Risk Assessment

Procedure	Use of LUNA-II Automated Cell Counter

Name(s) of person performing the work	Users (Lab manager & Lab Technician & Tenants & Licensee's)		
Name & position of assessor	Bushra Nawaz & Apprentice lab technician and Khwaja Islam & Lab Manager	**Signature**	
Date of assessment	29/07/2019	**RA Number**	BioE 0036

Outline of procedure / activity:

The LUNA-II Automated Cell Counter is an image-based cell-counting device that features an innovative autofocusing liquid lens and a proven counting algorithm, providing a fully automated solution for cell counting and viability analysis. Simply prepare a cell sample solution and the LUNA-II™ does the rest, doing away with the subjectivity and time expenditure of manual cell counting.

The LUNA-II provides:

- the total number of cells per mL,
- the number of live and dead cells per mL,
- the viability of cells (% live cells to total cells),
- cell images (optional: labelling live and dead cells as green and red circles, respectively),
- cell cluster maps (% of single cells, doublets, and triplets), and
- histograms of cell size distributions.

The LUNA-II automatically saves result as CSV files and both reusable and disposable slides can be used. Designed for cost-efficient an accurate cell counting, the LUNA reusable slide combines the economy of manual cell counting with speed, accuracy, and convenience of automated cell counting. The disposable LUNA cell counting slides maintain the highest standard of cell counting accuracy and offer the ultimate counting experience with no mess or clean-up.

The LUNA-II will be located on a benchtop in the TC lab (696.10.26) on the ground floor. The cell counter must not be operated by anyone other than trained Lab Personnel to ensure safe operation.

For quick start guide see appendix 1.

<u>Operating the Cell Counter:</u>
Ensure the cell counter is on a level surface away from vibrations of other devices. Connect the power cord to a suitable electrical outlet. Push the power button below the touchscreen to turn the instrument on. The

company logo will appear followed by the start-up screen. The main menu has four options; **count, review, protocol,** and **settings.**

Front and Back View of LUNA-II

Settings:

Select **settings** from the main menu; the **setting** screen displays:

- A home icon; press this to return to the main menu,
- A current protocol and date,
- The date and values of the most recent calibration,
- The date and version of the latest software update.

Setting options:

- [**Staining options**] select for the presence or absence of a stain (see below).
- [**Counting options**] select auto exposure to adjust light settings for each count.
- [**Date/time**] adjust the date and time of your instrument or record keeping purposes.
- **[Software update**] update software to the most recent version. Download the most recent version from the Logos Biosystems website (www.logosbio.com) into the root directory of a compatible USB drive. Press software update in the settings screen. Insert the USB drive with the downloaded file into the USB port. Press Start. Do not turn the instrument off during the update, the date and version of the software update will change automatically in the settings. Note; users must recalibrate the background after each software update.
- [**Background calibration**] perform background calibrations with each software update. The counting slide port should be empty for calibration step 1. If there is a slide in the port, remove it. Press start. Mix one part stain with an equal volume of distilled water, PBS, or plain medium. Put 10µL of the diluted stain into the chamber of a new LUNA™ cell-counting slide. A window will appear to indicate calibration step 2. Insert the slide face up and sample side first into the counting slide port. Do not insert the slide facedown. Press start. Press exit to return to the settings screen when calibration step 2 is complete. The background calibration value and data will have changed in the settings screen.

Settings: staining option

User scan select for the use or absence of a stain for cell counting with the LUNA-II.
The LUNA-II is optimized for use with trypan blue or Erythrosin B.

Turning On/Off:

1. To turn the To turn the instrument on, push the power button below the touchscreen.
2. It is unnecessary to turn the instrument off between uses as standby mode is activated after ten

minutes of inactivity. The touch screen will black out in standby mode.
3. Simply press the touchscreen or push the power button to start the LUNA-II up again.
4. Turn the instrument off at the end of each day.
5. To turn the instrument off, press the power icon in the main menu (see Section 2.2:Startup/Main Menu on manual)or push the power button for five seconds.

