

Standard Operating Procedure

SOP121

Title: USE AND MAINTENANCE OF THE CHEMOMETEC NC-3000 NUCLEOCOUNTER

Location: CBE LABORATORY UNIT

1. PURPOSE

To describe procedures for the use and maintenance of the Chemometec NC-3000 Nucleocounter for counting cells the optimal range is 5×10^4 – 5×10^6 cells/ml.

2. SCOPE

This SOP applies to operation and maintenance of the bench-top Chemometec NC-3000 Nucleocounter, located in the CBE Laboratory Unit at Holywell Park.

The Nucleocounter is a bench-top automated mammalian cell counter that performs cell count and viability measurements using the Via1-Cassette and NC-Slides. The system is based on staining mammalian cell DNA with fluorophores. The Via1-cassette, uses Acridine Orange (AO) and DAPI. The AO stains living and dead cells, while the DAPI stains only the dead cells, giving an accurate cell count. There are various assays which can be run on the NC-3000. These additional assays can only be done using the NC-slides and the COSHH must be done before ordering for each.

3. RESPONSIBILITES

The Operator shall:

- (i) Ensure that they are familiar with the requirements for the correct operation of the equipment and its controls, requirements, and emergency procedures by reference to this SOP, and the Manufacturer's Operating Instructions.
- (ii) Ensure that the equipment is suitable for the work they intend to carry out.
- (iii) Carry out routine cleaning and maintenance of the equipment, as required.
- (iv) Record alarms, adverse events, non-conformances, or malfunctions on the Preventative Maintenance Record and notify the Laboratory Manager/Responsible Person.

5.2. The Responsible Person/Laboratory Manager shall:

- (i) Ensure laboratory personnel are given suitable information, instruction, training or supervision in the safe use and maintenance of the equipment.
- (ii) Coordinate routine maintenance duties to be performed by laboratory personnel, as required (according to SOP004).
- (iii) Investigate and reported alarms, adverse events or non-conformances associated with equipment shaker usage.
- (iv) Schedule servicing and preventative maintenance with authorized service representatives. External maintenance and servicing of the equipment can only be performed after it has been

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suitably disinfected (see SOP003) and a 'Decontamination Certificate' has been issued (a proforma is available on the CBE Learn page).

4. EQUIPMENT AND MATERIALS

1. Chemometec NC-3000 Nucleocounter
2. Chemometec Nucleocassette or NC-slides A2 or A8.
3. Chemometec Solution 13
4. 70% IMS for cleaning

5. PROCEDURE

Operators should refer to the User Manual for a full description and specification of the Nucleocounter NC-3000 Automated Mammalian Cell Counter

5.1 Cell Counting Procedure

- (i) Log on to the laptop attached to the Nucleocounter.
- (ii) Open the Nucleocounter program by double clicking on the icon.
- (iii) The Nucleocounter is ready to use when the program is open and the light on the front of the Nucleocounter has turned to green.
- (iv) Select either Via-1 cassette, or A2 or A8- NC-slide, depending on which is being used. Then select the Cell Count and viability assay.

Enter the sample details.

Note: other assays can be run on the Nucleocounter.

- (v) Prepare the cell sample.
 - Via-1 cassette. The Via-1 cassette is pre-loaded with Solution 13. Pipette 200ul of cell suspension into an Eppendorf tube. Vortex the tube for 10 seconds. Load the Via-1 cassette. This is done by immersing the tip of the Via-1 Cassette directly into the cell suspension, ensuring that the bottom of the tip is covered, and then gently pressing the piston on the Via-1 Cassette to load the cell suspension into the cassette.
 - A2 or A8 NC-slide. Take a cell suspension sample and add an appropriate amount of Solution 13. (If you enter the amount of cell suspension taken on to the Nucleocounter it will tell you the amount of Solution 13 to add.) Vortex the sample and pipette the sample into the chamber of the slide. The A2 slide takes 30ul in each chamber and the A8 slide takes 10ul in each chamber.

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- (vi) The Via-1 Cassette or NC slide is then inserted into the NucleoCounter device by pressing the eject button, a drawer will come out and the slide or cassette can be inserted in.

CAUTION: Ensure that the NucleoCassette is correctly inserted into the device before pressing "Run"

- (vii) Press the eject button again to close the Nucleocounter.

CAUTION: The nucleocounter has to be closed, it will not perform a cell count until the drawer is closed.

- (viii) Once the cassette/slide has been loaded the measurement is activated by pressing the "Run" button.

- (ix) The analysis will begin after a few seconds and will take different lengths of time depending on what is being, a Via-1 cassette takes approximately 3 minutes while the slides take slightly longer.

- (x) Once complete, the total cell count number and the viability will be shown. If you want to see more detailed results, click on the results tab, and more information will be shown such as Live cells, dead cells, total cells, cell viability, cell diameter and aggregates.

