eppendorf

BIO NEWS

No. 36 – 2012

Eppendorf BioSpectrometer® – Simply Spectracular!

- Eppendorf PiezoXpert® in Action
- The ep*Motion*® GxP System Solution
- Premium Solutions for Forensics and Cell Care


Intracytoplasmic Sperm Injection with the Eppendorf PiezoXpert®

Easy Determination of Enzyme Activities with the Eppendorf BioSpectrometer® kinetic · Intracytoplasmic sperm injection with the Eppendorf PiezoXpert® · etc.

Dear Reader!

We wish you a great start into 2012! This new edition of Eppendorf BioNews offers you once again a wide variety of news and innovations from Eppendorf.

Small, compact and simply "spectracular" – the best way to describe our new Eppendorf BioSpectrometer basic and Eppendorf BioSpectrometer kinetic photometers – a spectrometer line specially designed for the modern life science laboratory (pages 4-5).

A giant leap for piezo-assisted micromanipulation is performed by the Eppendorf PiezoXpert – the new star of our Cell Technology product portfolio. Read more about its gentle force on page 9 and on pages 1-2 of the Application Notes!

Are you looking for a device for efficient and gentle vacuum concentration, then look no further than our new Concentrator plus! The 2011 winner of the "red dot design award" convinces with a variety of great features and more capacity than ever before (page 7).

We hope you enjoy reading this blend of editorial content, Application Notes, news and the ever-popular prize competition.

Your BioNews Editorial Team

IMPRINT

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Eppendorf BioNews Application Notes

TANJA MUSIOL, EPPENDORF AG

Eppendorf BioSpectrometer® – Simply Spectracular!

Photometric analyses are among the most frequently used applications in modern laboratories and are often found at the very beginning of the process chain. Follow-up tests are planned and new test series are prepared according to photometric determinations. If these determinations are based on incorrect data, the errors could accumulate exponentially throughout the process chain and, in worst case scenario, cause the tests to fail. That is precisely why dependable and accurate photometric measuring results are especially important.

Tradition meets innovation

For over 60 years, Eppendorf has produced and developed photometers. This broad range of expertise has been used to develop a new family of spectrometers, specially designed to meet the requirements of modern life science laboratories. The new Eppendorf BioSpectrometer product line is comprised of two models: the BioSpectrometer basic and the BioSpectrometer kinetic.

Whether for quantifying nucleic acids and proteins, optical turbidity measurement or more complex methods such as evaluating wavelength scans, multi-lambda applications or kinetic applications – just choose the device that fits your research needs best!

Both devices enable measurements in the UV/Vis range, and the recording of spectra or individual wavelengths in a range of 200 nm to 830 nm.

Tradition: Eppendorf has been developing and producing photometers for over 60 years.

The Eppendorf BioSpectrometer kinetic also features a cuvette shaft that can be temperature controlled. This allows you to determine enzyme and substrate kinetics right in the device without the need for separate accessories.

Small, compact and easy to operate

From simple analyses to complex applications – the new Eppendorf BioSpectrometer offers you a wide variety of applications in a small and compact device. Familiar elements featured in devices such as the BioPhotometer plus have been combined with the latest technology.

Although the Eppendorf BioSpectrometer offers a unique level of flexibility, it is extremely easy to use. The newly developed intuitive user guidance has been awarded the *"USEWARE Prize 2010 from the Association of German Engineers / Association of German Electrical Engineers and the Association for Measuring and Automation Technology for excellent user-friendly design of innovative technology."* The menu navigation guides you through the programming in a step-by-step process, thus minimizing the risk of errors or forgetting information to program. For each step, a help box will explain the operational sequences in the individual steps. Furthermore, the BioSpectrometer software also allows

New! *BioSpectrometer basic and BioSpectrometer kinetic*

users access to pre-programmed methods – a feature which has already proven its value many times over with Eppendorf photometers.

This unique combination of new and established technologies, and software components, ensures that the new Eppendorf BioSpectrometer delivers extremely dependable and reproducible results in an easy and convenient manner.

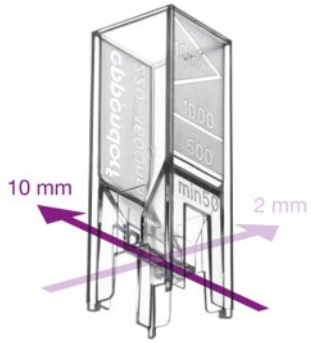
The combination makes the difference

Photometric measurements are only as good as the device in combination with the corresponding sample or measuring tube. Selecting the appropriate cuvette is just as important as choosing the right device. For example, choosing an optical light path which is too short for a low sample concentration can lead to incorrect results. Contamination or degradation of valuable sample material could have an even more dramatic effect. To ensure you easily find the perfect cuvette or microliter measuring cell for your application, volume or concentration, Eppendorf offers you the Cuvette Navigator.

The Cuvette Navigator can be found on our BioSpectrometer homepage at: www.eppendorf.com/biospectrometer.

Helpful hint!

You would like to learn more about the Eppendorf purity levels in order to select the perfect purity level for your valuable and sensitive samples (e.g., RNA)? You'll find everything you need at www.eppendorf.com/purities!



UVette®: Fully UV-transparent, disposable cuvette made of clear plastic with a light transmission between 220 nm and 1,600 nm

Eppendorf BioSpectrometer® • Ref. no. 242

News

Photometry Performance Plans

The optimal performance of your photometry precision instruments is an essential element for achieving continuously reproducible measuring results. Your instruments should be checked and adjusted at regular intervals to ensure they are running according to manufacturer specifications.

With our Photometry Performance Plans, we take care of the required cleaning, inspections, function tests, instrument adjustments and carry out software updates as necessary. With our Operational Qualification (OQ), comparison measurements are carried out using certified reference materials (acc. to NIST, Gaithersburg, MD, USA). Systematic and random errors plus particular wavelengths are tested. We confirm the verification with full documentation and a quality certificate.

epServices
for premium performance

The Performance Plans feature:

- Quality-tested, original Eppendorf spare parts
- Certified service reports and calibration reports

Your benefits:

- Reliably functioning system throughout the entire service life
- GLP conforming documentation
- Measurement traceability

Additional information is available at: www.eppendorf.com/epservices or on local websites.*

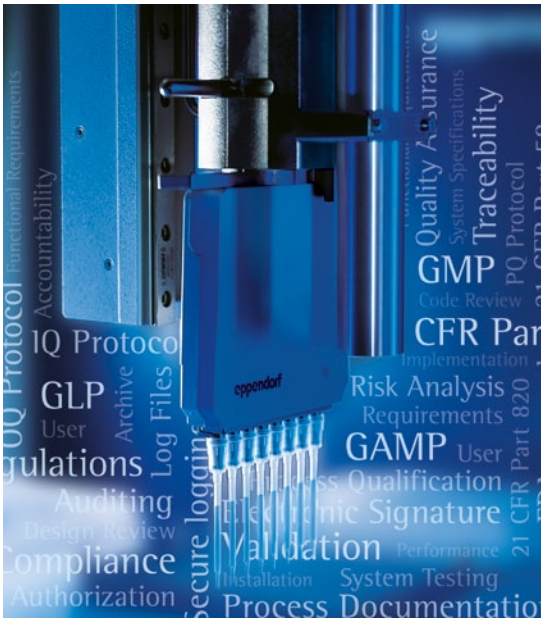
**Performance Plans are available in selected countries only and service offers may differ.*

CARSTEN BUHLMANN, EPPENDORF AG

High Standards for “Good Practice”

With GLP, GMP and GCP standards for regulated areas, global legislation has significantly increased the pressure on the pharmaceutical industry, biotechnology and clinical research. Although many liquid handling processes have been automated to make procedures faster and more precise, the necessary validation is very time consuming and labor-intensive. Conform to the strictest specifications, the new ep*Motion* GxP solution significantly reduces the validation process.

Regulated laboratories of pharmaceutical and biotechnology companies, their contractors, and clinical research companies are subject to regulations and guidelines that are becoming increasingly strict. The growing number of compliance topics companies are confronted with, both on the American and European markets, place high demands on the validation of processes and computer systems. The approval of any pharmaceutical or biopharmaceutical products on the American market require that the strict specifications of the CFR (Code of Federal Regulations) be observed. For the European Union, the EMA (European Medicines Agency) regulations apply. Interestingly enough, the EMA has prepared an update of the GMP (Good Manufacturing Practice): Appendix 11 on the use of computer systems and Chapter 4 on documentation. These revised requirements came into effect on June 30, 2011, for the EU, and are now comparable to the American 21 CFR Part 11 guidelines, which provide a binding regulation on handling electronic signatures and data which is stored and generated electronically.



