Quasi-Vivo® System: Tools for Physiologically Relevant *in vitro* Cell Culture

User Manual for QV500 Chamber System

Issue Number 5.2

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1. Scope of the document

This document describes the applications, setup and experimentation using the ***Quasi-Vivo***® cell culture chamber system. It is aimed at users with basic tissue culture experience; enabling the transition between current static culture to perfusion culture techniques. This manual provides new users with guidelines to begin perfusion culture; the methods described may be adapted to suit the specific application. We advise that you read this document *thoroughly* before starting an experiment. For more information on tissue culture and sterile techniques we suggest you refer to: *R. Ian Freshney, Culture of Animal cells, 2005*.

Please note that the current design enables the culture of any primary cell or cell line that grows as a monolayer. It is not currently designed for culture of cells in suspension. The system has been tested using human and rat primary hepatocytes, HepG2 (human liver carcinoma), C3A (human hepatoblastoma), NIH 3T3 (mouse embryonic fibroblast), adipocytes, NCI H292, HDMECs (Human Dermal Microvacular Endothelial Cells), HUVEC (human umbilical vein endothelial cells), human skin and oral fibroblasts, skin keratinocytes, osteoblasts and chondrocytes. These cells may be cultured on coverslips or scaffolds.

Training courses demonstrating the assembly and use of the ***Quasi-Vivo***® cell culture system are available, please contact us for more details.

2. Introduction

The Kirkstall ***Quasi-Vivo***® system has been developed in response to the need for small, inexpensive but reliable systems which can mimic the interactions between cells which occur in living organisms. The system enables quick and cost-efficient cytotoxicity testing and studies on metabolism with potential applications in the biotechnology, pharmaceutical, chemical, cosmetics and research industries. It allows the possibility of communication between different types of cells. This is achieved by a continuous flow of culture medium between the chambers. Figure 1 shows a flow chart explaining the practical implementation of the ***Quasi-Vivo***® system.