Option	Description
With Trypan Blue	This option is used when cell samples are mixed with trypan blue or Erythrosin B for regular bright field counting. This option generates cell viability data. Adjust the dilution factor in your set protocol appropriately.*****
Without Trypan blue**	When samples are not mixed with a stain, select this option and follow the directions in the message boxes. Adjust the dilution factor in your set protocol appropriately. *****

**The dilution factor will not change automatically. Failure to adjust the dilution factor will lead to inaccurate cell concentration calculations.*

********Low contrast from not using stain may lead to suboptimal results.*

Important – Users must recalibrate the background when switching stains (settings background calibration section 2.3.5 in the manual).

Protocol parameters:

The LUNA-II protocols have the following modifiable parameters:

Parameter	Range	Default	PBMC
Dilution factor	1-100	2	2
Noise reduction	1-10	5	5
Live Cell Sensitivity	1-9	1	7
Roundness (%)	0-100	60	60
Min. Cell Size (µm)	3-59	3	3
Min. Cell Size (µm)	4-60	60	30
Declustering level	None, Low, Medium, High	Medium	Medium

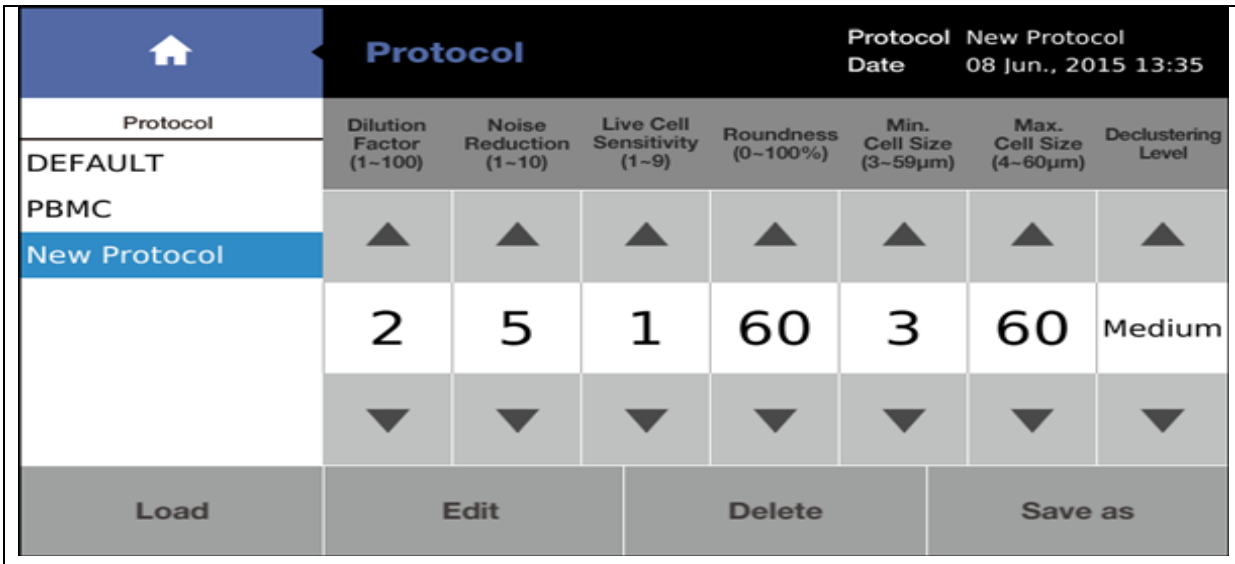
Refer to manual for explanation of each parameter listed above.

General Guidelines:

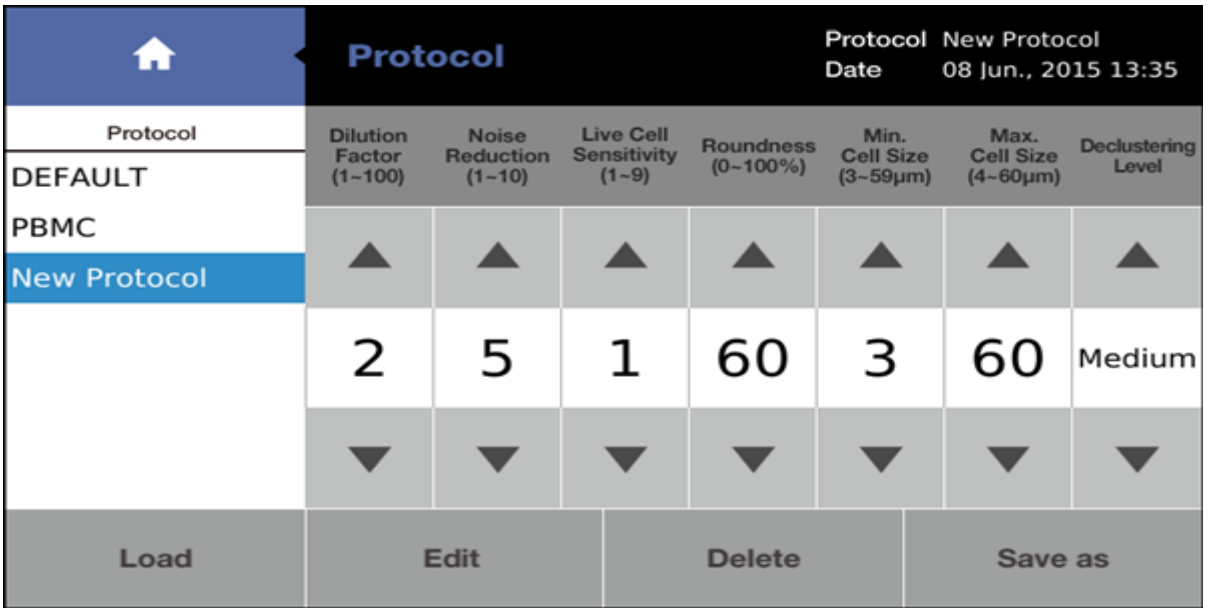
1. Hold slides by the edges to avoid touching the optical surface. Take care that the optical surfaces of the slide do not become smudged, damaged, or contaminated.
2. When staining cells with trypan blue, perform cell counting within three minutes of mixing samples for accurate cell viability measurements. If necessary, count your sample twice (duplicate readings) and take an average. Otherwise, use Erythrosin B for an alternative that is less toxic to your cells.
3. As the LUNA-II is calibrated before shipping, recalibration before use is not necessary.

Creating and Editing Protocols:

Select **protocol** from the main menu. The Protocol screen includes a list of saved protocols. The selected protocol is highlighted in blue. The parameters of the selected protocol are displayed in the right panel. The DEFAULT and PBMC protocol scan not be modified or deleted. To create a new protocol, select **New Protocol** and press **Load**. Press **Delete** to delete the selected protocol. Press **Edit** to modify the selected protocol. This will activate the arrows for each parameter, turning them a solid grey. Press the arrows to adjust the values of each parameter as desired.



Press **Edit** to modify the selected protocol.
This will activate the arrows for each parameter, turning them a solid grey.
Press the arrows to adjust the values of each parameter as desired.

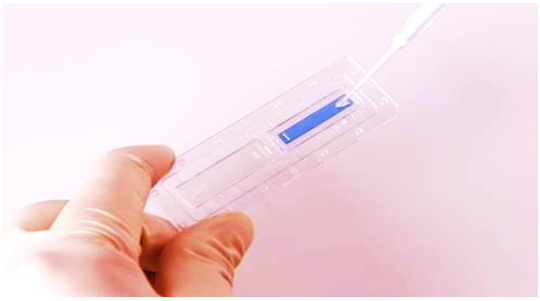


Press **Save as**.
Using the onscreen keyboard, name the protocol and press **Save**.
The newly created protocol will appear in the list of protocols in the Protocol screen.

Protocol Selection:
Select the desired protocol in the Protocol screen.
Press **Load** to apply the selected protocol.
Now the instrument is ready to count cells with the selected protocol.
Important! Merely selecting a protocol does not mean that it has been put into effect.
To apply the selected protocol, make sure to press **Load**.

Sample Preparation:

1. Prepare a cell suspension according to standard procedures. Mix gently but thoroughly to ensure that the suspension is homogenous.
2. Mix 10 µL cell suspension with 10 µL stain. Pipette gently.
3. Prepare a new LUNA™ Cell Counting Slide or a clean LUNA Reusable Slide. Hold the slide by its edges and place the pipette into the space between slide and coverslip at the edge of chamber, and fill/load 10-12 µL of the cell sample into a sample chamber. Do not lift the coverslip with pipette tip and do not overfill. For easy and accurate loading, hold the pipette at a 45-60° angle to the slide. Be careful not to over-load or under-load the sample chamber. Use only the provided coverslip with the LUNA Reusable Slide and cover slips.



