Comparative Testing of Single Use Pump Technologies Used In Downstream Processing For Watson-Marlow Fluid Technology Group

Conducted by
PDS Sandbox LLC

Version 2.0

PDS Sandbox

Abstract

After comprehensively reviewing the capabilities and performance of the Watson-Marlow Quantum 600 peristaltic pump, it is the position of PDS Sandbox that the data generated during these tests, demonstrates an excellent level of precision across a full range of pressures (up to 4 barg), with low shear that is approximately 50% lower than that of the Quattroflow QF1200SU pump from PSG Dover.

The test report also confirms that the Quantum 600 pump has minimal impact on CHO suspension cell cultures. This performance is better than would be expected with traditional peristaltic pumps and is comparable to the Levitronix PuraLev®200SU centrifugal pump but without the limitations of a strong pressure/flow dependency. PDS Sandbox has subsequently used the Quantum 600 pump for perfusion cell culture with great success.

Also, based upon the excellent accuracy of the Quantum 600 peristaltic pump, PDS Sandbox can recommend that this product is used for the delivery of target volumes, in many cases removing the need for secondary checks (load cells, scales, flow meters). This provides for a simple solution where, after calibration, the pump can be run at a set speed and time to deliver a desired volume accurately enough for many applications.

The overall conclusion of this report is that the Watson-Marlow Quantum 600 peristaltic pump successfully addresses many of the current limitations seen in single use pumps. It is accurate across a wide range of pressures and flows and exhibits very low shear. It also has a low pulsation characteristic that has been optimized for higher flows. This pump comes with a simple, intuitive control interface and is a robust flexible design that will deliver performance enhancements in a range of biopharmaceutical applications.

Introduction

At Process Design Solutions (PDS) we provide state of the art, best in class, process engineering solutions to the biotech, pharmaceutical and medical device industries. We operate a 10,000sq/ft facility to test feasibility, develop new processes/products/cycles, scale up, and troubleshoot/evaluate new technologies for both facility end users and suppliers at our headquarters in Hudson, New Hampshire.

We offer a wide, but focused, array of Single Use Engineering and Process Development services to a variety of clients from new start ups just entering the BioPharm Space to well established Single Use suppliers and everyone in-between.

Our services were commissioned by Watson-Marlow in order to compare the performance the following pump technologies used in biopharmaceutical processing.

Table 1: Products being compared in this trial

Manufacturer	*Product*	*Pump Technology*
Watson-Marlow	Quantum 600 Universal with ReNu SU Technology® cartridge (20/3P)	Peristaltic Pump
PSG Dover	Quattroflow QF1200SU	Quaternary diaphragm pump
Levitronix	PuraLev®200SU	Centrifugal pump

Quantum 600 Universal Pump

ReNu SU Technology® cartridge

The comparative testing included the following tests:

1. Mechanical shear test (A) using oil based emulsion and Sauter mean diameter measurement.
2. Mechanical shear test (B) using CHO suspension cell cultures.
3. Lifecycle metering performance testing.
4. Temperature testing – heating of equipment, process stream and reservoir
5. Pump Curve (Flow/Pressure and RPM/Flow)
6. Pulsation testing at different flow rates and pressures

1. Mechanical shear test (A) using oil based emulsion and Sauter mean diameter measurement.

1.1 Introduction

The purpose of this test was to compare the mechanical shear forces generated by different pump types by measuring the break down of fragile oil emulsion particles which were recirculated through the pumps.

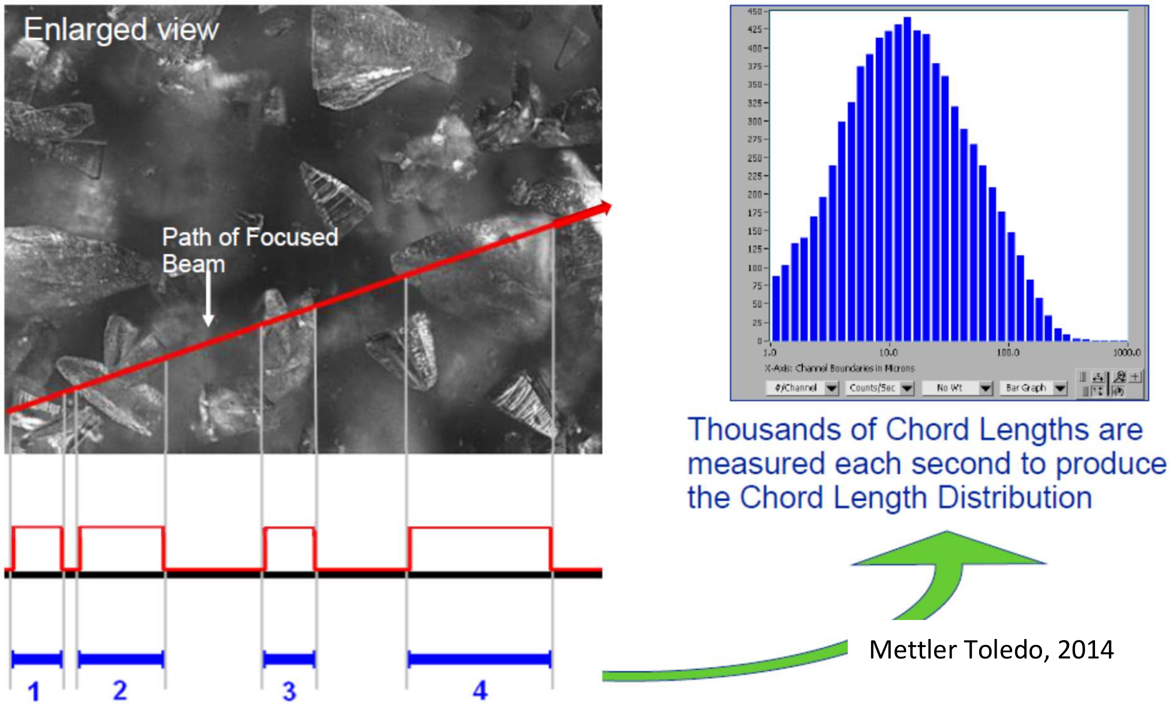
Since mechanical forces encountered by cells during normal manufacturing/operating conditions can result in variability to cell culture viability, cell culture health, product formation and quality, these shear forces are of great interest to researchers and manufacturers of biological products. Understanding and controlling the impact of these forces allows for greater manufacturing consistency of biologicals. Realizing the full impact of these forces and the resulting levels of shear caused by various single use pump technologies will aid in choosing the most appropriate/relevant technology for use within these processes. PDS Sandbox implemented a testing regime to test the Watson-Marlow Quantum 600 peristaltic pump and analyze the results against the previously tested pumping technology, the Quattroflow QF1200SU diaphragm pump.

Early testing utilizing an emulsion based Sauter Mean diameter testing by both Wollny and Maass et al., 2011, proved to be a reliable method of determining shear induced by mechanical stresses which were later employed by Dittler and Kaiser et al., 2014, to compare several Single Use (SU) pumps. These pumps were installed in a SU pump around loop that was indicative of what a current large scale SU mixing or Cell Culture system may be configured as under typical manufacturing conditions.

1.2 Test Equipment

Testing was conducted utilizing a Mettler Toledo FBRM, (Focused Beam Reflective Measurement), to determine cell shear by measuring the Sauter Mean diameter in relation to flow rate, pressure drop and pump selection. The FBRM Technology provides best in class real time measurement of droplet size and count within a short time at a resolution allowing for an accurate determination of stress induced droplet shear. Multiple parameters are monitored and reported by the iC FBRM 4.3 software for accurate recording of the experimental results.

During confirmation phase testing, the PVM in-line particle microscope technology served as an ancillary source for both sizing and count of emulsion droplets during testing. This technology also served to inform us of the presence of any entrained air bubbles that may be artifacts of fluid change over or additional entrainment of air bubbles in the liquid during set up and operation by providing distinguishing high resolution pictures of the emulsion droplets. All data collected from this technology were for informational purposes only and only reference pictures for supportive information are provided in this report.