In addition to the standards drafted by the CFR and EMA agencies, other quality regulations such as GLP (Good Laboratory Practice) or GCP (Good Clinical Practice) define general validation guidelines. GxP provides a summary of all guidelines for “good work practice”.

A wide range of laboratory equipment, instruments and analytical computer systems, including automated liquid handling systems such as the ep*Motion*, are used in regulated areas and are therefore subject to strict and time-consuming validation guidelines.

The new ep*Motion* GxP system solution was developed in accordance with GAMP 5 and fully aligned with the organizational and process-related requirements of 21 CFR Part 11, 58 (GLP), 211 (GMP) and 820 (QSR) and, as such, also to the GLP and GMP requirements for applications in Europe.

The ep*Motion* GxP system solution is made up of the automated ep*Motion* pipetting system, software, and services which are specifically designed to significantly reduce qualification and process validation procedures. As manufacturer, Eppendorf has already taken care of the major part of the regulatory required system validation and qualification, allowing users to fully concentrate on their application validation tasks (acceptance test).

Additional information (including our white paper on the precise technical implementation of requirements) can be found at: www.epmotion.com/gxp.

Or you can order our GxP solution brochure to learn more about new features like access control, complete electronic documentation, data security and integrity, electronic signature workflow management and more.

ep*Motion*® GxP system solution • Ref. no. 243

BJÖRN ARNOLD, EPPENDORF AG

Concentration the Easy Way

It's finally here! Available since July 2011, the new Eppendorf Concentrator plus has won over our customers with its modern design, compact size and simple operation. This exceptionally quiet instrument can be used for a wide variety of applications, in a volume range from 0.2 mL to 50 mL, as well as in microplates. With the new combi rotor F-35-6-30 for 15 mL and 50 mL conical tubes, fifteen rotors are now available.

Basic device or complete system

The new Concentrator plus is available in different configurations: as a complete system with integrated, service-free diaphragm pump or as a basic device for connection to an existing vacuum pump.

The complete "junction" system offers additional functions and increased flexibility. This means that the integrated vacuum pump can also be used separately – without needing to be removed from the system. Simply connect the port to the external device which needs a vacuum (e.g., gel dryer).

***New!** Concentrator plus: modern design, more capacity!*

***New!** F-35-6-30 rotor for 6 x 15 mL or 6 x 50 mL conical tubes*

Efficient and gentle vacuum concentration

The heating technology of the Concentrator plus optimizes the evaporation process. No matter which configuration you choose, you are assured of quick and efficient – yet gentle – vacuum concentration of your samples (e.g., DNA/RNA and proteins). The complete system includes a vapor condenser that purifies the exhaust air by up to 85 %, protecting you from unpleasant odors. Moreover, the automatic condensate drain extends the service life of all components that come into contact with vapors.

The Concentrator plus also features a centrifugation function for additional flexibility and a desiccator function to quickly dry, for example, filters. A wide range of fixed-angle rotors and the microplate rotor A-2-VC enable the Concentrator plus to be used for various applications in the laboratory.

Great features, safe and simple handling

- Four heating levels (room temperature, 30 °C, 45 °C, 60 °C) for safe and efficient concentration of a wide variety of samples
- Timer selection from 1 min to 9 h 59 min and ∞
- Mode/vent function: Choose between three different solvent modes (aqueous, alcohol, or high vapor pressure) for a processing time reduced by up to 20 %
- Chemical-resistant PTFE-coated diaphragm pump
- Chemical-resistant stainless steel chamber
- Brake function
- Imbalance stop
- Start/Stop of concentration process
- Extremely quiet operation, even with pump turned on
- Narrow width (33 cm) saves valuable bench space

Eppendorf Concentrator plus™ • Ref. no. 244

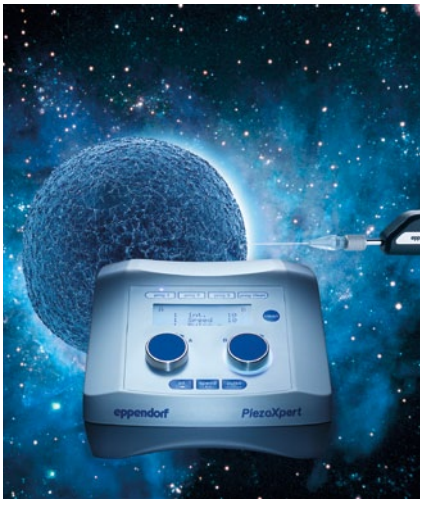
HEIDE NIESALLA, EPPENDORF AG

Our Expertise Orbits around Your Cells

In close collaboration with our customers, our engineers have developed a new device that allows for easier manipulation of cells. The Eppendorf PiezoXpert is used for piezo-assisted micromanipulation or microinjection. It enables highly precise and reproducible perforation of cell membranes. In cases where microinjection with a normal glass capillary is not possible (e.g., for injection of embryonic stem cells into mouse blastocysts or for mouse ICSI), the PiezoXpert is the ideal addition to your micromanipulation system.

The PiezoXpert convinces by "gentle force"

Our field testers were excited about the PiezoXpert. Especially the easy and reproducible installation was positively noted. But our test clients were equally impressed by the intuitive and ergonomic operation of the PiezoXpert; primarily the reproducible setting of the parameters Speed, Intensity and Pulse, as well as the simple triggering of the two sets of parameters via ergonomic foot control were appreciated.



Small (only 17x23 cm) but powerful: Eppendorf PiezoXpert®

The construction principle of the actuator, featuring direct transmission of the piezo impulses onto the microcapillary, ensures increased reproducibility for techniques such as mouse ICSI and injection of ES cells (please also see the Application Note in this issue, pp 1–2).

Three additional program keys on the PiezoXpert allow quick retrieval of saved parameter sets. The ultra-fast and powerful CLEAN function, used to remove debris from the capillary, helps speed up the work process.

Are you interested?

If you would like to learn more about the new PiezoXpert or about our micromanipulation systems, please visit our website

www.eppendorf.com/micromanipulation.

The brochure for the Eppendorf PiezoXpert is available from us and can be ordered by citing the ref. no. denoted below.

Eppendorf PiezoXpert® • Ref. no. 245

News

Customer Feedback on PiezoXpert

Interview with Dr. Irm Hermans-Borgmeyer (Head of the scientific service unit "Transgenic Animals", ZMNH, University Clinic Eppendorf, Hamburg, Germany)

You have tested the PiezoXpert intensively – for which applications do you use it?

We use the device to inject ES-cells into 4-cell and 8-cell, as well as into blastocyst stages.

Can you tell us what makes this device unique?

Firstly, it enables easy handling without the need to restructure our setup or use different capillaries. It is small and handy. I immediately felt comfortable with it – its operation is somewhat self-explanatory. Secondly, I find that it is extremely gentle on the embryos. We do not use the device exclusively for injections, but we also apply the CLEAN function to clean our injection capillary. This works really well.

How does the use of the PiezoXpert impact your work?

It makes our work a lot easier. We are much faster and also much more flexible. It saves us hours of work. When working with Balb mice, we often encounter the problem of obtaining embryos of different stages. With the PiezoXpert, we can simply inject into different stages on the same day without problems and without losing embryos.

The interview was conducted by Dr. Ilka Schneider (Eppendorf AG) and Dieter Knofe (Eppendorf Instruments GmbH).

Intracytoplasmic Sperm Injection with the Eppendorf PiezoXpert®

Michele Boiani, Max Planck Institute for Molecular Biomedicine, Münster, Germany

Here we describe the ICSI procedure for mouse using the new Eppendorf PiezoXpert and the Prime Tech piezo system (PMM), a piezo-drill device described elsewhere in combination with Eppendorf micromanipulators for mouse ICSI [2].

With the modelling of human disease through genetically modified mice, there is an increasing requirement for efficient and dependable techniques to preserve these often unique genomes. Cryopreservation of sperm, a much more efficient method than cryopreservation of embryos, allows for reduction of their number while preserving the genomes. Moreover, in some cases, the mouse genomic background or the genetic modification itself can lead to low values in sperm counts, viability or motility, meaning natural matings and *in vitro* fertilization (IVF) will not work well, so intracytoplasmic sperm injection (ICSI) of mouse oocytes offers a solution to overcome this problem. The combination of ICSI with sperm freezing and embryo transfer is a valuable resource for a research mouse facility and its service unit.

