Document Number:	**SASoM/EQUIP/112.v1**
Title:	**Use of the Biorad TC20 Automated Cell Counter.**
Version:	**v1**
Author:	**Peter Mullen**

Effective from:	14/12/2021
Valid to:	14/12/2026

SOP History		
Number	Date	Reason for Change
v1	14/12/2021	Original

1.0 Purpose –
The purpose of this SOP is to outline the principles and routine use of the BioRad TC20 Automated Cell Counter in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the routine use and maintenance of the BioRad TC20 Automated Cell Counter within the SASoM.

3.0 Responsibilities –
It is the responsibility of all users of the BioRad TC20 Automated Cell Counter within the SASoM to comply with this SOP.

4.0 Procedure –

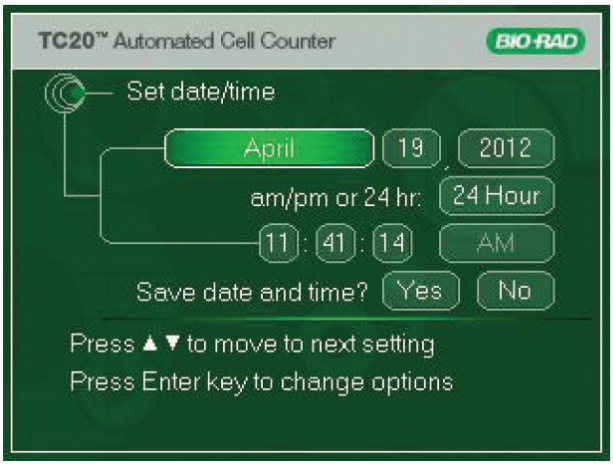
Unpacking the System Components

1. Unpack the TC20 automated cell counter carefully. Remove all packaging materials and store them for future use. Examine the instrument carefully for any damage incurred during transit. Ensure that all parts of the instrument including accessories listed above are included with the product. If any item is missing or damaged, contact your local Bio-Rad office.
2. Place the TC20 cell counter into upright position on a dry, leveled surface.
3. Insert the supplied power cord into the instrument.
4. Plug the power cord into the appropriate electrical outlet.
5. Turn on the instrument using the green power switch button. The **Home** screen appears.

Setting the Date and Time

The TC20 cell counter uses a date/time stamp to track cell count results stored on the instrument. The date and time should be set on the TC20 cell counter before the first count. Resetting the date/time stamp after using the TC20 cell counter will not affect stored results.

1. Press the **down** arrow key to go to **Options** from the **Home** screen, and press **Enter**.
2. Use the **down** arrow key to go to **Set date/time**, then press **Enter**.
3. Use the **up** or **down** arrow key to move through the settings until you reach the setting you want to modify (for example, month) (Figure 6). The press **Enter** to find the correct option (for example September, October).
4. Use the **up** or **down** key to move to the next setting (for example, day) to be modified, and press **Enter** to find the correct option within the setting.



Setting up Gating

TC20 cell counter has two counting modes, automated and user defined. In the automated mode (**User defined gates** are **Disabled**) the cell counting algorithm automatically identifies the cell population of interest. This mode does not require any user input and is suitable for immortal cell lines and samples that are composed of cells with similar cell size.

In the user defined mode (**User defined gates** are **Enabled**) the cell counter will count only objects within a user defined cell diameter range, this can be done by adjusting position of the low and high cell size gate (in µm). Only objects within the range will be analyzed as cells, those with diameters outside of that size range will be excluded from the cell count.

Enable user defined gates when counting samples containing multiple cell populations with a **wide range of cell diameters** from which you need to select a population of interest, for example fibroblasts with lymphocyte background.

User defined gates — select **Enabled** option to switch to the user defined mode or select **Disabled** option to switch to the automated mode. Once enabled, a histogram showing all objects found in the sample will be displayed at the beginning of each count. Move the size gates to select the range of cell diameters the TC20 cell counter will include in the count.

Use saved gates — the **Yes** option will save the position of the size gates from the previous count for the next count, thus saving time when multiple sample replicates are counted as you may need to move the size gates on the pre-count histogram only a few microns or not at all.

