EX1-PQ-001 SOP AND VERIFICATION OF PERFORMANCE QUALIFICATION FOR THE EXOID





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1 SCOPE

1.1 PURPOSE

Achieving accurate size and concentration measurements with the Exoid relies on correct operation of the entire system. This document:

- Records that maintenance checks have been undertaken, when and by whom.
- Defines a series of primary functionality checks that, if passed, confirm the system is operating correctly.
- Provides secondary checks in the event that the system fails any of the primary checks.
- Recommends maintenance procedures to rectify shortfalls in system performance.
- Records the state of repair of the equipment, and any maintenance steps that were undertaken to correct problems.

2 REQUIRED MATERIALS AND DOCUMENTS

2.1 MATERIALS

The following materials are required to perform the entire PQ protocol and are not provided by Izon:

- Izon Training Kit (or NP250, CPC200 and CPC400)
- Digital calipers
- Computer with the Exoid Control Suite software installed.
- Calibrated micropipettes 1 µL to 1 mL
- A vortex mixer
- Filtered deionised water
- Compressed nitrogen for drying. Alternatively, clean compressed air spray is also acceptable
- Lint-free tissue
- Standing racks for tubes (optional)
- Powder-free disposable gloves
- Refrigerator
- Digital Multimeter
- 10 MΩ reference cell

Hardware Details

Equipment type	S/N location	Serial number
Exoid Unit	Inside of rear 'foot' or on rear by plugs	
Fluid Cell	Engraved on the underside of upper and lower components	
Nanopore (NP200-300)	On long arm of nanopore	
Exoid Control Suite version	In the top left of the home screen	
Izon Data Suite version	In the top middle of the screen	

Computer requirements

Please refer to EX1-IQ-001 Section 9 for the computer minimum system and hardware requirements.

Acceptance Criteria	The computer meets the minimum requirements.	
Result satisfies AC? (Y/N)		Tester Initial/Date: Approver Initial/Date:
Notes		

2.2 DOCUMENTS

Confirm that the documents and drawings required to complete the PQ procedure are present and satisfactory (S) or unsatisfactory (US); and where they will be stored as a hard copy. Prior to Performance Qualification, confirm that the Installation Qualification and Operational Qualification have been completed and have these documents available for reference:

Documents Storage Location	Title	Initial	Date
	Tester		
	Approver		

Document Code	Document Title	Doc Status S/US	Initial	Date
EX1-IQ-001	SOP and Verification of Installation Qualification for the Exoid			
EX1-0Q-001	SOP and Verification of Operational Qualification for the Exoid			

2.3 DOCUMENT ACCEPTABILITY VERIFICATION

Acceptance Criteria	All listed documentation must be stored in a secure known location and controlled access for authorized personnel must be available. The scope of the documentation must be sufficient to ensure that the equipment can be installed, maintained and operated in accordance with cGMP requirements and vendor recommendations.		
Result satisfies AC? (Y/N)		Tester Initial/Date:	
		Approver Initial/Date:	
Notes			

3 IDENTIFICATION OF PERSONNEL PERFORMING PQ

The protocol may only be undertaken and verified by people who are qualified to do so:

Date of PQ		
Tester (Izon Certified)	Name	
	Position	
	Signature	
Approver (QA or	Name	
Representative)	Position	
	Signature	

4 FREQUENCY OF MAINTENANCE CHECKS

The maintenance protocol should be performed either to a schedule (annual by default, or a maximum of two years apart for infrequent users) – this is "Scheduled" maintenance OR whenever there is any doubt that the system is operating correctly – this is "Corrective" maintenance.

To ensure that the latest maintenance protocols are being used, request new documentation from Izon before undertaking formal maintenance checks. Record the date on which the maintenance procedure protocols were received from Izon and when they were performed, the dates should be less than 1 month apart.

The scheduled maintenance periods may be adjusted after corrective maintenance is undertaken (i.e. corrective maintenance can count as scheduled maintenance).

