List of Correspondences

Receipt No. / Issue No.	Subject	Type	Marked As	Attached On	Issued On	Pages	Remarks
2106425/2025/Research Section	AIIMS Website	Receipt		08/08/2025 03:54 PM		91-93	.
2106395/2025/Research Section	PAC Draft signed	Receipt		08/08/2025 03:47 PM		90-90	.
2100051/2025/Research Section	PAC Draft	Receipt		05/08/2025 11:48 AM		89-89	.
2094769/2025/BIOTECHNOLOGY	Submission of TSEC Meeting documents for the purchase of "Metabolic Analyzer"	Receipt		01/08/2025 11:18 AM		1-88	Attachment

To
Dean (Research)
All India Institute of Medical Sciences (AIIMS)
New Delhi

Dated: 30.07.2025

Subject: Submission of TSEC Meeting documents for purchase of **"Metabolic Analyzer"**

Project Code: I-1789

Dear **Sir**,

I am writing to kindly bring to your attention the details of the Technical Specifications and Evaluation Committee (TSEC) meeting conducted for the above-mentioned project, funded by the Indian Council of Medical Research (ICMR).

The meeting was held on Friday, July 22, 2025, at 10:30 AM in the Seminar Room, Second Floor, Department of Biotechnology, AIIMS, New Delhi. The agenda of the meeting was to finalize the technical specifications of **Metabolic Analyzer** for proposed high-value equipment procurement under the project (within the approved budget of ₹2.48 Crores).

The committee reviewed all proposed specifications in detail and approved them with minor modifications. All the changes were incorporated. The specifications are aligned with the project goals and scientific requirements. It was resolved that the PI may proceed to float tenders/e-procurement as per the institutional rules.

In compliance with institutional requirements, I hereby submit the meeting documentation for onward transmission to the **Store** and **Accounts Sections** for necessary processing.

Attachments:

1. Specification for High Throughput Single Cell Capture and Analysis system
2. Minutes of TSEC
3. Undertaking for General Specifications
4. TSEC attendance sheet
5. Fall Clause Certificate
6. Proprietary Article certificate (PAC)
7. Proprietary certificate from Agilent with US and Europe Patent
8. Quotation
9. Non-Blacklisted Declaration
10. Rate Reasonability certificate
11. GEM not ability certificate
12. Old PO
13. ICMR project fund ability document

Thanks

डॉ. रूपेश कुमार श्रीवास्तवा/Dr. RUPESH KUMAR SRIVASTAVA
अपर आचार्य/Additional Professor
जैव प्रौद्योगिकी विभाग/Dept. of Biotchnology
अ. भा. आ .सं., नई दिल्ली/A.I.I.M.S., New Delhi-110029

(Dr. Rupesh K. Srivastava)
Additional Professor
Room 202, Department of Biotechnology
AIIMS, Ansari Nagar New Delhi-29
Ph-9179567399

All India Institute of Medical Sciences (AIIMS), New Delhi

Department of Biotechnology

Minutes of the Technical Specifications and Evaluation Committee (TSEC) Meeting for Metabolic Analyzer

"Investigating the Diagnostic Potential of Gut-Associated Metabolites in Post-Menopausal Osteoporotic Women and Exploring Their Therapeutic Potential in Pre-Clinical Model of Osteoporosis: An Osteoimmunological Approach". Funding Agency: Indian Council of Medical Research ICMR funded project (F. No. EMDR/IG/13/2024-01-00842, Project code: I-1789

Meeting was held on Friday, July 22, 2025, Time: 10:30 AM at Seminar Room, Second Floor, Department of Biotechnology, AIIMS, New Delhi

Purpose: Finalization of technical specifications of **Metabolic Analyzer** for proposed high-value equipment procurement under the project (within the approved budget of (Estimated Cost INR 2.48 Crores).

The committee reviewed all proposed specifications in detail and approved them with minor modifications. All the changes were incorporated. The specifications are aligned with the project goals and scientific requirements. It was resolved that the PI may proceed to float tenders/e-procurement as per the institutional rules.

डॉ. भूपेन्द्र कुमार वर्मा/Dr. BHUPENDRA KUMAR VERMA
अपर आचार्य/Additional Professor
जैव प्रौद्योगिकी विभाग / Dept. of Biotchnology
अ. भा. आ. सं., नई दिल्ली/A.I.I.M.S., New Delhi-110029

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आचार्य एवं अध्यक्ष/Professor & Head
जैव रसायन विभाग
Department of Biochemistry
अखिल भारतीय आयुर्विज्ञान संस्थान, नई दिल्ली-29
All India Institute of Medical Sciences, New Delhi-29

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सह-आचार्य/Associate Professor
जैव प्रौद्योगिकी विभाग/Deptt. of Biotechnology
अ.भा.आ.सं., अंसारी नगर, नई दिल्ली-110029
AIIMS, Ansari Nagar, New Delhi-110029

Dr. Shilpi Minocha
Assistant Professor
Kusuma School of Biological Sciences
Indian Institute of Technology Delhi

Dr. JASWINDER SINGH MARAS
Associate Professor
Molecular & Cellular Medicines
of Liver & Biliary Sciences
Vihar, New Delhi-110070

Technical specification for Metabolic Analyzer

(Estimated Cost INR 2.48 Crores)

1. The instrument should be able to perform live cell, real-time analysis of cellular energy metabolism in 96-well microplate format
2. The instrument should be capable of simultaneously measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of living cells. OCR and ECAR data should be simultaneously calculated and displayed in real time.
3. Assays should be non-invasive and should not require the addition of dyes, labels, or reporters. Measurements should be reproducible to measure kinetic responses over several hours. Measurements within the same well should demonstrate a coefficient of variation of less than 5%, approximately equivalent to the instrument's background noise.
4. The instrument should be capable of analysing adherent cells without requiring trypsinisation.
5. The instrument should be capable of analysing suspension cells by adhering it to the well bottom.
6. The instrument should typically require only 5 x 10^3 to 200 x 10^3 cells per well in the 96-well platform.
7. The Low Limit of Detection (LLOD) with 95% confidence should be as below:
   i. OCR:13 pmol/min
   ii. PER: 30 pmol/min
8. The instrument should create a transient microchamber, make optical measurements and perform compound injections.
9. The instrument should be capable of adding up to four test compounds automatically to each well during the assay. Measurements may be performed before and after each compound is added.
10. The analyser should be able to maintain internal assay temperature from 16° to 42°C.
11. Rates should be automatically calculated and reported every 5-8 minutes. Data should be reported in calibrated rates of pmol/min or mpH/min
12. No cleaning should be required. All the parts that are in contact with the cells, media, or compounds should be disposable.
13. A cloud-based platform should be available for analysing and interpreting assay results.
14. The instrument should have Light Emitting Diodes (LEDs) as the monochromatic excitation source. LEDs should be operated at very low excitation energy densities to prevent detectable levels of photo bleaching. Usable life of the LED sources should be >10,000 hours.
15. The precision-moulded enclosures on the analyser should have speciality coatings and gaskets to shield the electronics and sample from ambient light, as well as block radiated and emitted electromagnetic interference
16. Convenient, easy-to-use test kits and reagents to simplify the study of cellular metabolism by providing pre-calibrated, pre-tested reagents for routine measurements of metabolic phenotype, mitochondrial respiration, glycolysis, fatty acid oxidation, and ATP production rates should be available from the manufacturer.
17. Standardised reagents, kits, and protocols designed and validated for use with the analyser should be available.
18. Optical sensors should not consume oxygen during the measurement and should not be in contact with the cells. Optical sensors should not be affected by compound or intracellular dye fluorescence.
19. Oxygen sensor should have a peak absorption of 530 nm (green), and peak emission of 650 nm (red). pH sensor peak should have a peak absorption of 470 nm (blue) and a peak emission of 530 nm (green).
20. Each sensor cartridge must be auto-calibrated at the beginning of the run.
21. An interface between the analyser and imager system should be available, which could apply cell count numbers directly to the data, enabling plate-to-plate, day-to-day and well-to-well comparisons.

22. Software required for complete post-acquisition analysis (to view assay results) and export in multiple file formats (MS® Excel, GraphPad Prism, or assay-specific reports) should be provided.
23. Total variability between wells from all sources (instrument background plus biological variability due to cells) for the average trained user should be <20%.
24. It should be provided with the following consumables to ensure efficient workflow. **These kits should be provided in a user-dependent, staggered manner over a period of 3 years**.
    i. 12 nos. of Seahorse XF DMEM assay medium pack (103680-100)
    ii. 12 nos. of Seahorse XF RPMI assay medium pack (103681-100)
    iii. 05 nos. of Seahorse XFe96/XF Pro FluxPak (103792-100)
    iv. 10 nos. of Seahorse XFe96/XF Pro PDL FluxPak Mini (103798-100)
    v. 08 nos. of Seahorse XF T cell Metabolic Profiling Kit (103772-100)
    vi. 08 nos. of Seahorse XF Mito Fuel Flex Test Kit (103260-100)
    vii. 08 nos. of Seahorse XF Glycolytic Rate Assay Kit (103344-100)
    viii. 08 nos. of Seahorse XF Real-Time ATP Rate Assay Kit (103592-100)
    ix. 08 nos. of Seahorse XF Cell Mito Stress Test Kit (103015-100)
    x. Compatible, branded high-quality 02 sets of pipettes (P10, P20, P200, P1000)

25. It should be provided with ONE high-end workstation for data analysis with the following specifications: 14" MacBook Pro with 14.2" Liquid Retina XDR display, nano-texture display, M4 Max chip with 14-core CPU, 32-core GPU, 16-core Neural Engine, 36GB unified memory, 2TB SSD storage, 96W USB-C Power Adapter, Multifunction (WiFi enabled) Color Laser Printer, 02 nos. of 10TB external SSD, with High performance 4-bay NAS System (Network Attached Storage).

26. **Additional conditions to be fulfilled:**

    i. The manufacturer should provide a comprehensive warranty of **03 (three) years (including all spares, labour and any software upgradation)** from the date of the installation certificate issued by the end user.
    ii. The rates for the Comprehensive Annual Maintenance Contract (CAMC) (including all spares, labour and any software upgradation) from the 4th to the 10th year should be provided.
    iii. A compatible online UPS with at least 60 minutes of backup, along with all furniture, etc, required for proper installation as per instrument requirement (02 no. 2 Ton 5 Star Split Air Conditioner, 02 nos. Chair, 02 nos. Stool) should be provided.
    iv. There should be more than **10 installations** of the system in the country, and the Principal company should have been operational in India for sales and after-sales support (service and training) for at least 5 years.
    v. The equipment should be installed and validated by the service engineer designated by the principal company.
    vi. On-site Application training should be provided, at least twice each year, for the first 03 years.
    vii. On-site technical support should be provided from start to end of each experiment, including planning, execution and data analysis, for a minimum of 3 years.
    viii. The supplier should have a training and application centre available in India, for carrying out training and help with troubleshooting.
    ix. The delivery date as stipulated should be strictly adhered to, failing which the institute reserves the right to refuse the supplies. The extension of the delivery date, if required, should be obtained before the expected delivery date.
    x. STANDARD clauses as applicable for all equipment procurement at AIIMS, New Delhi, including testimonials from users, standard down-time clause of 15 days; penalty to be added as extension of warranty.

DEPARTMENT OF BIOTECHNOLOGY
ALL INDIA INSTITUTE OF MEDICAL SCIENCES
ANSARI NAGAR, NEW DELHI

To whom it may concern

This is to certify that the enclosed specification of Metabolic Analyzer (Seahorse XF Pro Analyzer) is proprietary item. Pre-installation requirement such as site, power, water, space, manpower etc. are available in the Project investigator lab.

डॉ. भूपेन्द्र कुमार वर्मा/Dr. BHUPENDRA KUMAR VERMA
अपर आचार्य/Additional Professor
जैव प्रौद्योगिकी विभाग/Dept. of Biotchnology
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अ.भा.आ.सं., अंसारी नगर, नई दिल्ली-110029
AIIMS, Ansari Nagar, New Delhi-110029

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अपर आचार्य - अस्पताल प्रशासन
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अखिल भारतीय आयुर्विज्ञान संस्थान, नई दिल्ली
All India Institute of Medical Sciences, New Delhi

Dr. Shilpi Minocha
Assistant Professor
Kusuma School of Biological Sciences
Indian Institute of Technology Delhi
Hauz Khas, New Delhi-110016 (India)

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भारत सरकार/Govt. of India

DEPARTMENT OF BIOTECHNOLOGY
ALL INDIA INSTITUTE OF MEDICAL SCIENCES
Ansari Nagar, New Delhi – 110029

Dated: 22/07/2025

A meeting of the Technical Specification Committee is scheduled as follows:
Date: 22/07/2025 (Tuesday)
Time: 10:30 AM

Venue: Seminar Room, Second Floor, Department of Biotechnology, AIIMS, New Delhi – 110029

All concerned committee members are requested to attend the meeting on time to discuss and finalize the technical specifications for the proposed procurement of Metabolic Analyzer.

	Name	Signature
1.	**Dr. Rupesh Kumar Srivastava,** Additional Professor, Department of Biotechnology (Chairman)	
2.	**Dr. Bhupendra Kumar Verma**, Additional Professor, (HOD, Nominee)	
3.	**Dr. Angel Ranjan**, Additional Professor, Hospital Administration, AIIMS, Ansari Nagar, New Delhi (M.S Nominee (Member))	
4.	**Dr. Kalpana Luthra**, Professor & Head, Department of Biochemistry, AIIMS, Ansari Nagar, New Delhi (Member)	
5.	**Dr. Sumit Rathore**, Associate Professor, Department of Biotechnology, Member (store In-charge)	
6.	**Dr. Jaswinder Singh Maras**, Associate Professor, Department of Molecular and Cellular Medicine, Institute of Liver and Biliary Science, New Delhi (External Expert)	
7.	**Dr. Shilpi Minocha**, Assistant Professor, Kusuma School of Biological Sciences, INDIAN INSTITUTE OF TECHNOLOGY DELHI, Hauz Khas, New Delhi-110016, INDIA (External Expert)	
8.	**Dr. Amita Bali**, CMO, (SAG)-DDG (P), Dte. (DGHS Nominee)	

Chairman
(Technical Specification Committee)
Department of Biotechnology
AIIMS, New Delhi-110029

Dr. Shilpi Minocha
Assistant Professor
Kusuma School of Biological Sciences
Indian Institute of Technology
Hauz Khas, New Delhi-110016

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QUOTATION

THE DIRECTOR, ALL INDIA INSTITUTE OF MEDICAL SCIENCES - AIIMS - DELHI
ANSARI NAGAR, New Delhi-110029.
KIND ATTN : DR. RUPESH SRIVASTAVA

Quote No : QT12859
Quote Date : 29-05-2025
Valid Till: 29-06-2025

Sub: Your requirement of Seahorse XF Pro Analyzer with XF Discovery License.

Sr. No.	Cas No.	HSN No.	Applicable GST %	Product Name	Unit Price (INR)	Qty	Total Price (INR)
1	S7855A	90275090	18	Seahorse XF Pro Analyzer Agilent Seahorse XF Pro analyzer measures and reports the oxygen consumption rate (OCR), proton efflux rate (PER) or extracellular acidification rate (ECAR), as well as ATP production rates of live cells in a 96-well format. The XF Pro analyzer is also equipped with advanced software, standardized workflows, and advanced data analytics available in the Agilent Seahorse Analytics software. **Analyzer Application** - Cellular Energy Metabolism Studies, Phenotyping Screening, 2D Cultures, Spheroids **Assay Running Volume** - 160-300 µL/well **Microchamber volume** - 2.28 µL **Plate Format** - 96 well **Assay Wells** - 92 **Communication** - TCP/IP; USB; wireless **Controller Description** - Independent workstation with Windows 10 (64-bit) OS and touch-screen display, cable-connected to the analyzer, which allows full assay design, instrument control, and analysis capability **Dimensions (WxDxH)** - 38.74 cm x 45.72 cm x 58.42 cm **Memory** - 16 GB **Operating Environment Relative Humidity** - 20-80 % **Operating Environment Temperature** - 4-30 °C **Power Requirements** - 100-240 VAC, 9 A, 50/60 Hz **Sample Requirements** - 5000-250000 cells/well **Sample Temperature** - 16-42 °C	2,00,00,000.00	1	2,00,00,000.00

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Software - Wave Pro Controller software includes preloaded templates for quick experimental design, automatically calculates rates in real time, and provides exclusive cutting-edge data analysis Tools (e.g., multi-file analysis, dose-response curves, Z'), advanced template import feature and dose-response experiment setup

Weight - 22.2 kg

Best for - Phenotypic screening, Testing many conditions at once, Dose-response studies

General instrument performance - Highest-throughput analyzer, Experimental flexibility, Tested for hypoxia, Automation enabled, Advanced thermal control to reduce edge effect when used with XF Pro M plates

Measurement performance

– High Sensitivity

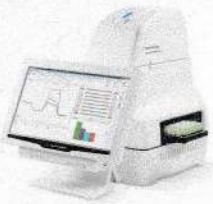
– Verified optimal measurement range (OCR of 13 to 350 pmol/min, PER of 50 to 950 pmol/min)

– Tight error in low ranges down to OCR of 13 pmol/min

Other features: Developed under ISO9001 certified compliant process

Suitable for spheroids

Compatible plate types - XF Pro M plate, XFe96/XF Pro plate, XFe96/XF Pro PDL plates and XFe96 spheroid plate



2	103793-100	38220090	12	Seahorse XFe96/XF Pro FluxPak Mini. For use with XFe96 and XF Pro Analyzer. Seahorse XFe96/XF Pro FluxPak Mini includes 6 XFe96/XF Pro sensor cartridges, 6 XFe96/XF Pro Cell Culture Microplates, and 1 bottle of Seahorse XF Calibrant Solution 500mL.	63,155.00	1	63,155.00
3	103344-100	38220090	12	Seahorse XF Glycolytic Rate Assay Kit	63,425.00	1	63,425.00
4	103681-100	38220090	12	Seahorse XF RPMI assay medium pack	24,060.00	1	24,060.00
5	103592-100	38220090	12	Seahorse XF Real-Time ATP Rate Assay Kit	44,875.00	1	44,875.00
6	103015-100	38220090	12	Seahorse XF Cell Mito Stress Test Kit	54,000.00	1	54,000.00
7	SYS-TS-4150-8D2	90279090	18	Extended Warranty - 2 yrs total	10,00,000.00	1	10,00,000.00

Total	2,12,49,515.00
Add : GST as applicable	38,09,942.00
Net F.O.R., Destination in Rupees	**2,50,59,457.00**

Amount In Words: INR : Two Crore Fifty Lakh Fifty Nine Thousand Four Hundred Fifty Seven INR Only.

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उप महानिदेशक (पी.)/Deputy Director General (P)
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सह-आचार्य/Associate Professor
of Biotechnology

TERMS & CONDITIONS:

1. Price quoted are F.O.R. Destination in Indian Rupees.
2. **GST prevailing at the time of invoicing shall be applicable extra.**
3. Payment Terms: 100% Advance in favour of; **"MEDISPEC INDIA LTD"**
   BANK DETAILS: ICICI BANK LIMITED
   UNIT NO.3 & 4, LINK HOUSE, LINK ROAD
   CHINCHOLI BUNDER, MALAD (W), MUMBAI - 400064
   BANK ACCOUNT NUMBER : 038805001003
   BANK MICR CODE : 400229211
   BANK IFSC CODE : ICIC0001959
4. Estimated Delivery: 15 weeks of order acceptance.
5. As materials are imported against orders, order once placed cannot be cancelled.
6. Since the materials are imported, prices are subject to change due to exchange rate variations and custom duty. Price quoted are based on the current Forex rate.
7. We reserve the right to readjust the prices without recourse to you at the time of delivery.
8. The terms of supplies are subject to Mumbai jurisdiction and are subject to Force Majeure clause.
9. Warranty of 12 months from the date of installation or 13 months from the date of delivery, whichever is earlier.
10. **MSME / UDYAM registration no. : UDYAM-MH-18-0032724**

For MEDISPEC (I) LTD

Phone : 09312698211 / 718810036 / 7718810037
Email : badonia@medispec.in, delhi@medispec.in, info@medispec.in
Web : www.medispec.in

ASHISH BADONI
Authorized Signatory

This is a computer generated Quotation, does not require signature.

डॉ. भूपेन्द्र कुमार वर्मा/Dr. BHUPENDRA KUMAR VERMA
अपर आचार्य/Additional Professor
जैव प्रौद्योगिकी विभाग/Dept. of Biotchnology
अ. भा. आ. सं., नई दिल्ली/A.I.I.M.S., New Delhi-110029

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Dr. KALPANA LUTHRA
आचार्य एवं अध्यक्ष/Professor & Head
जैव रसायन विभाग/Department of Biochemistry
All India Institute of Medical Sciences, New Delhi-29

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जैव प्रौद्योगिकी विभाग/Deptt. of Biotechnology
अ.भा.आ.सं., अंसारी नगर, नई दिल्ली-110029
AIIMS, Ansari Nagar, New Delhi-110029

डॉ. अमिता बाली/Dr. Amita Bali
उप महानिदेशक (पी.)/Deputy Director General (P)
स्वास्थ्य सेवा महानिदेशालय/Dte. G.H.S.
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
Ministry of Health & Family Welfare
भारत सरकार/Govt. of India

डॉ. एंजेल राजन सिंह
Dr. Angel Rajan Singh
अपर आचार्य - अस्पताल प्रशासन
Additional Professor - Hospital Administration
अखिल भारतीय आयुर्विज्ञान संस्थान, नई दिल्ली
All India Institute of Medical Sciences, New Delhi

Dr. JASWINDER SINGH MARAS
Associate Professor
Dept. of Molecular & Cellular Medicines
Institute of Liver & Biliary Sciences
D-1, Vasant Kunj, New Delhi-110070

Dr. Shilpi Minocha
Assistant Professor
Kusuma School of Biological Sciences
Indian Institute of Technology Delhi
Hauz Khas, New Delhi-110016 (India)

medispec
Products for Future of Science

Ref.: Quotation No.: QT12859 dated 29/05/2025

FALL CLAUSE CERTIFICATE

UNDERTAKING
TO WHOM SOEVER IT MAY CONCERN

We, **MEDISPEC INDIA LIMITED**, undertake that "The prices offered are the lowest and not higher than those offered to other organizations", in case it supplies or quotes a lower rate to other governments, public sector or private organization, it would reimburse the excess".

Your's Sincerely,
for MEDISPEC (I) LTD

Ashish Badoni
Authorized Signatory
Email: badonia@medispec.in, delhi@medispec.in

डॉ. कल्पना लूथरा
Dr. KALPANA LUTHRA
आचार्य एवं अध्यक्ष/Professor & Head
जैव प्रौद्योगिकी विभाग
Department of Biotechnology
अखिल भारतीय आयुर्विज्ञान संस्थान
All India Institute of Medical Sciences

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Associate Professor
Dept. of Molecular & Cellular Medicines
Institute of Liver & Biliary Sciences
D-1, Vasant Kunj, New Delhi-110070

डॉ. सुमित राठौर/Dr. SUMIT RATHORE
सह-आचार्य/Associate Professor
जैव प्रौद्योगिकी विभाग/Deptt. of Biotechnology
अ.भा.आ.सं., अंसारी नगर, नई दिल्ली-110029
AIIMS, Ansari Nagar, New Delhi-110029

डॉ. रूपेश कुमार श्रीवास्तव/Dr. RUPESH KUMAR SRIVASTAVA
अपर आचार्य/Additional Professor
जैव प्रौद्योगिकी विभाग/Dept. of Biotechnology
अ. भा. आ. सं., नई दिल्ली/A.I.I.M.S., New Delhi-110029

डॉ. भूपेन्द्र कुमार वर्मा/Dr. BHUPENDRA KUMAR VERMA
अपर आचार्य/Additional Professor
जैव प्रौद्योगिकी विभाग/Dept. of Biotchnology
अ. भा. आ. सं., नई दिल्ली/A.I.I.M.S., New Delhi-110029

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Additional Professor - Hospital Administration
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All India Institute of Medical Sciences, New Delhi

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उप महानिदेशक (पी.)/Deputy Director General (P)
स्वास्थ्य सेवा महानिदेशालय/Dte. G.H.S.
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
Ministry of Health & Family Welfare
भारत सरकार/Govt. of India

Dr. Shilpi Minocha
Assistant Professor
Kusuma School of Biological Sciences
Indian Institute of Technology Delhi
Hauz Khas, New Delhi-110016 (India)

MEDISPEC (I) LTD
A WING, 601, LOTUS CORPORATE PARK, JAY COACH, OFF W. E. HIGHWAY, GOREGAON EAST, MUMBAI – 400 063. INDIA
TEL : +91 7718810036 / 37 EMAIL : medispec@medispec.in WEB : www.medispec.in CIN : U24230MH1995PLC091242
AHMEDABAD - BANGALORE - CHANDIGARH - CHENNAI - DELHI - HYDERABAD - KOCHI - KOLKATTA - LUCKNOW

ALL INDIA INSTITUTE OF MEDICAL SCIENCES
ANSARI NAGAR, NEW DELHI – 110 029

Proprietary Article Certificate (PAC) (For Spare/Consumables etc.)

1.	The indented goods are manufactured by M/s	:	Supplier Name and Address: Medispec India Ltd, "A" Wing, 601, Lotus Corporate Park, Jay Coach, Off. W. E. Highway, Goregaon (East), Mumbai – 400 063. Manufacturer name: Agilent Technologies Singapore (International) Pte. Ltd., No. 1 Yishun Avenue 7, Singapore 768923
2.	Item Name	:	Seahorse XF Pro Analyzer
3.	Model/Part/Catalogue No.	:	Item: S7855A- Seahorse XF Pro Analyzer
4.	Reasons/Justification which makes the requirement Proprietary##		Sea horse is the only machine to measure ECAR & OCR simultaneously of living cells(attached is patent certificate

1. It is certified that market survey has been done and found that no other manufacturer is manufacturing similar / equipment specifications which can fulfill the vital requirements of end user.

2. It is certified that the purchase is proposed to be made on proprietary usage basis because any other material/equipment/instrument/Chemical etc. if used will lead to deviation and affect the integrity/accuracy, validity or analysis of the assignment/patient care.

##Note: 1.: TSEC should clearly mention the vital functional parameters requirements which end user essentially require and are manufactured by only one manufacturer mentioned in serial no.1.

Signature of Indenter
(Demanding Officer)

(Counter Signed)
(Head of the Department/PI)

डा. सुमित राठौर/Dr. SUMIT RATHORE
सह-आचार्य/Associate Professor
जैव प्रौद्योगिकी विभाग/Deptt. of Biotechnology
अ.भा.आ.सं., अंसारी नगर, नई दिल्ली-110029
AIIMS, Ansari Nagar, New Delhi-110029

डॉ. कल्पना लूथरा
Dr. KALPANA LUTHRA
आचार्य एवं अध्यक्ष/Professor & Head

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अपर आचार्य/Additional Professor
जैव प्रौद्योगिकी विभाग/Dept. of Biotchnology
अ. भा. आ. सं., नई दिल्ली/A.I.I.M.S., New Delhi-110029

डॉ. अमिता बाली/Dr. Amita B
उप महानिदेशक (पी.)/Deputy Director General (P.
स्वास्थ्य सेवा महानिदेशालय/Dte. G.H.S.
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
Ministry of Health & Family Welfare
भारत सरकार/Govt. of India

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All India Institute of Medical Sciences, New Delhi

Dr. Shilpi Mnocha

medispec
Products for Future of Science

Ref.: Quotation No.: QT12859 dated 29/05/2025

FALL CLAUSE CERTIFICATE

UNDERTAKING
TO WHOM SOEVER IT MAY CONCERN

We, **MEDISPEC INDIA LIMITED**, undertake that "The prices offered are the lowest and not higher than those offered to other organizations", in case it supplies or quotes a lower rate to other governments, public sector or private organization, it would reimburse the excess".

Your's Sincerely,
for MEDISPEC (I) LTD

Ashish Badoni
Authorized Signatory
Email: badonia@medispec.in, delhi@medispec.in

डॉ. रूपेश कुमार श्रीवास्तवा/Dr. RUPESH KUMAR SRIVASTAVA
अपर आचार्य/Additional Professor
जैव प्रौद्योगिकी विभाग/Dept. of Biotchnology
अ. भा. आ .सं., नई दिल्ली/A.I.I.M.S., New Delhi-110029

सत्यमेव जयते

INDIA NON JUDICIAL

Government of National Capital Territory of Delhi

e-Stamp

Certificate No.	:	IN-DL92286459176349X
Certificate Issued Date	:	29-May-2025 01:37 PM
Account Reference	:	IMPACC (IV)/ dl914903/ DELHI/ DL-SWD
Unique Doc. Reference	:	SUBIN-DLDL91490322689657063282X
Purchased by	:	MEDISPEC INDIA LTD
Description of Document	:	Article 4 Affidavit
Property Description	:	Not Applicable
Consideration Price (Rs.)	:	0 (Zero)
First Party	:	MEDISPEC INDIA LTD
Second Party	:	Not Applicable
Stamp Duty Paid By	:	MEDISPEC INDIA LTD
Stamp Duty Amount(Rs.)	:	10 (Ten only)

NOTARY PUBLIC ★ RAJKUMAR (ADVOCATE) Area: DELHI Regn. No. 6536 Expiry NOV.2027 ★ GOVT. OF INDIA

Please write or type below this line

NOTARY PUBLIC ★ RAJKUMAR (ADVOCATE) Area: DELHI Regn. No. 6536 Expiry NOV.2027 ★ GOVT. OF INDIA

Contd... 2

Statutory Alert:
1. The authenticity of this Stamp certificate should be verified at 'www.shcilestamp.com' or using e-Stamp Mobile App of Stock Holding

NOTARY PUBLIC
RAJKUMAR
(ADVOCATE)
Area: DELHI
Regn. No. 6536
Expiry NOV.2027
GOVT. OF INDIA

Affidavit

We, MEDISPEC INDIA LTD declaring:

- That there are no pending cases with CBI, ED, or FAM against the firm/organization.
- That MEDISPEC INDIA LTD is not blacklisted / debarred /banned by any government organization.

Your's Sincerely,
for MEDISPEC (I) LTD.

(Ref.: Quotation No.: QT12859 dated 29/05/2025)

MEDISPEC INDIA LTD. DELHI

Ashish Badoni
Authorized Signatory
Email: badonia@medispec.in, delhi@medispec.in

NOTARY PUBLIC
RAJKUMAR
(ADVOCATE)
Area: DELHI
Regn. No. 6536
Expiry NOV.2027
GOVT. OF INDIA

29 MAY 2025

ATTESTED

Notary Public, Delhi

medispec
Products for Future of Science

Ref.: Quotation No.: QT12859 dated 29/05/2025

RATE REASONABILITY CERTIFICATE

TO WHOM SOEVER IT MAY CONCERN

We, **MEDISPEC INDIA LIMITED**, certify that "the quoted rate are fair, reasonable and comparable to market rates".

Prices offered are the lowest and not higher than those offered to other organizations", in case it supplies or quotes a lower rate to other organization, it would reimburse the excess".

Your's Sincerely,

for MEDISPEC (I) LTD

Ashish Badoni
Authorized Signatory
Email: badonia@medispec.in, delhi@medispec.in

डॉ. राकेश कुमार श्रीवास्तव/Dr. RUPESH KUMAR SRIVASTAVA
अपर आचार्य/Additional Professor
जैव प्रौद्योगिकी विभाग/Dept. of Biotchnology
अ. भ. आ .सं., नई दिल्ली/A.I.I.M.S., New Delhi-110029

MEDISPEC (I) LTD
A WING, 601, LOTUS CORPORATE PARK, JAY COACH, OFF W. E. HIGHWAY, GOREGAON EAST, MUMBAI – 400 063. INDIA
TEL : +91 7718810036 / 37 EMAIL : medispec@medispec.in WEB : www.medispec.in CIN : U24230MH1995PLC091242
AHMEDABAD - BANGALORE - CHANDIGARH - CHENNAI - DELHI - HYDERABAD - KOCHI - KOLKATTA - LUCKNOW

भारतीय प्रौद्योगिकी संस्थान मद्रास
Indian Institute of Technology Madras
Chennai - 600 036

GSTIN : 33AAAAI3615G1Z6 STORES AND PURHCAS SECTION Phone : 044-2257 8287/88/85/90
E-Mail : adstores@iitm.ac.in Fax :

Purchase Order

Date 22-Mar-2024

Order No.: BT/2023/0267/SP/467

To,

M/s MEDISPEC INDIA LTD
A WING, 601, LOTUS CORPORATE PARK,
JAY COACH, OFF W.E. HIGHWAY,
GOREGAON (EAST), MUMBAI - 400 063.

With reference to your Tender No. GTED1/NATH/2024/25/METABO Dated 07.03.2024, please register our order and supply the following to BIO-TECHNOLOGY subject to the conditions overleaf. Please quote the above Purchase Order No and date in all packages correspondence bills etc.

S. No.	Description	Qty	Rate (In INR)	Total Cost (In INR)
1	Supply and Installation of METABOLIC GAS-ANALYZER FOR CO2 AND O2 - AUTOMATIC (Technical Specifications As Per Annexure)	1	2,58,27,761.00	2,58,27,761.00
	Total			2,58,27,761.00

Terms & Conditions:

- ✓ Price : Inclusive of taxes.
- ✓ Warranty : 3 Years
- ✓ Delivery : 10 weeks from the date of PO
- ✓ Payment Term: 100% payment in advance to M/s MEDISPEC (I) LTD by wire transfer against 20 % Bank Guarantee/FDR with validity upto the delivery period.
- ✓ Performance Bank Guarantee (PBG): 5% of PO Value to be furnished after the completion of Installation by M/s MEDISPEC (I) LTD.
- ✓ The validity of the PBG is from the date of Installation to the end of warranty period plus 60-days claim period.
- ✓ Other terms and conditions as per Tender.