If you select **No,** the size gates will flank what the TC20 cell counter presumes to be the population of interest.

Image preview — select **On** to display image of cells with diameters between the selected cell size gates. No image preview will be displayed if the **Off** option is selected.

To enable the **User defined** mode:

1. Press the **down** arrow key to go to **Gating setup** (Figure 7) from the **Home** screen, and press **Enter**.
2. Use the **up** or **down** arrow key to move through the settings (for example, User defined gates, Image preview) until you reach the setting you want to modify. Then press **Enter** until you find the correct option (for example, Enabled, Disabled).
3. Use the **up** or **down** arrow key to move to the next setting to be modified, press **Enter** to find the correct option for the setting.

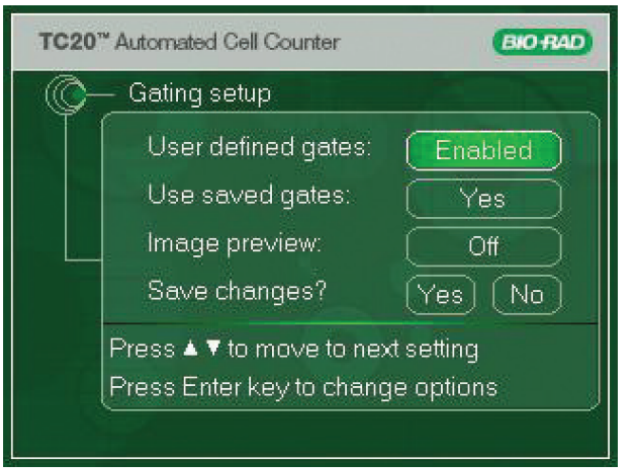


Fig. 7. Setting up gating.

4. Finally, when you have made all the modifications use the **up** or **down** key to reach **Yes** or **No** options for the **Save changes?** setting.
5. Press **Enter** to select **Yes** to save the changes and return to the **Home** screen, or select **No** to return to the **Home** screen without saving the changes.

Setting Up Automatic Data Export and Sample Naming

In this menu you can enable **automatic export of cell image** from the most recent count to a USB flash drive; the image can be viewed on your computer in JPEG format.

You can also use this menu to set sample naming preferences. Enabling **sample name** will allow you to select a name consisting of maximum eight alphanumeric characters. To save time the name selected for the most recent count will automatically be populated for the next cell count. You can then modify it, for example manually serialize it. When the automatic **name serialization** is enabled you can select a name consisting of maximum six characters, the last two characters are reserved for automatic numerical serialization. This unique sample name will be used as the JPEG file name and will be part of the result record listed in the **Previous counts.**

Important: At each new cell count the image from previous count is replaced by the image from the most recent count. When the instrument is switched off, the image from the last count is no longer kept in memory.

Performing Cell Counts:

Loading Slides

Handle the TC20™ counting slides using the edges and avoid touching the optical surface of the slides.

Fig. 9. Loading the counting slide.

Important: When loading the sample, place the pipet tip at a 45° angle at the bottom of the sample loading area (half circle at outer end of the chambers). Slide the tip along the surface and carefully touch the apex of the half circle (Figure 9). Once the tip is stopped, depress the plunger to begin the capillary loading process. Care should be taken to avoid visible bubble formation or back splatter. Do not overfill or underfill the chamber. Overfilling the chamber and possible resulting accidental spillage of sample inside the instrument could lead to biological contamination of the cell counter.

The cell counting slides cannot be reused. Dispose of used slides as biohazardous waste according to your local environmental health and safety regulations. To avoid injury do not break the counting slides.

Preparing Samples:

Preparing Samples without Trypan Blue Dye

1. Pipet 10 µl of the cell suspension into the outer opening of either chamber of the counting slide (Figure 9).

Preparing Samples with Trypan Blue

1. To determine cell viability, mix 1 part trypan blue dye and 1 part cell suspension: In a micro test tube or on Parafilm combine 10 µl of the cell suspension with 10 µl of trypan blue dye. Gently pipet up and down ten times to mix.
2. Pipet 10 µl of the mixture into the opening of either chamber on the counting slide.
3. When counting the sample in duplicate, combine 20 µl of the cell suspension with 20 µl of trypan blue dye, and then pipet 10 µl of the mixture into each chamber.

