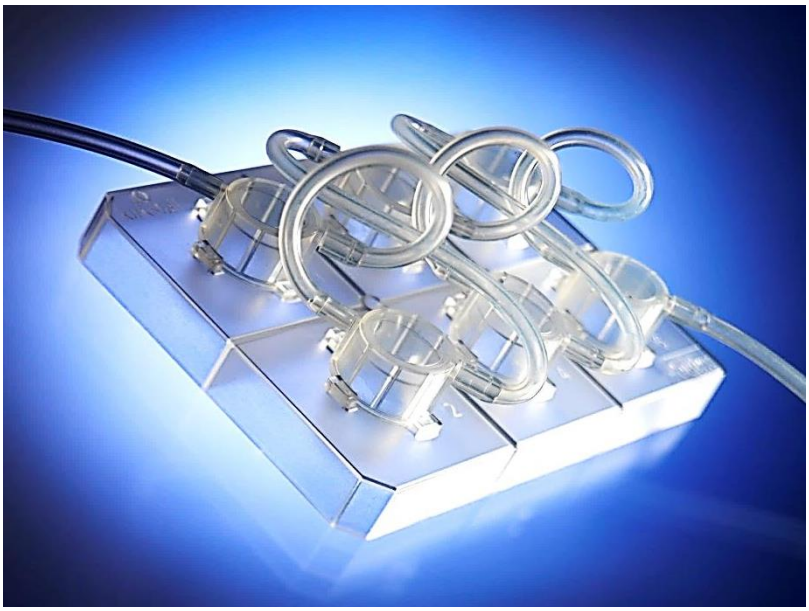
kirkstall

Quasi Vivo® System:

Tools for Physiologically Relevant *in vitro* Cell Culture



User Manual for QV900 Tray System

Issue Number 1.0

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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*.

The QV900 tray design enables the culture of any adherent primary cell or cell line, which can be grown as a monolayer or on a 3D support structure. It is not currently designed for culture of cells in suspension. ***Quasi Vivo***® 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.