In 1995 Kimura and Yanagimachi [1] introduced an improved way to microinject sperm into sensitive mouse oocytes, supported by a piezo device that drove a blunt-ended capillary in what resembled a 'drilling' of the oocyte. However, blunt-ended piezo-driven microcapillaries are difficult to use, not only because of the manufacturing, the need to fill them with heavy fluids such as mercury or Fluorinert (FC770), but also because of the difficulty of finding the 'right' settings on the control box of the piezo device. Common piezo devices show an unsatisfactory energy transfer, i.e. ability to drill the zona pellucida because the microcapillary oscillates not only axially (desired) but also laterally (undesired). In our field test we found that the microcapillary oscillations generated by the PiezoXpert are largely free of lateral oscillations and thereby facilitate mouse ICSI reliably.

Equipment
Inverted microscope Nikon TE2000 fitted with ELWD 4X and 40X DIC objectives (Nikon Corporation, Tokyo, Japan)
Micromanipulator (Narishige, Tokyo, Japan)
Micromanipulation chamber (homemade)
Eppendorf PiezoXpert
Prime Tech piezo system (PMM-150 FU, Prime Tech Ltd., Ibaraki, Japan)
CellTram vario microinjector (Eppendorf) for control of the ICSI microcapillary
Self-pulled capillaries for holding and injection
ThermoPlate (Tokai Hit, Shizuoka-ken, Japan) fitted to the stage of the microscope, room temperature 28–29 °C

Setup of the PiezoXpert

Connect the PiezoXpert to the CellTram vario microinjector. (In our laboratory the CellTram pressure circuit is filled with water.) Fit the capillary to the "actuator", which consists of the capillary holder and the Piezo element and install the PiezoXpert on the micromanipulator.

Push the mercury to the tip of the capillary using the CellTram vario. Lower the capillary into the medium containing 0.1 % PVP of the micromanipulation chamber.

Adsorb a small amount of medium via the front capillary opening, so that the samples will not get in contact with mercury or Fluorinert respectively.

Injection of single sperm heads into oocytes (Fig. 1 A–I)

Transfer approx. 0.5 µL of concentrated sperm suspension (10 x 10^6 sperm/mL) to the medium in the north sector of the micromanipulation chamber. Place about 10–30 oocytes in the medium in the south sector of the micromanipulation chamber. Each group should be processed within 15 min.

Decapitation of sperms: Using the CellTram vario, aspirate 100–200 sperms into the capillary while applying strong piezo pulses (for setting values see Table 1 on the next page). This way 10–20 % of the spermatozoa are 'decapitated', i.e. separated into head and midpiece plus tail.

Pick a single oocyte with the holding capillary and roll it using the ICSI capillary until the MII spindle is at 6 or 12 o'clock out of the injection's pathway.

Critical step: Avoid drilling the zona if the MII spindle is underneath. The cytoplasm of the MII spindle is harder than the rest of the cytoplasm, and if punctured it will decrease survival rates after ICSI.

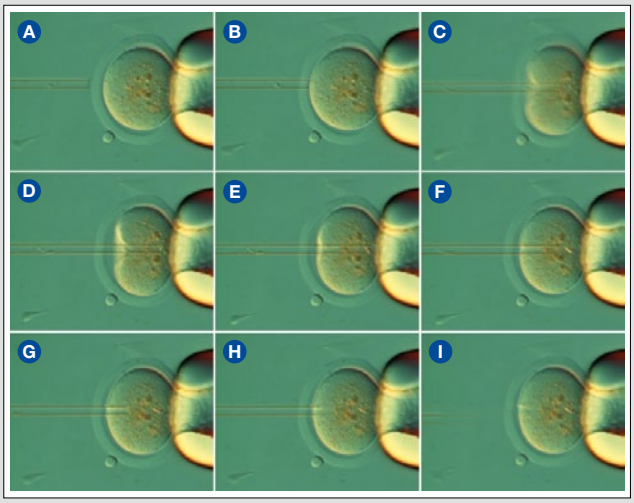


Fig. 1: *Sequence of events during ICSI of one mouse oocyte. A, B: Capillary drills the zona pellucida. C, D: Capillary penetrates through the zona pellucida and crosses the oocyte diameter. E: Rupture of the oolemma. F: Injection of a single sperm head. G–I: Retraction of the capillary*

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Intracytoplasmic Sperm Injection with the Eppendorf PiezoXpert®

Bring a single sperm head to a distance of 20–40 µm from the tip of the capillary. Juxtapose the tip of the capillary to the zona pellucida and apply 2–4 piezo impulses to pierce through it (A, B). Insert the capillary through the hole and cross the oocyte diameter without applying any pulse (C, D).

As the opposite side is reached, apply one single piezo pulse to puncture the oolemma at the capillary's tip, so as to make a hole (see Table 1).

Successful penetration of the oolemma is indicated by a rapid relaxation of the oolemma (E).

Critical step: Do not apply the piezo impulse until the capillary has reached the opposite side. If the piezo pulse is applied in the middle of the oocyte, the oocyte will lyse.

Release the sperm head inside the egg. (Keep the coinjected volume of medium to minimum.) Withdraw the capillary quickly but gently (G–I). Allow the injected oocytes 5–10 min recovery on the stage before returning them into the incubator.

Critical step: Do not let the detached sperm heads wait longer than 20 min before injecting them, as the sperm-borne oocyte activation factor (SOAF; [3]) may degrade.

Results and discussion

For our field test, 243 mouse oocytes were subjected to ICSI, using the PiezoXpert in combination with CellTram vario and mercury-filled microcapillaries. We compared the PiezoXpert results with those obtained in our routine application of the PMM in the same time period. Under the experimental conditions (Table 1), survival rates after microinjection with PiezoXpert were comparable to those afforded by the PMM. Mouse development after ICSI with PiezoXpert was successful: The fertilized mouse oocytes developed to blastocysts *in vitro*, and to full-term *in vivo* (Table 2).

Zona pellucida		Oolemma
Channel A	Parameters	Channel B
10	Intensity	9
5	Speed	5
∞	Pulse	∞

Table 1A

Sperm decapitation	
Channel A	Parameters
26	Intensity
10	Speed
∞	Pulse

Table 1B

Table 1A and 1B show different parameter settings for the drilling of zona pellucida and oolemma and the decapitation of sperm with the PiezoXpert. Values may vary depending on individual laboratory protocols, e.g. condition of cells, capillary or tube (injector) filling.

	n Oocytes	Survival rate	Blastocyst rate	Full term rate
PiezoXpert	243	0.82	0.56	0.13

Table 2: *Performance of the PiezoXpert at ICSI in the Boiani laboratory*

Sixty embryos produced with PiezoXpert were transferred to the genital tract of 3 pseudopregnant recipients, 2 of which became pregnant and delivered 8 pups.

Although it is difficult and premature to compare developmental rates across different piezo systems, some differences were apparent. We noted that the microinjection capillary driven by the PiezoXpert had lower extent of lateral oscillation than the PMM, as judged from the 'jumping' movement of the oocytes during drilling. Overall, we found that the PiezoXpert was easier to use, and had higher drilling efficiency than the PMM. At present there is little clarity as to the mode of action of piezo-driven micro-capillaries, as well as to the contribution of the mercury-filled capillary to the outcome. The way the piezo-driven capillary pierces through the zona pellucida and makes a hole in the oolemma may be due to the pressure burst, which is caused by the abrupt axial motion of the mercury column. However, evidence from high-speed camera imaging also points out the occurrence of lateral tip oscillations of the capillary when piezo impulses are applied [4], [5].

The addition of mercury in the capillary is intended to suppress the undesired lateral oscillations to improve the drilling efficiency [6]. We assume that the reduction of lateral oscillations may explain why the efficiency of zona drilling of the PiezoXpert was superior to the PMM.

In conclusion, while both PMM and PiezoXpert deliver satisfactory results in skillful hands, the PiezoXpert proved to be easier to use and more efficient at zona piercing and oolemma penetration, probably due to a lower extent of lateral oscillations.

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The complete version of this Application Note (No. 238) can be downloaded in PDF format at www.eppendorf.com/applications.

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Easy Determination of Enzyme Activities with the Eppendorf BioSpectrometer® kinetic

Martin Armbrecht, Eppendorf AG

All life, as we know it, is based primarily on biochemical processes, which depend on the actions of proteins, or enzymes, respectively. Enzymes are catalytically active proteins which, as catalysts, facilitate chemical reactions by reducing the energy required. It is enzymes which make certain metabolic pathways within an organism possible at all.