Important: The cell suspension must be loaded into the counting slides and counted immediately (within 5 minutes of mixing with trypan blue dye). Viable cells that are exposed to trypan blue dye for an extended period may start incorporating the dye, affecting the accuracy of the cell count.

Make sure the stock cell suspension is thoroughly mixed by pipetting or vortexing. Failure to do so can result in improper sample representation within the loaded counting chamber. Pipet the sample from the middle of the tube filled with stock cell suspension. Pipetting from the bottom or top can result in a sample with a higher or lower concentration, respectively.

If you work with adherent cells, use trypsin to get them into suspension. Using trypsin instead of scraping improves cell roundness and decreases the number of cell clusters.

Counting Cells on the TC20 Automated Cell Counter

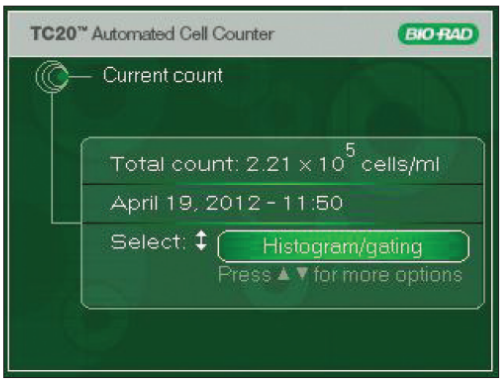
1. Simply inserting a counting slide into the slide slot of the TC20 cell counter will automatically initiate a cell count (Figure 10). The chambers are labeled A and B, with an arrow next to each chamber to indicate the direction of slide insertion. Make sure that the slide is completely inside the slide slot; otherwise the instrument does not detect the slide and counting does not start. Make sure the slide is inserted right side up.

Fig. 10. Inserting the counting slide into the TC20 cell counter.

2. The cell counter automatically detects the presence of the slide and initiates the count. The cell counter automatically detects the presence of trypan blue dye.

3. The count results appear on the **Current count** screen.

For samples without trypan blue dye — On the **Current count** screen, the instrument provides the total cell count per ml (Figure 11).



For samples with trypan blue dye — On the **Current count** screen, the instrument provides the total cell count per ml, live cell count per ml, and percentage of live cells (Figure 12). If the counted sample contains trypan blue, the instrument accounts for 1:1 dilution of trypan blue to cell suspension.

Important: If the number of cells is above or below the specified range of the TC20 automated cell counter, "Value out of range" is displayed on the **Current count** screen.

View the image of the cells (see **Viewing the Image of Cells from the Current Count** for details) and determine whether the sample should be diluted or concentrated. Then repeat the cell count.

Fig. 11. Cell count screen without trypan blue dye.

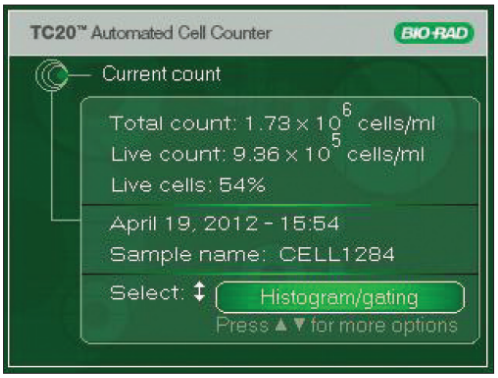


Fig. 12. Cell count screen with trypan blue dye.

If the result is between 5 x 10^4 and 5 x 10^5 cells/ ml, counting multiple sample replicates will increase accuracy. After performing replicate measurements calculate the average and use it as your result. If the result is above 5 x 10^5 cells/ml, only one measurement is needed.

4. Once the instrument completes the cell count, remove the slide from the slide slot. To exit the **Current count** screen, press **Home**.

5. If the slide is removed, results from the last count will remain on the screen until a new slide is inserted. When a new slide is inserted and the instrument starts counting, the previous result will be stored on the TC20 cell counter using a date/time stamp to track the count.