B K Shelja Sabar
ASSISTANT REGISTRAR
STORES AND PURCHASE SECTION
सहायक कुलसचिव / Assistant Registrar
भंडार एवं क्रय अनुभाग
Stores and Purchase Section

TERMS AND CONDITIONS

1. **Confirmation** : Supplier should confirm acceptance of the order immediately.

2. **Delivery** : Delivery should be made according to the stipulations in the order. The order will stand cancelled if the supply is not effected within the due date. The Institute will not be liable for deliveries made beyond the stipulated date of delivery without prior concurrence. If the delivery is not made by the date specified, the Institute may purchase the goods from elsewhere. Goods will be accepted by the Departments, where local delivery is involved between 8.00 a.m and 12.00 noon and 1.00 p.m to.4.30 p.m on all working days (from Monday through Friday) and between 9.00 a.m to 6.00 p.m (12.00 noon to 4.00 p.m. recess) for Hospital.

3. **Insurance** : The responsibility for insuring the goods will be that of the supplier.

4. **Advice of Despatch** : A despatch advice should be sent showing the details of items despatched, mode of despatch, date etc. Packing Notes/Delivery Notes bearing Purchase Order No. and date should accompany the goods.

5. **Acceptance of goods** : Goods will be subjected to inspection on receipt and will be accepted only if they comply with our requirements. The supplier will be responsible for replacements of the defective goods without any charge whatsoever. The goods if not accepted by us on inspection should be taken back by the supplier at his own risk and expense within 15 days from the date of communication from the concerned department/centre/section. Otherwise, it will be returned to the supplier at his risk and expenses. In case of shortages or damages in the goods supplied, if any should be made good and defective materials should be replaced by the supplier at free of cost.

6. **Prices** : The accepted rate inclusive of all charges unless otherwise agreed to.

7. **Invoice in Triplicate** : Original being pre-receipt on a Revenue Stamp should be sent to the concerned department/centre/section quoting the Purchase Order No. and Date along with the delivery details. Invoice should be supported by cash receipts or freight, insurance if any. Net amount of the invoice may be rounded off to the nearest rupee. Raise all your invoice in the name of the INDIAN INSTITUTE OF TECHNOLOGY MADRAS, SARDAR PATEL ROAD, CHENNAI - 600 036.

8. **Declaration** : That Supplier will be deemed to have declared his acceptance of the above conditions by acceptance of the order.

9. **Disputes and Jurisdiction** : Any legal dispute arising out of any breach of contract pertaining to this order shall be settled in the court of competent jurisdiction located within the City of Chennai in Tamil Nadu.

MEDISPEC (I) LTD

Offering Product for future of Science ...

SERVICE REPORT

☑ INST ☐ PMS ☐ CL — INST-001240

Date : 29/05/2024

☐ AHMEDABAD ☐ BANGALORE ☐ CHANDIGARH ☑ CHENNAI ☐ DELHI ☐ HYDERABAD ☐ KOLKATA ☐ LUCKNOW ☐ MUMBAI ☐ PUNE

Institution : Indian Institute of Technology, Madras
Sardar Patel Road,
Chennai, Tamilnadu

City : Chennai

Pincode : 600036

Attn : Dr. Shantanu Pradhan

Department : Biotechnology

Tel. : 9330351348

Email : spradhan@iitm.ac.in

Order Ref. : BT/2023/0267/SPL/467

PO Date : 22/03/2024

Model	Serial No.		
(S7850A) XF Pro	US00450414	Mainboard Base code :	1.49
XF ProController 10.20	HC035891C8	CoBoard1 Version :	2.10
Wave Pro 10.20	–	CoBoard2 Assaycode :	2.10
s) UPSIKVA	Luminous	PIC24 Version :	1.43

Principals: ☐ BioTek ☐ Syngene ☐ NuAire ☐ Synbiosis ☐ GeSiM ☐ WTA ☐ Biospherix ☑ Agilent

Service Complaint : Installation of XF Pro

Service Type: ☐ Inspection ☑ Installation ☐ U / Warranty Service ☐ U / AMC Service ☐ Preventive Maintenance ☐ Paid Service / Calibration ☐ Add-on / Complementary

Observation : * Instrument & its accessories (Consumables & Reagents) received as per Purchase Order in good condition.

Service Details :
* Verification (Physical Verification) of Instrument & its accessories done
* Unboxing of Equipment & Accessories completed.
* Installation of XF Pro Controller & Analyser completed.
* Operational, Maintenance, Calibrant checks completed.

Utilities Check List: ☑ Electric Supply : 230VAC ☑ Ground / Earthing ☑ Voltage Stabiliser / UPS ☑ Air Conditioning ☑ Dust Free Environment ☑ Computer ☐ Printer ☑ Interface : Serial / USB ☐ COM Port : ☐ Gas Supply ☐ Distilled Water ☐ ☐

Details of Test Equipments / Test Jigs / QC Test Solutions / Materials / Parts used :

No.	Part No.	Serial No.	Batch No.	Name / Description	Valid upto Date
2	103792-100	—	0006790696	Seahorse XFe96/XF Pro Fluxpak	
3	102905-100		0006776141	Seahorse XFe96 Spheroid Fluxpak	
4	103015-100		18099038	XF Cell Mito stress Test Kit	
5	103592-100		18077166	XFp Real-Time ATP Rate Assay Kit	
6	103674-100		18136166	Seahorse XF Glutamine Oxidation stress Test kit	
7	103344-100		18094925	Seahorse XF Glycolytic Rate Assay Kit.	

Test Result : Installation of Seahorse XF Pro completed satisfactorily

Note / Remarks : Installation completed. Demo with samples to be done.

For MEDISPEC (I) LTD. : [signature] 29/05/2024 [stamp: MEDISPEC INDIA LTD MUMBAI]

Engineer's Name : Shirish Dhamale

For Customer's Approval / Acceptance : [signature] Pradhan

Signature & Seal :

A WING, 601, LOTUS CORPORATE PARK, JAY COACH, OFF W. E. HIGHWAY, GOREGAON EAST, MUMBAI - 400 063, INDIA
TEL : +91 77188 10036 / 37 EMAIL : support@medispec.in WEB : www.medispec.in

Supply Order

ALL INDIA INSTITUTE OF MEDICAL SCIENCES
ANSARI NAGAR, NEW DELHI - 110029
STORE SECTION (DO)

S.O. No. 61/SO(DO)/Biotech/2020-21/FSC-I **Dated: 04.02.2021**

M/s. LABMATE (ASIA) PVT. LTD.,
Baid Mehta Complex,
C-Block, 1st Floor, No. 183,
Mount Road, Saidapet,
Chennai – 600015 (India)

Date of Delivery: 30.04.2021
Or Earlier

Sir,

With reference to your Proforma Invoice No. **LA/AIIMS/P.I./15092020** dated **15.01.2021** against our Tender Ref. No. **47/Stores(DO)/Biotech/PAC/2019-20/FSC**. You are requested to arrange supplies of under mentioned items at the price Marked against each of deliver the same to the Store Officer, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, on terms and Conditions stated as Annexure-I (On behalf of the Director, AIIMS, New Delhi).

S.No.	Description of Goods	Qty	Rate	Amount
1.	**XFe96 Extracellular Flux Analyzer-01 no.** **Make: Agilent Technologies, USA**	1	1,89,86,612	1,89,86,612
	Consists of following:			
1.1	**S7800A XFe96 Extracellular Flux Analyzer:** a) 102601-100 XFe 96 FluxPak Mini - 2 nos. b) 103015-100 XF Cell Mito Stress Test Kit For 6 nos. 96 well plates - 1 no. c) 103020-100 XF Glycolysis Stress Test Kit For 6 nos of 96 well plates - 1 no d) 103193-100 XF Base Medium (100 ml)-1 no.			
	Consumables :			
2.	102416-100 Seahorse XFe96 FluxPak	6	1,33,507	8,01,045
3.	102504-100 Seahorse XF Plasma Membrane Permeabilizr	6	36,792	2,20,751
4.	103015-100 Seahorse XF Cell Mito Stress Test Kit	9	52,606	4,73,454
5.	103020-100 Seahorse XF Glycolysis Stress Test Kit	12	78,102	9,37,225
6.	103260-100 Seahorse XF Mito Fuel Flex Test Kit	6	78,102	4,68,613
7.	103344-100 Seahorse XF Glycolytic Rate Assay Kit	6	63,691	3,82,147
8.	103592-100 XF Real-Time ATP Rate Assay Kit	6	44,215	2,65,289
			Sub Total	**2,25,35,136**
			GST @ 5%	**11,26,757**
			Total Price	**2,36,61,893**

4/2/2021
वरिष्ठ भण्डार अधिकारी (नि.दा.)
Sr. Stores Officer (D.O.)
अ.भा.आ.सं., नई दिल्ली-110029
AIIMS, New Delhi-110029

17/02/21

1. Items Eight only.
2. Rupees Two Crore Thirty Six Lac Sixty One Thousand Eight Hundred Ninety Three only.
3. Warranty:- 5 Years comprehensive warranty from the date of Satisfactory Installation
4. Post Warranty Comprehensive Annual Maintenance Contract Charges will be applicable @ Rs. 9,49,331/- per year + GST extra for 6th to 10th Year, after expiry of 05 years comprehensive warranty.
5. The above given consumables prices will be fixed till 31st March 2022. After 31st March 2022, the increase of 5% will be applicable on consumables items for the period of next 4 years - subject to the increase in custom duty charges.
6. Other Terms and Conditions as per Annexure-I Attached

Sr. Stores Officer (DO)

Sr. Stores Officer (D.O.)
अ.भा.आ.सं., नई दिल्ली-110029
A.I.I.M.S., New Delhi-110029

Copy to:

1. Store Account Section
2. Central Store Depot
3. Firm Copy
4. HOD, Biotechnology

ANNEXURE - I

ALL INDIA INSTITUTE OF MEDICAL SCIENCES
ANSARI NAGAR, NEW DELHI – 110029

1.	Delivery terms	:	**FOR AIIMS NEW DELHI-110 029** The goods are to be delivered in Department of Biotechnology at AIIMS. (Quantity: 01 No.)
2.	Last date of delivery	:	ON OR BEFORE: **30.04.2021** for delivery of the equipment including Installation and commissioning and satisfactory handing over the department.
3.	Supply order No.	:	S.O. NO. 61/SO(DO)/Biotech/2020-21/FSC-I
4.	Comprehensive Warranty	:	The warranty will be for 05 (five) years Comprehensive (INCLUDING ALL SPARE AND LABOR) from the date of installation certificate issued by the user department.
5.	Taxes:	:	The price mentioned in the supply order is inclusive of all taxes/ GST.
6.	PAYMENT TERMS	:	Payment shall be made through Electronics Transfer in NEFT/ RTGS subject to recoveries if any by way of Liquidated damages or any other charges as per term & condition of contract. Payment will be made in Indian Rupees in the following manner: a) **On Delivery:** 75% (Seventy Five %) payment of the contract price of the equipments/goods shall be paid on receipt of equipments /goods in good condition and upon the submission of the following documents: (i) Four copies of supplier's invoice showing contract number, goods description, quantity, unit price and total amount; (ii) Inspection Note in original of having received the goods issued by the authorized representative of the consignee; (iii) Two copies of packing list identifying contents of each package. b) **After Installation:** Balance 25% (Twenty Five %) payment of the contract price shall be paid after successful installation, conditioning and handing over of the complete system, subject to submission of Performance Bank Guarantee as detailed on S.No. 07 below and the submission of the following documents: (i) Four copies of supplier's invoice showing contract number, goods description, quantity, unit price and total amount; (ii) Inspection Note in original about satisfactory installation and commissioning of the complete system by the authorized representative of the consignee.

वरिष्ठ भण्डार अधिकारी (वि.का.
Sr. Stores Officer (D.O.
अ.भा.आ.सं., नई दिल्ली-11002
AIIMS, New Delhi-11002

7.	Performance Security	:	Within fifteen (15) days of receipt of Supply Order, the supplier, shall furnish performance security to the Purchaser/Consignee for an amount equal to three percent (03%) of the total value of the contract, valid up to sixty (60) days after the date of completion of all contractual obligations by the supplier, including the warranty obligations, initially valid for a period of minimum 63 months from the date of Notification of Award The Performance security shall be denominated in Indian Rupees or in the currency of the contract as detailed below: It shall be in any one of the forms namely Account Payee Demand Draft or Fixed Deposit Receipt drawn from any Scheduled bank in India or Bank Guarantee issued by a Scheduled bank in India, in the prescribed form as provided in section XV of this document in favour of the Purchaser/Consignee. The validity of the Fixed Deposit receipt or Bank Guarantee will be for a period up to sixty (60) days beyond Warranty Period.
8.	Comprehensive AMC (including all spares and Labor)	:	**The rates for Comprehensive Annual Maintenance Contract for XFe96 Extracellular Flux Analyzer-01 no. (including all spares and labor also) will be applicable @ Rs.9,49,331/- per year per unit + GST Extra or 6th to 10th year, after expiry of five years comprehensive warranty.** This contract shall be effective immediately after the expiry of the warranty period. Hence, bidder has to submit a fresh proposal of approved rates for preparing CMC agreement. Before commencement of CMC period, the supplier has to furnish a Performance Bank Guarantee for 2.5 % of the cost of the equipment valid till 2 months extra after expiry of entire CMC period. The payment of CMC will be made on yearly basis after satisfactory completion of said period duly certified by end user. The Performance Bank Guarantee for CMC will be applicable in case of equipment cost is more than Rs.10 lakhs. During warranty and CMC period, Institute will not provide any exemption documents for clearance from custom or maintain the equipment.
9.	Installation	:	Installation of the equipment will be done by the trained Engineers of supplier free of cost.

वरिष्ठ भण्डार अधिकारी (नि.)
Sr. Stores Officer (D.C.)
अ.भा.आ.सं., नई दिल्ली-110029
A.I.I.M.S., New Delhi-110029

10.	Training	:	If applicable, necessary training to Doctors/Technicians/Staff must be provided by Principal/Indian Agent at free of cost for smooth operation and maintenance of the equipment to the satisfaction of the user department.
11.	Liquidated damage of delay in supply	:	The delivery date as stipulated should be strictly adhered to failing which the Institute reserves the right to refuse the supplies. The extension of the date of delivery, if required should be obtained before the expected delivery date. If the supplier fails to deliver any or all of the goods within prescribed time frame(s) incorporated in the supply order, the Purchaser/Consignee shall, without prejudice to other rights and remedies available to the Purchaser/Consignee under the contract, deduct from the contract price, as liquidated damages, a sum equivalent to 0.5% per week of delay or part thereof on delayed supply of goods and/or services until actual delivery subject to a maximum of 10% of the contract price. Once the maximum is reached Purchaser/Consignee may consider termination of the contract as per. In case of non-supply, the order shall be cancelled and the penalty shall be levied at the discretion of the Director, AIIMS or his authorized representative as per Institute rules.
12.	Up –time Guarantee	:	The firm should provide uptime guarantee of 95% (24 hrs/day basis) will be ensured both during warranty and during CAMC.
13.	Downtime Penalty Clause	:	The firm should provide uptime guarantee of 95% (24 hrs/day basis) will be ensured both during warranty and during CAMC. If downtime exceeds the 5% limit, extension of the warranty period will be twice the excess down time period. In addition a penalty equal to amount of 0.25% of the total cost of equipment per day will be liveable for the excess downtime period subject to Maximum of 10% of Cost of the Equipment. Complaints should be attended properly, maximum within 8 hrs. The vendor must undertake to supply all spares for optimal upkeep of the equipment for at least FIVE YEARS after handing over the unit to the Institute. If accessories/other attachment of the system are procured from the third party, then the vendor must produce cost of accessory/other attachment and the CMC from the third party separately along with the main offer and the third party will have to sign the CMC with the Institute if required.

4/2/2025

वरिष्ठ भण्डार अधिकारी (नि.का.
Sr. Stores Officer (
अ.भा.आ.सं., नई दिल्ली-11002
AIIMS, New Delhi-11002

14.	**In case of Indian Rupees payment:** The supplier has to submit the following information invariably to make payment through Electronic mode i.e. RTGS/NEFT and the charges incurred for affecting such electronic transfers will be borne by the vendors. The details of present charges for NEFT/RTGS are as under: **NEFT: Up to Rs.1 lakh --Rs.5/- per transaction** **Rs. 1 lakh and above –Rs.25/- per transaction** **RTGS: Upto Rs.5 lakh –Rs.25/- per transaction** **Rs.5 lakh and above --Rs.50/- per transaction** **To make payment through above said mode, the vendor/supplier/contracts have to submit the following information invariably along with their bills/challans:** **i. Name of the Beneficiary** **ii. Account No. of the beneficiary.** **iii. IFCS Code of the Bank/Branch.** **"Failing which their bills/challans will not be accepted/entertained for making payment."**
15.	**Resolution of Dispute/Arbitration:** If dispute or difference of any kind shall arise between the Purchaser/Consignee and the supplier in connection with or relating to the contract, the parties shall make every effort to resolve the same amicably by mutual consultations. If the parties fail to resolve their dispute or difference by such mutual consultation within twenty-one days of its occurrence, then, unless otherwise provided in the SCC, either the Purchaser/Consignee or the supplier may give notice to the other party of its intention to commence arbitration, as hereinafter provided the applicable arbitration procedure will be as per the Arbitration and Conciliation Act, 1996 of India. In the case of a dispute or difference arising between the Purchaser/Consignee and a domestic Supplier relating to any matter arising out of or connected with the contract, such dispute or difference shall be referred to the sole arbitration of an officer in the Ministry of Law and Justice, appointed to be the arbitrator by the Director, AIIMS. The award of the arbitrator shall be final and binding on the parties to the contract subject to the provision that the Arbitrator shall give reasoned award in case the value of claim in reference exceeds Rupees One lakhs (Rs.1,00,000/-).
16.	Force Majeure: Any failure or omission to carry out the provisions of this contract/supply order by the supplier shall not give rise to any claim by the supplier and the Institute, one against the other, if such failure or omission arises from the act of God, which shall include all acts of natural calamities such as fire, floods, civil strike, compliance with any statute regulations of the Govt., Lockouts, and strikes, riots, embargoes, or from any political or other reason beyond control of supplier and their Indian agent including war (whether declared or not) civil war or state of insurrection, provided notice of the occurrence of any event by either party to the other shall be given within two weeks from the date of occurrence of any event which could be attributed to Force Majeure conditions.

वरिष्ठ भण्डार अधिकारी (नि.का.)
Sr. Stores Officer (D.C.)
अ.भा.आ.सं., नई दिल्ली-110029
A.I.I.M.S., New Delhi-110029

17.	**Venue of Arbitration:** The venue of arbitration shall be New Delhi, India.
18.	In addition to the above, in case of distributor changed by OEM during warranty/guarantee, the accepted liabilities by Indian agent will be completed/accepted by manufacturer or their new appointed Indian agent.
19.	In addition to the above, the other terms & conditions mentioned in the PAC proposal /bid documents & AIIMS Purchase Manual 2018 vide **No. 47/Stores(DO)/Biotech/2019-20/FSC-I** will also apply & binding on the supplier/OEM.
20.	**Correction/omission,** if any, will be considered within seven days from the date of issue of supply order only.

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4/2/2025

वरिष्ठ भण्डार अधिकारी (नि.का.)
Sr. Stores Officer (D.C.)
अ.भा.आ.सं., नई दिल्ली-110029
A.I.I.M.S., New Delhi-110029

Agilent

Sole Source Letter – Agilent Seahorse XF Technology

Dear Researcher,

Thank you for your interest in the Agilent Seahorse XF Analyzers. Seahorse XF metabolic analyzers are the most cited and most used technology platform for measuring cell metabolism in live cells in real time. Seahorse XF analyzers are the only commercially available instruments for scientific research that simultaneously measure the two major energy producing pathways of the cell – mitochondrial respiration and glycolysis – in living cells, in a microplate, in real time. Researchers are utilizing Agilent Seahorse XF technology for faster, better, and more accurate measurements of cellular metabolism.

Agilent Technologies is the sole manufacturer and developer of Seahorse XF Analyzers, XF sensor cartridges, and XF technology. Seahorse XF users have published >6,000 peer-reviewed articles in peer-reviewed journals, demonstrating that Seahorse XF technology is the gold standard for metabolic measurements in live cells.

Seahorse products are sold in exclusively by Agilent Technologies in the following countries: Austria, Belgium, Denmark, Finland, France, Germany, Ireland, Netherlands, Spain, Sweden and the United Kingdom. There are no authorized distributors for Seahorse XF Analyzers and XF consumables in these countries.

Seahorse XF technology is patented in many countries; specifically, EU Patent 1664740 covers the microchamber technology that makes this platform unique.

The following pages list technical features, many of which are found only in Seahorse XF technology, that justify Agilent Seahorse XF Analyzers and XF consumables for a sole source purchase.

Sincerely,

1/13/2021

X

William Jastromb
Product Manager, Seahorse XF Instruments
Signed by: William Jastromb

Dr. JASWINDER SINGH MARAS
Associate Professor
Dept. of Molecular & Cellular Medicines
Institute of Liver & Biliary Sciences
D-1, Vasant Kunj, New Delhi-110070

डॉ. एंजेल राजन सिंह
Dr. Angel Rajan Singh
अपर आचार्य – अस्पताल प्रशासन
Additional Professor - Hospital Administration
अखिल भारतीय आयुर्विज्ञान संस्थान, नई दिल्ली
All India Institute of Medical Sciences, New Delhi

डॉ. अमिता बाली/Dr. Amita Bali
उप महानिदेशक (पै.)/Deputy Director General (P)
स्वास्थ्य सेवा महानिदेशालय/Dte. G H.S.
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
Ministry of Health & Family Welfare
भारत सरकार/Govt. of India

डॉ. सुमित राठौर/Dr. SUMIT RATHORE
सह-आचार्य/Associate Professor
जैव प्रौद्योगिकी विभाग/Deptt. of Biotechnology
अ.भा.आ.सं., अंसारी नगर, नई दिल्ली-110029
AIIMS, Ansari Nagar, New Delhi-110029

Dr. RUPESH KUMAR SRIVASTAVA
Additional Professor
Deptt. of Biotchnology
A.I.I.M.S., New Delhi-110029

डॉ. कल्पना लूथरा
Dr. KALPANA LUTHRA
Professor & Head
Department of Biochemistry

Dr. Shilpi Minocha
Assistant Professor

Dr. BHUPENDRA KUMAR VERMA

15 September 2020

Agilent

Unique Features of Agilent Seahorse XF Technology

Sole Source Letter (continued)

Agilent Seahorse XF Technology

- Simultaneously measures oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of living cells. Using these measurements, glycolytic Proton Efflux Rate (PER) is easily calculated as an accurate measurement of glycolysis rates in real time. Quantitative ATP production rates can also be calculated in real time using well-validated proprietary algorithms.
- Performs a non-invasive measurement that does not require the addition of dyes, labels, or reporters.
- Measurements may be repeated to measure kinetic responses over several hours without causing unhealthy hypoxic or acidic conditions.
- Measures adherent cells without requiring trypsinization; suspension cells can be measured by attachment to the well bottom.
- Typically requires only $1x10^4$ to $1x10^6$ cells per well in the 24-well system; $5x10^3$ to $5x10^5$ per well in the 96-well and 8-well platform.
- Cells/microplate remain viable following an Agilent Seahorse XF assay and may be used for another assay.
- Measurement technology is covered by US patent # 7,276,351, EU Patent 1664740, and other issued and pending patents throughout the world.
- For Research Use Only. Not for use in diagnostic procedures.

Agilent Seahorse XFe and XF HS Mini Analyzers

- Instrument creates transient microchamber, makes optical measurements, and performs compound injections.
- Up to four test compounds may be added automatically to each well during the assay. Measurements may be performed before and after each compound is added.
- Temperature controlled measurement chamber is maintained at 37°C ± 0.5°C; alternative temperatures supported. XFe analyzers are also supported to for analysis in a hypoxic chamber.
- OCR and ECAR data are simultaneously calculated and displayed in real time. OCR and ECAR enable for calculations of glycolytic PER and ATP production rates.
- Rates are automatically calculated and reported every 5-8 minutes. Data are reported in calibrated rates of pmol/min or mpH/min.
- No cleaning is required. All the parts that are in contact with the cells, media, or compounds are disposable.
- Data analysis is done with Seahorse Analytics, a cloud-based platform designed expressly for analyzing and interpreting Seahorse XF assay results.
- Wave Desktop software provides an alternative way to view assay results and export into MS® Excel, Graphpad Prism, or Seahorse assay-specific reports.

Excitation and Emission

- Light Emitting Diodes (LEDs) are used as the monochromatic excitation source. LEDs are operated at very low excitation energy densities to prevent detectable levels of photobleaching.
- The usable life of the LED sources is >10,000 hours.

- Narrow band pass filters control spectral adherence.

Enclosure

- The precision-molded enclosures on Agilent Seahorse XFe and XFp Analyzers have specialty coatings and gaskets to shield the electronics and sample from ambient light, as well as block radiated and emitted electromagnetic interference.

Agilent Seahorse XF Assay Kits and Reagents

- Convenient, easy-to-use Seahorse XF test kits and Seahorse XF reagents simplify the study of cellular metabolism by providing pre-calibrated, pre-tested reagents for routine measurements of metabolic phenotype, mitochondrial respiration, glycolysis, and fatty acid oxidation, and ATP production rates.
- Standardized reagents, kits, and protocols are designed and validated for use with Agilent Seahorse XF analyzers.
- Convenient, quality-controlled kits provide standardized metabolic measurements in an easy-to-use format.
- Kits are simple, cost-effective and support reproducible results.

Agilent Seahorse XF Sensor Cartridges and Microplates

- Only Agilent Seahorse XF sensor cartridges are compatible with Agilent Seahorse XF instruments and must be used during an Agilent Seahorse XF assay.
- Agilent Seahorse XF Cell Culture Microplates and Miniplates have a unique geometry required for the XF measurements and are tissue culture treated for optimal cell growth.
- Optical sensors do not consume oxygen during the measurement and are not in contact with the cells.
- Optical sensors are not affected by compound or intracellular dye fluorescence.
- Oxygen sensor peak absorption = 530 nm (green). Oxygen peak emission = 650 nm (red).
  pH sensor peak absorption = 470 nm (blue). pH sensor peak emission = 530 nm (green).
- Each sensor cartridge is auto-calibrated at the beginning of the run.
- Sample preparation steps are automation compatible with an Agilent Bravo automated liquid handler or similar device.

Data Quality

- Because Agilent Seahorse XF assays are non-invasive, multiple measurements may be made to monitor samples over several hours. Measurement-to-measurement within the same well demonstrates a coefficient of variation of 5%, approximately equivalent to the instruments' background noise.
- Total variability between wells from all sources (instrument background plus biological variability due to cells) for the average trained user is <20%.

Normalization

An interface between a Seahorse XFe Analyzer and a BioTek Cytation 1 or Cytation 5 Imager is available, which applies cell count numbers directly to XF data enabling plate-to-plate, day-to-day, and well-to-well comparisons.

US007276351B2

(12) **United States Patent**
Teich et al.

(10) **Patent No.: US 7,276,351 B2**
(45) **Date of Patent: Oct. 2, 2007**

(54) **METHOD AND DEVICE FOR MEASURING MULTIPLE PHYSIOLOGICAL PROPERTIES OF CELLS**

(75) Inventors: **Jay S. Teich**, Weston, MA (US); **Andy C. Neilson**, Groton, MA (US); **Michael R. Sweeney**, Pelham, NH (US); **Geoff Uhl**, Cambridge, MA (US)

(73) Assignee: **Seahorse Bioscience**, Billerica, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 357 days.

(21) Appl. No.: **10/688,791**

(22) Filed: **Oct. 17, 2003**

(65) **Prior Publication Data**

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Related U.S. Application Data

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(51) **Int. Cl.**
G01N 1/30 (2006.01)
G01N 33/48 (2006.01)

(52) **U.S. Cl.** **435/40.51**

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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Primary Examiner—Ralph Gitomer
Assistant Examiner—Bin Shen
(74) *Attorney, Agent, or Firm*—Goodwin Procter LLP

(57) **ABSTRACT**

A method of analyzing cells disposed in media within a vessel includes the steps of providing a vessel having an original volume of media about the cells, reducing the original volume of media about at least a portion of the cells to define a reduced volume of media, and analyzing a constituent related to the cells within the reduced volume of media. An apparatus for analyzing cells includes a stage adapted to receive a vessel holding cells and a volume of media, a plunger adapted to receive a barrier to create a reduced volume of media within the vessel including at least a portion of the cells, the barrier adapted for insertion into the vessel by relative movement of the stage and the plunger, and a sensor in sensing communication with the reduced volume of media, wherein the sensor is configured to analyze a constituent disposed within the reduced volume.

