

RLQ System Instructions

Definitions:

Test area

Test area is an area located in the middle of a test line.

Control area

Control area is an area located in the middle of a control line.

Background area

Background area is an area on a test strip located anywhere except areas of test and control lines. Usually background area is an area between test and control lines.

Colour

Every Colour can be represented by mixture of three base colours red, green, and blue (RGB). The intensity of each base colour is represented on a scale from 0 to 255, where 0 value is the darkest and 255 value is the brightest.

Grey

A grey value is defined as the average of RGB values.

T value

T value is colour intensity of a Test area (T-line).

C value

C value is colour intensity of a Control area (C-line).

B value

B value is colour intensity of a Background area.

Calculation Type

Calculation type an equation, which includes T, C and/or B values.

Final value

Final value is the result obtained when applying the calculation type to the T, C and/or B values.

Function

Methods of calculating a calibration curve: linear, piecewise linear, spline and logistic.

General considerations

The RLQ system is designed to work with a resolution of between 300 and 600 dpi. It is important the images are scanned and processed in the same manner each time. Most scanners will normally automatically adjust the light setting for each image. This function should be over ruled manually adjusted back to a standard setting for the test for each scan. The RLQ system is designed for use with any scanner however it is important to note that the test and control lines are positioned around 2 mm above the glass during scanning. Due to this the image obtained from some scanners is out of focus, these scanners can still be used as the effect is constant, however it has been found in general due to the optics used, that HP scanners give a sharp image of the test and control lines. It is not recommended to carry out any editing of the images as this has been found to in some cases affect the results obtained. The RLQ system is designed to work with images where the gRADs are at any angle, however it is recommended that you scan the gRADs at a constant angle and position to overcome possible variations in the image obtained. Although in theory it is possible to process up to 50 gRADs in the one image, in practice around 10 gRADs is the upper limit of what can be achieved while keeping within the time limits of the test time and image size for downloading.

Calibration

is carried out when setting up a new test or you have altered the imaging system. Calibration can be carried out with gRADs run with known analyte concentrations or with a calibration card with assigned concentrations (discussed in a later section).

1. Choose **Test code (gRAD batch number)** of the test you are using.
2. Write down comments, which can include the analyte, and test reagent batch. This will be recorded in a calibration file and displayed when a quantification is carried out.
3. **Analyte**, enter the name of the analyte to be tested and the units used.
4. **Colour**, in most cases green will be selected as this gives the most sensitive reading assay for the red gold colloid used in most gRAD assays. However grey may be used if a less sensitive assay is required. If different coloured Latex beads are used then you can select the other colours. (This parameter should be optimised once all the data has been entered)
5. **Calculation Type**, the RLQ system allows you to calculate the **Final** result based on a number of different combinations of the **T**, **C** and **B** values obtained (based on the selected colour), the optimal option is very much dependent on the specific assay. In general for gRAD assays the B-T or (B-T)/(B-C) options are the most appropriate. (This parameter should be optimised once all the data has been entered)
6. **Function**, the RLQ software is able to fit the **Final** results obtained vs assigned concentrations, the most appropriate curve is dependent on the assays of interest. (This parameter should be optimised once all the data has been entered)
7. Double click on the first available **Image** cell, a browser window will appear, select the image file to be processed. The chosen image will then be displayed, by left-clicking within the window and dragging your mouse, you are able to crop the image. It is important that both the **T** and the **C** marks present on the gRAD are visible and complete in the selected area, (**NOTE: the RLQ system will not recognise the gRAD if these marks are not clear, so it is important not to write over these marking**) it is also a good idea to include any sample information written on the gRAD.
8. Wait for the software to upload the image to the server. The RLQ program scans the image and assigns a separate image row for each gRAD found. A row is selected by clicking on it, the selected **T**, **C** and **B** areas are outlined in blue indicating the assigned gRAD. The **T**, **C**, **B** and **Final** values obtained for the gRAD are indicated in the appropriate cells.

