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## SHORT COMMUNICATION

# Dissolved Oxygen Concentration in Culture Medium: Assumptions and Pitfalls

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Oxygen is a key factor in the regulation of cytotrophoblast differentiation, proliferation and invasion in early pregnancy. Abnormalities in oxygen concentration have also been linked to a number of pregnancy disorders. Cell culture models have been used to study the effect of oxygen on cytotrophoblast behaviour in vitro, however, there is often little or no validation of oxygen levels in these cell culture systems. In this study, dissolved oxygen levels in culture medium maintained in standard culture conditions (18% $O_2$) measured 18%. On transfer to a low oxygen environment (2% $O_2$), oxygen levels decreased to 6–8% after 4 h and reached 2% only after 24 h in culture. Culture medium pre-gassed with nitrogen to remove dissolved oxygen quickly absorbed oxygen when exposed to ambient air during dispensing and required further incubation in a 2% oxygen environment before dissolved oxygen levels equilibrated to 2%. Thus, cultured cells placed in a low oxygen environment would be exposed to varying levels of oxygen before the desired level of oxygen exposure is reached. This study highlights the importance of validation of oxygen levels and potential problems associated with in vitro studies on the regulatory effects of oxygen.
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## INTRODUCTION

A number of important clinical conditions such as pre-eclampsia, fetal growth restriction and spontaneous miscarriage have been linked to the placenta being exposed to abnormal oxygen concentrations [1]. Women living at high altitude are exposed to chronic hypoxia and they too have increased risk of complications of pregnancy [2], underscoring the involvement of oxygen. Cytotrophoblast proliferation and differentiation appears, at least in part, to be regulated by oxygen tension. These observations have been the driving force for investigators to develop in vitro models to determine the effects of different oxygen concentrations on cell culture models such as primary trophoblast cultures and placental explant culture [3–7]. One of the key issues in studies of this nature is the actual oxygen levels which cultured cells are exposed to during incubation in different oxygen environments. Some authors report specific methods to control the oxygen environments to which the cells are exposed and/or the measurement of oxygen in culture medium [3,5,7,8], while others make no mention of oxygen levels in their culture systems other than in the incubator environment [6,9,10]. The aim of this study was to define the oxygen environments encountered in our cell culture systems and to address some of the issues and potential problems associated with studies of this nature.

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## METHODS

### Cell culture incubators

Studies were carried out using two Forma Scientific water-jacketed incubators (Forma Scientific, Inc. USA), one set at 5% $CO_2$ in air for standard culture conditions with the sensor on the incubator reading approximately 18.2% $O_2$ and one set at 5% $CO_2$/93% $N_2$ with the oxygen sensor reading approximately 2% $O_2$ for low oxygen conditions. Oxygen levels in each incubator environment were measured using a Fyrite Gas Analyser.

### Measurement of dissolved oxygen levels

There are many commercially available oxygen probes and meters, of varying specifications, for the measurement of dissolved oxygen in a liquid environment but few are ideal for the measurement of dissolved oxygen in cell culture systems

such as those used in trophoblast and explants cultures. A Jenway 970 Portable Dissolved Oxygen/°C Meter and Electrode (Jencons Scientific Ltd, UK) was chosen for the measurement of dissolved oxygen in this study due to the cost and the suitability of the technical specifications, in particular the integral temperature compensation. Issues relating to the measurement of oxygen in cell culture systems and the factors that influenced the choice of oxygen probe used in this study are discussed in more detail later in this communication.

The oxygen meter was calibrated, according to the manufacturer's instructions, to 21% oxygen in water saturated air and to 0% oxygen using the zero salts supplied with the meter. In order to satisfy the requirements of the oxygen electrode, experiments were carried out using approximately 125 ml of M199 (supplemented with 10% fetal bovine serum and 1% antimycotic/antibiotic solution) (GibcoBRL Life Technologies, UK) in a T75 vented-lid cell culture flask. This was necessary so that the depth of the culture medium in the upright flask was sufficient to cover the temperature compensating element of the electrode and allowed stirring of the culture medium during measurement. The flask was placed flat in the incubators to allow maximum surface area for gaseous exchange. The surface area/volume in the T75 flask was 75 $cm^2$/125 ml and this compares to 8 $cm^2$/4 ml in 35 mm culture dishes, 1.9 $cm^2$/0.4–1 ml in 24 well plates and 0.6 $cm^2$/200 µl in the Millicell inserts used by many investigators for trophoblast and explant culture experiments. All experiments were performed in triplicate and repeated three times.

*Experiment 1*

Culture medium was warmed to 37 °C in T75 flasks in standard culture conditions (5% $CO_2$ in air). Dissolved oxygen levels were measured and the flasks were then transferred to low oxygen conditions at 37 °C for up to 24 h. Dissolved oxygen levels in the culture medium were measured at intervals during the incubation period. Separate flasks were used for each time point to prevent the medium being affected by atmospheric oxygen.

*Experiment 2*

Nitrogen gas (Cryoservice, UK) was bubbled through culture medium (2–3 psi) (125 ml) in a T75 flask for 30 min to eliminate dissolved oxygen in the culture medium. The opening to the flask was sealed with parafilm through which there was an inlet for the pipette delivering the nitrogen gas and an outlet to allow gas to escape. The flask was immediately transferred to a 2% oxygen environment at 37 °C. Dissolved oxygen levels in the culture medium were measured after 1 h and following overnight incubation.

*Experiment 3*

Nitrogen gas was bubbled through culture medium (125 ml) in a T75 flask for 30 min to eliminate dissolved oxygen in the culture medium, as described above. The culture medium was then dispensed into smaller volumes (5 × 25 ml) to mimic culture medium changes (total dispensing time 4 min) and then returned to the T75 flask. Dissolved oxygen in the culture medium was measured immediately in the 125 ml of culture medium and the flask placed in the low oxygen incubator at 37 °C. Dissolved oxygen levels were measured at intervals up to 16 h.

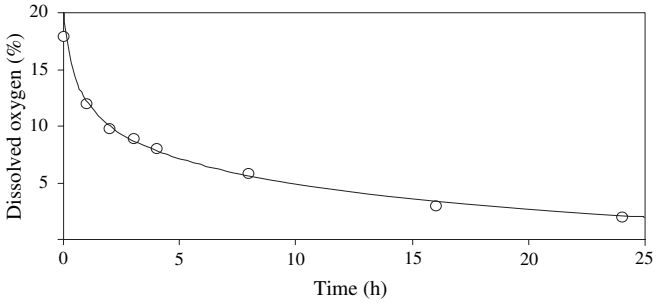


**Figure 1.** Dissolved oxygen concentration (% $O_2$) in test culture medium incubated at 37 °C in hypoxic conditions for up to 24 h. Oxygen levels were measured using a Jenway Model 970 Dissolved Oxygen Meter and Electrode (Jencons Scientific Ltd, UK).

## RESULTS

Oxygen levels, measured using a Fyrite Gas Analyser, were 18–20% in the standard incubator and 2–3% in the low oxygen incubator.

### Experiment 1

The dissolved oxygen concentration in culture medium maintained in standard culture conditions measured approximately 18% ($pO_2$ 140 mmHg). When culture medium was transferred to the low oxygen incubator, oxygen levels decreased gradually to measure 6–8% (46–61 mmHg) after 4 h, 7% (53 mmHg) after 8 h, 3% (23 mmHg) after 16 h and 2–3% (15–23 mmHg) after 24 h of incubation (Figure 1). The pH was unaffected by the changes in oxygen concentration (pH 7.2–7.4).

### Experiment 2

When nitrogen gas was bubbled through culture medium, dissolved oxygen levels fell from 18–20% to approximately 1.5% (11 mmHg) after 15 min and to 0% after 30 min. When pre-gassed culture medium (0%) was placed directly into the incubator with the 2% oxygen environment, oxygen levels in the culture medium equilibrated to approximately 2% after 1 h and remained at 2–3% (15–23 mmHg) following overnight incubation.

### Experiment 3

When culture medium pre-gassed with nitrogen to eliminate oxygen (0%) had been dispensed in ambient air, dissolved oxygen levels increased to 8.5% (65 mmHg). Further incubation of the cell culture medium in a hypoxic environment