Flow Chart for using the Quasi-Vivo® System

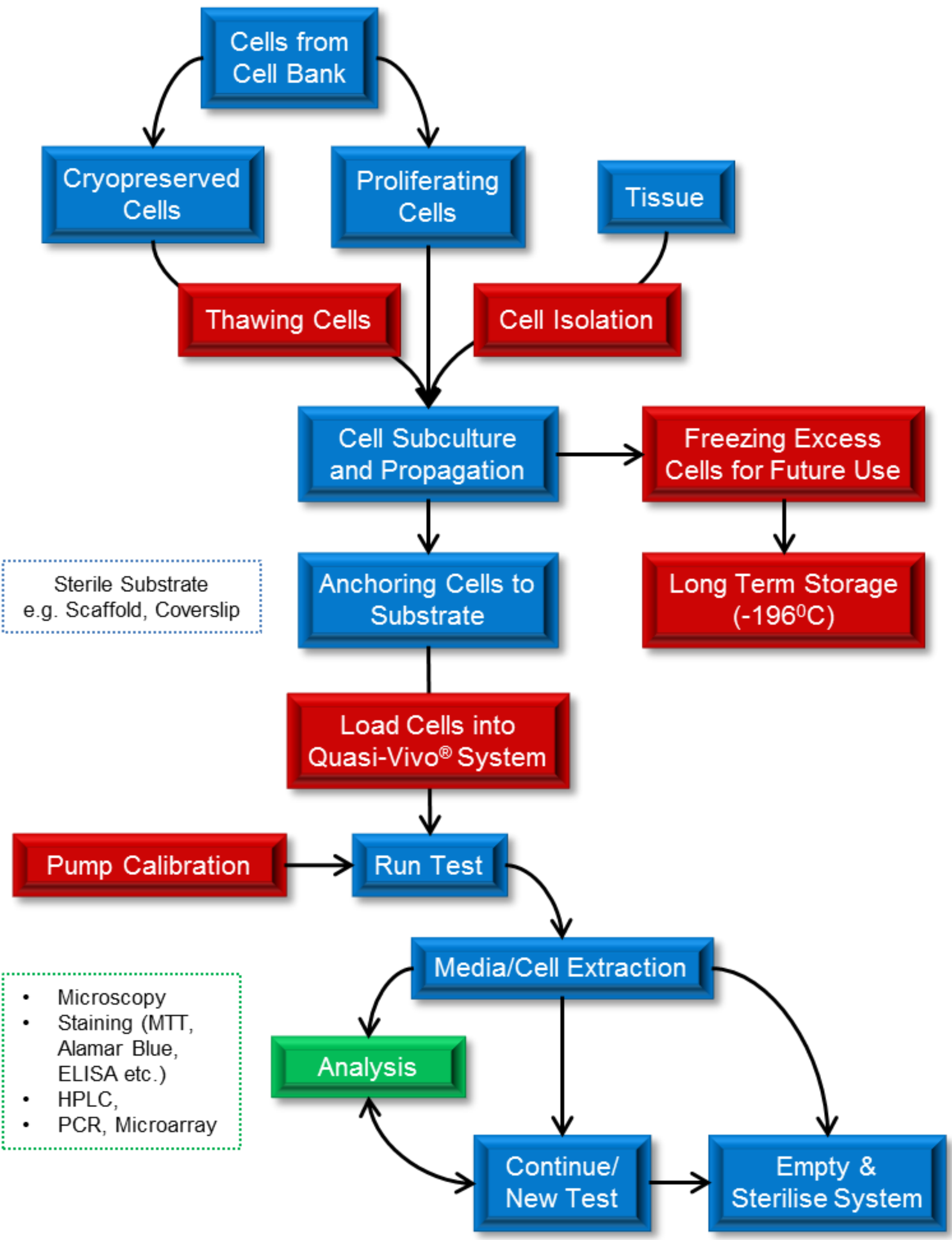

Figure 1. Flow chart explaining the practical implementation of the ***Quasi-Vivo®*** *system, and how it fits into current tissue culture protocol. It is recommended that you should calibrate your pump before starting an experiment. Refer to sections 4 and 11 for more information.*

3. Outline description of silicone cell culture chambers

The silicone chamber (Figure 2) is the basic building block of the ***Quasi-Vivo***® system. ***Quasi-Vivo***® chambers are made of poly-dimethyl-siloxane (PDMS), a biocompatible silicone polymer. The external dimensions of ***Quasi-Vivo***® chambers are approximately 23 mm in height and extending to 37 mm in diameter. The internal volume of each chamber is approximately 2 ml and designed to accommodate 12-14 mm diameter coverslips.

Figure 2. The ***Quasi-Vivo***® *chamber.*

Ethylene oxide gas-plasma sterilization is used to sterilise the chambers at the end of the manufacturing process. This is the most efficient method which maintains the unique properties of silicone; flexibility and breathability. The chambers are supplied double wrapped in sealed packs, each of which contains a colour-change indicator which turns from purple to green when the sterilization process is complete.

Chambers can be re-used if washed with 70% ethanol and thoroughly rinsed with PBS after each experiment. The chambers are autoclave safe; however autoclaving more than 3 times is not recommended as the silicone will lose flexibility and become discoloured.

Sterile silicone chambers are used for cell culture in non-therapeutic and non–diagnostic applications. Each chamber is made of two parts: an upper body and a base as shown in Figure 3.

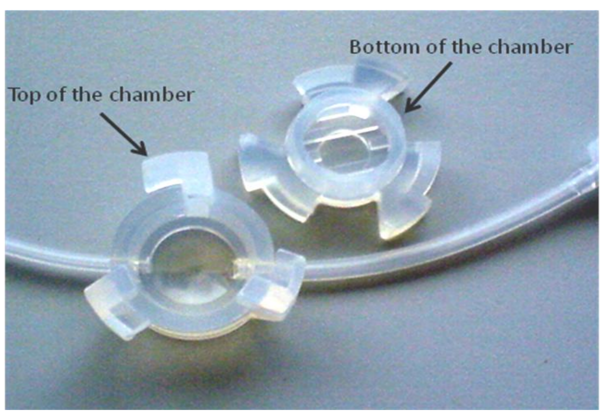

Figure 3. Illustration of the chamber's component parts. Cells are seeded or attached to a coverslip or scaffold which is placed in the bottom of the chamber.

Your ***Quasi-Vivo***® system is delivered as a kit which includes:

- 3 silicone chambers with tubing and connectors
- 1 reservoir bottle
- 100 glass coverslips, 12 mm diameter
- A user manual
- 6 x 22 cm extension tubing with luer connectors
- A 0.2 μm filter

We recommend the use of translucent polypropylene luer connectors, as supplied with the kit. Other connectors may be used but we found that the silicone tubing may slip and disconnect from the connector when placed in the incubator over 24 h incubation periods.

Spare tubing and connectors can be supplied, as well as additional items such as holding trays; please contact Kirkstall for more information.

4. Choosing a pump

The ***Quasi-Vivo***® system must be connected to a peristaltic pump to enable perfusion of the chambers. Kirkstall is happy to provide advice and support in choosing the pump which will suit customers' needs. Table 1 below shows examples of different types of pumps which can be provided by Kirkstall. See Figure 4 for a demonstration of the system setup including the pump.