Mettler Toledo, 2014

The vessel used was a Sartorius Palletank 50 L Levmix Tote, equipped with a 50 L bottom to bottom mixing bag equipped with 0.75"ID X 1.0"OD Silicone tubing for both supply and return lines. All other lines used were either 0.75"ID x 1.0" OD braided silicone STHT-R for high pressure segments or C-Flex 374 TPE where pressure was not likely to be encountered. Flow was measured via a Levitronix Leviflow LFSC-22D Clamp on Sensor, and confirmed through periodic volumetric sampling. Pressure was measured by a Pendotech Inline SU pressure sensor to a PMAT-4 transmitter and Ashcroft 0-60 PSI pressure gauge for FRO confirmation and the reducing valve utilized was an ITT 1" diaphragm valve completed the loop components.

1.3 Test Materials

Mobile EAL Arctic 22 synthetic oil and Triton X-100 are the primary flocculent components when mixed at a concentration of 1.28 ml and 0.18 ml per liter of purified water respectively. All other materials and disposable items are typical to most research and development labs.

1.4 Test Method

The mixing system was attached to the pump for the designated set of conditions performed. The 50L SU bag was filled with 22 liters of purified water as measured on the integrated load cell indicator after having been properly tared prior to addition. The pump was set to a suitable rpm or percentage output to obtain a vigorous flow (5.0-7.0LPM) but being careful to not cause undue cavitation or entrainment of bubbles while ensuring that all air in the lines are purged from the system. This step is important to remove confounding air bubbles from the flow path thus maintaining an accurate measurement of emulsion spheres.

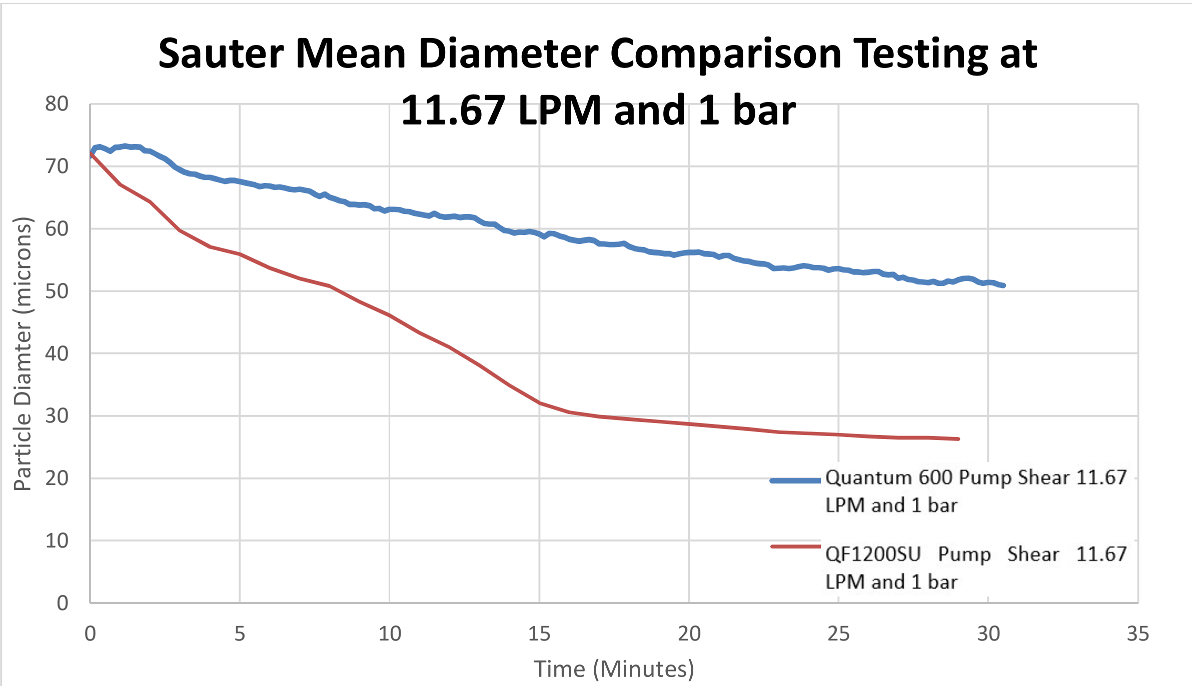
Once having purged the system of air the pump was stopped. The Levmixer was then started and adjusted to a mix rate of 150 rpm. The appropriate volume of Triton X-100 was added and allowed to mix for 1 minute. The Mobile Arctic EAL oil was added at the appropriate concentration and the emulsion mixture was allowed to mix for a total of 15 minutes to obtain a target mean Sauter Diameter of between 55-75uM. This mix time was determined by earlier experiments in which a suitable starting diameter was sought while maintaining a reasonable bubble count and distribution. The distribution and sizing were monitored and measured within the mixing vessel with both the FBRM.

Having reached the required mix time the Levmixer was turned off and the experimental pump was engaged at the appropriate rpm or percentage output as to reach the required flow rate. The flow was allowed to steady and then the pressure was adjusted to reach the called for differential pressure. The emulsion mixture was allowed to continue to recirculate at the target conditions for 30 minutes. During this time the Sauter Mean diameter is recorded along with the pressure flux. Having completed the test period the system is drained and rinsed with ~ 30 liters of water to remove residual material and ensure a repeatable base line reading. This system is then re-filled with the appropriate amount of water and re-circulated to remove air. This was repeated for all shear tests.

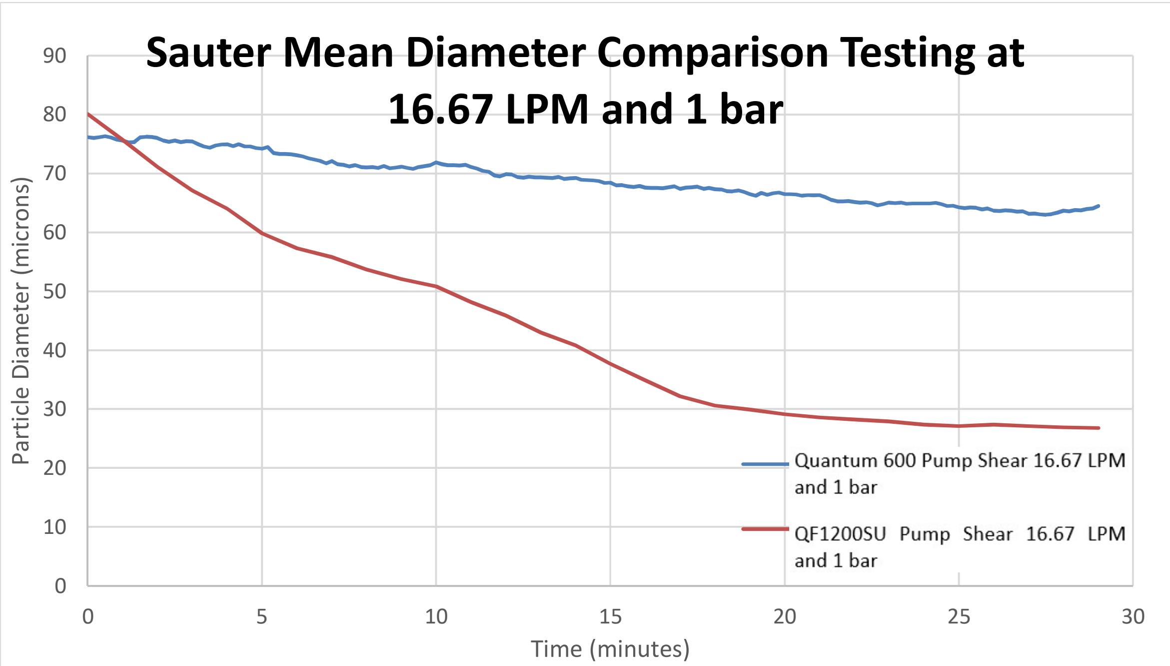
1.5 Test Results - Mechanical Shear Testing (A)

The results for the Watson-Marlow Quantum 600 pump and the Quattroflow QF1200SU pump are shown in Graphs 1 and 2. This degree of accuracy in starting droplet size is difficult to obtain due to a larger effect that a limited amount of large bubbles can have on the resultant starting Sauter Mean Diameter. Please note after testing was engaged, and a brief delay in establishing greater uniformity, the results provided a relatively stable curve. Graph 1 shows the resultant change in the Sauter Mean diameter of the pump tested.

Graph 1: Watson-Marlow Quantum and Quattroflow QF1200SU pumps Sauter Mean Diameter shear testing at 11.67 LPM and 1 bar



Graph 2: Watson-Marlow Quantum and Quattroflow QF1200SU pumps Sauter Mean Diameter shear testing at 16.67 LPM and 1 bar



As shown by Graphs 1 and 2, the pump tested exhibited a defined rate or slope of decay in the Sauter mean diameter of the emulsion droplets. By evaluating the decrease in diameter, it is evident that under the same operating conditions this pump shows its own decay profile. While it is understood that droplet size and distribution will have an effect on the rate of decay as larger drops become smaller, the rate of the decay should diminish as the pump reaches the maximal shear zone.