Counting Samples with Multiple Cell Populations

When counting samples with multiple cell populations that vary greatly in size (for example, fibroblasts with lymphocyte background) enable the **User defined gates** (Figure 7). A histogram (Figure 13) will be displayed at the beginning of the cell count; you can adjust the size gates to select the population of interest.

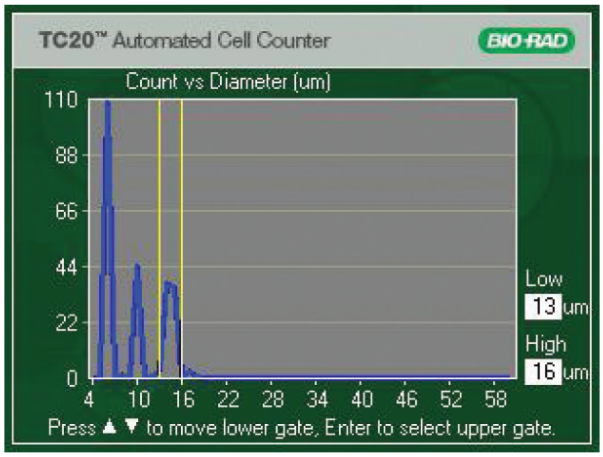


Fig. 13. Counting samples with multiple cell populations.

Important: This histogram displayed at the beginning of the count shows all objects present in the sample. After you have confirmed cell size range to be analyzed (delineated by position of the cell size gates) objects within that specified cell size range are analyzed as cells, those with diameters outside of that size range are excluded from the cell count.

If **User defined gates** are **Disabled**, the TC20 cell counter will automatically (without input from the user) determine what the population of interest is.

If unsure which part of the histogram represents your population of interest, enable the manual mode and move the size gates to the extremes of the histogram. Then open the exported JPEG on your computer using the **TC20 data analyzer** and move the size gates until only the cells you are interested in are annotated in the image. Use the displayed size gate positions (in microns) as a guide for subsequent counts on the TC20 cell counter.

To select a population on the histogram displayed at the beginning of the cell count:

1. When the histogram is displayed the low size gate is flashing and positions of gates (in microns) are displayed in the lower right corner.
2. Use the **up** or **down** arrow keys to move the low gate to the desired position.
3. Press **Enter** to select the high gate. Use the **up** or **down** arrow key to move the high gate to the desired position.
4. Press **Enter** to confirm position of both gates.
5. Press **Enter** to proceed with the count, or press **Home** to start over. Press **Home** anytime during the process to start over.
6. Press **Home** twice to return to the **Home** screen.

Sample Naming

When sample naming is enabled (see **Setting Up Automatic Data Export and Sample Naming** for details) you will be prompted to select a sample name for each cell count. You can select a name containing up to eight alphanumeric characters.

This unique sample name will be used as the JPEG file name and will be part of the result record listed in the **Previous counts.**

To save time, the sample name you selected for the previous count will automatically be populated for the next count and can be modified, for example manually serialized.

If automatic name serialization is enabled, you can select a name consisting of maximum six characters, the last two fields are reserved for automatic numerical serialization.

Underscore symbol — create a space in the sample name

Dot (.) — indicates the end of the name, cursor will move to the **Use name?** field

To name a sample:

1. Insert a counting slide into the slide slot of the TC20 cell counter to initiate a cell count.
2. At the end of the count you will be prompted to select a sample name (Figure 14). Use the **up** or **down** arrow key to scroll through the list of characters until you find the correct one. Then press **Enter** to confirm the selection.
3. Cursor then moves on to the next character field to be populated. Repeat this until you have selected all characters you need.
4. Cursor then moves onto the **Use name?** field. Select **Yes** to use the sample name, **Rename** to make modifications, or **No** to not use the sample name (the default CELLxxx. will be used instead). Then press **Enter** to confirm.

