User guide – ELISA app

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Contents

[Preface 3](#_Toc112155880)

[Audience 3](#_Toc112155881)

[Scope 3](#_Toc112155882)

[Software 3](#_Toc112155883)

[Setup process 4](#_Toc112155884)

[First time installation 4](#_Toc112155885)

[App startup 4](#_Toc112155886)

[Navigation 4](#_Toc112155887)

[Contents and Function 5](#_Toc112155888)

[Main function 5](#_Toc112155889)

[Screens 5](#_Toc112155890)

[Navigation bar 5](#_Toc112155891)

[Homepage 6](#_Toc112155892)

[Input Data page 7](#_Toc112155893)

[Plate Layout page 8](#_Toc112155894)

[Dilutions page 9](#_Toc112155895)

[Visualize Data page 11](#_Toc112155896)

[Cut-off page 12](#_Toc112155897)

[Intermediate Results page 13](#_Toc112155898)

[End Results page 14](#_Toc112155899)

[Known problems and solutions 15](#_Toc112155900)

[Screens 15](#_Toc112155901)

[Visualize data 15](#_Toc112155902)

# Preface

## Audience

The ELISA App is developed for technicians at the rheumatology department of the LUMC.

## Scope

The function of the ELISA App is to process ELISA-reader results in a clear and reproducible manner. The processing happens in seven steps: loading in the ELISA-reader data, loading in the plate layout, loading in the dilutions, visualizing and curating the plates & determining the linear part of the standard curve, calculating the cut-off, determining the upper and lower detection limits, and generating a final result.

## Software

The app is developed for Microsoft Windows using python version 3.10, which is not installed on Windows by default. Table 1 contains all python libraries used in this app, next to the python standard library. These extra libraries can also be found in “requirements.txt” in the app files.

|  |  |
| --- | --- |
| Package | Versie |
| django | 4.0.5 |
| openpyxl | 3.0.10 |
| seaborn | 0.11.2 |
| pandas | 1.4.2 |
| matplotlib | 3.5.2 |
| numpy | 1.22.4 |
| scipy | 1.8.1 |

## Setup process

### First time installation

To deploy the app, first install python 3 from (<https://www.python.org>) and choose to add it to your PATH in the installation prompt.

(For more advanced users, if you’re using a venv, execute the root/scripts/activate.bat script to activate your venv). Then install the required libraries using **python -m pip install – r requirements.txt** through the windows console **cmd**. (should also work without python -m depending on path)

Once all libraries are successfully installed, the application setup is complete!

### App startup

To start the app, run the command **python ELISA\_app/manage.py runserver**

A link should be visible as output: “Starting development server at <http://127.0.0.1:8000/>”

To access the app, open your browser and go to <http://127.0.0.1:8000/>.

## Navigation

For a global view of the user guide, refer to the "Contents” page at the start of this document. This contains all parts of the user guide and its corresponding page number.

For detailed descriptions of all the screens in the app, refer to the chapter “Contents and Function, Screens”. This chapter is divided with separate paragraph headers for each part of the app.

For known bugs, refer to “Known problems and solutions”. This chapter contains all known bugs, once again divided per screen, and any known workarounds.

To rapidly look up a subject in the user guide, you can refer to the index and look up a keyword. The index contains keywords used in the user guide and their corresponding page numbers.

# Contents and Function

## Main function

The purpose of this application is to analyse and visualize ELISA data. The user can adjust this data as they see fit.

## Screens

### Navigation bar

A screenshot of a phone

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1

8

7

6

5

3

4

2

*Figure 1: All interactions in the navigation bar labelled 1 to 8.*

The Navigation bar is present on every page on the left side of the screen. The menu is in the order of data processing. When a button (1 to 8) is pressed, the user is directed to that page.

### Homepage

Graphical user interface, text, application

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*Figure 2: The layout of the homepage.*

The homepage is the starting page of the application. Here information is displayed about the use of the application and the contact info.

### Input Data page

Graphical user interface, text, application

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3

2

1

*Figure 3: All interactions in the Input Data page labelled 1 to 3.*

The Input Data page is where the ELISA-reader data will be submitted.

When choosing a file (1) the format must be a txt or xlsx. Multiple files can be selected, but each file can only contain data of one plate. Each cell in the ELISA-reader data must be filled. When a file is chosen, it can be uploaded to the program (2). If an incorrect file is submitted or the file contains the wrong information, an error message will be shown on screen explaining what went wrong.

To empty the data from the entire application, click the empty database button (3). This could be used when incorrect data has been uploaded.

### Plate Layout page

A picture containing graphical user interface

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9

6

5

11

12

13

14

10

8

7

2

1

4

3

*Figure 4: All interactions in the Plate Layout page labelled 1 to 14.*

The Plate Layout page is where the plate layout will be submitted.

The user must first select whether they are using modified and non-modified peptide data (1) or only modified peptide data (2). The next step is the option whether to use a cut-off (3) or not (4).

Next you need to fill in where the ST values are located. If they are located in a row, select the row option (5) and fill in the row (7) in which the ST values are located. If the ST values are located in columns, select the column option (6) and fill in the columns (8) in which the ST values are located. The row input (7) must only receive one number while the column input (8) must receive two numbers in this format: 1, 2 or 3, 4 etc.

After selecting these options, the data file can now be uploaded. When choosing a file (9) the format must be a xlsx. Only one file can be uploaded, but multiple plates can be present in the file. When a file is chosen, it can be uploaded to the program by pressing the upload data button (10). After uploading the data, the plates from the document and the ST value input are shown on the page.

