INTRODUCTION
Quinton® isotonic solution produced by Laboratoire Quinton (France), is a saline solution of seawater for medical purposes. It is known that seawater contains many mineral elements like zinc, silicon, selenium, chlorine, sodium, calcium, magnesium and many others. Many of these elements, like sodium or chlorine are essential for the proper functioning of body cells, including cells involved in immune response processes. The solution also contains other nutrients and minerals such as calcium or magnesium than although less abundant are equally important and closely related to the mechanisms by the immune system uses under different conditions such as defensive functions or homeostatic balance.

OBJECTIVES

To analyse in vitro effect of the solution on PBMNc morphological changes, cell viability, and cell aggregation.

To check a potential in vivo immunomodulatory activity of Quinton® isotonic solution on isolated human PBMNc.

MATERIALS, METHODS AND SAMPLES

Sample: PBMNc of three healthy volunteers were isolated by density gradient centrifugation (Ficoll® Hypaque) from 10 ml of anticoagulated blood (EDTA).

Methods:

1. Cell Culture: Isolated PBMNc were cultured in 96 well plates (200,000 cells/well) under the following conditions:
   - RPMI, Quinton® isotonic with ISO+ or saline solution (SS+), all of them supplemented with 1% antibiotic, 1% glutamine and 1% FCS.
   - RPMI, Quinton® isotonic without any supplement (ISO-) was also tested.
   - Cells were stimulated for 4 days in a 5% CO2 incubator with PHA, PMA + Ionomicin or anti-CD3 + anti-CD28 and the different culture conditions were compared to each other regarding parameters below (2-4).

2. Cell viability, cell morphological changes and presence of types of aggregates were analysed by optical-inverted microscope. For cell viability, trypan blue solution was also used.

3. Released haemoglobin from erythrocytes in every condition was measured by a modified Drabkin procedure.

4. Flow Cytometry: CFSE assay and different fluorochrome-conjugated (PE, FITC, PECy5) monoclonal antibodies such as anti-CD3, anti-CD4, anti-CD19, anti-CD25 (Becton-Dickinson®) were used to analyse proliferation and activation on several lymphocyte subtypes (EPICS XL, Coulter®).

RESULTS
Concerning general cell parameters (morphology, viability and aggregation) the most important aspect to be mentioned is that Quinton® isotonic solution ISO+ (supplemented just with the same supplements antibiotic, fetal calf serum and glutamine that are usually added to conventional culture media), had a similar behaviour to RPMI, that undoubtedly is one of the most used culture media worldwide. The presence of some small aggregates in unstimulated cells (CONTROL), when using ISO+ or RPMI, ISO+ and SS+ (not shown) can reflect a certain ability of the solution itself to stimulate cells in vitro (Figure 1).

Concerning cell activation, Figure 3 also plots showing percentages of unstimulated and anti-CD3+ anti-CD28 stimulated CD3+ T cells excepting a stimulation with anti CD3 + anti-CD28 on CD3+ cells (only for the CD3 subset; not shown), although as lower extent than RPMI; percentages are always indicated in the upper right quadrants of each square from Figure 3. The highest activation of RPMI cells was expected if taking into account that RPMI apart from the above mentioned substances (antibiotic, fetal calf serum and glutamine) is enriched with many other nutrients that are absent in Quinton solution.

When regards to cell proliferation, Figure 1 dot plots are showing proliferation (measured in amount of CFSE+ cells) of unstimulated and anti-CD3+ anti-CD28 stimulated CD3+ lymphocytes in the different media (RPMI, ISO+ and SS+). Again the highest proliferation (upper left quadrants of each square) was shown in RPMI (conventional), as expected, when compared with both ISO and SS cultured cells. However, after looking carefully at the right histogram displayed in Figure 4 a shift towards the left side is observed in ISO+ stimulated CD3+ cells (curve in green colour) versus ISO- stimulated CD3+ cells (curve in blue colour); this displacement, although lesser than that observed for RPMI (curve in red colour), is also indicating a proliferation of cells that can be perfectly linked to the aforementioned activation found with ISO+ on CD3+ cells.

Regarding haemoglobin, released haemoglobin from ISO+ erythrocytes remained constant all along the 96 hours of culture, being always lower than that observed for RPMI, in which a constant increase of haemoglobin was detected since 48h reaching its highest value at 96h.

CONCLUSIONS
Quinton® isotonic solution is very well tolerated by cultured cells; morphology and cell viability is perfectly conserved all along the culture.

When minimally supplemented, is able to emulate many of the results observed when using conventional culture mediums such as RPMI, in terms of activation, proliferation and aggregation. Furthermore, it seems to exert a possible protective effect on erythrocytes, avoiding or delaying spontaneous lysis.

REFERENCES