In general, the action of enzymes may be pictured as such: The chemical compound which is converted by the enzyme is called the substrate. The substrate binds to the enzyme via the substrate-binding pocket and is subsequently converted, presumably by a change in shape of the enzyme.

The result is the product of the enzymatic reaction (Fig. 1).

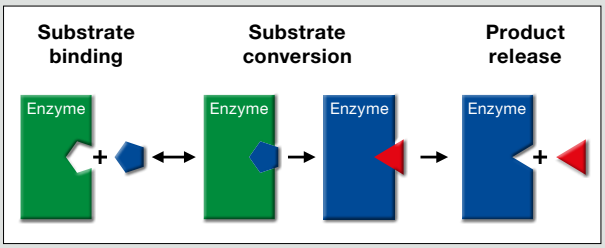

Fig. 1: *Schematic sequence of events during an enzymatic reaction*

Important information about their role in metabolic processes can be gained from measuring enzyme activity. Generally, the increase in product, or the decrease in substrate concentration are measured photometrically by changes in absorption over a defined time interval (Fig. 2).

Enzyme activity is calculated from the change in absorbance over a defined time interval:

$$\text{Activity} = \frac{\Delta A}{\Delta t} = \frac{A2-A1}{t2-t1}$$

As shown in Fig. 2, in the beginning absorbance increases in a linear fashion. At this time, sufficient substrate is available in the reaction solution. However, over time the amount of substrate will decrease, and the conversion rate of the enzyme will slow down. The curve begins to flatten. For this reason, measurement of enzyme activity is restricted to the linear range of absorbance increase.

The reaction speed of an enzyme is dependent on different factors besides substrate concentration: Ambient temperature and pH, as well as certain helper and co-factors influence enzyme activity significantly.

Unfortunately, many products or substrates of enzymatic reactions cannot be directly determined by photometric means. In these cases it is of great advantage that certain co- or helper factors, e.g., NAD(P) or NAD(P)H_2 are involved in almost all enzymatic conversions in central metabolism. These factors which serve as either electron donor or electron recipient are easily detectable by photometry.

Enzyme activity is determined via increase or decrease in NAD(P)H_2, respectively, depending on whether NAD(P)H_2 is generated or used up during a given enzymatic reaction. Both NAD(P) and NAD(P)H_2 have an absorbance maximum at 260 nm. However, NAD(P)H_2 shows an additional maximum at 340 nm (Fig. 3), thus providing a means to differentiate between NAD(P)H_2 and NAD(P) by photometric methods.

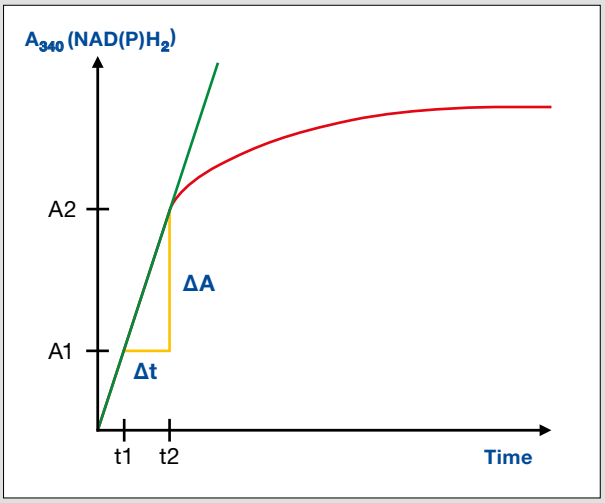

Fig. 2: *Absorbance curve of an enzymatic reaction, as captured by a spectrophotometer*

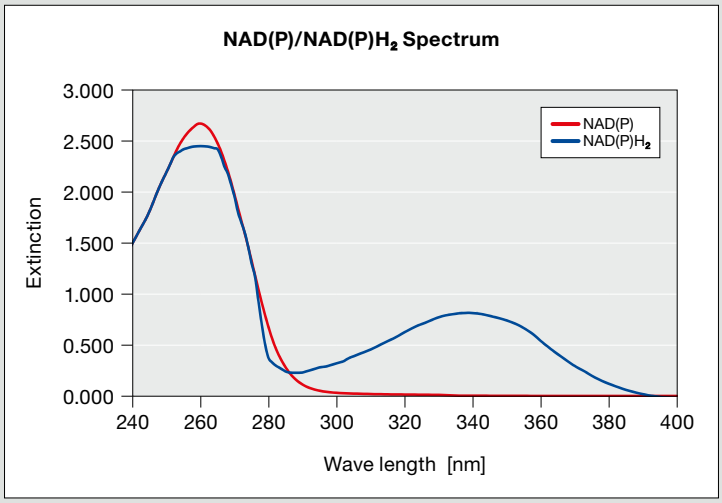

Fig. 3: *Absorbance spectra of NAD(P)/NAD(P)H_2. Both components can be easily differentiated by their absorbance at 340 nm. Hence, the activities of NAD(P)/NAD(P)H_2-dependent enzymes are determined by a change in absorbance at 340 nm.*

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Easy Determination of Enzyme Activities with the Eppendorf BioSpectrometer® kinetic

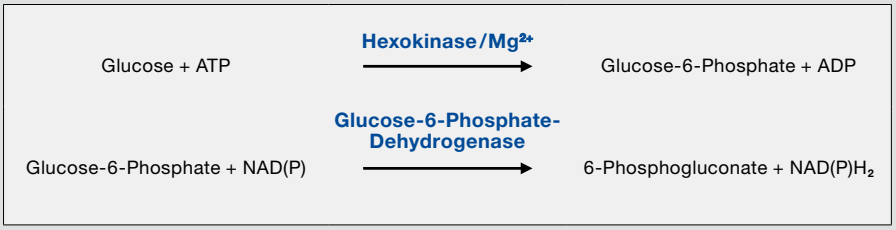

Fig. 4: *Hexokinase activity is determined via a coupled second reaction during which $NAD(P)H_2$ is generated.*

Fig. 4 shows the catalytic conversion of hexokinase, an enzyme from central metabolism.

Linear regression analysis on the BioSpectrometer kinetic during activity measurements

The Eppendorf BioSpectrometer kinetic enables comfortable and reproducible determination of enzyme activities. The temperature can be set accurately via an integrated Peltier element, thus ensuring that the measurement performed in the BioSpectrometer occurs at the optimal temperature for the enzyme to be studied.

Furthermore, end point and two point determinations, along with linear regression, offer three options for evaluation of enzyme activity. It is to be emphasized that during evaluation via linear regression, the start and end points of the compensation curve may be set retroactively to allow precise capture of the linear range of the enzyme kinetic.

Prior to measurement, the parameters need to be determined in the Eppendorf BioSpectrometer kinetic (Fig. 5).

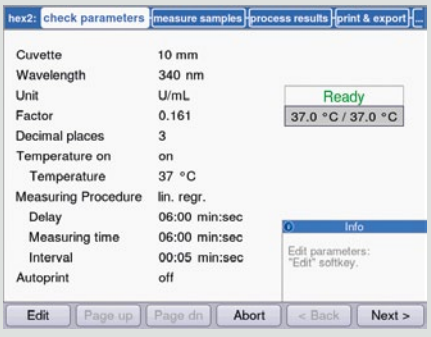

Fig. 5: *Parameter settings for an enzymatic measurement in the Eppendorf BioSpectrometer® kinetic*

In addition to the temperature at which the measurement is to be conducted, cuvette path length and the wavelength, the option of calculating the enzyme activity directly via a compensation factor exists.

This factor is derived from the absorption coefficient of the substrate or product to be measured. Furthermore, a pre-incubation step may be programmed using the “Delay” function. This function ensures that measurement is not initiated until the final temperature is reached inside the reaction solution. In order to save time, the measurement time may be exactly defined (“Measuring time”). Also, depending on the speed of the reaction, the interval between the points of measurement may be varied (“Interval”).

If enzyme activity is determined using linear regression, the compensation curve will be overlaid across the entire range following completion of the measurement. If the compensation curve does not correspond to the linear range, the result will be shown in “red”. An additional warning will be shown in a “help box” (Fig. 6).