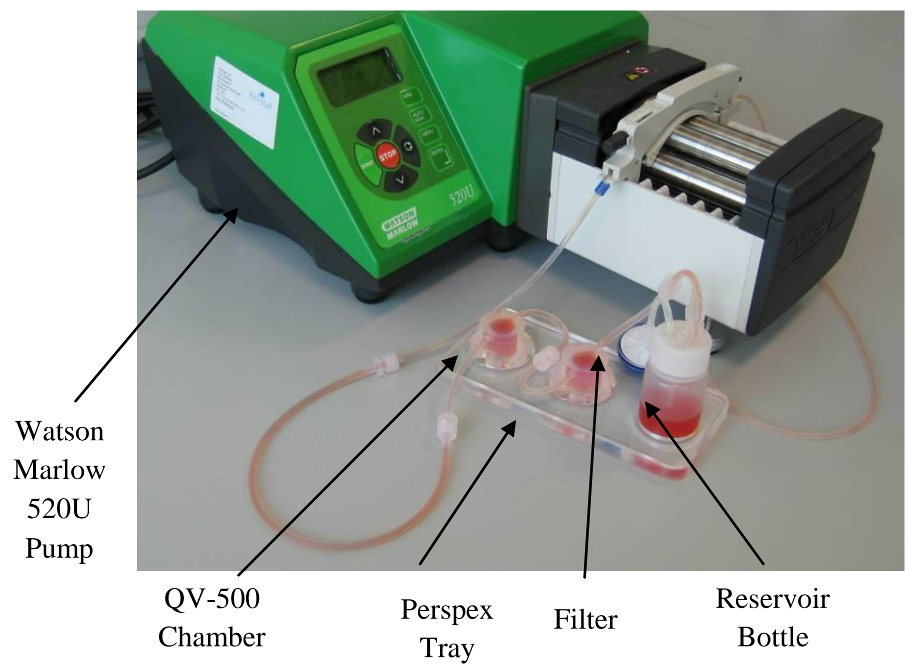

Figure 4. ***Quasi-Vivo***® *system setup connected to the Watson Marlow 520U peristaltic pump.*

	Manufacturer / Model	Weight [kg] / Dimensions [cm]	Advantages	Disadvantages	Pump Cost [£]	Manifold Tubing Cost [£]
	Watson Marlow / 520 UN	Weight: 12.3 Dimensions: h: 15.5 w 28 d:53	• Very reliable • Fully water resistant • Fits up to 8 cassettes or more • 5 year guarantee	• Very large, heavy, difficult to fit in some incubators • Designed for higher flow- fewer increments at low range • Channel flow not independently controlled	2800 Approx	68.58 (x6)
	Ismatec / IPC-N	Weight: 5.1 Dimensions: h: 12.5 w 15 d:22	• Small and light • Easily fit in dry incubator • Fits up to 8 cassettes • Ultra low flow rates, small increments	• Shouldn't use in humid incubator • Can't vary cassette number • Channel flow not independently controlled	2700 Approx	28.89 (x12)
	Parker Hannifin / QV PF22X0103	Weight: <1.0 Dimensions: h: 10.5 w 19.5 d:17	• Low cost • 2 independently controlled pump heads • Small and light • Easily fit in wet/dry incubator • Ideal for ALI experiments • Ideal for 1 or 2 channel experiments	• Only 2 connections • No digital display • Manually determine flow rate	1050 Approx	Standard tubing with collars supplied

Table 1. Descriptions of suitable pumps

5. General comments

All procedures which involve cell culture (seeding cells on coverslips, opening sterile chambers, opening and closing reservoir bottles, disconnecting and connecting the tubing) should be carried out in accordance with aseptic techniques. It is necessary to ensure that all cell culture procedures are performed to a standard that will prevent contamination from bacteria, fungi, mycoplasma and cross contamination with other cell lines. All work should be done in a Class II tissue culture hood, wearing gloves and laboratory coats. After assembly the completed ***Quasi-Vivo***® system can be transferred into a CO_2 incubator and left for the duration of the experiment.

6. Preparing the system for the first use

The reservoir bottle and chambers are supplied sterile and with the luer connectors attached. The extra luer connectors and tubing are not sterile. Initially the supplied luer connectors should be attached to the tubing in the required configuration; for consistency tube inlets should have female connectors and tube outlets should have male connectors. The thinner (1/16” internal diameter (ID)) tubing requires the connectors of this size, while the thicker (3/32” ID) tubing requires the connectors of this size. These assembled components should then be sterilised (we recommend autoclaving).

The whole system should now be connected under aseptic conditions in the configuration shown in figure 5. The reservoir bottle has three tubes connected to it; the longest tube (1/16” ID) is the outlet and should be submerged in medium, the middle length tube (3/32” ID) is the reservoir inlet and should be just above the level of the medium to avoid foaming. Finally, the shortest tube (3/32” ID) is for gas exchange and should not be touching the medium. A filter should be connected to this gas exchange tube.

Figure 6. Illustration of the chamber inlet and outlet.