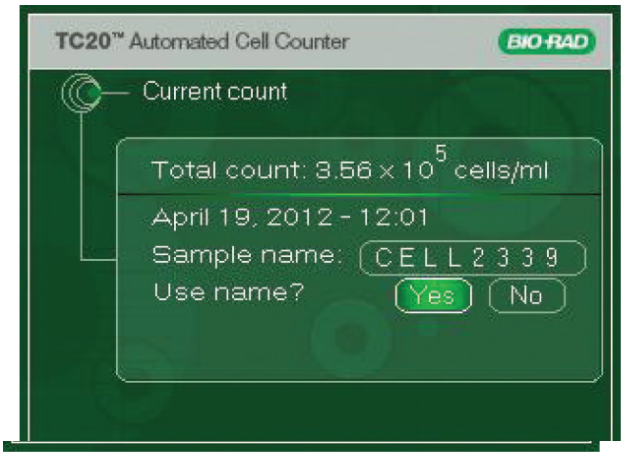


Fig. 14. Sample naming.

Using the Dilution Calculator

The dilution calculator is used to calculate volume adjustments needed to achieve the cell concentration required for the next experiment. If trypan blue was used during the count, the instrument accounts for the 1:1 dilution with the dye in the calculation results, and only the live cell concentration will be used for calculations.

To use the recent count as the starting cell concentration, use the following instructions:

1. From the **Current count** screen, use the **down** arrow key to select **Dilution calculator**.
2. Press **Enter** to continue.
3. Use the **up** or **down** arrow key to select a value, and press **Enter** to confirm the selection. If the value of the desired cell count is higher than the starting cell count, "Invalid dilution" is displayed on the screen.
4. Repeat step 3 until all the parameters have been set.
5. To recalculate the dilution, continue pressing **Enter** to go back to the field to be modified. Subsequent changes to values automatically recalculate output results.
6. Once all the parameters have been set, the curser lands on the **Print dilution results** field. To print the dilution results, use the **down** arrow key to select **Yes** and then press **Enter**.

Viewing the Image of Cells from the Current Count

To see the image from the most recent count:

1. Use the **down** arrow key to select **View image** from the **Current count** screen.
2. Press **Enter**.
3. Use the **up** arrow key to zoom in on an image and examine cells in detail. To zoom out, use the **down** arrow key (Figure 15).
4. To return to the **Current count** screen, press **Enter**.

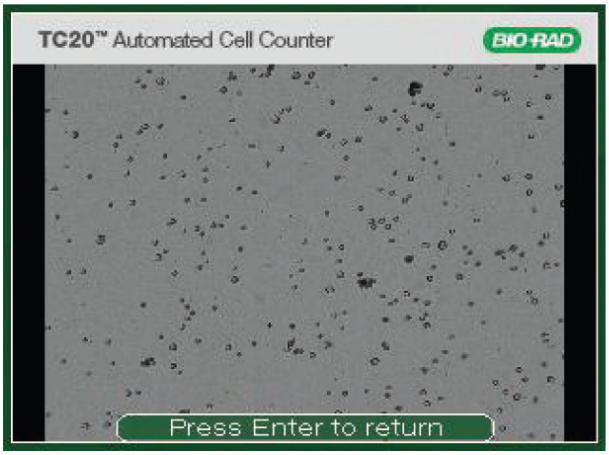


Fig. 15. Image of cells.

Exporting the Image of Cells from the Current Count

The image from the most recent count can be downloaded onto a USB flash drive and viewed on your computer in JPEG format and can be further analyzed using the TC20 data analyzer software. If the **Data autosave** is **Enabled**, the image from the most recent count is automatically exported to USB flash drive. If the **Data autosave** is **Disabled**, then the image can be exported directly from the current count screen or by using the **Export data** menu on the **Home** screen.

To export the image from the **Current count** screen:

1. Use the down arrow key to select **Export image** from the **Current count** screen, and press **Enter** to confirm.
2. If a USB flash drive is already inserted into USB port A, the export starts automatically. If there is no USB flash drive inserted into the instrument, "Insert USB flash drive into Port A to export current image. Press Enter to return" is displayed on the screen. Insert a USB flash drive into USB port A.
3. A bar showing the progress of the image download is displayed on the screen. When the image export is complete, "File saved as CELLxxx.JPG. Press Enter to return" is displayed.
4. To exit, press **Home**.