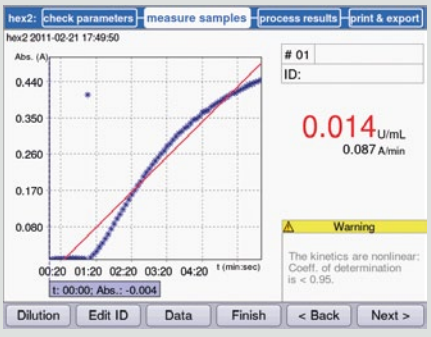

Fig. 6: *Linear regression directly after measurement*

By resetting the start and end points of the compensation curve, the linear regression may be refitted. The quality of the refitted compensation curve is defined by the determination constant ($R^2 > 0.95$), see Fig. 7

Fig. 7: *Refitting of the linear regression*

Following the fitting of the linear regression, there is the option to have all information pertaining to the coefficient of determination, i.e. start and end point of the measurement, as well as the formula for the compensation curve, displayed clearly in table format (Fig. 8).

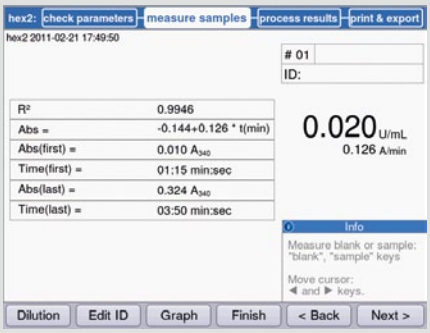

Fig. 8: *Overview of all important parameters in table format*

Conclusion

Through simple adaptation of the parameters, such as measurement window for the linear regression, setting of the temperature optimum and the option of pre-incubating the reaction solution, the Eppendorf BioSpectrometer kinetic offers comfortable tools for easy and reproducible determination of enzyme activity.

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Technical Report: The Eppendorf Research® plus Pipette – Full Autoclavability, Easy Adjustment, Quick and Simple Maintenance

Kornelia Ewald, Eppendorf AG

Introduction

In order to meet the high requirements of today's laboratories, modern piston stroke pipettes should be partly or fully autoclavable as well as UV-resistant. The new Eppendorf Research plus pipettes can be decontaminated either by UV-light or by autoclaving the entire instrument. Thus, cleaning following use with infectious or contaminated samples is easily possible.

Furthermore, changing the pipette's adjustment is an important feature when liquids of a density different from water are dispensed, or when it is found, during calibration, that the factory adjustment of the pipette has been changed by certain external influences. The possibility of adjusting the Research plus pipette allows the correct and easy dispensing of liquids with different densities.

Re-adjustment for specific liquids or geographical altitudes

During production, piston stroke pipettes are adjusted to distilled water under certified measuring conditions. To indicate the adjustment, all Research plus pipettes carry an adjustment seal. If necessary, re-adjustment for specific liquids or for altitude can be carried out easily. The red adjustment seal, which is applied to the adjustment opening following re-adjustment, serves to visualize a change of adjustment (Fig. 1). A novel feature of the Research plus pipettes is the additional indication of a change to the factory adjustment through the adjustment window (Fig. 2). Here, the exact change of adjustment is immediately visible (Fig. 3), even if the original seal was removed and the adjustment opening is possibly open. Using the adjustment window, changing the adjustment back to the original setting is possible. In every case, a change in adjustment needs to be verified gravimetrically.

Settings for user adjustment

Note: The setting values in Table 1 are for orientation only as the systematic and random errors are affected by the operation, the tip used and other factors (e. g., temperature). The data were calculated for wall dispensing. The tips were not pre-wetted. For PCR master mix (5 PRIME HotMasterMix) settings epT.I.P.S. LoRetention were used.

A new tip was used for each pipetting. The work was carried out relatively quickly and thus under realistic conditions. It is essential to check the data according to your own work method.

To change the factory adjustment please read the operating manual for the Eppendorf Research plus (www.eppendorf.com/researchplus).

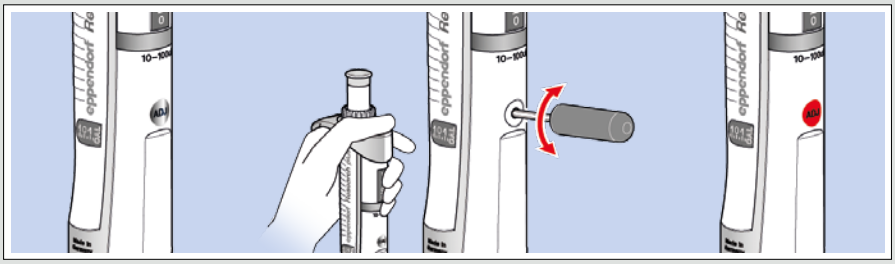

Fig. 1: *Original adjustment seal* *Adjustment change* *Adjustment seal following adjustment change*

Fig. 2: *Factory adjustment*

Fig. 3: *Change of adjustment*

Adjustment change procedure

If the adjustment is changed, the volume changes by a certain value. Note: the change only applies to the testing volume.

Example: You readjust a 10–100 µL pipette with a volume setting of 100 µL by 1 µL (1 µL = 1 %). If the volume setting is 10 µL, the pipette is also adjusted by 1 µL (= 10 %).

1. ***Remove the grey calibration seal.***
2. ***Keep the ejector pressed.***
3. ***Insert the adjustment tool (from the delivery package).***
4. ***Turn the adjustment tool until the desired value is displayed on the adjustment display.***
5. ***Carry out weighings to verify accuracy and precision.***
6. ***After the checks, stick the red adjustment seal (from the delivery package) on.***

If the adjustment is meant for a specific liquid, mark the pipette accordingly. Use the labeling area on the pipette for this purpose and write down the liquid and the volume. Carry out a gravimetric test for each change to the adjustment. Follow the test procedures of EN ISO 8655-2 and 8655-6.

An SOP (Standard Operation Procedure) for the test is available on our website www.eppendorf.com/sop.

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	2–20 µL grey	2–20 µL yellow	10–100 µL yellow	20–200 µL yellow	30–300 µL orange	100–1.000 µL blue	0,5–5 mL violet	1–10 mL turquoise
DMSO 99.8 %	-4.5 to -3.5	-4.5 to -3.5	-4.5 to -3.5	-4.5 to -3.5	-4.5 to -3.5	-2.5 to -1.5	-2.5 to -1.5	-0.5 to 0.5
H_2SO_4 98 %	-0.5 to 0.5	-0.5 to 0.5	-0.5 to 0.5	-0.5 to 0.5	-0.5 to 0.5	1.5 to 2.5	4.5 to 5.5	7.5 to 8.0
H_3PO_4 85 %	-0.5 to 0.5	-0.5 to 0.5	0.5 to 1.5	1.5 to 2.5	1.5 to 2.5	1.5 to 2.5	4.5 to 5.5	7.5 to 8.0
PEG 400 40 %	-0.5 to 0.5	-0.5 to 0.5	-0.5 to 0.5	-0.5 to 0.5	-0.5 to 0.5	-0.5 to 0.5	-0.5 to 0.5	-0.5 to 0.5
Glycerol 50 %	0.5 to 1.5	0.5 to 1.5	0.5 to 1.5	0.5 to 1.5	0.5 to 1.5	0.5 to 1.5	0.5 to 1.5	1.5 to 2.5
NaOH 40 %	2.5 to 3.5	4.5 to 5.5	-0.5 to 0.5	1.5 to 2.5	2.5 to 3.5	0 to 1.0	3.5 to 4.5	5.5 to 6.5
Cesium chloride 45 %	6.0 to 7.0	6.0 to 7.0	2.5 to 3.5	2.0 to 3.0	2.0 to 3.0	1.5 to 2.5	1.0 to 2.0	4.5 to 5.5
PCR master mix	7.5 to 8.0	7.5 to 8.0	7.5 to 8.0	7.5 to 8.0	7.5 to 8.0	7.5 to 8.0	not tested	not tested
Ethanol 99.8 %	7.5 to 8.0	7.5 to 8.0	7.5 to 8.0	7.5 to 8.0	7.5 to 8.0	7.5 to 8.0	7.5 to 8.0	7.5 to 8.0