48 Claims, 8 Drawing Sheets

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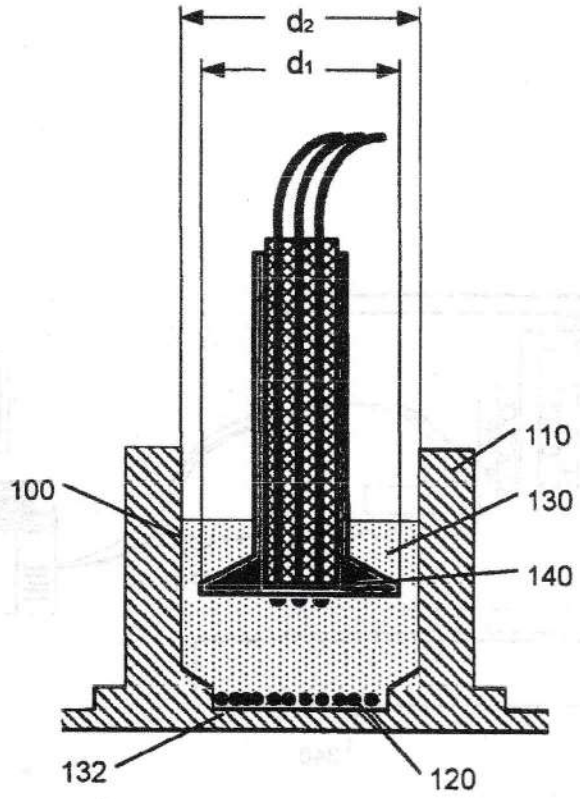

Fig. 1

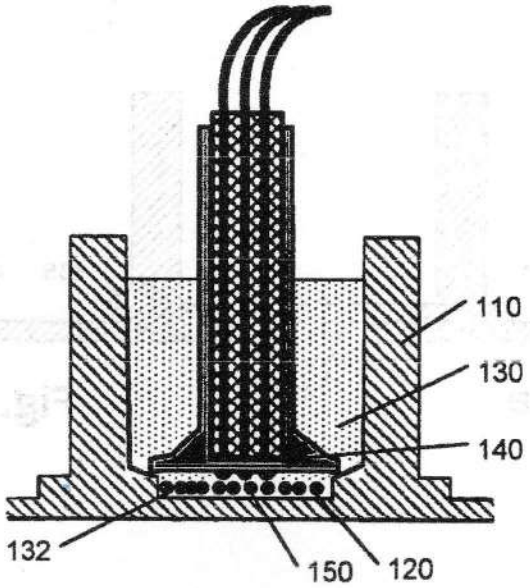

Fig. 2

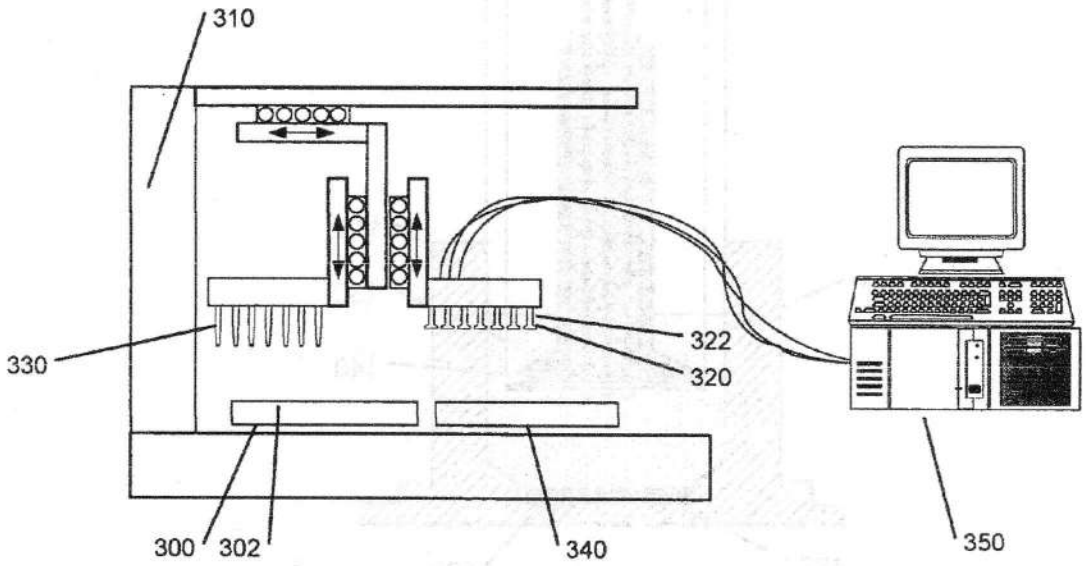

Fig. 3

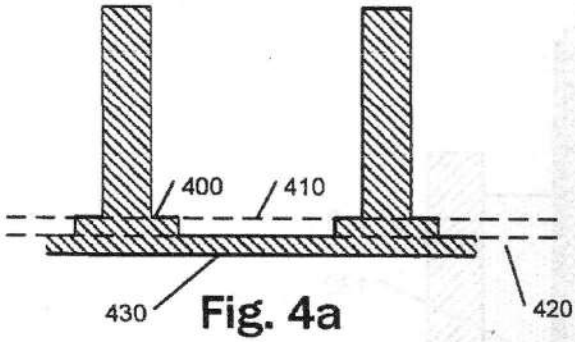

Fig. 4a

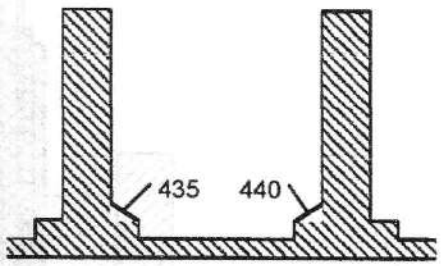

Fig. 4b

Fig. 5a

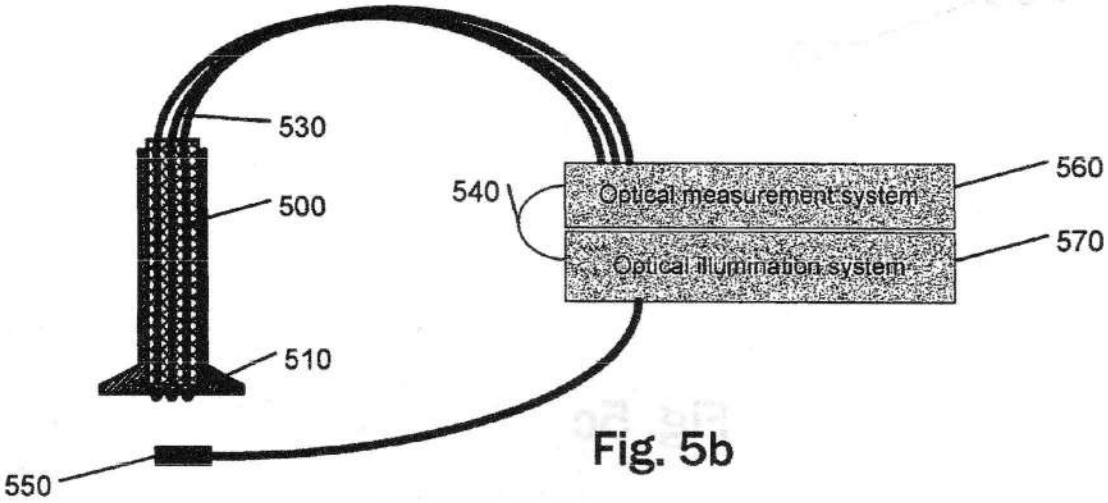

Fig. 5b

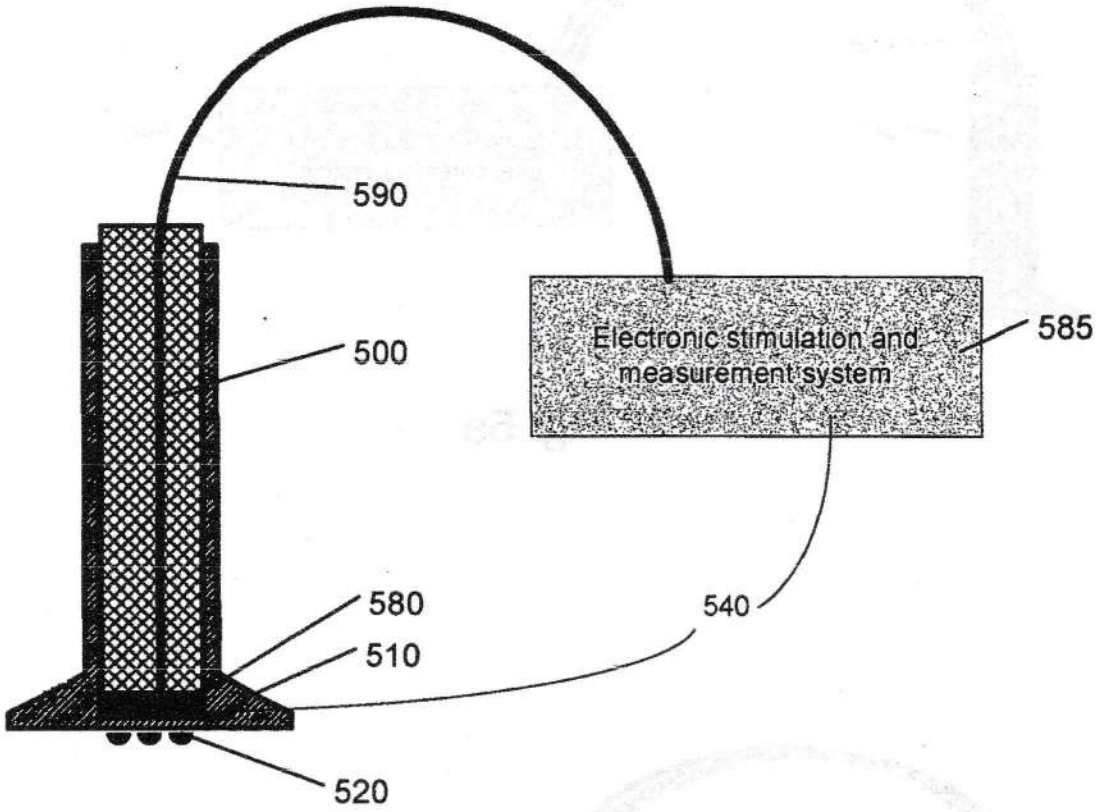

Fig. 5c

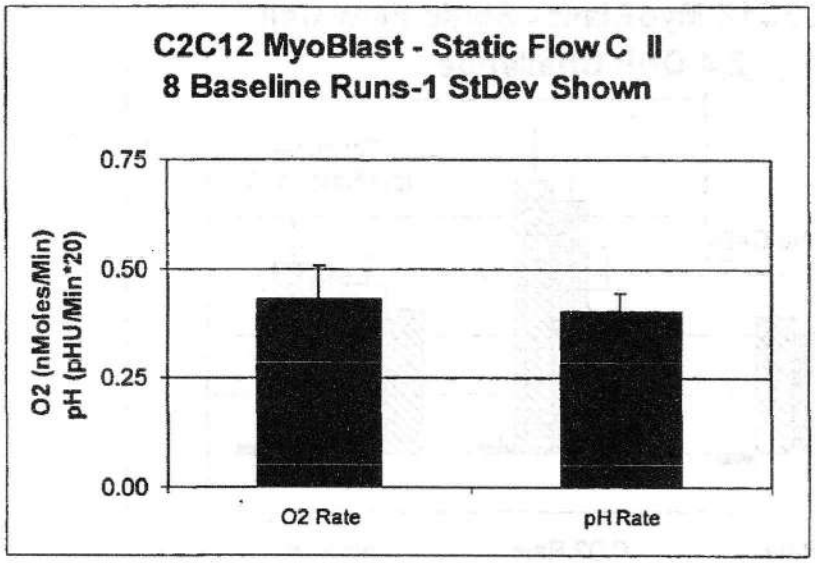

FIG 6

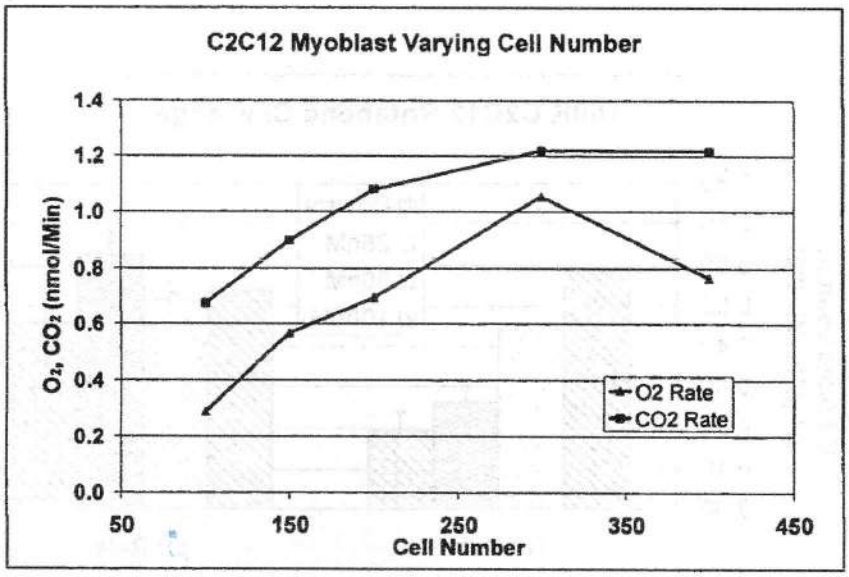

FIG 7

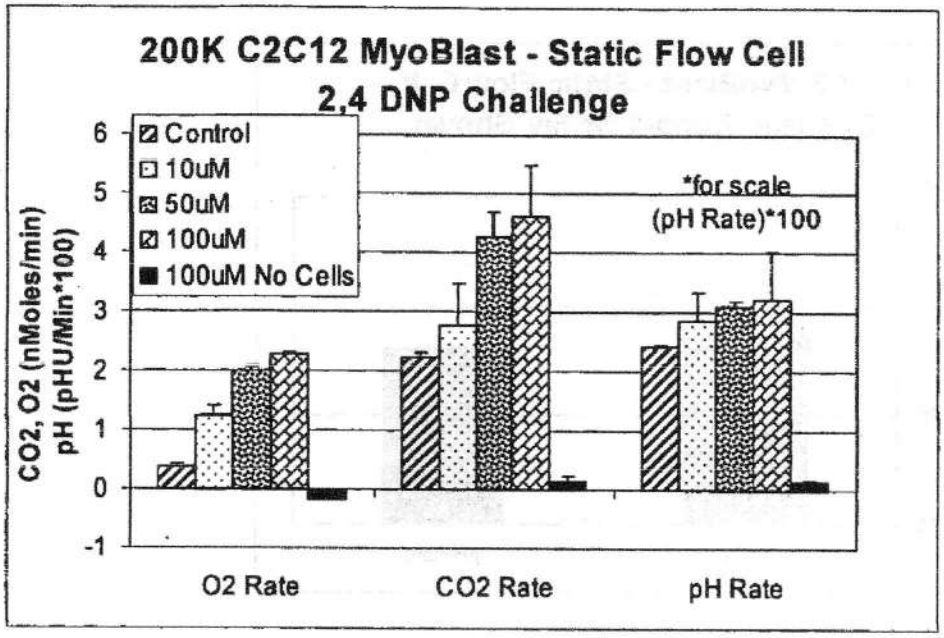

FIG 8

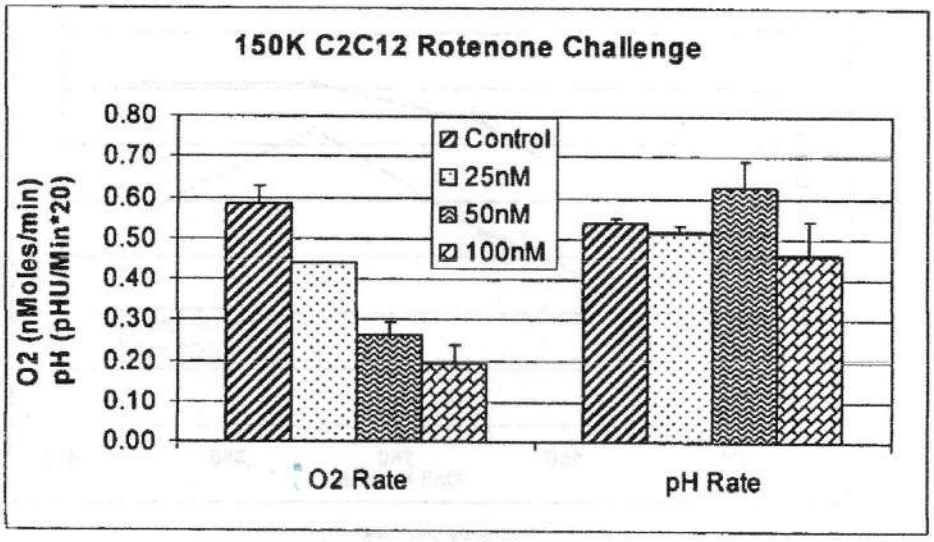

FIG 9

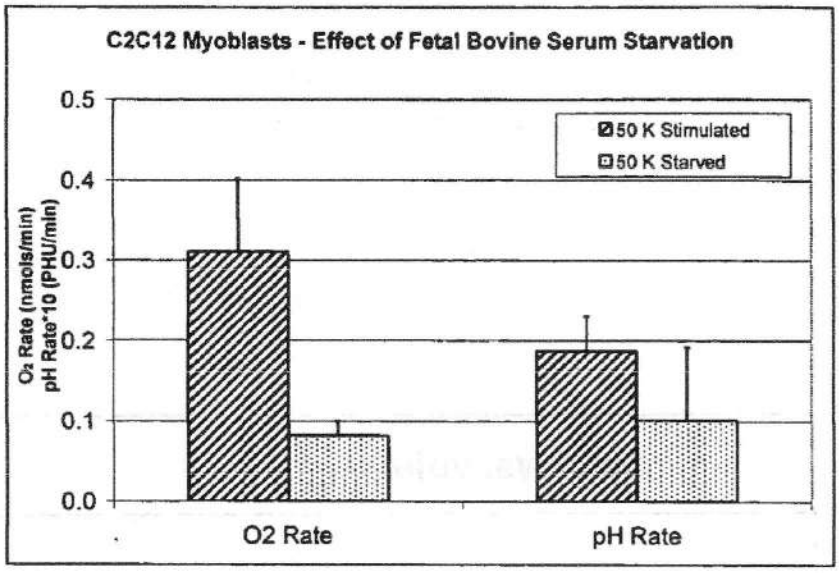

FIG 10

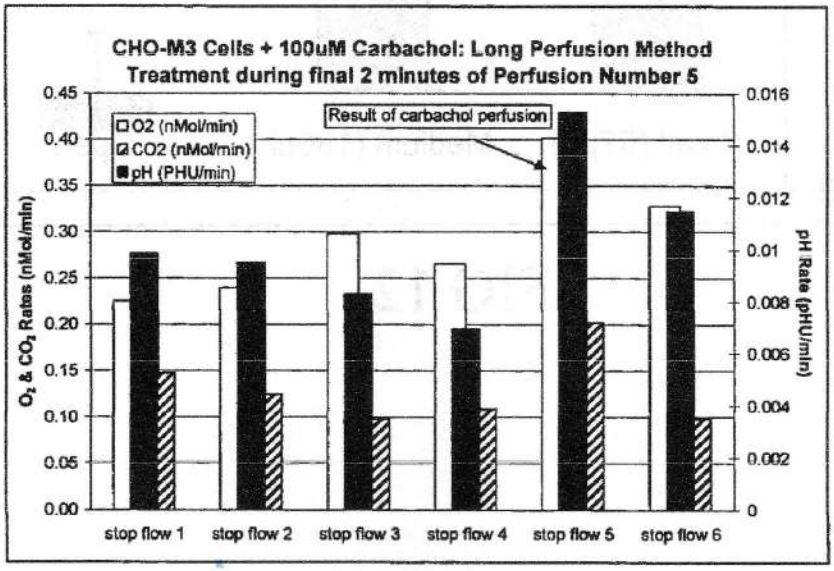

FIG 11

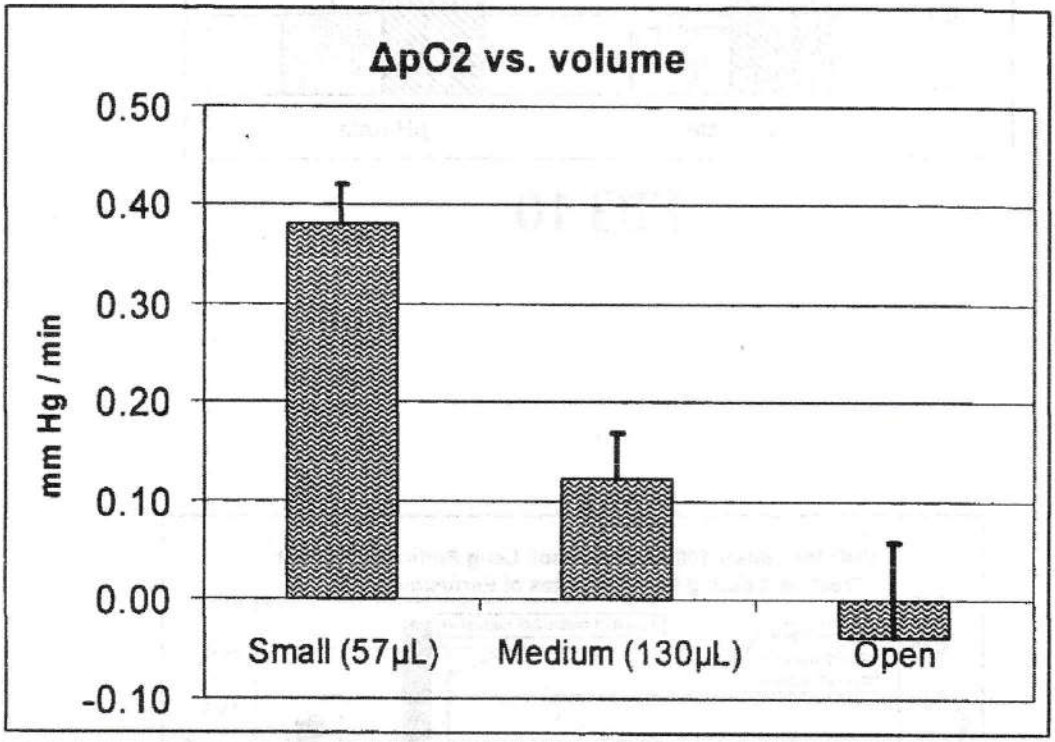

FIG 12

METHOD AND DEVICE FOR MEASURING MULTIPLE PHYSIOLOGICAL PROPERTIES OF CELLS

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Ser. No. 60/502,417, filed on Sep. 10, 2003, the entire disclosure of which is hereby incorporated by reference.

FIELD OF THE INVENTION

This application relates generally to high throughput screening techniques and, more specifically, to the measurement of the constituents (analytes) of an extracellular medium surrounding living cells. All of the patents, articles, and other references cited herein form a part of this patent application and their respective disclosures are incorporated herein by reference in their entirety.

BACKGROUND

Living cells typically consume nutrients and oxygen from the surrounding medium, and return metabolic byproducts, including ions, carbon dioxide, lactate, and various proteins, to this extracellular environment. The rate of uptake and excretion of these analytes can provide valuable information regarding the metabolic processes underway inside the cells.

Conventional biological assays inherently exhibit significant limitations. An ideal biological assay is homogeneous (i.e., does not require the introduction of a foreign agent such as a dye), non-invasive (i.e., has no deleterious effect on the biological process), and rapid.

Many tools have been developed to probe the mechanistic processes of cells using internalized reporters such as fluorescent dyes. A device that is able to measure extracellular analytes using a non-invasive, homogeneous assay performed within a container that is compatible with existing invasive tools would be particularly useful.

Some previous approaches relate to oxygen flux rate measurements, since respiration can be deemed to be a basic measure of cell viability. Many devices have been developed to monitor respiration in vitro, through determination of the rate of depletion of oxygen in the extracellular medium. The earliest instruments relied on the change in total gas pressure in a sealed vessel, using the assumption that this change was primarily due to oxygen consumption.

In the 1960s, the Clark electrode (Clark, L. C. Jnr. *Ann. NY Acad. Sci.* 1962; 102:29-45), and later the miniaturized Clark electrode, enabled a more specific measure of oxygen partial pressure. The relative complexity of the Clark design, and the fact that the electrode itself consumed oxygen, may have hindered its incorporation in a highly parallel instrument suitable for widespread use. However, these devices were deemed successful enough to measure cell viability (Gesinski R M, Morrison J H, Toepfer J R. "Measurement of oxygen consumption of rat bone marrow cells by a polarographic method." *J Appl Physiol.* 1968; 24(6):751-754), to profile the toxic effects of drugs and chemicals (Shenoy M A, Biaglow J E, Varnes M E, Hetzel F W. "Inhibition of cultured human tumor cell oxygen utilization by chlorpromazine." *Adv Exp Med Biol.* 1983;159:359-68), and to show the effect of agents such as insulin on cellular metabolic processes (Panten U and Klein H. "O_2 consumption by isolated pancreatic islets, as measured in a Microincubation system with a Clark-type electrode." *Endocrinology* 1982; 111:1595-1600).

More recently, several oxygen sensors have been developed that can enable the design of a non-invasive, homogeneous readout of cellular respiration. Fluorescent compounds, whose response is diminished by the phenomenon of oxygen-quenching, are now available. These compounds can be embedded in an oxygen permeable membrane and exposed to cell media, and can be read using low cost, fiber coupled, semiconductor light sources and sensors (Wolfbeis O S, 2002. "Fiber-Optic Chemical Sensors and Biosensors." *Annal of Chem.* 2002; 74:2663-2678).

An ion-sensitive field-effect transistor (ISFET), whose gate region can be exposed to a liquid analyte, has been adapted to measure oxygen pressure using enzyme catalyzed conversion of oxygen (O_2) to H^+ ions that can be detected by this sensor (Lehmann, M, Baumann W, Brischwein M, Gahle H-J, Freund I, Ehret R, Dreschler S, Palzer H, Kleintges M, Sieben U and Wolf B. "Simultaneous measurement of cellular respiration and acidification with a single CMOS ISFET. 2001." *Biosensors & Bioelectronics.* 2001;16:195-203).

Devices have been described and/or demonstrated that incorporate oxygen-quenched fluorophores, ISFETs and other oxygen sensors within sample chambers containing bacteria or mammalian cells for the purpose of measuring respiration rate, viability, or the effect of drugs or toxins. These devices range in size from fluorescent patches attached to the interior wall of large cell culture bottles (Tolosa L, Kostov Y, Harms P, Rao G. "Noninvasive measurement of dissolved oxygen in shake flasks." *Biotechnol Bioeng* 2002 Dec. 5;80(5):594-7), to fluorescent sensors embedded within microscopic flow cells fabricated using microfluidics technology (Lähdesmäki I, Scampavia L D, Beeson C, and Ruzicka J. "Detection of Oxygen Consumption of Cultured Adherent Cells by Bead Injection Spectroscopy." *Anal. Chem.* 1999; 71: 5248-5252), to microtitre plates with fluorescent compounds suspended within (O'Riordan T C, Buckley D., Ogurtsov V, O'Connor R., Papkovsky D B "A cell viability assay based on monitoring respiration by optical oxygen sensor." *Anal. Biochem.* 2000; 278(2):221-227) or deposited upon the wells (Woodnicka M, Guarino R D, Hemperly J J, Timmins M R, Stitt D, Pitner J B. "Novel fluorescent technology platform for high throughput cytotoxicity and proliferation assays." *Journal of Biomolecular Screening.* 2000; 5:141-152).

Some patents describe a device for monitoring cells using an oxygen-quenched fluorescent compound that is placed in contact with a broth containing bacteria or mammalian cells. A fluorescence measurement of cells treated with a drug or toxin may be compared to a reference, purportedly to determine the effect of the compound on cellular respiration. In an embodiment, cells are contained within a microplate that is exposed to ambient air. Cells are maintained at a low density in order to maintain viability in this configuration, because high cell density would likely result in anoxia, acidification of the media, and contact inhibition. Measurement times may, therefore, typically be tens of hours or days. In addition, the influx of ambient oxygen and lack of control of sample volume may allow only relative measurement to control to be made. In another embodiment, to limit ambient oxygen influx, mineral oil is placed above the cell media. Because cell density is typically quite low, long measurement times are typically required.

A number of patents and publications describe oxygen flux measurement systems incorporating small, closed sample chambers containing high densities of cells. In these devices, an active perfusion system is used to intermittently restore normal levels of dissolved oxygen, pH, and nutrients.

None of these systems are designed or configured to enable the user to easily culture cells, maintain their viability, run experiments in parallel with high throughput, or run other types of assays without detaching and moving the cells.

There have also been approaches to measuring cellular acidification rate. Living cells produce protons (H^+ ions) as a byproduct of various metabolic processes, including both aerobic and anaerobic respiration. Protons are also produced when ion exchange pumps on the surface of eukaryotic cells are activated as a result of binding of a ligand with a transmembrane receptor or ion channel. In a fixed volume of extracellular media, this proton flux causes a gradual acidification that can be measured using a pH sensor. Thus, an indication of metabolic rate and/or receptor activation can be determined from a precise measurement of extracellular acidification rate.

A number of pH sensors can be applied to the measurement of cell media. In addition to fluorescent and ISFET sensors similar to those described previously, a light addressable potentiometric sensor has been incorporated in an instrument for rapid measurement of proton flux (Parce W, Owicki J, Kercso K, Sigal G, Wada H, Muir V, Bousse L, Ross K, Sikic B, and McConnell H. 1989. "Detection of Cell-Affecting Agents with a Silicon Biosensor." *Science*. 1989; 246(4927):243-247).

One patent describes a device employing a method for measurement of extracellular acidification (pH) as an indicator of cellular metabolism. In this device, a small sample chamber containing a high density of cells is intermittently perfused with media and closed to allow measurement of the pH change resulting from cellular proton excretion. A series of repetitive stop/flow cycles provides kinetic metabolic rate data. Because the sample chamber, once assembled, is fixed in size and contains a high density of cells, active perfusion is required to prevent cell death from the rapid acidification and depletion of oxygen from the media. The addition of a perfusion system to the device results in the need for relatively complex tubing, pumps, and other features, that create cleaning and sterilization problems for the user. In addition, when cells are to be treated with a drug using this device, the drug may need to be perfused over the cells for a relatively long period of time, thereby consuming large quantities of typically scarce and expensive compounds.

Other extracellular analytes can be measured using non-invasive techniques. Carbon dioxide evolution can be determined from the measurement of carbon dioxide (CO_2) partial pressure in the media using various fluorescent sensors (Pattison R, Swamy J, Mendenhall B, Hwang C, and Frohlich B. "Measurement and Control of Dissolved Carbon Dioxide in Mammalian Cell Culture Processes Using an in Situ Fiber Optic Chemical Sensor." 2000. Biotechnology Prog. 16:769-774)(Ge X, Kostov Y, and G Rao. High Stability non-invasive autoclavable naked optical CO_2 sensor. 2003. Biosensor and Bioelectronics 18:pp.857-865).

Other ions and chemical constituents can be measured using non-invasive techniques based on optical or semiconductor sensors. In addition, larger molecules such as proteins can be measured using non-invasive techniques that are sensitive to the binding of these molecules to antibodies that are attached to sensors exposed to the extracellular media (Flora K and J Brennan. Comparison of Formats for the Development of Fiber-Optic Biosensors Utilizing Sol-Gel Derived Materials Entrapping Fluorescently-Labeled Proteins. Analyst, 1999, 124, 1455-146).

Other physical phenomenon that support such sensors are surface plasmon resonance (Jordan & Corn, "Surface Plasmon Resonance Imaging Measurements of Electrostatic Biopolymer Adsorption onto Chemically Modified Gold Surfaces," Anal. Chem., 69:1449-1456 (1997), grating couplers (Morhard et al., "Immobilization of antibodies in micropatterns for cell detection by optical diffraction," Sensors and Actuators B, 70, p. 232-242, 2000), ellipsometry (Jin et al., "A biosensor concept based on imaging ellipsometry for visualization of biomolecular interactions," Analytical Biochemistry, 232, p. 69-72, 1995), evanescent wave devices (Huber et al., "Direct optical immunosensing (sensitivity and selectivity)," Sensors and Actuators B, 6, p. 122-126, 1992), reflectometry (Brecht & Gauglitz, "Optical probes and transducers," Biosensors and Bioelectronics, 10, p. 923-936, 1995) and Wood's anomaly (B. Cunningham, P. Li, B. Lin, J. Pepper, "Colorimetric resonant reflection as a direct biochemical assay technique," Sensors and Actuators B, Volume 81, p. 316-328, Jan. 5, 2002).

In general, the utility of devices incorporating these sensing technologies for the purpose of measuring secretion of proteins by cells is limited by detection sensitivity. Sensitivity can be increased, typically by increasing cell density in the region proximal to the sensor surface. However, cellular health declines rapidly as cell density increases, due to anoxia, acidification of the media, and contact inhibition. It is possible, but generally undesirable, to adhere cells directly to the sensor surface.

A need exists for the provision of a high cell density for measurement of analytes and a low density for maintenance of cell health and growth. While many devices have been developed for the purpose of measuring flux rates of extracellular analytes, there exists a need to meet requirements that may enable widespread use in the fields of biological research, drug discovery and clinical diagnostics. A need exists for devices with high throughput and ease of use. A parallel configuration may be desirable. Preferably, a tradeoff between long assay times and the length of time to prepare the sample would be eliminated. Lack of these attributes may result in low sample throughput and therefore incompatibility with modern drug discovery and diagnostic activities.

In addition, there is a need for an instrument that can be used to measure extracellular flux rates of cells in a non-invasive manner within a vessel that is commonly used for other high throughput assays, thereby allowing the use of the flux rate measurement as a quality control or complementary measurement to existing assays.

In summary, there is a need for a device that can meet the goals of data quality, compatibility with existing experimental practices, and ease-of-use, thereby enabling widespread adoption of a new technology.

SUMMARY

New methods and apparatus have been conceived and developed for providing high cell density for measurement of analytes and low cell densities for maintenance of cell health and growth. The instant invention can determine the flux rates of various extracellular analytes in minutes, can provide quantitative rather than relative readings, can be used without adversely affecting the physiological state of the cells under test, and does not require an active perfusion or agitation system.

One feature of the invention is the temporary creation of a substantially closed sample chamber within a vessel containing a low density mixture of cells and media, and a sensor or plurality of sensors for measurement of analytes. Since a temporary sample chamber is created within a larger vessel, media containing high levels of dissolved oxygen

and other analytes, and normal pH, is supplied to the cells prior to, and immediately after a measurement is made. Using this feature, cells can be grown, maintained for extended periods, treated with drug compounds, and assayed using any of a variety of methods, while being periodically assayed for viability and respiration rate, without compromising the cells.

Furthermore, the media containing cells need not be removed from the vessel; it is only displaced temporarily. Therefore, a minimal quantity of drug compound is required.

In addition, by precisely controlling the dimensions of the temporary sample chamber, a quantitative flux rate for extracellular analytes can be determined easily. Therefore, an external reference is not required; a change in the flux rates of cells in a vessel can be determined from multiple readings of this one vessel.

Elements of one embodiment of the invention include:

1. Temporary formation of a small, relatively impermeable sample chamber (containing one or more cells, one or more sensors, and a small amount of cell media) within a larger media-filled vessel.
   This configuration assists with:
   - increasing the rate of change of analytes in the media so that a sensitive measurement can be made in a reasonably short time, i.e., minutes vs. hours for some of the prior art methods;
   - eliminating the need for a reference, by overcoming the following limitations of the prior art:
     a. Low sensitivity (low cell density in the measurement broth and therefore a small signal that may need to be measured);
     b. Unknown sample volume (user variability in fill level of each well and evaporation); and
     c. O_2 influx from the surrounding environment (unless the entire well is sealed with, e.g., a mineral oil coating as suggested by the prior art, which results in a terminal experiment);
   - eliminating the need for complex fluidic systems to provide intermittent perfusion to a flow cell, since a high ratio of cells/media is only created temporarily in accordance with the invention; and
   - development of a high sensitivity cell-based assay system for other types of sensors, including SPR, SRU, etc., where the analyte affected by the cells is affected at a low rate that is difficult to measure;
2. The specific design of a device to accomplish the above, including a stepped well and inverted, mushroom-shaped probe with optical sensors on the bottom surface; and
3. Temporary insertion of the sensor described above into a variety of vessels (including clear-bottom microplates) containing cells.
   This enables the use of substantially all conventional assays, without the need to move cells or disturb their adhesion to the vessel surface; and
   Sensors can be cleaned and reused in minutes.

It is one object of this invention to provide a rapid, non-invasive, and easy-to-use method for determining various physiological properties of living cells. In particular, a device and method are described that can measure overall cellular metabolic and respirative rates, the relative proportion of aerobic to anaerobic respiration, the relative rates of consumption of various metabolic substrates, the effect of stimulation of certain transmembrane and other cellular receptors, the rates of production of various secreted factors, and cell viability.

The device and method can be applied in a variety of fields, including biological research, drug discovery, and clinical diagnostics. The device can be used as a stand-alone instrument or in conjunction with existing assay methods. For example, as a drug discovery tool, the device can be used to screen various compounds for an effect on cellular metabolism, protein secretion, or intra/extra cellular ion exchange. In addition, the device can be used to replace more complex, invasive, and time consuming methods for determining the toxic effects of compounds on cells or tissue samples. For this purpose, the device eliminates the need for the addition of dyes and incubation of cells. The device can also be used to determine the health of cells or tissue both before and after a conventional assay is performed, thereby improving the performance of such an assay.

In one aspect, the invention includes a method of analyzing cells disposed in media within a vessel. The method includes providing an original volume of media about the cells, reducing the original volume of media about at least a portion of the cells to define a reduced volume of media, and analyzing a constituent related to the cells within the reduced volume of media.