We found during previous testing that the rate of increased shear can, in part, be explained by the additional shear of the valve used to create the recirculation line pressure but it is also a direct result of increased velocity of the driving mechanism needed to overcome the additional system pressure as a result of restricting flow in the loop. At pressures of 2 bar and above the flow loop was providing the majority of the mechanical shear due to the flow across the valve and as a result this is not a true indication of the amount of shear from the pump alone. In essence, at 2bar and above, the flow loop conditions and valve selection will be critical to ensure the lowest amount of shear.

The point approximately as the mean particle diameter becomes stable was used to compare Watson-Marlow Quantum 600 Pump and the Quattroflow QF1200SU pump. Table 2 compares the Watson-Marlow Quantum 600 Pump and the Quattroflow QF1200SU pumps.

Table 2: Comparison of shear test results between the Watson-Marlow Quantum and Quattroflow QF1200SU Pumps

	Flow Rate (LPM)	Pressure (bar)	Approx. Final Stable Point (µm)
Quantum Pump	11.67	1	50
Quantum Pump	16.67	1	48
QF1200SU	11.67	1	27
QF1200SU	16.67	1	22

Several findings can be concluded from Table 2. First, there is very little difference between 11.67 and 16.67 LPM at 1 bar for each pump type. This is to be expected since shear stress is defined in terms of applied force straining on the material. If the pressure is the same, the force applied to the conduit or in other words the driving force causing shear stress is the same. This result lends to the validity of the testing methodology. Second, the final stable point when the shear force dividing the emulsion droplets balances with their tendency to combine into larger droplets clearly shows a difference in shear force. Assuming the droplets combine at a consistent rate for each test since the concentrations of oil and surfactant are the same, the final droplet size has a strong correlation to shear.

1.6 Conclusion - Mechanical Shear Testing (A)

Based on the test metrics, the oil emulsion mean diameter results indicate that the Watson-Marlow Quantum 600 Pump produces half the shear stress on the process stream as the Quattroflow QF1200SU Diaphragm Pump. This is a direct indicator of the products ability to pump delicate shear sensitive biological materials without causing damage.

2. Mechanical shear test (B) using CHO suspension cell cultures

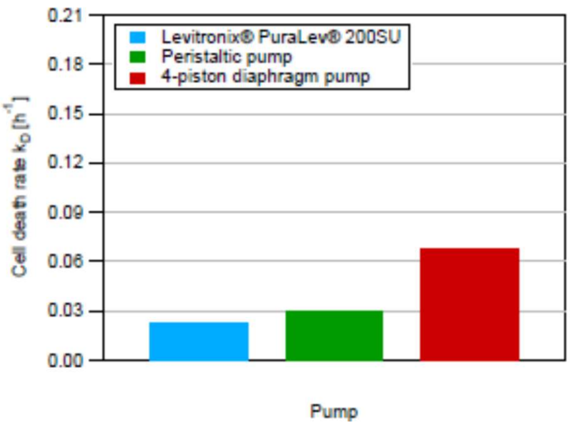
2.1 Introduction

This study explores the mechanical shear stress caused to CHO (Chinese Hamster Ovary) cell culture when using the Quantum 600 pump.

When it comes to utilizing pumps in upstream biologics manufacturing operations, mechanical shear stress must be carefully evaluated. A study conducted by Levitronix, ref. Blaschczok et al. 2013, entitled "*Investigations into Mechanical Stress Caused to CHO Suspension Cells by Single-Use Magnetically Levitated, Bearingless Centrifugal Pumps*" identified that the use of a Levitronix PuraLev®200SU centrifugal pump had better performance with

k_D=0.023 h^{-1} to that of a competitor peristaltic pump k_D=0.03 h^{-1} which represents a difference of roughly 23%. The following is directly from the aforementioned Levitronix testing:

Graph 3: Investigations on Mechanical Stress Caused to CHO Suspension Cells by Standard and Single-Use Pumps, (see Blaschzok et al. 2013).



This testing was recreated to evaluate the direct comparison between the peristaltic and diaphragm pumps utilized, as well as, an evaluation of the impact to CHO Suspension Cell Cultures utilizing the PuraLev®200SU centrifugal pump. What was measured in both the Levitronix testing and the testing conducted at PDS Sandbox was an evaluation of the mechanical shear stress on the CHO suspension cell cultures through a measurement of the Viable Cell Density immediately following the recirculating pumping process. PDS Sandbox also evaluated any longer term effects from the mechanical shear forces by continuing the cell culture for a period of 5 days to evaluate any longer term effects on the rate of cell death through lactate dehydrogenase (LDH) testing. These values were compared to both historical and a satellite culture data during the experiment. In comparison to the testing executed in the aforementioned Levitronix paper, it should be noted the flow loop design ensured nothing else aside from the pump was being evaluated as the pump inlet and outlet are ¾" and the system flow path design minimized ancillary shear impact. Since CFG pumps are not positive displacement, there are inherent shear forces that are exerted into the cell culture as result of the need to pump against a pressure drop to ensure priming. This is evident in the "*Investigations into Mechanical Stress Caused to CHO Suspension Cells by Single-Use Magnetically Levitated, Bearingless Centrifugal Pumps*" by Levitronix.

2.2 Test Equipment

The setup of the pump loop test equipment was as follows:

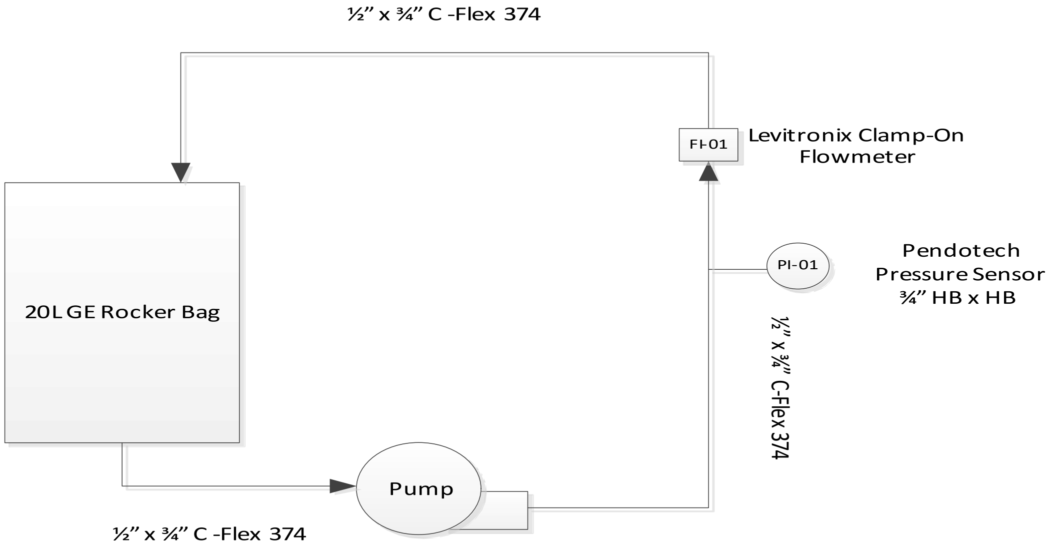


Table 3: Cell Expansion - All testing was conducted using CHO-K1 Cells. The following parameters and strategy were followed for the cell cultivation:

Parameter	250mL Shake Flask	2 x 1000mL Shake Flask	20L Rocker Bag
Temperature	37C	37C	37C
Agitation Speed	125 RPMs	180 RPMs	22 Rocks per Min
Rocker Angle	N/A	N/A	12 Degrees
CO2	5%	5%	5%
Target Seeding Density	2.50E+05	2.50E+05	2.50E+05
Target Harvest Density	1.00E+07	1.00E+07	1.00E+07
Target Final Volume	125mL	500mL	10L
SU Supplier	Corning	Corning	GE
Cell Culture Media	Ham's F12 with 2mM Glutamine and 10%FBS	Ham's F12 with 2mM Glutamine and 10%FBS	Ham's F12 with 2mM Glutamine and 10%FBS

Table 4: Analytical Equipment Used for Test Analysis included the following:

Test Instrument	Supplier	Model
Cell Counter	Nova Biomedical	Bioprofile Flex
Osmolality Analyzer	Nova Biomedical	Bioprofile Flex
LDH (Cell Death) Assay	Roche	Cedex Bio Analyzer
Sterile Sealer	Sartorius	BioSealer

2.3 Test Method

Following the **Cell Expansion** methodology outlined above, every stage of the inoculum preparation CHO cells were cultured until they reached a density of >1.0E6 until the inoculation of the 20L scale. The cell expansion methodologies were conducted in triplicate, as well as parallel, to allow for an independent assessment of cell cultures exposed to pumping mechanical stresses from those cultures which had not been pumped. Testing was conducted utilizing the Quantum 600 pump as speeds of 100RPMs and 300RPMs, (Flowrates of 5LPM and 15LPM) each for 12 hours in duration. Samples were taken every hour to measure the Current Viable Cell Density, against the Starting Viable Cell Densities. After the recirculation testing was complete, all recirculation loops were given a double sterile seal and the cell cultures were allowed to grow for another period of 5 days. 2x a day, the cell cultures were sampled for Viable Cell densities and lactate dehydrogenase (LDH) concentration. These values were compared against the cell culture which did not undergo any of the recirculation pumping mechanical shear stress testing. When cell membranes are damaged, lactate dehydrogenase (LDH) (an enzyme found inside every living cell) is released into the surrounding extracellular space. LDH is only released when cell membrane integrity is compromised so the presence of this enzyme can be used as an effective cell death marker for cell culture. The relative amounts of live and dead cells within the medium can then be quantitated by measuring the amount of released LDH using the Roche Cedex Bioanalyzer.

2.4 Test Results - Mechanical Shear Testing (B) using CHO suspension cell cultures

The starting inoculum cell culture train data and averages were as recorded in table 5 below.

Table 5: Starting inoculum cell culture data

Process Data	SB-WM-QR-001	SB-WM-QR-002	SB-WM-QR-003
Cryo Vial Numbers	CHO-K1 #1, #2, #3	CHO-K1 #1, #2, #3	CHO-K1 #1, #2, #3
Elapse Time (days)	3 Days	3 Days	3 Days
Expansion Container Type	Shaker Flask	Shaker Flask	Rocker Bag
Expansion Container Size	250mL	1000mL	20L
Volume	125mL	500mL	10L
Temperature (C)	37	37	37
Shaker RPM	125	180	22
Seeding Density Target SP	2.50E+05	2.50E+05	2.50E+05
Avg. Seeding Density Target Actual	2.63E+05	2.44E+05	2.52E+05
Harvest VCD SP (cells/mL)	1.00E+06	1.00E+06	1.00E+06
Avg. Harvest VCD Actual (cells/mL)	1.32E+06	1.10E+06	1.03E+06
Avg. Starting Viability	95.2%	96.1%	98.2%
Avg. Ending Viability	98.7%	99.0%	99.1%

Once each of the Cell Cultures were at their target VCDs, recirculation testing commenced utilizing a gamma irradiated flow path incorporating the Watson-Marlow Quantum 600 with ReNu SU Technology® cartridge 20/3P. Testing conditions are listed in table 6 and do not utilize a restriction in the line but rather the use of gravity coupled with velocity to achieve adequate pressure in the flow path. The process data results are shown in table 6.

Table 6: Recirculation cell culture test results

Process Data	SB-WM-QR-003-1	SB-WM-QR-003-2	SB-WM-QR-003-3
Wave Rocker Number	Wave #1	Wave #2	Wave #3
Pumped?	No	Yes	Yes
Pump RPM SP	N/A	100	300
Pump Flow Actual	N/A	5.1 LPM	15.2 LPM
Pumping Time	N/A	12 Hours	12 Hours
Recirculation Loop Pressure	N/A	0.2 bar	0.5 bar
Starting 12 Hour Recirc Viability	98.7%	99.0%	99.1%
End 12 Hour Recirc Viability	99.1%	96.1%	95.7%
Starting 12 Hour Recirc VCD	1.03E+06	1.12E+06	1.08E+06
End 12 Hour Recirc VCD	1.81E+06	1.56E+06	1.24E+06
LDH Values At Beginning of 5 Day Hold	103.54U/L	140.71U/L	178.04U/L
LDH Values at End of 5 Day Hold	371.20U/L	401.88U/L	380.69U/L
VCD at End of 5 Day Hold	7.80E+06	7.33E+06	7.42E+06
Viability at End of 5 Day Hold	97.7%	96.2%	98.1%

Wave #1 results show cell viability of a control sample which was not recirculated through a pump.
Wave #2 results show cell viability change when recirculating at 5.1 LPM through the Quantum 600 pump.
Wave #3 results show cell viability change when recirculating at 15.2 LPM through the Quantum 600 pump.

There seems to be little impact on the VCD after the 5 day hold and all VCDs and Viabilities were within the performance tolerance of the equipment (Nova BioProfile Flex) being utilized to measure VCD and Viability. The LDH values were taken from the Roche Cedex BioAnalyzer and show that right after the 12 hours of recirculation that there was a slight increase of LDH which appears to correlate directly to agitation speed. Please note Time 1 testing, after 1 hour of recirculation was within 2.3% of the End of day values for both pump speeds.

The recirculation of CHO-K1 cell culture can be supported by the Watson-Marlow Quantum 600 pump. Having the cells continue to proliferate during the recirculation at a rate of 1 vessel volume every 2 minutes (for the 100 RPM Test) for 12 hours and 1 Vessel volume every 40 seconds (For the 300 RPM Test) for 12 hours as long as you keep the recirculation path as continuous a loop as possible without sudden changes in pressure. Testing shows, the ReNu SU Technology® cartridge (20/3P) pump head does have a statistically relevant impact to CHO-K1 based cell cultures under the conditions testing at PDS Sandbox.

2.5 Conclusion – Mechanical Shear Testing (B) using CHO suspension cell cultures

The results of this test, which replicates the methodology used by Blaschzok et al. in 2013, demonstrate that the Quantum 600 Pump with the ReNu SU Technology® cartridge (20/3P), has minimal impact on the cells during 12 hours of constant recirculation, (see table 6).

The impact of the Quantum 600 pump on cell viability is not long lasting as every one of the cell cultures were able to overcome the initial mechanical shear impact, where the resulting data does not show any outliers of statistical significance. The change in cell viability recorded is not just attributable to the pump but also the velocity during the recirculation test and its impact on the cell culture. Pulsation also has an impact on sensitive cell culture and the low pulsation Quantum 600 pump has a measurable benefit over traditional peristaltic technologies.

These CHO suspension shear results are comparable to what would be expected from a centrifugal pump and indicate that the graph 3 on page 8 would now reflect the Quantum 600 and the centrifugal pump to be on a similar level and much better than traditional peristaltic pumps.

3. Lifecycle Metering Performance Testing

3.1 Introduction

The purpose of this testing was to determine the performance capabilities in meter volume consistency for both the Watson-Marlow Quantum 600 pump and the Levitronix PuraLev®200SU centrifugal pump. This was designed to highlight the difference in pressure/flow performance between peristaltic and centrifugal pump technologies. These tests were designed to calculate both the range of variability within each test and between tests to calculate full range variability.

3.2 Test Method

Life cycle performance testing was conducted at set RPMs, at random durations run with varied/random backpressures in triplicate to establish the initial dataset. After the initial dataset was generated, both the Quantum 600 pump and PuraLev®200SU centrifugal pump from Levitronix were set to the maximum RPM output (400RPM for Quantum 600, 9000RPM for PuraLev®200SU) in continuous operation for a period of 40 hours at 2 bar pressure.

