QN1-OQ-002 SOP AND VERIFICATION OF OPERATIONAL QUALIFICATION FOR THE qNANO

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CONTENTS

1 / SCOPE

PURPOSE

This Operational Qualification (OQ) for the qNano defines the responsibilities and the procedures that must be complied with to ensure that the OQ of this equipment is successfully completed. It also outlines the materials and tasks required to perform the OQ procedure.

Prior to this procedure being carried out the Installation Qualification for the qNano must have already been performed.

The successful completion of this protocol verifies that the operational performance of this equipment complies with the relevant guidelines and rules contained within the Company Quality Standard (ISO 13485) and 21 CFR part 11 software regulations (where relevant).

OBJECTIVES

To ensure that:

- The equipment functionality is in accordance with requirements of the Functional Specification.
- The equipment operates in a manner safe to operational staff.
- The equipment operates in manner safe to the product.
- That there are documented procedures covering all aspects of the use of this equipment.

2 / REQUIRED MATERIALS AND DOCUMENTS

MATERIALS

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

- Computer (must meet minimum specifications) with Control Suite Software installed and verified.
- Calibrated micropipettes - 1 µL to 1 mL
- A vortex mixer
- Filtered deionised water (for cleaning)
- Compressed nitrogen for drying. Alternatively, clean compressed air spray is also acceptable
- Lint-free tissue for drying
- Standing racks for tubes (optional)
- Powder-free disposable gloves
- Refrigerator

DOCUMENTS

Confirm that these documents required to complete the OQ procedure are present. They will be stored in the same location as outlined for the documents in QN1-IQ-002.

Document Reference	Document Title	Present?	Initial	Date
QN1-IQ-002	Verification of IQ Protocol for the qNano (COMPLETED)			
QN1-OQ-002	Standard Protocol and Verification of OQ for the qNano			
QN1-OQ-007	21 CFR Part 11 Software Regulation Guidelines			

DOCUMENTATION ACCEPTABILITY VERIFICATION

If the documents meet the Acceptance Criteria, fill out below

Acceptance Criteria	All listed documentation must be stored in a secure known location. 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)		Executor Initial/Date:
		Reviewer Initial/Date:
Notes		

3 / IDENTIFICATION OF PERSONNEL PERFORMING OQ

Enter the details of the people involved with this Operational Qualification. The training records for all Izon Science, Ltd. personnel are held on file and can be inspected by the QA department.

Date of OQ		
Executor (Izon Staff)	Name	
	Position	
	Signature	
Reviewer (Customer QA Rep)	Name	
	Position	
	Signature	

4 / CALIBRATION PARTICLE PREPARATION

BACKGROUND INFORMATION

Particle size and concentration are converted from a blockade magnitude (nA) and blockade rate (blockades per minute) into a diameter (nm) and concentration (particles per mL) using calibration particles of known diameter and concentration. Different calibration particles are used depending on the nanopore size selected. For this OQ procedure, particle details are as follows:

Catalogue Number	Analysis Range (nm)	Izon Calibration Particle	Target Particle Conc (/mL)
NP250	110-630	CPC200/400	$2.0x10^9$

Calibration particles are supplied in concentrated form and should be diluted in the same electrolyte as the sample particles, to achieve the target concentration shown above.

REQUIRED TECHNIQUES

The SOP executor must have understanding and experience with Forward Pipetting Technique. Forward pipetting should be used in ALL sample preparation. For more information on pipetting techniques, please visit: https://support.izon.com/pipetting-techniques

Great care should be taken during particle preparation – any errors in the dilution process will relate directly to an error in calculated particle concentration. Below are some tips for particle preparation:

- All particles must be diluted immediately before use. Forward pipetting should be used for sample preparation to give the most accurate dilutions and to avoid wastage.
- Take care to mix fluids homogenously before and after dilution (larger particles will tend to sediment and can be seen on the bottom of the vial prior to mixing).
- Avoid transferring excess calibration particles on the outside of the pipette tip.
- Incorrect pipetting technique or using a non-calibrated pipette will affect the final concentration of the prepared sample.
- Use tips that are clean and do not have filters in them

PARTICLE PREPARATION CHECKLIST

Complete the following checklist to verify that calibration particles have been correctly prepared:

Step	Requirement			Complete?
a	Measurement Electrolyte has been freshly filtered through a 0.22 µm filter.			
b	Pipettes are all calibrated (if not, ensure that they are used at exactly the same settings for preparing the different samples).			
c	Pipette exactly 500 µL of filtered electrolyte into two 1.5 mL Eppendorf tubes and label each vial as "TS" and "CAL". Write the dilution factor of 1:51.			
d	Prepare calibration particles: • Vortex Training Particles (TKP-200) on medium speed for 15 seconds. Ensure no sediment can be seen. • Pipette exactly 10 µL of Training Particles into the vials labelled "CAL"			
e	Prepare sample particles: • Vortex Solution S (Training Sample) on medium speed for 15 seconds. Ensure no sediment can be seen. • Pipette exactly 10 µL of Solution S into the vials labelled "TS"			
f	Vortex the diluted particle vials "CAL" and "TS" on medium speed for 15 seconds.			
g	Record the details of TKP200 and Sol S:			
	Particle Details	Mean Diameter (nm)	Concentration (Particles/mL)	
	TKP200			
	Solution S			

PARTICLE PREPARATION VERIFICATION

When Particle Preparation is complete, fill in the following sign-off box:

Task	Name	Signature	Date
Executed			
Reviewed			

5 / PARTICLE SIZE ANALYSIS

RECORD A CALIBRATION FILE

Complete the following checklist to verify that the calibration file has been correctly recorded

Step	Requirement	Complete?
a	A stable baseline current with RMS noise of <15 pA has been established.	
b	Set the voltage by clicking the '+' or moving the slider to achieve a current of around 100 nA (±5 nA).	
c	Ensure that no blockades are seen when the filtered electrolyte is in the top fluid cell – blockades may indicate dirty vials or micro bubbles in the electrolyte.	
d	Replace the upper fluid cell with 35 µL of the diluted Training Particles "CAL" • Remove the upper fluid cell and add an aliquot of electrolyte on the top of nanopore. • Rinse the upper fluid cell with deionised water and dry with compressed gas • Gently wipe the top of the nanopore with a lint-free tissue • Fit the upper fluid cell into place • Load 35 µL of the next sample immediately	
e	Refer to the Relative Particle Size (%) plot. Improve the signal-to-noise ratio by: • Changing the nanopore stretch • Increasing voltage to achieve a current of 120 nA. • Repeat until an average relative particle size of 0.25% is achieved.	
f	Record the below values: • Nanopore Stretch (mm): • Voltage (V): • Average Baseline Current (nA): • Average RMS Noise (pA):	
g	Record >500 blockades events.	
h	Save recording with the correct information entered in the SAVE DATA window as "Calibration" with file name "Training Calibration TKP200". Make sure the "Mean" diameter is entered as the particle size. Click OK.	

RECORD A SAMPLE FILE

Complete the following checklist to verify that sample file has been correctly recorded and calibrated:

Step	Requirement	Complete?
a	Replace the upper fluid cell with 35 µL of the diluted Training Sample "TS" • Remove the upper fluid cell and add an aliquot of electrolyte on the top of nanopore. • Rinse the upper fluid cell with deionised water and dry with compressed gas • Gently wipe the top of the nanopore • Fit the upper fluid cell into place • Load 35 µL of the next sample immediately	
b	Ensure that the baseline current and RMS noise levels have not changed significantly (within 5%) from the calibration particles' run. • Nanopore Stretch (mm): • Voltage (V): • Average Baseline Current (nA): • Average RMS Noise (pA):	
c	Refer to the Relative Particle Size (%) plot. The blockades should be visible. If not, refresh the sample in the upper cell.	
d	Record >500 blockades events.	
e	Recording saved with the correct information entered in the SAVE DATA window as "Sample" with file name "Training sample, TS". Click OK.	

PROCESS DATA (SIZING)

Complete the following checklist to verify that all recorded files have been correctly processed and calibrated:

Step	Requirement	Complete?
a	Process both files in the Analyse tab	
b	Calibrate the "Training Sample, TS" file using "CAL" file. The "Training Sample, TS" file now displays size in nm in the data plots and reports	
c	Save the files in a group called "Test File 1"	
d	Review the measurement. In order for sample particle size to be correctly calculated using calibration particles: • The settings (stretch and voltage) must not be changed between both recordings. • Baseline current between calibration and sample recording should be within 5%. • Both calibration and sample particles must have a blockade magnitude sufficiently larger than the RMS noise.	
e	Confirm calculated size meets acceptance criteria: Given Mean Diameter (GMD) x 0.15 = Allowable Deviation (AD) _____ x 0.15 = _____ GMD – AD < Calibrated Mean Diameter < GMD + AD _____ < _____ < _____	

Acceptance Criteria	• Solution S (Training Sample), "TS" particle size distribution histogram shows an approximate normal distribution • The calibrated MEAN diameter of Training Sample particles is within ±15% of the value shown on the label	
Result satisfies AC? (Y/N)		Executor Initial/Date:
		Reviewer Initial/Date:
Notes		

6 / PARTICLE CONCENTRATION ANALYSIS

In order to get the most accurate concentration measurements, particle translocation rates are recorded at multiple pressures for the calibration and sample particles. The pressure is applied externally using the Variable Pressure Module (VPM). Three pressure recordings will be made (e.g.: P=4, 6, 8 mbar).

Complete the following checklist to verify that concentration protocol has been performed correctly.

Step	Requirement	Complete?
a	Refresh "TS" sample in the upper fluid cell	
b	Apply the lowest pressure value. User may choose a suitable pressure depending on the nanopore setting. • Set the system to VENT (vent hole visible) • Pull the plunger to "0" • Connect the PM2 nozzle to the upper fluid cell • Set the system to SEAL (vent hole closed) • Push the plunger to the desired value.	
c	Baseline current is stable. If not, refresh sample in the upper fluid cell	
d	Record >500 blockade events.	
e	Save the data. Call the file "TS, PX". (X being the value of the applied pressure)	
f	Without opening the VPM vent valve, increase the pressure, record >500 blockades and save the (sample) file as "TS, PX" (X being the new value of applied pressure).	
g	Without opening the VPM valve, further increase the pressure, record >500 blockades and save the (sample) file as "TS, PX" (X being the newest value of applied pressure).	
h	Replace the upper fluid cell with 35 µL of the diluted "CAL" particles by performing the sample changeover protocol: • Release the applied pressure and disconnect the PM2 nozzle. • Remove the upper fluid cell and add an aliquot of electrolyte on the top of nanopore. • Rinse the upper fluid cell with deionised water and dry with compressed gas • Gently wipe the top of the nanopore with a lint free tissue • Fit the upper fluid cell into place • Load the next sample immediately	
i	Ensure that the baseline current and RMS noise levels have not changed significantly (within 5%) from the sample particles' run.	
j	Repeat Steps (b) – (g), saving these as CALIBRATION. Check that: • The dilution factor and concentration are correctly entered. • Identical pressure values are used.	
k	Release the pressure once all three calibration recordings have been saved.	

PROCESS DATA (CONCENTRATION)

Complete the following checklist to verify that all recorded files have been correctly processed and calibrated:

Step	Requirement	Complete?
a	Process all six files in the Analyse Data tab	
b	• Use the three sample files and the three calibration files to perform a "Multi-Point Concentration" calibration. All "TS" files now display size in nm in the data plots and reports	
c	Save the files in a group called "Test File 2"	
d	Review the measurement. For sample particle's concentration to be correctly calculated using calibration particles: • The settings (stretch and voltage) must not be changed between both recordings. • Baseline current between calibration and sample recording should be within 5% • A linear rate plot is achieved.	
e	Confirm calculated concentration meets acceptance criteria: Given Concentration (GC) x 0.25 = Allowable Deviation (AD) _____ x 0.25 = _____ GC – AD < Calibrated Concentration < GC + AD _____ < _____ < _____	

Acceptance Criteria	The calculated Solution S (Training Sample), "TS" particle concentration is within ±25% of the value shown on the label.	
Result satisfies AC? (Y/N)		Executor Initial/Date:
		Reviewer Initial/Date:
Notes		

7 / NANOPORE CLEANING

At the end of an experiment it is critical to wash out the pore before removal of the pore from the qNano instrument. Removal of the pore with electrolyte and salts within it will shorten its lifespan. Salt crystals will form within the pore often rendering it impossible to wet or with reduced resolving capability when next used.

Complete the following checklist to verify that the cleaning protocol has been performed correctly.

Step	Requirement	Complete?
a	Stretch nanopore to 48 mm and apply 0.1 V	
b	Replace upper and lower fluid cells with electrolyte	
c	Connect the PM2 nozzle and apply maximum pressure for 1 minute, ensuring there are no significant blockades present.	
d	Once completed, repeat Step b and c with filtered DI water. Tips: 1. During fluid changeover, add an aliquot of filtered DI water to the top of nanopore to avoid drying. You can see the baseline current drop to <2 nA. 2. Repeat until baseline current drops to <2 nA. Flushing for 10 min is recommended. 3. If the current is still high repeat with fresh addition of DI water to upper and lower cell.	
e	Take nanopore off, wash with DI water, and dry.	
f	Rinse the fluid cells with DI water and dry with a lint-free tissue followed by compressed gas.	

Acceptance Criteria	The baseline current drops to a value approximately 0 nA (<5 nA)	
Result satisfies AC? (Y/N)		Executor Initial/Date:
		Reviewer Initial/Date:
Notes		

8 / 21 CFR PART 11 SOFTWARE REGULATION

Work through and approve QN1-OQ-007 21 CFR Part 11 Software Regulation Guidelines.

Acceptance Criteria	The Control Suite Software passes the tests laid out in QN1-OQ-007.	
Result satisfies AC? (Y/N)		Executor Initial/Date:
		Reviewer Initial/Date:
Notes		

9 / CONCLUSION / COMPLETION OF OQ

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 OQ.

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 OQ 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 **OQ** 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 OQ have been accounted for in the OQ Report. This OQ has therefore been completed.

OPERATIONAL QUALIFICATION COMPLETION

Name	Title	Signature	Date