PQ Type	PQ Docs Received Date	PQ Date	Next PQ Due Date
(Scheduled / Corrective)	(dd/mm/yy)	(dd/mm/yy)	

Acceptance Criteria	The PQ documentation used was obtained from Izon less than 1 month ago.	
Result satisfies AC? (Y/N)	Tester Initial/Date:	
	Approver Initial/Date:	
Notes		

5 PREPARING THE SOLUTIONS

5.1 ELECTROLYTE PREPARATION

Follow the instructions in <u>Section 7 of EX1-IQ-001</u> to prepare the Measurement Electrolyte (ME). Fill out the table below to record the ME batch details used in this performance qualification.

Electrolyte Type	
Electrolyte Origin (Izon/Customer)	
Electrolyte Batch/Date of Preparation	
Electrolyte Filtered? (Y/N)	
Filtered Electrolyte is Labelled Correctly?	

5.2 WETTING SOLUTION

Follow the instructions in <u>Section 7 of EX1-IQ-001</u> to prepare the wetting solution.

Wetting Solution and ME are filtered into the Falcon tubes through a 0.22 μm filter and are labelled correctly.		
Tester Initial/Date:		
Approver Initial/Date:		
lle		

5.3 CALIBRATION PARTICLE PREPARATION

View <u>Section 4 of EX1-OQ-001</u> for important information regarding the preparation of calibration particles. For this PQ protocol, either Training Particles (TKP 200) and Training Sample (Solution S) provided in the Training Kit or CPC200 and CPC400 can be used.

Particle Type (Circle one)	TKP200 or CPC200	Solution S or CPC400
Particle Batch Number		
Expiry Date		
Concentration (particles/mL)		
Dilution Factor		
Diluted Particles Vortexed >15 seconds? (Y/N)		
Diluted Particles Labelled Correctly? (Y/N)		

FUNCTIONALITY CHECKS 6

Successful functionality checks will confirm that the system and components are working as intended. If any of the checks do not meet the acceptance criteria, additional checks and remedial actions are specified -(details of these checks and fixes can be found in Section 7 of this document).

6.1 CHECK PRESSURE SYSTEM FOR LEAKS

Load a nanopore with liquid in the upper fluid cell, stretch to 47 mm before closing the ECS software and opening the "APS tester" from the computer Start menu. Navigate to the "Testing" tab and select the correct COM port in the dropdown menu on the bottom left of the window and click connect on the bottom right. Populate the tests as shown below as well as the Exoid Serial Number, APS Serial Number and Tester Name, and check the "Run Test" boxes. No other tests available need to be completed.

Pressure Hold Test										
Run Test 🔽	Min. Pressure:	-2500 🗘	Pa	Max. Pressure:	2500	🗘 Pa	Repeats: 1 🗘	Timeout:	10 🗘 minutes	
Pressure Ramp										
Run Test 🔽	Min. Pressure:	-2000 🗘	Pa	Max. Pressure:	2000	🗘 Pa	Pressure Step	s: 1000 🗘	Test Time: 5 🗘	minutes

Select "Run tests" below the test parameters window.

Fill out the following checklist to ensure that the test is being performed accurately:



The ECS is closed.

The APS nozzle is connected to the upper fluid cell with liquid inside and a stretched nanopore underneath.

If the acceptance criteria are not met or it is not possible to pass the APS test after multiple attempts, proceed to later sections in this document for trouble shooting, listed below:



Upper Fluid Cell check 1



Once the above checks have been performed, carry out the above tests again. If the system still does not pass the criteria, please contact Izon Support for further assistance.

Acceptance Criteria	APS tests have a "PASS" status.	
Result satisfies AC? (Y/N)		Tester Initial/Date:
		Approver Initial/Date:
Notes		

6.2 CHECK STRETCHER UNIT FOR ACCURACY

Load a nanopore onto the stretcher unit. Make sure the ECS software is closed and open the "Delta tester" from the computer Start menu. Select the correct COM port in the dropdown menu on the bottom left of the window and click connect on the bottom right. On the "Raw" tab, select "GoTo" in the command dropdown. Enter the desired stretch in the green "Args" box and click "Send." Wait for the stretcher to stop moving and then measure the distance across the outside of the jaws using a set of callipers. Record the values in the table below:

Target value (mm)	Measured value (mm)	Remedial Action Required?	Final Check
42			
44			
46			
48			
50			
49			
47			
45			
43			

Fill out the following checklist to ensure that the test is being performed accurately:



The ECS is closed.

The calipers are zeroed, and the stretch is being measured correctly across the outside of the jaws.

The stretching mechanism should run smoothly without any squeaking, rattling or crunching. If the mechanism is making excessive noise, contact Izon Support. Do not continue to attempt to use the stretch mechanism as this could cause permanent internal damage.

If the acceptance criteria are not met or it is not possible to pass the stretcher test after multiple attempts, proceed to later sections in this document for trouble shooting, listed below:

Exoid check 2

Once the above checks have been performed, carry out the stretcher accuracy check again. If the system still does not pass the criteria, please contact Izon Support for further assistance.

All measured values are withir	n 0.2 mm of the target value.
Result satisfies AC? (Y/N)	Tester Initial/Date:
	Approver Initial/Date:
	All measured values are within

6.3 CHECK RESTING BASELINE CURRENT AND RMS NOISE

Using the baseline trace in the "Start Analysis" tab, this check is undertaken without the nanopore or electrolyte present.

Fill out the following checklist to ensure that the test is being performed accurately:

- Dry Upper and Lower fluid cell with compressed gas.
- Lower fluid cell fully pushed into base fitting.
- Upper Fluid Cell and Fluid Cell Cap fitted.
 - Signal trace turned on with zero voltage applied.

Resting Baseline Checks	Initial Check	Remedial Action Required?	Final Check
Record Current (nA)			
Record RMS Noise Typical (pA)			

If the acceptance criteria are not met, proceed to later sections in this document for trouble shooting, listed below:

- System check 1
- Lower Fluid Cell checks 2 and 3
- Upper Fluid Cell check 1
- Exoid Check 1

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Once the above checks have been performed, carry out the resting baseline current and RMS noise check again. If the system still does not pass the criteria, please contact Izon Support for further assistance.

Acceptance Criteria	 Current is 0±3 nA RMS noise < 7 pA 	
Result satisfies AC? (Y/N)		Tester Initial/Date:
		Approver Initial/Date:
Notes		

6.4 VCA CHECK

Using the VCA tester, install a 10 M Ω reference cell and perform a VCA baseline sweep from -1000 mV to 1000 mV in steps of 100 mV at a period of 30 seconds. Choose an output folder before beginning.

Fill out the following checklist to ensure that the test is being performed accurately:



10 MΩ reference cell installed.

Instrument is not disturbed during the course of the test.

Open the baseline sweep (if it does not open automatically) and check the status.

If the status is "FAILED" contact Izon Support for assistance.

Acceptance Criteria	Baseline sweep has a "PASS" status.	
Result satisfies AC? (Y/N)		Tester Initial/Date:
		Approver Initial/Date:
Notes		

6.5 CHECK CURRENT SYMMETRY

Using the baseline trace in the "Setup System" tab, set up the nanopore with a stable baseline current as outlined in <u>EX1-IQ-001 Section 8</u>.

Fill out the following checklist to ensure that the test is being performed accurately:

Nanopore fitted and stretched to

Upper Fluid Cell and Fluid Cell Cap fitted.

ME in the upper and lower fluid cell with a stable baseline established,

Baseline doesn't drift by more than 0.1 nA in 30 seconds.

47 mm.

RMS noise <10 pA

Apply a voltage so as the current is ~100 nA and record the current, RMS noise and voltage values in the table below. Invert the voltage (e.g. 240 mV to -240 mV) and allow the system to stabilise, record the current, RMS noise and voltage values in the table on the next page.

Current Checks	Initial Check	Remedial Action Required?	Final Check
Positive Current Value "Ipos" (nA)		-	
Positive Voltage Value (mV)		-	
Positive Current RMS noise (pA)		-	
Negative Current Value "Ineg" (nA)		-	
Negative Voltage Value (mV)		-	
Negative Current RMS noise (pA)		_	
Calculate Ineg/Ipos			

If the acceptance criteria are not met or it is not possible to establish a stable baseline current after multiple attempts and different nanopores, proceed to later sections in this document for trouble shooting, listed below:

Exoid check 1

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Fluid Cell maintenance checks

Once the above checks have been performed, carry out the current symmetry check again. If the system still does not pass the criteria, please contact Izon Support for further assistance.

Acceptance Criteria	 Stable baseline is achievable RMS noise <10 pA for both positive and negative voltage values 0.9 < (Ineg/Ipos) < 1.1 	
Result satisfies AC? (Y/N)		Tester Initial/Date:
		Approver Initial/Date:
Notes		

6.6 FUNCTIONAL STABILITY

Replace the electrolyte in the upper fluid cell with the diluted CPC200 or TKP200 Calibration Particles. Apply 500 Pa pressure and optimise the applied voltage and stretch so that the blockades are around 0.2 nA.

Fill out the following checklist to ensure that the test is being performed accurately:

Baseline current ~100 nA.

Particle rate of 500-1500 particles/min (Increase applied pressure if necessary).

Capture a three pressure recording calibration set. Process the files and view the blockade histogram and particle rate plot.

If the acceptance criteria are not met or it is not possible to hold a linear rate plot after multiple attempts, proceed to later sections in this document for trouble shooting, listed below:

APS checks 1 and 2

Once the above checks have been performed, carry out the functional stability check again. If the system still does not pass the criteria, please contact Izon Support for further assistance.

Acceptance Criteria	 Particles are resolved. Particle rate plot is linear. No irregular signs in the signal trace e.g. positive spikes. 	
Result satisfies AC? (Y/N)		Tester Initial/Date:
		Approver Initial/Date:
Notes		

7 SYSTEM MAINTENANCE CHECKS

The checks listed below need only be performed if one or more of the Functionality Checks in Section 6 do not meet the acceptance criteria. Once these checks and remedial actions are complete, repeat the failed functionality checks to confirm that the system meets the required acceptance criteria.

7.1 ASSEMBLY MAINTENANCE CHECKS

These checks are to confirm that the component parts are working correctly when assembled into a system.

Issues with electrical connections can generally be rectified by cleaning the contact areas – refer to the details in the components checks below.

System Check 1 - Isolation of the fluid cell assembly from the Exoid

- Check that the lower fluid cell is pushed firmly into place.
- Fit the upper fluid cell and shielding cap.
- The continuity between the Exoid body and fluid cell assembly can be measured at the test points shown to the right using a digital resistance meter.

When using a multimeter to check for continuity the machine will beep when the probes are touched together. The beep indicates continuity between the two points.

If the multimeter beeps during the test then contact Izon Support.



Acceptance Criteria	Results	Results Meets AC? (Y/N)
Multimeter should NOT beep during continuity test		

7.2 PRESSURE SYSTEM HARDWARE MAINTENANCE CHECKS

APS Check 1 - Nozzle O-Ring Seal

The nozzle O-ring seal which connects the APS nozzle to the upper fluid cell can suffer mechanical or, with incompatible fluids, chemical damage.

- Check that the O-ring is still flexible and that the sealing surface has no significant damage.
- The nozzle O-ring should normally be dry and NOT have any grease applied.
- If in doubt, request a replacement O-ring from Izon (size MR003x1 material S70):
 - Remove the old O-ring by sliding a tool down the side and levering it up.
 - Press the replacement O-ring into place.

Take care to avoid scratching the nozzle component when replacing the O-ring.

Acceptance Criteria	Results	Results Meets AC? (Y/N)
O-ring in good condition.		

APS Check 2 – Connection of the APS Nozzle to the Fluid Cell

- Connect the APS nozzle into the upper fluid cell.
- Check that there is a slight resistance as the nozzle is inserted it should neither feel tight, nor fall into position.
- If the APS nozzle is slightly tight, it may be possible to accidentally disconnect the upper fluid cell when removing the nozzle. To avoid accidental fluid cell disconnection, it is good practice to avoid rotating the nozzle when removing or inserting it from the upper fluid cell.
- If the nozzle is too loose, contact Izon Support for assistance.