One or more of the following features may be included. The reduced volume of media about the cells may be increased to substantially the original volume. A first concentration of the constituent may be determined, and a second concentration of the constituent may be determined at a predetermined time interval from the determination of the first concentration. A flux rate of the constituent may be calculated based on the first concentration and the second concentration.

The reduced volume may include, for example about 5-50% of the original volume, preferably about 5-20% of the original volume. In some embodiments, the reduced volume may be less than about 5% of the original volume.

The cells may include bacteria, fungus, yeast, a prokaryotic cell, a eukaryotic cell, an animal cell, a human cell, and/or an immortal cell. At least a portion of the cells may be attached to a surface of the vessel. At least a portion of the cells may be suspended in the media. At least a portion of the cells may include living tissue.

The constituent being analyzed may include a dissolved gas (e.g., O_2, CO_2, NH_3), an ion (e.g., H^+, Na^+, K^+, Ca^{++}), a protein (e.g., cytokines, insulin, chemokines, hormones, antibodies), a substrate (e.g., glucose, a fatty acid, an amino acid, glutamine, glycogen, pyruvate), a salt, and/or a mineral. The constituent may be extracted from the media by at least a portion of the cells. The constituent may be secreted into the media by at least a portion of the cells.

Analyzing the constituent may include sensing the presence and/or the concentration of the constituent. Analyzing the constituent may include by sensing a first concentration of a first constituent, sensing a second concentration of a second constituent, and determining a relationship between the first concentration and the second concentration. Analyzing the constituent may include sensing a rate of change of a concentration of the constituent.

A sensor in contact with the media within the reduced volume may be used. The sensor may be a fluorescent sensor, a luminescent sensor, an ISFET sensor, a surface plasmon resonance sensor, a sensor based on an optical diffraction principle, a sensor based on a principle of Wood's anomaly, an acoustic sensor, or a microwave sensor.

Analyzing the constituent may include determining a parameter such as cell viability, cell number, cell growth rate, response to at least one of a drug, a toxin or a chemical, detection of an entity, and internalization.

The method may include perfusing additional media through the vessel and/or replenishing the media in the vessel.

Reducing the volume of media may include disposing a barrier in the vessel, typically not causing displacement of the media out of the vessel. At least a portion of the barrier may include a sensor. Alternatively or additionally, the reduced volume of media may include a sensor, such as a fluorophore. At least a portion of the vessel may include a sensor.

The environment of at least a portion of the cells may be altered prior to reducing the original volume of media. The environment may be altered by, e.g., exposing at least a portion of the cells to at least one of a drug, a chemical, or a toxin.

The environment of at least a portion of the cells may be altered after reducing the original volume of media.

The method may include covering the vessel, sealing the vessel, and/or stirring at least a portion of the original volume of media in the vessel.

In another aspect, the invention features an apparatus for analyzing cells. The apparatus includes a stage adapted to receive a vessel holding cells and a volume of media; a plunger adapted to receive a barrier to create a reduced volume of media within the vessel including at least a portion of the cells, the barrier adapted for insertion into the vessel by relative movement of the stage and the plunger, and a sensor in sensing communication with the reduced volume of media, wherein the sensor is configured to analyze a constituent disposed within the reduced volume.

One or more of the following features may be included. The sensor may be configured to analyze the constituent without disturbing the cells. The vessel may include a well disposed in a microplate. The well may include a step. The barrier may be adapted to stir the media prior to analysis of the constituent.

The sensor may be, for example, a fluorescent sensor, a luminescent sensor, an ISFET sensor, a surface plasmon resonance sensor, a sensor based on an optical diffraction principle, a sensor based on a principle of Wood's anomaly, an acoustic sensor, or a microwave sensor. At least a portion of the vessel may include the sensor, the reduced volume of media may include the sensor, and/or at least a portion of the barrier may include the sensor.

The apparatus may include an automated electro-optical measurement system. The apparatus may also include a computer, with the automated electro-optical measurement system being in electrical communication with the computer.

The barrier may be biased relative to the plunger.

In another aspect, the invention features an apparatus for analyzing cells. The apparatus includes a vessel for holding cells and a volume of cell media; a plunger adapted to receive a barrier to create a reduced volume of media within the vessel including at least a portion of the cells, the barrier adapted for insertion into the vessel by relative movement of the stage and the plunger without disturbing the cells, such that the reduced volume is less than about 50% of the volume of media; and a sensor in sensing communication with the reduced volume of media, wherein the sensor is configured to analyze a constituent disposed within the reduced volume.

In another aspect, the invention features a plate including multiple wells for holding media and cells. Each of at least a portion of the wells includes a seating surface for receiving a barrier a reduced volume.

One or more of the following features may be included. A shape of the seating surface may be generally planar, arcuate, contoured, tapered, conical, stepped, or interlocking. The reduced volume within each of the wells may vary by less than about 10% of a mean volume of the wells, preferably by less than about 5% of the mean volume of the wells, more preferably by less than about 1% of the mean volume of the wells. The seating surfaces of the wells may each include a step disposed about an inner periphery of a respective well. The steps may lie in a step plane disposed above a bottom plane defined by bottoms of respective wells. The step plane and the bottom plane may be parallel planes. A height of the step plane may be less than about 1 millimeter (mm) above the bottom plane, preferably less than about 200 μm above the bottom plane, more preferably less than about 50 μm above the bottom plane.

A fluorescent sensor may be disposed within at least one of the wells. At least one of the wells may include a transparent bottom. At least one of the wells may include an opaque wall.

In another aspect, the invention features a barrier for analysis of cells disposed in media in a vessel. The barrier includes a body portion for insertion into the vessel, the body portion having a barrier surface for mating with a first surface of the vessel to create a reduced volume.

One or more of the following features may be included. A shape of the barrier surface may be generally planar, arcuate, contoured, tapered, conical, stepped, or interlocking. The barrier may include a cover for mating with a second surface of the vessel.

A sensor may be disposed on the barrier surface for analyzing a constituent of a media disposed about at least a portion of the cells. The sensor may include an optical sensor. The optical sensor may be adapted to sense a fluorophore.

A conductor may be coupled to the sensor and configured to conduct signals therefrom. The conductor may include an optical fiber and may be disposed at least partially in the body portion. The barrier may include a readout for transmitting a signal from the sensor. The readout may be visual, fiber, electronics on a post, and/or a plate reader from the bottom.

The barrier may include a plurality of barriers arranged to be received within a plurality of wells in a microplate.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a partial cross-sectional view of one embodiment of the present invention, where the vessel is formed by a single well within a multi-well microplate, the cover and sensor assembly being shown in a pre-measurement position;

FIG. **2** is a cross-sectional view of the cover and sensor assembly of FIG. **1** in the measurement position;

FIG. **3** is a schematic illustration of a complete measurement system, in accordance with one embodiment of the invention;

FIGS. **4***a* and **4***b* are schematic cross-sectional views of wells with different seating surfaces;

FIGS. **5***a*-**5***c* are schematic cross-sectional views of barriers that include both a sensor assembly and a readout;

FIG. **6** is a graph showing the result of a study of the oxygen consumption and extracellular acidification rates of typical mammalian cells, depicting the mean and standard deviation of a series of eight separate measurements using one embodiment of the invention;

FIG. **7** is a graph showing the result of a study of the oxygen consumption and carbon dioxide evolution rates of various numbers of typical mammalian cells using one embodiment of the invention;

FIG. **8** is a graph showing the result of a study of the effect of the chemical compound 2,4, DNP on the rates of oxygen consumption, carbon dioxide evolution, and extracellular acidification of typical mammalian cells using one embodiment of the invention;

FIG. **9** is a graph showing the result of a study of the effect of the chemical compound Rotenone on the rates of oxygen consumption and extracellular acidification of typical mammalian cells using one embodiment of the invention;

FIG. **10** is a graph showing the result of a study of the effect of cell proliferation on oxygen consumption and extracellular acidification using one embodiment of the invention;

FIG. **11** is a graph showing the result of a study of the effect of the chemical compound Carbachol on the rate of extracellular acidification of typical mammalian cells using one embodiment of the invention; and

FIG. **12** is a graph showing a comparison of the measured rates of oxygen consumption of typical mammalian cells in a vessel with, and without, the formation of a small, enclosed sample chamber using one embodiment of the invention.

DETAILED DESCRIPTION

This invention enables the temporary creation of a highly concentrated volume of cells within a larger volume of cell media, in order to allow sensitive measurements of the change in constituents of the media that result from biological activity of the cells. By temporarily, rather than permanently, reducing the media volume (and therefore concentrating the cell/media mixture), cells are exposed to a non-normal environment for only a brief period of time and are therefore not adversely affected by the measurement process.

In one embodiment of the invention, cells are grown or placed on the bottom of a vessel containing sufficient type and volume of media to support growth for an extended period of time. A sample chamber is formed in the bottom of the vessel, consisting of the bottom of the vessel and vertical walls, such that the enclosed volume is sufficient to contain the cells plus a reduced volume of media.

A barrier, having a diameter slightly less than the inside diameter of the vessel, is located above the sample chamber on a movable actuator. Upon actuation, the barrier may be raised above the level of liquid in the vessel, or lowered into the liquid and on to the vessel walls, forming a sample chamber that is relatively impervious to the diffusion of analytes from the sample chamber to and from the bulk media now above the cover.

A cross-sectional view of a representative embodiment is shown in FIG. **1**. The drawing details a vessel **100** that is typical of one well **110** within a multi-well microplate. The walls of this single well **110** form the vessel **100** that contains live cells **120** and cell growth media **130**. Cells may or may not adhere to a bottom surface **132** of the vessel, and the bottom surface may be treated or coated to encourage adherence. Alternatively, cells may be suspended within the media and may be forced to the bottom of the vessel using gravity or centrifugal force.

A barrier **140**, having a diameter slightly less than an inside diameter of the vessel **100**, is used to form a cover that defines a sample chamber **150** within the vessel. Barrier **140** may have a diameter d_1 of, e.g., 6 mm, and vessel **100** may have an inside diameter d_2 of, e.g., 7 mm. In FIG. **1**, the barrier **140** is shown in a pre-measurement position within the vessel. To effect a measurement, a manual or motorized plunger (actuator) can then be used to reposition the barrier **140** slightly above the bottom surface **132** of the vessel **100** as shown in FIG. **2** by lowering the barrier **140** or raising the well **110**. Orienting the barrier to the position shown in FIG. **2** prior to measurement defines the sample chamber **150** having a reduced volume of media, thereby enhancing measurement sensitivity.

A single vessel of nearly any size may be fabricated, or multiple vessels may be fabricated in a one- or two-dimensional arrangement. In one embodiment, a two-dimensional pattern of vessels corresponding to the pattern and dimensions of a microplate, as described by the Society for Biomolecular Screening standards for microplates ("SBS-1 Footprints" and "SBS-4 Well Positions," both full proposed standards updated May 20, 2003), and containing a total of 12, 24, 96, 384, 1536, or any other number of individual wells may be fabricated.

The vessel and sample chamber may typically be formed using plastic material such as, for example, polystyrene or polypropylene, with the bottom clear and the sides colored black to reduce optical cross-talk from one well to another.

A variety of types of barriers may be employed to temporarily reduce the volume of media about the cells without causing displacement of media out of the vessel, such as a simple planar cover lowered vertically, a sliding cover extended horizontally, or a pair of disks with cutouts that can be rotated to act as a valve. It is desirable that the barrier not disturb, i.e., not move, the cells or the media proximal to the cells, in order to reduce the required settling time prior to a measurement.

A complete measurement system can be assembled using the components shown in FIG. **3**. A vessel **300**, e.g., a plate such as a microplate including a plurality of wells **302**, is placed upon a translation stage **310**. The microplate is disposed beneath an array of barriers **320** disposed on a plunger **322** adapted to receive the barriers and an array of pipettors **330**. Each of at least a portion of the wells includes a seating surface (see, e.g., FIGS. **4***a* and **4***b*) adapted to receive one of the barriers. Barriers **320** may include sensors. An original volume of media may be disposed in the wells. Using manual or motorized actuation, the barriers and pipettors may be lowered into the microplate wells to create a reduced volume of media within the wells. The reduced volume may be less than, e.g., 50%, of the original volume of media. The barriers are adapted for insertion into the vessel, i.e., into the wells, by relative movement of the stage **310** and the plunger **322**. The barriers and pipettors may also be lowered into one of several fluid reservoirs **340** containing wash buffers and calibrants. When the barriers create the reduced volume of media within the vessels, sensors may be in sensing communication with the reduced volume of media and may be configured to analyze one or more constituents disposed within the reduced volume. The sensors may be interrogated by an optical interface consisting of illumination sources (e.g., light emitting diodes) and light detectors (e.g., photodiodes), with appropriate band-limiting filters interspersed between the optical elements. A computer and software **350** perform actuation, calibration and measurement functions.

A change in the temperature of the media within the sample chamber may result in unwanted measurement errors from at least two sources. First, the capacity of the media to hold dissolved gasses changes with temperature, and there-

fore a change in temperature may cause an apparent change in dissolved gas concentration as the media seeks equilibrium with the surrounding environment. Second, the measurement properties of many types of sensors changes with temperature.

To ensure accurate and repeatable measurements, the temperature of a reduced volume of media in the vessel may be controlled or a correction factor may be applied to the measurement. Because evaporation induces cooling of the liquid media, control of evaporation may be desired to reduce thermal drift, thermal gradients, and gas exchange.

Providing environmental and temperature control for the sample chamber may reduce unwanted impact on the measurement process. For example, uncontrolled temperature changes of the media surrounding the cells can directly impact the rate of apparent oxygen consumption. Oxygen will naturally off-gas from media as it warms, thus introducing the appearance of a change in cellular respiration when, in fact, the rate change observed is a natural function of dissolved gas seeking equilibrium as the temperature increases. Similarly, any evaporation from the media due to other uncontrolled environmental conditions such as humidity or exposure to air currents can artificially impact the measurements made from various sensors including those of dissolved gases, ions, and temperature.

Using this measurement system, an assay cycle is initiated by mating the sensors/barriers with the vessel walls to form closed sample chambers with reduced volume of media containing the cells. The rate and pattern of actuation of the barriers may be programmed to prevent rapid motion of the media that may disturb the cells, i.e., displace the cells by or cause shear stress on the cells, and may be alternated to provide fluid motion for stirring of the media, as desired.

Additionally, the barriers may be independently biased, for example, by using springs or other force elements, to ensure adequate seating of the covers in all of the wells, simultaneously.

The electro-optical interface and computer are then used to measure the change in response of the sensor or sensors resulting from the change in concentration of extracellular analytes. The rates of consumption or production of analytes may be determined by making multiple readings over a period of minutes and then calculating the slope between selected measurement points. Once the measurement sequence is completed, the sensor/covers are retracted to expose the cells to the full volume of media within each vessel.

The measurement system may include provisions for single or multiple-point calibration of the analyte sensors. For example, two reservoirs containing liquid of known, but different pH, oxygen, CO_2, or other analyte levels may be incorporated, and a two-point (gain and offset) calibration may be performed periodically. Alternatively, "factory" pre-calibration of the sensors may be used to eliminate the need for field calibration, or to reduce the calibration to a single point (offset) correction.

Referring to FIG. **4***a*, in one embodiment, a microplate is used to provide a plurality of measurement vessels in a standardized pattern. By incorporating a seating surface **400** in each well, a precise reduced volume of media can be maintained about the cells during the measurement period. The reduced volume within each of the wells disposed in a plate may vary by less than about 10% of a mean volume of the wells. In some embodiments, the reduced volume may vary by less than 5% of the mean volume of the wells, and in some embodiments, the reduced volume may vary by less than 1% of the mean volume of the wells. The seating surface **400** or steps may lie in a step plane **410** disposed above a bottom plane **420** defined by bottoms **430** of respective wells, with the step plane **410** and the bottom plane **420** being parallel planes. The height of the step plane is generally less than about 1 mm above the bottom plane and typically less than 50 μm to 200 μm above the bottom plane.

Referring to FIG. **4***b*, in another embodiment, a sloped surface **435** is incorporated to prevent the adhesion of cells on the seating surface **440**. Any of a variety of alternative mating cover and seating surfaces can be employed, in various combinations and permutations, including those that are generally planar, arcuate, contoured, tapered, conical, stepped, interlocking, etc. What is generally desired is that mating features reliably and repeatably isolate the reduced volume from the original volume, such that the reduced volume has a generally predetermined or known capacity. Auxiliary seating components, such as O-rings, or resilient or compliant sealing lips, flaps, or other features may be employed on the covers or in the wells to enhance the seal, as desired.

The barrier can be fabricated to include a sensor assembly and a readout for transmitting a signal from the sensor assembly. FIG. **5***a* shows a cross-sectional view of a barrier formed from the combination of a tubular solid support **500** and a removable cover **510** or sheath, having an enlarged distal end that forms a structure upon which one or more optically-coupled sensors **520** are attached. In one embodiment, the sheath may be fabricated from a material that is either disposable or sterilizable in order to prevent contamination of the cell media. A readout **530** may be in the form of optical fibers disposed within the tubular support for communication between the sensors and an electro-optical measurement system **540**. The electro-optical measurement system **540** may incorporate a source of illumination, an optical detector, spectral filters, and signal processing components. The electro-optical measurement system **540** may be automated. In some embodiments, the electro-optical measurement system **540** may be in electrical communication with computer **350** (see FIG. **3**).

FIG. **5***b* shows an alternative arrangement in which the sensors are illuminated by an external light source **550**. Electro-optical measurement system **540** may include separate components, i.e., an optical measurement system **560** and an illumination system **570**. The optical measurement system **560** and illumination system **570** may be automated. In some embodiments, the optical measurement and illumination systems **560**, **570** may be in electrical communication with computer **350**. Referring to FIG. **5***c*, in an alternative embodiment, the electro-optical measurement system **540** includes optical and measurement components **580** located within the tubular support **500** and an external electronic measurement system **585**. The optical and measurement components may communicate with the external electronic measurement system **585** through a cable **590**.

Any form of signal communication can be employed, as desired. Such forms of signal communication might include simple visual interrogation of a signal change such as a change in color; fiber optic signal communication coming from any side of the vessel; a laser or CCD-based plate reader interrogating the signal from the bottom of a transparent vessel.

In practice, many different configurations of vessels, barriers, and sensors may be employed. The total vessel volume may range from many liters to a fraction of a microliter (ml), but is generally less than about 1 ml. The ratio of the reduced volume of media enclosed within the

temporary sample chamber to an original volume of media provided in the vessel may range from about 50% to less than about 5% and even as low as less than about 1%, but is typically in the range of 5-20%.

Many different types and numbers of cells can be analyzed, including bacteria, fungus, yeast, prokaryotic and eukaryotic cells, animal or human cells, etc. Cells may adhere to the vessel wall or may be suspended within the media. Immortalized cells, native and primary cells, and homogenized or sliced tissue may be analyzed. A centrifuge may be used to concentrate cells within the sample chamber region of the vessel.

Any number of constituents of the media may be analyzed, including dissolved gasses, ions, proteins, metabolic substrates, salts and minerals. These constituents may be consumed by the cells (such as O_2), or may be produced by the cells either as a byproduct (such as CO_2 and NH_3) or as a secreted factor (such as insulin, cytokines, chemokines, hormones or antibodies). Ions such as H^+, Na^+, K^+, and Ca^{++} secreted or extracted by cells in various cellular metabolism processes may also be analyzed. Substrates either consumed or produced by cells such as glucose, fatty acid, amino acids, glutamine, glycogen and pyruvate may be analyzed. Specialized media may be used to improve the sensitivity of the measurement. For example, the change in pH resulting from extracellular acidification can be increased by using a media with reduced buffer capacity, such as bicarbonate-free media.

The analysis performed using this method may simply detect the presence of a constituent in the media, or may quantitatively analyze the amount and change in concentration, volume, or partial pressure of a constituent. With the incorporation of multiple sensors, one or more ratios of constituents may be analyzed. As an example, the ratio of anaerobic to aerobic respiration utilized by the cell can be determined from a calculation of the ratio of oxygen consumption to extracellular acidification rate that is enabled by a measurement of changes in oxygen partial pressure and pH of the extracellular media. Analysis may include sensing a first concentration of a first constituent, sensing a second concentration of a second constituent, and determining a relationship between the first concentration and the second concentration.

The type of sensors utilized include oxygen sensors, such as oxygen-quenched fluorescent sensors, enzyme-coupled ISFET sensors, miniature Clark electrodes, or other oxygen sensors; pH sensors, including fluorescent sensors, ISFET sensors, pH sensitive dye sensors, LAP sensors, or other pH sensors; CO_2 sensors, including bicarbonate buffer coupled and ammonium dye coupled fluorescent sensors as well as other CO_2 sensors; various ion and small molecule sensors; large molecule sensors including surface plasmon resonance sensors and sensors exploiting the principle of Wood's anomaly; acoustic sensors; and microwave sensors.

The method may be used to measure any number of attributes of cells and cellular function. For example, cell viability and metabolic rate may be determined from measurements of oxygen consumption rate, extracellular acidification rate, or other metabolic analyte fluxes. By comparison of one or more analyte flux rates to a known rate per cell, cell number may be determined and therefore growth rates can be monitored.

The number of sensors used may range from one to many hundreds. Sensors for dissolved gasses may be placed within the sample chamber, but not in direct contact with the media. Other sensors, however, should be in direct contact with the media and in close proximity to the cells. This may be accomplished by mixing an indicator compound, e.g., a fluorophore, with the cell media, or by embedding the indicator in a compound that is permeable to the analyte to be measured. The embedded indicator may then be attached to any surface of the sample chamber region of the vessel.

In one embodiment, one or more sensors may be attached to the lower surface of the barrier, so as to be exposed to the extracellular media upon lowering of the barrier. One example of a sensor for this purpose is a fluorescent indicator, such as an oxygen-quenched fluorophore, embedded in an oxygen permeable substance, such as silicone rubber.

Sequential measurements of a single group of cells may be made at predetermined time intervals to analyze the effect of changes in the extracellular environment on their function, for example to examine the effect of exposure to a drug, chemical, or toxin. In this method, the volume of media surrounding the cells is first reduced, the constituents of the media are measured, and the volume is restored to its original value, as previously described. The environment surrounding the cells is then altered, such as by adding a chemical that activates a transmembrane receptor, changing the dissolved oxygen level, or adding a nutrient. One or more additional measurement cycles are then performed using the temporarily reduced volume method, to analyze the effect of the altered extracellular environment.

At any time during the sequence of measurements, the cell media may be replenished. In this way, sequential measurements can be made over a period of minutes, hours, or days. Any one of several different approaches may be followed to replenish the media. Media may be replenished by substantially removing part or all of the media within the full volume of the vessel using standard manual or automated pipetting instruments. Alternatively, media may be replenished only within the reduced volume of the vessel when a barrier is lowered into position. In the latter method, media may be replenished by fluidic extraction and delivery from a top side of the vessel through a portal in a plunger mechanism or through a portal built into any one of the sides or bottom of the vessel.

The introduction of an environment altering constituent such as a chemical, dissolved gas or nutrient may also be applied either to the full volume of the vessel as noted above or alternatively to only the reduced volume of the vessel. In the latter embodiment, the volume of media surrounding the cells is first reduced, the constituents of the media are measured, and the volume is restored to its original value, as previously described. The volume is then again reduced and the environment immediately surrounding the cells within only the reduced volume is then altered, by the addition of a constituent through a portal in the plunger or elsewhere in the vessel defining the reduced volume. One or more measurements are made in the presence of the constituent. After this measurement cycle, the media within the reduced volume may be exchanged one or more times to flush out the constituent before exposing the cells once again to the full original volume. This approach may provide a benefit of reducing the volume of compound required. It may also provide the possibility of studying isolated effects without contaminating the entire volume, thereby, in effect, simulating a flow system in wellplate format.

EXAMPLES

The following examples illustrate certain exemplary and preferred embodiments and applications of the instant invention, but are not intended to be illustrative of all embodiments and applications.

Example 1

Repetitive Measurement of the Basal Respiration and Acidification Rates of C2Cl2 Myoblasts:

A prototype device was fabricated in order to evaluate various properties and potential applications of the invention.

The device included a cylindrical vessel, fabricated from polycarbonate membrane, and designed to receive a 12 mm diameter, polycarbonate membrane assembly (Corning SNAPWELL™ P/N 3802) with a pore size of approximately 3 μm. A cylindrical polycarbonate cover could be temporarily inserted into the vessel to form a smaller sample chamber, approximately 1.5 mm high, with a volume of about 160 microliter (μl). A series of bores around the perimeter of the vessel allowed the insertion of three 500 μm diameter optical fibers. The distal tip of each optical fiber was coated with a fluorescent sensing material to form a biosensor.

The three biosensors were designed to measure the partial pressures of O_2 and CO_2, and the pH of the media contained within the vessel. One fiber tip was coated with a matrix of Ruthenium dyes, encapsulated in oxygen permeable silicone rubber, to provide a readout of dissolved oxygen concentration. A second fiber tip was coated with a complex of Fluoroscene dye encapsulated in silicone rubber, to provide a readout of H^+ ion concentration (pH). A third biosensor was fabricated by using a CO_2 permeable membrane to create a small reservoir of $NaHCO_3$ surrounding a Hydroxy-Pyrene Trisodium Salt (HPTS) pH sensitive dye. A change in CO_2 concentration in the cell media would then cause a change in pH of this encapsulated reagent resulting in a measurable change in the fluorescent properties of the pH sensitive dye, and this change was calibrated to provide quantitative CO_2 concentration data.

Light emitting diodes were used to illuminate the three optical sensors at various wavelengths as shown in Table 1, in terms of nanometers (nm). Also shown in Table 1 are the wavelengths used to sense the fluorescent emission of each sensor. In each case, both analyte sensitive ("sensor"), and analyte insensitive ("reference") fluorescent properties of the dyes were measured to minimize unwanted drift and interference. Dichroic splitters were used to couple individual fiber/dye assemblies to a pair of photodiodes/filter sets (O_2 sensor) or a pair of LED/filter sets (pH and CO_2).

TABLE 1

Analyte sensor excitation and emission wavelengths

	Sensor Excitation	Reference Excitation	Sensor Emission	Reference Emission
Oxygen	488 nm	488 nm	610 nm	535 nm
PH	464 nm	435 nm	530 nm	530 nm
CO_2	460 nm	415 nm	530 nm	530 nm

Each sensor was calibrated once using multiple measurement points and a polynomial regression method to establish a nonlinear calibration curve.

Sensors were then recalibrated daily using a two-point calibration method. pH sensors were calibrated by sampling the optical response while submerged in a buffer solution with pH of 6.0 for 2 minutes, then in a solution with pH 8, each for two minutes. Oxygen and CO_2 sensors were calibrated using data points acquired while both sensors were submerged for two minutes in a saline bath purged with room air, followed by a bath purged with 10% CO_2/90% N_2.

During a typical assay, approximately 1.5×10^5 cells were placed in the vessel along with 500 μl of liquid media, resulting in a cell density of 3×10^5 cells/ml. To perform a measurement, the cylindrical cover was temporarily inserted into the vessel. The cover displaced liquid media, but not cells, to form a smaller sample chamber with a volume of 160 μl and a therefore a cell density of approximately 1×10^6 cells/ml. This resulted in more than a 6× increase in the rate of change of analytes within the media in proximity to the biosensors.

In order to evaluate the ability of the prototype device to reproducibly measure extracellular analytes flux rates, 1.5×10^5 undifferentiated C2Cl2 murine skeletal muscle cells (obtained from ATCC, Manassas, Va.) were seeded on each of eight separate 12 mm diameter polycarbonate membranes which were then incubated at 37° C. for a period of 12 hours.

In a sequence of tests, wells were removed from the incubator, inspected visually, and placed into the measurement device. 160 μl of bicarbonate ($NaHCO_3$)-free DMEM Medium (obtained from Specialty Media, Phillipsburg, N.J.) was then added, and the device was assembled to form an enclosed sample chamber. The concentration of each analyte (partial pressures of O_2 and CO_2, and pH as an indicator of proton concentration) was then measured every 8 seconds for a period of 20 minutes, and the average rate of change of each analyte was calculated over a four minute period from t=12 minutes to t=16 minutes.

To determine the extracellular flux rates of O_2 and CO_2, the rates of change of partial pressures were divided by volume of each analytes available in the media (moles) to result in a value expressed in nmol/minute. The rate of acidification was expressed in mpH units/min (multiplied by 20 for scaling on the chart), but can easily be shown as protons per minute by calculating the number of available electrons in the media buffer within the known sample volume.

The mean and standard deviation of the dissolved oxygen and pH decay rates for the series of eight tests are shown in FIG. **6**. As shown, these flux rates are highly reproducible in the prototype device.

Example 2

Measurement of Basal Respiration and Acidification Rates for Various Cell Densities:

The experimental device described in Example 1 was used to investigate the relationship between cell number and oxygen and CO_2 flux rates. Varying numbers (1.0×10^5-4.0×10^5) of C2Cl2 myoblasts were seeded on 12 mm diameter polycarbonate membranes (Corning SNAPWELL™) which were then incubated at 37° C. for a period of 12 hours.

Wells were then removed from the incubator, inspected visually, and placed into the measurement device. 150 μl of $NaHCO_3$-free DMEM Medium (obtained from Specialty Media, Phillipsburg, N.J.) was then added, and the device was assembled to form an enclosed sample chamber. The concentration of each analyte was then measured every 5 seconds for a period of 20 minutes, and the average rate of change from t=10 minutes to t=20 minutes from start was computed. The resulting flux rates are shown in Table 2 and in graphical form in FIG. **7**.

TABLE 2

Measuring Metabolic Analytes from Varying Titrations of C2C12 Myoblasts

Cell # (000)	O_2 Rate	CO_2 Rate	pH Rate	CO_2/O_2	O_2/pH
400	0.77	1.22	0.023	0.88	0.33
300	1.06	1.22	0.021	1.15	0.50
200	0.70	1.08	0.019	0.93	0.35
150	0.57	0.90	0.021	1.63	0.26
100	0.29	0.68	0.013	2.36	0.22

The data in Table 2 shows, as expected, that increasing cell density increases analyte flux rates in a near-linear fashion for most cell densities. Above a density of 3×10^5 cells, oxygen flux did not increase as rapidly, presumably due to contact inhibition and crowding effects.

The device can therefore be used to evaluate the effect of high cell densities on metabolic rates.

Example 3

The Effect of 2,4 DNP on C2C12 Myoblasts:

The chemical compound 2,4 DNP can be used to uncouple mitochondrial respiration from ATP synthesis by disassociating the linkage between the respiratory chain and the phosphorylation system. In the presence of this compound, it is known that oxygen consumption will increase dramatically, while proton flux remains relatively constant.

In this experiment, C2C12 myoblasts were seeded on 12 mm diameter polycarbonate membranes and incubated for 12 hours. Wells were then removed from the incubator, inspected visually, and placed into the measurement device. 160 µl of $NaHCO_3$-free DMEM medium was then added, and the device was assembled to form an enclosed sample chamber.

The dissolved concentrations of O_2 and CO_2, and the pH in the media were then measured every 5 seconds for a period of 20 minutes in order to determine a control baseline for each analyte flux. Once the baseline was established, a sequence of experiments were performed where varying doses of 2,4 DNP (obtained from Sigma, St. Louis Mo.) were added to the cell media and a 20 minute measurement of analyte flux rates was performed. A control experiment was also performed using the highest dose of 2,4 DNP, but without cells. The data from the dose response is shown in Table 3 and FIG. **8**.

TABLE 3

Effect of 2,4DNP on C2C12 Myoblasts

2,4 DNP Dose (µM)	O_2 Rate (nM/min)	CO_2 Rate (nM/min)	pH Rate (pH/min)	CO_2/O_2	O_2/pH
0	0.38	2.22	0.024	5.83	0.16
10	1.26	2.78	0.028	2.18	0.46
50	1.99	4.24	0.031	2.13	0.64
100	2.30	4.59	0.032	2.00	0.73
100 No Cells	−0.18	0.15	0.001	−0.84	−1.30

The data in Table 3 shows that as predicted, treatment with 2,4 DNP causes a dose-dependent increase in O_2 consumption rates while having little effect on extracellular acidification.

Example 4

The Effect of Rotenone on C2C12 Myoblasts:

Rotenone is known to inhibit cellular respiration by blocking NADH dehydrogenase in the respiratory chain. C2C12 Myoblasts were used to show this effect. 1.5×10^5 C2C12 myoblasts were seeded on membranes, incubated and placed in the measurement system along with 150 µl $NaHCO_3$-free DMEM medium of as described in Example 3.

The dissolved concentrations of O_2 and CO_2, and the pH in the media were then measured every 5 seconds for a period of 20 minutes in order to determine a control baseline for each analyte flux. Once the baseline was established, a sequence of experiments were performed where varying doses of Rotenone (obtained from Sigma, St. Louis Mo.) were added to the cell media and a 20 minute measurement of analyte flux rates was performed. A control experiment was also performed using the highest dose of Rotenone, but without cells. The data from the dose response is shown in Table 4 and FIG. **9**.

TABLE 4

Effect of Rotenone on C2C12 Myoblasts

Rotenone Dose	O_2 Rate	CO_2 Rate	pH Rate	CO_2/O_2	O_2/pH
0	0.58	1.50	0.027	2.56	0.22
25	0.44	1.76	0.026	4.00	0.17
50	0.26	2.08	0.031	8.02	0.08
100	0.19	2.34	0.023	12.18	0.09
200	0.04	2.24	0.027	69.84	0.02
200-No Cells	−0.07	0.14	0.001	7.51	−0.51

The data in Table 4 demonstrates that, as expected, treatment with Rotenone causes a dose-dependent decrease in O_2 consumption rate in these cells.

