

Luminex Xponent: Plate-level Quality Control

Sahal Thahir & Jeff Bailey

2024-06-04

Contents

Aim	1
---------------	---

Aim

This code aims to perform plate-level quality control analysis for Luminex studies through bead counts, Background MFI, and Standard curve analysis.

Instructions

1. Modify YAML Parameters:

- Open this document in RStudio.
- Edit the YAML parameters at the top of the document:
 - `input_file`: Path to your input CSV file.
 - `min_beadcount`: Set the minimum bead count threshold.
 - `min_Rsquared`: Set the minimum R-squared value for standard curve QC.
 - `file_path`: Specify the output directory for plots and PDFs.
 - ‘

2. Run the Analysis:

- After modifying the YAML parameters, click the *knit* button at the top of the page to generate the report.

Code Explanation

Dependencies

- R version 4.3.1 (2023-06-16)
 - `tidyverse` (v. 2.0.0)
 - `here` (v. 1.0.1)
 - `gridExtra` (v. 2.3)

This code chunk will ensure these packages are installed and loaded.

Import and Data Extraction

1. Raw data import: Import raw data CSV file into R.

```
# No input required
#YAML extraction
# Read parameters from YAML header
input_file <- params$input_file
min_beadcount <- params$min_beadcount
min_Rsquared <- params$min_Rsquared
file_path <- params$file_path
```

```

background_col <- params$background

# Raw data import
lines <- readLines(input_file)

## Warning in readLines(input_file): incomplete final line found on
## 'data/raw/luminex/RTSS_Ahero_IgG3_3.21.24_Lowbeadtest.csv'

2. Extract dataframes: find median MFI and bead count data. This includes a check to ensure the
   median MFI and beadcount dataframes align.

## [1] "Column names are consistent between the median MFI and bead count data frames."

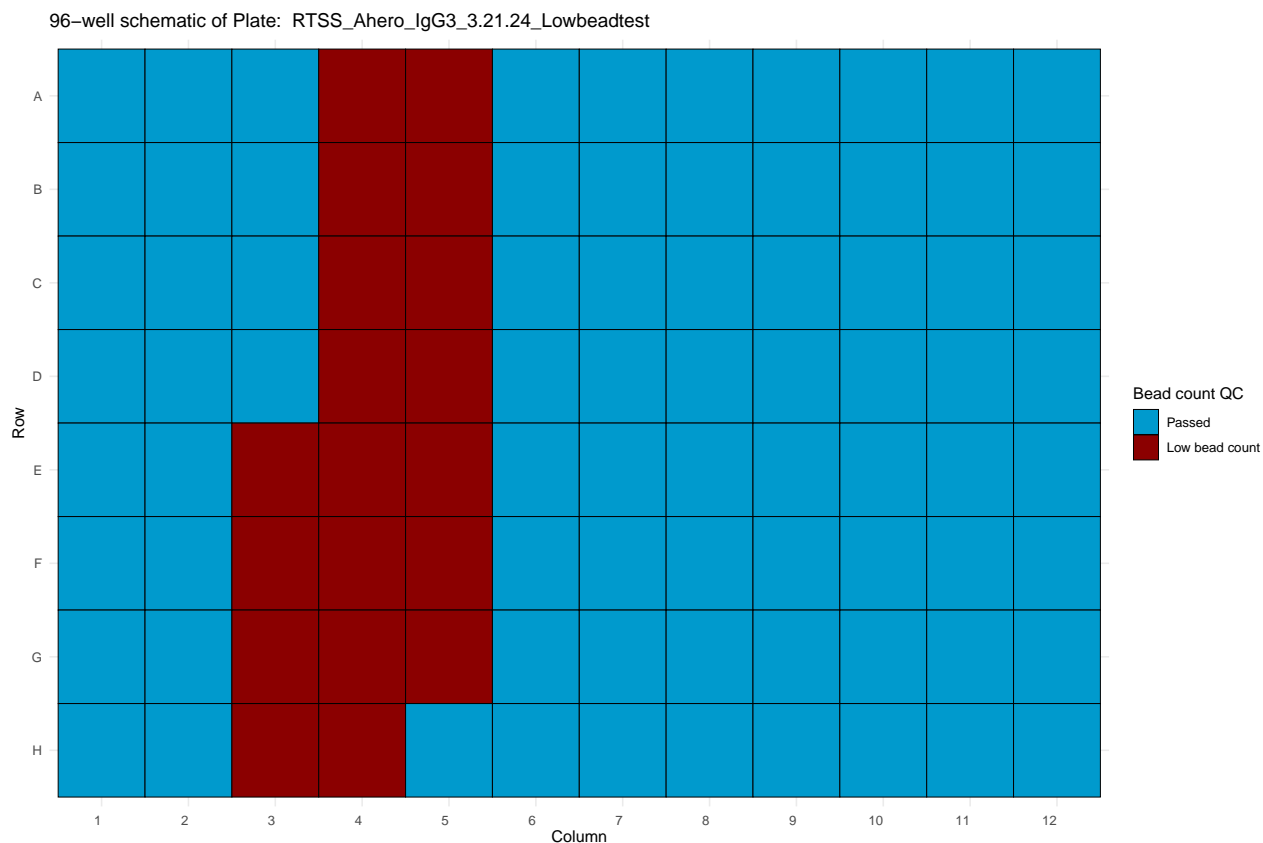
3. Identify low bead count wells: this searches the beadcount data frame for well-antigen data with
   low bead count, as set in the YAML.This will export a csv file of the samples which will need to be
   rerun.

## The bead QC list has been exported to data/qc/RTSS_Ahero_IgG3_3.21.24_Lowbeadtest_beadqc_low_df.csv