Acceptance Criteria	Results	Results Meets AC? (Y/N)
Slight resistance to nozzle insertion, not too tight and not too loose.		

7.3 FLUID CELL MAINTENANCE CHECKS

Fluid cell maintenance checks focus mainly on the electrical contact areas. These can become dirty or oxidised over time, which degrades the signal quality. Lift the fluid cell out of the Exoid and separate it into its three sections (lower fluid cell, upper fluid cell and shielding cap).

Lower Fluid Cell Check 1 – The Lower Electrode

The lower electrode consists of either a ring of silver wire recessed in the plastic component, which is coated with an Ag/AgCl electrode paste, or a sintered electrode. The colour of the paste can vary from being very similar to the plastic to quite a lot darker, depending on usage.

Check that the electrode is still present and has not been damaged by incompatible cleaning agents or physical abrasion (the example electrode here is in good working order). If in doubt, send a photo of the electrode to Izon. Isopropyl Alcohol/Isopropanol/IPA is NOT compatible with the electrode and should not be used to clean the upper or lower fluid cells.



Acceptance Criteria	Results	Results Meets AC? (Y/N)
Electrode in good condition.		

Lower Fluid Cell Check 2 –Lower Fluid Cell to Lower Fluid Cell Fitting Contact Area

When the lower fluid cell is pushed into the Exoid unit, there are three silver plated electrical pins (circled) that contact the lower fluid cell fitting.



Check that the pins are clean, if not then use a lint free tissue with a SMALL amount of 70% EtOH to gently clean these areas. Take care to blow away any residual liquid with compressed air.

Acceptance Criteria	Results	Results Meets AC? (Y/N)
The contact areas are clean and free of debris.		

Lower Fluid Cell Check 3 – The Lower Fluid Cell Bayonet Contact Area

The electrical connection between the lower and upper fluid cells is made via the two gold plated pogo-pins on the lower fluid cell.

- Check that the contact areas are clean, if not then blow away any particulates with compressed air.
- Check that the ball bearings in the top of the pogo-pins are moving freely. They should spring back up when pushed down carefully.

If the ball bearings do not push down easily, gently push down on them.



Acceptance Criteria	Results	Results Meets AC? (Y/N)
The contact areas are clean and free of debris.		
The ball bearings in the pogo pin move freely and spring back up.		

Upper Fluid Cell Maintenance Check 1 – The Bayonet Contact Area

The electrical connection between the lower and upper cells is made via two contact pin sockets on the underside of the upper fluid cell.

Check that the contact areas are clean, if not then use a lint free tissue with a SMALL amount of 70% EtOH to gently clean these areas. Take care to blow away any residual liquid with compressed air.



Acceptance Criteria	Results	Results Meets AC? (Y/N)
The contact areas are clean and free of debris.		

Upper Fluid Cell Maintenance Check 2 – The Upper Fluid Cell to Nanopore Sealing Area

A sliding seal is maintained between the upper fluid cell and the nanopore, which allows the nanopore to be stretched and relaxed without any leakage of fluid.



A damaged seal will result in leakage of fluid from the upper cell, especially when pressure is applied, or when working at low stretches. Contact Izon Support if a replacement part is required.



Acceptance Criteria	Results	Results Meets AC? (Y/N)
The sealing area is in good condition		

7.4 EXOID MAINTENANCE CHECKS

Exoid Check 1 - The Lower Fluid Cell Fitting Area

When the fluid cell is pushed into the lower fluid cell fitting, the three silver plated pins fit into three gold plated pin holes. It is critical that there is no debris or liquid inside these holes. Blow the holes out with clean compressed air if there is any doubt.



Acceptance Criteria	Results	Results Meets AC? (Y/N)
The pin holes are clean and free of debris or liquid.		