Note: If a new slide is inserted while the instrument is exporting an image, the instrument queues the count until it completes the export. The instrument then detects the slide and asks if it should start counting the sample in the new slide. If a new slide is inserted after the image is exported, the instrument starts counting the sample in the new slide.

Viewing Histogram and Gating from the Current Count

The histogram of cell size distribution from the most recent count shows the approximate cell diameters (in µm) on the X axis and the cell counts on the Y axis. The cell counts are based on the 4 mm^2 imaged area. For samples without trypan blue, the plot shows the total cell count (Figure 16). For samples with trypan blue, two plots are used to show the number of live and dead cells (Figure 17).

Important: The TC20 cell counter accurately counts samples with single or mixed cell populations. If you work with mixed cell population samples containing cells that vary greatly in size you should enable the **User defined gates** in the **Gating setup** menu. The histogram only shows approximate cell diameters; it is not intended to provide precise cell diameters.

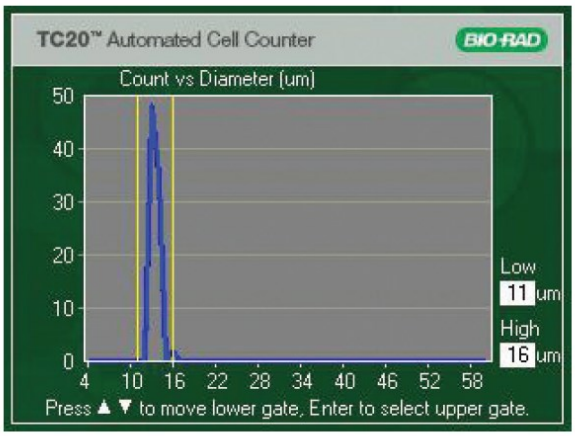


Fig. 16. Histogram for total cell count (sample without trypan blue dye).

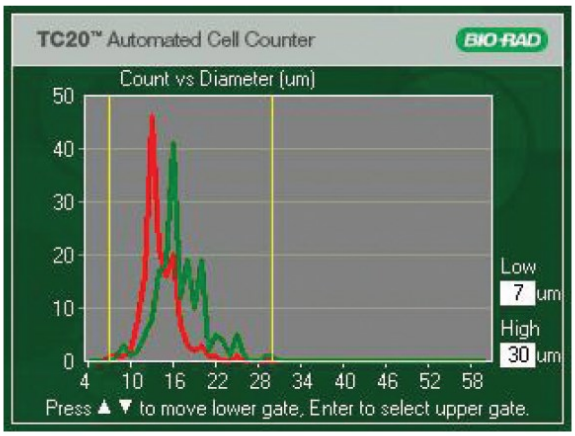


Fig. 17. Histogram for live and dead cell count (sample with trypan blue dye).

To gate the histogram from the most recent count:

1. Use the **down** arrow key to select **Histogram/gating** from the **Current count** screen, then press **Enter**.
2. When the histogram is displayed the low size gate is flashing and positions of gates are displayed in microns in the lower right corner.
3. Use the **up** or **down** arrow keys to move the low gate to the desired position.
4. Press **Enter** to select the high gate. Use the **up** or **down** arrow key to move the high gate to the desired position.
5. Press **Enter** to confirm position of both gates.
6. Gated cell count is displayed, press **Enter** to save it or press **Home** to start over. Press **Home** anytime during the process to start over.
7. Press **Enter** to return to the **Current count** screen.

Maintenance and Troubleshooting

Performing the TC20 System Verification Test

The TC20™ automated cell counter is calibrated at the time of manufacture and does not require further adjustment by the customer. A system verification slide can be purchased to validate the functionality and performance of the TC20 cell counter. It is a printed glass slide with a defined number of circles printed on each side.