Table 1: *Settings for user adjustment*

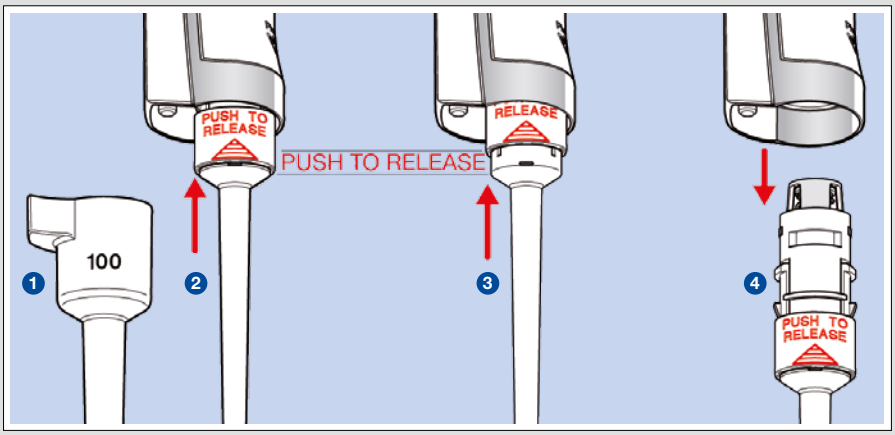

Fig. 4

Autoclaving and UV-sterilization

Modern piston stroke pipettes are either fully autoclavable, or the parts which become contaminated during improper use can be autoclaved. Thus, remaining doubts of the user regarding sterility can be dispelled, opening up new fields of application. Autoclaving of air cushion pipettes and pipette tips (with the exception of filter tips) is normally performed at 121 °C at an excess pressure of 1 bar (100 kPa) for 20 min.

The new pipette Research plus can be fully autoclaved without becoming discolored. Following autoclavation, the pipette needs to dry completely and cool down. In case the pipette was autoclaved in parts, all parts need to cool completely prior to re-assembly. Otherwise, plastic parts may be overexpanded and damaged. Greasing of the pipette piston following autoclaving is not necessary with Eppendorf pipettes.

UV-resistance of the plastic materials used in the production of a pipette is of central importance for many areas of application. UV-resistant pipettes, such as the Research plus, can remain in areas of cell culture labs without risk, since the UV-light used to disinfect these work areas will not have any adverse effects on the pipette material, nor on the function of the pipette.

For decontamination using UV-light, a 30 Watt low-pressure mercury-vapor lamp with a wavelength of 254 nm is to be used. The optimal distance between lamp and pipette is appr. 60 cm.

The following methods for cleaning or decontamination of the Research plus pipette may not be combined:

- Disinfectants, DNA-/RNA-decontaminating agents or sodium hypochlorite with additional
- Steam autoclaving or UV-irradiation.

Specifics regarding maintenance and cleaning

The Research plus pipettes are easy to clean. The lower part should be cleaned on a regular basis, in accordance with the frequency of use (Fig. 4). Push down the ejection button and pull off the ejector sleeve (1); then slide up the ring on the lower part labeled “PUSH TO RELEASE” (2) by appr. 5 mm (3) until the lower part is released. Take the lower part out of the upper part (4).

Detailed instructions on the maintenance and cleaning of multi-channel pipettes can be found in Application Note 198 (p. 9) that you can download at www.eppendorf.com/applications.

Outlook

In today’s research environment, function and handling forces of a pipette play important roles. However, additional deciding factors include simple cleaning and maintenance procedures, as well as decontamination by autoclaving and UV-irradiation. The simple and secure adjustment of a piston stroke pipette is one further demand during daily laboratory routine.

The new Research plus pipettes meet these criteria in every respect and are thus the ideal instrument for everyday pipetting in the laboratory.

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Time Savings as well as Improved Reproducibility through Centrifugation at 30,000 x *g*

Heike Frerichs, Stephanie Max, Natalie Worku
Institute for Hygiene and Environment, Hamburg, Germany

Abstract

Protocol optimization can contribute substantially to increased laboratory productivity. During sample preparation, centrifugation steps claim a large proportion of the available time. Therefore, these are ideally suited for optimization. During the course of analysis of veterinary drugs, the Centrifuge 5430 R, in combination with Eppendorf Safe-Lock tubes, achieved a reduction in centrifugation time of up to 75 % at 30,130 x g. Furthermore, the measured values showed improved reproducibility compared to standard protocols.

Introduction

This Application Note describes standard sample preparations, which were employed to test the use of the Centrifuge 5430 R (Eppendorf) in comparison with a laboratory centrifuge by a different manufacturer. The analysis protocols performed herein serve the detection of pharmacologically active substances (veterinary drugs) in food of animal origin, per LC/MSMS (liquid chromatography, tandem mass spectrometry). Specifically, sample preparation protocols for the detection of metabolites of nitrofurans are introduced. These are substances with antibiotic activity.

Materials and methods

Experiment I:
Sample preparation for the detection of nitrofuran metabolites in muscle meat (salted chicken muscle; fortified samples)

For this method, the four nitrofuran metabolites 3-amino-2-oxazolidinone (AOZ), 1-aminohydantoin (AHD), 3-amino-5-morpholinomethyl-1,3-oxazolidin-2-one (AMOZ) and semicarbazide (SEM) are derivatized using 2-nitrobenzaldehyde. Following pH adjustments, the derivatives are extracted with 20 mL ethyl acetate and centrifuged at 3,220 x *g* for 15 min. The supernatant is transferred to a test tube and dried using a parallel evaporator (Syncore; Büchi, New Castle, DE, USA). The residue is solubilized in 200 µL methanol/water and mixed carefully.

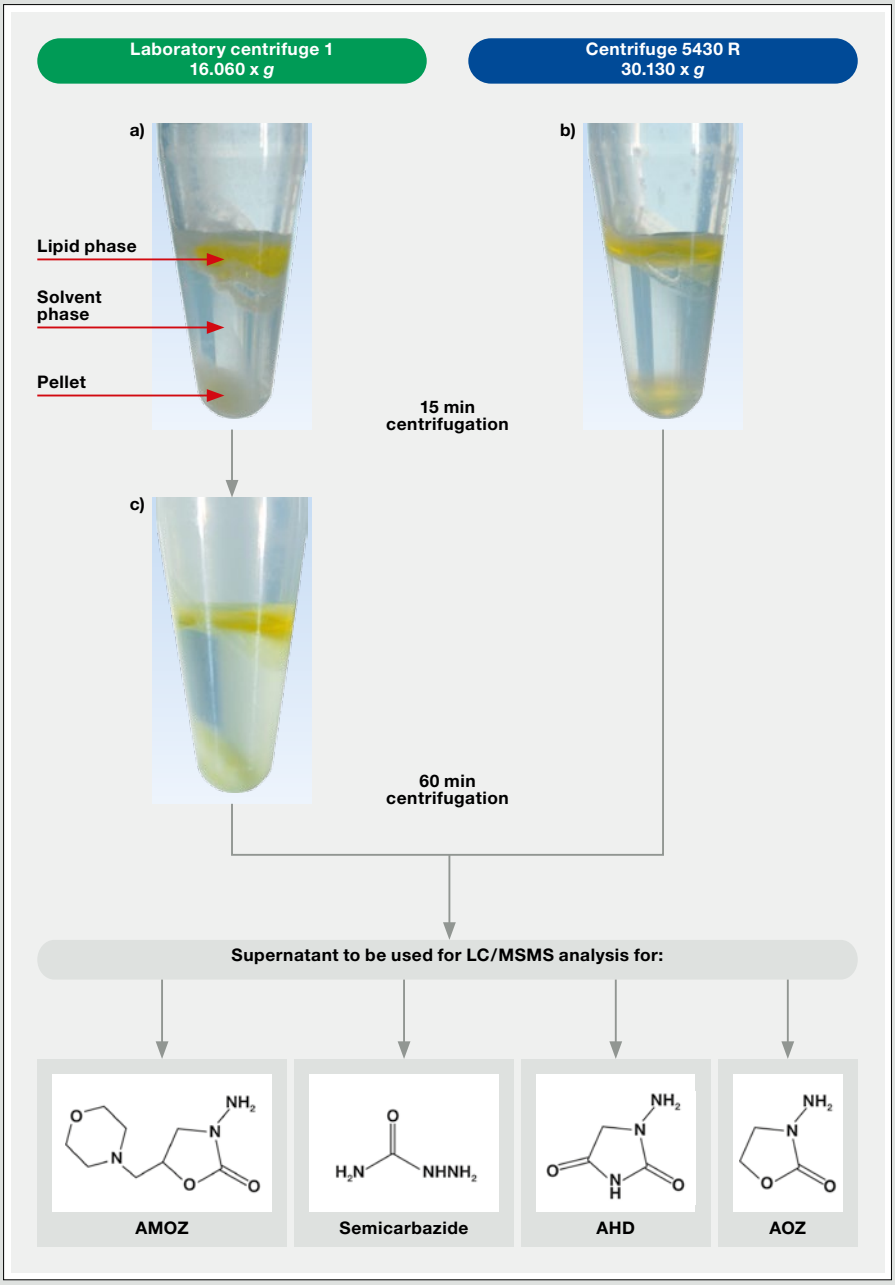

Fig. 1: *Matrix salted chicken muscle, extracted with experimental protocol IIa. Following 15 min centrifugation in laboratory centrifuge 1 (a) and Eppendorf Centrifuge 5430 R (b); following 60 min centrifugation in laboratory centrifuge 1 (c). Photos: T. Krenz.*

This solution is transferred to a 1.5 mL Eppendorf Safe-Lock Tube and centrifuged at maximum g-force (laboratory centrifuge 1: 16,060 x *g*, Centrifuge 5430 R: 30,130 x *g*) at 4 °C until a clear intermediate phase can be removed.

