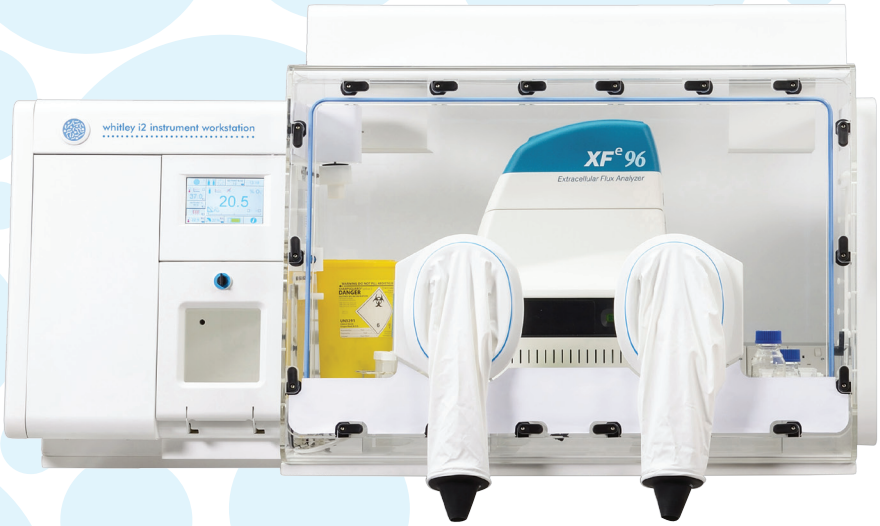

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Novel Modified Atmosphere Cellular Handling System Allows Housing of Seahorse Analyser under Variable Oxygen Tensions

Daniel Secker

ABSTRACT

A Seahorse Biosciences Extracellular Flux Analyser (Seahorse XF Analyser) allows researchers to measure mitochondrial respiration and glycolysis in experimental cell populations. Previously, solutions for housing such an instrument in a variable oxygen atmosphere have been limited, with many users deciding not to pursue research after deeming current solutions unsuitable for generation of appropriate data.

To this end, Don Whitley Scientific and The University of Manchester undertook a collaborative project to develop a novel system for cellular processing and Seahorse XF Analysis within a closed environment which allows oxygen variability.

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INTRODUCTION

Seahorse XF Analysers allow researchers to analyse the metabolic phenotype of a cell population. Mitochondrial respiration is determined by measuring the oxygen consumption rate (OCR) and the glycolytic activity by measuring the extra-cellular acidification rate (ECAR). This data allows users to compare aerobic vs anaerobic respiration of a cell population throughout a series of stress tests.

Dr Ian Stratford and his group in the Pharmacy School at the University of Manchester wished to use a modified atmosphere workstation to interrogate gynaecological cancer cells using their Seahorse XF Analyser at reduced oxygen tensions. The use of low oxygen tensions allows simulation of the hypoxic tumour microenvironment these cells would experience in vivo.

Oxygen availability can have a strong influence on the metabolic activity of gynaecological cancer cells, as demonstrated by increased rates of glycolysis and lactate production. These traits have been suggested to predict an increased likelihood of metastasis, resistance to therapy, and reduced survival in affected individuals. Lactate transport in cancer cells is carried out by members of the monocarboxylate transporter (MCT) family, notably MCT1 and 4. Thus, it is hypothesised that pharmacologic inhibition of MCTs could improve treatment outcome by reducing the glycolytic potential of these tumour cells. Dr Stratford's group tested this hypothesis by supplementing culture media with Simvastatin, a potential MCT inhibitor.

EXPERIMENTAL

A novel workstation, comprising two chambers linked by a transfer tunnel, allows the use of the Seahorse analyser under variable oxygen tensions and thus provides a unique platform for metabolic analysis of cell lines in hypoxia as well as air. With this equipment, cell lines are first cultured in the HEPA-filtered first chamber (Whitley H35 HEPA Hypoxystation) under user selectable conditions incorporating O_2 tension, CO_2 tension, temperature and humidity.

The cells are then transferred (without exposure to ambient laboratory conditions) through the transfer tunnel and into the second chamber (Whitley i2 Workstation), purpose-designed to accommodate a Seahorse XF analyser. This second chamber houses the Seahorse analyser at user defined O2 tension, but at room temperature (required by Seahorse) and in an absence of CO2 (required for accurate Seahorse analyser operation as CO2 in its aqueous form, carbonic acid, can interfere with ECAR readings). This second chamber also incorporates a built in incubation compartment to allow incubation of cells at user defined temperature and in an absence of CO2 pre-analysis, as per seahorse analyser manufacturer recommendations.

Fig.1. Working inside an i2 Instrument Workstation

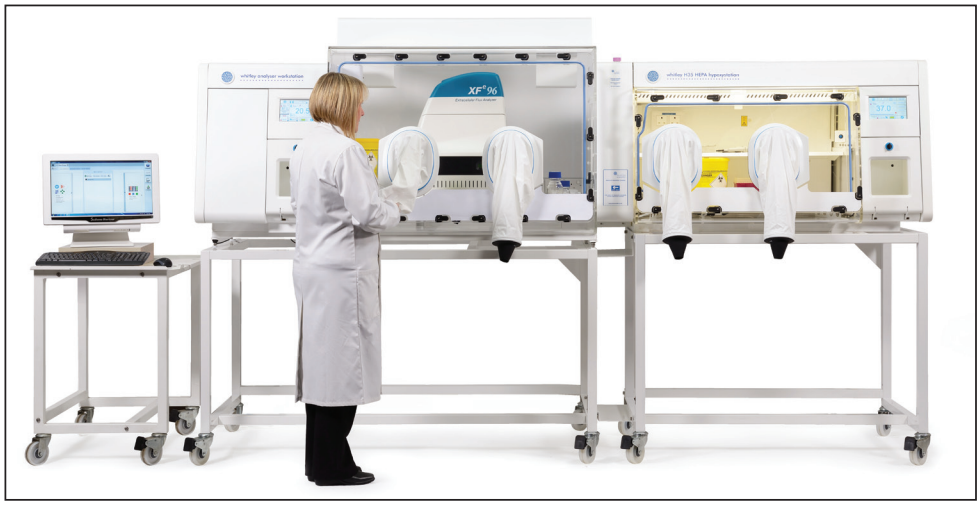
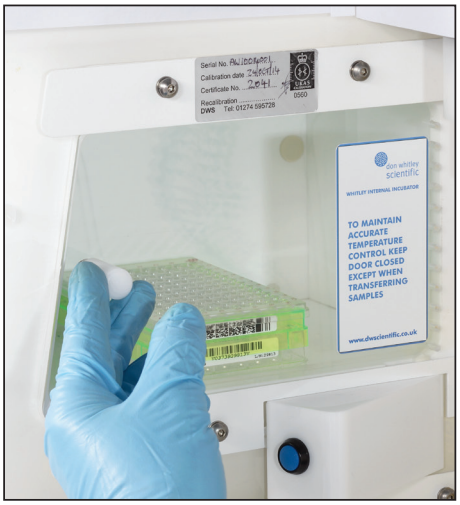


Fig.2. Incubation Compartment



CONCLUSION

The combination of a Whitley H35 HEPA Hypoxystation and a Whitley i2 Instrument workstation (Don Whitley Scientific) provides a suitable hypoxic environment in which a Seahorse XF96 Extracellular Flux Analyser can be used to measure cell metabolism at 3% oxygen concentration without exposure to ambient laboratory oxygen at any point during the experimental procedure.

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