; SV2: 0 ml; SV2: 500 ml.

IN
EX
V1
V2
end Ex

**Example 2 – Sample volume until end expiration**

Vges: 1000; SolV: 1000ml; SV1: 100ml; SV1: 500 ml; SV2: 0 ml; SV2: 1500 ml.

IN
EX
V1
V2
end Ex
**EBC-Collector**

*ECoScreen 2*

**Technical Data**

- **Size (H x W x D):** 50 x 18.5 x 37 cm
- **Weight:** approx. 17 kg
- **Voltage:** 220-230 V (115V on request), 50-60 Hz
- **Power consumption:** 150 VA

**Ambient specifications**

- **Temperature range:** 5-30°C / 41-86 °F
- **Flow measurement with Venturi principle**
- **Flow range:** up to 10 L/s
- **Accuracy:** ± 5 %
- **Temperature control:** ± 1 °C

**Spare parts**

- collection bags one way
- Valve unit
- Calibration pump
- below valve
- other please ask

**Option**

file transfer via USB connection

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**EBC-Analyzer**

*ECoCheck*

**Technical Data**

- **Size:** 15.5 x 12.5 x 30 cm
- **Weight:** 3.0 kg
- **Voltage:** 220-230 V (115V on request), 50-60 Hz
- **Power consumption:** 50 VA

**Ambient specifications**

- **Temperature range:** 5-30°C / 41-86 °F
- **Storage of H2O2 Sensors:** 2 - 8 °C
- **Storage of Lactat Sensors:** -10 to –25°C
- **Storage of Calibration-Kit:** 2-8 °C
- **Sensitivity:** 30 – 3,000 nmol/L
- **Accuracy:** ± 5 %

**Spare parts**

- H2O2 biosensors
- Lactate biosensors
- pH-probe
- buffer kits,
- Calibration kits

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