```

Visualization of bead count data

This code generates a well schematic identifying wells identified as having low bead counts. as well as a heatmap of antigen/well locations and the quantity of beads, to assist in determining the cause of low bead counts (low antigen concentration in mastermix, vortexing between wells, etc.)



```

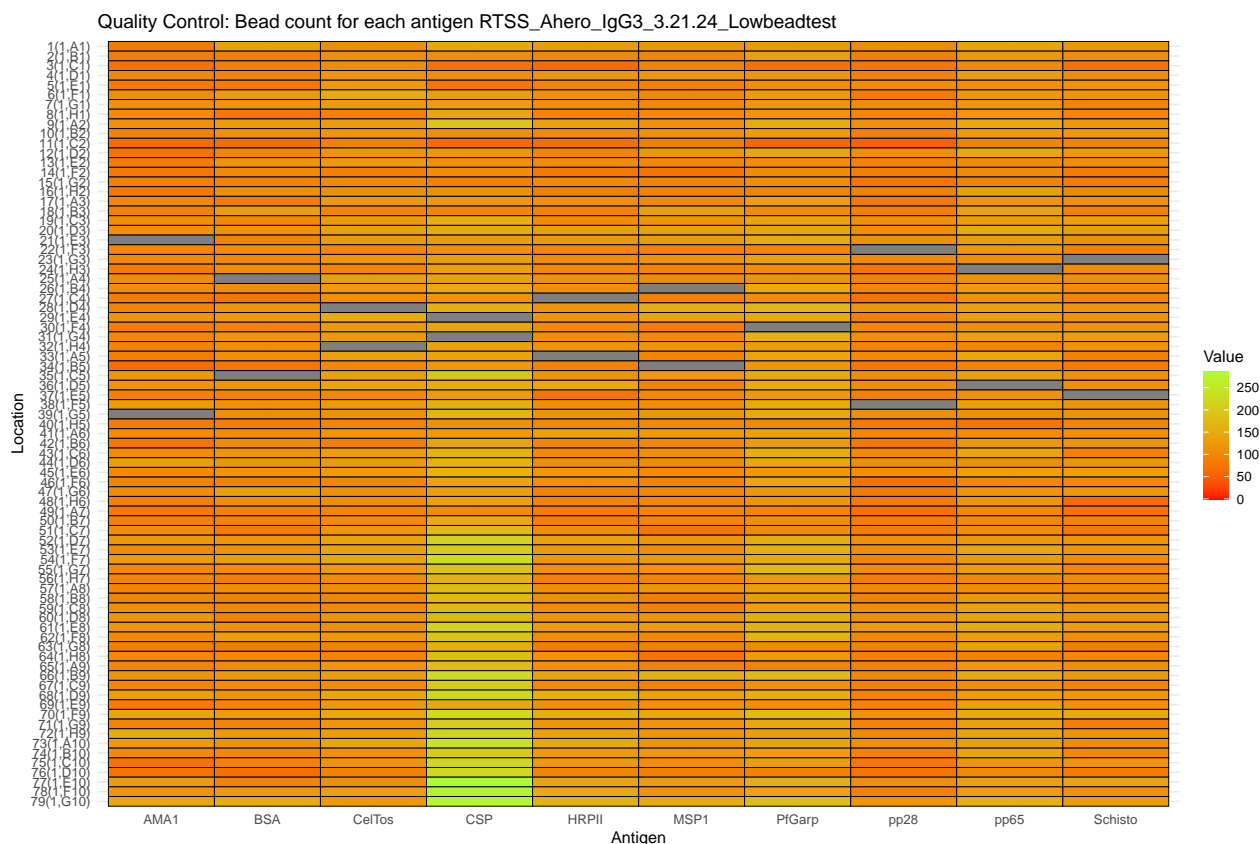
## # A tibble: 19 x 4
##   Location Sample      Antigen Plate
##   <chr>    <chr>      <chr>  <chr>
## 1 21(1,E3) RAM-002    AMA1   RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 2 22(1,F3) RAC-002-T01 pp28    RTSS_Ahero_IgG3_3.21.24_Lowbeadtest