Example 5

Measurement of Respiration and Acidification Rate Changes Resulting from Cell Proliferation:

The experimental device described in Example 1 was used to investigate the relationship between cell proliferation and oxygen, CO_2 and proton flux rates. 5.0×10^4 C2C12 myoblasts were seeded on 12 mm diameter polycarbonate membranes and then incubated at 37° C. 12 hours after being seeded, cells were placed in DMEM serum-free media (Gibco, Carlsbad, Calif.) to inhibit proliferation. After 24 hours, half of the cells were switched to DMEM serum-containing media to stimulate proliferation, while the other half were maintained in serum-free media.

Wells were then removed from the incubator, inspected visually, and placed into the measurement device. 57 µl of $NaHCO_3$-free DMEM Medium (obtained from Specialty Media, Phillipsburg, N.J.) was then added and the device was assembled to form an enclosed sample chamber. The concentration of each analytes was then measured every 8 seconds for a period of 20 minutes, and the average rate of decay from t=10 minutes to t=20 minutes from start was computed. The resulting flux rates are shown in Table 5 and in graphical form in FIG. **10**.

TABLE 5

Effect of cell proliferation on extracellular analyte fluxes

	O2 Rate (nMoles/min)	PH Rate * 10 (PHU/min)
50 K Stimulated	0.311 +/- 0.091	0.188 +/- 0.020
50 K Starved	0.082 +/- 0.019	0.102 +/- 0.050

The data in Table 5 demonstrates that, as expected, cell proliferation results in an increase in oxygen consumption and the rate of extracellular acidification.

Example 6

Measurement of G-Protein Coupled Receptor Activation in CHO-M3 Cells:

Previous studies have shown that stimulation of transmembrane receptors often causes a rapid increase in extracellular acidification rate, resulting primarily from acute activation of ion exchange pumps. In this experiment, the prototype device was used to detect a change in extracellular acidification rate following treatment of cells with a receptor agonist.

Chinese hamster ovary (CHO) cells were transfected to over-express the muscarinic receptor subtype m3. The prototype device described in Example 1 was then used to monitor O_2 consumption, CO_2 production, and extracellular acidification rates, following treatment with the well-known, general acetylcholine receptor agonist, Carbachol.

Materials and Methods: Cell culture reagents were obtained from Gibco BRL (Grand Island, N.Y.). Carbachol was purchased from Sigma Chemical Co. (St. Louis, Mo.). Bicarbonate-free DMEM medium was obtained from Specialty Media (Phillipsburg, N.J.). Polycarbonate membrane SNAPWELL™s (12 mm diameter, 3 μm pore size) were obtained from Corning (Corning, N.Y.). CHO cells expressing m3-muscarmnic receptors (CHO-M3 cells) were obtained from the American Type Tissue Culture (ATCC; Manassas, Va.). Cells were cultured in Ham's F-12 medium supplemented with 10% fetal bovine serum (Hyclone), 1% GlutaMax and 0.1% Gentamicin and were maintained in a 5% CO_2 incubator. Cells were subcultured when they reached 80% confluency. CHO-M3 cells were seeded at a density of 2×10^5 onto a SNAPWELL™ 24 hours prior to use. Immediately prior to testing, cells on SNAPWELL™ were switched to bicarbonate-free DMEM medium combined with 3.7 g/l NaCl to maintain osmolarity (medium pH 7.4-7.5).

Protocol Description: Probes were calibrated immediately prior to testing. The bottom of the test vessel was filled with bicarbonate-free medium. The SNAPWELL™ was removed from a 5% CO_2 incubator, and the regular growth medium (Ham's F-12) was replaced with bicarbonate-free DMEM medium. Thereafter, the SNAPWELL™ was placed into the test vessel. Bicarbonate-free medium was pipetted onto the top of the SNAPWELL™, and the cover piece of the test vessel was placed gently on top of the SNAPWELL™ and screwed into place, compressing the assembly. The probe software was started, and the pH, CO_2 and O_2 analytes were measured over the next 3.5 hours. Following the initial 1.5 hours of perfusion at a rate of 78 μl/min, a series of stop flow (10 minutes each) and medium re-perfusion (10 minutes each, 78 μl/min) cycles were started. During the last 2 minutes of medium re-perfusion cycle number 5, 100 μM carbachol was perfused across the SNAPWELL™. During re-perfusion number 6, bicarbonate-free DMEM medium was once again perfused across the SNAPWELL™. A rate of change for the analytes was calculated during each stop flow cycle.

Results:

CHO-M3 Baseline Followed by 100 μM Carbachol Treatment

The first four series of perfusion/stop flow cycles were done to establish a noise band on the three analytes prior to carbachol treatment during perfusion number 5. Bicarbonate-free medium re-perfusion during per perfusion number 6 and the rates calculated during stop flow number 6 were to assess potential continuing post-carbachol treatment effects on analytes' rates. Resulting data are shown below in Table 6 and in FIG. 11.

TABLE 6

Effect of carbachol exposure on oxygen consumption, carbon dioxide evolution, and extracellular evolution

Rate Summary	O_2 (nMol/min)	CO_2 (nMol/min)	pH (PHU/min)	O_2/CO_2 Ratio	O2/pH Ratio
Stop Flow 1	0.23	0.15	0.01	1.53	0.23
Stop Flow 2	0.24	0.12	0.01	1.93	0.25
Stop Flow 3	0.30	0.10	0.01	3.04	0.36
Stop Flow 4	0.27	0.11	0.01	2.45	0.38
Stop Flow 5	0.40	0.20	0.02	1.99	0.26
Stop Flow 6	0.33	0.10	0.01	3.34	0.29

Average baseline values compared to treatment values illustrate that a 2-minute exposure of CHO-M3 cells to carbachol resulted in a doubling in the pH rate (0.01 PHU/min vs 0.02 PHU/min), though O_2 and CO_2 rates also demonstrated increases above baseline levels (see below). Post-treatment rates generated during stop flow period number 6 essentially returned to pre-treatment values.

Example 7

Comparison of Measured Analyte Fluxes with and without the Temporary Formation of a Sample Chamber:

The temporary formation of a sample chamber within a larger vessel, and containing an effectively high concentration of cells, is a characteristic for the instant invention.

In order to demonstrate this principle, a cylindrical vessel of approximately 12 mm in diameter and 10 mm in height was constructed from polycarbonate material. Fluorescent sensors capable of measuring partial pressures of O_2 and CO_2, and a sensor capable of measuring pH, were installed in the bottom of the vessel and calibrated as described previously. A cylindrical cover was also fabricated from polycarbonate material, with a diameter to accommodate insertion into the vessel, in order to provide a gas-impermeable cover and to reduce the enclosed volume of the vessel as required. A cylindrical spacer of 0.5 mm in height was also fabricated, with a diameter to accommodate insertion in the bottom of the vessel, thus providing a stop for the cover at a precise location.

Approximately 1×10^5 C2Cl2 myoblast cells was prepared as described previously. The cells were placed within the vessel along with approximately 1 ml of cell growth media. The partial pressure of oxygen within the cell media was measured continuously using a calibrated fluorescent probe.

The rate of change of pO_2 was then calculated from the difference between the pO_2 values at 12 and 16 minutes from the start of the experiment.

A cylindrical cover was then placed on the surface of the cell media in order to inhibit the influx of oxygen from the ambient air. This also reduced the volume of media exposed to the cells to approximately 130 μl. Again, the pO_2 values at 12 and 16 minutes from the start of the experiment were measured and recorded.

The cylindrical cover was then lowered within the vessel (to rest on the spacer) so as to reduce the volume of media exposed to the cells to approximately 57 μl. Again, the pO_2 values at 12 and 16 minutes from the start of the experiment were measured and recorded.

The measured rates of change of pO_2 within the cell media for the three conditions are shown in tabular form in Table 7 and in graphical form in FIG. **12**.

TABLE 7

Oxygen depletion rates in a four minute interval, with and without the formation of an enclosed sample chamber (n = 3)

Volume	ΔpO2	s.d.	% CV
Small (57 μL)	0.38	0.04	10%
Medium (130 μL)	0.12	0.05	38%
Open	−0.04	0.10	n/a

This experiment demonstrates that the formation of an enclosed sample chamber, sealed from ambient air and containing a high density of cells, generates flux rates sufficient to provide a rapid measurement with a high signal-to-noise ratio.

It is apparent that many modifications and variations of this invention as hereinabove set forth may be made without departing from the spirit and scope of the present invention and the above examples are not intended to in any way to limit the present invention but are merely exemplary. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein.

What is claimed is:

1. A method of determining a cell constituent extracted from or secreted into a volume of medium (**130**) in a vessel (**100**) by a sample comprising cells (**120**), the method comprising:

(a) placing said sample in a vessel in an apparatus comprising:

the vessel, including a chamber containing an original volume of medium and, within the chamber, a surface to hold or contain the sample;

a moveable barrier (**140**) which alters the volume of the medium in contact with the sample; and

a sensor in sensing contact with the medium;

(b) moving the barrier to reduce the volume of the chamber and thereby the medium, forming a reduced-volume measuring chamber (**150**) in contact with the sample;

(c) determining a cell constituent in the medium in the reduced volume measuring chamber with the sensor; and

(d) moving the barrier to increase the reduced volume of medium about the cells to substantially the original volume.

2. The method of claim **1**, further comprising the step of repeating steps b) and c).

3. The method of claim **1**, further comprising the steps of:

determining a first concentration of the constituent; and

determining a second concentration of the constituent at a predetermined time interval from determining the first concentration.

4. The method of claim **3** further comprising the step of calculating a flux rate of the constituent based on the first concentration and the second concentration.

5. The method of claim **1** wherein the reduced volume is in a range of about 5-50% of the original volume.

6. The method of claim **5** wherein the reduced volume is in a range of about 5-20% of the original volume.

7. The method of claim **1** wherein the reduced volume is less than about 5% of the original volume.

8. The method of claim **1** wherein the cells comprise a cell selected from the group consisting of bacteria, fungus, yeast, a prokaryotic cell, a eukaryotic cell, an animal cell, a human cell, and an immortal cell.

9. The method of claim **1** wherein at least a portion of the cells are attached to said surface of said chamber.

10. The method of claim **1** wherein at least a portion of the cells are suspended in the media.

11. The method of claim **1** wherein at least a portion of the cells comprise living tissue.

12. The method of claim **1** wherein the constituent comprises a material selected from the group consisting of a dissolved gas, an ion, a protein, a substrate, a salt, and a mineral.

13. The method of claim **12** wherein the dissolved gas is selected from the group consisting of O_2, CO_2, and NH_3.

14. The method of claim **1** wherein the constituent comprises a material extracted from the media by at least a portion of the cells.

15. The method of claim **1** wherein the constituent comprises a material secreted into the media by at least a portion of the cells.

16. The method of claim **1** wherein determining the cell constituent comprises sensing a presence of the constituent.

17. The method of claim **1** wherein determining the cell constituent comprises sensing a concentration of the constituent.

18. The method of claim **1** wherein determining the cell constituent comprises sensing a first concentration of a first constituent, sensing a second concentration of a second constituent, and determining a relationship between the first concentration and the second concentration.

19. The method of claim **1** wherein determining the cell constituent comprises sensing a rate of change of concentration of the constituent.

20. The method of claim **1** wherein the sensor comprises a sensor selected from the group consisting of a fluorescent sensor, a luminescent sensor, an ISFET sensor, a surface plasmon resonance sensor, a sensor based on an optical diffraction principle, a sensor based on a principle of Wood's anomaly, an acoustic sensor, and a microwave sensor.

21. The method of claim **1** wherein determining the cell constituent comprises determining a parameter selected from the group consisting of cell viability, cell number, cell growth rate, response to at least one of a drug, a toxin, and a chemical, detection of an entity, and internalization.

22. The method of claim **1**, further comprising the step of perfusing additional media through the vessel.

23. The method of claim **1**, further comprising the step of replenishing the media in the vessel.

24. The method of claim **1** wherein the barrier is disposed in the vessel without causing displacement of media out of the vessel.

25. The method of claim 1 wherein at least a portion of the barrier comprises the sensor.

26. The method of claim 1 wherein the sensor is disposed on a surface of said barrier in contact with said reduced volume measuring chamber.

27. The method of claim 26 wherein the sensor comprises a fluorophore.

28. The method of claim 1 wherein at least a portion of the vessel comprises a sensor.

29. The method of claim 1 comprising the additional step of altering an environment of at least a portion of the cells prior to reducing the original volume of media.

30. The method of claim 29 wherein altering the environment comprises exposing at least a portion of the cells to at least one of a drug, a chemical, and a toxin.

31. The method of claim 1, further comprising the step of altering an environment of at least a portion of the cells after step b).

32. The method of claim 1 comprising the additional step of covering the vessel.

33. The method of claim 1 comprising the additional step of stirring at least a portion of the original volume of media in the vessel.

34. The method of claim 1 comprising the additional step of sealing the vessel.

35. The method of claim 1 wherein the cells comprise an animal cell, a human cell, or an immortal cell.

36. The method of claim 1 wherein said vessel comprises a well in a plate including multiple wells for holding media and cells.

37. A method of analyzing cells disposed in media within a vessel by analyzing constituents extracted from or secreted into the media, the method comprising the steps of:

(a) providing a vessel having an original volume of media about the cells;

(b) disposing a barrier into the vessel to reduce the original volume of media about at least a portion of the cells in the vessel and to define a reduced volume of media; and

(c) analyzing a constituent related to the cells within the reduced volume of media using a sensor in contact with the reduced volume of media.

38. The method of claim 37 wherein the cells are selected from the group consisting of animal cells, human cells, and immortal cells.

39. The method of claim 37 wherein the sensor is disposed on a surface of said barrier in contact with the reduced volume.

40. The method of claim 37 wherein the sensor comprises a fluorophore.

41. The method of claim 39 wherein the sensor comprises a fluorophore.

42. The method of claim 37 comprising the additional step of:

d) increasing the reduced volume of media about the cells in said vessel to substantially the original volume.

43. The method of claim 37 wherein the constituent comprises a material selected from the group consisting of a dissolved gas, an ion, a protein, a substrate, a salt, and a mineral.

44. The method of claim 37 wherein the constituent comprises a dissolved gas selected from the group consisting of O_2, CO_2, and NH_3.

45. The method of claim 37 wherein the sensor comprises a sensor selected from the group consisting of a fluorescent sensor, a luminescent sensor, an ISFET sensor, a surface plasmon resonance sensor, a sensor based on an optical diffraction principle, a sensor based on a principle of Wood's anomaly, an acoustic sensor, and a microwave sensor.

46. The method of claim 37 wherein the barrier is disposed in the vessel without causing displacement of media out of the vessel.

47. The method of claim 37 comprising the additional step of repeating steps b) and c) without removing said cells from said media.

48. The method of claim 37 wherein said vessel comprises a well in a plate including multiple wells for holding media and cells.

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(54) **METHOD AND DEVICE FOR MEASURING MULTIPLE PHYSIOLOGICAL PROPERTIES OF CELLS**

VERFAHREN UND VORRICHTUNG ZUR MESSUNG MEHRERER PHYSIOLOGISCHER EIGENSCHAFTEN VON ZELLEN

METHODE ET DISPOSITIF PERMETTANT DE MESURER LES MULTIPLES PROPRIETES PHYSIOLOGIQUES DES CELLULES

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(73) Proprietor: **Agilent Technologies, Inc.**
Santa Clara, CA 95051 (US)

(72) Inventors:
- **TEICH, Jay, S.**
Weston, MA 02493 (US)
- **NEILSON, Andy, C.**
Groton, MA 01450 (US)
- **SWEENEY, Michael, R.**
Pelham, NH 03076 (US)
- **UHL, Geoff**
Cambridge, MA 02139 (US)

(74) Representative: **Grünecker Patent- und Rechtsanwälte**
PartG mbB
Leopoldstraße 4
80802 München (DE)

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Description

Field of the Invention

[0001] This application relates generally to high throughput screening techniques and, more specifically, to the measurement of the constituents (analytes) of an extracellular medium surrounding living cells. All of the patents, articles, and other references cited herein form a part of this patent application and their respective disclosures are incorporated herein by reference in their entirety.

Background

[0002] Living cells typically consume nutrients and oxygen from the surrounding medium, and return metabolic by-products, including ions, carbon dioxide, lactate, and various proteins, to this extracellular environment. The rate of uptake and excretion of these analytes can provide valuable information regarding the metabolic processes underway inside the cells.

[0003] Conventional biological assays inherently exhibit significant limitations. An ideal biological assay is homogeneous (i.e., does not require the introduction of a foreign agent such as a dye), non-invasive (i.e., has no deleterious effect on the biological process), and rapid.

[0004] Many tools have been developed to probe the mechanistic processes of cells using internalized reporters such as fluorescent dyes. A device that is able to measure extracellular analytes using a non-invasive, homogeneous assay performed within a container that is compatible with existing invasive tools would be particularly useful.

[0005] Some previous approaches relate to oxygen flux rate measurements, since respiration can be deemed to be a basic measure of cell viability. Many devices have been developed to monitor respiration *in vitro,* through determination of the rate of depletion of oxygen in the extracellular medium. The earliest instruments relied on the change in total gas pressure in a sealed vessel, using the assumption that this change was primarily due to oxygen consumption.

[0006] In the 1960s, the Clark electrode (Clark, L.C. Jnr. Ann. NY Acad. Sci. 1962; 102:29-45), and later the miniaturized Clark electrode, enabled a more specific measure of oxygen partial pressure. The relative complexity of the Clark design, and the fact that the electrode itself consumed oxygen, may have hindered its incorporation in a highly parallel instrument suitable for widespread use. However, these devices were deemed successful enough to measure cell viability (Gesinski RM, Morrison JH, Toepfer JR. "Measurement of oxygen consumption of rat bone marrow cells by a polarographic method." J Appl Physiol. 1968; 24(6):751-754), to profile the toxic effects of drugs and chemicals (Shenoy MA, Biaglow JE, Varnes ME, Hetzel FW. "Inhibition of cultured human tumor cell oxygen utilization by chlorpromazine." Adv Exp Med Biol.1983;159:359-68), and to show the effect of agents such as insulin on cellular metabolic processes (Panten U and Klein H. "O2 consumption by isolated pancreatic islets, as measured in a Microincubation system with a Clark-type electrode." Endocrinology 1982; 111:1595-1600).

[0007] More recently, several oxygen sensors have been developed that can enable the design of a non-invasive, homogeneous readout of cellular respiration. Fluorescent compounds, whose response is diminished by the phenomenon of oxygen-quenching, are now available. These compounds can be embedded in an oxygen permeable membrane and exposed to cell media, and can be read using low cost, fiber coupled, semiconductor light sources and sensors (Wolfbeis OS, 2002. "Fiber-Optic Chemical Sensors and Biosensors." Annal of Chem. 2002; 74:2663-2678).

[0008] An ion-sensitive field-effect transistor (ISFET), whose gate region can be exposed to a liquid analyte, has been adapted to measure oxygen pressure using enzyme catalyzed conversion of oxygen (O_2) to H^+ ions that can be detected by this sensor (Lehmann, M, Baumann W, Brischwein M, Gahle H-J, Freund I, Ehret R, Dreschler S, Palzer H, Kleintges M, Sieben U and Wolf B. "Simultaneous measurement of cellular respiration and acidification with a single CMOS ISFET. 2001." Biosensors & Bioelectronics. 2001;16:195-203).

[0009] Devices have been described and/or demonstrated that incorporate oxygen-quenched fluorophores, ISFETs and other oxygen sensors within sample chambers containing bacteria or mammalian cells for the purpose of measuring respiration rate, viability, or the effect of drugs or toxins. These devices range in size from fluorescent patches attached to the interior wall of large cell culture bottles (Tolosa L, Kostov Y, Harms P, Rao G. "Noninvasive measurement of dissolved oxygen in shake flasks." Biotechnol Bioeng 2002 Dec 5;80(5):594-7), to fluorescent sensors embedded within microscopic flow cells fabricated using microfluidics technology (Lähdesmäki I, Scampavia LD, Beeson C, and Ruzicka J. "Detection of Oxygen Consumption of Cultured Adherent Cells by Bead Injection Spectroscopy." Anal. Chem. 1999; 71: 5248-5252), to microtitre plates with fluorescent compounds suspended within (O'Riordan TC, Buckley D., Ogurtsov V, O'Connor R., Papkovsky DB "A cell viability assay based on monitoring respiration by optical oxygen sensor." Anal. Biochem. 2000; 278(2):221-227) or deposited upon the wells (Woodnicka M, Guarino RD, Hemperly JJ, Timmins MR, Stitt D, Pitner JB. "Novel fluorescent technology platform for high throughput cytotoxicity and proliferation assays." Journal of Biomolecular Screening. 2000; 5:141-152).

[0010] Some patents describe a device for monitoring cells using an oxygen-quenched fluorescent compound that is

placed in contact with a broth containing bacteria or mammalian cells. A fluorescence measurement of cells treated with a drug or toxin may be compared to a reference, purportedly to determine the effect of the compound on cellular respiration. In an embodiment, cells are contained within a microplate that is exposed to ambient air. Cells are maintained at a low density in order to maintain viability in this configuration, because high cell density would likely result in anoxia, acidification of the media, and contact inhibition. Measurement times may, therefore, typically be tens of hours or days. In addition, the influx of ambient oxygen and lack of control of sample volume may allow only relative measurement to control to be made. In another embodiment, to limit ambient oxygen influx, mineral oil is placed above the cell media. Because cell density is typically quite low, long measurement times are typically required.

[0011] A number of patents and publications describe oxygen flux measurement systems incorporating small, closed sample chambers containing high densities of cells. In these devices, an active perfusion system is used to intermittently restore normal levels of dissolved oxygen, pH, and nutrients. None of these systems are designed or configured to enable the user to easily culture cells, maintain their viability, run experiments in parallel with high throughput, or run other types of assays without detaching and moving the cells.

[0012] There have also been approaches to measuring cellular acidification rate. Living cells produce protons (H^+ ions) as a byproduct of various metabolic processes, including both aerobic and anaerobic respiration. Protons are also produced when ion exchange pumps on the surface of eukaryotic cells are activated as a result of binding of a ligand with a transmembrane receptor or ion channel. In a fixed volume of extracellular media, this proton flux causes a gradual acidification that can be measured using a pH sensor. Thus, an indication of metabolic rate and/or receptor activation can be determined from a precise measurement of extracellular acidification rate.

[0013] A number of pH sensors can be applied to the measurement of cell media. In addition to fluorescent and ISFET sensors similar to those described previously, a light addressable potentiometric sensor has been incorporated in an instrument for rapid measurement of proton flux (Parce W, Owicki J, Kercso K, Sigal G, Wada H, Muir V, Bousse L, Ross K, Sikic B, and McConnell H. 1989. "Detection of Cell-Affecting Agents with a Silicon Biosensor." Science. 1989; 246(4927):243-247).

[0014] One patent describes a device employing a method for measurement of extracellular acidification (pH) as an indicator of cellular metabolism. In this device, a small sample chamber containing a high density of cells is intermittently perfused with media and closed to allow measurement of the pH change resulting from cellular proton excretion. A series of repetitive stop/flow cycles provides kinetic metabolic rate data. Because the sample chamber, once assembled, is fixed in size and contains a high density of cells, active perfusion is required to prevent cell death from the rapid acidification and depletion of oxygen from the media. The addition of a perfusion system to the device results in the need for relatively complex tubing, pumps, and other features, that create cleaning and sterilization problems for the user. In addition, when cells are to be treated with a drug using this device, the drug may need to be perfused over the cells for a relatively long period of time, thereby consuming large quantities of typically scarce and expensive compounds.

[0015] Other extracellular analytes can be measured using non-invasive techniques. Carbon dioxide evolution can be determined from the measurement of carbon dioxide (CO_2) partial pressure in the media using various fluorescent sensors (Pattison R, Swamy J, Mendenhall B, Hwang C, and Frohlich B. "Measurement and Control of Dissolved Carbon Dioxide in Mammalian Cell Culture Processes Using an in Situ Fiber Optic Chemical Sensor." 2000. Biotechnology Prog. 16:769-774)(Ge X, Kostov Y, and G Rao. High Stability non-invasive autoclavable naked optical CO2 sensor. 2003. Biosensor and Bioelectronics 18:pp.857-865).

[0016] Other ions and chemical constituents can be measured using non-invasive techniques based on optical or semiconductor sensors. In addition, larger molecules such as proteins can be measured using non-invasive techniques that are sensitive to the binding of these molecules to antibodies that are attached to sensors exposed to the extracellular media (Flora K and J Brennan. Comparison of Formats for the Development of Fiber-Optic Biosensors Utilizing Sol-Gel Derived Materials Entrapping Fluorescently-Labeled Proteins. Analyst, 1999, 124, 1455-146).

[0017] Other physical phenomenon that support such sensors are surface plasmon resonance (Jordan & Corn, "Surface Plasmon Resonance Imaging Measurements of Electrostatic Biopolymer Adsorption onto Chemically Modified Gold Surfaces," Anal. Chem., 69:1449-1456 (1997), grating couplers (Morhard et al., "Immobilization of antibodies in micro-patterns for cell detection by optical diffraction," Sensors and Actuators B, 70, p. 232-242, 2000), ellipsometry (Jin et al., "A biosensor concept based on imaging ellipsometry for visualization of biomolecular interactions," Analytical Bio-chemistry, 232, p. 69-72, 1995), evanescent wave devices (Huber et al., "Direct optical immunosensing (sensitivity and selectivity)," Sensors and Actuators B, 6, p. 122-126, 1992), reflectometry (Brecht & Gauglitz, "Optical probes and transducers," Biosensors and Bioelectronics, 10, p. 923-936, 1995) and Wood's anomaly (B. Cunningham, P. Li, B. Lin, J. Pepper, "Colorimetric resonant reflection as a direct biochemical assay technique," Sensors and Actuators B, Volume 81, p. 316-328, Jan. 5, 2002).

[0018] In general, the utility of devices incorporating these sensing technologies for the purpose of measuring secretion of proteins by cells is limited by detection sensitivity. Sensitivity can be increased, typically by increasing cell density in the region proximal to the sensor surface. However, cellular health declines rapidly as cell density increases, due to anoxia, acidification of the media, and contact inhibition. It is possible, but generally undesirable, to adhere cells directly

to the sensor surface.

[0019] A need exists for the provision of a high cell density for measurement of analytes and a low density for maintenance of cell health and growth. While many devices have been developed for the purpose of measuring flux rates of extracellular analytes, there exists a need to meet requirements that may enable widespread use in the fields of biological research, drug discovery and clinical diagnostics. A need exists for devices with high throughput and ease of use. A parallel configuration may be desirable. Preferably, a tradeoff between long assay times and the length of time to prepare the sample would be eliminated. Lack of these attributes may result in low sample throughput and therefore incompatibility with modern drug discovery and diagnostic activities.

[0020] In addition, there is a need for an instrument that can be used to measure extracellular flux rates of cells in a non-invasive manner within a vessel that is commonly used for other high throughput assays, thereby allowing the use of the flux rate measurement as a quality control or complementary measurement to existing assays.

[0021] International patent application WO 03/059518 describes an assay device comprising a base and glass plate lid. The base has an array of shallow microwells each having a flat rim, all rims being co-planar. When the lid is placed on the base a thin capillary gap is formed on each rim, acting as a liquid seal for a microwell chamber. The liquid is excess sample liquid and further excess is accommodated in overspill cavities between the microwells. Because of the liquid seal and shallow configuration the benefits of microfluidic devices are achieved together with the handling convenience and use of conventional detection equipment of conventional microplate devices.

[0022] International patent application WO 98/15645 discloses the preamble of appended claims 1 and 34. It describes a biological indicator test utilizing oxygen sensing within a microenvironment as a means for determining the presence of viable microorganisms capable of growth, a method for biological indicator testing for determining the completeness of a sterilization cycle utilizing oxygen sensing as the means for determining oxygen content in a biological indicator assay vessel containing an optical oxygen sensor, and a method and device for determining antimicrobial drug resistance or sensitivity to a contaminated sample.

[0023] In summary, there is a need for a device that can meet the goals of data quality, compatibility with existing experimental practices, and ease-of-use, thereby enabling widespread adoption of a new technology.

Summary

[0024] According to an aspect of the present invention, there is provided a method in accordance with claim 1 herein. The new method has been conceived and developed for providing high cell density for measurement of analytes and low cell densities for maintenance of cell health and growth. The instant invention can determine the flux rates of various extracellular analytes in minutes, can provide quantitative rather than relative readings, can be used without adversely affecting the physiological state of the cells under test, and does not require an active perfusion or agitation system.

[0025] One feature of the invention is the temporary creation of a substantially closed sample chamber within a vessel containing a low density mixture of cells and media, and a sensor or plurality of sensors for measurement of analytes. Since a temporary sample chamber is created within a larger vessel, media containing high levels of dissolved oxygen and other analytes, and normal pH, is supplied to the cells prior to, and immediately after a measurement is made. Using this feature, cells can be grown, maintained for extended periods, treated with drug compounds, and assayed using any of a variety of methods, while being periodically assayed for viability and respiration rate, without compromising the cells. Furthermore, the media containing cells need not be removed from the vessel; it is only displaced temporarily. Therefore, a minimal quantity of drug compound is required.

[0026] In addition, by precisely controlling the dimensions of the temporary sample chamber, a quantitative flux rate for extracellular analytes can be determined easily. Therefore, an external reference is not required; a change in the flux rates of cells in a vessel can be determined from multiple readings of this one vessel.

[0027] Elements of one embodiment of the invention include:

1. Temporary formation of a small, relatively impermeable sample chamber (containing one or more cells, one or more sensors, and a small amount of cell media) within a larger media-filled vessel.
This configuration assists with:

» increasing the rate of change of analytes in the media so that a sensitive measurement can be made in a reasonably short time, i.e., minutes vs. hours for some of the prior art methods;

» eliminating the need for a reference, by overcoming the following limitations of the prior art:

a. Low sensitivity (low cell density in the measurement broth and therefore a small signal that may need to be measured);

b. Unknown sample volume (user variability in fill level of each well and evaporation); and

c. O_2 influx from the surrounding environment (unless the entire well is sealed with, e.g., a mineral oil coating as suggested by the prior art, which results in a terminal experiment);

» eliminating the need for complex fluidic systems to provide intermittent perfusion to a flow cell, since a high ratio of cells/media is only created temporarily in accordance with the invention; and

» development of a high sensitivity cell-based assay system for other types of sensors, including SPR, SRU, etc., where the analyte affected by the cells is affected at a low rate that is difficult to measure;

2. The specific design of a device to accomplish the above, including a stepped well and inverted, mushroom-shaped probe with optical sensors on the bottom surface; and

3. Temporary insertion of the sensor described above into a variety of vessels (including clear-bottom microplates) containing cells.

» This enables the use of substantially all conventional assays, without the need to move cells or disturb their adhesion to the vessel surface; and

» Sensors can be cleaned and reused in minutes.

[0028] It is one object of this invention to provide a rapid, non-invasive, and easy-to-use method for determining various physiological properties of living cells. In particular, a device and method are described that can measure overall cellular metabolic and respirative rates, the relative proportion of aerobic to anaerobic respiration, the relative rates of consumption of various metabolic substrates, the effect of stimulation of certain transmembrane and other cellular receptors, the rates of production of various secreted factors, and cell viability.

[0029] The device and method can be applied in a variety of fields, including biological research, drug discovery, and clinical diagnostics. The device can be used as a stand-alone instrument or in conjunction with existing assay methods. For example, as a drug discovery tool, the device can be used to screen various compounds for an effect on cellular metabolism, protein secretion, or intra/extra cellular ion exchange. In addition, the device can be used to replace more complex, invasive, and time consuming methods for determining the toxic effects of compounds on cells or tissue samples. For this purpose, the device eliminates the need for the addition of dyes and incubation of cells. The device can also be used to determine the health of cells or tissue both before and after a conventional assay is performed, thereby improving the performance of such an assay.

[0030] In one aspect, the invention includes a method of analyzing cells disposed in media within a vessel. The method includes providing an original volume of media about the cells, reducing the original volume of media about at least a portion of the cells to define a reduced volume of media, and analyzing a constituent related to the cells within the reduced volume of media.

[0031] One or more of the following features may be included. The reduced volume of media about the cells may be increased to substantially the original volume. A first concentration of the constituent may be determined, and a second concentration of the constituent may be determined at a predetermined time interval from the determination of the first concentration. A flux rate of the constituent may be calculated based on the first concentration and the second concentration.

[0032] The reduced volume may include, for example about 5-50% of the original volume, preferably about 5-20% of the original volume. In some embodiments, the reduced volume may be less than about 5% of the original volume.

[0033] The cells may include bacteria, fungus, yeast, a prokaryotic cell, a eukaryotic cell, an animal cell, a human cell, and/or an immortal cell. At least a portion of the cells may be attached to a surface of the vessel. At least a portion of the cells may be suspended in the media. At least a portion of the cells may include living tissue.

[0034] The constituent being analyzed may include a dissolved gas (e.g., O_2, CO_2, NH_3), an ion (e.g., H^+, Na^+, K^+, Ca^{++}), a protein (e.g., cytokines, insulin, chemokines, hormones, antibodies), a substrate (e.g., glucose, a fatty acid, an amino acid, glutamine, glycogen, pyruvate), a salt, and/or a mineral. The constituent may be extracted from the media by at least a portion of the cells. The constituent may be secreted into the media by at least a portion of the cells.

[0035] Analyzing the constituent may include sensing the presence and/or the concentration of the constituent. Analyzing the constituent may include by sensing a first concentration of a first constituent, sensing a second concentration of a second constituent, and determining a relationship between the first concentration and the second concentration. Analyzing the constituent may include sensing a rate of change of a concentration of the constituent.