Following the system round in a loop; the outlet tube of the reservoir is connected to 1/16” ID extension tube (22 cm in length). This is connected to the pump manifold tubing which is in turn connected to the 3/32” ID extension tube (22 cm in length). This is connected to the chambers which are then connected to the reservoir inlet. Chamber inlets must be the 1/16” ID tube and the outlets must be the 3/32” ID tube (see Figure 6). Figure 5 shows three chambers connected in series, however, the

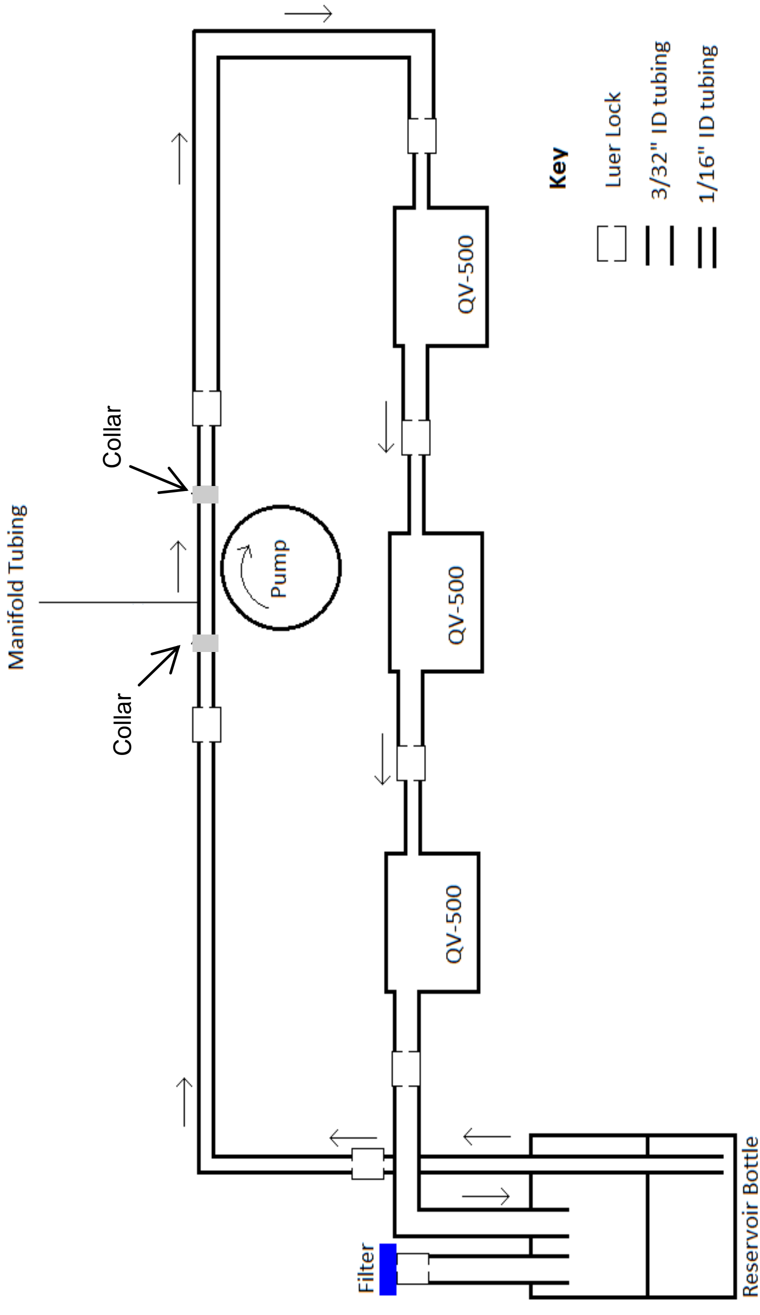

Figure 5. Schematic diagram of the system.

number of chambers employed may be adjusted for the particular experiment. Once the system is connected in this way it is recommended not to disconnect the tubes or chambers as this will increase the risk of infection.

The connected system should be washed through with sterile PBS by filling the reservoir bottle with sterile PBS and connecting the closed system to the peristaltic pump at a flow rate of 1 ml/minute for at least 20 minutes. The PBS should be removed from the reservoir bottle under aseptic conditions using a sterile pipette and disposed. The PBS in the chambers and tubes should also be removed; this can be achieved by lifting and tilting the chambers to allow the liquid to return to the reservoir through the 1/16" ID tube (normally this is the outlet from the reservoir) where it can be removed and disposed. Finally the system should be primed with the culture medium to be used in the experiment, in the same manner as the PBS wash, and removed. The system is now ready to be used in an experiment.

7. Preparation of cells

Cells should be seeded no less than 2 hours before the experiment, onto either coverslips or 3D scaffolds. If required, coverslips can be pre-treated by coating with collagen I (from rat tail) or any other coating protein for a minimum of 1 hour in the incubator or overnight at 4°C. The concentration of collagen depends on the type of cells and should be devised experimentally. As an example, for keratinocytes and fibroblasts the optimum concentration of collagen I is 0.2 mg/ml, and for human hepatocytes: 0.1 mg/ml. After incubation, the coverslips should be washed three times with PBS and either used immediately or stored in fridge for up to 1 week.