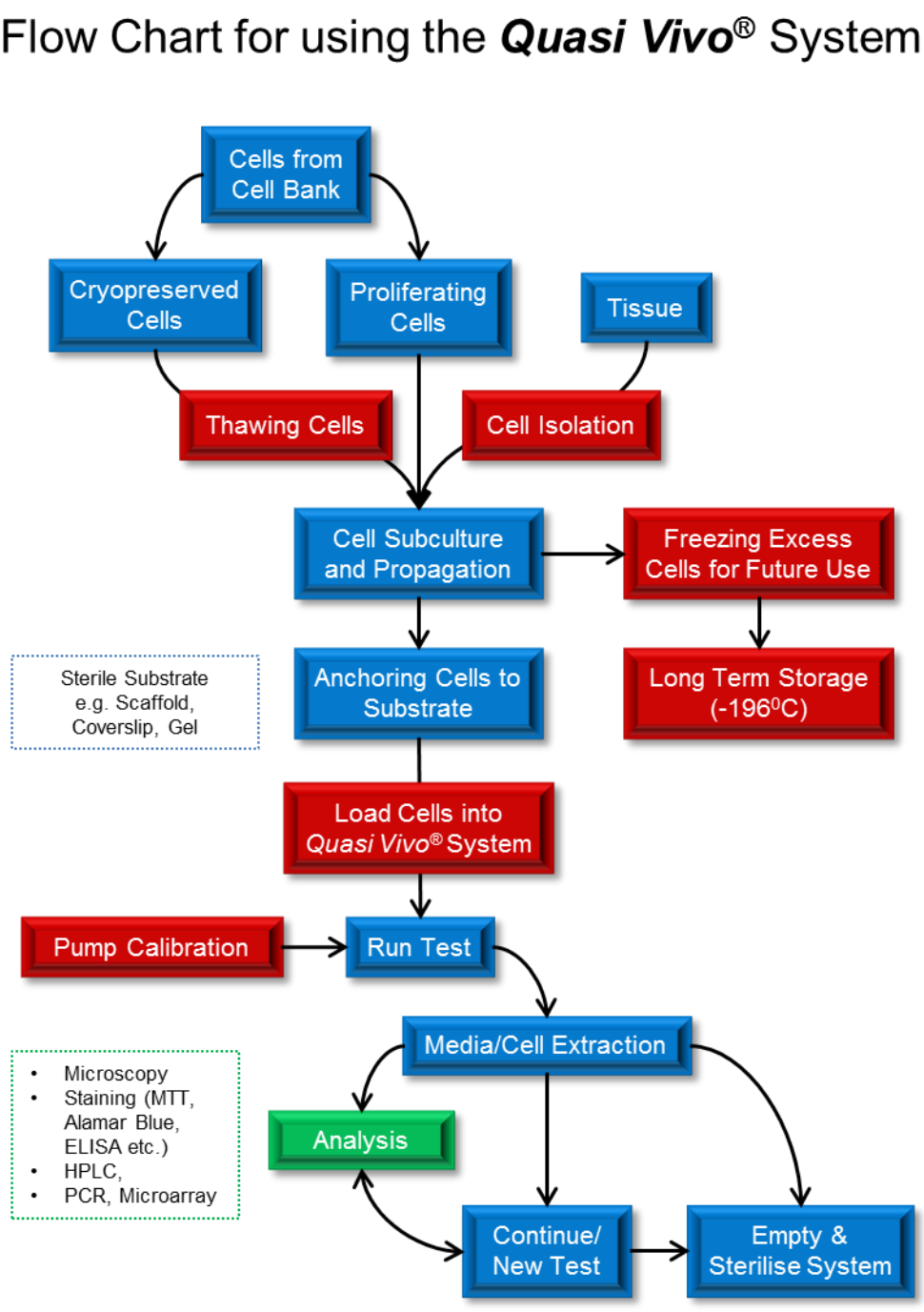

*Figure 1. Flow chart explaining the practical implementation of the **Quasi Vivo®** system, and how it fits into current tissue culture protocol. It is recommended that you should calibrate your pump before starting an experiment. Refer to sections 4 and 11 for more information.*

3. Outline description of cell culture chambers

The plastic tray contains 6 individual chambers, and forms the core of the ***Quasi Vivo®*** system. ***Quasi Vivo®*** trays are made of Altuglas® a medical grade, optically clear plastic. The external dimensions of the tray are 85.5mm x 127.8mm, equal to those of standard laboratory well plates, and each chamber has an internal volume of 4.3ml. The chambers are designed to accommodate 12-14mm diameter coverslips and are compatible with various 3D scaffolds.

WARNING:

IMS solutions, used in many laboratories, react with the plastic used in QV900 trays, resulting in clouding and damage to the tray. Where the experimental procedure requires sterilisation of the exterior of the tray (such as returning to a sterile hood after incubation), we recommend the use of Ethanol or Isopropl Alcohol, which should not affect the trays.

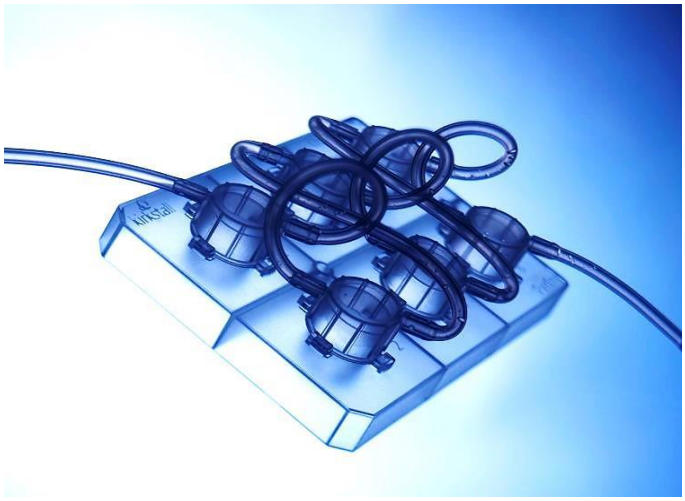

*Figure 2. The **Quasi-Vivo®** 900 Tray*

The QV900 tray system is designed as a single-use disposable product, and is not suitable for autoclaving. Each tray is sterilised using gamma radiation following manufacture and is supplied sterile in sealed packaging. Tubing and reservoir bottles are autoclave safe; however repeated autoclaving will cause the silicone components to lose flexibility and become discoloured. We thus recommend replacing these components after three autoclave cycles.

The sterile QV900 trays are used for cell culture in non-therapeutic and non–diagnostic applications. Each tray contains a base unit and six separate chamber lids, as shown in Fig. 3.

Quasi Vivo® QV900 trays are available in a range of packs, as well as complete starter and evaluation kits, which in addition to the trays and connecting tubing contains two reservoir bottles with air filters and loading trays, and extension tubing, luer connectors and a user guide.

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 for incubation periods of 24 hours or longer.

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

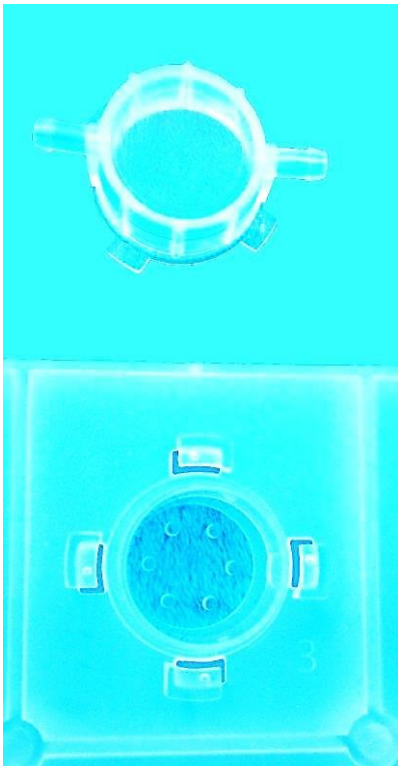

Figure 3. Components of a QV900 tray.
Upper: Chamber Lid.
Lower: QV900 tray, incorporating chamber.

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 Fig 4. for a demonstration of the system setup including the pump.

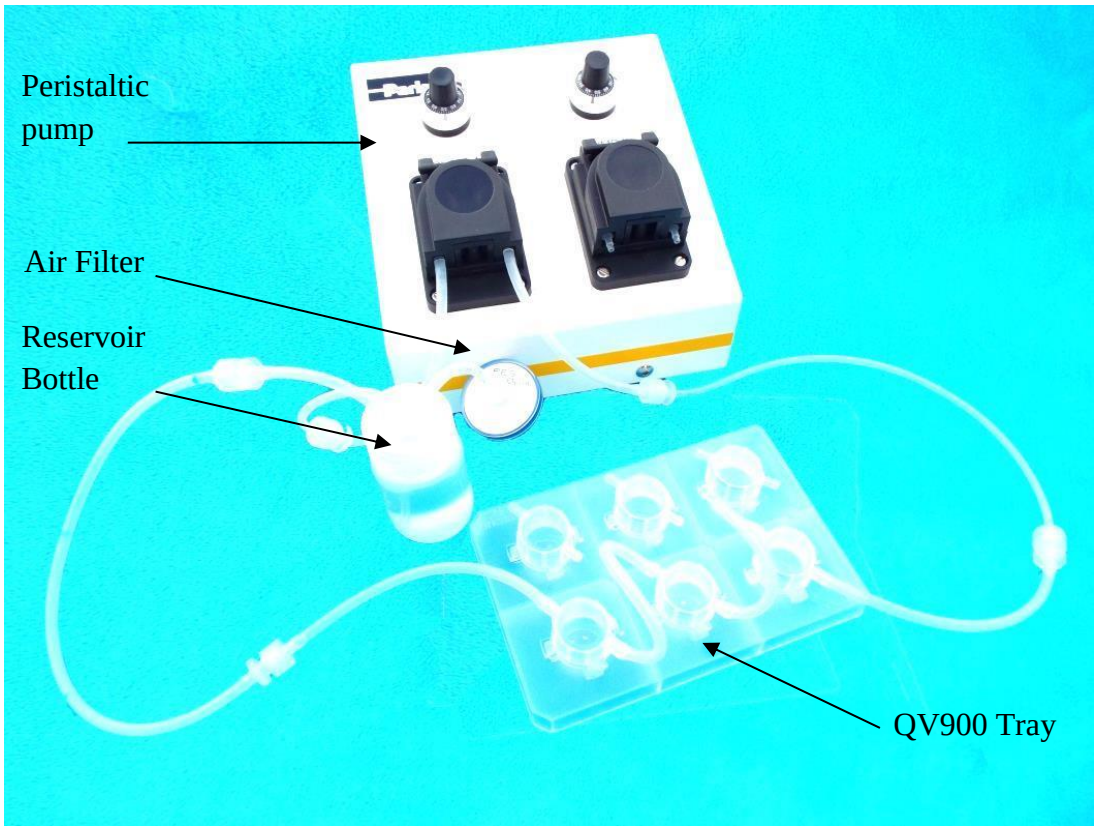

Figure 4. **QV900** *system setup connected to* ***Parker PF22X0103*** *peristaltic pump.*

	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: 14.5 d:14.5	• Low cost • Designed for wet/dry incubator • 2 independently controlled pump heads • Small and light • Ideal for 1 or 2 channel experiments	• Only 2 connections • No digital display • Manually determine flow rate	1050	Standard tubing with collars supplied

Table 1. Descriptions of suitable pump

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 bottles are supplied sterile and with the luer connectors attached. The QV900 trays and associate tubing are also supplied sterile, but require assembly into the required configuration under aseptic conditions alongside the rest of the system. 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 barbed ends of the luer connecters are a push fit into the tubing with the same listed internal diameter (ID) as the connector size, i.e. 1/16" connectors fit the 1/16" ID tubing. These assembled components should then be sterilised (we recommend autoclaving).

The reservoir bottle has three tubes connected to it; the longest tube (1/16" ID) is the inlet tubing and should be submerged in medium, the middle length tube (3/32" ID) is the reservoir outlet tubing 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 syringe filter should be connected to this gas exchange tube.

Following the system round in a loop: the inlet 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 connector tubing (3/32" ID, 10cm in length) with female leur, the other end of which is attached to inlet of first chamber, connector tubing without luers is used to outlet on one chamber to the inlet of the next chamber in series. The chamber lid is sloped, with the inlet lower than the outlet, as demonstrated in fig. 6.

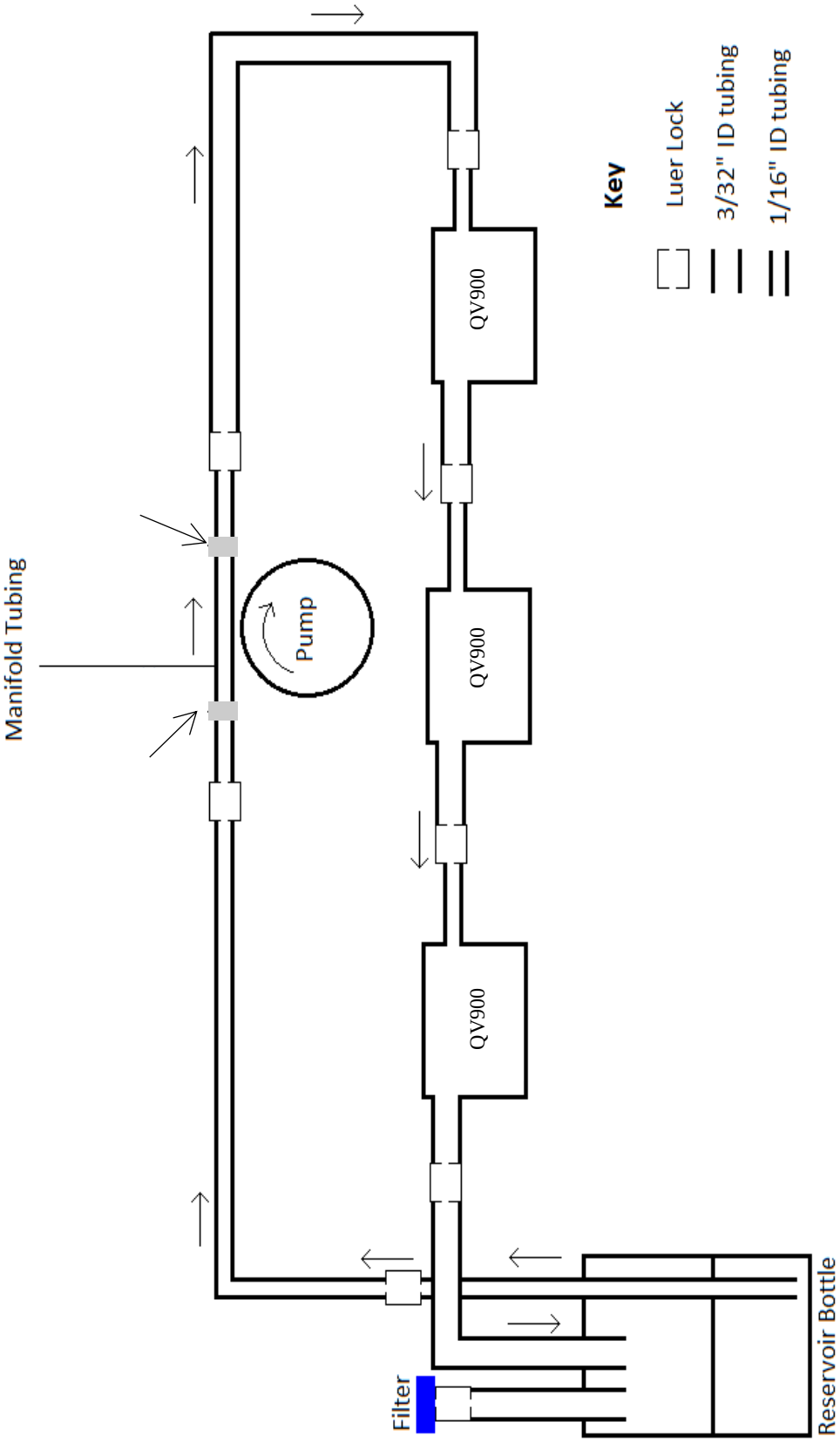

Figure 5. Schematic diagram of the system.

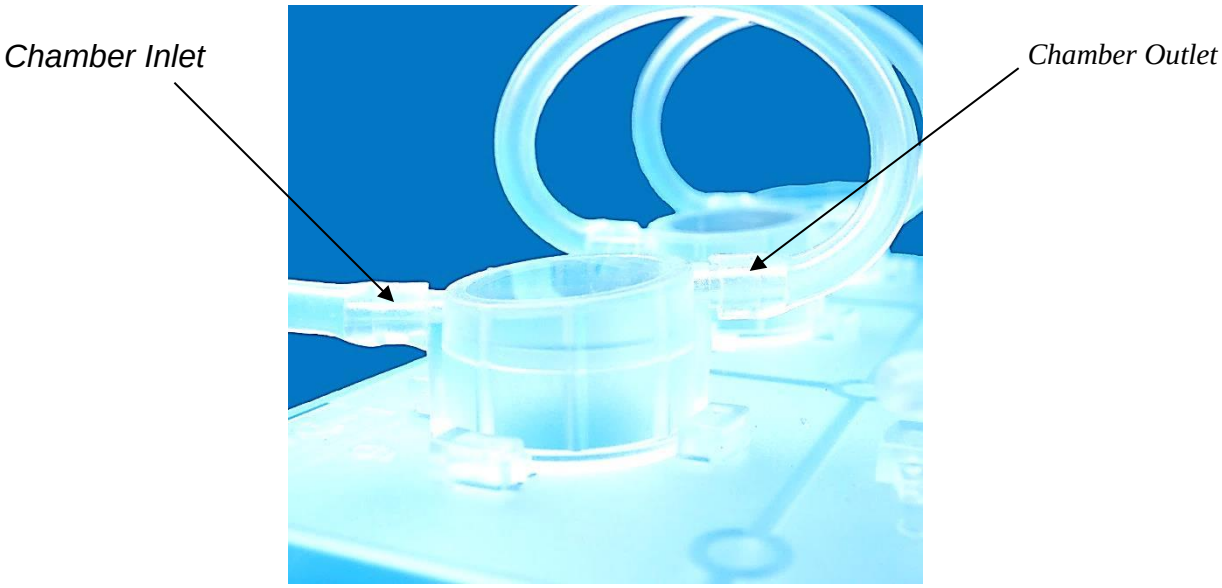

Figure 6. Illustration of the chamber inlet and outlet.

The outlet of last chamber is connected to connector tubing with male leur attached, which is used to connect to the 3/32" ID extension tube (22 cm in length). This extension tubing is connected to the outlet tubing of the reservoir, allowing for the return of medium to the reservoir. The tubing supplied in the standard tray tubing pack allows the setup of either two series of 3 chambers or one series of 6 chambers.

Figure 5 shows three chambers connected in series, however, the 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 during the experiment, as this will increase the risk of contamination.

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. QV900 chamber configurations

The circular design of the chamber means that the experimental environment will be the same for each chamber regardless of the lid orientation permitting that flow always runs in the direction of inlet to outlet. Any number of chambers on the tray can be utilised for an experiment, depending on experimental design.

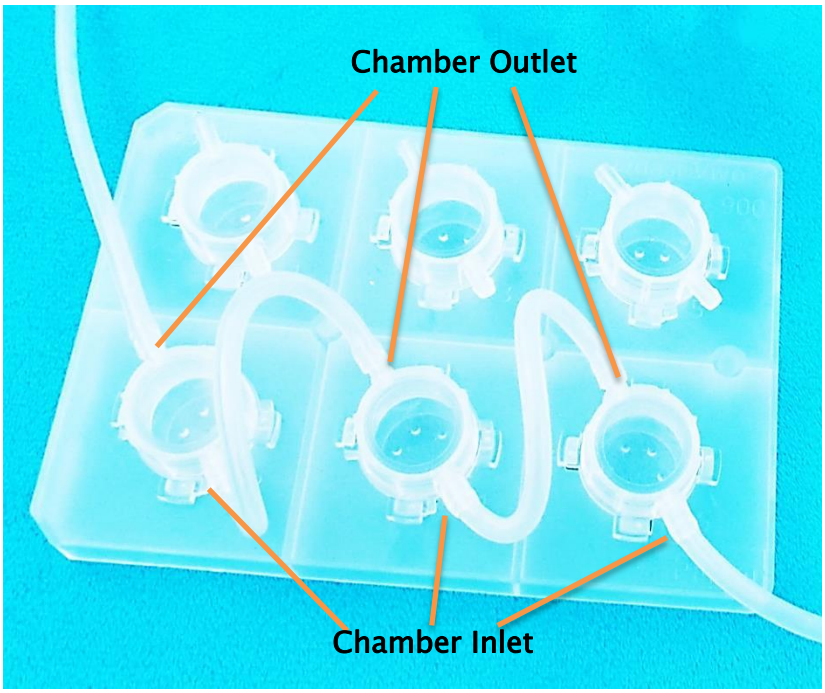

Figure 7a) 3 chambers of QV900 connected in series

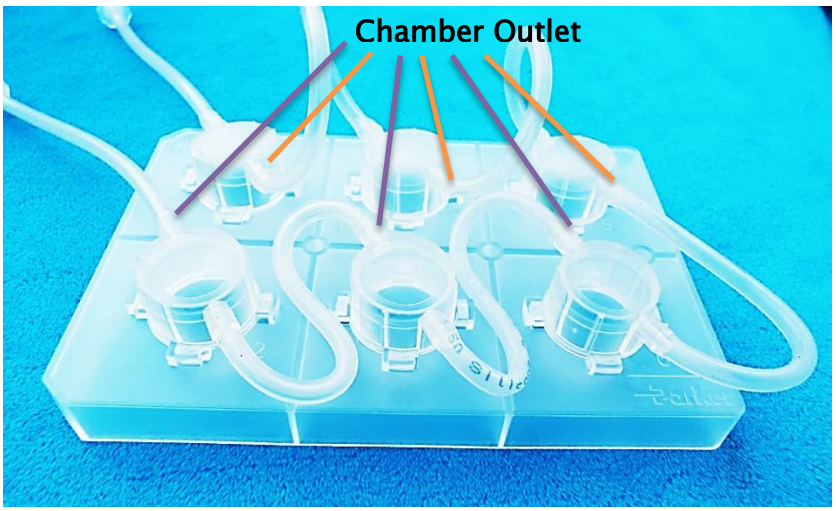

Figure 7b) All 6 chambers of QV900 connected in series

Figure 7 shows optimum tubing configurations and lid orientations for both 3 & 6 chambers in series. When running 2 repeats of 3 chambers in series it is recommended that all chamber lids are in the same orientation to the tray base as shown in Fig. 7 a. When using the tray with all 6 chambers in series it is recommended that the outlets face the centre divider of the tray base (that runs between chambers 1, 3, & 5 and chambers 2, 4, & 6) as shown in Fig. 7b.

8. 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 ECM 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 optimum concentration of collagen I for keratinocytes and fibroblasts: 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.

Similarly cells can be seeded directly onto the optically-clear base if the QV900 chambers, after coating with ECM proteins as per the method described for culture on coverslips.

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.

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.

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.

9. Opening and closing the chamber

a) To open the chambers grip the side of the chamber lid between thumb and forefinger, and twist in an anti-clockwise direction until the retaining lugs are clear of their housings on the tray, and lift off the chamber lid.

   Note: When gripping the chamber, be careful to hold the ridges on the side, **not** the inlet & outlet of the chamber, as using these to twist the lid may damage the lid.

b) To close the chamber, hold the lid by the sides and push onto the chamber with the inlet and outlet approximately 45 degrees anti-clockwise of the desired orientation. The lid will ‘click’ as the internal sealing ring of the lid engages with the lower chamber. Twist the top of the chamber in a clockwise direction, until the external lugs are engaged.

10. 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. 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). Add approximately 2 ml of the required medium to each chamber, this will prevent cells from drying out when using the low flow rates required. 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 15-20 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.

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.

Note: Medium volume will also depend on length of tubing in the system; pumps kept outside the incubator require additional tubing and therefore a greater volume of medium.

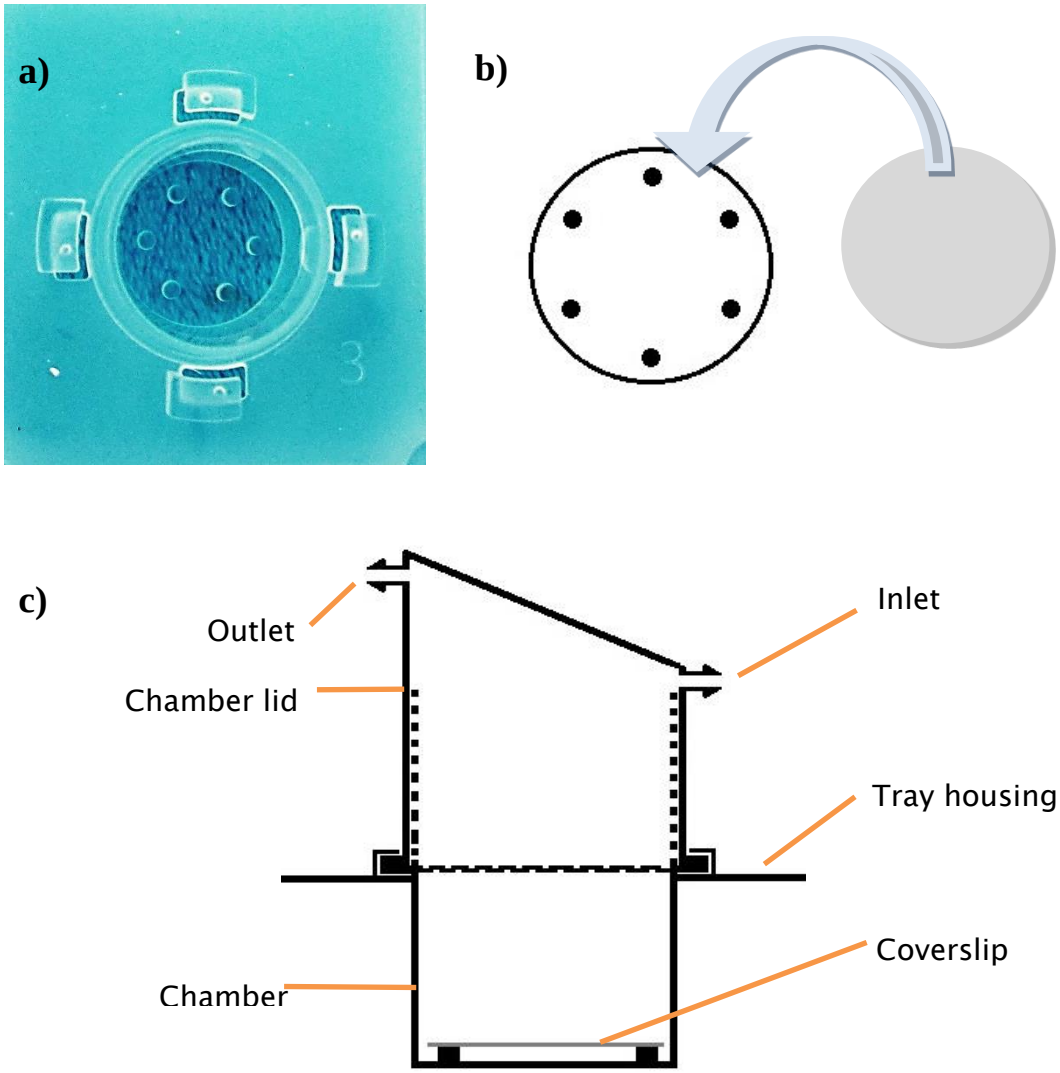

Figure 7. a) Base of the chamber with posts on the bottom, b) Putting the 12 mm cell seeded coverslip onto the bottom of the chamber, c) Schematic representation of the assembled chamber. Cells are on the top of the coverslip.

11. 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 , the length of the tubing 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.

12. 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 5-10 minutes from the final extension tubing before the reservoir. At each speed setting, measure the volume of liquid circulated by the increase in mass of the collection container per 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.

13. 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, and remove the tray from the system. Open the chambers and transfer coverslips/ scaffolds to a fresh 24 well plate for analysis, if cells have been seeded directly onto QV900 then continue analysis in tray.

14. 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.

15. Sterilisation and cleaning procedures

The trays are intended as single use disposable products, and after use should be disposed of in the same manner as other cell culture plasticware.

Tubing, connectors, and reservoir bottles can be stored after opening. In this case, tubing and connectors should be washed with 70% ethanol; this should be circulated on a high flow rate for minimum of 1hr.

Reservoir bottles are sterilised by using standard autoclaving procedure (121ºC, 15 psi, 15 min). Silicone parts can also be sterilised by gas-plasma and γ-irradiation. However, to maintain reliability of the system we advise not to exceed more than 3 sterilsation cycles for any component.

If the system is to be stored without autoclaving, a final wash with de-ionised water should be performed and the system left to dry out thoroughly before storing. If using this cleaning method, it is recommended that 70% ethanol should be circulated through re-used components before the priming steps of the subsequent experiment.

Also, we advise users NOT TO wash whole system with sodium azide which can accumulate on the surfaces and leak out during the experiment. The translucent polypropylene leur connectors supplied with the kit are autoclave safe, but can be also sterilsed by gamma radiation.

16. 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; the lower barb should be the inlet. 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.

Residual moisture around the inside of the chamber lid can cause continual slow leakage from the chambers. If this is the case, ensure the contact area between the chamber and lid is completely dry before reassembling the system.

3. There is a suspected contamination.

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

17. 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?*

The QV900 tray cannot be reused. Reservoir bottles, tubing and Leur Locks can however 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 2mL 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 the ***Quasi Vivo***® 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 cells 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 QV900 tray, however it is possible to use inserts to adapt our QV600 ALI chamber for this purpose.

14. *Does the pump go outside or inside the incubator?*

It can be either; this will depend on investigator preference and pump choice. 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.

15. *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.

16. *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.

17. *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.

18. *Can the chambers represent organs?*

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

19. *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.

20. *How many systems can fit in an incubator?*

This will depend on the size of the incubator and whether the pump is kept inside or outside. Using other ***Quasi Vivo***® products, we have fitted the equivalent of 6 trays into one incubator containing two pumps.

21. 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 contamination. 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.

22. 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.

18. Glossary

Autoclave	Pressurized device designed to heat aqueous solutions above their boiling point at normal atmospheric pressure to achieve sterilsation. Typical autoclaving cycle in tissue culture labs is 121 °C for 15 minutes or 134 °C for 3 minutes, although longer sterilsation cycles (134 °C for 18 minutes) may be necessary for removal of some bacterial strains or prions.
Chemical sterilsation	Alternative to heat sterilsation used to protect heat-sensitive materials. Low temperature gas sterilsers function by exposing the articles to be sterilsed 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 sterilsed is chemically compatible with the sterilant being used.
Ethylene Oxide	(EO or EtO) gas is commonly used to sterilse 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 sterilse than any heat treatment and also requires a period of post-sterilsation aeration to remove toxic residues.

Gas plasma sterilsation	This form of chemical sterilsation is also known as low-temperature gas plasma (LTGP) sterilsation. An aqueous hydrogen peroxide solution is boiled in a heated vaporizer and then flows as a vapor into a sterilsation chamber at low pressure and low temperature. Exposure to the hydrogen peroxide vapor and plasma for a controlled time completes the sterilsation procedure.
Quasi Vivo® Systems	Multichamber bioreactor or Inter-connected Cell culture system, general term for the flow system.
Radiation Sterilsation	Methods of sterilsation 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.
Sterilsation	Process of killing or eliminating transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) Sterilsation can be achieved through application of heat, chemicals, radiation, high pressure or filtration.

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