Exoid Check 2 - Stretching Mechanism Calibration

The Exoid system should automatically keep the stretcher calibrated, however in the event that it is consistently incorrect by more than 0.2 mm the stretcher can be manually calibrated using the Delta tester. Make sure the ECS is closed, and then open the Delta Tester from the windows start menu. Select the correct COM Port on the bottom left, and click "Connect" on the bottom right. Choose "Calibrate" from the command dropdown. Measure the stretch applied to the nanopore by measuring across the outside of the jaws and enter it into the "Args" box. Click "Send >>" and then use the GoTo command to check the accuracy of the calibration.

It is best to calibrate the stretcher at a lower stretch when performing this calibration manually. Calibrate at 42 mm stretch, use the Exoid stretcher buttons to achieve this.

Acceptance Criteria	Results	Results Meets AC? (Y/N)
The stretcher is calibrated correctly, with reported values within 0.2 mm of the target values.		

Exoid Check 3 – The Power Supply

Check your power supply type, it should have a three-pin (earthed) power cable. A three-pin earthed power supply provides better protection against electrical noise from external sources such as the computer, lab equipment, or lighting. Please contact Izon Support if you do not have a three-pin power supply.

Acceptance Criteria	Results	Results Meets AC? (Y/N)
The power supply is three-pin.		

8 FUNCTIONALITY AND MAINTENANCE SIGN-OFF

Once Sections 6 and 7 have been completed, fill in the appropriate boxes following the results of the previous sections.

Maintenance Sign-Off (complete one row below)	Date	Initial
Functionality checks passed without remedial maintenance actions.		Tester:
System Approved for Ongoing Use.		Approver:
Functionality checks passed with remedial maintenance actions.		Tester:
System Approved for Ongoing Use.		Approver:
Functional checks not all passed. Consult with Izon Support to rectify remaining issues before continuing use.		Tester:
		Approver:

9 PARTICLE SIZE AND CONCENTRATION ANALYSIS

Follow Sections 5 and 6 in <u>EX1-OQ-001</u> to obtain size and concentration data for Solution S (or CPC400s). Review the particle size distribution histogram for this sample and ensure that it shows a normal distribution, the measured mean diameter (MMD) is within 10% of the certified value (CMD, shown on the original vial), and that the measured particle concentration (MC) is within 25% of the certified value (CC).

Once this section has been completed, clean the nanopore in accordance with Section 7 of EX1-OQ-001.

Acceptance Criteria	Results		
Mean Diameter	(MMD)	(CMD)	CMD x 0.1 = Acceptable Variance (AV) MMD must fall within the range of CMD±AV
Concentration	(MC)	(CC)	CC x 0.25 = Acceptable Variance (AV) MC must fall within the range of CC±AV

Acceptance Criteria	 Sample particle size distribution histogram shows an approximate normal distribution. The measured mean diameter of sample particles is within ±10% of the certified value. The measured sample particle concentration is within ±25% of the certified value. 		
Result satisfies AC? (Y/N)	Tester Initial/Date:		
	Approver Initial/Date:		
Notes			

10 CONCLUSION / COMPLETION OF PQ

List any discrepancies between anticipated/accepted and actual results of the previously described sections. Describe any corrective actions that are required to certify execution of the PQ.

Protocol Section	Further Action Required	Are There GMP Implications?	Corrective Action Number

Circle the appropriate answer from the bolded words below:

According to the information collected and reviewed as a result of this PQ process, it is our opinion that the required work **has been / has not been** completed and satisfactory results **have been / have not been** obtained, with the exception of those related to the following items on which corrective action is required:

Number of items requiring Corrective Action: —

This PQ cannot be approved if any of the outstanding Corrective Actions (CA) could compromise the company's cGMP procedures or standards.

The number of outstanding CAs is: —

Do these CAs have cGMP implications: YES / NO

IF YES THEN THIS DOCUMENT CANNOT BE SIGNED OFF AS COMPLETED.

Signing this block below confirms that all variations and failures listed within this PQ have been accounted for in the PQ Report. This PQ has therefore been completed.

10.1 PERFORMANCE QUALIFICATION COMPLETION

Name	Title	Signature	Date

NOTES

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