TP_031

BLOOD TRAUMA, CELL DEPLETION AND FIBRINOGEN DEPOSITION TEST

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00	**---**	**First issue.**	**15 Sept 2018**
01		**Inclusion of centrifugal blood pump devices**	**27 Mar 2019**

1. Scopo/Scope

This test is performed to evaluate blood trauma and the effects of device-blood interaction of Qura devices throughout their intended use. More specifically, the test quantifies haemolytic effects and platelet and/or white blood cell depletion during blood recirculation and the presence of blood clot and/or fibrinogen deposits on blood contacting surfaces after recirculation with blood. Results are compared those obtained on predicate commercial devices subjected to the same preliminary and test conditions.
In particular, the test applies to:

Code	Description
1. Qura Heat Exchangers	
HX11V-S0	Standard Heat Exchanger Low Flow HX-S LF
HX22V-S0	Standard Heat Exchanger Medium Flow HX-S MF
HX55V-S0	Standard Heat Exchanger High Flow HX-S HF
HX22V-C0	Cardioplegia Heat Exchanger Low Flow HX-C HF
HX32V-C0	Cardioplegia Heat Exchanger High Flow HX-C LF
2. Qura Centrifugal Pumps	
CP37V-V0	Centrifugal blood pump High Flow CP HF

CP22V-V0	Centrifugal blood pump Medium Flow CP MF

2. Documenti e norme applicabili/Applicable documents and standards

2.1 Esterni/External:

ISO 7199:2009 - Cardiovascular implants and artificial organs - Blood-gas exchangers (oxygenators)
EN ISO 18242:2016 – Cardiovascular implants and extracorporeal systems - Centrifugal blood pumps
ASTM F1841 2017 Standard Practice for Assessment of Haemolysis in Continuous Flow Blood Pumps
ASTM F756 2017 Standard Practice for Assessment of Haemolytic Properties of Materials
3-55 ASTM F1830-97 (Reapproved 2017) Standard Practice for Selection of Blood for In Vitro Evaluation of Blood Pumps

2.2 Interni/Internal:

General documents	
TP_012 “Accelerated aging test”	
TP_003 “Leak Test”	
OI_034 “Sample size for R&D product testing”	
OI_900 “Lab Procedure for handling of biological fluids”	
Specific documents	
Device Code	
1. Qura Heat Exchangers	
HX11V-S0; HX22V-S0; HX55V-S0; HX22V-C0; HX32V-C0	OI_401 “Process Controls Heat Exchanger Processing”
HX22V-C0; HX32V-C0	OI_012 “Guide for HX-C Production”
HX11V-S0; HX22V-S0; HX55V-S0	OI_026 “Guide for HX-S Production”
2. Qura Centrifugal Pumps	
CP37V-V0; CP22V-V0	OI_044 “Guideline for Pump production”

3. Responsabilità/Responsibility

The R&D Manager is responsible to plan and coordinate the testing activity. The test executor is responsible to conduct and document the test according to the present protocol; any deviation from the present document and related method have to be noted and referred by the test executor in order to be properly considered. R&D manager is responsible to approve the test results.

4. Razionale/Rationale

The purpose of this test is to evaluate the variation of plasma free hemoglobin concentration, white blood cells (WBCs) and platelets (thrombocytes, THR) in vitro, on the Qura devices over a period of 6 hours (equivalent to the max claimed intended use period).

5. Numerosità del campione/Sample size

The sample size is determined according to Risk Analysis and relevant confidence/reliability table reported in OI_034 “Sample size for R&D product testing” and is reported in the following table.

Device Code	Sample size
1. Qura Heat Exchangers	
HX11V-S0; HX22V-S0; HX55V-S0	30
HX22V-C0; HX32V-C0	
2. Qura Centrifugal Pumps	
CP37V-V0; CP22V-V0	x

Samples of similar commercial predicate devices should be used as a comparative reference.