To perform the TC20 system test:

1. Place the TC20 cell counter on a dry, level surface.
2. Insert the supplied power cord into the instrument.
3. Plug the power cord into the appropriate electrical outlet.
4. Turn on the instrument using the green power switch.
5. When performing the system test, **User defined gates** must be disabled. From the Home screen arrow down to **Gating setup**; press **Enter**.
6. Arrow down to **User defined gates** and press **Enter** until **Disabled** is selected (Figure 19).
7. Arrow down to **Yes** in the Save changes setting and press **Enter**. The Home screen appears.
8. Arrow down to **Options** and press **Enter**.
9. From the Options screen, arrow down to **System test**; press **Enter**.

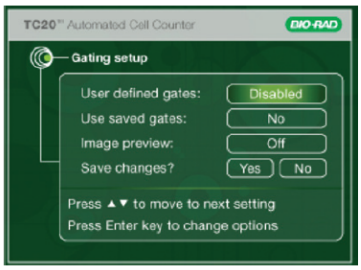


Fig. 19. Disabling user defined gates.

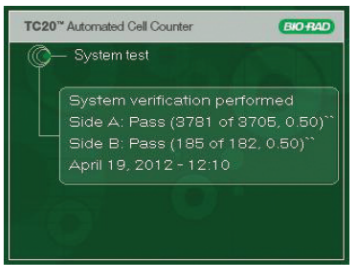


Fig. 20. System verification results.

10. When prompted, insert side A of the verification slide into the slide port. Press **Enter**.
11. When prompted, remove the slide from the instrument and insert side B of the slide into the port. Press **Enter**.
12. Pull out the verification slide and press **Enter**. This checks for debris on the optics.
13. The results of the system test are displayed on the System test screen (Figure 20). For each sample, a Pass or Fail result is reported, along with the number of objects found and expected, and the ratio of live objects (example: Pass 218 of 221, 0.50). The number expected can change each time the test is repeated, due to slight variations in slide position.
14. System test results can be exported via USB using the **Export previous counts** function. (See Exporting Data from Previous Counts for details.)
15. Save the verification slide for future use.

Important: If the cell counter fails the system test procedure, a reason for the failure will be displayed on the System test screen. The TC20 counter will indicate either cleaning the verification slide and repeating the test or contacting Bio-Rad Technical Support.

Do not insert the verification slide if it is damaged. It could damage the instrument.

Testing the system once a month is recommended but can be done as often as daily. Neither test frequency will modify the slide in any way.

Cleaning the TC20 Automated Cell Counter

The TC20 cell counter requires little maintenance but with long and constant use it requires some cleaning and other maintenance. The instrument should be cleaned on a regular schedule to remove debris or dirt that might interfere with proper function.

Cleaning the case — always turn off the instrument and disconnect the power cable before cleaning the case. Wipe the outer case with a soft, lint-free cloth and deionized water. Avoid wetting the power switch or jack.

> **Warning!** Never pour or spray water or other solutions directly on the instrument. Wet components can cause electrical shock when the cell counter is plugged in.

Cleaning the LCD screen — always turn off the instrument and disconnect the power cable before cleaning the LCD screen. Wipe the screen with a soft, lint-free cloth lightly moistened with LCD cleansing detergent. Excessive force can damage the screen. Wipe the screen dry immediately.

> **Warning!** Do not use abrasive detergents or rough material, as they may scratch the control panel and display.

Decontaminating the instrument — always turn off the instrument and disconnect the power cable before decontaminating the unit. Wipe the outer case with a soft, lint-free cloth and 70% alcohol.

> **Warning!** If you use a 10% bleach solution it may leave a residue of bleach crystals that over time may scratch the surface. Avoid touching or cleaning the LCD screen and control panel. Follow by wiping down with a damp cloth to remove any excess bleach.

Do not spill liquids inside the cell counter, for example by overfilling the slides or tipping the cell counter over while a slide with sample is in the sample port. This could contaminate the internal structure of the unit and would require professional decontamination.

5.0 Personal protection –
Howie coat must be worn at all times.

6.0 Training –
All users have to be trained by a designated person before using the Instrument.

7.0 Related documents –
Please read the online manual for further information.

8.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 14/12/2021

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

Signature:  Date: 14/12/2021

QA release by:

Name: Claire Sneddon

Position: QA Manager

Signature:  Date:15/12/2021

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE