

Toxicology, Immunotoxicity, Suspension Cells, PBMCs

## Evaluating agarose-embedded suspension cells (lymphocytes) using the **Bionas Discovery™ 2500 system**

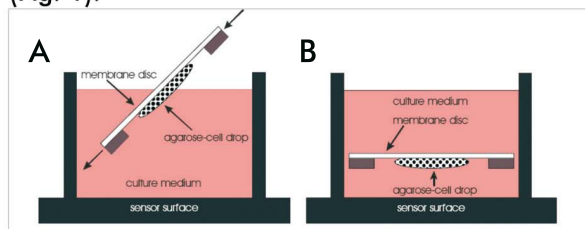
### INTRODUCTION

The **Bionas Discovery™ 2500 system** is a multi-parametric, non-invasive and label-free device to monitor and investigate metabolic parameters like extracellular oxygen consumption and acidification. Until now, the method has been very valuable for the investigation of adherent cell lines and primary cells. Cells from the immune system and their progenitors typically grow in suspension cultures. The requirements for the measurement of those cells are very much the same as for adherent cells with only some differences in the preparation. For using cells that do not attach to the chip surface, a biocompatible, low-buffering matrix that permits movement of fluids but prevents immobilized cells from floating is required. Therefore, the aim of this study was to establish a suitable method for the measurement of suspension cells with the **Bionas® 2500 analyzing system**.

### MATERIALS & METHODS

**Cell culture.** Peripheral blood mononuclear cells (PBMC), provided by ProBioGen AG were cultured in RPMI-1640 + 10% FCS.

**Preparation of agarose-embedded cells.** For measurement, cells were suspended in 2% agarose ( $3,75 \times 10^6$  cells/chip). After calculating the appropriate cell number, 200  $\mu$ l of cell suspension were mixed with 100  $\mu$ l agarose. 10  $\mu$ l of this cell/matrix-suspension were transferred into the ring's opening of the membrane disc. After solidification of the agarose, the membrane was inserted upside down onto the chips, which was moistened with medium before (Fig. 1).

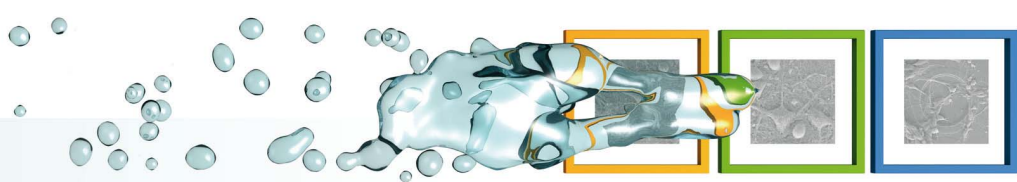


**Fig.1:A:** Inserting membrane disc with an agarose-cell-drop. The agarose drop is placed between sensor surface and membrane disc. **B:** Final position of the membrane disc with agarose-cell-drop.

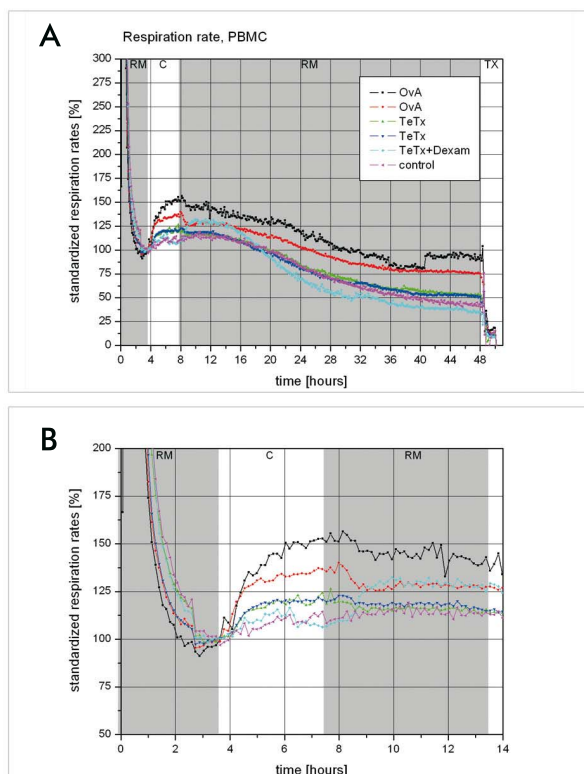
**Test compounds.** The substances were exposed with concentrations of 10  $\mu$ g/ml Ovalbumin (OvA), 250 ng/ml Tetanus Toxoid (TeTx) and 1  $\mu$ M Dexamethasone, as a control chip medium without compound was applied.