[0036] A sensor in contact with the media within the reduced volume may be used. The sensor may be a fluorescent

sensor, a luminescent sensor, an ISFET sensor, a surface plasmon resonance sensor, a sensor based on an optical diffraction principle, a sensor based on a principle of Wood's anomaly, an acoustic sensor, or a microwave sensor.

[0037] Analyzing the constituent may include determining a parameter such as cell viability, cell number, cell growth rate, response to at least one of a drug, a toxin or a chemical, detection of an entity, and internalization.

[0038] The method may include perfusing additional media through the vessel and/or replenishing the media in the vessel.

[0039] Reducing the volume of media includes disposing a barrier in the vessel, typically not causing displacement of the media out of the vessel. At least a portion of the barrier includes a sensor. Additionally, the reduced volume of media may include a sensor, such as a fluorophore. At least a portion of the vessel may include a sensor.

[0040] The environment of at least a portion of the cells may be altered prior to reducing the original volume of media. The environment may be altered by, e.g., exposing at least a portion of the cells to at least one of a drug, a chemical, or a toxin.

[0041] The environment of at least a portion of the cells may be altered after reducing the original volume of media.

[0042] The method may include covering the vessel, sealing the vessel, and/or stirring at least a portion of the original volume of media in the vessel.

[0043] In another aspect, the invention relates to an apparatus for determining a constituent extracted from or secreted by a sample comprising cell in accordance with claim 34.

[0044] One or more of the following features may be included. The sensor may be configured to analyze the constituent without disturbing the cells. The vessel may include a well disposed in a microplate. The well may include a step. The barrier may be adapted to stir the media prior to analysis of the constituent.

[0045] The sensor may be, for example, a fluorescent sensor, a luminescent sensor, an ISFET sensor, a surface plasmon resonance sensor, a sensor based on an optical diffraction principle, a sensor based on a principle of Wood's anomaly, an acoustic sensor, or a microwave sensor.

[0046] An example features a plate including multiple wells for holding media and cells. Each of at least a portion of the wells includes a seating surface for receiving a barrier a reduced volume.

[0047] One or more of the following features may be included. A shape of the seating surface may be generally planar, arcuate, contoured, tapered, conical, stepped, or interlocking. The reduced volume within each of the wells may vary by less than about 10% of a mean volume of the wells, preferably by less than about 5% of the mean volume of the wells, more preferably by less than about 1% of the mean volume of the wells. The seating surfaces of the wells may each include a step disposed about an inner periphery of a respective well. The steps may lie in a step plane disposed above a bottom plane defined by bottoms of respective wells. The step plane and the bottom plane may be parallel planes. A height of the step plane may be less than about 1 millimeter (mm) above the bottom plane, preferably less than about 200 μm above the bottom plane, more preferably less than about 50 μm above the bottom plane.

[0048] A fluorescent sensor may be disposed within at least one of the wells. At least one of the wells may include a transparent bottom. At least one of the wells may include an opaque wall.

[0049] An example features a barrier for analysis of cells disposed in media in a vessel. The barrier includes a body portion for insertion into the vessel, the body portion having a barrier surface for mating with a first surface of the vessel to create a reduced volume.

[0050] One or more of the following features may be included. A shape of the barrier surface may be generally planar, arcuate, contoured, tapered, conical, stepped, or interlocking. The barrier may include a cover for mating with a second surface of the vessel.

[0051] A sensor is may disposed on the barrier surface for analyzing a constituent of a media disposed about at least a portion of the cells. The sensor may include an optical sensor. The optical sensor may be adapted to sense a fluorophore.

[0052] A conductor may be coupled to the sensor and configured to conduct signals therefrom. The conductor may include an optical fiber and may be disposed at least partially in the body portion. The barrier may include a readout for transmitting a signal from the sensor. The readout may be visual, fiber, electronics on a post, and/or a plate reader from the bottom.

[0053] The barrier may include a plurality of barriers arranged to be received within a plurality of wells in a microplate.

Brief Description of the Drawings

[0054]

FIG. 1 is a partial cross-sectional view of one embodiment of the present invention, where the vessel is formed by a single well within a multi-well microplate, the cover and sensor assembly being shown in a pre-measurement position;

FIG. 2 is a cross-sectional view of the cover and sensor assembly of FIG. 1 in the measurement position;

FIG. 3 is a schematic illustration of a complete measurement system, in accordance with one embodiment of the invention;

FIGS. 4a and 4b are schematic cross-sectional views of wells with different seating surfaces;

FIGs. 5a - 5c are schematic cross-sectional views of barriers that include both a sensor assembly and a readout;

FIG. 6 is a graph showing the result of a study of the oxygen consumption and extracellular acidification rates of typical mammalian cells, depicting the mean and standard deviation of a series of eight separate measurements using one embodiment of the invention;

FIG. 7 is a graph showing the result of a study of the oxygen consumption and carbon dioxide evolution rates of various numbers of typical mammalian cells using one embodiment of the invention;

FIG. 8 is a graph showing the result of a study of the effect of the chemical compound 2,4, DNP on the rates of oxygen consumption, carbon dioxide evolution, and extracellular acidification of typical mammalian cells using one embodiment of the invention;

FIG. 9 is a graph showing the result of a study of the effect of the chemical compound Rotenone on the rates of oxygen consumption and extracellular acidification of typical mammalian cells using one embodiment of the invention;

FIG. 10 is a graph showing the result of a study of the effect of cell proliferation on oxygen consumption and extracellular acidification using one embodiment of the invention;

FIG. 11 is a graph showing the result of a study of the effect of the chemical compound Carbachol on the rate of extracellular acidification of typical mammalian cells using one embodiment of the invention; and

FIG. 12 is a graph showing a comparison of the measured rates of oxygen consumption of typical mammalian cells in a vessel with, and without, the formation of a small, enclosed sample chamber using one embodiment of the invention.

Detailed Description

[0055] This invention enables the temporary creation of a highly concentrated volume of cells within a larger volume of cell media, in order to allow sensitive measurements of the change in constituents of the media that result from biological activity of the cells. By temporarily, rather than permanently, reducing the media volume (and therefore concentrating the cell/media mixture), cells are exposed to a non-normal environment for only a brief period of time and are therefore not adversely affected by the measurement process.

[0056] In one embodiment of the invention, cells are grown or placed on the bottom of a vessel containing sufficient type and volume of media to support growth for an extended period of time. A sample chamber is formed in the bottom of the vessel, consisting of the bottom of the vessel and vertical walls, such that the enclosed volume is sufficient to contain the cells plus a reduced volume of media.

[0057] A barrier, having a diameter slightly less than the inside diameter of the vessel, is located above the sample chamber on a movable actuator. Upon actuation, the barrier may be raised above the level of liquid in the vessel, or lowered into the liquid and on to the vessel walls, forming a sample chamber that is relatively impervious to the diffusion of analytes from the sample chamber to and from the bulk media now above the cover.

[0058] A cross-sectional view of a representative embodiment is shown in FIG. 1. The drawing details a vessel 100 that is typical of one well 110 within a multi-well microplate. The walls of this single well 110 form the vessel 100 that contains live cells 120 and cell growth media 130. Cells may or may not adhere to a bottom surface 132 of the vessel, and the bottom surface may be treated or coated to encourage adherence. Alternatively, cells may be suspended within the media and may be forced to the bottom of the vessel using gravity or centrifugal force.

[0059] A barrier 140, having a diameter slightly less than an inside diameter of the vessel 100, is used to form a cover that defines a sample chamber 150 within the vessel. Barrier 140 may have a diameter d_1 of, e.g., 6 mm, and vessel 100 may have an inside diameter d_2 of, e.g., 7 mm. In FIG. **1**, the barrier 140 is shown in a pre-measurement position within the vessel. To effect a measurement, a manual or motorized plunger (actuator) can then be used to reposition the barrier 140 slightly above the bottom surface 132 of the vessel 100 as shown in FIG. **2** by lowering the barrier 140 or raising the well 110. Orienting the barrier to the position shown in FIG. **2** prior to measurement defines the sample chamber 150 having a reduced volume of media, thereby enhancing measurement sensitivity.

[0060] A single vessel of nearly any size may be fabricated, or multiple vessels may be fabricated in a one- or two-dimensional arrangement. In one embodiment, a two-dimensional pattern of vessels corresponding to the pattern and dimensions of a microplate, as described by the Society for Biomolecular Screening standards for microplates ("SBS-1 Footprints" and "SBS-4 Well Positions," both full proposed standards updated May 20, 2003), and containing a total of 12, 24, 96, 384, 1536, or any other number of individual wells may be fabricated.

[0061] The vessel and sample chamber may typically be formed using plastic material such as, for example, polystyrene or polypropylene, with the bottom clear and the sides colored black to reduce optical cross-talk from one well to another.

[0062] A variety of types of barriers may be employed to temporarily reduce the volume of media about the cells without causing displacement of media out of the vessel, such as a simple planar cover lowered vertically, a sliding cover extended horizontally, or a pair of disks with cutouts that can be rotated to act as a valve. It is desirable that the barrier not disturb, i.e., not move, the cells or the media proximal to the cells, in order to reduce the required settling time prior to a measurement.

[0063] A complete measurement system can be assembled using the components shown in FIG. **3**. A vessel 300, e.g., a plate such as a microplate including a plurality of wells 302, is placed upon a translation stage 310. The microplate is disposed beneath an array of barriers 320 disposed on a plunger 322 adapted to receive the barriers and an array of pipettors 330. Each of at least a portion of the wells includes a seating surface (see, e.g., FIGs. 4a and 4b) adapted to receive one of the barriers. Barriers 320 may include sensors. An original volume of media may be disposed in the wells. Using manual or motorized actuation, the barriers and pipettors may be lowered into the microplate wells to create a reduced volume of media within the wells. The reduced volume may be less than, e.g., 50%, of the original volume of media. The barriers are adapted for insertion into the vessel, i.e., into the wells, by relative movement of the stage 310 and the plunger 322. The barriers and pipettors may also be lowered into one of several fluid reservoirs 340 containing wash buffers and calibrants. When the barriers create the reduced volume of media within the vessels, sensors may be in sensing communication with the reduced volume of media and may be configured to analyze one or more constituents disposed within the reduced volume. The sensors may be interrogated by an optical interface consisting of illumination sources (e.g., light emitting diodes) and light detectors (e.g., photodiodes), with appropriate band-limiting filters interspersed between the optical elements. A computer and software 350 perform actuation, calibration and measurement functions.

[0064] A change in the temperature of the media within the sample chamber may result in unwanted measurement errors from at least two sources. First, the capacity of the media to hold dissolved gasses changes with temperature, and therefore a change in temperature may cause an apparent change in dissolved gas concentration as the media seeks equilibrium with the surrounding environment. Second, the measurement properties of many types of sensors changes with temperature.

[0065] To ensure accurate and repeatable measurements, the temperature of a reduced volume of media in the vessel may be controlled or a correction factor may be applied to the measurement. Because evaporation induces cooling of the liquid media, control of evaporation may be desired to reduce thermal drift, thermal gradients, and gas exchange.

[0066] Providing environmental and temperature control for the sample chamber may reduce unwanted impact on the measurement process. For example, uncontrolled temperature changes of the media surrounding the cells can directly impact the rate of apparent oxygen consumption. Oxygen will naturally off-gas from media as it warms, thus introducing the appearance of a change in cellular respiration when, in fact, the rate change observed is a natural function of dissolved gas seeking equilibrium as the temperature increases. Similarly, any evaporation from the media due to other uncontrolled environmental conditions such as humidity or exposure to air currents can artificially impact the measurements made from various sensors including those of dissolved gases, ions, and temperature.

[0067] Using this measurement system, an assay cycle is initiated by mating the sensors / barriers with the vessel walls to form closed sample chambers with reduced volume of media containing the cells. The rate and pattern of actuation of the barriers may be programmed to prevent rapid motion of the media that may disturb the cells, i.e., displace the cells by or cause shear stress on the cells, and may be alternated to provide fluid motion for stirring of the media, as desired.

[0068] Additionally, the barriers may be independently biased, for example, by using springs or other force elements, to ensure adequate seating of the covers in all of the wells, simultaneously.

[0069] The electro-optical interface and computer are then used to measure the change in response of the sensor or sensors resulting from the change in concentration of extracellular analytes. The rates of consumption or production of analytes may be determined by making multiple readings over a period of minutes and then calculating the slope between selected measurement points. Once the measurement sequence is completed, the sensor/covers are retracted to expose the cells to the full volume of media within each vessel.

[0070] The measurement system may include provisions for single or multiple-point calibration of the analyte sensors. For example, two reservoirs containing liquid of known, but different pH, oxygen, CO_2, or other analyte levels may be incorporated, and a two-point (gain and offset) calibration may be performed periodically. Alternatively, "factory" pre-calibration of the sensors may be used to eliminate the need for field calibration, or to reduce the calibration to a single

point (offset) correction.

[0071] Referring to FIG. 4a, in one embodiment, a microplate is used to provide a plurality of measurement vessels in a standardized pattern. By incorporating a seating surface 400 in each well, a precise reduced volume of media can be maintained about the cells during the measurement period. The reduced volume within each of the wells disposed in a plate may vary by less than about 10% of a mean volume of the wells. In some embodiments, the reduced volume may vary by less than 5% of the mean volume of the wells, and in some embodiments, the reduced volume may vary by less than 1% of the mean volume of the wells. The seating surface 400 or steps may lie in a step plane 410 disposed above a bottom plane 420 defined by bottoms 430 of respective wells, with the step plane 410 and the bottom plane 420 being parallel planes. The height of the step plane is generally less than about 1 mm above the bottom plane and typically less than 50 μm to 200 μm above the bottom plane.

[0072] Referring to FIG. 4b, in another embodiment, a sloped surface 435 is incorporated to prevent the adhesion of cells on the seating surface 440. Any of a variety of alternative mating cover and seating surfaces can be employed, in various combinations and permutations, including those that are generally planar, arcuate, contoured, tapered, conical, stepped, interlocking, etc. What is generally desired is that mating features reliably and repeatably isolate the reduced volume from the original volume, such that the reduced volume has a generally predetermined or known capacity. Auxiliary seating components, such as O-rings, or resilient or compliant sealing lips, flaps, or other features may be employed on the covers or in the wells to enhance the seal, as desired.

[0073] The barrier can be fabricated to include a sensor assembly and a readout for transmitting a signal from the sensor assembly. FIG. 5a shows a cross-sectional view of a barrier formed from the combination of a tubular solid support 500 and a removable cover 510 or sheath, having an enlarged distal end that forms a structure upon which one or more optically-coupled sensors 520 are attached. In one embodiment, the sheath may be fabricated from a material that is either disposable or sterilizable in order to prevent contamination of the cell media. A readout 530 may be in the form of optical fibers disposed within the tubular support for communication between the sensors and an electro-optical measurement system 540. The electro-optical measurement system 540 may incorporate a source of illumination, an optical detector, spectral filters, and signal processing components. The electro-optical measurement system 540 may be automated. In some embodiments, the electro-optical measurement system 540 may be in electrical communication with computer 350 (see FIG. 3).

[0074] FIG. 5b shows an alternative arrangement in which the sensors are illuminated by an external light source 550. Electro-optical measurement system 540 may include separate components, i.e., an optical measurement system 560 and an illumination system 570. The optical measurement system 560 and illumination system 570 may be automated. In some embodiments, the optical measurement and illumination systems 560, 570 may be in electrical communication with computer 350. Referring to FIG. 5c, in an alternative embodiment, the electro-optical measurement system 540 includes optical and measurement components 580 located within the tubular support 500 and an external electronic measurement system 585. The optical and measurement components may communicate with the external electronic measurement system 585 through a cable 590.

[0075] Any form of signal communication can be employed, as desired. Such forms of signal communication might include simple visual interrogation of a signal change such as a change in color; fiber optic signal communication coming from any side of the vessel; a laser or CCD-based plate reader interrogating the signal from the bottom of a transparent vessel.

[0076] In practice, many different configurations of vessels, barriers, and sensors may be employed. The total vessel volume may range from many liters to a fraction of a microliter (ml), but is generally less than about 1 ml. The ratio of the reduced volume of media enclosed within the temporary sample chamber to an original volume of media provided in the vessel may range from about 50% to less than about 5% and even as low as less than about 1%, but is typically in the range of 5-20%.

[0077] Many different types and numbers of cells can be analyzed, including bacteria, fungus, yeast, prokaryotic and eukaryotic cells, animal or human cells, etc. Cells may adhere to the vessel wall or may be suspended within the media. Immortalized cells, native and primary cells, and homogenized or sliced tissue may be analyzed. A centrifuge may be used to concentrate cells within the sample chamber region of the vessel.

[0078] Any number of constituents of the media may be analyzed, including dissolved gasses, ions, proteins, metabolic substrates, salts and minerals. These constituents may be consumed by the cells (such as O_2), or may be produced by the cells either as a byproduct (such as CO_2 and NH_3) or as a secreted factor (such as insulin, cytokines, chemokines, hormones or antibodies). Ions such as H^+, Na^+, K^+, and Ca^{++} secreted or extracted by cells in various cellular metabolism processes may also be analyzed. Substrates either consumed or produced by cells such as glucose, fatty acid, amino acids, glutamine, glycogen and pyruvate may be analyzed. Specialized media may be used to improve the sensitivity of the measurement. For example, the change in pH resulting from extracellular acidification can be increased by using a media with reduced buffer capacity, such as bicarbonate-free media.

[0079] The analysis performed using this method may simply detect the presence of a constituent in the media, or may quantitatively analyze the amount and change in concentration, volume, or partial pressure of a constituent. With

the incorporation of multiple sensors, one or more ratios of constituents may be analyzed. As an example, the ratio of anaerobic to aerobic respiration utilized by the cell can be determined from a calculation of the ratio of oxygen consumption to extracellular acidification rate that is enabled by a measurement of changes in oxygen partial pressure and pH of the extracellular media. Analysis may include sensing a first concentration of a first constituent, sensing a second concentration of a second constituent, and determining a relationship between the first concentration and the second concentration.

[0080] The type of sensors utilized include oxygen sensors, such as oxygen-quenched fluorescent sensors, enzyme-coupled ISFET sensors, miniature Clark electrodes, or other oxygen sensors; pH sensors, including fluorescent sensors, ISFET sensors, pH sensitive dye sensors, LAP sensors, or other pH sensors; CO_2 sensors, including bicarbonate buffer coupled and ammonium dye coupled fluorescent sensors as well as other CO_2 sensors; various ion and small molecule sensors; large molecule sensors including surface plasmon resonance sensors and sensors exploiting the principle of Wood's anomaly; acoustic sensors; and microwave sensors.

[0081] The method may be used to measure any number of attributes of cells and cellular function. For example, cell viability and metabolic rate may be determined from measurements of oxygen consumption rate, extracellular acidification rate, or other metabolic analyte fluxes. By comparison of one or more analyte flux rates to a known rate per cell, cell number may be determined and therefore growth rates can be monitored.

[0082] The number of sensors used may range from one to many hundreds. Sensors for dissolved gasses may be placed within the sample chamber, but not in direct contact with the media. Other sensors, however, should be in direct contact with the media and in close proximity to the cells. This may be accomplished by mixing an indicator compound, e.g., a fluorophore, with the cell media, or by embedding the indicator in a compound that is permeable to the analyte to be measured. The embedded indicator may then be attached to any surface of the sample chamber region of the vessel.

[0083] In one embodiment, one or more sensors may be attached to the lower surface of the barrier, so as to be exposed to the extracellular media upon lowering of the barrier. One example of a sensor for this purpose is a fluorescent indicator, such as an oxygen-quenched fluorophore, embedded in an oxygen permeable substance, such as silicone rubber.

[0084] Sequential measurements of a single group of cells may be made at predetermined time intervals to analyze the effect of changes in the extracellular environment on their function, for example to examine the effect of exposure to a drug, chemical, or toxin. In this method, the volume of media surrounding the cells is first reduced, the constituents of the media are measured, and the volume is restored to its original value, as previously described. The environment surrounding the cells is then altered, such as by adding a chemical that activates a transmembrane receptor, changing the dissolved oxygen level, or adding a nutrient. One or more additional measurement cycles are then performed using the temporarily reduced volume method, to analyze the effect of the altered extracellular environment.

[0085] At any time during the sequence of measurements, the cell media may be replenished. In this way, sequential measurements can be made over a period of minutes, hours, or days. Any one of several different approaches may be followed to replenish the media. Media may be replenished by substantially removing part or all of the media within the full volume of the vessel using standard manual or automated pipetting instruments. Alternatively, media may be replenished only within the reduced volume of the vessel when a barrier is lowered into position. In the latter method, media may be replenished by fluidic extraction and delivery from a top side of the vessel through a portal in a plunger mechanism or through a portal built into any one of the sides or bottom of the vessel.

[0086] The introduction of an environment altering constituent such as a chemical, dissolved gas or nutrient may also be applied either to the full volume of the vessel as noted above or alternatively to only the reduced volume of the vessel. In the latter embodiment, the volume of media surrounding the cells is first reduced, the constituents of the media are measured, and the volume is restored to its original value, as previously described. The volume is then again reduced and the environment immediately surrounding the cells within only the reduced volume is then altered, by the addition of a constituent through a portal in the plunger or elsewhere in the vessel defining the reduced volume. One or more measurements are made in the presence of the constituent. After this measurement cycle, the media within the reduced volume may be exchanged one or more times to flush out the constituent before exposing the cells once again to the full original volume. This approach may provide a benefit of reducing the volume of compound required. It may also provide the possibility of studying isolated effects without contaminating the entire volume, thereby, in effect, simulating a flow system in wellplate format.

EXAMPLES

[0087] The following examples illustrate certain exemplary and preferred embodiments and applications of the instant invention, but are not intended to be illustrative of all embodiments and applications.

Example 1

Repetitive measurement of the basal respiration and acidification rates of C2C12 Myoblasts:

[0088] A prototype device was fabricated in order to evaluate various properties and potential applications of the invention.

[0089] The device included a cylindrical vessel, fabricated from polycarbonate material, and designed to receive a 12 mm diameter, polycarbonate membrane assembly (Corning Snapwell™ P/N 3802) with a pore size of approximately 3 μm. A cylindrical polycarbonate cover could be temporarily inserted into the vessel to form a smaller sample chamber, approximately 1.5 mm high, with a volume of about 160 microliter (μl). A series of bores around the perimeter of the vessel allowed the insertion of three 500 μm diameter optical fibers. The distal tip of each optical fiber was coated with a fluorescent sensing material to form a biosensor.

[0090] The three biosensors were designed to measure the partial pressures of O_2 and CO_2, and the pH of the media contained within the vessel. One fiber tip was coated with a matrix of Ruthenium dyes, encapsulated in oxygen permeable silicone rubber, to provide a readout of dissolved oxygen concentration. A second fiber tip was coated with a complex of Fluoroscene dye encapsulated in silicone rubber, to provide a readout of H^+ ion concentration (pH). A third biosensor was fabricated by using a CO_2 permeable membrane to create a small reservoir of $NaHCO_3$ surrounding a HydroxyPyrene Trisodium Salt (HPTS) pH sensitive dye. A change in CO_2 concentration in the cell media would then cause a change in pH of this encapsulated reagent resulting in a measurable change in the fluorescent properties of the pH sensitive dye, and this change was calibrated to provide quantitative CO_2 concentration data.

[0091] Light emitting diodes were used to illuminate the three optical sensors at various wavelengths as shown in Table 1, in terms of nanometers (nm). Also shown in Table 1 are the wavelengths used to sense the fluorescent emission of each sensor. In each case, both analyte sensitive ("sensor"), and analyte insensitive ("reference") fluorescent properties of the dyes were measured to minimize unwanted drift and interference. Dichroic splitters were used to couple individual fiber/dye assemblies to a pair of photodiodes/filter sets (O_2 sensor) or a pair of LED/filter sets (pH and CO_2).

TABLE 1

Analyte sensor excitation and emission wavelengths				
	Sensor Excitation	Reference Excitation	Sensor Emission	Reference Emission
Oxygen	488 nm	488 nm	610 nm	535 nm
PH	464 nm	435 nm	530 nm	530 nm
CO_2	460 nm	415 nm	530 nm	530 nm

[0092] Each sensor was calibrated once using multiple measurement points and a polynomial regression method to establish a nonlinear calibration curve.

[0093] Sensors were then recalibrated daily using a two-point calibration method. pH sensors were calibrated by sampling the optical response while submerged in a buffer solution with pH of 6.0 for 2 minutes, then in a solution with pH 8, each for two minutes. Oxygen and CO_2 sensors were calibrated using data points acquired while both sensors were submerged for two minutes in a saline bath purged with room air, followed by a bath purged with 10% CO_2 / 90% N_2.

[0094] During a typical assay, approximately $1.5x10^5$ cells were placed in the vessel along with 500 μl of liquid media, resulting in a cell density of $3x10^5$ cells/ml. To perform a measurement, the cylindrical cover was temporarily inserted into the vessel. The cover displaced liquid media, but not cells, to form a smaller sample chamber with a volume of 160 μl and a therefore a cell density of approximately $1x10^6$ cells/ml. This resulted in more than a 6X increase in the rate of change of analytes within the media in proximity to the biosensors.

[0095] In order to evaluate the ability of the prototype device to reproducibly measure extracellular analytes flux rates, $1.5x10^5$ undifferentiated C2C12 murine skeletal muscle cells (obtained from ATCC, Manassas, VA) were seeded on each of eight separate 12 mm diameter polycarbonate membranes which were then incubated at 37 °C for a period of 12 hours.

[0096] In a sequence of tests, wells were removed from the incubator, inspected visually, and placed into the measurement device. 160 μl of bicarbonate ($NaHCO_3$) -free DMEM Medium (obtained from Specialty Media, Phillipsburg, NJ) was then added, and the device was assembled to form an enclosed sample chamber. The concentration of each analyte (partial pressures of O_2 and CO_2, and pH as an indicator of proton concentration) was then measured every 8 seconds for a period of 20 minutes, and the average rate of change of each analyte was calculated over a four minute period from t=12 minutes to t=16 minutes.

[0097] To determine the extracellular flux rates of O_2 and CO_2, the rates of change of partial pressures were divided by volume of each analytes available in the media (moles) to result in a value expressed in nmol/minute. The rate of

acidification was expressed in mpH units/min (multiplied by 20 for scaling on the chart), but can easily be shown as protons per minute by calculating the number of available electrons in the media buffer within the known sample volume.

[0098] The mean and standard deviation of the dissolved oxygen and pH decay rates for the series of eight tests are shown in FIG. 6. As shown, these flux rates are highly reproducible in the prototype device.

Example 2

Measurement of basal respiration and acidification rates for various cell densities:

[0099] The experimental device described in Example 1 was used to investigate the relationship between cell number and oxygen and CO_2 flux rates. Varying numbers ($1.0x10^5$ - $4.0x10^5$) of C2C12 myoblasts were seeded on 12 mm diameter polycarbonate membranes (Corning Snapwell™) which were then incubated at 37 °C for a period of 12 hours.

[0100] Wells were then removed from the incubator, inspected visually, and placed into the measurement device. 150 µl of $NaHCO_3$-free DMEM Medium (obtained from Specialty Media, Phillipsburg, NJ) was then added, and the device was assembled to form an enclosed sample chamber. The concentration of each analyte was then measured every 5 seconds for a period of 20 minutes, and the average rate of change from t=10 minutes to t=20 minutes from start was computed. The resulting flux rates are shown in Table 2 and in graphical form in FIG. 7.

TABLE 2

Measuring Metabolic Analytes from Varying Titrations of C2C12 Myoblasts

Cell # (000)	O_2 Rate	CO_2 Rate	pH Rate	CO_2/O_2	O_2/pH
400	0.77	1.22	0.023	0.88	0.33
300	1.06	1.22	0.021	1.15	0.50
200	0.70	1.08	0.019	0.93	0.35
150	0.57	0.90	0.021	1.63	0.26
100	0.29	0.68	0.013	2.36	0.22

[0101] The data in Table 2 shows, as expected, that increasing cell density increases analyte flux rates in a near-linear fashion for most cell densities. Above a density of $3x10^5$ cells, oxygen flux did not increase as rapidly, presumably due to contact inhibition and crowding effects.

[0102] The device can therefore be used to evaluate the effect of high cell densities on metabolic rates.

Example 3

The Effect of 2,4 DNP on C2C12 Myoblasts:

[0103] The chemical compound 2,4 DNP can be used to uncouple mitochondrial respiration from ATP synthesis by disassociating the linkage between the respiratory chain and the phosphorylation system. In the presence of this compound, it is known that oxygen consumption will increase dramatically, while proton flux remains relatively constant.

[0104] In this experiment, C2C12 myoblasts were seeded on 12 mm diameter polycarbonate membranes and incubated for 12 hours. Wells were then removed from the incubator, inspected visually, and placed into the measurement device. 160 µl of $NaHCO_3$-free DMEM medium was then added, and the device was assembled to form an enclosed sample chamber.

[0105] The dissolved concentrations of O_2 and CO_2, and the pH in the media were then measured every 5 seconds for a period of 20 minutes in order to determine a control baseline for each analyte flux. Once the baseline was established, a sequence of experiments were performed where varying doses of 2,4 DNP (obtained from Sigma, St. Louis MO) were added to the cell media and a 20 minute measurement of analyte flux rates was performed. A control experiment was also performed using the highest dose of 2,4 DNP, but without cells. The data from the dose response is shown in Table 3 and FIG. **8**.

TABLE 3

Effect of 2,4DNP on C2C12 Myoblasts

2,4 DNP Dose (µM)	O_2 Rate (nM/min)	CO_2 Rate (nM/min)	pH Rate (pH/min)	CO_2/O_2	O_2/pH
0	0.38	2.22	0.024	5.83	0.16
10	1.26	2.78	0.028	2.18	0.46

(continued)

Effect of 2,4DNP on C2C12 Myoblasts

2,4 DNP Dose (μM)	O_2 Rate (nM/min)	CO_2 Rate (nM/min)	pH Rate (pH/min)	CO_2/O_2	O_2/pH
50	1.99	4.24	0.031	2.13	0.64
100	2.30	4.59	0.032	2.00	0.73
100 No Cells	-0.18	0.15	0.001	-0.84	-1.30

The data in Table 3 shows that as predicted, treatment with 2,4 DNP causes a dose-dependent increase in O_2 consumption rates while having little effect on extracellular acidification.

Example 4

The Effect of Rotenone *on C2C12 Myoblasts:*

[0106] Rotenone is known to inhibit cellular respiration by blocking NADH dehydrogenase in the respiratory chain. C2C12 Myoblasts were used to show this effect. $1.5x10^5$ C2C12 myoblasts were seeded on membranes, incubated and placed in the measurement system along with 150 μl $NaHCO_3$-free DMEM medium of as described in Example 3.

[0107] The dissolved concentrations of O_2 and CO_2, and the pH in the media were then measured every 5 seconds for a period of 20 minutes in order to determine a control baseline for each analyte flux. Once the baseline was established, a sequence of experiments were performed where varying doses of Rotenone (obtained from Sigma, St. Louis MO) were added to the cell media and a 20 minute measurement of analyte flux rates was performed. A control experiment was also performed using the highest dose of Rotenone, but without cells. The data from the dose response is shown in Table 4 and FIG. **9**.

TABLE 4

Effect of Rotenone on C2C12 Myoblasts

Rotenone Dose	O_2 Rate	CO_2 Rate	pH Rate	CO_2/O_2	O_2/pH
0	0.58	1.50	0.027	2.56	0.22
25	0.44	1.76	0.026	4.00	0.17
50	0.26	2.08	0.031	8.02	0.08
100	0.19	2.34	0.023	12.18	0.09
200	0.04	2.24	0.027	69.84	0.02
200-No Cells	-0.07	0.14	0.001	7.51	-0.51

The data in Table 4 demonstrates that, as expected, treatment with Rotenone causes a dose-dependent decrease in O_2 consumption rate in these cells.

Example 5

Measurement of respiration and acidification rate changes resulting from cell proliferation:

[0108] The experimental device described in Example 1 was used to investigate the relationship between cell proliferation and oxygen, CO_2 and proton flux rates. $5.0x10^4$ C2C12 myoblasts were seeded on 12 mm diameter polycarbonate membranes and then incubated at 37 °C. 12 hours after being seeded, cells were placed in DMEM serum-free media (Gibco, Carlsbad, CA) to inhibit proliferation. After 24 hours, half of the cells were switched to DMEM serum-containing media to stimulate proliferation, while the other half were maintained in serum-free media.

[0109] Wells were then removed from the incubator, inspected visually, and placed into the measurement device. 57 μl of $NaHCO_3$-free DMEM Medium (obtained from Specialty Media, Phillipsburg, NJ) was then added and the device was assembled to form an enclosed sample chamber. The concentration of each analytes was then measured every 8 seconds for a period of 20 minutes, and the average rate of decay from t=10 minutes to t=20 minutes from start was computed. The resulting flux rates are shown in Table 5 and in graphical form in FIG. **10**.

TABLE 5

Effect of cell proliferation on extracellular analyte fluxes		
	O2 Rate (nMoles/min)	PH Rate* 10 (PHU/min)
50 K Stimulated	0.311 +/- 0.091	0.188 +/- 0.020
50 K Starved	0.082 +/- 0.019	0.102 +/- 0.050

The data in Table 5 demonstrates that, as expected, cell proliferation results in an increase in oxygen consumption and the rate of extracellular acidification.