Type of cells	Number of cells	Confluence
Dermal keratinocytes	$5x10^4$	50%
	$1x10^5$	100%
Dermal fibroblasts	$2.5x10^4$	50%
	$5x10^4$	100%
Osteoblasts	$1x10^5$	100%
Chondrocytes	$1x10^5$	100%
Hepatocytes	$2.4x10^5$	100%
HepaRG	$4.2x10^5$	100%
HDMEC	$1x10^4$	100%

Table 2. Guidelines for growing different cell types.

Cells should be seeded on coverslips, typically in a 24-well plate. The number of cells depends on their type. Table 2 shows examples of seeding densities for different cell types.

Some cells, such as hepatocytes, are sensitive to flow and need to be coated by a thin, permeable upper layer, which reduces cell stress. The most commonly used coatings are collagen I and alginate. To coat the cells with collagen it is necessary to prepare rat tail collagen I solution (BD Bioscience, cat # 354236) at a concentration of 1 mg/ml 1 hour before the experiment. For detailed instruction, refer to supplier's product specification sheet and 'Alternate Gelation Procedure for Rat Tail Collagen I'. 200 µL of this solution is applied to each glass slide to cover the seeded cells, and then incubated at 37 ºC for 30 min - 1 h. The coverslips are now ready to be used in an experiment. When removing the coverslips from the plate, ensure that the collagen layer on the top of the cells is left undisturbed, and transfer the coverslips to static plates or cell culture chambers.

Note: You should take care and prevent any contact of your cells with alcohol, which might be used to sterilise coverslips or forceps. If coverslips are sterilised with alcohol, a sufficient length of time should be allowed for these to dry completely. Forceps should be additionally rinsed with PBS before use.

8. Opening and closing the chamber

a) To open the chamber grip the bottom of the chamber between three fingers, one on each of the lugs and twist the top of the chamber in an anti-clockwise direction. Lift off the top of the chamber.

b) To close the chamber push the top onto the bottom then grip the bottom of the chamber between three fingers, one on each of the lugs and twist the top of the chamber in a clockwise direction, locking the chamber when the external lugs are fully engaged.

9. Setting up an experiment

After you have cleaned and primed the system, as described in Section 6, it is ready for use. Open each of the chambers under aseptic conditions in the Class II tissue culture hood. Add approximately 1 ml of the required medium to each chamber. Gently lift the seeded coverslips or scaffolds from the wells of the static culture plate,

with sterile forceps, and transfer each one to the bottom of a separate chamber, with the cells uppermost (Figure 7). Lock the chamber as previously described.

Fill the reservoir bottle with the medium of your choice; approximately 4 ml per chamber plus some extra medium. For example, for 2 chambers we suggest adding between 10-20 ml of medium. The volume of medium used depends on the length of the culture period and can be optimised for the specific cell type. It will also depend on the length of the tubing in your system. If your pump is kept outside the incubator, your system may have more tubing and therefore require a greater volume of medium. Carefully screw on the top of the reservoir bottle. If highly proliferative cells are used, it is advisable to use medium with no serum added, to prevent excessive cell proliferation. The system is now closed and the experiment may begin; the system should be placed inside a CO_2 incubator and connected to a peristaltic pump.

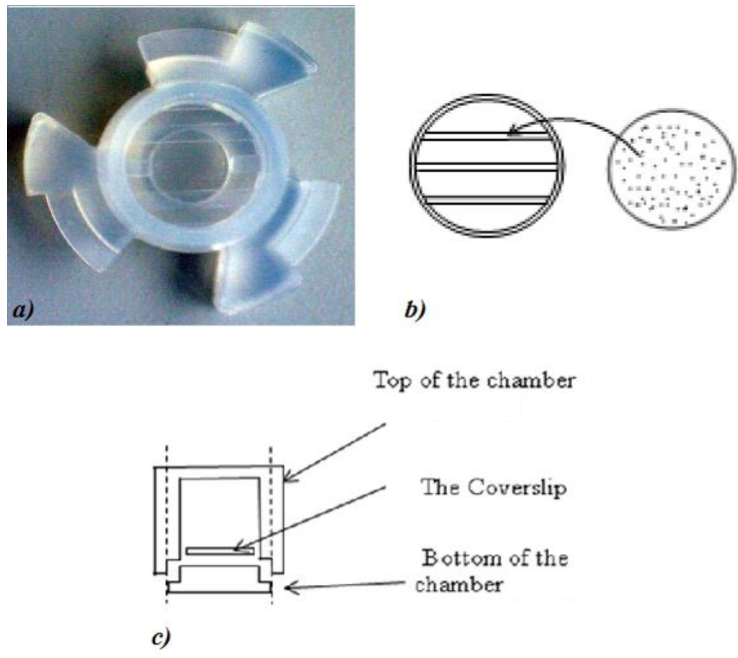

Figure 7. a) Base of the chamber with visible ridges on the bottom, b) Putting the 12 mm cell seeded coverslip onto the bottom of the chamber, c) Schematic representation of the assembled chamber. Cells are on the top of the coverslip.