- (xi) After recording the data, remove and discard the Cassette or NC-slide appropriately as bio hazardous waste. **Do not reuse the Cassette or Slide.**

- (xii) At this point, the NucleoCounter is ready for another sample. If you are not using the instrument, close the Nucleocounter program and log off. Please do not shut down the Computer or turn the Nucleocounter off.

If other assays are to be done on the Nucleocounter, please see the Chemometec website, an also the NC-3000 Nucleocounter manual.

5.3. Spill Procedure

- (i) If potentially infectious material or any other liquid is spilled inside the equipment, do not use the equipment. Contact the Responsible Person.
- (ii) If potentially infectious material is spilled on the outside of the equipment, the unit should be decontaminated by wiping the outer surface with a cloth sprayed with 70% IMS, in accordance with procedures outlined in SOP004 and SOP038.
- (iii) All spills should be recorded in the Spill Record.

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5.4. Maintenance, cleaning, troubleshooting and equipment malfunction

5.4.1 Maintenance and cleaning

CAUTION: When cleaning the cassette insertion area, great care must be taken against introducing any liquid or dust into the insertion slit of the NucleoCounter. Any liquid that enters the interior of the NucleoCounter can damage the optical parts and thus compromise the quality of the cell counts.

NOTE: The presence of a dust particle will normally not influence the counting of cells. The NucleoCounter will distinguish between cells and contaminants since a cell is generally significantly smaller.

5.4.2 Equipment Malfunction

- (i) If any part of the equipment fails or malfunctions, contact the Laboratory Manager/Responsible Person. With permission of the Laboratory Manager or Responsible Person, consult the Operator Instruction Manuals to access fault finding, error displays and troubleshooting procedures.
- (ii) All problems and corrective actions should be recorded in the Equipment Maintenance Record.
- (iii) If the equipment fails to work or malfunctions and cannot be rectified according to troubleshooting procedures detailed in the Operator and Users Manuals the Laboratory Manager must be informed and a "Do Not Use" notice should be posted on the equipment. Contact the manufacturer for advice and coordinate with the Lab Manager for external maintenance and servicing.
- (iv) External maintenance and servicing of the equipment can only be performed after it has been suitably disinfected (refer to SOP003 for further details) and a 'Decontamination Certificate' has been issued (a proforma is available on the CBE LEARN page).

5.4.3 Troubleshooting

- (i) If the error message "**No valid cassette**" appears, this is due to one of the following situations:
 - The cassette has been analysed previously
 - The cassette is not inserted properly into the NucleoCounter
 - There is no cassette inserted into the NucleoCounter
- (ii) If the error message "**Analysis Aborted**" appears, this is due to the stained mixture inside the cassette not reaching the chamber within a given time. This is often due to an insufficient volume of lysate mixture being loaded into the cassette. Load a new cassette and repeat the

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analysis. This error can also occur if the cassette has not been inserted properly into the NucleoCounter. In this situation, take the cassette out, insert it properly and run the analysis again.

- (iii) If the error message “**Sample could not be analysed**” appears this is either due to the concentration of stained nuclei inside the cassette chamber being far too high – i.e. when the sample is not sufficiently diluted.
- (iv) There is also an error, when the number of cells is not within the optimal range, and cannot give an accurate cell count number, It will tell you whether the amount is too high or too low. This means that you may need to dilute the sample more or centrifuge the sample and resuspend in a smaller volume.
- (v) If the error message “**Sensor error**” appears, samples can still be analysed but the accuracy of the results cannot be guaranteed since the volume of the measurement chamber may not be read correctly. Contact ChemoMetec should this situation arise.
- (vi) If the NucleoCounter cannot be turned on, please verify that the power supply is connected to the Nucleocounter and a working power plug. If the NucleoCounter still does not turn on, consult the user manual, and notify the lab manager.
- (vii) All problems and corrective actions should be recorded in the Equipment Maintenance Log

5.5 Waste Disposal

Refer to SOP003 and SOP039 for details on disposal of biological waste, waste chemicals and solvents, respectively.

- (i) Solid Waste** such as the NucleoCassette after usage and micro-centrifuge vials containing lysate mixtures should be disposed of via the orange stream solid waste i.e. safe to autoclave according to manufacturer’s instruction (refer to SOP003 for further information of disposal of such waste).
- (ii) NC-slides** are glass, these are to be disposed off in sharps boxes.

6. DOCUMENTATION

The following records are outputs of this SOP:

1. QS-Form-009 Decontamination of equipment certificate
2. Equipment maintenance record

These records shall be filed in the Equipment File and stored in the CBE Office or otherwise archived for future review or retrieval.

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SOP Version History

| Version Reviewed | Date Revised/ Reviewed | Revision Summary | New Version Number |
|-------------------------|-----------------------------------|---|-----------------------------------|
| 1.0 | 01.10.12 P.Hourd | Annual Review – Format revised | 2.0 |
| 2.0 | 13/08/2020 J.Bowdrey | Updated from Nucleocounter NC-100 to NC-3000 Updated with the use of Via-1 cassettes and NC- slides A2 and A8. Updated how to do a cell count. | 3.0 |
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