



*Cellomatoics*  
*BioSciences*

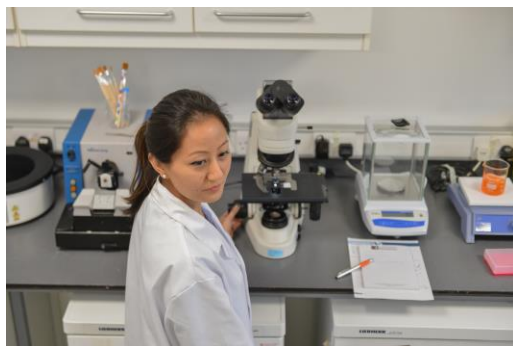
Revolutionising Laboratory Services



Respiratory Capabilities

# Who are we?

- Cellomatics Biosciences Ltd is a Specialised laboratory-based Contract Research Organisation (CRO)
- We support our customers in every aspect of their Preclinical in vitro R&D studies
- With an experienced team of scientists and clinical professionals, we understand your requirements and can deliver innovative and practical solutions

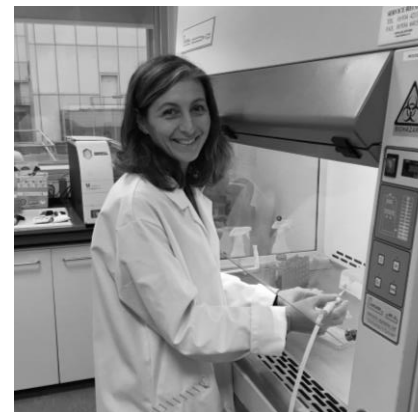


# Our team



**Dr. Shailendra Singh**  
**Founder and CEO**

Shailendra's scientific background lies in target identification and validation for the management of respiratory diseases. Overall, he has over 14 years of experience in research and development within the academia and industry together with clinical diagnostics.



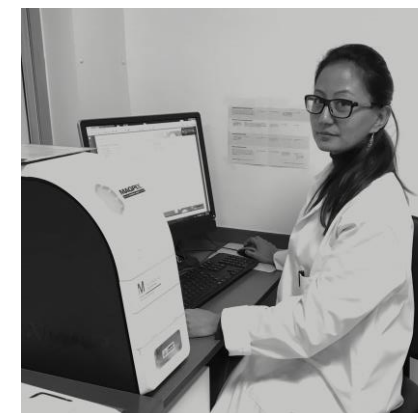
**Dr. Federica Riu**  
**Bioassay Study Manager**

Federica holds a MSc in Cellular Biology and a PhD in Clinical and Experimental Microbiology. She has over 10 years of experience in Molecular, Cellular and Vascular Biology, Microbiology and Stem Cells.



**Camille Hetez, MSc**  
**Head business development**

Camille holds a MSc in Biotechnology Engineering from Paris and an MBA Entrepreneurship. She has experience in various Business Development positions and also 3 years experience as a Co-founder and CEO in a innovative biotechnology company.



**Dr. Anushuya Tamang**  
**Bioassay Study Scientist**

Anushuya holds a MSc in Biotechnology , MRes in Cancer Biology and PhD in Developmental Biology. She has more than 6 years experience in Molecular and Cellular biology as well as Histology.

# The company

- Operating out of Biocity, Nottingham
- Fully serviced laboratory
- Specialised personnel
- National and International partners

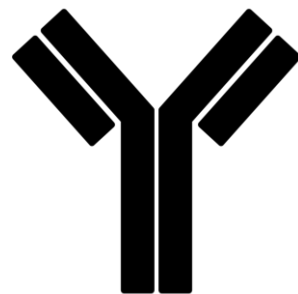


***“We provide a fully integrated service from study design, logistics support, performing experiments, and data package delivery”***

# Area of expertise



Oncology



Immunology



Cardiovascular



Respiratory

# Overview of our services



**Target validation & qualification**



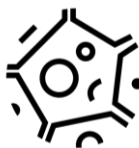
**Multiplex nucleic acid & proteins testing**



**Toxicity assays**

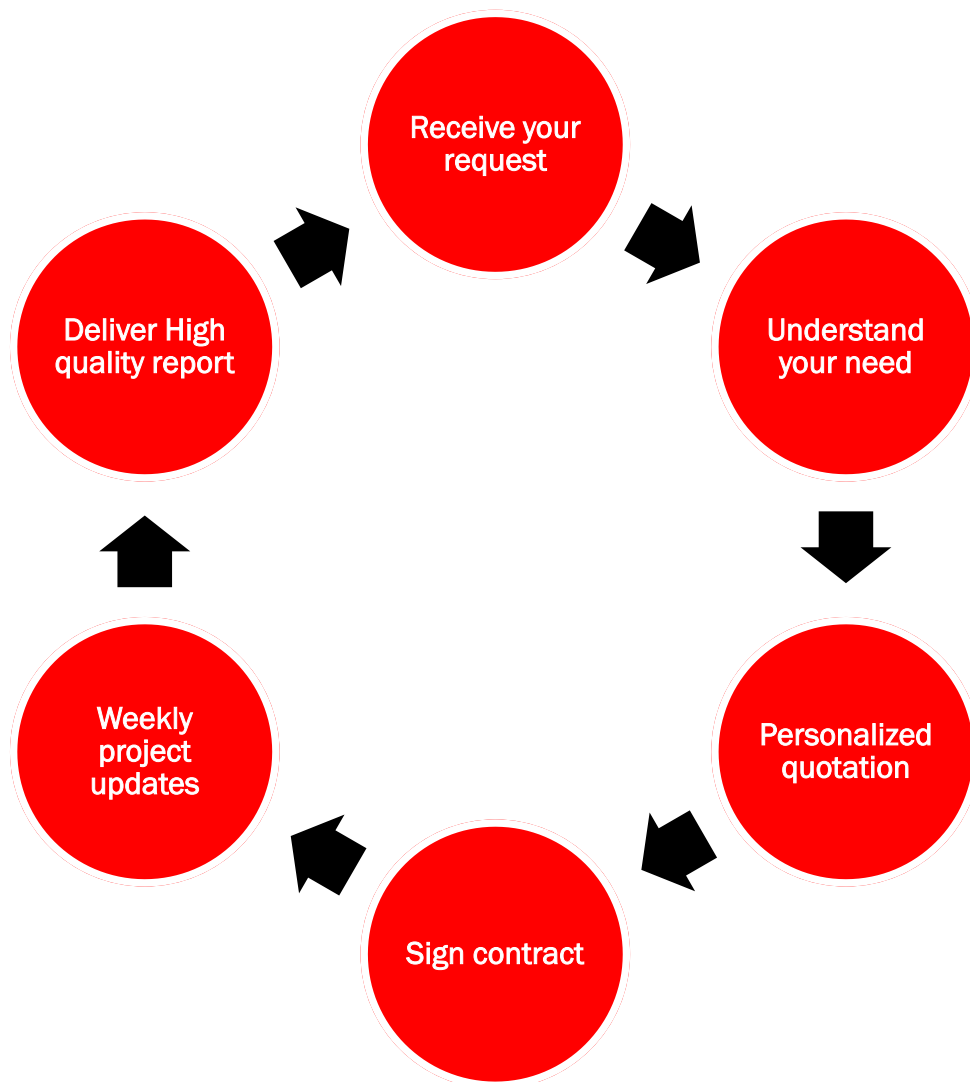


**Biomarker assays**



***In vitro* Cell-based assays**

# Our approach



## We are committed to

1. *Understanding your needs and tailoring our services to meet your requests*
2. *Meeting your timelines*
3. *Providing interactive and efficient customer support*
4. *Delivering high quality services at a very cost effective price*

# Respiratory Diseases

- Respiratory diseases have been identified to be an immense health burden worldwide with approximately 1 billion persons suffering from chronic respiratory conditions.
- Estimates suggest that:
  - 235 million people suffer from asthma
  - more than 200 million people have chronic obstructive pulmonary disease (COPD)
  - 65 million endure moderate-to-severe COPD
  - 8.7 million people develop tuberculosis (TB) annually
  - millions live with pulmonary hypertension and
  - more than 50 million people struggle with occupational lung diseases
  - each year, 4 million people die prematurely from chronic respiratory disease

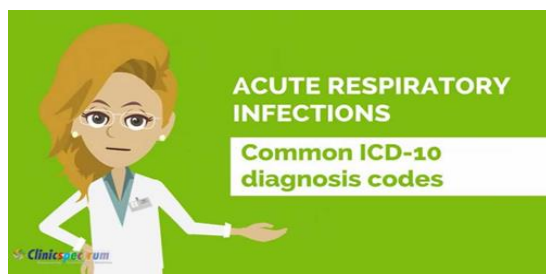
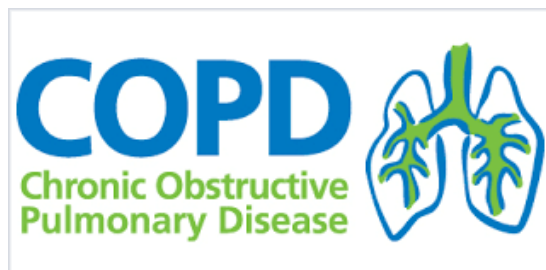


# Respiratory Diseases

- Asthma is the most common chronic disease, affecting about 14% of children globally and rising
- COPD is the fourth leading cause of death worldwide
- The most common lethal cancer in the world is lung cancer, which kills more than 1.4 million people each year
- Respiratory tract infections caused by influenza kill 250,000 – 500,000 people and cost 71–167 billion US dollars annually.



# The BIG 5 Respiratory Diseases



# Available airway cell-based models

## ■ Monocultures

- Primary Human bronchial epithelial cells (HBEC; healthy and diseased)
- Primary Human airway smooth muscle cells (HASM; healthy and diseased)
- Primary Human lung fibroblasts (HLF)
- Primary Human microvascular endothelial cells - lung (HMVEC-L)
- Human Dendritic cells
- Immortalised Human Mast Cell Line (LUVA), LAD2, HMC-1
- Immortalised bronchial epithelial cells - BEAS-2B
- HMC-1 - BEAS2B co-culture: in direct contact
- Immortalized human cystic fibrosis bronchial epithelial cell line (CFT1)
- Immortalised Human bronchial smooth muscle cell (CFT1)
- Peripheral blood mononuclear cells (PBMCs) including monocytes
- Peripheral blood granulocytes including eosinophils, neutrophils

# Available cell-based models

## ■ 3D Airway Model

- A highly physiological, three-dimensional cellular system of Human Bronchial Epithelial Cells (HBEpC) differentiated into a pseudostratified epithelium on transwell inserts with a liquid/air interface
- *3DAirwayALI™* tissue uses primary human airway epithelial cells and fibroblasts grown on an electrospun 3D biological scaffold. These have appropriate epithelial cell barrier function and differentiate into a mucociliated phenotype
- Primary HASM cells printed into *3DBioRing™* tissues
- 3D HASM (airway smooth muscle) cultures: HASM cells embedded in collagen I
- 3D HASM-mast cell co-culture: HASM and mast cells embedded in collagen I
- HMC-1 – BEAS 2B 3D co-culture: BEAS 2B were grown to confluence in the top of a Transwell membrane and HMC-1 added to the bottom chamber.

*3DAirwayALI™ and 3DBioRing™ are 3D models developed by Aspect Biosystems*

# Respiratory assays

## ■ Target validation, qualification

- Real time PCR
- Gene silencing/knockdown assays
- Western Blotting
- Immunocytochemistry

## ■ Proliferation

- MTT: indicator of mitochondrial metabolic activity
- BrdU: detects 5-bromo-2'-deoxyuridine (BrdU) incorporated into cellular DNA during cell proliferation

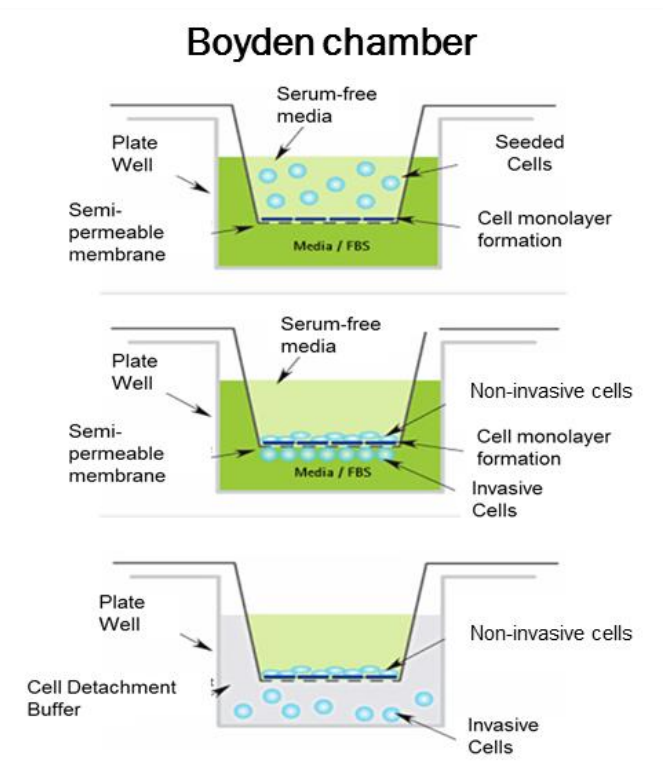
# Respiratory assays

## ■ Cytotoxicity/Viability/Apoptosis

- Green Cyanine dye assay: based on the principles of measuring the membrane integrity that occur as a result of cell death
- Alamar Blue assay: designed to measure cell proliferation quantitatively by incorporating an oxidation-reduction (REDOX) indicator
- LDH Cytotoxicity assay: measures release of a cytosolic enzyme, lactate dehydrogenase, from dead cells
- MTT assay: indicator of mitochondrial metabolic activity
- Caspase3/7 assay: an assay to detect caspase 3/7 activity as an indicator of apoptosis

# Respiratory assays

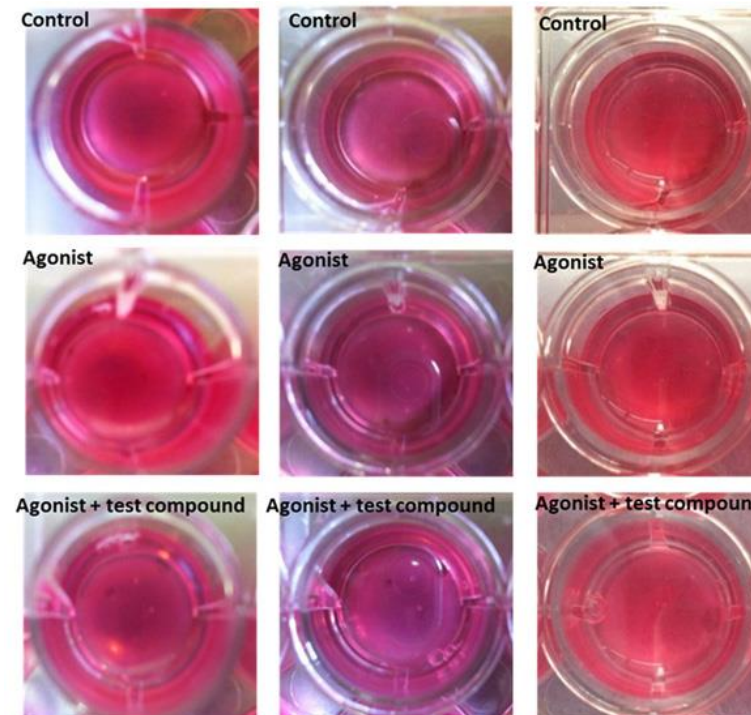
- Migration and Invasion Assays
- Boyden chamber
  - Cells seeded at the top chamber and chemoattractant or different cell line in the bottom chamber.
    - End point: Number of cells migrated to the bottom chamber, compared to controls.