Fill in the highest value of the standard curve (11) and the number the value should be divided by each step (12). Fill in the name of the unit relevant to the study (13) and update the table using the update table button (14). This will replace all the ST values with the correct values (for instance, A: 60, B: 30, C: 15, etc…).

Dilutions pageGraphical user interface, text

Description automatically generated with medium confidence

2

1

*Figure 5: All interactions in the first step of the Dilutions page labelled 1 and 2.*

The Dilutions page is where the dilution for the plates will be submitted. When choosing a file (1) the format must be a xlsx. Only one file can be uploaded, but this file can contain either only one dilution plate or multiple dilution plates. If a file with only one plate is submitted, this dilution is applied to every plate. When a file with an equal amount of dilution plates as the number of plates uploaded in ‘input data’ is submitted, the plates will be linked with the plate names (Plate 1 – Plate 1, Plate 33 – Plate 33 etc…). When a file is chosen, it can be uploaded to the program by pressing the upload data button (2).

After uploading the data, the dilution plates from the document are shown on the page.

Table

Description automatically generated*Figure 6: All interactions in the first step of the Dilutions page labelled 1 and 2.*

4

3

When the uploaded file contains only one dilution plate, you get the option to submit the names of the plates the dilution plate belongs to.

Table

Description automatically generated

7

6

5

*Figure 7: All interactions in the first step of the Dilutions page labelled 1 and 2.*

When the names are filled in (3) and submitted (4), you can select (5) a second dilution file which contains the rest of the dilution plates needed. The plates which should be linked to these new dilutions should be inputted (7) in the same way as with the previous plate select input (3). Then you can submit (6) the data.

### Visualize Data page

Chart

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5

4

3

2

1

*Figure 8: All interactions in the Visualize Data page labelled 1 to 5.*

The Visualize Data page is where the plate data is shown. Per plate it shows the background subtracted ODs for each sample and a graph with the calibration line. The user can select the top (1) and bottom (2) points of what correctly encompasses the linear part of the calibration line. If the user judges an entire plate to be faulty, they can remove the plate from future calculations by checking the checkbox (3) corresponding to that plate. The healthy donor plate can be selected (4) and submitted (5) to be used in future calculations. The selection of a healthy donor plate is mandatory.

### Cut-off page

Chart, box and whisker chart

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*Figure 9: All interactions in the Cut-off page labelled 1 to 6.*

If the ‘Modified/Non-modified ELISA’ option was selected in the Plate layout page, only the modified datapoints will be shown. If the ‘General ELISA’ option was selected in the Plate layout page, all the datapoints will be shown.

The Cut-off page is where the swarm plots of the healthy donor OD’s (background subtracted) are shown, and the user can determine the cut-off value. When entering the page, only the left side is visible.

First, HD outliers must be detected and removed from the dataset. The formula for determining the outliers is shown, and the user can alter the inputs for the mean (1) and standard deviation (2). After the formula is filled in, the ‘outlier value’ is given by the program. ODs higher than the outlier value are considered outliers.

By pressing the create new swarm plot button (3), a new swarm plot is shown in which the data points outside the outlier value are removed.

Now, the cut-off can be calculated. The formula for calculating the cut-off is shown and the user can alter the values of the inputs mean (4) and standard deviation (5). When the formula is filled out, the cut-off can be calculated by pressing the submit cut-off button (6). The calculated cut-off value is now displayed above the submit cut-off button (6).

If the ‘I don’t want to calculate a cut-off’ option was selected in the Plate layout page, this page will be skipped and an error page will take its place to signal the user to move on to the next page.

 *Figure 10: The error page shown from the Cut-off page if the no cut-off option is selected.*

### Intermediate Results page

Table

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*Figure 11: All interactions in the first part of the Intermediate Results page labelled 1 and 2.*

The intermediate results page is where the upper and lower detection limit will be determined for the entire experiment.

Two tables are shown. The left table, which contains the bottom 20 below samples and the top 20 linear samples. The table displays the sample ID, the arbitrary units, and the location on the plate-specific standard curve. The user can determine the correct experiment-specific lower detection limit by looking at the transition from below to linear, input the value (1) and submit the data (2).

When the button is pressed, the page switches to where the linear/above table is now shown instead of the below/linear. The input also changes from lower detection to upper detection. The actions stay the same.

Table

Description automatically generated *Figure 12: The second part of the Intermediate Results page.*

### End Results page

Graphical user interface, table

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*Figure 12: All interactions in the End Results page labelled 1 to 10.*

The end results page is where the final rule is applied which determines whether a sample is positive or negative and where information about the samples is displayed.   
Different rules can be selected to change the positive and negative outcome. There are four rules: rule one, the modified OD should be the given value (1) times higher than the non-modified OD. Rule two, the modified OD should be higher than the given value (3) plus the non-modified OD. Rule 3, the value of the unit of measurement should be above the given value (5). Rule 4, no rule should be applied.

When a rule is chosen and filled in, the update table button (2, 4, 6, 7) belonging to that rule should be pressed. When a rule is submitted, the table is shown.

It is possible to combine rules one and three, and rules two and three. Rules one and two cannot be combined.

If the user wants to download the table, they can do so by entering the desired name for the file in the field (8) and clicking the download button (9).

# Known problems and solutions

In this section known problems which could occur when using the application are addressed and solutions are presented.

## Screens

### Visualize data

Problem: If you click on the visualize data page but instead get referred to an error page, the data in the database probably wasn’t deleted before submitting new data.

Solution: Delete the data from the database in the input page and restart the application.