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Time Savings as well as Improved Reproducibility through Centrifugation at 30,000 x *g*

Experiment II:
Comparison of data reproducibility

After one hour centrifugation at 4 °C, the solvent phase (see experiment I) is pipetted into an amber glass vial with micro-insert and measured with LC/MSMS (HP 1100; Agilent Technologies, Santa Clara, CA, USA. Quattro micro; Waters Corporation Milford, MA, USA). Separation was achieved with a Luna C18 column (Phenomenex, Torrance, CA, USA), (150 x 2 mm, 5 µm particle size) with methanol/ammonium acetate buffer as the mobile phase (flow rate: 0.2 mL/min, injection volume 10 µL). The LC/MSMS was operated in MRM mode (ESI pos.), the following transitions were detected: AOZ: m/z 236 >104; AMOZ: m/z 335 > 262; AHD: m/z 249 >134; SEM: m/z 209 >166.

The reproducibility of the concentrations of the nitrofuran metabolites was tested via comparison of the coefficients of variation.

Results and discussion

Experiment I: The sample preparation for experiment I describes the final centrifugation step prior to analysis of nitrofuran metabolites in salted chicken muscle meat. Following extraction and centrifugation, a lipid phase is visible on top. The intermediate layer is the solvent phase to be analyzed. The bottom of the tube holds the solid matrix components which are present in a more or less compact pellet. Fig. 1 shows the results after 15 or 60 min centrifugation time (laboratory centrifuge 1) and after 15 min centrifugation time (Centrifuge 5430 R), respectively.

In the Centrifuge 5430 R (Fig. 1b) the matrix components were enriched in a more compact and tight pellet. After only 15 min, the intermediate solvent phase could be removed without the risk of carrying over pellet components which could potentially contaminate subsequent analysis instruments (e.g., LC/MSMS).

Metabolite	Preparation	Laboratory centrifuge 1	Centrifuge 5430 R
		Coefficient of variation [%]	
AOZ	A	14.4	5.6
	B	12.9	1.2
AMOZ	A	4.4	1.1
	B	6.0	1.9
AHD	A	5.5	3.8
	B	2.2	3.0
SEM	A	4.6	2.0
	B	4.3	3.4

Table 1: *Summary of the coefficients of variation for all four metabolites, analyzed by the same method (AOZ, AHD, AMOZ, SEM) following sample preparation II; calculated for laboratory centrifuge 1 and the Centrifuge 5430 R, respectively.*

For the laboratory centrifuge 1, this step was only possible after 60 min centrifugation (Fig. 1c). Thus, the time required for the centrifugation step of the matrix to be analyzed could be reduced by up to 75 % for experiment I as well.

Experiment II: For sufficient phase separation to be achieved, centrifugation with laboratory centrifuge 1 took one hour in experiment I. Therefore, for the purpose of comparison of reproducibility, the measured results from both centrifuges were compared after this maximum centrifugation time (= 1 h). This experimental design was used to test whether the qualitatively visible improved phase separation achieved with the Centrifuge 5430 R at 30,130 x *g* would also lead to improved reproducibility at the data analysis level. Peak areas, as well as parameter concentrations calculated from matrix standard curves, were compared.

To this end, quadruple determination of two separate preparations (A and B) of a sample of salted chicken muscle meat were considered, with regards to the following metabolites: 3-amino-2-oxazolidinone (AOZ), 1-aminohydantoin (AHD), 3-amino-5-morpholinomethyl-1,3-oxazolidin-2-one (AMOZ) and semicarbazide (SEM) [1].

The coefficient of variation, which is smaller for more reproducible values, was used as a measure of reproducibility (Table 1).

The use of the Centrifuge 5430 R (30,130 x *g*) considerably improves reproducibility of the residuals of the four tested antibiotic metabolites (only exception: preparation B, measurement of AHD). For laboratory centrifuge 1, the coefficient of variation is between 2.2 % and 14.4 %, and for Centrifuge 5430 R the coefficient is between 1.1 % and 5.6 %. Considering the fact that the varied centrifugation is only one of many steps during sample preparation, while all other preparation steps were performed with identical instruments, the gain in reproducibility is remarkable.

Conclusion

The system of Centrifuge 5430 R and the 1.5 mL Eppendorf Safe-Lock tubes enables centrifugation at 30,130 x *g*. During the analysis of veterinary drugs, this system led to improved phase separation performance. Thus, centrifugation times could be reduced by up to 75 % for different protocols. Furthermore, data reproducibility could be improved. Further protocol optimizations using the Centrifuge 5430 R and the 1.5 mL Safe-Lock tubes are planned.

Literature

[1] Application Note 240:
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PETER SCHREINER, EPPENDORF AG

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**Frequency-controlled refrigeration (Patent DE 19932721C1, US. Pat. No. 6,866,621)*

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TEA GRUNZ, EPPENDORF AUSTRIA GMBH, VIENNA

Eppendorf Austria Customer Receives Coveted Award

On May 24, 2011, the Unilever Hygiene Award which includes an endowment of 4,400 Euros, was awarded to Dr. Ariane Pietzka during the 21st Dosch Symposium of the Austrian Society for Hygiene, Microbiology and Preventative Medicine (ÖGHMP) in Bad Ischl, Austria.

Ariane Pietzka, of the Austrian Agency for Health and Food Safety (AGES) received the honor for her work in developing a procedure for rapid typing of *Listeria monocytogenes*. Rapid typing of listeria germs allows for the appropriate diagnosis and more efficient treatment of listeriosis which can be life-threatening for risk groups such as immunocompromised patients, pregnant women and newborns.

Listeria monocytogenes is a bacterium found in the environment which can be transmitted to food. Consuming contaminated food, in turn, can cause Listeriosis. To clarify epidemiological issues, all listeria isolates (food and patient samples) are typed in Austria using DNA analysis – and then a genetic fingerprint is made of each listeria isolate. To identify contaminated food as quickly as possible, it is necessary to work with a fast typification-method.

During the development of the typing procedure, the reaction samples were pipetted using an ep*Motion* from Eppendorf. On behalf of Eppendorf, our colleague Peter Weissmann congratulated Ariane Pietzka on her award and talked with her about her work.

PW: On behalf of Eppendorf, I would like to congratulate you on winning the hygiene award and your successful research. Can you tell us a bit about your research work?

AP: In 2009, a listeriosis outbreak caused by a sour milk cheese infected with listeria led to eight deaths and numerous illnesses in Austria and Germany. There was a great deal of media interest during the outbreak, which created an enormous amount of pressure to quickly deliver accurate results using a new method (PFGE). Because PFGE typing takes several days, we considered the outbreak an opportunity to further pursue our screening method (high-resolution melting, HRM) that was already in development. In January 2011, the method was published in the *Journal of Molecular Diagnostics* [1], for which we then received the hygiene award.

Dr. Ariana Pietzka (center) with her colleagues Dr. Werner Ruppitsch and Anna Stöger

PW: What makes listeria so dangerous?

AP: Listeria are very undemanding, resistant bacteria. They have the unusual ability to grow at low temperatures and are primarily transmitted to humans via contaminated food such as raw meat or raw milk. The bacteria are especially dangerous for pregnant women, newborns and immunocompromised individuals.

PW: Which procedures has the ep*Motion* helped you most with?

AP: I have primarily pipetted PCR/HRM samples, with 10 µL sample volumes, in 384-well plates. The obvious advantage is that fewer pipetting errors occur than with manual handling, and other errors are easier to identify.