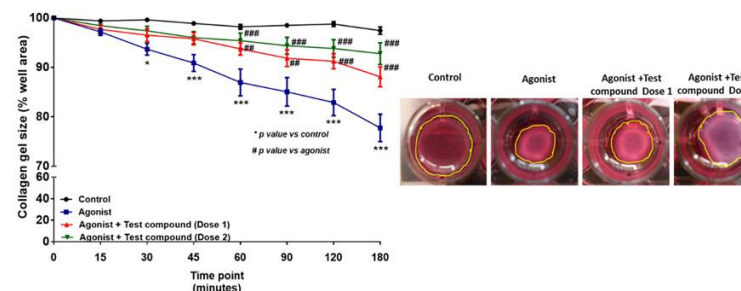
# Respiratory assays

## ■ Collagen gel contraction

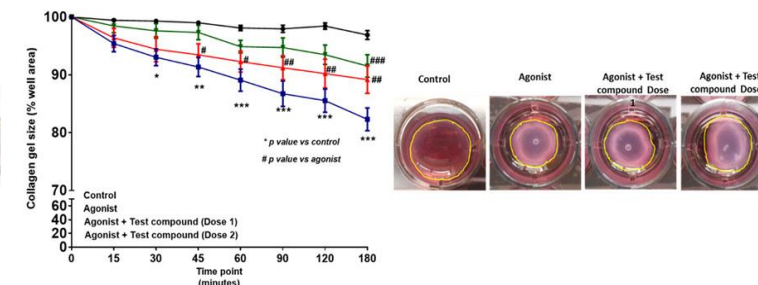
- To assess contractile properties of human airway smooth muscle cells (HASM)
- Collagen gels impregnated with HASM cells are treated in the presence/absence of agonist/antagonist
- Gels were photographed at specific time points and the gel size was measured and quantified as an indicator of airway smooth muscle contraction



Data Example 1



Data Example 2



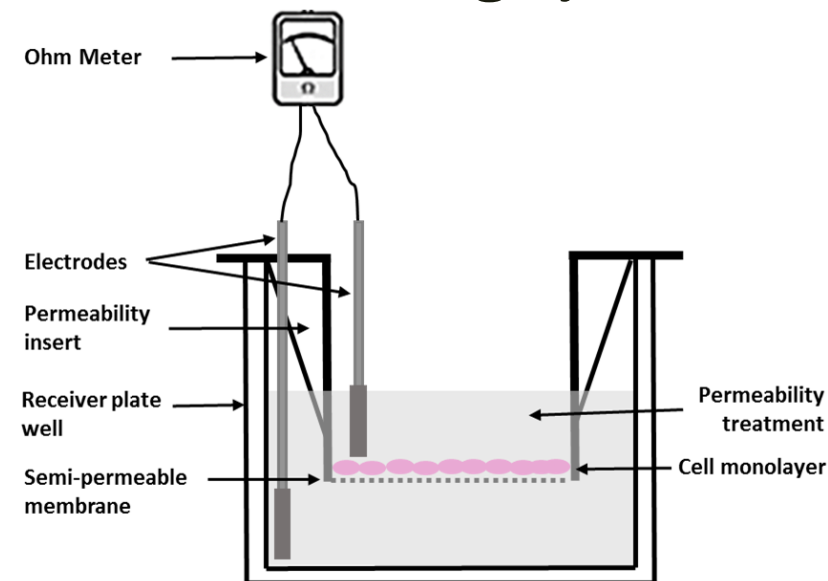


## ■ Oxidative stress/DNA damage

- 8-oxo-dG immunoassay: biomarker of oxidative DNA damage and oxidative stress
- Superoxide Dismutase Assay: a colorimetric assay to detect superoxide Dismutase (SOD) activity in cell and tissue extracts as an indicator of oxidative stress

# Respiratory assays

- Trans-endothelial/trans-epithelial electrical resistance TEER is the measurement of electrical resistance across a cellular monolayer, and a very sensitive and reliable method to confirm the integrity and permeability of the monolayer.
- End-point
  - Quantitative data: Ohm meter readings.

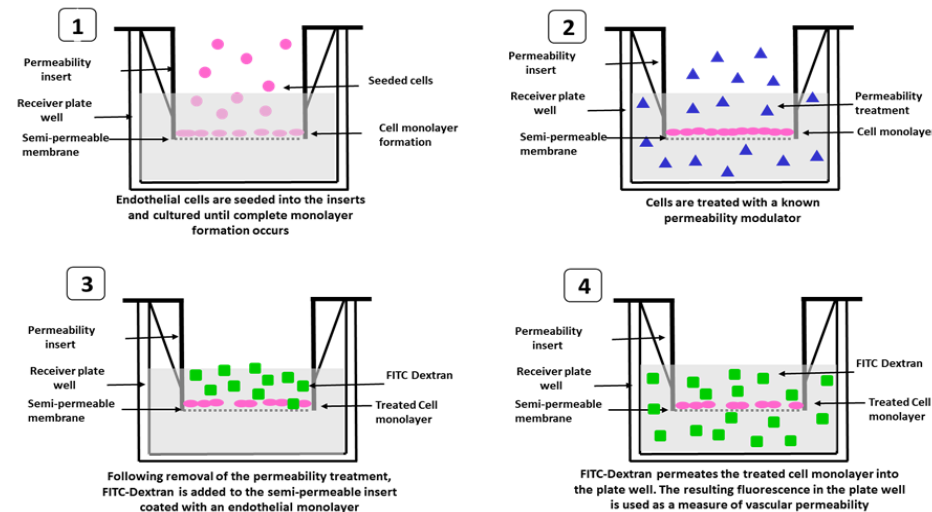


### TEER measurement with chopstick electrodes.

The total electrical resistance includes the ohmic resistance of the cell layer  $R_{TEER}$ , the cell culture medium  $R_M$ , the semipermeable membrane insert  $R_I$  and the electrode medium interface  $R_{EMI}$ .

# Respiratory assays

- FITC-Dextran molecules leak through cell monolayers where the permeability is compromised.
- End-point
  - Quantitative data: Determined by measuring the fluorescence of the receiver plate well solution.
  - Qualitative data: Staining of the monolayer after FITC-Dextran assay.



Representation of FITC-Dextran permeability assay.

# Respiratory assays

- Vascular Permeability assays (airway endothelial cells)/Membrane Integrity Assays (airway epithelial cells)
  - Tight junction protein expression (V-Cadherin, occludins and claudins) by immunofluorescence
  
- Measurement of Mucin production
  - Human airway epithelial cells are differentiated using air-liquid interface (ALI) culture method to form mucociliated epithelial cells
  - Cells are treated in the presence or absence of the test compound and mucus production is then measured by immunoassays

# Respiratory assays

## ■ Respiratory toxicity and irritation *in vitro* model

- Using differentiated airway epithelial *in vitro* model characterised by pseudo-stratified epithelium with tight junction formation, numerous apical cilia and apical mucin production
- Positive Controls: Bleomycin (Irritation); Triton X-100 (Cell Death)
- Biochemical Endpoints: MTT, LDH
- Gene and Protein Expression Endpoints: IL-1 $\alpha$ , IL-6, IL-8, TNF $\alpha$ , TGF $\beta$
- Other Endpoints: Oxidative Stress, Apoptosis

# Respiratory assays

## ■ Mast cell degranulation assays

- HBEC/BEAS 2B and HMC-1 Co-culture model
- Sensitisation using either 2.5 µg/ml human IgE or Calcium Ionophore to induce mast cell degranulation
- Measure the mast cell tryptase activity using a spectrophotometric method as an indicator of mast cell degranulation
- Measure the release of other mediators of allergy and inflammation including histamine, lipoxin A4 by immunoassays
- Measure the release of β-hexosaminidase

## ■ B lymphocytes IgE release assay

- Stimulation of B lymphocyte cell line with IL4, IL5 and IL13
- Measure IgE release by immunoassays

# Respiratory assays

## ■ Pathway activation biomarkers

- Measure phosphorylation of downstream signalling events
  - Multiplexing immunoassays
  - Western Blot
  - In-Cell ELISA

## ■ Disease Biomarkers

- Measure release of cytokines, chemokines, growth factors and inflammatory mediators (mRNA and protein)
  - Multiplexing immunoassays
  - ELISA



*“At Cellomatics Biosciences, we believe in growing together. We welcome the opportunity to partner and achieve excellence together”*

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