Example 6

Measurement of G-Protein Coupled Receptor Activation in CHO-M3 Cells:

[0110] Previous studies have shown that stimulation of transmembrane receptors often causes a rapid increase in extracellular acidification rate, resulting primarily from acute activation of ion exchange pumps. In this experiment, the prototype device was used to detect a change in extracellular acidification rate following treatment of cells with a receptor agonist.

[0111] Chinese hamster ovary (CHO) cells were transfected to over-express the muscarinic receptor subtype m3. The prototype device described in Example 1 was then used to monitor O_2 consumption, CO_2 production, and extracellular acidification rates, following treatment with the well-known, general acetylcholine receptor agonist, Carbachol.

[0112] Materials and Methods: Cell culture reagents were obtained from Gibco BRL (Grand Island, NY). Carbachol was purchased from Sigma Chemical Co. (St. Louis, MO). Bicarbonate-free DMEM medium was obtained from Specialty Media (Phillipsburg, NJ). Polycarbonate membrane snapwells (12 mm diameter, 3 μm pore size) were obtained from Corning (Corning, NY). CHO cells expressing m3-muscarinic receptors (CHO-M3 cells) were obtained from the American Type Tissue Culture (ATCC; Manassas, VA). Cells were cultured in Ham's F-12 medium supplemented with 10% fetal bovine serum (Hyclone), 1% GlutaMax and 0.1% Gentamicin and were maintained in a 5% CO_2 incubator. Cells were subcultured when they reached 80% confluency. CHO-M3 cells were seeded at a density of $2x10^5$ onto a snapwell 24 hours prior to use. Immediately prior to testing, cells on snapwells were switched to bicarbonate-free DMEM medium combined with 3.7 g/l NaCl to maintain osmolarity (medium pH 7.4 - 7.5).

[0113] Protocol Description: Probes were calibrated immediately prior to testing. The bottom of the test vessel was filled with bicarbonate-free medium. The snapwell was removed from a 5% CO2 incubator, and the regular growth medium (Ham's F-12) was replaced with bicarbonate-free DMEM medium. Thereafter, the snapwell was placed into the test vessel. Bicarbonate-free medium was pipetted onto the top of the snapwell, and the cover piece of the test vessel was placed gently on top of the snapwell and screwed into place, compressing the assembly. The probe software was started, and the pH, CO_2 and O_2 analytes were measured over the next 3.5 hours. Following the initial 1.5 hours of perfusion at a rate of 78 μl/min, a series of stop flow (10 minutes each) and medium re-perfusion (10 minutes each, 78 μl/min) cycles were started. During the last 2 minutes of medium re-perfusion cycle number 5, 100 μM carbachol was perfused across the snapwell. During re-perfusion number 6, bicarbonate-free DMEM medium was once again perfused across the snapwell. A rate of change for the analytes was calculated during each stop flow cycle.

Results:

[0114] CHO-M3 Baseline followed by 100 μM Carbachol Treatment

[0115] The first four series of perfusion / stop flow cycles were done to establish a noise band on the three analytes prior to carbachol treatment during perfusion number 5. Bicarbonate-free medium re-perfusion during per perfusion number 6 and the rates calculated during stop flow number 6 were to assess potential continuing post-carbachol treatment effects on analytes' rates. Resulting data are shown below in Table 6 and in FIG. 11.

Table 6

Effect of carbachol exposure on oxygen consumption, carbon dioxide evolution, and extracellular evolution					
Rate Summary	O_2 (nMol/min)	CO_2 (nMol/min)	pH (PHU/min)	O_2/CO_2 Ratio	O2/pH Ratio
Stop Flow 1	0.23	0.15	0.01	1.53	0.23
Stop Flow 2	0.24	0.12	0.01	1.93	0.25

(continued)

Effect of carbachol exposure on oxygen consumption, carbon dioxide evolution, and extracellular evolution					
Rate Summary	O_2 (nMol/min)	CO_2 (nMol/min)	pH (PHU/min)	O_2/CO_2 Ratio	O2/pH Ratio
Stop Flow 3	0.30	0.10	0.01	3.04	0.36
Stop Flow 4	0.27	0.11	0.01	2.45	0.38
Stop Flow 5	0.40	0.20	0.02	1.99	0.26
Stop Flow 6	0.33	0.10	0.01	3.34	0.29

Average baseline values compared to treatment values illustrate that a 2-minute exposure of CHO-M3 cells to carbachol resulted in a doubling in the pH rate (0.01 PHU/min vs 0.02 PHU/min), though O_2 and CO_2 rates also demonstrated increases above baseline levels (see below). Post-treatment rates generated during stop flow period number 6 essentially returned to pre-treatment values.

Example 7

Comparison of measured analyte fluxes with and without the temporary formation of a sample chamber:

[0116] The temporary formation of a sample chamber within a larger vessel, and containing an effectively high concentration of cells, is a characteristic for the instant invention.

[0117] In order to demonstrate this principle, a cylindrical vessel of approximately 12 mm in diameter and 10 mm in height was constructed from polycarbonate material. Fluorescent sensors capable of measuring partial pressures of O_2 and CO_2, and a sensor capable of measuring pH, were installed in the bottom of the vessel and calibrated as described previously. A cylindrical cover was also fabricated from polycarbonate material, with a diameter to accommodate insertion into the vessel, in order to provide a gas-impermeable cover and to reduce the enclosed volume of the vessel as required. A cylindrical spacer of 0.5 mm in height was also fabricated, with a diameter to accommodate insertion in the bottom of the vessel, thus providing a stop for the cover at a precise location.

[0118] Approximately 1 x 10^5 C2C12 myoblast cells was prepared as described previously. The cells were placed within the vessel along with approximately 1 ml of cell growth media. The partial pressure of oxygen within the cell media was measured continuously using a calibrated fluorescent probe. The rate of change of pO_2 was then calculated from the difference between the pO_2 values at 12 and 16 minutes from the start of the experiment.

[0119] A cylindrical cover was then placed on the surface of the cell media in order to inhibit the influx of oxygen from the ambient air. This also reduced the volume of media exposed to the cells to approximately 130 µl. Again, the pO_2 values at 12 and 16 minutes from the start of the experiment were measured and recorded.

[0120] The cylindrical cover was then lowered within the vessel (to rest on the spacer) so as to reduce the volume of media exposed to the cells to approximately 57 µl. Again, the pO_2 values at 12 and 16 minutes from the start of the experiment were measured and recorded.

[0121] The measured rates of change of pO_2 within the cell media for the three conditions are shown in tabular form in Table 7 and in graphical form in FIG. **12**.

Table 7

Oxygen depletion rates in a four minute interval, with and without the formation of an enclosed sample chamber (n=3)			
Volume	ΔpO2	s.d.	%CV
Small (57µL)	0.38	0.04	10%
Medium (130µL)	0.12	0.05	38%
Open	-0.04	0.10	n/a

[0122] This experiment demonstrates that the formation of an enclosed sample chamber, sealed from ambient air and containing a high density of cells, generates flux rates sufficient to provide a rapid measurement with a high signal-to-noise ratio.

17. The method of claim 1 wherein determining the constituent comprises sensing a concentration of the constituent.

18. The method of claim 1 wherein determining the constituent comprises sensing a first concentration of a first constituent, sensing a second concentration of a second constituent, and determining a relationship between the first concentration and the second concentration.

19. The method of claim 1 wherein determining the constituent comprises sensing a rate of change of concentration of the constituent.

20. The method of claim 1 wherein the sensor comprises a sensor (520) selected from the group consisting of a fluorescent sensor, a luminescent sensor, an ISFET sensor, a surface plasmon resonance sensor, a sensor based on an optical diffraction principle, a sensor based on a principle of Wood's anomaly, an acoustic sensor, and a microwave sensor.

21. The method of claim 1 wherein determining the constituent comprises determining a parameter selected from the group consisting of cell viability, cell number, cell growth rate, response to at least one of a drug, a toxin, and a chemical, detection of an entity, and internalization.

22. The method of claim 1, further comprising the step of perfusing additional media (130) through the vessel.

23. The method of claim 1, further comprising the step of replenishing the media (130) in the vessel.

24. The method of claim 1 wherein the barrier (140) is disposed in the vessel (100) without causing displacement of media (130) out of the vessel (100).

25. The method of claim 1 wherein the sensor (520) comprises a fluorophore.

26. The method of claim 1 comprising the additional step of altering an environment of at least a portion of the cells (120) prior to reducing the original volume of media.

27. The method of claim 26 wherein altering the environment comprises exposing at least a portion of the cells (120) to at least one of a drug, a chemical, and a toxin.

28. The method of claim 1, further comprising the step of altering an environment of at least a portion of the cells (120) after step b).

29. The method of claim 1 comprising the additional step of covering the vessel (100).

30. The method of claim 1 comprising the additional step of stirring at least a portion of the original volume of media (130) in the vessel (100).

31. The method of claim 1 comprising the additional step of sealing the vessel (100).

32. The method of claim 1 wherein the cells (120) comprise an animal cell, a human cell, or an immortal cell.

33. The method of claim 1 wherein said vessel (120) comprises a well (302) in a plate (300) including multiple wells (302) for holding media and cells (120).

34. Apparatus for determining a constituent extracted from or secreted by a sample comprising cells (120), the apparatus comprising:

a. a vessel (100) for holding a medium (130) and having a surface (132) to hold or contain the sample within the medium (130);
b. a moveable barrier (140) defining a sample chamber (150) within the vessel (100);
c. a sensor (520) for determining said constituent, said sensor (520) being disposed on the barrier (140) arranged to be in sensing contact with the medium (130) within said sample chamber (150) when the vessel (100) contains the medium (130), said apparatus being **characterized in that** it further comprises:
d. an actuator to position said barrier (140) within the vessel (100); and

e. a computer and software (350) for repeatedly moving the barrier (140) within the vessel to reduce an original volume of the medium (130) in contact with the sample, forming a reduced-volume measuring chamber in contact with the sample;

determining a constituent in the medium (130) in the reduced-volume measuring chamber with the sensor (520) and moving the barrier (140) to increase the reduced volume of medium about the cells (120) to substantially the original volume.

Patentansprüche

1. Verfahren zum Bestimmen einer Komponente, die durch eine Zellen umfassende Probe (120) aus einem Volumen von Medium (130) in einem Gefäß (100) extrahiert oder in dieses hinein sezerniert wird, wobei das Verfahren umfasst:

   a. Einbringen besagter Probe in eine Vorrichtung, umfassend ein Gefäß (100), welches ein ursprüngliches Volumen von Medium innerhalb des Gefäßes (100) enthält, wobei die Gefäß (100) eine Oberfläche (132) hat, um die Probe aufzunehmen oder zu enthalten, eine bewegliche Barriere (140), die das Volumen des Mediums (130), das mit der Probe in Kontakt ist, verändert und einen Sensor (520), der auf der beweglichen Barriere (140) in messendem Kontakt mit dem Medium (130) angeordnet ist,
   b. Bewegen der Barriere (140), um das Volumen des Mediums (130), das mit der Probe in Kontakt ist, zu reduzieren, wobei eine volumenreduzierte Messkammer in Kontakt mit der Probe gebildet wird,
   c. Bestimmen einer Komponente im Medium (130) in der volumenreduzierten Messkammer mit dem Sensor (520),
   d. Bewegen der Barriere (140), um das reduzierte Volumen von Medium um die Zellen (120) auf das im Wesentlichen ursprüngliche Volumen zu steigern, dadurch charakterisiert, dass mindestens ein zusätzlicher Messzyklus, der Schritt (b) und (c) umfasst, nach Schritt (d) durchgeführt wird.

2. Verfahren nach Anspruch 1, weiter nach Schritt (d) das Modifizieren des Mediums (130) vordem Durchführen des mindestens einen Messzyklus umfassend.

3. Verfahren nach Anspruch 2, wobei das Modifizieren des Mediums (130) das Einbringen einer das Milieu verändernden Komponente umfasst.

4. Verfahren nach Anspruch 1, weiter umfassend die Schritte des
   Bestimmens einer ersten Konzentration der Komponente und
   des Bestimmens einer zweiten Konzentration der Komponente zu einem vorbestimmten Zeitintervall ab dem Bestimmen der ersten Konzentration.

5. Verfahren nach Anspruch 4, weiter den Schritt des Berechnens einer Flussrate der Komponente basierend auf der ersten Konzentration und der zweiten Konzentration umfassend.

6. Verfahren nach Anspruch 1, wobei das reduzierte Volumen in einem Bereich von etwa 5-50 % des ursprünglichen Volumens liegt.

7. Verfahren nach Anspruch 6, wobei das reduzierte Volumen in einem Bereich von etwa 5-20 % des ursprünglichen Volumens liegt.

8. Verfahren nach Anspruch 1, wobei das reduzierte Volumen weniger als etwa 5 % des ursprünglichen Volumens beträgt.

9. Verfahren nach Anspruch 1, wobei die Zellen Bakterien, Pilze, Hefe, prokaryotischen Zellen, eukaryotische Zellen, tierische Zellen, menschliche Zellen oder unsterbliche Zellen sind.

10. Verfahren nach Anspruch 1, wobei mindestens ein Teil der Zellen (120) an besagte Oberfläche (132) des besagten Gefäßes (100) angeheftet ist.

11. Verfahren nach Anspruch 1, wobei mindestens ein Teil der Zellen (120) in den Medien (130) suspendiert ist.

12. Verfahren nach Anspruch 1, wobei mindestens ein Teil der Zellen (120) lebendes Gewebe umfasst.

13. Verfahren nach Anspruch 1, wobei die Komponente ein Material umfasst, das aus der Gruppe, die aus einem gelösten Gas, einem Ion, einem Protein, einem Substrat, einem Salz und einem Mineral besteht, ausgewählt ist.

14. Verfahren nach Anspruch 13, wobei das gelöste Gas aus der Gruppe, die aus O_2, CO_2 und NH_3 besteht, ausgewählt ist.

15. Verfahren nach Anspruch 1, wobei die Komponente ein Material umfasst, das von mindestens einem Teil der Zellen (120) aus den Medien (130) extrahiert wird.

16. Verfahren nach Anspruch 1, wobei die Komponente ein Material umfasst, das von mindestens einem Teil der Zellen (120) in die Medien (130) sezerniert wird.

17. Verfahren nach Anspruch 1, wobei das Bestimmen der Komponente das Erfassen einer Konzentration der Komponente umfasst.

18. Verfahren nach Anspruch 1, wobei das Bestimmen der Komponente das Erfassen einer ersten Konzentration einer ersten Komponente, das Erfassen einer zweiten Konzentration einer zweiten Komponente und das Ermitteln einer Beziehung zwischen der ersten Konzentration und der zweiten Konzentration umfasst.

19. Verfahren nach Anspruch 1, wobei das Bestimmen der Komponente das Erfassen einer Geschwindigkeit der Konzentrationsänderung der Komponente umfasst.

20. Verfahren nach Anspruch 1, wobei der Sensor einen Sensor (520) umfasst, der aus der Gruppe, die aus einem Fluoreszenzsensor, einem Lumineszenzsensor, einem ISFET-Sensor, einem Oberflächenplasmonresonanzsensor, einem auf einem optischen Beugungsprinzip basierenden Sensor, einem auf einem Prinzip von Woods Anomalie basierenden Sensor, einem akustischen Sensor und einem Mikrowellensensor besteht, ausgewählt ist.

21. Verfahren nach Anspruch 1, wobei das Bestimmen der Komponente das Bestimmen eines Parameters umfasst, der aus der Gruppe, die aus Zelllebensfähigkeit, Zellzahl, Zellwachstumsrate, Reaktion auf mindestens ein Arzneimittel, ein Toxin und eine Chemikalie, Detektion einer Entität und Internalisierung besteht, ausgewählt ist.

22. Verfahren nach Anspruch 1, weiter den Schritt des Perfundierens zusätzlicher Medien (130) durch das Gefäß umfassend.

23. Verfahren nach Anspruch 1, weiter den Schritt des Nachfüllens der Medien (130) im Gefäß umfassend.

24. Verfahren nach Anspruch 1, wobei die Barriere (140) in dem Gefäß (100) angeordnet ist, ohne eine Verdrängung von Medien (130) aus dem Gefäß (100) zu verursachen.

25. Verfahren nach Anspruch 1, wobei der Sensor (520) ein Fluorophor umfasst.

26. Verfahren nach Anspruch 1, den zusätzlichen Schritt des Änderns eines Milieus mindestens eines Teils der Zellen (120) vordem Reduzieren des ursprünglichen Volumens der Medien umfassend.

27. Verfahren nach Anspruch 26, wobei das Ändern des Milieus es umfasst, mindestens einen Teil der Zellen (120) mindestens einem von einem Arzneimittel, einer Chemikalie und einem Toxin auszusetzen.

28. Verfahren nach Anspruch 1, weiter den Schritt des Änderns eines Milieus mindestens eines Teils der Zellen (120) nach Schritt (b) umfassend.

29. Verfahren nach Anspruch 1, den zusätzlichen Schritt des Bedeckens des Gefäßes (100) umfassend.

30. Verfahren nach Anspruch 1, den zusätzlichen Schritt des Rührens mindestens eines Teils des ursprünglichen Volumens der Medien (130) in dem Gefäß (100) umfassend.

31. Verfahren nach Anspruch 1, den zusätzlichen Schritt des Verschließens des Gefäßes (100) umfassend.

32. Verfahren nach Anspruch 1, wobei die Zellen (120) eine tierische Zelle, eine menschliche Zelle oder eine unsterbliche Zelle umfassen.

33. Verfahren nach Anspruch 1, wobei besagtes Gefäß (120) eine Vertiefung (302) in einer Platte (300), die mehrere Vertiefungen (302) enthält, zum Aufnehmen von Medien und Zellen (120) umfasst.

34. Vorrichtung zum Bestimmen einer Komponente, die aus einer Zellen umfassenden Probe (120) extrahiert oder von dieser sezerniert wird, wobei die Vorrichtung umfasst:

a. ein Gefäß (100) zum Aufnehmen eines Mediums (130) und mit einer Oberfläche (132) zum Aufnehmen oder Enthalten der Probe innerhalb des Mediums (130),
b. eine bewegliche Barriere (140), die eine Probenkammer (150) innerhalb des Gefäßes (100) abgrenzt,
c. einen Sensor (520) zum Bestimmen der besagten Komponente, wobei besagter Sensor (520), der auf der Barriere (140) angeordnet ist, eingerichtet ist, um in messendem Kontakt mit dem Medium (130) innerhalb der besagten Probenkammer (150) zu sein, während das Gefäß (100) das Medium (130) enthält, wobei die besagte Vorrichtung dadurch charakterisiert ist, dass sie weiter umfasst:
d. einen Stellantrieb, um die besagte Barriere (140) innerhalb des Gefäßes (100) zu positionieren und
e. einen Computer und eine Software (350) zum wiederholten Bewegen der Barriere (140) innerhalb des Gefäßes, um ein ursprüngliches Volumen des Mediums (130) im Kontakt mit der Probe zu verringern, zum Bilden einer volumenreduzierten Messkammer in Kontakt mit der Probe,

zum Bestimmen einer Komponente im Medium (130) in der volumenreduzierten Messkammer mit dem Sensor (520) und zum Bewegen der Barriere (140), um das reduzierte Volumen von Medium um die Zellen (120) auf das im Wesentliche ursprüngliche Volumen zu steigern.

Revendications

1. Procédé de détermination d'un constituant extrait de ou sécrété dans un volume de milieu (130) dans un récipient (100) par un échantillon comprenant des cellules (120), le procédé comprenant :

a. le placement dudit échantillon dans un appareil comprenant un récipient (100) contenant un volume d'origine de milieu à l'intérieur du récipient (100), le récipient (100) ayant une surface (132) pour recevoir ou contenir l'échantillon ; une barrière mobile (140) qui change le volume du milieu (130) en contact avec l'échantillon ; et un capteur (520) disposé sur la barrière mobile (140) en contact de détection avec le milieu (130) ;
b. le déplacement de la barrière (140) pour réduire le volume du milieu (130) en contact avec l'échantillon, ce qui forme une chambre de mesure à volume réduit en contact avec l'échantillon ;
c. la détermination d'un constituant dans le milieu (130) dans la chambre de mesure à volume réduit avec le capteur (520) ;
d. le déplacement de la barrière (140) pour augmenter le volume réduit de milieu (130) autour des cellules (120) sensiblement jusqu'au volume d'origine ;

caractérisé en ce que
au moins un cycle de mesure additionnel comprenant l'étape (b) et (c) est effectué après l'étape (d).

2. Procédé selon la revendication 1, comprenant en outre, après l'étape (d), la modification du milieu (130) avant de réaliser l'au moins un cycle de mesure.

3. Procédé selon la revendication 2 dans lequel la modification du milieu (130) comprend l'introduction d'un constituant modifiant l'environnement.

4. Procédé selon la revendication 1, comprenant en outre les étapes consistant à : déterminer une première concentration du constituant ; et
déterminer une seconde concentration du constituant à un intervalle de temps déterminé par rapport à la détermination de la première concentration.

5. Procédé selon la revendication 4 comprenant en outre l'étape consistant à calculer un taux de flux du constituant sur la base de la première concentration et de la seconde concentration.

6. Procédé selon la revendication 1 dans lequel le volume réduit est dans une plage d'environ 5 à 50 % du volume d'origine.

7. Procédé selon la revendication 6 dans lequel le volume réduit est dans une plage d'environ 5 à 20 % du volume d'origine.

8. Procédé selon la revendication 1 dans lequel le volume réduit est inférieur à environ 5 % du volume d'origine.

9. Procédé selon la revendication 1 dans lequel les cellules (120) sont des bactéries, un champignon, de la levure, des cellules procaryotes, des cellules eucaryotes, des cellules animales, des cellules humaines, ou des cellules immortelles.

10. Procédé selon la revendication 1 dans lequel au moins une partie des cellules (120) sont fixées à ladite surface (132) dudit récipient (100).

11. Procédé selon la revendication 1 dans lequel au moins une partie des cellules (120) sont en suspension dans le milieu (130).

12. Procédé selon la revendication 1 dans lequel au moins une partie des cellules (120) comprennent du tissu vivant.

13. Procédé selon la revendication 1 dans lequel le constituant comprend un matériau choisi dans le groupe constitué d'un gaz dissous, d'un ion, d'une protéine, d'un substrat, d'un sel et d'un minéral.

14. Procédé selon la revendication 13 dans lequel le gaz dissous est choisi dans le groupe constitué d'O_2, de CO_2 et de NH_3.

15. Procédé selon la revendication 1 dans lequel le constituant comprend un matériau extrait du milieu (130) par au moins une partie des cellules (120).

16. Procédé selon la revendication 1 dans lequel le constituant comprend un matériau sécrété dans le milieu (130) par au moins une partie des cellules (120).

17. Procédé selon la revendication 1 dans lequel la détermination du constituant comprend la détection d'une concentration du constituant.

18. Procédé selon la revendication 1 dans lequel la détermination du constituant comprend la détection d'une première concentration d'un premier constituant, la détection d'une seconde concentration d'un second constituant et la détermination d'une relation entre la première concentration et la seconde concentration.

19. Procédé selon la revendication 1 dans lequel la détermination du constituant comprend la détection d'un taux de changement de concentration du constituant.

20. Procédé selon la revendication 1 dans lequel le capteur comprend un capteur (520) choisi dans le groupe constitué d'un capteur fluorescent, d'un capteur luminescent, d'un capteur ISFET, d'un capteur à résonance plasmonique de surface, d'un capteur basé sur un principe de diffraction optique, d'un capteur basé sur un principe d'anomalie de Wood, d'un capteur acoustique et d'un capteur à micro-ondes.

21. Procédé selon la revendication 1 dans lequel la détermination du constituant comprend la détermination d'un paramètre choisi dans le groupe constitué de la viabilité des cellules, du nombre de cellules, du taux de croissance des cellules, de la réponse à au moins l'un d'un médicament, d'une toxine et d'un produit chimique, la détection d'une entité et l'internalisation.

22. Procédé selon la revendication 1, comprenant en outre l'étape consistant à perfuser du milieu (130) additionnel à travers le récipient.

23. Procédé selon la revendication 1, comprenant en outre l'étape consistant à remettre à niveau le milieu (130) dans le récipient.

24. Procédé selon la revendication 1 dans lequel la barrière (140) est disposée dans le récipient (100) sans causer de déplacement de milieu (130) hors du récipient (100).

25. Procédé selon la revendication 1 dans lequel le capteur (520) comprend un fluorophore.

26. Procédé selon la revendication 1 comprenant l'étape additionnelle consistant à modifier un environnement d'au moins une partie des cellules (120) avant de réduire le volume d'origine du milieu.

27. Procédé selon la revendication 1 dans lequel la modification de l'environnement comprend l'exposition d'au moins une partie des cellules (120) à au moins l'un d'un médicament, d'un produit chimique et d'une toxine.

28. Procédé selon la revendication 1, comprenant en outre l'étape consistant à modifier un environnement d'au moins une partie des cellules (120) après l'étape b).

29. Procédé selon la revendication 1 comprenant l'étape additionnelle consistant à couvrir le récipient (100).

30. Procédé selon la revendication 1, comprenant l'étape additionnelle consistant à agiter au moins une partie du volume d'origine de milieu (130) dans le récipient (100).

31. Procédé selon la revendication 1 comprenant l'étape additionnelle de fermeture étanche du récipient (100).

32. Procédé selon la revendication 1 dans lequel les cellules (120) comprennent une cellule animale, une cellule humaine ou une cellule immortelle.

33. Procédé selon la revendication 1, dans lequel ledit récipient (120) comprend un puits (302) dans une plaque (300) incluant de multiples puits (302) destinés à contenir du milieu et des cellules (120).

34. Appareil de détermination d'un constituant extrait de ou sécrété dans un échantillon comprenant des cellules (120), l'appareil comprenant :

a. un récipient (100) destiné à recevoir un milieu (130) et ayant une surface (132) pour recevoir ou contenir l'échantillon à l'intérieur du milieu (130) ;
b. une barrière mobile (140) définissant une chambre d'échantillon (150) à l'intérieur du récipient (100) ;
c. un capteur (520) destiné à déterminer ledit constituant, ledit capteur (520) étant disposé sur la barrière (140) de façon à être en contact de détection avec le milieu (130) à l'intérieur de ladite chambre d'échantillon (150) quand le récipient (100) contient le milieu (130), ledit appareil étant **caractérisé en ce qu'**il comprend en outre :
d. un actionneur pour positionner ladite barrière (140) à l'intérieur du récipient (100) ; et
e. un ordinateur et un logiciel (350) destinés à déplacer de façon répéter la barrière (140) à l'intérieur du récipient afin de réduire un volume d'origine du milieu (130) en contact avec l'échantillon, ce qui forme une chambre de mesure à volume réduit en contact avec l'échantillon ; déterminer un constituant dans le milieu (130) dans la chambre de mesure à volume réduit avec le capteur (520) et déplacer la barrière (140) pour augmenter le volume réduit de milieu (130) autour des cellules (120) sensiblement jusqu'au volume d'origine.