Slide Insertion:

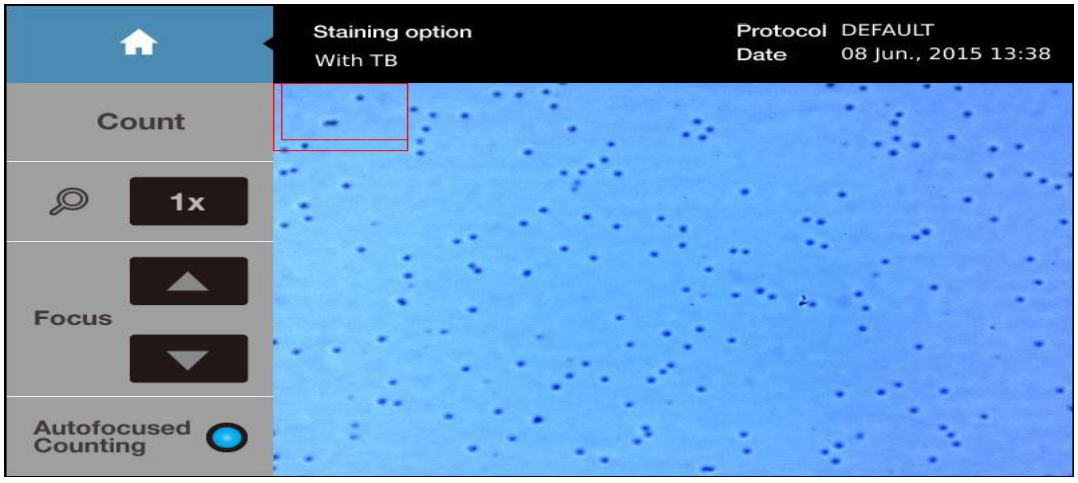
4. Insert the slide face up into the sliding port of LUNA-II. The LUNA-II can only analyse the inserted chamber. **Important**! Do not insert the slide facedown. A live image will appear on the screen. If not, the slide may be inserted incorrectly.

Focusing:

5. The LUNA-II has an autofocusing algorithm that works in tandem with a focusing mechanism to rapidly obtain the Z position of the sample by applying a small voltage to a liquid lens. The elimination of mechanical parts in the focusing mechanism removes noise and significantly reduces the need for servicing.

Autofocusing:

6. Press the circle next to **[Autofocused Counting]**. The circle will turn blue when the autofocus is activated.



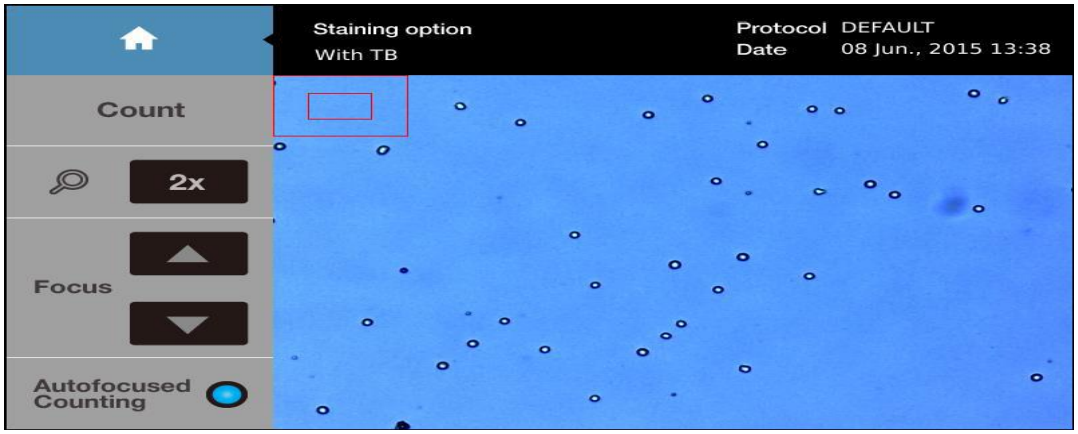
Manual focusing:

7. User scan adjust the focus manually by simply pressing the **[Focus]**arrow heads (up or down) with

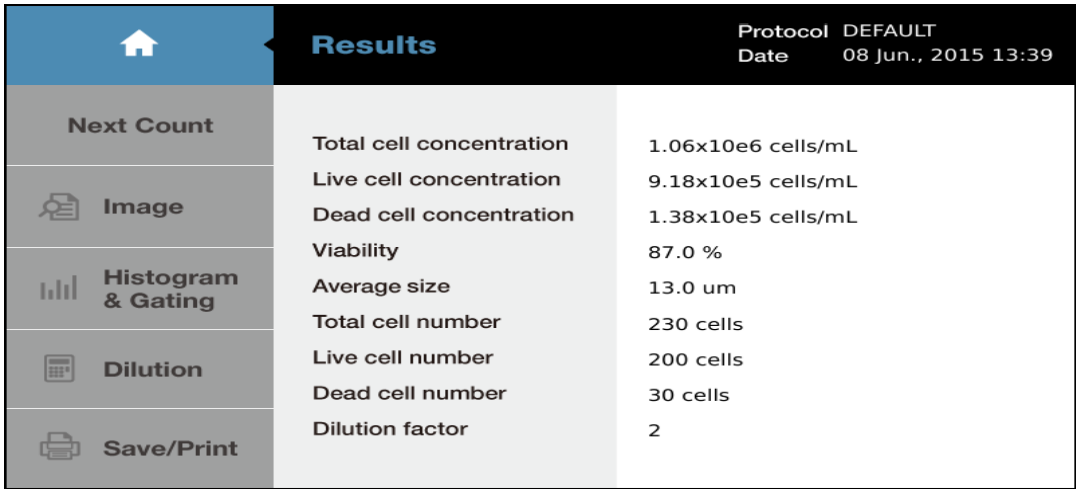
the autofocus function on or off.

Cell Counting:

8. Use a finger or a stylus to navigate the image. The red outer box inthe topleft corner of the image represents the entire counting area and the inner box is the current field of view. The size and location of the inner box will change with the magnification and movement of the screen.



9. Press the magnifier button to zoom in and out of the image.
10. Press **[Count]**to start counting.
11. The LUNA-II™counts the cells in 0.5 µL, which is comparable to five (1 mm x 1 mm) squares on a standard hemocytometer.
12. Counting time will vary with protocol, cell size,and cell concentration. With the DEFAULT protocol, cell samples with a concentration of ~1x 106cell/mL will take at minimum 10seconds to count without autofocusing or 15 seconds with autofocusing.
13. Cell count and viability results will appear.



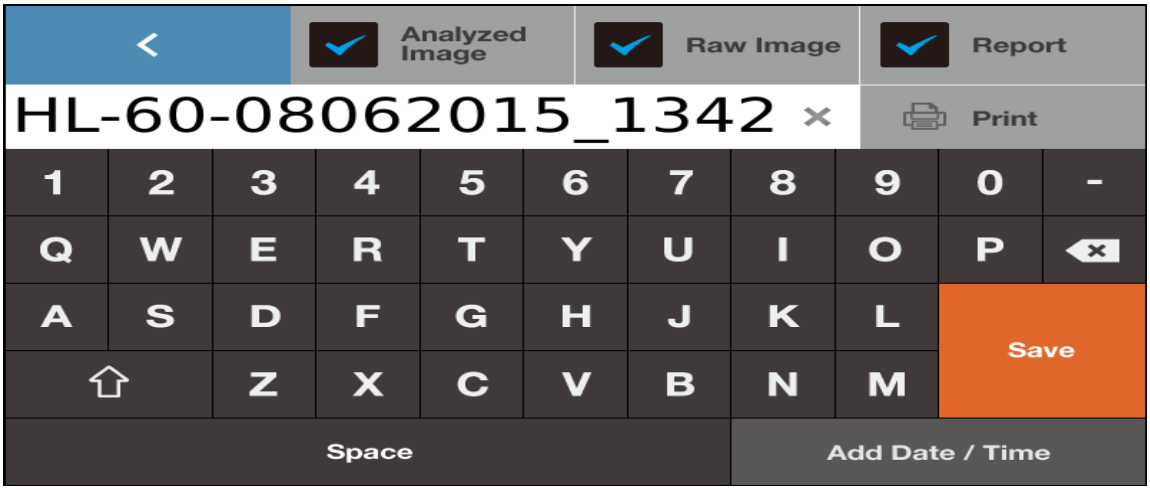
Results:

14. The LUNA-I has on board data analysis software that allows users to analyze cell count and viability data immediately.
15. Press **[Image]** to view the captured image of the analyzed cell sample.
16. Use a finger or a stylus to navigate the image.
17. The **Tag** and magnifier buttons are to the right of the image.
18. Press the magnifier button to zoom in and out of the saved image.