Literature

[1] Ariane T Pietzka, Anna Stöger, Steliana Huhulescu, Franz Allerberger, Werner Ruppitsch.
Gene Scanning of an Internalin B Gene Fragment Using High-Resolution Melting Curve Analysis as a Tool for Rapid Typing of *Listeria monocytogenes*. J Mol Diagn. 2011 Jan;13(1):57-63.

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CAROLYN TAUBERT & BERRIT HOFF, EPPENDORF AG

Welcome to Eppendorf

Christopher Gregg (left) and Peter Stern

The Canadian scientist **Dr. Christopher Gregg**, winner of the 2010 *Eppendorf & Science Prize for Neurobiology*, visited Eppendorf headquarters in Hamburg in June of 2011. Dr. Gregg was accompanied by Dr. Peter Stern, Senior Editor at *Science*/AAAS and Chair of the Prize Selection Committee.

On this occasion Dr. Gregg gave a talk about his ground-breaking work on maternal and paternal gene expression in the brain. His research focuses on genes that alter their expression in the brains of offspring according to whether they were inherited from the father versus the mother.

Earlier in 2011, Dr. Gregg joined the Department of Neurobiology and Anatomy at the University of Utah as Assistant Professor with his own research group.

The international US$ 25,000 *Eppendorf and Science Prize for Neurobiology* is awarded jointly by Eppendorf and the journal *Science*.

For more information visit www.eppendorf.com/prize.

Geoff Marsh (left) and Suzan Rooijakkers

In October 2011 *Eppendorf Young Investigator Award Winner 2011*, **Assistant Professor Suzan Rooijakkers**, PhD (University Medical Center Utrecht, Department of Medical Microbiology, Utrecht, Niederlande) visited Eppendorf AG. Suzan Rooijakkers received the 15.000 € prize for her discoveries of how the pathogen *Staphylococcus aureus* evades immune attack to survive in the human host.

Suzan Rooijakkers also gave a talk about her research and met with Geoff Marsh (Podcast Editor of *Nature* Publishing Group, London) for an interview for a *Nature* podcast. You can listen to it at www.eppendorf.com/awardpodcast.

For the first time the prize ceremony had taken place at the EMBL Advanced Training Centre in Heidelberg, Germany, in May 2011. A podcast from the event as well as a video with the highlights of all speeches can be found on the Award homepage (see below).

The 15,000 Euro *Eppendorf Award for Young European Investigators* is presented in partnership with *Nature*.

For more information visit www.eppendorf.com/award.

News

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We provide certification documents for your quality and regulatory management that are in accordance with national and international legal and regulatory requirements.

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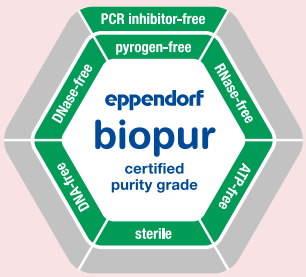
New: For easier, faster identification of the correct purity level all Eppendorf consumables are now labeled with color-coded quality seals.



Biopur consumables now also PCR inhibitor-free and DNase-free!

Prize Competition

Win online at www.eppendorf.com/bn-service

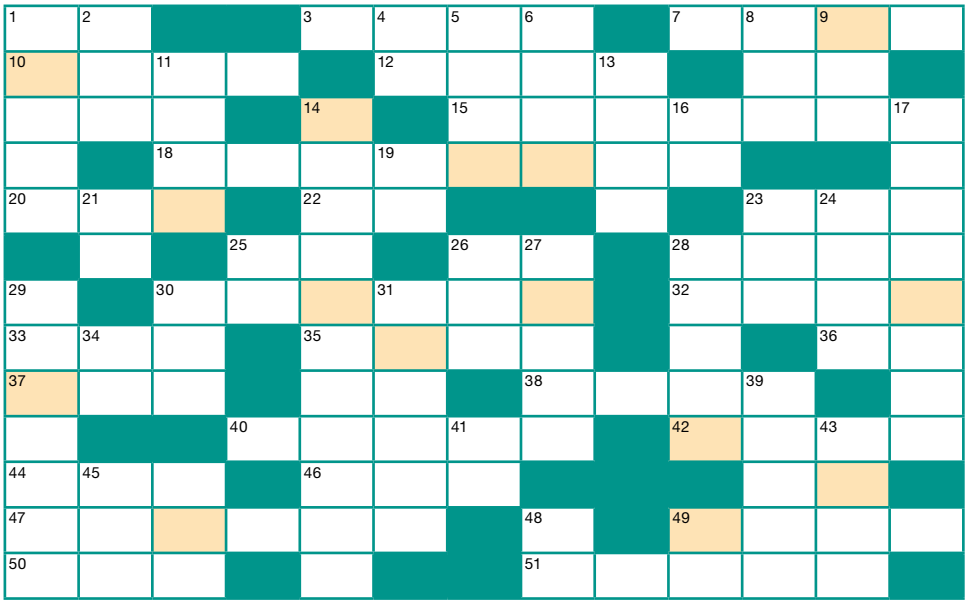
The solution of the prize competition of BioNews No. 34 was "Captain Eppi". Dr. Sidney Shaw (University of Bern, Switzerland) won the first prize, an Eppendorf Xplorer pipette.

Have fun in our new crossword!

How to find out the solution: Simply arrange the encircled letters of the crossword in the correct order. Send us the solution until **30th June 2012**.

You can either use the reply fax (p. 15), send us an e-mail to bionews@eppendorf.de, or participate online at www.eppendorf.com/bn-service.

All correct answers will be considered for a prize. Winners will be notified in writing. Cash payment of the prize is not possible. No recourse to legal action. The judges' decision is final. Eppendorf employees and their families may not participate. The winner of the first prize will be published in BioNews No. 38.



1st prize:
1 Eppendorf Research® plus 8-channel pipette 10–100 µL

2nd to 5th prize:
1 Amazon® Voucher for the value of 50,00 Euro

6th to 15th prize:
200 bonus ep-points each

ACROSS

1 Name affix of Barcelona and Bayern München
3 Round object used in sports or games
7 Urbi et ...
10 Genuine, not artificial
12 Public violence, tumult
15 Pilot of an aircraft
18 Decorative element in architecture
20 Type of beer
22 US "sunshine state" (abbr.)
23 Badge, label, sign
25 Board game
26 US state and river (abbr.)
28 Indonesian island
30 Shape, size, general makeup
32 Graphic mark, emblem
33 Liquid Crystal Display
35 Opposite of stereo
37 And so on ...
38 Skilled, experienced cook
40 Used by drummers (sing.)
42 Music genre in Memphis and "Motown"
44 Sick, bad
46 Electrically charged particle
47 Native name for Japan
49 Gang, clique, crowd
50 Positioning system
51 Collision, conflict, confrontation

DOWN

1 Ms Kahlo's first name
2 Chief Executive Officer (abbr.)
4 Chemical symbol for argon
5 Male given name, short form of William
6 All you need is ... (according to the Beatles)
8 Long-tailed rodent
9 Greek prefix for "life"
11 Flowering succulent plant
13 Female given name (short form)
14 Data, facts, knowledge
16 ISO country code of Austria
17 Pertaining to a specific district
19 Chemical symbol für aluminum
21 Chemical symbol for lithium
23 Path, way (Chin. philosophy)
24 It's got "rithm"
26 Island in the Irish Sea
27 Inventory, supply
28 The B in R&B
29 Discoverer of penicillin
31 Combinable with ep, E or Slow
34 Postal abbreviation for Connecticut
39 Inappropriate or unfair act in sports (pl.)
41 ISO country code of China
43 Its capital is Salt Lake City
45 Part of the mouth
48 Carbon copy (abbr.)
49 West Coast US state (abbr.)

To Eppendorf AG, Hamburg, Germany, Attn. Anton Janott, Fax (+49) 40 - 5 38 01-8 40

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○	242	Eppendorf BioSpectrometer®	***I have comments on the BioNews No. 36:***	
○	243	ep*Motion*® GxP solution		
○	244	Eppendorf Concentrator plus™		
○	245	Eppendorf PiezoXpert®		
○	246	Go Green. Join the Centrifuge Evolution!		
○	247	Rolling Cabinet Centrifuges 58xx		
○	Eppendorf General Catalog 2012/2013 		***I have suggestions/topics for the next BioNews:***	

Solution of BioNews No. 36 prize competition:

			S			C					E			R

Closing date: 30th June 2012 (Visit www.eppendorf.com/bn-service to participate online!)