10. Flow rates

The flow rate for each cell type and system configuration must be carefully chosen. Most cells prefer a flow rate between 25-600 μL/min, but the optimum flow rate should be determined for each experimental set-up.

Therefore, when using the system it is strongly advisable to calibrate and measure the flow rate of the liquid circulating in the system. The flow rate will vary depending on the number of chambers, the diameter of the tubing (Figure 8), the length of the tubing and the position of the chambers and reservoir in relation to the pump (for instance, placing on a higher shelf in the incubator) and most importantly – on the type of pump used. The flexibility of the system allows a wide range of options, e.g. attachment of different combinations of chambers and tubing. Any modification should be followed by calibration of the system. Figure 8 shows the relationship between the volume flow rate and pump speed depending on the diameter of the tubing used.

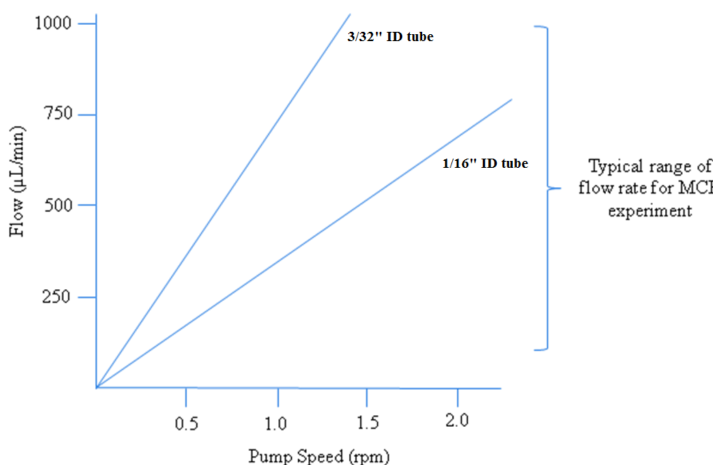

Figure 8. The relationship between the volume flow rate and pump speed for the two diameters of tubing used.

11. Calibrating the system

To calibrate the system, run the required configuration with sterile PBS at various pump speed settings and collect the liquid output over 1 minute from the final chamber. At each speed setting, measure the volume of liquid circulated by the increase in mass of the collection container after 1 minute. It is important that the air is expelled from the system before starting to measure the flow rate. Repeat this procedure three times for each setting to obtain a mean value.

12. Disassembling the *Quasi-Vivo*® system

Remove the ***Quasi-Vivo***® system from the incubator and switch off the pump. Remove the medium from the reservoir, chambers and connecting tubes. Open the chambers and remove the coverslips, placing them in a fresh 24 well plate for analysis. Lock the chambers so the system is ready for cleaning.

13. Cleaning the *Quasi-Vivo*® system

Ensure that all the chambers and tubing are securely connected together, as for an experiment, and fill the reservoir bottle with sterile PBS which can be supplemented with antibiotics. Circulate the PBS through the whole system at a high speed (1 – 2 ml/min) for a few hours. It is important that the PBS should circulate a minimum of two times throughout the whole system. The time required for this will depend on the number of chambers and hence volume of the system.

14. Sterilisation and cleaning procedures

The chambers can be used for more than one experiment without autoclaving, if the rules of aseptic techniques to prevent contamination are strictly followed. In this case it is advisable to circulate 70% ethanol through the whole system and rinse **VERY** thoroughly with sterile PBS (ideally overnight) to remove any traces of ethanol which might affect cell viability. Tubing, connectors, chambers and reservoir bottles can be stored after opening for longer periods of time. In this case, tubing and connectors should be stored submerged in 70% ethanol; chambers and reservoir bottles should be rinsed with 70% ethanol followed by sterile PBS (long exposure to ethanol can damage the surface of silicone chambers), emptied and stored until needed.

Chambers, tubing and reservoir bottles are gas-plasma sterilised which is the optimum and least harmful sterilization method for silicone chambers and tubing. Silicone parts can also be sterilised by using standard autoclaving procedure (121ºC, 15 psi, 15 min) and also γ-irradiation. However, to maintain reliability of the system we advise not to exceed more than 3 sterilization cycles for any component. Also, we advise users NOT TO wash whole system with sodium azide which can accumulate on the surfaces of chambers and leak out during the experiment. The translucent polypropylene connectors supplied with the kit are autoclave safe, but can be also sterilized by gamma radiation.

15. Troubleshooting

1. An air bubble has entered the system.

If this occurs as the system is filling up, this is normal and any bubbles will be removed in time by the slant in the roof of the chamber. Ensure that the chamber is connected the correct way round; thin tube at the inlet, thick tube at the outlet. Bubbles will be removed from the system when they enter the reservoir.

2. The system starts to leak.

This should not happen in normal operation. Check all the connections between tubes and ensure the chambers are correctly assembled. Check the tubing for breaks due to fatigue.

3. There is a suspected infection.

Remove coverslips and inspect under a microscope. Save a sample of the media and keep it in the incubator to establish whether an infection develops. Clean the system thoroughly with ethanol and autoclave.

4. The chambers are stiff to open.

Take the chamber in the palm of your hand and squeeze. This should loosen the silicone. After opening and closing the chambers a few times they should become less stiff and easier to work with.

16. FAQ

1. How do I optimise the flow rate for my cell type?

Use the guidelines we have given in this booklet to find a range of possible flow rates. Set up the system and compare the viability of cells cultured for 24 hours (or some other appropriate time scale for your cell type) at each flow rate in this range. Coverslips of cells can be transferred from the chambers into a 24 well plate and assays such as alamarBlue® or MTT may be performed to assess viability. The flow rate which produced the highest viability compared to the other flow rates and the static control should be used.

2. How do I assemble the system?

The system is provided as a modular kit that can be connected in any configuration that you choose, to allow a variety of experiments to be devised. Please read

sections 6, 8 and 9 of this booklet which provides instructions on the setup of the system. To supplement this document, regular training courses are also available to give detailed instruction and advice on using the system.

3. Can you reuse the system?

QV500 chambers can be autoclaved up to 3 times. The reservoir bottle, tubing and Leur Lock components can be autoclaved repeatedly.

4. What happens if the cells detach?

The silicone used in the system discourages cells from attaching, so even if cells are dislodged by flow, they will not attach elsewhere in the system.

5. How do you fill the system?

We recommend transferring coverslips from static well plates to 1mL new medium in each chamber. The rest of the medium is added to the reservoir bottle, 4 ml per each chamber.

6. How do you get cells to adhere to the cover slip?

Different types of cells adhere under different conditions – some cells prefer glass coverslips (such as HDMEC's [human dermal microvascular endothelial cells] or NCI's [cancer cells]), whereas others prefer plastic coverslips (such as hepatocytes). In addition, cells may require a layer of cell matrix coating in order to encourage their attachment such as collagen, for optimal seeding conditions for a certain cell type, it is best to refer to the literature.

7. Is the media recirculating or single pass?

The system is designed to use recirculating media, which allows cells to condition the medium with growth factors and signalling molecules, and therefore improves growth, viability, and the system's ability to model the in vivo environment. However the system can easily be set up to allow single pass where required.

8. Can you use the system to maintain cell lines under flow?

Yes, primary human hepatocytes have been maintained for up to a month after isolation using this system.

9. Is there sufficient metabolite to do Western Blot?

This would depend on the protein to be studied. Each chamber will contain between

250K-300K cells depending on cell types. Highly-expressed proteins such as those in ribosomes should be detectable in a small system of 2 chambers, but for proteins with lower expression, more chambers may be required.

10. *Can you do enzymatic studies?*

Yes. The amount of cell material needed will depend on the enzyme studied, its relative expression and the nature of the study (see ‘7’).

11. *Are there some genes not stimulated by flow?*

So far the focus of gene study has been on a small number of important genes in hepatocytes, all of which were found to be upregulated under flow compared to static.
There may be some genes whose regulation is unaffected by the presence of flow per se, but the cell and culture as a whole will benefit from both the nutrients being refreshed and toxins/metabolic products taken away.

12. *Is cell morphology different when cultured under flow?*

There are subtle differences such as hepatocytes appearing more obviously binucleate when grown under flow.

13. *Can you create models of barriers formed by tight junctions in Caco2 cells?*

Not in the QV500 chambers, however it is possible to use inserts to adapt our QV600 ALI chamber for this purpose.

14. *What is the difference between endothelial and epithelial cells?*

Epithelium is one of the four basic types of animal tissue, along with connective tissue, muscle tissue and nervous tissue. Epithelial tissues line the cavities and surfaces of structures throughout the body, and also form many glands. Functions of epithelial cells include secretion, selective absorption, protection, transcellular transport and detection of sensation. In Greek "Epi" means, "on, upon," and "Theli" meaning "tissue."
The **endothelium** is the thin layer of cells that lines the interior surface of blood vessels, forming an interface between circulating blood in the lumen and the rest of the vessel wall. These cells are called **endothelial cells**. Endothelial cells line the entire circulatory system, from the heart to the smallest capillary. These cells reduce turbulence of the flow of blood, allowing the fluid to be pumped farther.
Endothelial tissue is an epithelial tissue.

15. Does the pump go outside or inside the incubator?

It can be either; this will depend on investigator preference. According to our users, the inside/outside split is about 50:50. We can make recommendations for pumps that will work in humid or non-humid incubators if needed.

16. Do you need to coat the coverslips before you put them in the chambers?

Yes, we advise that coverslips are coated with 1mg/ml collagen both before and after cell seeding.

17. Why do you coat them in collagen, what properties does it have?

The collagen coating shields cells from laminar flow, and prevents them from detaching.

18. How many chambers can be connected to the same reservoir bottle?

Up to 6 chambers (1 tray) can be connected to a single 30 ml reservoir bottle. At the time of printing, the maximum number of chambers used in an experiment, in multiple circuits, was 32.

19. Can the chambers represent organs?

Yes, cells from different tissues can be cultured separately and connected together, to model organ interaction.

20. What cell types have we used and how do we use them?

For types used, you can refer to the leaflet. For how, this will be dependent on the cell type. If it isn’t a cell type that we’ve worked with (which includes rat primary hepatocytes, NCI H292, HepaRG and HDMECs) then it is best that they refer to the literature for how cells have been cultured.

21. How many systems can fit in an incubator?

Depends on the size of the incubator and whether the pump is kept inside or outside. We have fit 18 2-or-more chamber systems into one incubator containing two pumps.

22. How do you set up a typical experiment?

Cells are seeded onto coverslips in a static 24-well plate and cultured until confluent.

System components (chambers, reservoir bottle and tubing) are autoclaved, and systems are connected the day before, and washed with PBS then primed with antibiotic-free medium overnight to check for infection. Then cells on coverslips are coated with 1mg/ml collagen for 30min, and transferred to either chambers or static controls. Cells are incubated under flow or static conditions for as long as is required.

23. How long can cells be cultured for?

Hepatocytes have been cultured for a month and have retained their phenotype and CYP gene expression. The length of the experiment can be varied from 1 to 3 day experiments, to week or month-long studies and potentially longer.

17. Glossary

Autoclave	Pressurized device designed to heat aqueous solutions above their boiling point at normal atmospheric pressure to achieve sterilization. Typical autoclaving cycle in tissue culture labs is 121 °C for 15 minutes or 134 °C for 3 minutes, although longer sterilization cycles (134 °C for 18 minutes) may be necessary for removal of some bacterial strains or prions.
Chemical sterilization	Alternative to heat sterilization used to protect heat-sensitive materials. Low temperature gas sterilizers function by exposing the articles to be sterilized to high concentrations (typically 5 - 10% v/v) of very reactive gases (alkylating agents such as ethylene oxide or oxidizing agents such as hydrogen peroxide and ozone). While the use of gas and liquid chemical sterilants/high level disinfectants avoids the problem of heat damage, users must ensure that the article to be sterilized is chemically compatible with the sterilant being used.
Ethylene Oxide	(EO or EtO) gas is commonly used to sterilize objects sensitive to temperatures greater than 60°C such as plastics, optics and electrics. Ethylene oxide treatment is generally carried out between 30°C and 60°C with relative humidity above 30% and a gas concentration between 200 and 800 mg/L for at least 3 hours. Ethylene oxide is highly effective, however is highly flammable, requires a longer time to sterilize than any heat treatment and also requires a period of post-sterilization aeration to remove toxic residues.

Gas plasma sterilization	This form of chemical sterilization is also known as low-temperature gas plasma (LTGP) sterilization. An aqueous hydrogen peroxide solution is boiled in a heated vaporizer and then flows as a vapor into a sterilization chamber at low pressure and low temperature. Exposure to the hydrogen peroxide vapor and plasma for a controlled time completes the sterilization procedure.
Quasi-Vivo® Systems	Multichamber bioreactor or Inter-connected Cell culture system, general term for the flow system.
Radiation Sterilization	Methods of sterilization by using radiation such as electron beams, X-rays, gamma rays, or subatomic particles. Irradiation with X-rays or gamma rays does not make materials radioactive. Irradiation with particles may make materials radioactive, depending upon the type of particles and their energy, and the type of target material. Neutrons and very high-energy particles can make materials radioactive, but have good penetration, whereas lower energy particles (other than neutrons) cannot make materials radioactive, but have poorer penetration.
rpm	Revolutions per minute (abbreviated rpm, RPM, r/min, or $r \cdot min^{-1}$) is a unit of frequency: the number of full rotations completed in one minute around a fixed axis.
Shear stress	In this context: the sideways force per unit area on a surface due to flow of medium across that surface.
Sterilization	Process of killing or eliminating transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) Sterilization can be achieved through application of heat, chemicals, radiation, high pressure or filtration.

Kirkstall Ltd
3 Aspen Court, Aspen Way
Centurion Business Park
Rotherham
S60 1FB
Tel: 01709 361 241
www.kirkstall.org