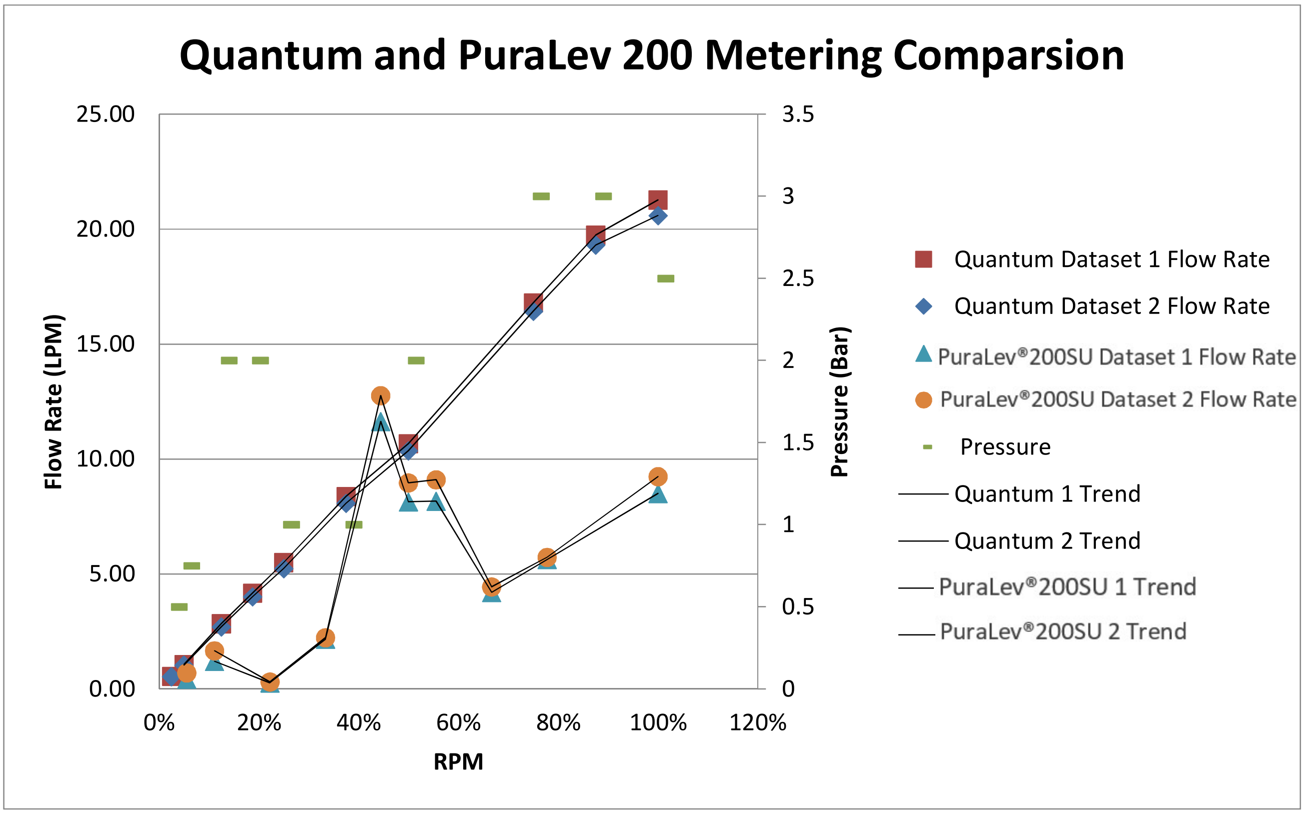
After 40 hours, the initial testing was repeated and each of the datasets were compared independently, as well as, against each other. This was to assess if the was any flow decay or diminished flow performance resulting from extended operation and effects such as tubing break-in.

3.3 Test Results

Lifecycle metering performance testing results for the Quantum 600 Pump, as outlined in the testing data section, show no statistically relevant reduction in flow during both the individual RPM/Backpressure tests, as well as, between the 2 executed test, using the same ReNu SU Technology® cartridge post 40 hours of operation at 400RPMs at 2 bar pressure and 14^{o}C temperature control of the reservoir for the recirculation. The difference between the two datasets was between 2 and 5%.

Lifecycle metering performance testing results for the PuraLev®200SU centrifugal pump, as outlined in the testing data section, shows greater variability in flow for each individual RPM/Backpressure tests, as well as, between the two executed test, using the PuraLev®200SU centrifugal pump head post 40 hours of operation at 400RPM at 2 bar pressure and 14^{o}C temperature control of the reservoir for the recirculation. Pressure greatly impacts pump performance due to the type of pump (centrifugal) compared to the positive displacement design of a peristaltic pump. The difference between the 2 datasets was between 2 and 62%.

Graph 4: Metering comparison results. Quantum 600 pump verses Levitronix PuraLev®200SU



3.4 Conclusion – Lifecycle Metering Performance Tests

These results demonstrate that the Quantum 600 pump is a suitable product for use in metering applications. It displayed excellent output consistency over this extended life test (40 hours) and the flow performance was virtually independent of back pressure.

The PuraLev®200SU centrifugal pump results showed a significant variation in metering performance and a strong dependency on system back pressure.

The slight fall in metering performance of the Quantum 600 pump ReNu SU Technology® cartridge is likely to be associated with the 'breaking-in' of the thermoplastic polyurethane (TPU) tubing. That said, the design of the ReNu SU Technology® cartridge does not allow for uncontrolled break-in of the tubing and due to the physical characteristics of the TPU tubing, there is a high degree of resilience and noticeable lack of tubing deformity as often seen in other long term usage peristaltic pumping applications.

4. Temperature testing – heating of equipment, process stream and reservoir

4.1 Introduction
This test was designed to compare the heating effects of the Quantum 600 pump with the Quattroflow QF1200SU pump.

4.2 Test Method
Testing was performed on the temperature of the pump head, pump body, and impact, if any, on the process stream. The temperature was measured at 15 minute intervals utilizing an infra-red thermometer to obtain a reasonable span of time for loop circulation to have an effect on the reservoir temperature, if at all. While we see localized heating on both the pumps tested it is considered to be of a moderate nature that will not affect most processes and application.

4.3 Test Results
The results from testing on the Watson-Marlow Quantum 600 Pump and the Quattroflow QF1200SU pump are summarized in Table 5.

The temperature did not increase more than 10°C during one hour of testing except the front of the Watson-Marlow Quantum pump head for either pump. The front panel location rose to 35.5°C (11.8°C increase).

Graph 5: Watson-Marlow Quantum and Quattroflow QF1200SU pump fluid temperature testing results comparison

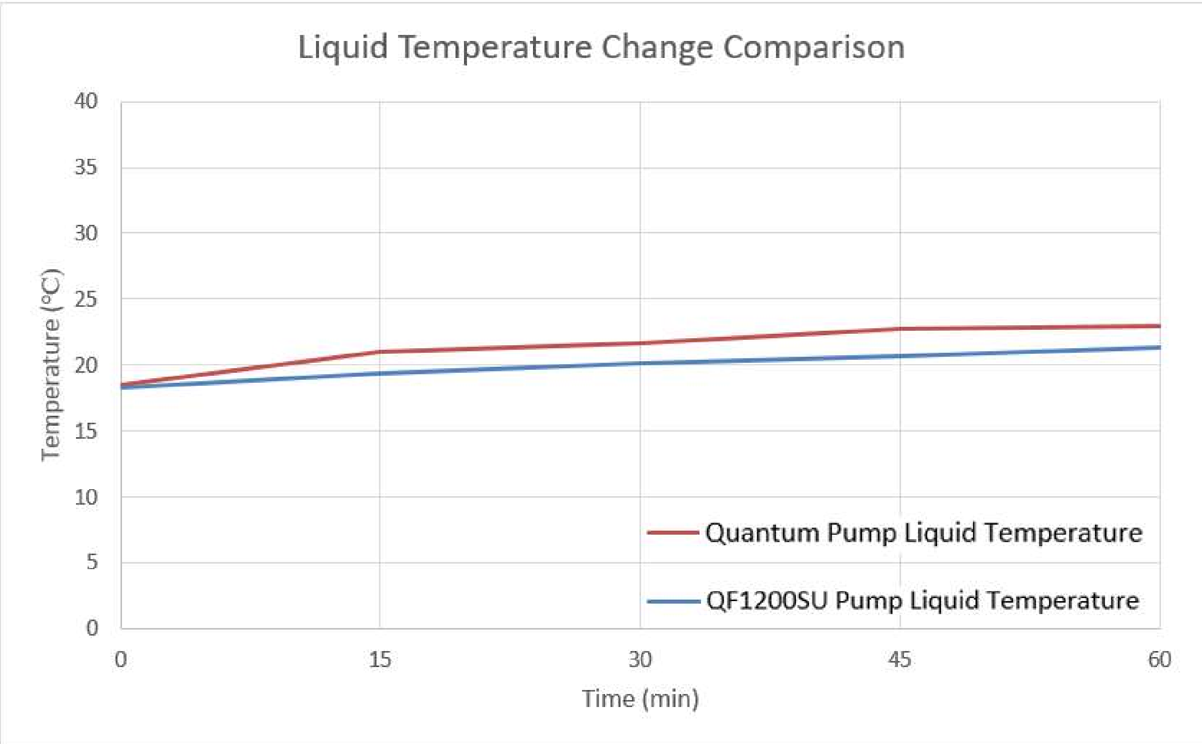


Table 7: Summary comparing the fluid temperature testing results for the Watson-Marlow Quantum Pump and the Quattroflow QF1200SU pump. Note the final fluid temperature is after 60 minutes of continuous pumping.

	Starting Fluid Temperature (°C)	Final Fluid Temperature (°C)	Temperature Change (°C)
Quantum Pump	18.5°C	23°C	4.5°C
QF1200SU	18.2°C	21.4°C	3.2°C

4.4 Conclusion

The results show that the measured fluid temperature increase was greater when using the Quantum 600 pump. The difference in final fluid temperature was small (1.3°C) and in the vast majority of applications this will have no material impact on the transfer media. On the Watson-Marlow Quantum 600 Pump itself, only one component exceeded 35°C. This was the front plate of the pump housing.

5. Pump Curve (Flow/Pressure and RPM/Flow)

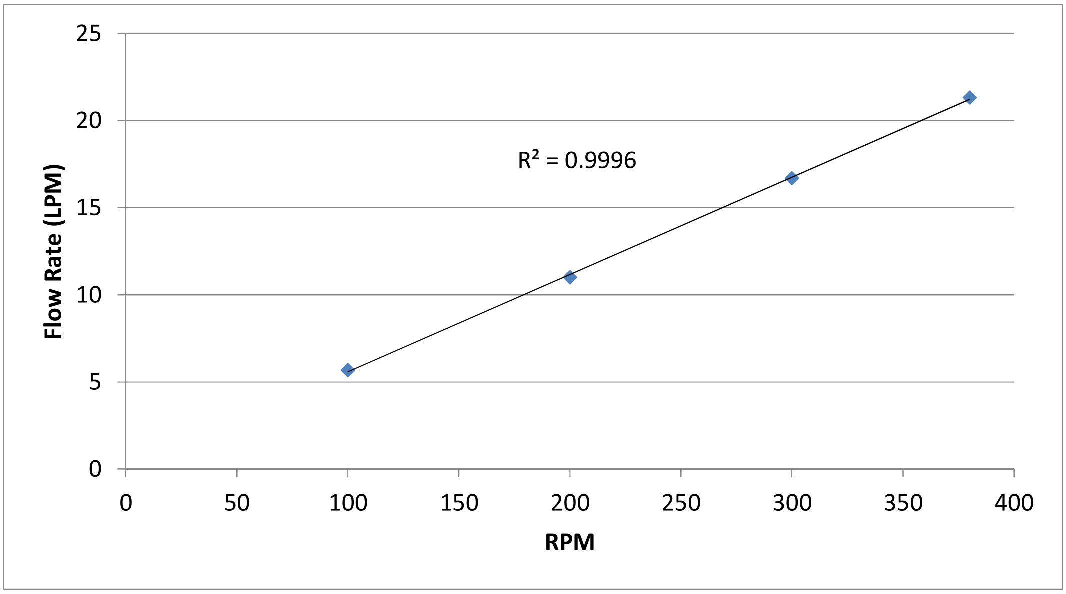
5.1 Introduction

Pump curve testing was conducted on the Watson-Marlow Quantum 600 Pump to evaluate the impact of pressure on known flow rates as well as alignment between RPMs and Pump Flow Rates.

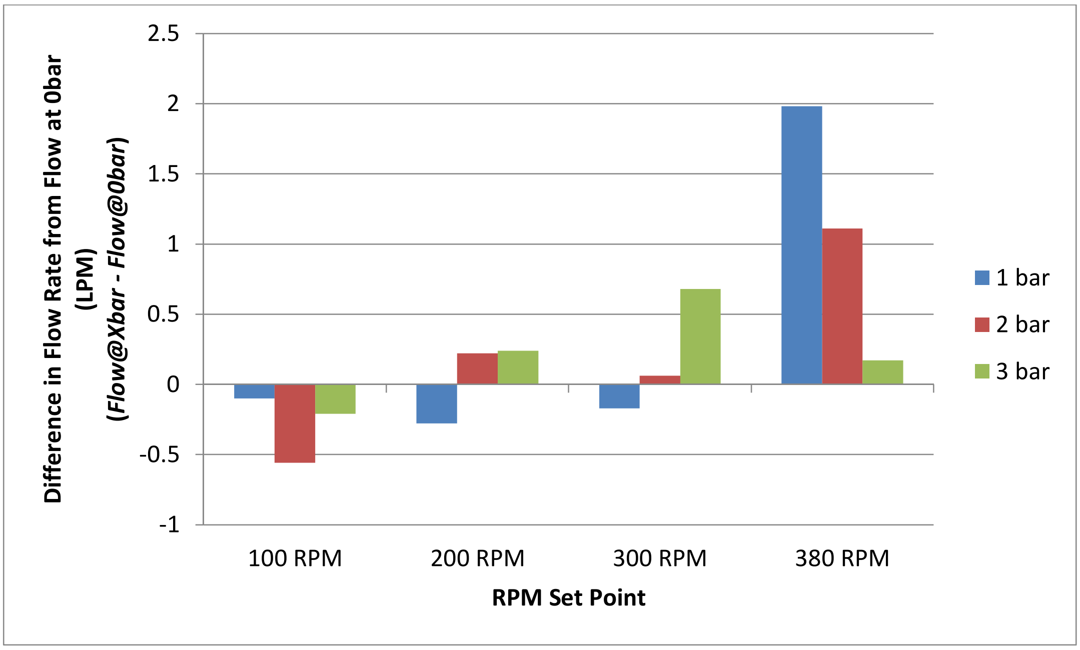
5.2 Test Results

The flow rate to RPM curve in Graph 6 is very linear and demonstrates that there is little, if any, loss of efficiency as you increase the pump speed. In Graph 7, this comparison between the flow rate losses, relative to the pressure, demonstrates that at the higher RPMs there is a greater impact to flow rate than at the lower RPMs. There is some variability at lower pump flow set points under pressure with a significant reduction in flow at higher pump set points.

Graph 6: Watson-Marlow Quantum 600 Pump flow rate versus RPM testing results



Graph 7: Watson-Marlow Quantum Pump difference in flow rate at various flow rates and pressures



5.3 Conclusion

These results show that the Quantum 600 pump delivers a high degree of accuracy across a wide range of pressures and flows. Flow variation is smallest at high pressure and flow.

This means that this product is suitable for applications that need to deliver target volumes of fluid by operating the pump for a set speed and time without the need for a secondary check, (load cell, scale or flow meter).

6. Pulsation testing at different flow rates and pressures

6.1 Introduction

The objective of this test was to evaluate and compare the pulsation performance at various flow rates and pressures for of the Quantum 600 pump and the Quattroflow QF1200SU pump.

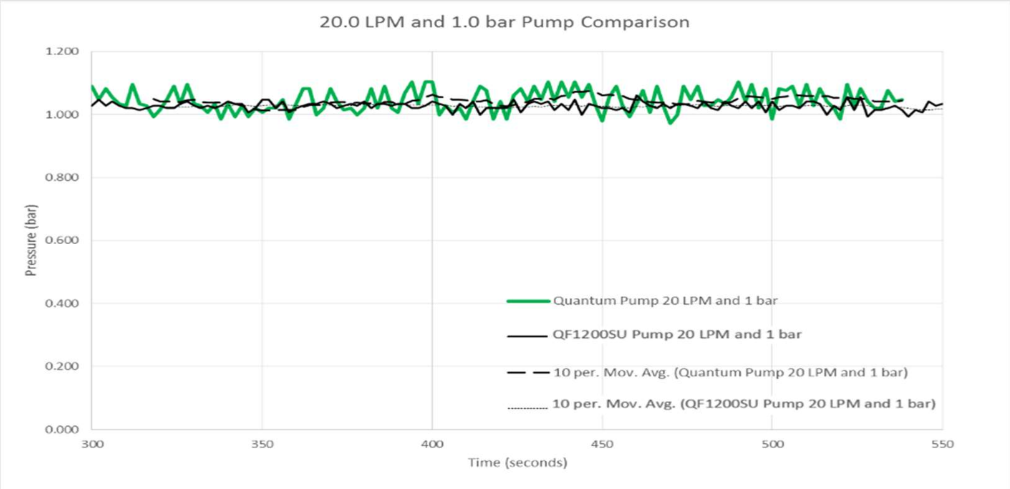
6.2 Test Method

Utilizing a Pendotech PMAT-2 pressure sensor, pressure was monitored during shear testing execution to obtain pressure pulsation across the span of conditions observed. Also the standard deviation of pressure readings by the Pendotech pressure sensor were plotted against the pressure set points. Two flow setpoints were chosen representing mid-range and maximum flow.