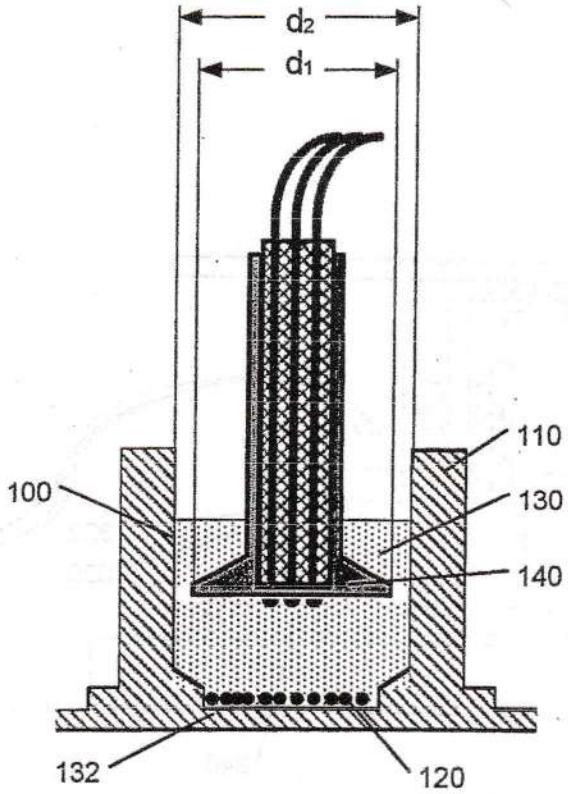

Fig. 1

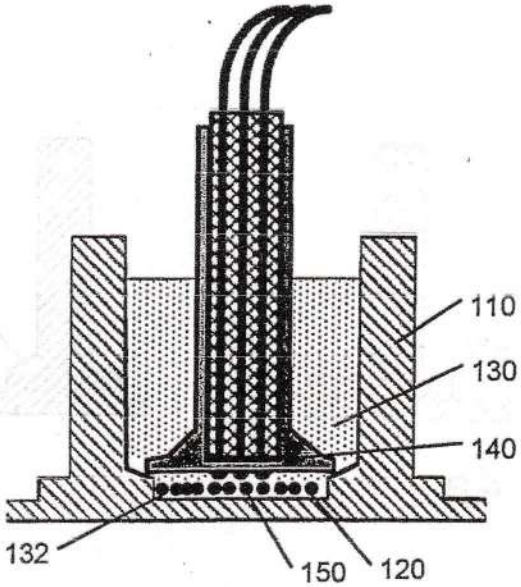

Fig. 2

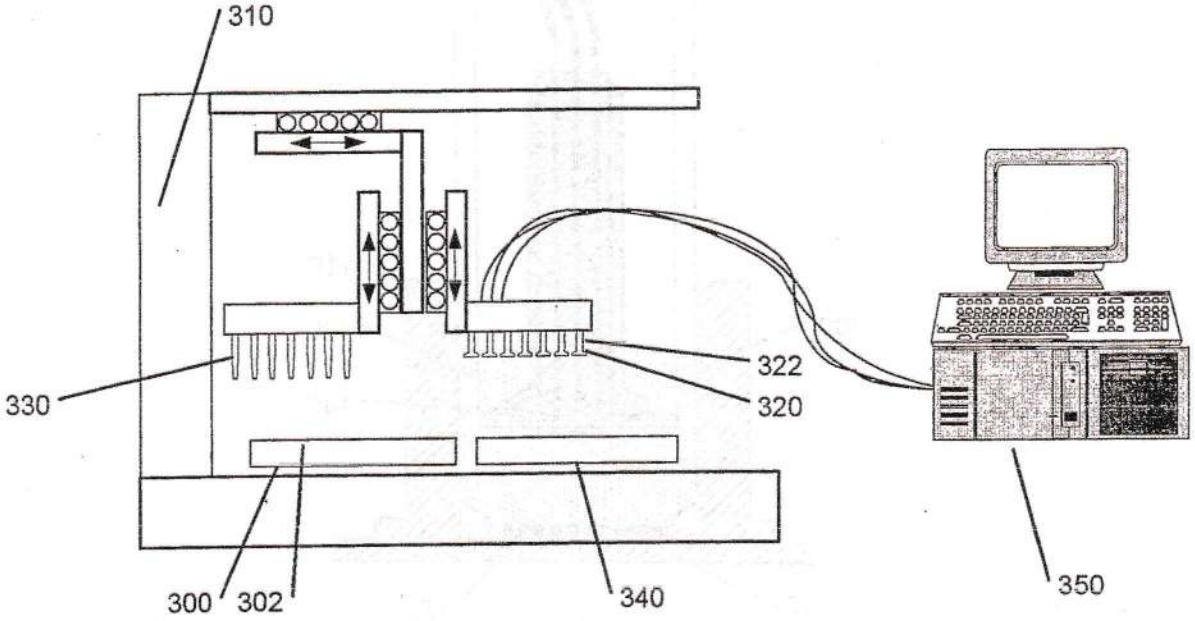

Fig. 3

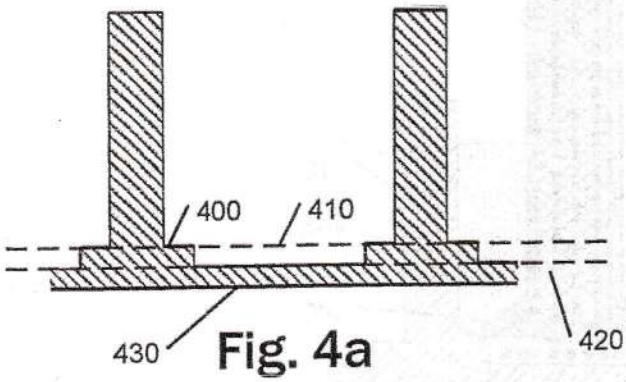

Fig. 4a

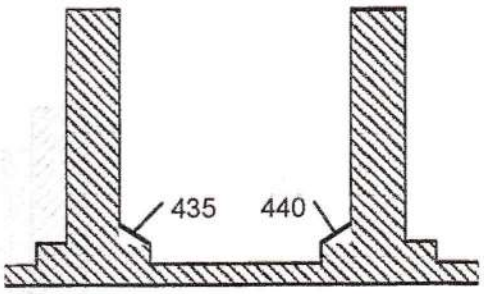

Fig. 4b

Fig. 5a

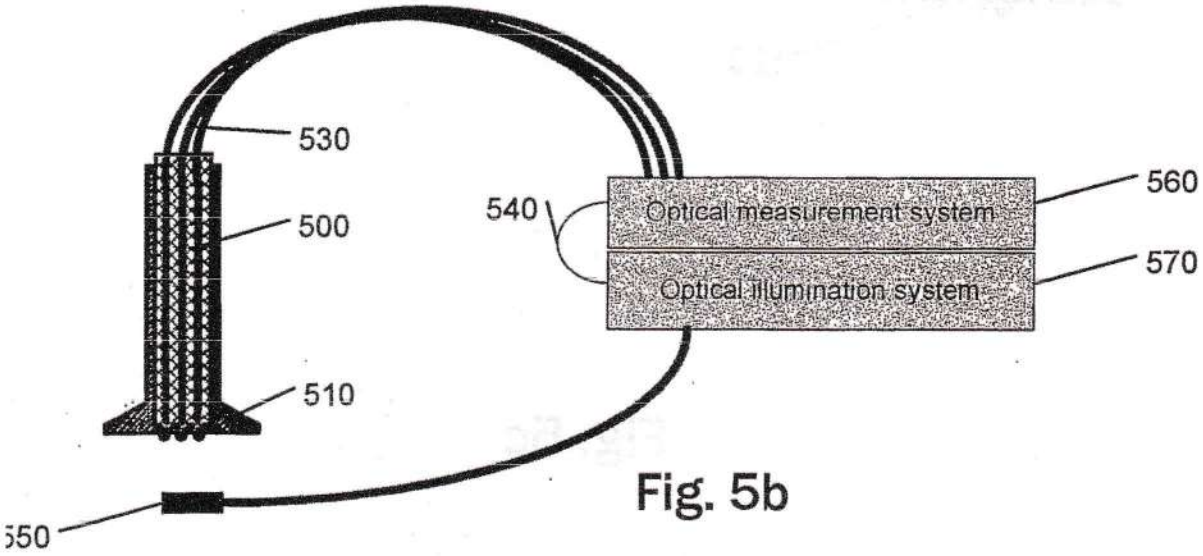

Fig. 5b

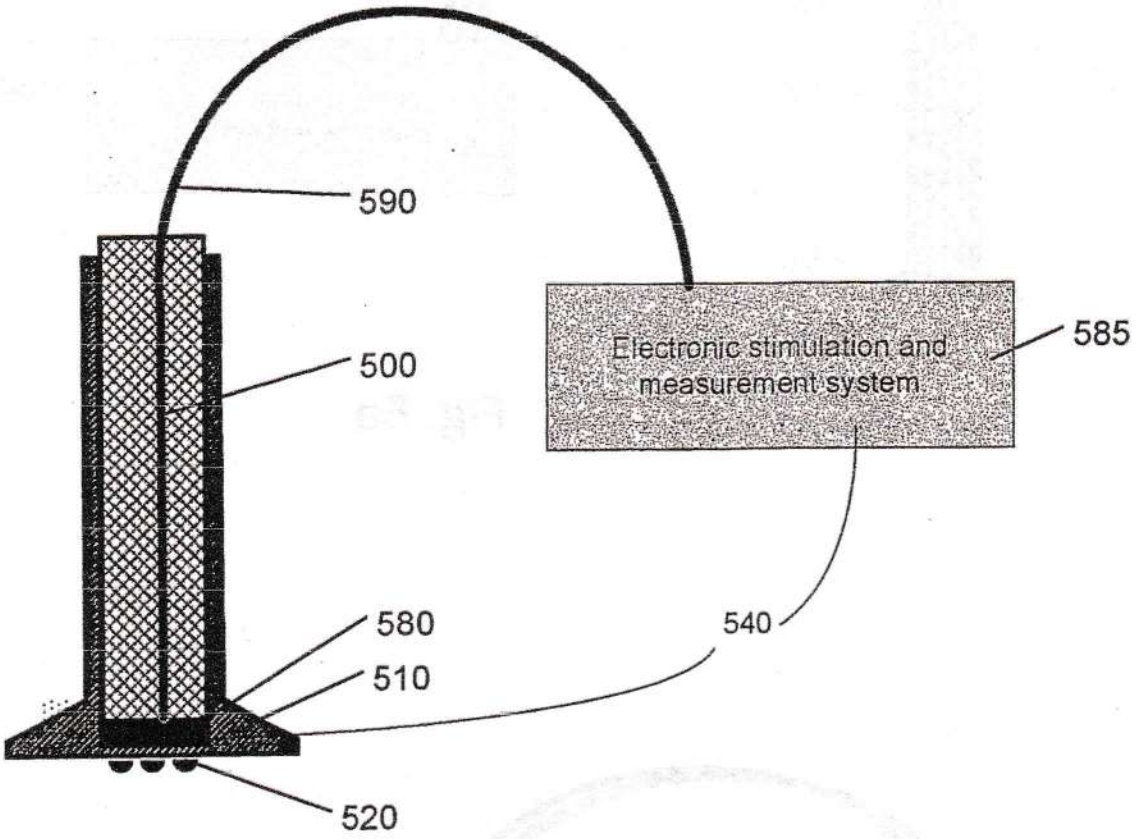

Fig. 5c

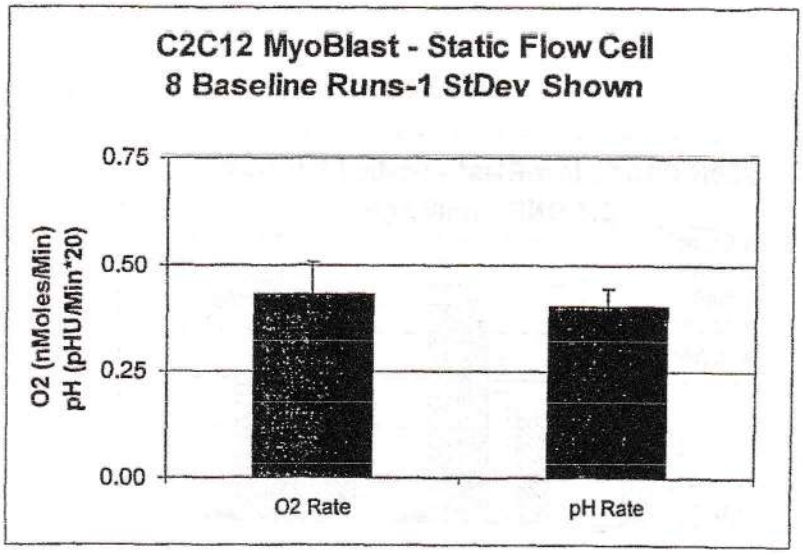

FIG 6

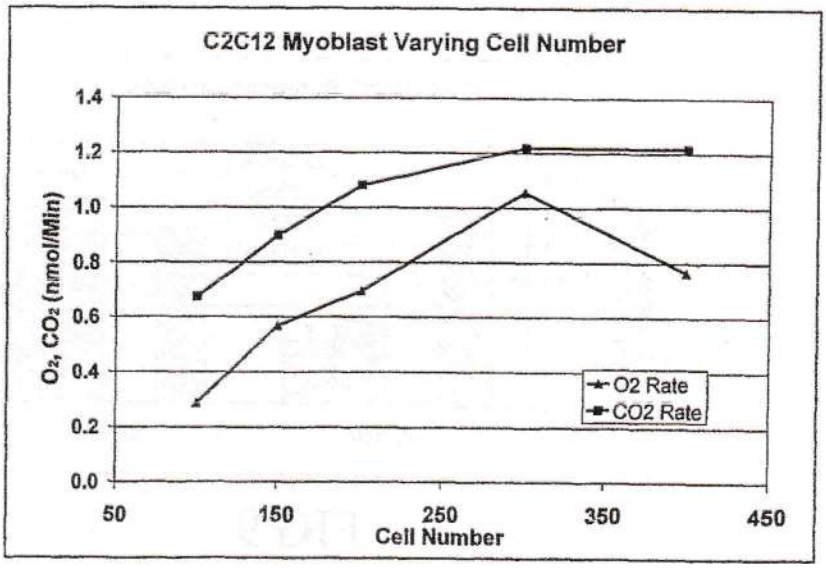

FIG 7

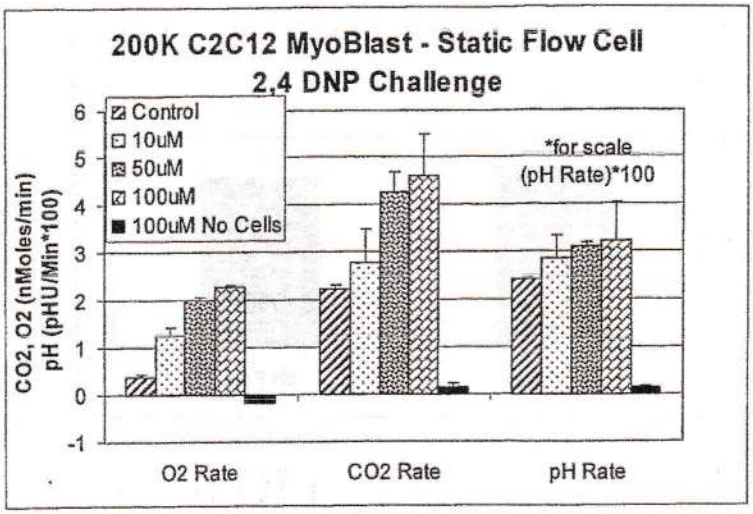

FIG 8

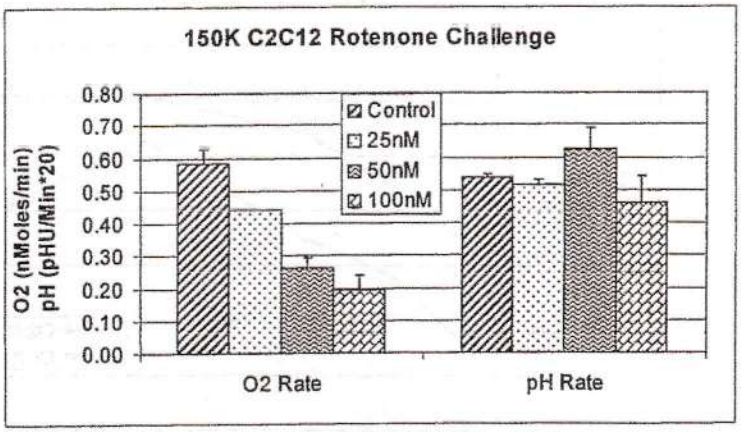

FIG 9

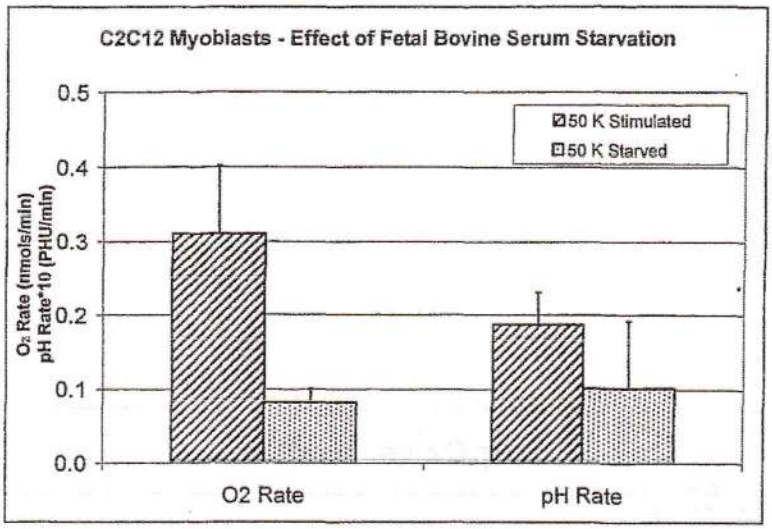

FIG 10

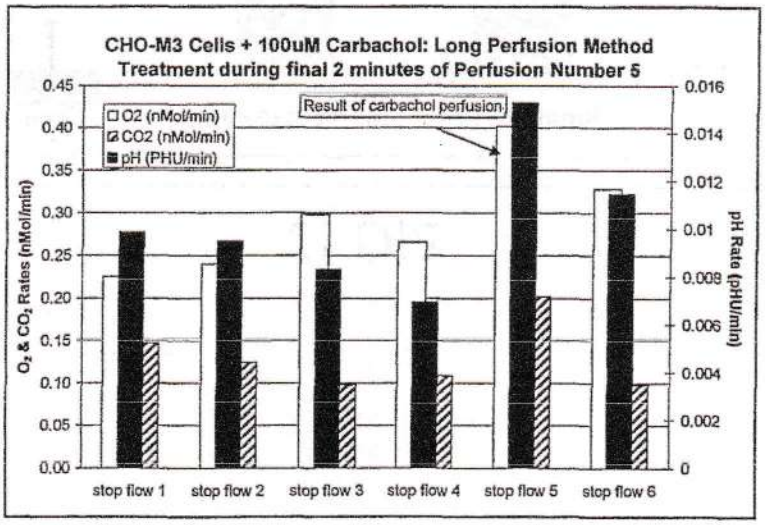

FIG 11

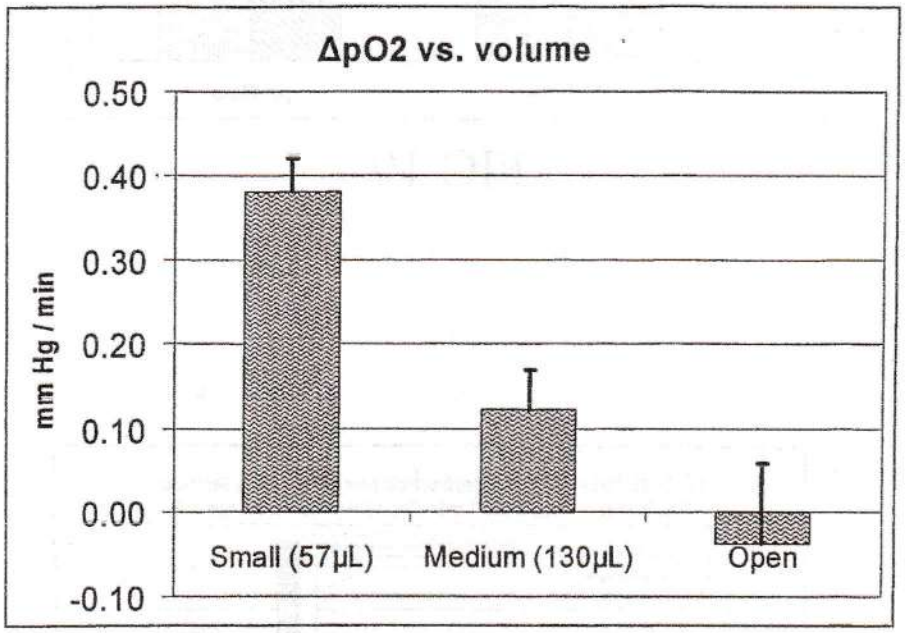

FIG 12

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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- WO 03059518 A **[0021]**
- WO 9815645 A **[0022]**

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Report ID: GEM/GARPTS/22072025/37FAXXYFCVVP
Report Name: Metabolic Analyzer
Generated By: Bhupendra Kumar Verma , Department of Health and Family Welfare , Ministry of Health and Family Welfare

Generated On: 22/07/2025
Valid till: 21/08/2025

GeM Availability Report and Past Transaction Summary

GeM Availability Report and past transaction summary report is generated based on the specifications searched by the Buyer. The specification may be modified appropriately for searching relevant categories on GeM. Buyer may navigate to GeM category page by clicking on the category link to view category specifications and products/services available in the category.

Order Count and Order Value displayed is on a cumulative basis since GeM inception.

1. Search String: Metabolic Analyzer

Search type: Product

1. There are categories available on GeM matching your requirements (as listed here). You can create a bid on GeM with a product closest matching your required specifications and add additional parameters in specifications through Corrigendum using RMS functionality.
2. If you feel that category TP needs updating you can submit category updating request also through RMS.
3. If you do not want to use any of the above option and want to proceed for procurement outside GeM, please suggest the specifications of the required product for creation of new category on GeM for future procurement.

Search Result: Category available/suggested on GeM but marked as "not matching requirements" by the buyer with undertaking as under:

It is certified that I have thoroughly checked all probable categories suggested by GeM and I am satisfied that the product required is not covered / does not fall in any of the suggested categories and can not be procured under any of these categories even after inclusion of List of Values(LOV) wherever possible in category specifications of suggested categories. It is also certified that the technical specification requirement are such that these can not be covered even by adding specification parameters using ATC in any of the GeM suggested categories. This is a one-time requirement hence new category creation is not proposed / or requirement is recurring but request for new category creation will be submitted separately post generation of GeMARPTS.

Category Name	Catalog Count	Order Count			Order Value (in Lakhs)		
		Direct Purchase	Reverse Auction	Bid	Direct Purchase	Reverse Auction	Bid
logic analyzer	15	38	2	5	39	45	58
Water Quality Meters / Analyzers	1,821	3,615	15	256	2,687	46	802
Signal Analyzer	65	113	4	10	220	186	247
Biogas analyzer	10	2	1	5	4	2	27
Visual Field Analyzer (V2)	5	0	0	0	0	0	0
ESR Analyzer (V2)	64	43	3	9	214	43	94
Fully Automatic Biochemistry Analyzer (V2)	904	298	37	164	1,776	388	2,431

Category Name	Catalog Count	Order Count			Order Value (in Lakhs)		
		Direct Purchase	Reverse Auction	Bid	Direct Purchase	Reverse Auction	Bid
HBA1C/Hemoglobinopathy Analyzer	18	4	0	17	37	0	705
Spectrum Analyzers and Vector Network Analyzers	1,227	103	109	240	417	6,045	8,053
Automatic Fat Analyzer	55	73	1	11	249	2	120

Z.21011/1/2023-Admin-I
Government of India
Ministry of Health and Family Welfare
Directorate General of Health Services
[Administration-I Section]

Nirman Bhawan, New Delhi.
Dated the 02nd June, 2025

To

The Director,
(Kind Attn: Prof. M Srinivas)
AIIMS, Ansari Nagar, New Delhi-110029
Email- director@aiims.gov.in

Subject: Nomination for Deputing DGHS Nominee for Finalization of Technical Specifications for Procurement of Equipment under ICMR Project (F. No. EMDR/IG/13/2024-01-00842, Project code:1-1789) at AIIMS-regarding

Sir/Ma'am,

I am directed to refer to your letter dated 27.05.2025 enclosed therein Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), New Delhi letter dated 19.05.2025 (copy enclosed) on the subject cited above and to say that *Dr. Amita Bali, CMO (SAG)-DDG(P) Dte. GHS, New Delhi* is nominated for the above said purpose.

This issues with the approval of DGHS.

Yours faithfully,

02/06/2025

(Arindam Banerjee)
Deputy Director
Tel. No. (011)-23063539

Copy to,

1. PPS to DGHS
2. Dr. Amita Bali, CMO (SAG)-DDG(P) Dte.GHS (HQ) along with a copy of communication referred above.

अखिल भारतीय आयुर्विज्ञान संस्थान
अन्सारी नगर, नई दिल्ली-११००२६ (भारत)
ALL INDIA INSTITUTE OF MEDICAL SCIENCES
ANSARI NAGAR, NEW DELHI - 110029 (INDIA)

Ph. 011-26594805/4800, Email: director@aiims.gov.in
दिनांक/Dated :.....27.05.2025.

स्वास्थ्य सेवा महानिदेशक का कार्यालय/O/o DGHS
एफ.डी.एस.सं./FTS NO. 42642
दिनांक/Dated 30.5.25

To

The Director General of Health Services,
Government of India,
Ministry of Health & Family Welfare,
Nirman Bhawan,
Room No. 446, A-Wing,
New Delhi-110001.
Email- dghs@nic.in

Subject: Forwarding of Request for Deputing DGHS Nominee for Finalization of Technical Specifications for Procurement of Equipment under ICMR Project (F. No. EMDR/IG/13/2024-01-00842, Project code: I-1789) at AIIMS.

Respected Madam,

Please find enclosed a request from the Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), New Delhi, regarding the nomination of a representative from the Directorate General of Health Services (DGHS) to participate in the Technical Specification and Evaluation Committee (TSEC) for the finalization of specifications and procurement of equipment (Metabolic Analyzer and Single Cell Capture System) under the ICMR-funded project titled "Investigating the Diagnostic Potential of Gut-Associated Metabolites in Post-Menopausal Osteoporotic Women and Exploring Their Therapeutic Potential in Pre-Clinical Model of Osteoporosis: An Osteoimmunological Approach" (Project Code: I-1789).

The request is forwarded for your kind consideration and necessary action.

With regards,

Yours Sincerely

27/5/2025

[Prof. M. Srinivas]
Director

Dr Nomination of DGHS
DR Amita Bali SSO(P)
DY. Div. (CA).
30/05

L 1440 TLL
19.05.2025

Department of Biotechnology

All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi-110029

To

Dated: 19-05-2025

The
Director
All India Institute of Medical Sciences
Ansari Nagar, New Delhi-29

27 MAY 2025
RECEIVED

Subject: Request for deputing **DGHS nominee** for the TSEC committee for ICMR funded project (F. No. EMDR/IG/13/2024-01-00842, Project code: I-1789)

Respected **Sir**,

We are implementing a ICMR project (I-1789), entitled "Investigating the Diagnostic Potential of Gut-Associated Metabolites in Post-Menopausal Osteoporotic Women and Exploring Their Therapeutic Potential in Pre-Clinical Model of Osteoporosis: An Osteoimmunological Approach". In this project we have budget of the following two equipment's:
1. Metabolic Analyzer
2. Single Cell capture system
The estimated cost of the both equipment's is more than 1 crore each (F. No. EMDR/IG/13/2024-01-00842; Project code: I-1789). Therefore, we need a committee comprising two external experts (related discipline) and DGHS nominee.

You are requested to approve the committee including **external experts** (consents attached) for the technical specifications and evaluation committee (TSEC) of above-mentioned purchase.

Below is the suggested name for the committee

1. **Dr. Anushree Gupta**, Additional Professor & **Head**, Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi-110029.
2. **Dr. Rupesh K. Srivastava**, Additional Professor, Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi-110029. **(End User Faculty member & PI of the project).**
3. **Dr. Sumit Rathore**, Associate Professor and **Store In-charge**, Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi-110029.
4. **Dr. Angel Ranjan,** Additional Professor, Hospital Administration, AIIMS, Ansari Nagar, New Delhi-110029. **(MS nominee)**
5. **Dr. Kalpana Luthra**, **Professor & Head**, Department of Biochemistry, All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi-110029. **(Head of Department of the related discipline)**
6. **Dr. Jaswinder Singh Maras**, Associate Professor, Department of Molecular and Cellular Medicine, Institute of Liver and Biliary Science, New Delhi **(External expert).**
7. **Dr. Shilpi Minocha**, Assistant Professor, Kusuma School of Biological Sciences, INDIAN INSTITUTE OF TECHNOLOGY DELHI, Hauz Khas, New Delhi- 110016, INDIA **(External expert).**
8. Ministry of Family and Health Welfare, Nirman Bhawan, New Delhi. **(DGHS nominee)**

Thanking you

Best regards

Dr. रूपेश कुमार श्रीवास्तव
Dr. RUPESH KUMAR SRIVASTAVA
अपर आचार्य/Additional Professor
जैव प्रौद्योगिकी विभाग/Deptt. of Biotechnology
भारतीय आयुर्विज्ञान संस्थान, नई दिल्ली-29
All India Institute of Medical Sciences, New Delhi-29

(Dr. Rupesh Kr. Srivastava)
Additional Professor,
Translational Immunology, Osteoimmunology & Immunoporosis Lab (TIOIL)
An ICMR-Collaborating Centre of Excellence in Bone Health
Department of Biotechnology,
All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi-110029.
Ph: 09179567399

DEPARTMENT OF BIOTECHNOLOGY
ALL INDIA INSTITUTE OF MEDICAL SCIENCES
Ansari Nagar, New Delhi – 110029

Dated: 09/07/2025

A Technical Specification Committee Meeting has been fixed on **22/07/2025 at 10.30 AM** in the Seminar Room, Second Floor, Department of Biotechnology, AIIMS, New Delhi -29.

S. No.	Name of the Items	Approx. Cost
1.	Metabolic Analyzer	< 1Cr
2.	Single Cell Capture System	< 1Cr

The following members of the **Departmental Store Technical Specification Committee** are requested to attend the above said meeting.

1.	**Dr. Rupesh Kumar Srivastava,** Additional Professor, Department of Biotechnology	Chairman
2.	**Dr. Anushree Gupta**, Additional Professor & Head, Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi-110029.	Head, Department of Biotechnology (Member)
3.	**Dr. Angel Ranjan**, Additional Professor, Hospital Administration, AIIMS, Ansari Nagar, New Delhi-110029	M.S Nominee (Member)
4.	**Dr. Kalpana Luthra**, Professor & Head, Department of Biochemistry, All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi-110029.	Head of Department of the related discipline (Member)
5.	**Dr. Sumit Rathore**, Associate Professor, Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi-110029.	Member (store In-charge)
6.	**Dr. Jaswinder Singh Maras**, Associate Professor, Department of Molecular and Cellular Medicine, Institute of Liver and Biliary Science, New Delhi	External Expert
7.	**Dr. Shilpi Minocha**, Assistant Professor, Kusuma School of Biological Sciences, INDIAN INSTITUTE OF TECHNOLOGY DELHI, Hauz Khas, New Delhi- 110016, INDIA	External Expert
8.	**Dr. Amita Bali**, CMO, (SAG)-DDG (P), Dte.	DGHS Nominee

I nominate Dr. Bhupendra to attend the meeting.
Anushree
14/07/25

डॉ. रूपेश कुमार श्रीवास्तव/Dr. RUPESH KUMAR SRIVASTA
अपर आचार्य/Additional Professor
जैव प्रौद्योगिकी विभाग/Dept. of Biotchnolog
अ. भा. आ. सं., नई दिल्ली/A.I.I.M.S., New Delhi-1100

Chairman
(Technical Specification Committee)
Department of Biotechnology
AIIMS, New Delhi – 110029

Distribution as above:

डॉ. अनुश्री गुप्ता/Dr. Anushree Gupta
अपर आचार्य एवं अध्यक्ष/Additional Professor & Head
जैव प्रौद्योगिकी विभाग
Department of Biotechnology
अखिल भारतीय आयुर्विज्ञान संस्थान, नई दिल्ली-29
All India Institute of Medical Sciences, New Delhi-29

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V. RAMALINGASWAMI BHAWAN, ANSARI NAGAR, POST BOX 4911, NEW DELHI - 110 029

F.No. EMDR/IG/13/2024-01-00842

Date 01.01.2025

The Director,
All India Medical Science,
New Delhi-110029.

Subject: - "Investigating the Diagnostic Potential of Gut-Associated Metabolites in Post-Menopausal Osteoporotic Women and Exploring Their Therapeutic Potential in Pre-Clinical Model of Osteoporosis: An Osteoimmunological Approach"

Sir/Madam,

The Director General of the ICMR sanctions the above mentioned research scheme initially for the period of one year initially from subject to extension up to the total duration specified in para 4 below:-

1. The Director General of the ICMR also sanctions the budget allotment of **Rs.5,47,23,724/-** as detailed in the attached statement for the period from **2024-25.** The grant-in-aid will be given subject to the following conditions.
2. The payment of the grant will be made in lump-sum to the Head of the Institute. The first installment of the grant will be paid generally as soon as report regarding appointment of the staff is received by the Council. The Staff appointed on the project should be paid as indicated in the budget statement.
3. The staff on the project will be recruited as per the rules and procedure of the host institute and second part of the undertaking be obtained from the employees of the project. The staff grant will not be released unless the required undertaking [part-II] from Head of the Institute is received in this office.
4. The demand for payment of the subsequent installment of the grant should be placed with the Council in the prescribed proforma. The approved duration of the scheme is Three Year. The annual extension will be given after review of the work done on the scheme during the previous year.
5. Five copies of the annual progress report should be submitted to the ICMR every year after completion of ten months of the project giving complete actual details of the research work done. Failure to submit the report in time may lead to termination of project.
6. Subject to the condition that the grant will be utilize after following the provisions laid down in the GFRs-2017 & TA Rules Please keep the fund in the separate saving Bank Account opened for ICMR funded Research projects so that interest earned thereon is credited into the accounts

The receipt of this letter may please be acknowledged.

Yours faithfully,

01/01/25

Jaibir Singh
Sr. Administrative Officer

Copy together with a copy of the budget statement forwarded to information

1. Dr. Rupesh Srivastava, Associate Professor, All India Institute of Medical Sciences, New Delhi-110029.
2. Accounts. V. for information.
3. Copy together with the budget forwarded to Budget Section [Finance Section] for compilation of the ICMR Budget
4. IRIS Cell No. **EMDR/IG/13/2024-01-00842**

डॉ. रुपेश कुमार श्रीवास्तव/Dr. RUPESH KUMAR SRIVASTAVA
अपर आचार्य/Additional Professor
जैव प्रौद्योगिकी विभाग/Dept. of Biotchnology
अ. भा. आ. सं., नई दिल्ली/A.I.I.M.S., New Delhi-110029

BUDGET STATEMENT

(2024-25)

Subject: Research project entitled. "Intermediate Grants Adhoc" Payment of 1st Year for the research project entitled "Investigating the Diagnostic Potential of Gut-Associated Metabolites in Post-Menopausal Osteoporotic Women and Exploring Their Therapeutic Potential in Pre-Clinical Model of Osteoporosis: An Osteoimmunological Approach" under Dr. Rupesh Srivastava, Associate Professor, All India Institute of Medical Sciences, New Delhi-110029

S. No	Item	1st Year
1.	**Staff** Project Research Scientist- I (Non-Medical)@ 56,000/- pm + 30% HRA @/-=16,800/-=72,800/- Salary increased for 3rd year 5%	8,73,600
2.	Project Research Scientist - II **(Non Medical)@67000/-** pm + 30% HRA @/-=20,100/-=87,100/- Salary increased for 3rd year 5%	10,45,200 3,12,000
3.	Project Technical Support - II **@20,000/-** pm + 30% HRA @/-=6,000/-=26,000/- Salary increased for 3rd year 5%	
2.	Recurring (Contingencies)	2,00,000
3.	Consumables	1,40,00,000
4.	Non- recurring (Equipment)	3,75,00,000
5.	Travel	3,00,000
6.	Overhead charges 3%	4,92,924
	Total	**5,47,23,724**

Total budget allotment of **Rs.5,47,23,724/-(Rupees Five Crores Forty Seven Lakhs Twenty Three Thousand Seven Hundred Twenty Four Only)**

F. No. EMDR/IG/13/01-2024-00842

8/1/25

(Jaibir Singh)
Sr. Administrative Officer
For Director General

डॉ. रूपेश कुमार श्रीवास्तवा/Dr. RUPESH KUMAR SRIVASTAVA
अपर आचार्य/Additional Professor
जैव प्रौद्योगिकी विभाग/Dept. of Biotchnology
अ.भा.आ.सं.; नई दिल्ली/A.I.I.M.S., New Delhi-110029

All-India Institute of Medical Sciences
Ansari Nagar, New Delhi-29
(RESEARCH SECTION)

Ref. No. 10/Prop/BioT/R.K.S/25-26/RS **Dated: -_____**

Subject: **Procurement of Metabolic Analyzer.**

****

The request has been received **Dr. Rupesh Kumar Srivastava, Additional Prof. Dept. of Biotechnology, AIIMS** to Purchase the subject item from **M/s Medispec Inida Ltd., Mfg:- M/s. Agilent Technologies Singapore (International) Pte. Ltd.)** on proprietary basis. The proposals submitted by **M/s Medispec Inida Ltd., Mfg:- M/s. Agilent Technologies Singapore (International) Pte. Ltd.)** and Performa Invoice and Departmental PAC certifications are attached.

The above documents are being uploaded for open information to submit objections, nature of the equipment/item within issue of 15 days **10/Prop/BioT/R.K.S/25-26/RS.** The comments should be received by office of Stores Officer (RS), Research Section at AIIMS on or before _____ **upto12:00 p.m.,** failing which it will be presumed that any other vendor is having no comment to offer and case will be decided on merits.

STORES OFFICER (RS)

Encl: Related documents enclosed.

1. **PAC Certificate enclosed.**
2. **Performa Invoice.**

2106395/2025/Research Section

E-office → 3314615

All-India Institute of Medical Sciences
Ansari Nagar, New Delhi-29
(RESEARCH SECTION)

Ref. No. 10/Prop/BioT/R.K.S/25-26/RS **Dated: -** 08-08-2025

Subject: Procurement of Metabolic Analyzer.

****

The request has been received **Dr. Rupesh Kumar Srivastava, Additional Prof. Dept. of Biotechnology, AIIMS** to Purchase the subject item from **M/s Medispec Inida Ltd., Mfg:- M/s. Agilent Technologies Singapore (International) Pte. Ltd.)** on proprietary basis. The proposals submitted by **M/s Medispec Inida Ltd., Mfg:- M/s. Agilent Technologies Singapore (International) Pte. Ltd.)** and Performa Invoice and Departmental PAC certifications are attached.

The above documents are being uploaded for open information to submit objections, nature of the equipment/item within issue of 15 days **10/Prop/BioT/R.K.S/25-26/RS.** The comments should be received by office of Stores Officer (RS), Research Section at AIIMS on or before 23-08-2025 **upto12:00 p.m.,** failing which it will be presumed that any other vendor is having no comment to offer and case will be decided on merits.

08/08/2025

STORES OFFICER (RS)

Encl: Related documents enclosed.

1. **PAC Certificate enclosed.**
2. **Performa Invoice.**

Procurement of Whole Metabolic Analyzer

Last Updated On : 08 Aug 2025

VIEW TENDER DETAILS

Tender No	Procurement of Whole Metabolic Analyzer.",
(10/Prop/BioT/R.K.S/25-26/RS)	
Title	**Date of Uploading**
08/08/2025	
Date of Closing	
23-08-2025	
Document	**Date of Opening**
23-08-2025	

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All India Institute of Medical Sciences

Ansari Nagar, New Delhi - 110029

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