19. Press **Tag** to label what was counted as live cells with green circles and dead cells with red circles. The Tag function allows users to verify the instrument's counting accuracy immediately.
20. Press **Tag** again to remove the labels.
21. Press **[Histogram & Gating]** to see a graphical representation of the cell count results. User scan review the distribution of cells according to their sizes. Green bars represent live cells and red bars represent dead cells. The **Total/on** button indicates that live and dead cells are both represented.
22. Press **Total/on** to change it to **Live/on** and display the size distribution of only live cells.
23. Press **Live/on** to change it to **Dead/on** and display the size distribution of only dead cells.
24. The LUNA-II provides a gating function that can be controlled by the gating bar on the bottom of the screen. Select the desired light grey limit icon. The selected icon will become red.
25. Press the arrows on either end of the size to alter the minimum and maximum size limits. The gating function is helpful for monitoring co-cultured cells with distinct sizes and the exclusion of non-cellular particles.
26. Press **Apply** to set the size gating limits. The count results will adjust accordingly.
27. Press **Cell Number** to change the Y-axis to **Cell Concentration**.
28. Press **Cluster Map/off** to change it to **Cluster Map/on** and show the distribution of cell clusters.
29. Press **[Dilution]** and the dilution calculator will appear. Users may use the on board dilution calculator to compute dilutions for subsequent experiments.

Saving and Printing:

30. The LUNA-II provides the option of saving and/or printing results.
31. Press **[Save/Print]** in the Results screen.
32. The Save/Print screen has three saving options.
33. Select the desired saving options. The selected options will be marked with a blue.
34. Using the onscreen keyboard, name the count as desired.
35. Users may add the date to the name by pressing the **Add Date/Time** button.
36. Press **Save** to save to a USB drive. A folder of the same name will be created to contain all the files generated.
37. A summary of each count performed is automatically saved to the LUNA-II.
38. The LUNA-II stores up to 1000 counts on board.



Saving options	Description
Analyzed Image	Tagged image of live and dead cells
Raw Image	Untagged image of cells
Report	PDF report with count data and histograms

Review Previous Results:

39. The LUNA-II allows users to review previous results.
40. Select **review** from the main menu.
41. The review screen has two options: **[Review Files]** and **[Previous Counts]**.**[Review Files]** brings up data from a USB drive and **[Previous Counts]** looks up data stored directly on the LUNA-II.
42. Insert a USB drive into the LUNA-II. Press **[Review Files]** to select a LUNA-II-generated folder from the USB drive. The cell count results and corresponding image will appear on the right side of the screen.
43. If available in the folder, a tagged image will appear below the results. Tap on the image to make it full size. The image may be magnified with the magnifier.
44. Press **[Previous Counts]** to see a list of up to 1000 previous counts and their summarized results. Data can be exported to a USB drive as individual CSV files.

Product Care:

1. Rinse the LUNA Reusable Slide and Coverslip with tap water followed by 70% ethanol. Handle gently to avoid damage.
2. Dry gently and thoroughly with Kim wipes.
3. Store in the container provided.
   Do not press down on the LUNA Reusable slide and coverslip.
   Do not apply excessive force to the slide chamber when handling.
   Do not leave the LUNA Reusable slide and coverslip in solvents for extended periods of time.
   Do not sonicate the LUNA Reusable slide and coverslip.

Cleaning:

1. Turn the LUNA-II off and disconnect the power cable before cleaning. Make sure that liquids do not enter any part of the instrument during cleaning.
2. Clean the surfaces of the instrument with a soft cloth dampened with distilled water. Wipe dry immediately. Do not pour or spray liquids directly onto the instrument. Do not wet electrical wires or connections in order to avoid electrical shock or damage.
3. Clean the touchscreen with a soft cloth lightly dampened with an authorized LCD cleansing detergent. Wipe dry immediately. Do not exert excessive force or pressure as this can damage the resistive touchscreen.
4. Do not use abrasive cloths or bleach solutions as this can cause topical damage.