6. Descrizione del test/Test description

6.1 Reagenti e materiali/Reagents and materials

Material Code	Description	Lot (to fill in the TR)
Device code (to fill in the TR)	Device under test (to fill in the TR)	
Predicate device (to fill in the TR)	Predicate device (to fill in the TR)	
Supplies	**Description**	
N/A	Bovine blood	
N/A	Saline solution	
TBD	Hematocrit capillaries	
TBD	15 ml plastic vials	
TBD	1 ml plastic syringes	
TBD	Reservoir	
TBD	PVC Tubing	
TBD	Silicone pump tubing	
TBD	PC tube connectors	

TBD	Stopcocks	
TBD	Adjustable tube clamp	
TBD	Cell coulter disposable tubes	
TBD	Disposable plastic cuvettes (for use with spectrophotometer)	

6.2 Attrezzature e strumenti/Equipment and instruments

Instruments/Equipment	**Calibration expiry date (to fill in the TR)**	**ID number (to fill in the TR)**
Cell coulter		
Blood Gas Analyzer ABL		
UV/Vis spectrophotometer		
Microhematocrit centrifuge		
Microhematocrit reader		
Centrifuge		
Flow meter system		
Flow meter probe		
Peristaltic pump		
Thermostatic bath		
Pressure transducer/manometer		
Decimal temperature sensor/thermometer		
Scanning electron microscope (SEM)		
Environment		
	Environment temperature:	
All tools and instruments (when applicable) used for testing and verification activities are calibrated and maintained under control as per Qura Quality System		

6.3 Preparazione del campione/Preparation of test samples

Samples should be prepared according to Qura manufacturing and packaging processes. All samples must be identified with production lot/batch number and progressive number if applicable. Samples must have passed Leak Test.

6.4 Condizionamento del campione/Sample conditioning

Test should be performed on devices subjected to two ETO sterilization cycles and to accelerated aging cycle simulating shelf life aging according to TP_012 “Accelerated Aging Test”.

6.5 Procedura/Procedure

Preliminary operations

When operating with bovine blood, the OI_900 “Lab Procedure for handling of biological fluids” must be followed by the operator.

Blood should be prepared according to the following indications.

- Collect fresh bovine blood in 1:20 (v:v) acid citrate dextrose-A solution and add heparin at a concentration of 8000 UI/5 l right after blood collection.
- Filter blood using a < 100 µm screen size filter previously washed with saline solution. It is recommended to perform this operation by gravity filling.
- After filtration, collect at least 20 ml blood sample to perform haemogas, hematoctrit, cell coulter and free haemoglobin measurements (refer to Blood collection, analyses protocol and hemolysis evaluation for detailed procedures).
- Depending on haemogas measurements, adjust filtered blood (dilute by addition of saline solution and correct base excess and pH values by adding sodium bicarbonate 5% w/v) in order to meet the following conditions. Prepare the pool of blood necessary to perform all tests (considering the priming volumes of the circuit and the number of parallel circuits to be tested).
- NOTE: during the blood dilution, consider the saline solution already present in the circuit (see the Test set-up and test protocols paragraph).

Total blood haemoglobin concentration	12 ± 0.2 g/dl
Haematocrit	30 ÷ 36 %
pH	7.1 ÷7.4
Blood glucose concentration	10 ± 5 mmol/l
Base excess	± 5 mmol/l

Table 1. Blood conditions required for performing blood trauma tests.

Test set-up and test protocols – Heat Exchangers

1 Set up different circuits according to the following scheme (figure 1):

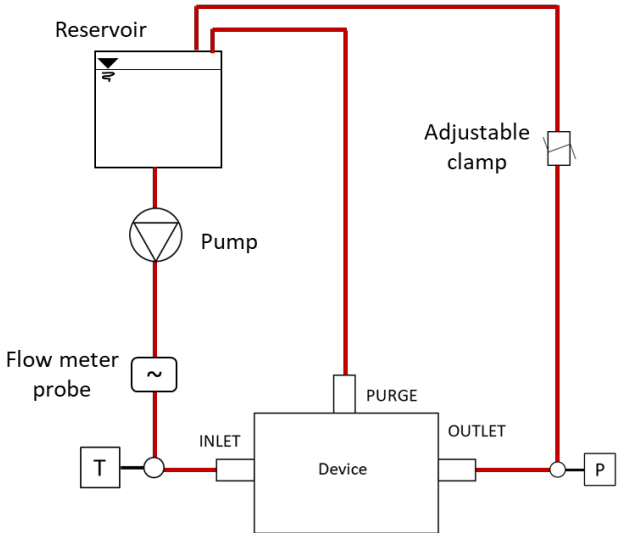

Figure 1. Layout of the hydraulic circuit for blood trauma test of the Heat Exchangers

For each test, at least a blank circuit or a circuit composed of a predicate commercial device should be prepared and tested as a reference. All circuits must have the same priming volume. The blank circuit shall compensate the device priming volume with longer tubing sets.
NOTE: all circuits must be washed with saline solution before performing blood trauma tests.