9. If necessary after your image shows up in the image window you can move it by simultaneously holding the **Shift** button and dragging your mouse with the left-click button. You can also rotate it or adjust its size by simultaneously holding the **Ctrl** or **Alt** button respectively, and dragging your mouse with the left-click button. You are only allowed to edit in values that are included in the **calculation** equation.
10. If for some reason the RLQ program cannot find a particular gRAD in the image or you wish to edit the selected area you may assign correct areas manually by clicking on the **Test, Bkg** or **Ctrl** cells and drawing box. The selected area can also be moved, rotated or resized by pressing **Shift, Ctrl** or **Alt** buttons respectively while dragging your mouse with the left-click button. This will load colour intensity results to the corresponding **Test, Bkg** and **Ctrl** cells.
11. The **analyte** being tested must be selected for each row. This function is mostly relevant for tests with multiple test lines like the Multi3 device (currently in development).
12. The tested or assigned concentration (**Conc**) must be entered in the column for each of the gRADs analysed.
13. Once you have entered all the information and all status cells show "OK" or "skipped" the **calibrate** button will darken allowing you to click on it and proceed with the calibration. The RLQ software produces a calibration curve which replaces the image in the right hand window.
14. You are able to optimise the **colour, calculation type** and **function** settings to your particular test. The results of a particulate the setup can be saved as an image file for future reference.
15. Save the calibration by clicking the **Save calibration** button and selected an appropriate file name and folder as it is this file that will be used for the routine analysis.

Quantify

for the routine analysis of unknown samples using the calibration file obtained in the above section.

1. Click on the **Browse** button and choose your calibration file. It should show up in the Calibration bar.
2. **Analyte, Colour, Calculation Type**, and **Function** will appear automatically in the upper table.
3. You are able to add any comments into the comments section which will be recorded in a results file.
4. Double click on the first available **Image** cell, a browser window will appear, choose the image of interest. The chosen image will then be displayed, by left-clicking within the window and dragging your mouse you are able to crop the image. It is important that both the T and the C marks present on the gRAD are visible and complete in the selected area, (NOTE: the RLQ system will not recognise the gRAD if these marks are not clear, so it is important not to write over these markings) it is also a good idea to include any sample information written on the gRAD.
5. Wait for the software to upload the image to the server. The RLQ program scans the image and assigns a separate image row for each gRAD found. A row is selected by clicking on it, the selected **T, C** and **B** areas are outlined in blue indicating the assigned gRAD. The **T, C, B** and **Final** values are obtained for the gRAD are indicated in the appropriate cells.
6. If necessary after your image shows up in the image window you can move it by simultaneously holding the Shift button and dragging your mouse with the left-click button. You can also rotate it or adjust its size by simultaneously holding the Ctrl or Alt button respectively, and dragging your mouse with the left-click button. You are only allowed to edit in values that are included in the calculation equation.
7. If for some reason the RLQ program cannot find a particular gRAD in the image or you wish to edit the selected area you may assign correct areas manually by clicking on the **Test, Bkg** or **Ctrl** cells and drawing box. The selected area can also be moved, rotated or resized by pressing Shift, Ctrl or Alt buttons respectively while dragging

- your mouse with the left-click button. This will load colour intensity results to the corresponding **Test**, **Bkg** and **Ctrl** cells.
8. The **analyte** being tested must be selected for each row. This function is mostly relevant for tests with multiple test lines like the Multi3 device (currently in development).
 9. The calculated concentration is then indicated in the **Conc.** column of the table.
 10. If for some reason the RLQ program cannot find a particular gRAD in the image or you wish to edit the selected area you may assign correct areas manually by clicking on the **Test**, **Bkg** or **Ctrl** cells and drawing box. The selected area can also be moved, rotated or resized by pressing **Shift**, **Ctrl** or **Alt** buttons respectively while dragging your mouse with the left-click button. This will load colour intensity results to the corresponding **Test**, **Bkg** and **Ctrl** cells and recalculate the **Final** value and the **concentration**.
 11. You are able to save the results as an image file by clicking on the **snapshot** button or as a text file by pressing the **save report** button.

Calibration card

To allow others to carry out your test without the need for developing a calibration set you can use a calibration card. RapidAssays supplies a 4 point card (four separate gRADs) calibration card. Once you have produced your calibration file you are then able to scan and process the calibration card assigning a concentration for each of the four gRADs. The assigned concentrations are then included on or with the card and included with the developed gRAD assay. The calibration card is then used to produce a new calibration file for the new imaging system. The new calibration file is then used for the gRAD assay quantification on this system. The calibration card can also be used as a control card to check for variations in the system.