[SOP AFT/CG] AQIX® FLUID TECHNOLOGY
- APPLICATION OF CARBOGEN GAS

1. AQIX® STORAGE SOLUTIONS

a) Previous trials have shown that optimal preservation of the functionality of isolated animal and human biopsy samples are achieved when AQIX® RS-I and RS-S solutions are pre-aerated with *carbogen* gas [95% O_2 / 5% CO_2].

b) *Carbogen* gas cylinders come in 10L sizes which will suffice for this application. A 2 – 4 psi 'Regulator' valve will be required and set to 1-2 psi to deliver a slow flow of the *carbogen* gas.

c) The carbogenation of AQIX® RS-I and RS-S solutions is achieved by simply passing *carbogen* gas from the carbogen cylinder via a 0.22µm filter into suitable tubing attached to a 1.0 mL sterile pipette and then into these solutions for 10 – 15 minutes [within suitable, 125mL, 250, or 1.0L gas impermeable container] and then tightly sealing the lid closure of the container (see Fig. 'CG', below).

d) The sealed, gassed containers can be stored for up to 24 weeks at 3 – 8 ºC under dark conditions prior to being dispatched to the tissue biopsy procurement site.

e) The carbogenated AQIX® RS-I and RS-S solutions can be transported to the procurement site over 'wet' ice [0 - 4 ºC] or at ambient temperatures [< 25 ºC].

f) At the procurement site, the sealed containers are opened and the tissue biopsy sample inserted and the containers re-sealed quickly to prevent the outward diffusion of oxygen and carbon dioxide.

g) The sealed containers of either AQIX® RS-I and RS-S solutions are then transported back to the investigative laboratory over 'wet' ice [0 - 4 ºC] or at ambient temperatures [< 25 ºC].

h) Transportation times of 12 – 96 hours are achievable for biopsies stored over 'wet' ice [0 - 4 ºC] and 12 – 48 hours for biopsies stored at ambient temperatures [< 25 ºC].

i) At the investigative laboratory, the tissue biopsies should be examined immediately using (i) normothermic, isolated tissue perfusion techniques [see Section **2**] or (ii) incubated under conventional Tissue Culture conditions **but** using ***humidified, carbogen* gas** at 37 ºC [see Section **3**].