2 Carefully adjust the occlusion of the roller pumps by following standard clinical perfusion protocols (i.e. setting the pump occlusion so that a 100 cmH_2O column of saline solution drops at about 1 cm/min).

3 Prime the circuits with saline solution, and carefully remove air from the circuit.

4 Prime the circuits with the blood pool. Refer to Table 1 for values of priming volumes defined for different Qura devices. It is recommended to perform this operation by gravity filling from the blood pool container.
NOTE: all circuits must be primed with the same blood pool and tested in parallel under the same conditions. During priming, purge line (if present) should be kept open and blood outlet must be clamped.

5 Start the pumps and slowly prime the devices, making sure that air correctly purges (tapping may be needed).

6 When no air bubbles pass through the purge line, open the clamp at blood outlet and close the purge line with a clamp (where applicable).
When the circuit is completely primed with blood, collect the baseline sample (for sample collection operations refer to Blood collection, analyses protocol and hemolysis evaluation)

7 Set the flow rate according to Table 2.

Device	Code	Max flow rate [l/min]	Priming volume [l]
HX-S	HX55V-S0	8	4
	HX22V-S0	3	1.5
	HX11V-S0	1	0.5

HX-C	HX32V-C0	1	0.5
	HX22V-C0	0.5	0.25

Table 2. Flow rate and priming volume for performing blood trauma test of the Heat Exchangers .

8 Set the adjustable clamp to reach a back pressure at blood outlet (P) equal to:

- 300 ± 10 mmHg for HX-S
- 150 ± 10 mmHg for HX-C.

9 Start the 6 hours test. Verify that no liquid leakage occurs during the test by visual inspection.

10 At specified time intervals perform blood sample collection and analyses as described in Blood collection and analyses protocol.

11 At the end of the test devices shall be fixed with glutaraldehyde for the determination of the fibrinogen deposition as described in Glutaraldehyde fixation and SEM analyses

Test set-up and test protocols – Centrifugal blood pump

1 Set up different circuits according to the following scheme (figure 2):

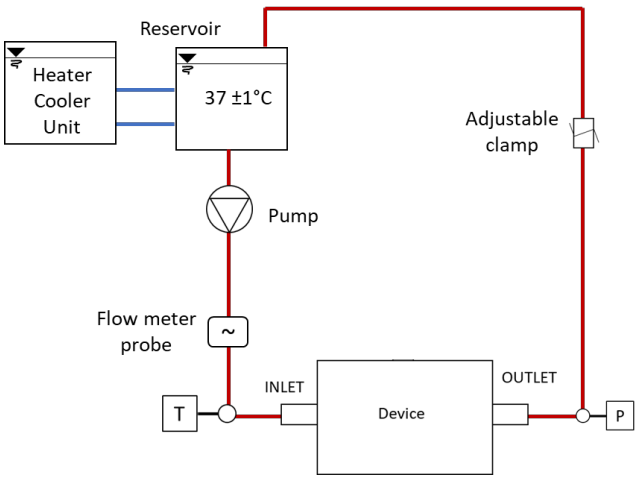

Figure 2. Layout of the hydraulic circuit for blood trauma test of the Centrifugal Pumps

For each test, at least a blank circuit or a circuit composed of a predicate commercial device should be prepared and tested as a reference. All circuits must have the same priming volume. The blank circuit shall compensate the device priming volume with longer tubing sets.
NOTE: all circuits must be washed with saline solution before performing blood trauma tests.

2 Prime the circuits with saline solution, and carefully remove air from the circuit.

3 Prime the circuit with the blood pool. Refer to Table 3 for values of priming volumes defined for different Qura devices. It is recommended to perform this operation by gravity filling from the blood pool container.
NOTE: all circuits must be primed with the same blood pool and tested in parallel under the same conditions.

4 Start the pumps and slowly increase the RPM to reach the desired flow rate and back pressure. Set the back pressure at blood outlet (P, figure 2) equal to 300 ± 10 mmHg. Set the flow rate according to Table 3.

Device	Code	Max flow rate [l/min]	Priming volume [l]
CP	CP37V-V0	8	4
	CP22V-V0	8	4

Table 3. Flow rate and priming volume for performing blood trauma test of the Centrifugal Pumps

5 When the circuit is completely primed with blood, collect the baseline sample (for sample collection operations refer to Blood collection, analyses protocol and hemolysis evaluation)

6 Start the 6 hours test and verify that no liquid leakage occurs during the test by visual inspection.

7 At specified time intervals perform blood sample collection and analyses as described in Blood collection and analyses protocol.

8 At the end of the test devices shall be fixed with glutaraldehyde for the determination of the fibrinogen deposition as described in Glutaraldehyde fixation and SEM analyses

Blood collection, analyses protocol and hemolysis evaluation

To perform blood analyses the operator shall collect 10 ml of blood samples from the reservoir using a pipette, or by gravity when using blood bags as reservoirs. Samples shall be collected in disposable 15 ml plastic tubes for subsequent operations.
Collection shall be performed from all circuits under test at the time intervals defined in table 4:

-

Time after initiation of the test [min]	HX-S	HX-C	CP
0 (baseline)	X	X	X
10	X	X	
30	X	X	X
90	X	X	
180	X	X	X
270	X	X	
360	X	X	X

Table 4

For each blood sample, the following parameters shall be measured:

- haematocrit, using the capillary centrifuge and haematocrit reader
- total blood haemoglobin and pH value, using the blood gas analyzer
- red blood cells (RBCs), WBCs and THR concentration, using the cell coulter

After the analyses performed on whole blood, separate plasma from RBCs by centrifuging the sample at 2000 g for 15 minutes (slow brake). After centrifuge, transfer al least 2 ml of plasma sample into a disposable spectrophotometer cuvette and measure:

- plasma free haemoglobin concentration, using the spectrophotometer

In addition, at each time interval and for each circuit under test the operator shall record:

- blood flow rate (l/min)
- blood temperature
- backpressure
- RPM only for CP testing

At the end of the test calculate the normalized index of hemolysis (NIH) for each tested device/circuit as follows:

$$NIH\left(\frac{g}{100l}\right) = \Delta f_{Hb} \times V \times \frac{100 - Hct}{100} \times \frac{100}{Q \times \Delta t}$$

where:

Δf_{Hb} (g/l) is the increase of plasma free hemoglobin at 360', calculated as $f_{Hb360} - f_{Hb0}$

V (l) is the priming volume of the circuit at 360' (corrected by subtracting collected blood volume)

Hct (%) is blood hematocrit at 360'

Q (l/min) is blood flow rate at 360'

Δt (min) is the duration of the test (360')

Glutaraldehyde fixation and SEM analyses

At the end of the test devices should be prepared for further evaluation as follows:

- rinse the device extensively with saline solution,
- fill the device with glutaraldehyde solution 2.5% v/v in ddH_2O and let it stand for 30 minutes to allow fixation of biological residues on the device surfaces
- drain the device and dry it completely by blowing air into it
- open the device to expose blood contacting surfaces. Visually inspect the surfaces for the presence of visible fibrinogen deposits or blood cell aggregates. Take pictures of the open device.
- collect samples for SEM analyses from the most critical points (i.e., the deposits or aggregates if present)

7. Test report

Verification must be performed with proper equipment and instruments as per section 6.
Test results shall be recorded on TR_XXX_YY "Test report" form, dated and signed by the operator who executed test. The completed test reports in PDF format are filed in the company server in the proper directory.

7.1 Presentazione dei risultati/Results reporting

Results should be presented as follows. Documents with raw data acquired on each device can be attached to the test report.
For each device/circuit fill the following table.

BLOOD TRAUMA, CELL DEPLETION AND FIBRINOGEN DEPOSITION TEST									
Device		**Lot**			**SN**	**Leackage**			
----		----			----	Yes ☐		No ☐	
Hemolysis Evaluation									
Blood data						**Circuit data**			
Time (min)	**WBC (10^3/µl)**	**THR (10^3/µl)**	**ctHb (g/dl)**	**Hct (%)**	**Hb free (mg/dl)**	**Q (l/min)**	**T (°C)**	**P (mmHg)**	**RPM**
0									
10									
30									
90									
180									
270									
360									
NIH (g/100l)									
Acceptability criteria*			**Pass**			**Fail**			
$NIH_{Qura} \leq NIH_{Pred}$			☐			☐			
Fibrinogen Deposition Evaluation									
Visible Deposits ☐		Yes ☐	No		**Location**				
SEM analysis									
Notes									
Always specify from which part of the device the samples have been collected									
Date_____ Operator_____ Signature_____									

Table 3. Result table of blood trauma, cell depletion and fibrinogen deposit for each Qura device.

*Not applicable for predicate and blank.

7.2 Criteri di accettabilità/Acceptability criteria

The devices passes the test if $NIH_{Qura} \leq NIH_{Pred}$.

8. Analisi statistica/Statistical analysis

N/A