```

```
## 3 23(1,G3) RAC-002-T07 Schisto RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 4 24(1,H3) RAC-002-T08 pp65 RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 5 25(1,A4) RAM-005 BSA RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 6 26(1,B4) RAC-005-T01 MSP1 RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 7 27(1,C4) RAC-005-T08 HRPII RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 8 28(1,D4) RAM-007 CelTos RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 9 29(1,E4) RAC-007-T01 CSP RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 10 30(1,F4) RAC-007-T07 PfGarp RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 11 31(1,G4) RAC-007-T08 CSP RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 12 32(1,H4) RAM-008 CelTos RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 13 33(1,A5) RAC-008-T01 HRPII RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 14 34(1,B5) RAC-008-T07 MSP1 RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 15 35(1,C5) RAC-008-T08 BSA RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 16 36(1,D5) RAM-009 pp65 RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 17 37(1,E5) RAC-009-T01 Schisto RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 18 38(1,F5) RAC-009-T07 pp28 RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 19 39(1,G5) RAC-009-T08 AMA1 RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
```

The bead QC list has been exported to data/qc/RTSS_Ahero_IgG3_3.21.24_Lowbeadtest_beadqc_low_df.csv

The bead QC report has been exported to data/qc/RTSS_Ahero_IgG3_3.21.24_Lowbeadtest_beadqc.png



4. **Modify Median MFI data:** All plate locations with a low beadcount will be switched to a NA value for standard curve evaluation

Median MFI data has been modified for low bead count wells.