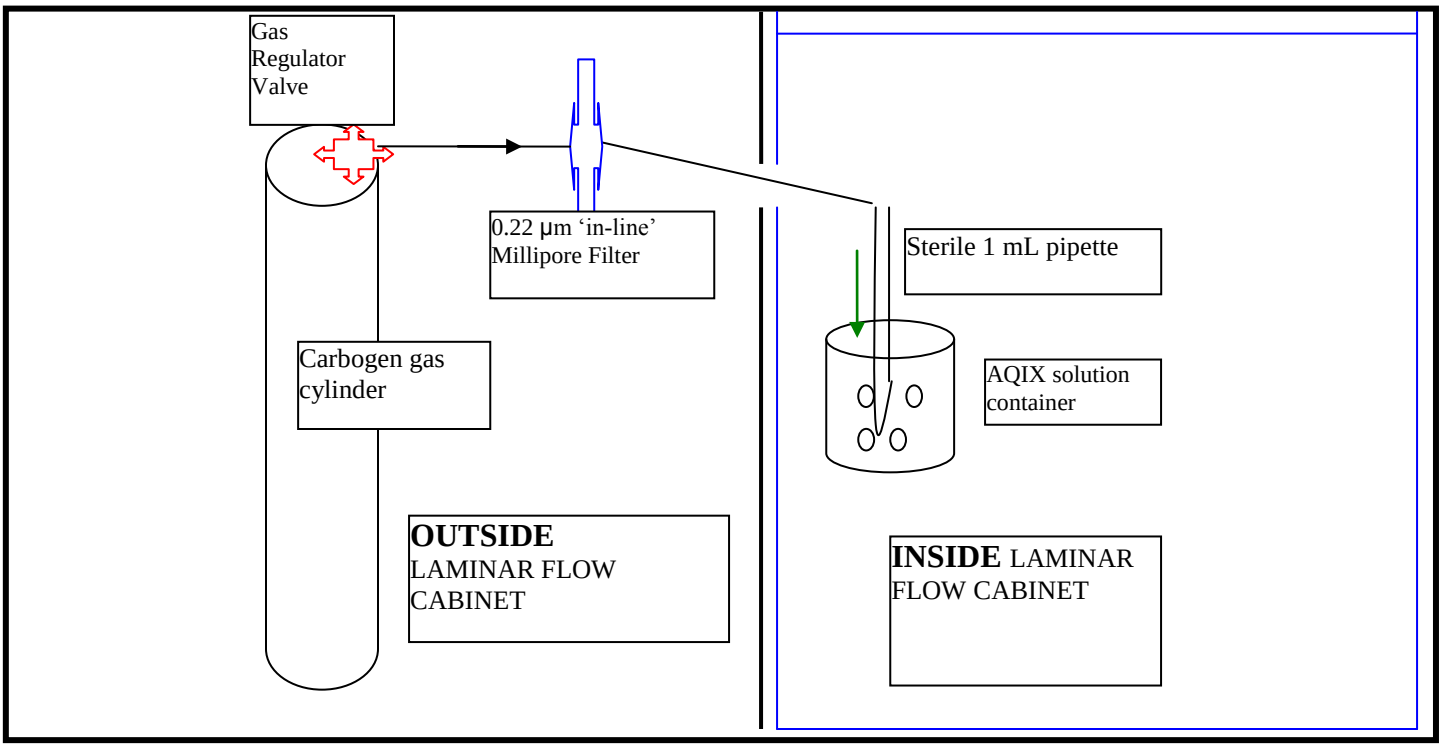

Fig. 'CG' – Set-up for the carbogenation of AQIX solutions under sterile conditions

2. AQIX® RS-I PERFUSION SOLUTION

a) Tissue biopsy samples previously stored in AQIX® RS-I or RS-S solutions are re-animated using *carbogenated* AQIX® RS-I solution during the continuous perfusion of AQIX® RS-I at 1 – 4 mL/min at 32 - 38 ºC.

b) AQIX® RS-I solution is placed in a suitable reservoir container and then continuously aerated with *carbogen* gas during the total experimental time.

c) The AQIX® RS-I solution within the experimental tissue chamber or bath is continuously *carbogenated* and replenished with fresh, *carbogenated* AQIX® RS-I solution at 1 – 4 mL/min at 32 - 38 ºC.

d) Tissue preparations may be examined under 'non-perfused' conditions (e.g., drug applications) at 32 - 38 ºC for up to 30 minutes while maintaining continuous aeration with *carbogen* gas and then returned to perfused conditions and allowed 15 – 30 minutes to re-equilibrate.

3. AQIX® SOLUTIONS UNDER TISSUE CULTURE CONDITIONS

a) AQIX® RS-I /RS-S/RS-C(cardioplegic) perfusion/storage solutions can be used under TC-conditions but require to be incubated using humidified,

5% Carbon Dioxide gas, in order to establish the P_{CO_2} / HCO_3^- buffering level at 37 ºC to maintain a pH of 7.42 ± 0.04.

b) AQIX® fluids require to be replenished every 24 hours with fresh fluids.

c) AQIX® fluids require to be stored at 3 - 8 ºC under dark conditions prior to use.

4. General Comments

4.1 Pre-aerated AQIX® RS-I and RS-S solutions are packaged in sealed, non-diffusible containers and 'ready-to-use' at the site of tissue procurement.

4.2 Assembly of 'ready-to-use' AQIX® RS-I and RS-S solutions from 100mL vials of [10x] concentrates requires the simple addition of 2.1g/L of Sodium Hydrogen Carbonate and 900 mL of Ultra Pure water [see, SOP-12; 'Instructions for Use'].