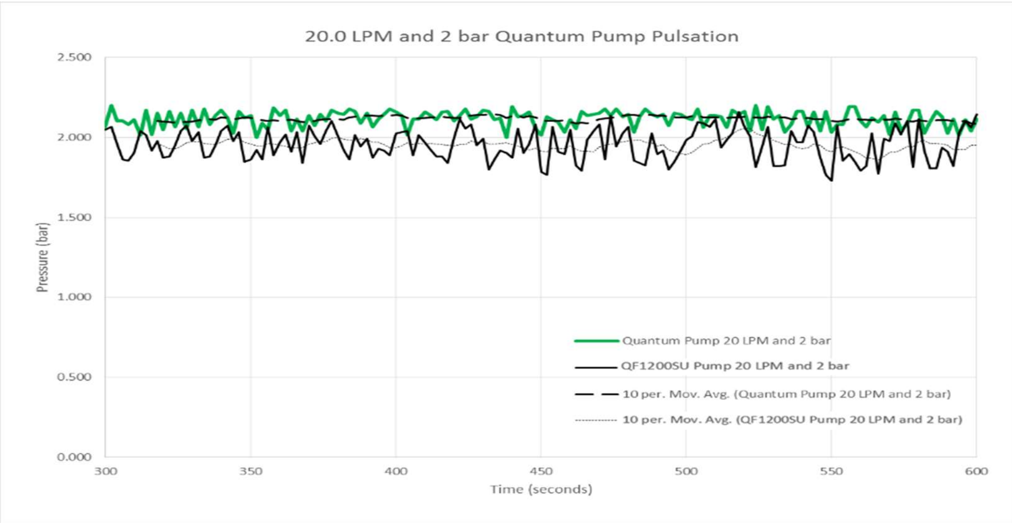
6.3 Test Results

Graphs 8 to 12 on the following pages show the pulsation test results for a range of flows and pressures for the time period of between 5 and 10 minutes. Original data and a 10 point rolling average trend line are depicted.

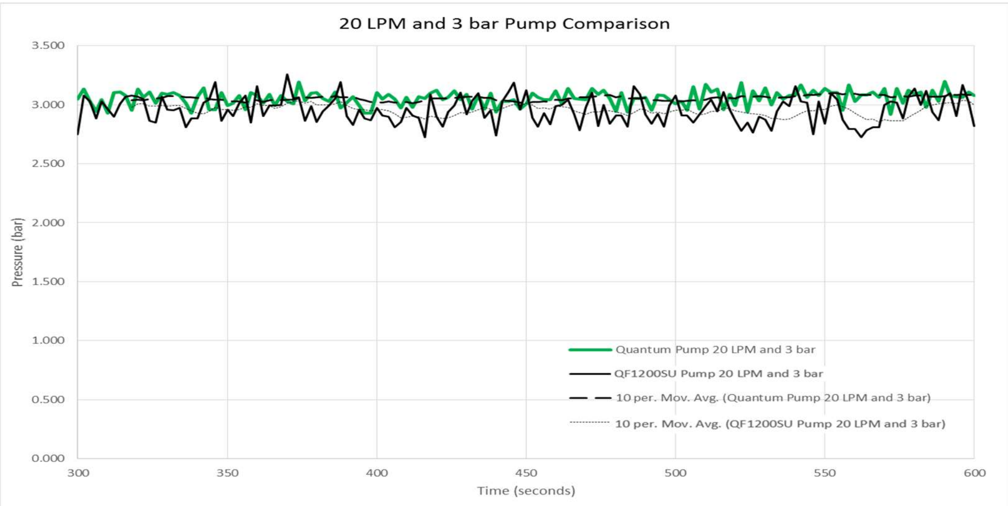
Graph 8: Test conditions: flow 20 LPM and pressure 1 Bar.



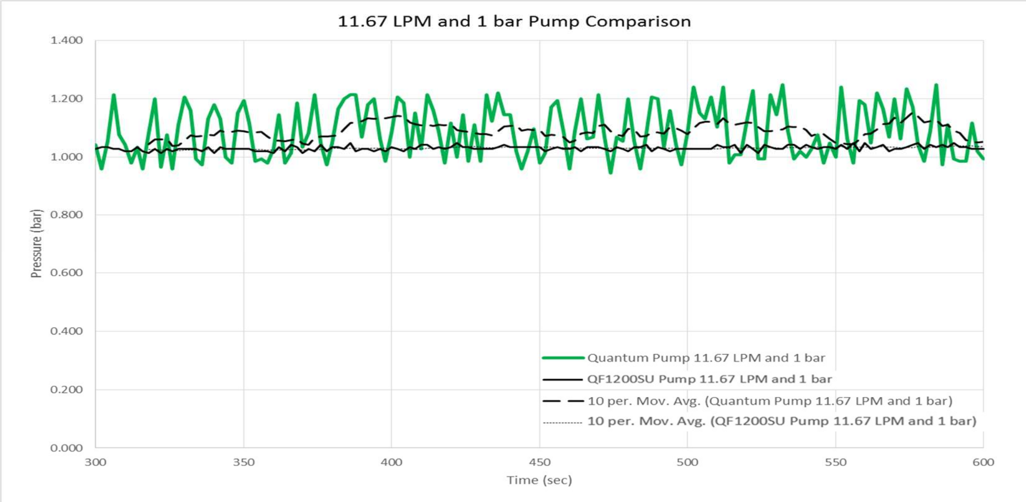
Graph 9: Test conditions: flow 20 LPM and pressure 2 Bar.



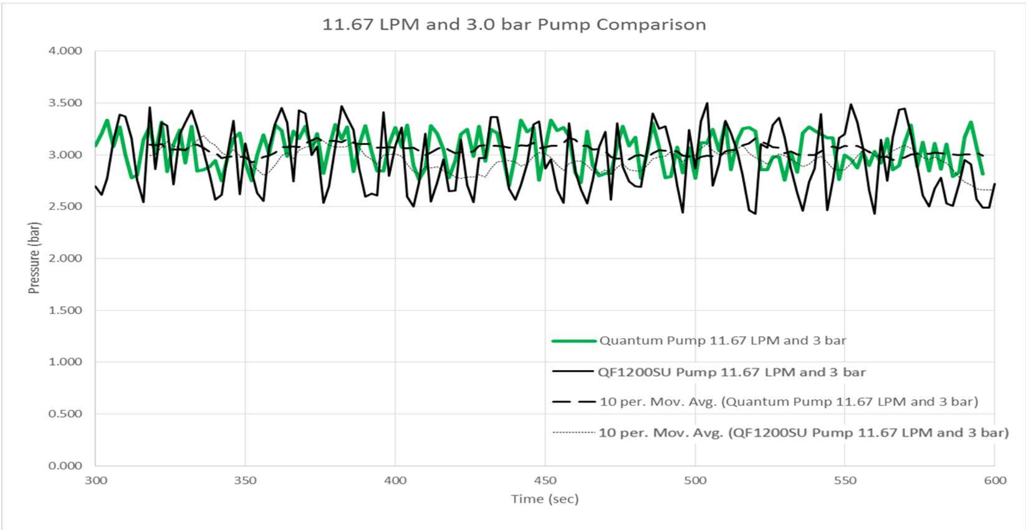
Graph 10: Test conditions: flow 20 LPM and pressure 3 Bar.



Graph 11: Test conditions: flow 11.67 LPM and pressure 1 Bar.