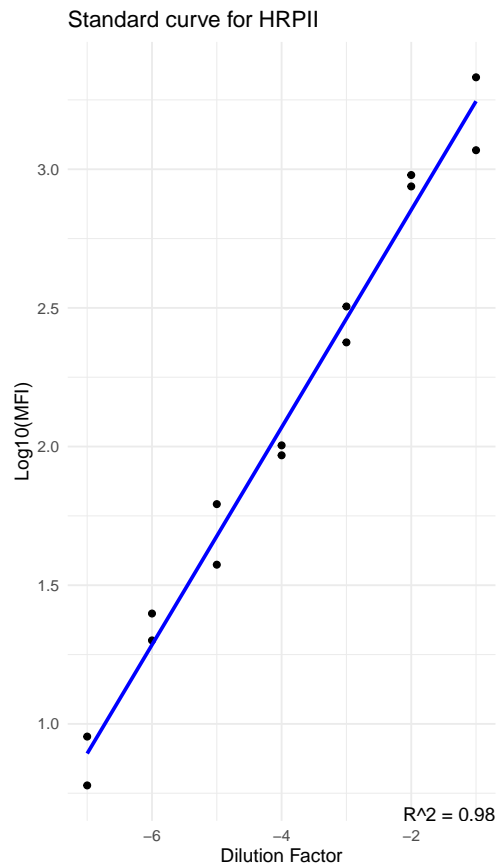
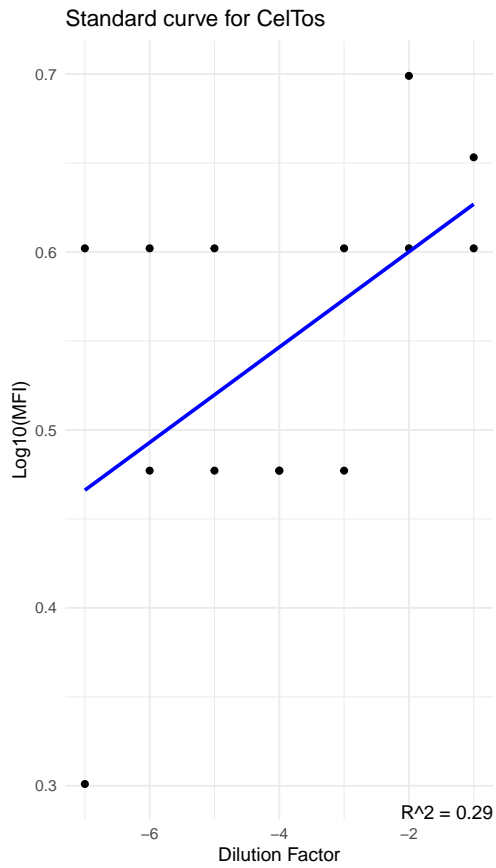
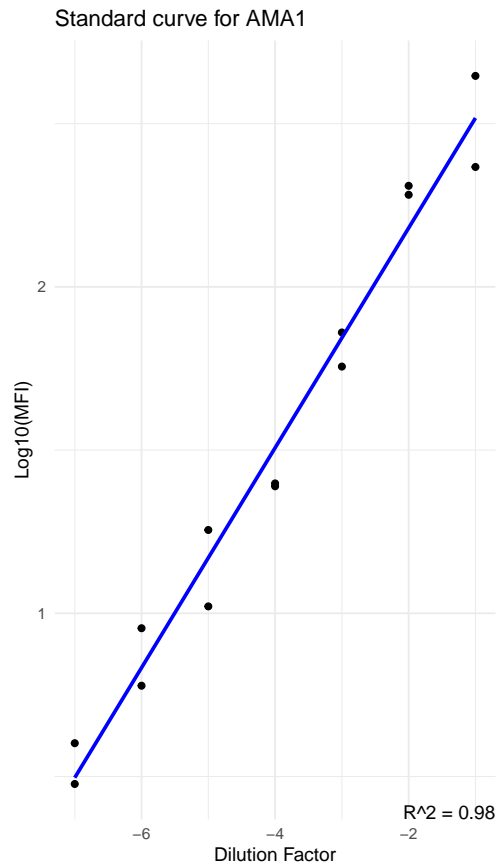
5. **Evaluate standard curves:** Make sure the background and standard analytes are defined in the YAML params

This first chunk conducts background MFI subtraction, based on the analyte defined in the YAML.

```
## Background MFI subtraction completed.
```

This code chunk

```
## 'geom_smooth()' using formula = 'y ~ x'  
## 'geom_smooth()' using formula = 'y ~ x'  
## 'geom_smooth()' using formula = 'y ~ x'
```



```
## TableGrob (2 x 2) "arrange": 3 grobs
##   z      cells   name      grob
## 1 1 (1-1,1-1) arrange gtable[layout]
## 2 2 (1-1,2-2) arrange gtable[layout]
## 3 3 (2-2,1-1) arrange gtable[layout]

##   Analyte R_squared
## 1   AMA1 0.9756595
## 2  CelTos 0.2923485
## 3  HRPII 0.9840924

##   Analyte R_squared min_Rsq
## 1   AMA1 0.9756595   TRUE
## 2  CelTos 0.2923485  FALSE
## 3  HRPII 0.9840924   TRUE

## [1] "This plate has passed plate-level quality control for standard curve linearity."
## [1] "At least one analyte meets the criteria for the preset minimum R-squared (see min_Rsquared in Y
## [1] "Median MFI from this plate will be included in the compiled study dataframe."
```

Create quality controlled Median MFI data

```
##   Analyte R_squared min_Rsq
## 1   AMA1 0.9756595   TRUE
## 2  CelTos 0.2923485  FALSE
## 3  HRPII 0.9840924   TRUE

## [1] "This plate has passed plate-level quality control for standard curve linearity."
## [1] "At least one analyte meets the criteria for the preset minimum R-squared (see min_Rsquared in Y
## [1] "Median MFI from this plate will be included in the compiled study dataframe."

## Plate passed QC. All NA values have been replaced with 'Failed QC (bead)'.

## The cleaned median MFI data has been exported to data/clean/luminex/RTSS_Ahero_IgG3_3.21.24_Lowbeadt
```

Developer notes

->