**Analysis in the Bionas Discovery™ 2500 system.** Medium with 0.1% FCS (running medium, RM) was pumped to the cells with a flow rate of 56  $\mu$ l/min in flow-on, flow-off cycles, which were repeated throughout the experiment. After receiving a stable baseline for measurement, immunogenic substances in absence or presence of an immunosuppressant (Ovalbumin or Tetanus Toxoid with/without Dexamethasone) were applied for 4h. The exposure was followed by a recovery phase and a final killing of the cells by adding Triton X-100 to the RM.

In the pre-running phase (Fig. 2, grey, 1-4h), cells adapt to the flow rate and to the buffer-reduced medium of the system to ensure stable sensor signals. Next, cells are exposed to immunogenics with or without immunosuppressive substances for 4h, diluted in RM (white, C). OvA (black and red) increases the respiration rate to 150%. The addition of TeTx (green and blue) or the combination of TeTx with Dexamethasone (cyan) results in a 25% increase of the oxygen consumption. After the removal of TeTx in the combinatory application, the curve continues to increase but shows a rapid decline 6h later to 30%. TeTx alone behaves similarly to the control curve (magenta) and decreases to about 50% of the respiration activity. The OvA exposed cells show the highest activity after 48h with 75% - 100%.



## OXYGEN CONSUMPTION OF EMBEDDED PBMC



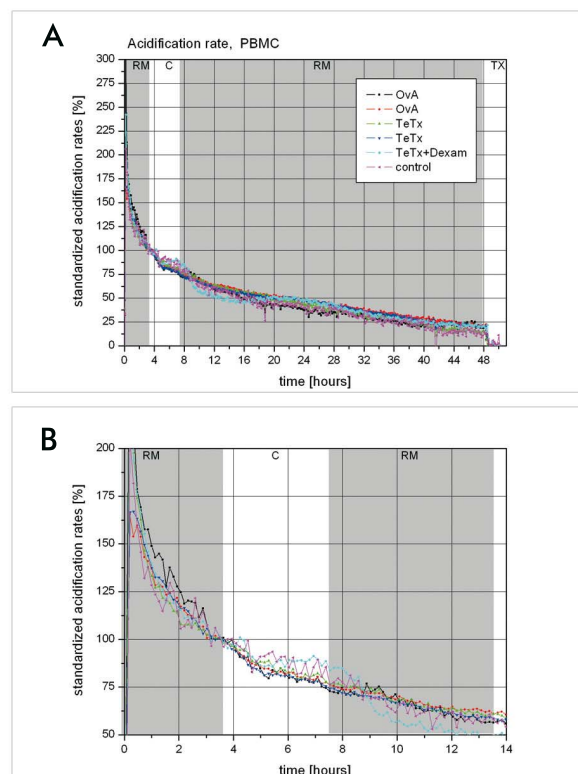
**Fig. 2:** Respiration rates of primary human lymphocytes (A: overview of total run, B: close-up, 0-14h)

During incubation (4-8h, C), cells are exposed to the tested compounds (white). In all conditions tested, the acidification rates for the tested substances are not different from control cells and are not influenced by the applied substances (Fig. 3). In the regeneration phase (total of 40h), the cells are again exposed to RM. A continuous decrease of acidification rates can be seen up to 40h of measurement, followed by a constant level of around 25%, indicating reduced glycolysis of the cells. Within the first 8h after substance removal, the combination of TeTx with Dexamethasone (cyan) demonstrates a reduced acidification which is linked to an increased respiration during that time.

## CONCLUSION

The effect of the two immunogenic substances on the respiration rates could be analyzed successfully by the **Bionas Discovery™ 2500 system**. The immunosuppressant modulates the effect of the immunogenic substances by decreasing the respiration with the start of exposure and increasing levels of respiration during the regeneration phase while the acidification was not affected.

## ACIDIFICATION RATES OF EMBEDDED PBMC



**Fig. 3:** Acidification rates of primary human lymphocytes (A: overview of total run, B: close-up, 0-14h)

These results show that the usage of agarose-embedded suspension cells is a valuable tool for a multi-parametric data collection.

## KEYWORDS

suspension cells, immunotoxicity, membrane inserts, agarose

## AUTHORS

S. Ortinau, PhD, Bionas GmbH  
C. Giese, PhD, ProBioGen AG  
A. Lubitz, Dipl.-Ing. (FH), ProBioGen AG

## CONTACT

Bionas GmbH, Friedrich-Barnewitz-Straße 3  
18119 Rostock, +49 (0) 381 5196 442  
www.bionas-discovery.com  
sales@bionas-discovery.com

**ProBioGen**

Supporting Biopharmaceutical Visions

ProBioGen AG, Goethestrasse 54, 13086 Berlin  
+ 49 (0) 30-924 00 60  
www.probiogen.de, info@probiogen.de