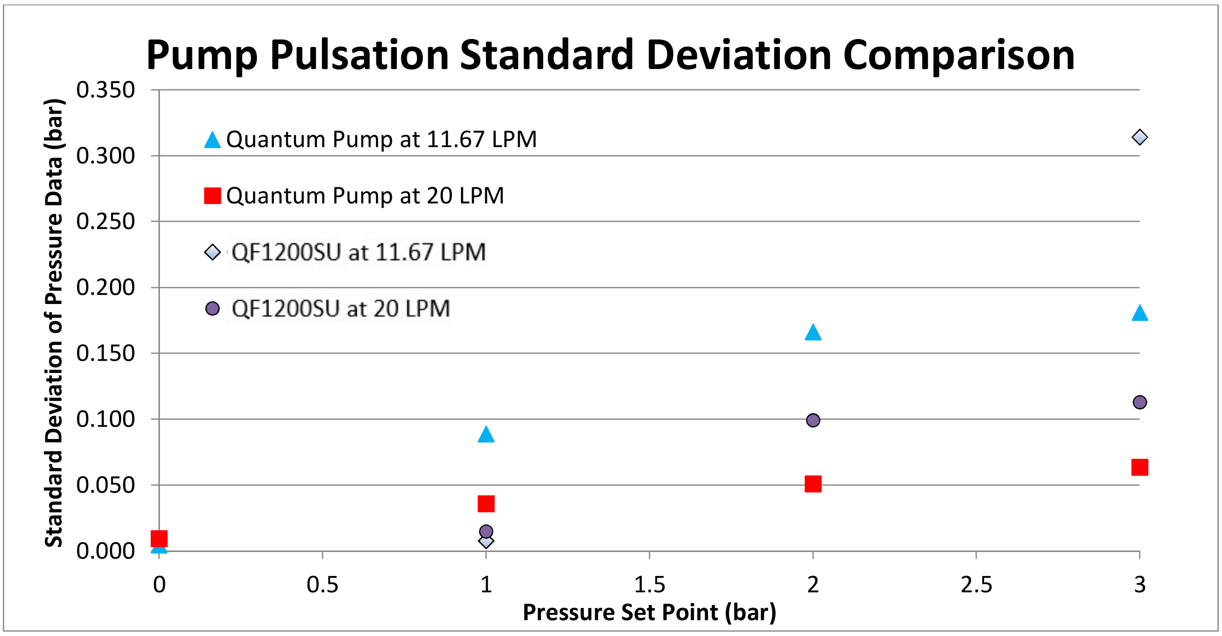


Graph 12: Test conditions: flow 11.67 LPM and pressure 3 Bar.



For both flow rates, the pulsation increases with pressure. There is greater pulsation at 11.67 LPM over 20 LPM. Appendix 1 has the Pulse Data Summary detailed results across the Watson-Marlow Quantum 600 pump and Quattroflow QF1200SU Pump at each set point.

Graph 13: Standard deviation of pressure readings from pulsation testing on the Watson-Marlow Quantum and Quattroflow QF1200SU Pump at various flow rates and pressures



6.4 Conclusion – Pulsation Tests

These results show that the Quantum 600 pump exhibits very low levels of pressure pulsation on its outlet side. Optimum performance is achieved at higher pressures and flows where most customers are likely to want to operate in order to maximize productivity.

In this test the pulsation produced by Quantum 600 pump was less than that exhibited by the Quattroflow QF1200SU pump for outlet pressures of greater than 1 Bar. It is also much lower than is typical for traditional peristaltic pumps.

The high frequency operation of the Quattroflow QF1200SU pump means that a high sample rate is required to accurately capture the variation in output pressure.

7. Report Summary

The testing performed shows that the Watson-Marlow Quantum 600 Pump produces less shear than the Quattroflow QF1200SU Pump. Creating a metric for evaluating the shear test data and summarizing it in a table demonstrated that the Watson-Marlow Quantum Pump significantly out-performed the Quattroflow QF1200SU pump. The shear created by the pump was more easily isolated by testing at and comparing different flow rates at the same pressure.

These results were further corroborated by the CHO cell viability tests which showed that the Quantum 600 pump performance was comparable to the Levitronix PuraLev®200SU centrifugal pump and better than traditional peristaltic pumps. This provides users with a modern peristaltic pump option for shear sensitive processes without the drawbacks of a strong inlet pressure dependency.

It was found that the pressure pulsation of the Watson-Marlow Quantum 600 Pump was less than that of the Quattroflow QF1200SU at pressures of above 1 bar (Graph 13). Temperature testing on the Watson-Marlow Quantum 600 pump was similar to the Quattroflow QF1200SU Pump. Fluid temperature increased by a nominal amount from 21°C to 23°C and is considered negligible.

The Quantum 600 pump is also considered a suitable product for metering applications. The degree of accuracy across the full range of pressures, once tested/confirmed/calibrated, means that the Watson-Marlow Quantum 600 Pump can replace other pumping solutions where there is a secondary need for flow or weight confirmation. Meaning, the accuracy for some applications, could lend itself to setting the pump at a specific RPM for a preset amount of time to achieve a specific target volume without a secondary check (load cell, scale or flow meter).

8. Recommendations

The applications for the Watson-Marlow Quantum 600 pump are varied but it would be the recommendation for the use of the Quantum 600 pump, at a minimum, in the following applications:

- Tangential Flow Filtration (TFF) including Ultrafiltration and Microfiltration
- Depth Filtration
- Viral Filtration (VF)
- Chromatography
- Metering Pump Applications at High Pressure

The Watson-Marlow Quantum 600 Pump now provides the industry with an option for shear sensitive materials that materially surpasses the Quattroflow QF1200SU from PSG Dover and is also an alternative to Levitronix CFG pumps.

In fact, based on this study and analysis PDS Sandbox would recommend the Quantum 600 Pump for cell culture operations where relevant such as the following applications:

- Harvest Operations including:
    - Perfusion Operations
    - Centrifugation Operations

References

[1] Maaß, S., Wollny, S., Voigt, A. and Kraume, M., 2011. Experimental comparison of measurement techniques for drop size distributions in liquid/liquid dispersions. *Experiments in Fluids*, *50*(2), pp.259-269.

[2] Dittler, I., Kaiser, S.C., Blaschczok, K., Löffelholz, C., Bösch, P., Dornfeld, W., Schöb, R., Rojahn, J., Kraume, M. and Eibl, D., 2014. A cost-effective and reliable method to predict mechanical stress in single-use and standard pumps. *Engineering in Life Sciences*, *14*(3), pp.311-317.

[3] Blaschczok, K., Kaiser, S.C., Löffelholz, C., Imseng, N., Burkart, J., Bösch, P., Dornfeld, W., Eibl, R. and Eibl, D., 2013. Investigations on Mechanical Stress Caused to CHO Suspension Cells by Standard and Single-Use Pumps. *Chemie Ingenieur Technik*, *85*(1-2), pp.144-152.

Appendix 1 Pulse Data Summary

Pump	Flow Rate (LPM)	Pressure Set Point (bar)	Standard Deviation of Pressure	Pressure Range	Inter Quartile Pressure Range	Min Pressure	Max Pressure	Mean Pressure	Median Pressure	Q25	Q75
Quantum Pump	11.67	0	0.004	0.014	0.007	0.000	0.014	0.005	0.007	0.000	0.007
Quantum Pump	11.67	1	0.089	0.303	0.169	0.945	1.248	1.087	1.076	1.000	1.169
QF1200 Pump	11.67	1	0.008	0.034	0.007	1.014	1.048	1.030	1.027	1.027	1.034
Quantum Pump	11.67	2	0.166	0.531	0.324	1.868	2.399	2.130	2.130	1.965	2.289
Quantum Pump	11.67	3	0.181	0.634	0.345	2.703	3.337	3.048	3.068	2.868	3.213
QF1200 Pump	11.67	3	0.314	1.069	0.569	2.434	3.503	2.942	2.910	2.672	3.241
Quantum Pump	20	0	0.009	0.041	0.014	-0.014	0.028	0.002	0.000	-0.007	0.007
Quantum Pump	20	1	0.036	0.131	0.062	0.972	1.103	1.044	1.038	1.020	1.082
QF1200 Pump	20	1	0.015	0.076	0.017	0.986	1.062	1.026	1.027	1.017	1.034
Quantum Pump	20	2	0.051	0.200	0.076	1.999	2.199	2.117	2.130	2.082	2.158
QF1200 Pump	20	2	0.099	0.427	0.165	1.731	2.158	1.956	1.958	1.875	2.041
Quantum Pump	20	3	0.064	0.283	0.090	2.916	3.199	3.055	3.061	3.013	3.103
QF1200 Pump	20	3	0.113	0.531	0.162	2.723	3.254	2.953	2.951	2.868	3.030
Quantum Pump	20	4	0.072	0.324	0.107	3.951	4.275	4.120	4.123	4.071	4.178

Color for greater value in comparison