Safety precautions:

1. Read the manual carefully before you begin to use this instrument to ensure that you know how to operate it safely and correctly. Use the instrument as specified by Logos Biosystems. Keep the manual in an easily accessible location for future reference.
2. Install the instrument on a sturdy and level surface. Avoid vibrations from other devices.
3. Do not touch any components with wet hands.
4. Operate the instrument in the conditions described in the Environmental Conditions for Operation.
5. Use the components provided or authorized by Logos Biosystems. If the proper combination of components are not used, product safety performance cannot be guaranteed.
6. Always use the power cord and AC adapter and provided by Logos Biosystems. If the proper power cord and AC adapter are not used, the electrical safety of the product cannot be guaranteed.
7. Ensure that the input voltage is compatible with the instrument's power supply voltage.
8. Ensure that the grounding terminal of the instrument and electrical outlet are properly connected. If the instrument is not grounded, the electrical safety of the product cannot be guaranteed.

9. Turn the instrument on only after connecting the power cord and AC adapter to the power source and the instrument. Turn the instrument off before disconnecting the power cord or moving the instrument.
10. Disconnect the power cord after operation or in the case of abnormalities.
11. Do not disassemble the instrument in any event. If the instrument is malfunctioning or broken, please contact your local distributor or Logos Biosystems. Disassembling the instrument invalidates its warranty.
12. When connecting the USB drive to a computer, be careful not to be infected by computer viruses.
13. When disposing of this instrument, check and observe the rules and regulations of your local government.
14. Wear proper personal protective equipment (PPE) when handling stains and cell samples to avoid exposure.
15. Do not reuse LUNA Cell Counting Slides. Used slides must be disposed as biohazardous waste according to the rules and regulations of your local rules.

Potential hazards

Substance or item handled	Associated Hazard (s)	Existing Control Measures	Risk (L/M/H)	Further Action required	Risk (L/M/H)
Handling of Luna II Automated cell counter	Electrical hazard - Electrical shock – danger of death.	Only switch on the device if the device and power cable are undamaged. Cell counter is earth protected (RCD protected) with a 13A plug fitted. Regular visual checks of power cord for faulty, fraying or wear and tear. Do not remove the housing of the device. Only trained personal allowed to use the cell counter. Annual pat testing and service contract. Regular visual checks of power cords for fault, fraying or wear and regular electrical safety check. Any faults must be reported and repaired before continued use.	L	No further action required if the existing control measures are adhered to.	L
Glass slides (reusable slide or disposable slide)	Biohazard from cell samples and stain used.	COSHH assessment provided by users. Gloves, safety glasses and lab coat worn.	L	No further action required if the existing control measures are adhered to.	L

Glass slides and glass cover slips (reusable slide or disposable slide)	Possibility of cuts from broken microscope slides and covers slides.	Carry and store slides/cover slips in an appropriate container. Dispose of any broken glass immediately into a clinical sharps bin provided picking up the pieces with forceps. Gloves, safety glasses and lab coat worn.	L	No further action required if the existing control measures are adhere to.	L
Product care	Ethanol	Gloves, safety glasses and lab coat worn. Refer to BioE 003 COSHH assessment.	L	No further action required if the existing control measures are adhere to.	L

Persons potentially at risk:

Only the user or others near by

Action in event of an accident or emergency:

1. **Fire**: raise the fire alarm and evacuate the area. Use correct fire extinguisher if you have been trained and it is safe to do so.

Arrangements for monitoring effectiveness of control:

Daily inspection of equipment by lab technician.

Annual preventative maintenance by external contractor (Luna).

Instruction and training given to all operators, which is reviewed annually.

Existing operators receive annual refresher training.

Annual pat testing by external contractor (Janus).

Review of the Risk Assessment:

Date of review		Name of reviewer	
Date of next review		Signature	

Have the control measures been effective in controlling the risk?

Yes	No

Have there been any changes in the procedure or in the information available which affect the estimated level of risk from the listed substances

Yes	No

What changes to the control measures are required?

Declaration by Tenant/Licensee/Technician:

I confirm that I have read this Risk Assessment and that I understand the hazards and risks involved and will follow all of the safety procedures stated. Where PPE has been identified as a control measure, I will ensure that it is worn.

Declaration by Laboratory Manager (LM):

I confirm that the tenant/licensee/technician who has signed below is competent to undertake the work. My counter-signature indicates that I am happy for the work to proceed.

Name (Please print)	Signature	LM Countersignature	Date

Name (Please print)	Signature	LM Countersignature	Date