Luminex Xponent: Plate- level Quality Control

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Aim

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

R Markdown

This is an R Markdown document, areas that between the `{r}` frames that are require manual entries as described in the **bolded text**. When entries are completed, press the *knit* button at the top of the page.

Dependencies

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

Required Inputs

1. Confirm working directory:

This is the project folder where the project is found. All folders and files added to the code will be within this folder. If needed you can set the working directory using the `setwd()` function.

Working directory: /Users/sahal/Documents/Research/R Projects/RTSS_Kisumu_Schisto

2. Choose file (input required):

Chose the raw plate file from working directory by adding what folder within (in the quotes). In the example below, the plate csv is in the `Project/data/raw/luminex/` folder.

3. Set minimum bead count:

Set the **minimum bead count** as `min_beadcount`. In this example, the standard we have set here is `50`. All bead counts <50 will be identified.

4 Define the `file_path`:

For example, here "Project/data/qc" folder is where the *PlateName_beadqc_df.csv will be saved*

```r
# Choose the input file
input_file <- "data/raw/Ahero_IgG1_Test.csv"
# Minimum bead count for analysis
min_beadcount <- 50

#Output file path
file_path <- "data/qc"
```
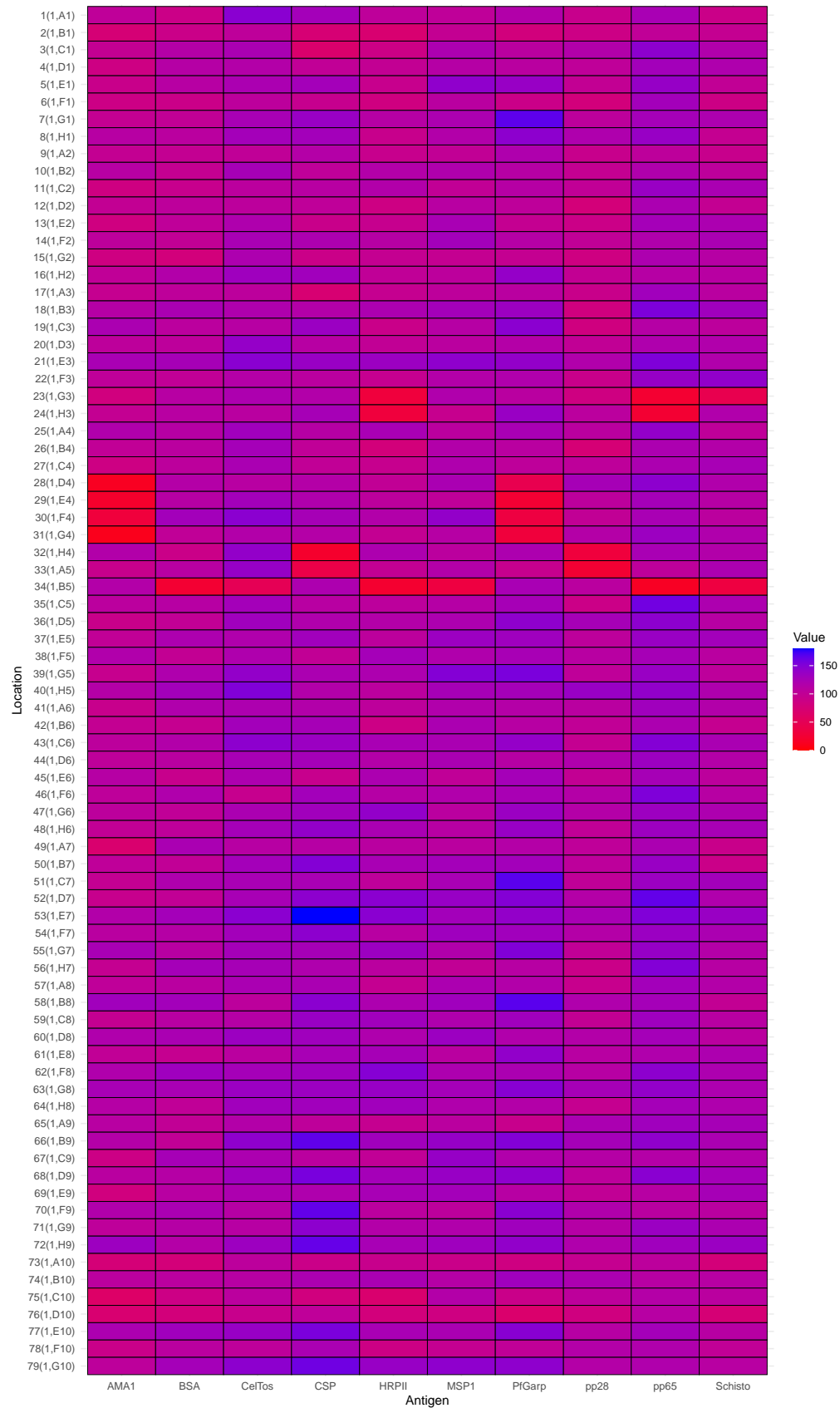
Quality Control

Bead count per Antigen

This code assists in check if low bead counts are associated with specific antigens. Note the plot below is not oriented as a 96-well plate given it displays multiple antigens per well.

```
## The bead QC report has been exported to data/qc/Ahero_IgG1_Test_beadqc.png
```

Quality Control: Bead count for each antigen Ahero_IgG1_Test

Bead Count: per well

This code results in a 96 plate- well schematic indicating wells with low wells. A :ist of wells, sample IDs, and analytes that are less than the minimum bead count set. This file is exported to `file_path` (chosen above) as *PlateName_beadqc_low_df.csv*. Values in this list will be `Na` in the Median MFI dataframe. `file_path`: data/qc (this can be edited in the **Bead Count** section)

96–well schematic of Plate: Ahero_IgG1_Test
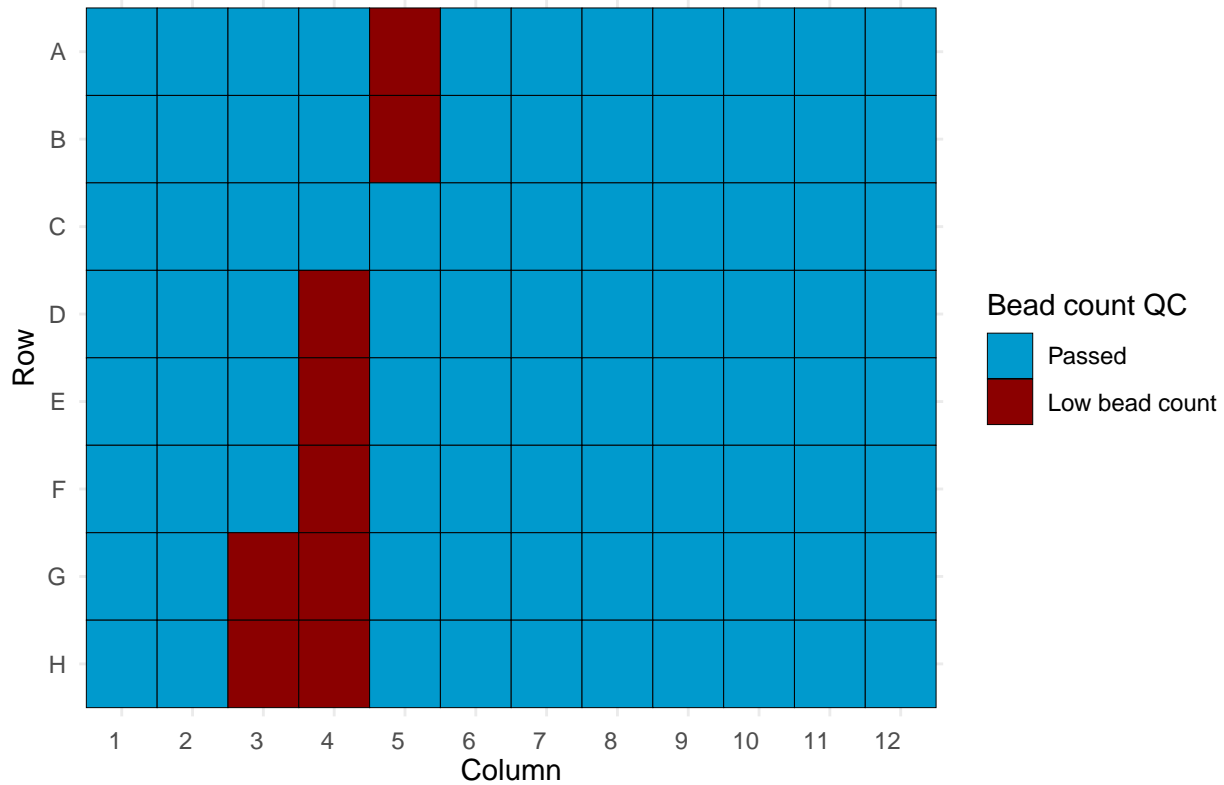

```
##      Location        Sample Antigen          Plate
## 1  28(1,D4)      RAM-007    AMA1 Ahero_IgG1_Test
## 2  29(1,E4) RAC-007-T01    AMA1 Ahero_IgG1_Test
## 3  30(1,F4) RAC-007-T07    AMA1 Ahero_IgG1_Test
## 4  31(1,G4) RAC-007-T08    AMA1 Ahero_IgG1_Test
## 5  32(1,H4)      RAM-008    pp28 Ahero_IgG1_Test
## 6  33(1,A5) RAC-008-T01    pp28 Ahero_IgG1_Test
## 7  23(1,G3) RAC-002-T07 Schisto Ahero_IgG1_Test
## 8  34(1,B5) RAC-008-T07 Schisto Ahero_IgG1_Test
## 9  23(1,G3) RAC-002-T07    pp65 Ahero_IgG1_Test
## 10 24(1,H3) RAC-002-T08    pp65 Ahero_IgG1_Test
## 11 34(1,B5) RAC-008-T07    pp65 Ahero_IgG1_Test
## 12 34(1,B5) RAC-008-T07     BSA Ahero_IgG1_Test
## 13 34(1,B5) RAC-008-T07    MSP1 Ahero_IgG1_Test
## 14 23(1,G3) RAC-002-T07   HRPII Ahero_IgG1_Test
## 15 24(1,H3) RAC-002-T08   HRPII Ahero_IgG1_Test
## 16 34(1,B5) RAC-008-T07   HRPII Ahero_IgG1_Test
## 17 34(1,B5) RAC-008-T07  CelTos Ahero_IgG1_Test
## 18 32(1,H4)      RAM-008     CSP Ahero_IgG1_Test
## 19 33(1,A5) RAC-008-T01     CSP Ahero_IgG1_Test
```

```
## 20 28(1,D4)     RAM-007  PfGarp Ahero_IgG1_Test
## 21 29(1,E4) RAC-007-T01  PfGarp Ahero_IgG1_Test
## 22 30(1,F4) RAC-007-T07  PfGarp Ahero_IgG1_Test
## 23 31(1,G4) RAC-007-T08  PfGarp Ahero_IgG1_Test

## The bead QC list has been exported to data/qc/Ahero_IgG1_Test_beadqc_low_df.csv
##  This file will be added to in data/qc/qc_compile.csv
```

QC of median MFI data

This extracts the median MFI data, with all well-analyte combinations <50 beads/well replaced with *NA*. It completes the BSA MFI subtraction as well

note: negative values are kept

Background MFI

This code averages the median MFI values for all background and non-background samples. This requires all Background samples to have "Background" within the characters in the `Sample` column
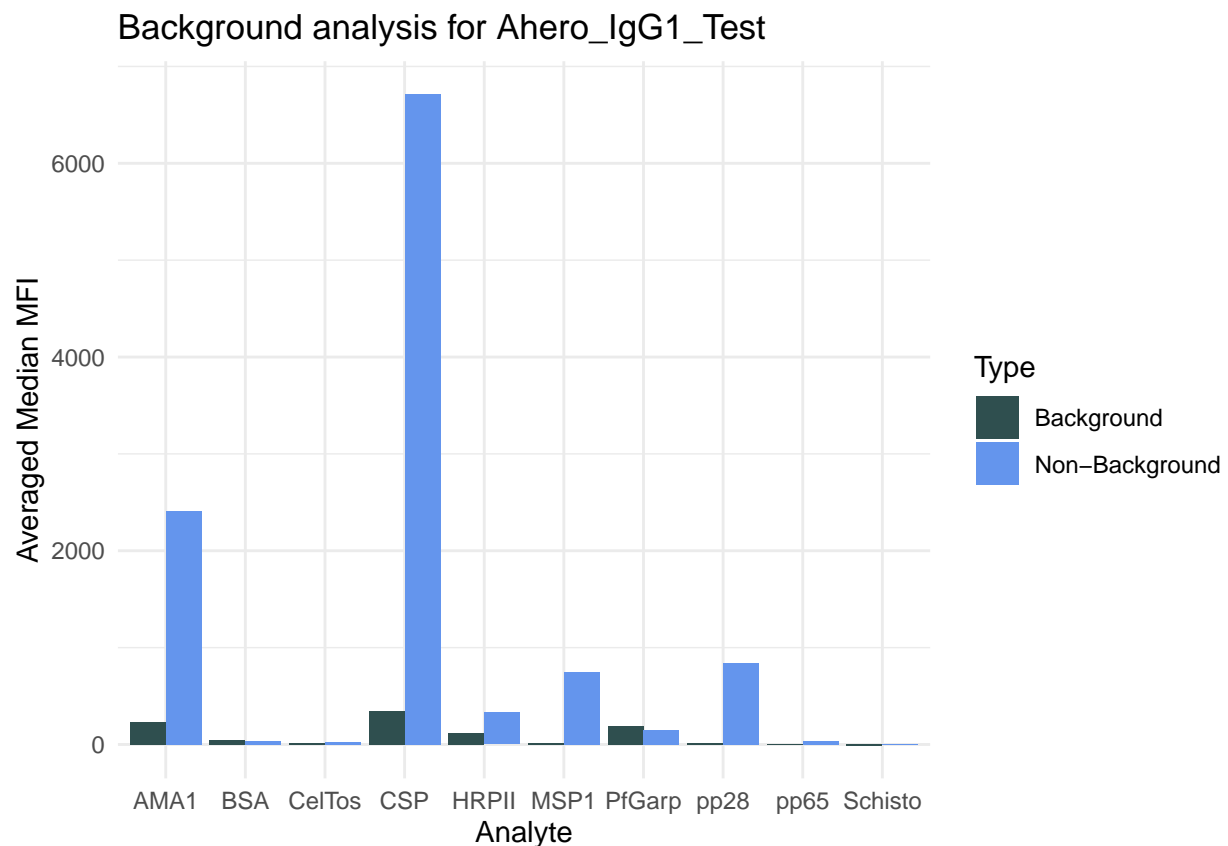
Standard curve

This code will attempt to create standard curves. This requires the samples have "Standard" within the characters in the `Sample` column. This will extraction `Dilution_Factor` as the number within the name (x -1).

For example: *Standard 2* will have a `Dilution_factor` of -2
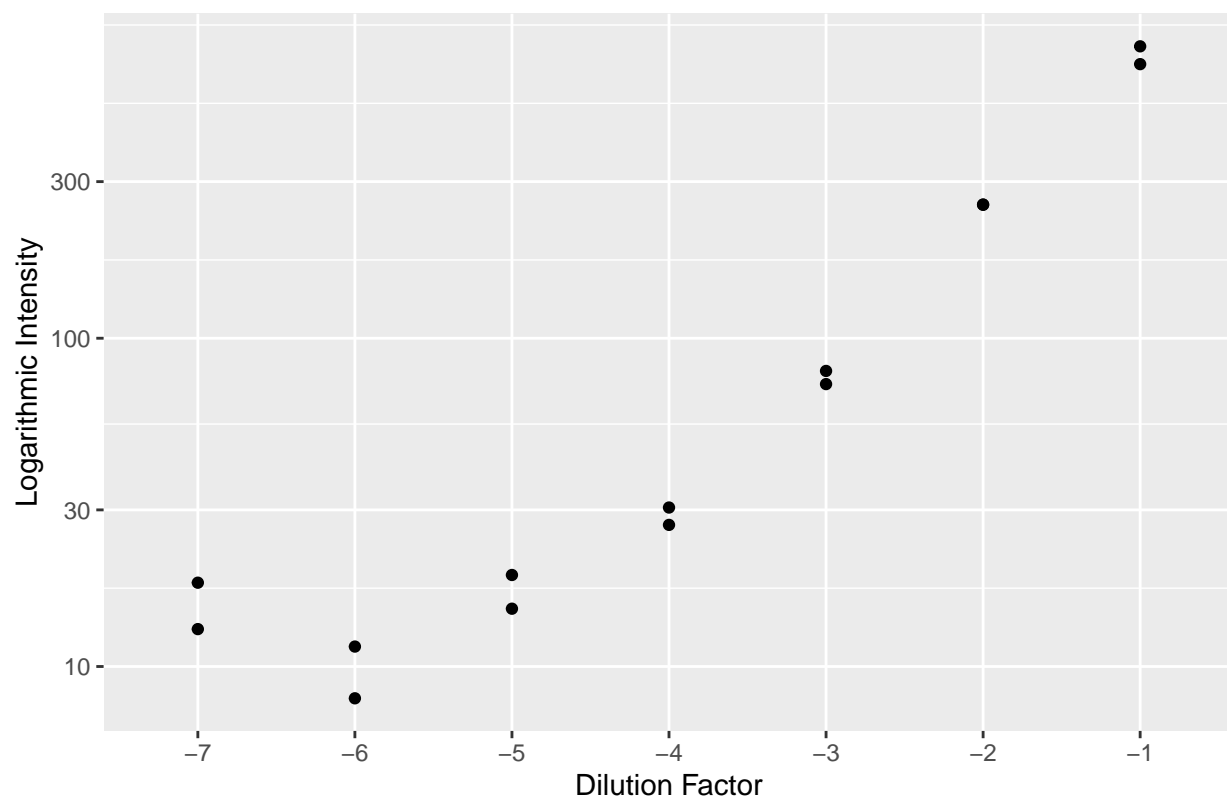
This code will run for all `analyte`
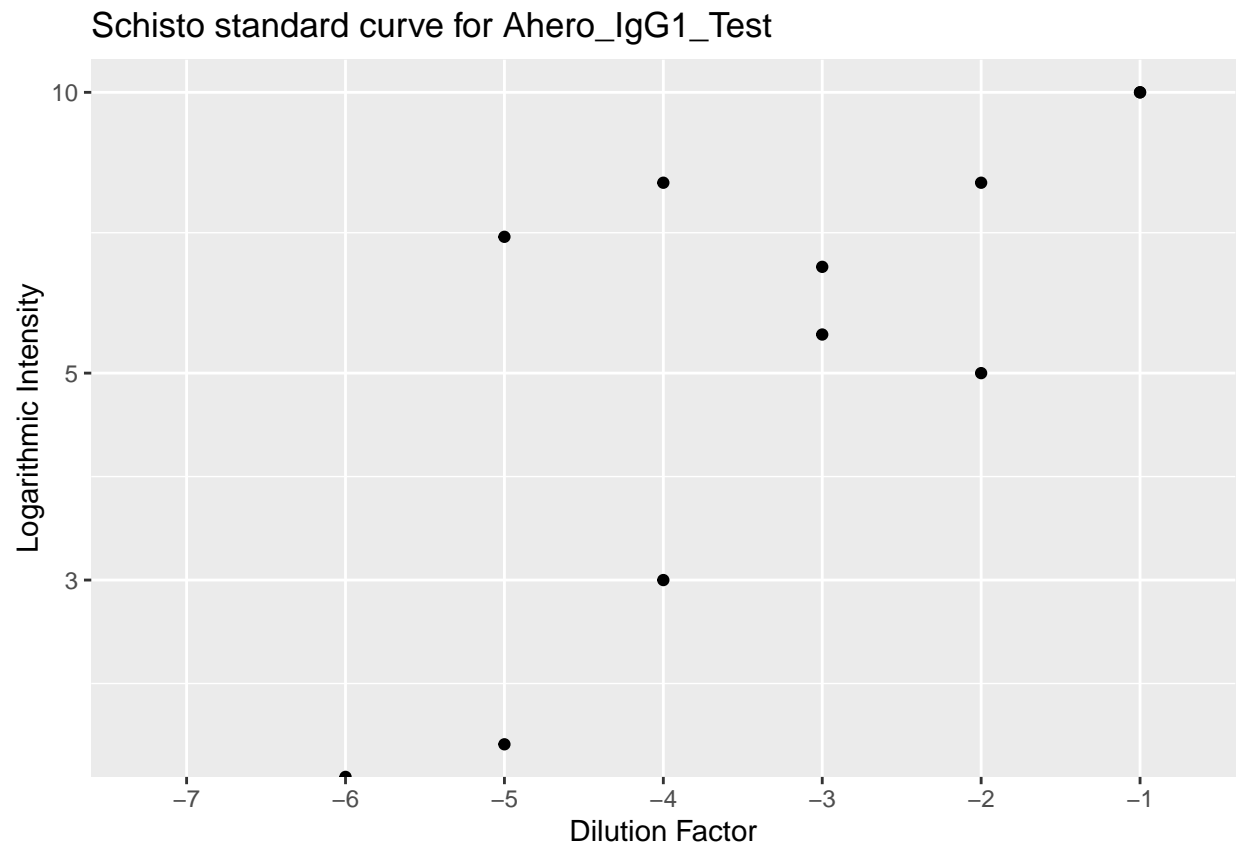
[[1]]
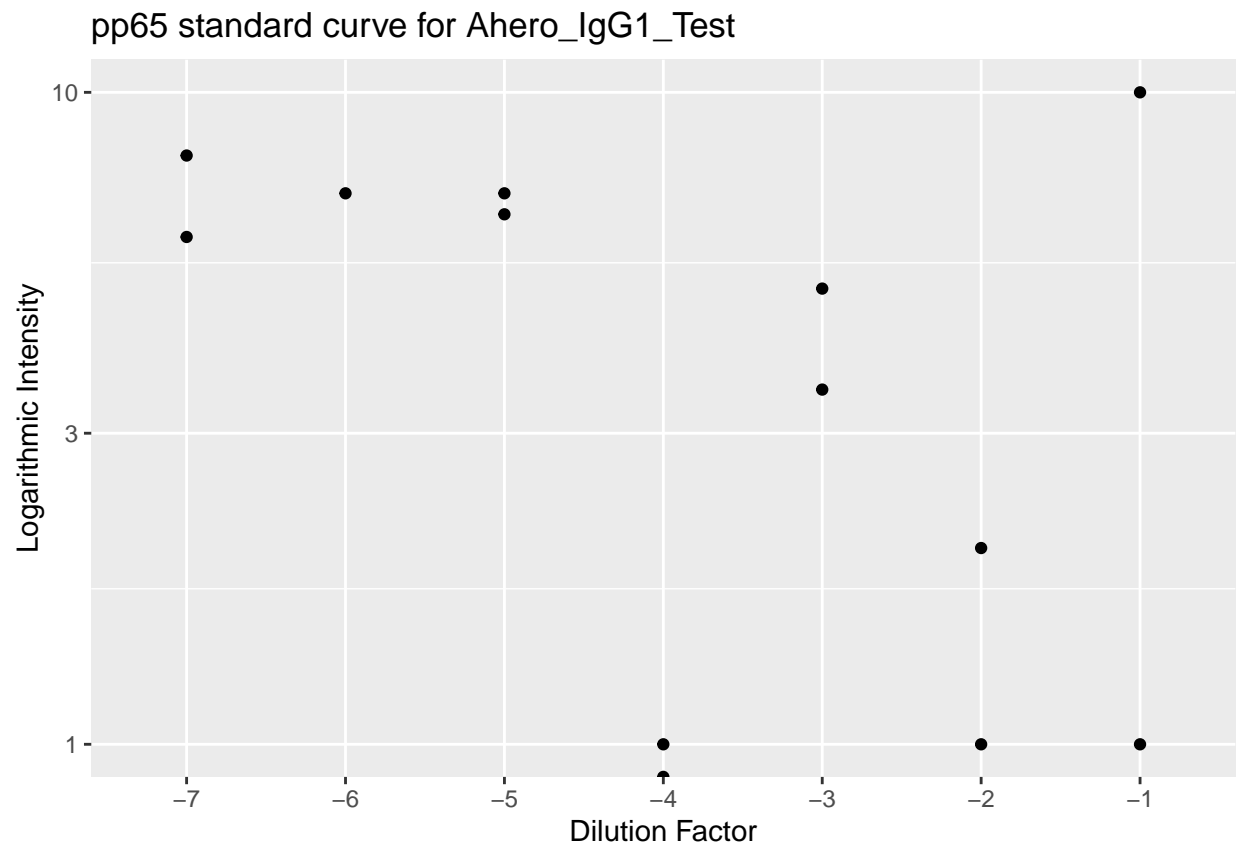
AMA1 standard curve for Ahero_IgG1_Test
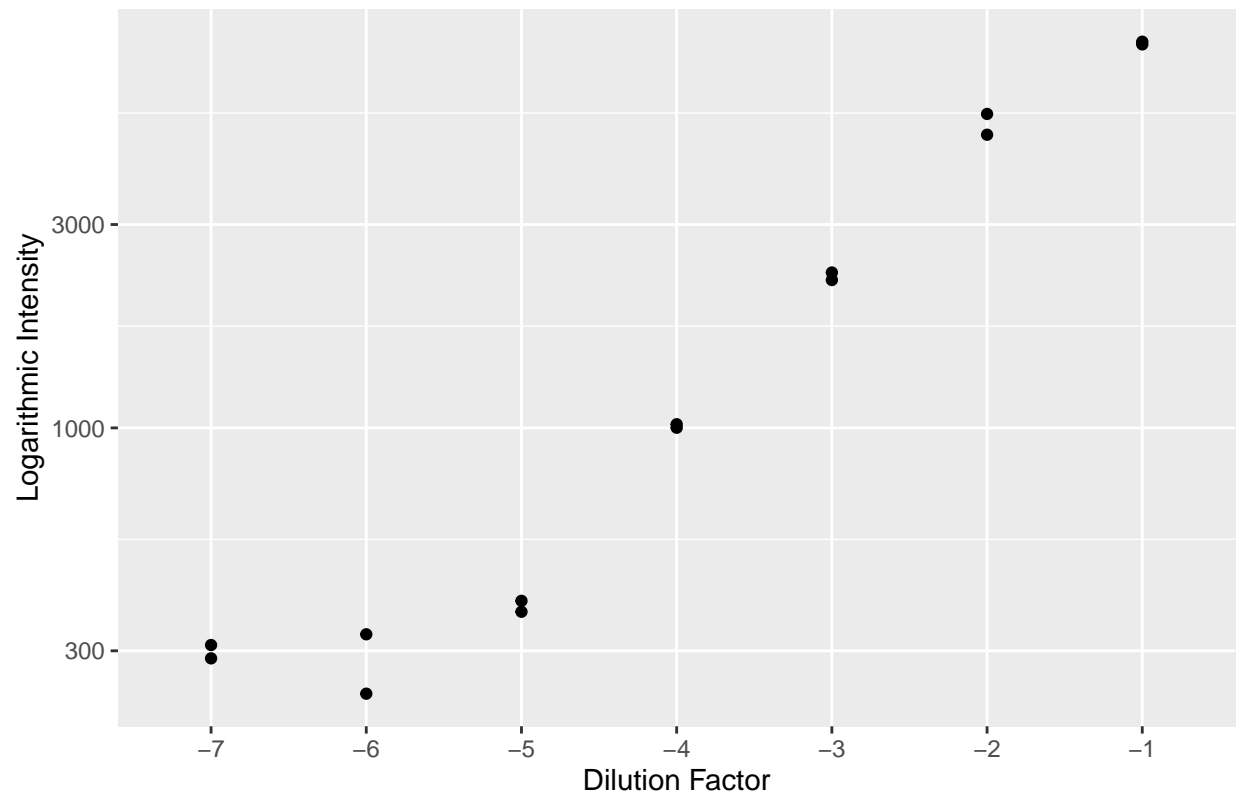
##
[[2]]

pp28 standard curve for Ahero_IgG1_Test


```
## 
## [[3]]
```

Schisto standard curve for Ahero_IgG1_Test

```
## 
## [[4]]
```

pp65 standard curve for Ahero_IgG1_Test
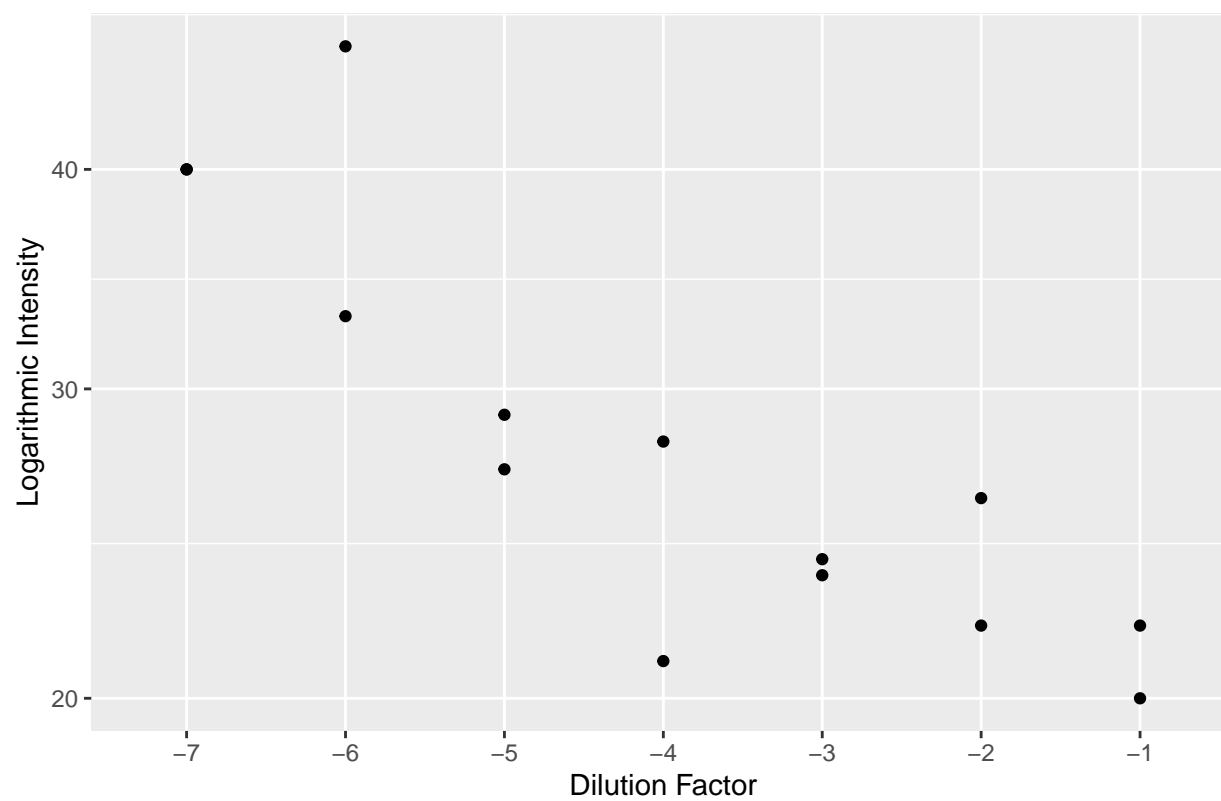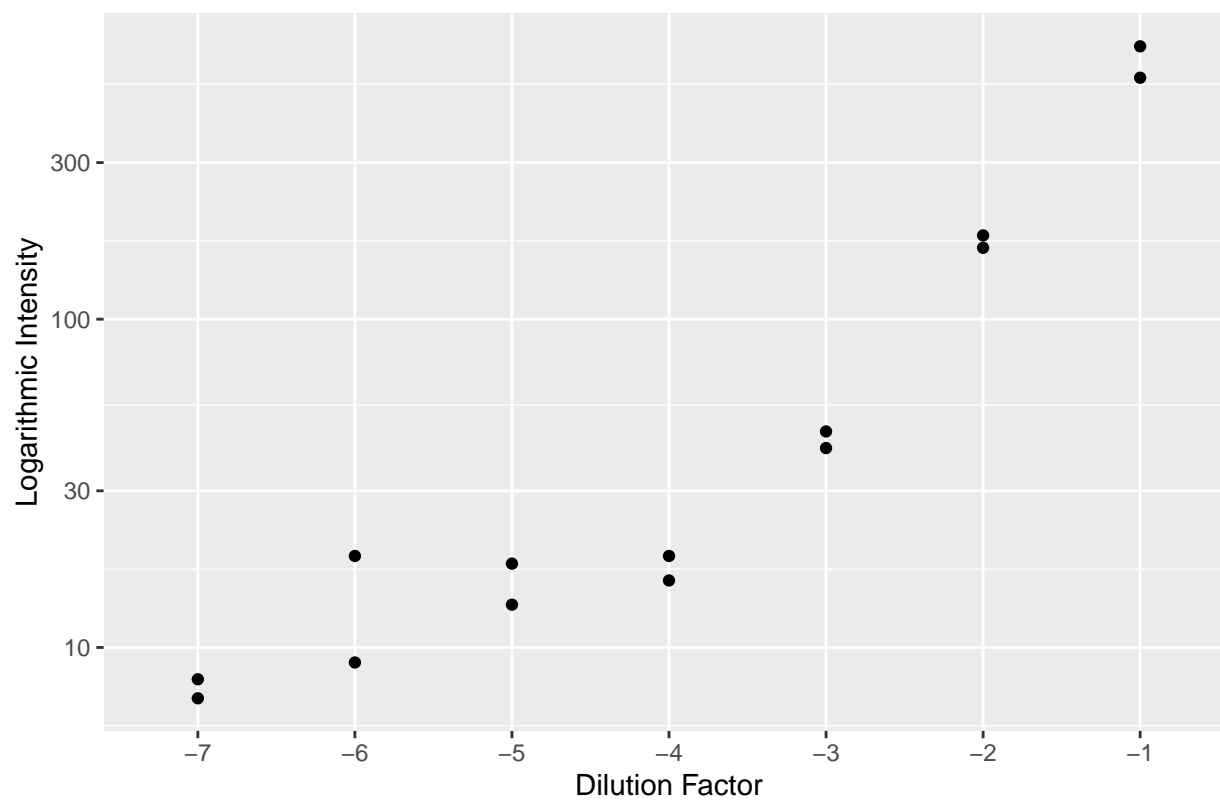
```
## 
## [[5]]
```

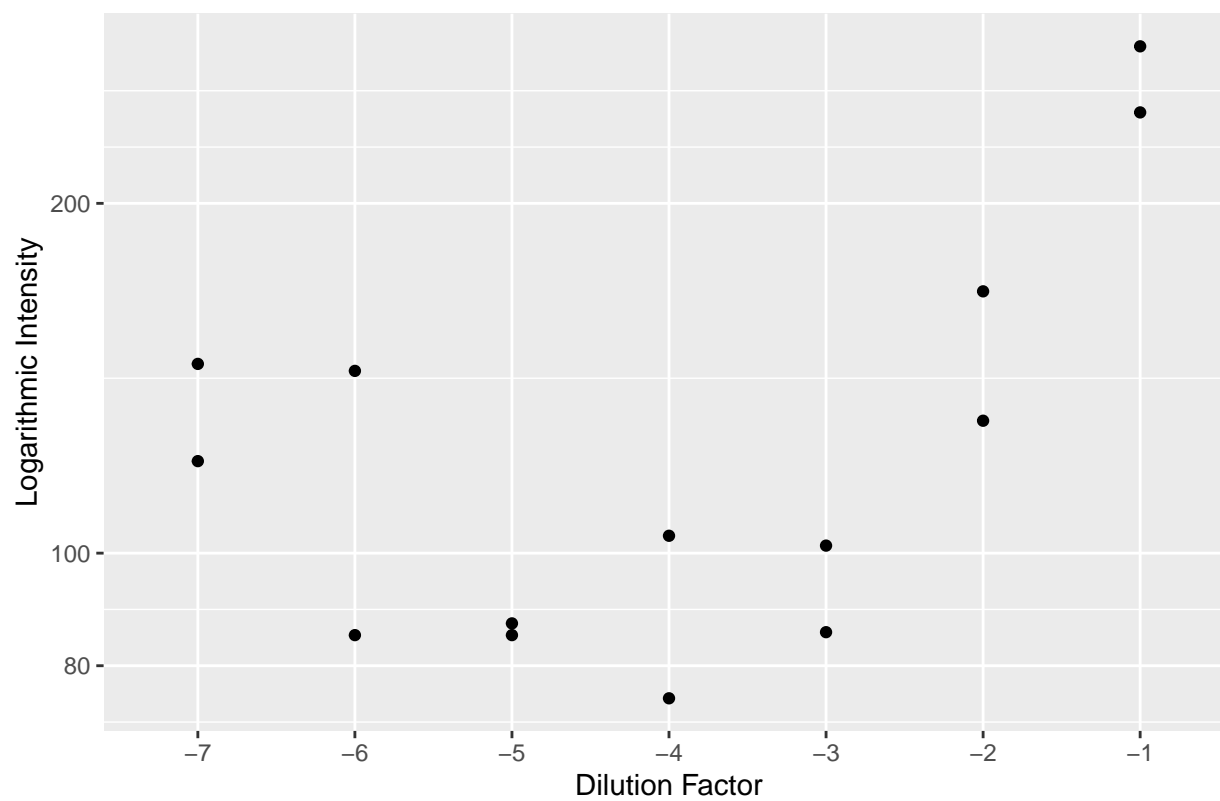## BSA standard curve for Ahero_IgG1_Test


```
##
## [[6]]
```

MSP1 standard curve for Ahero_IgG1_Test

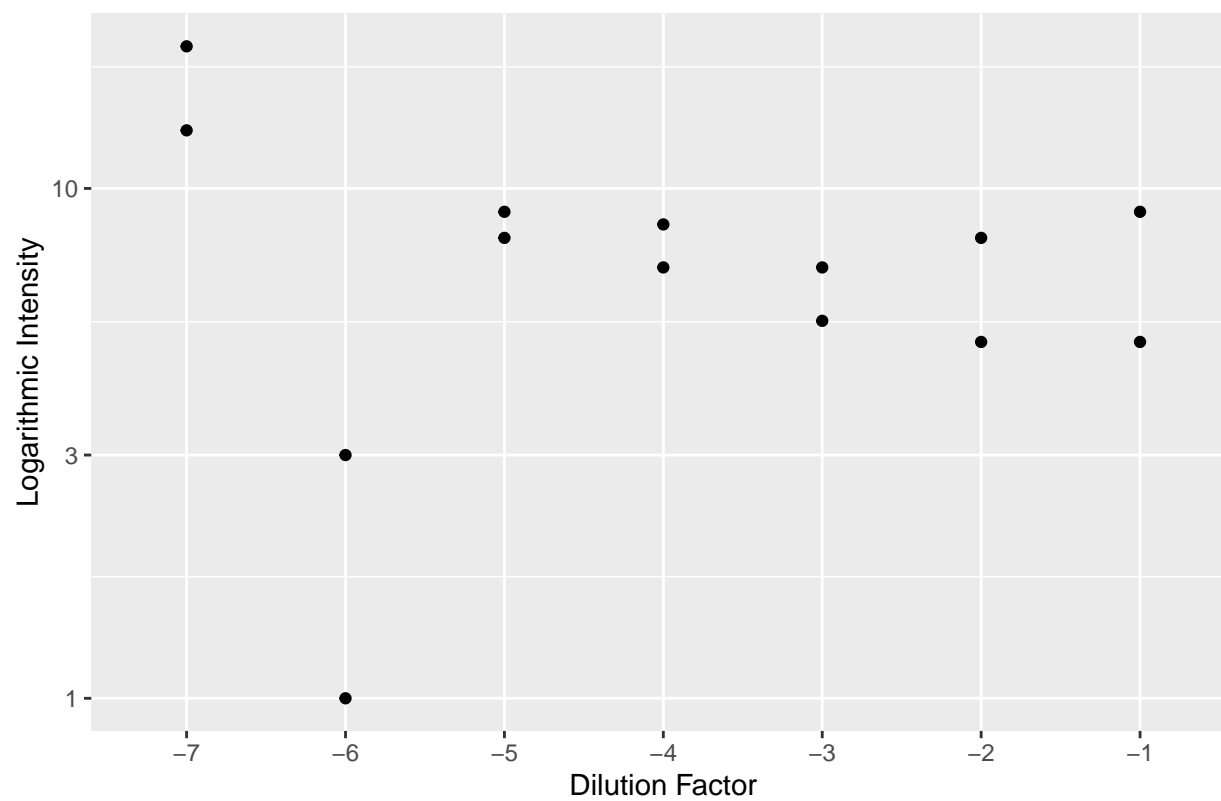
```
##
## [[7]]
```
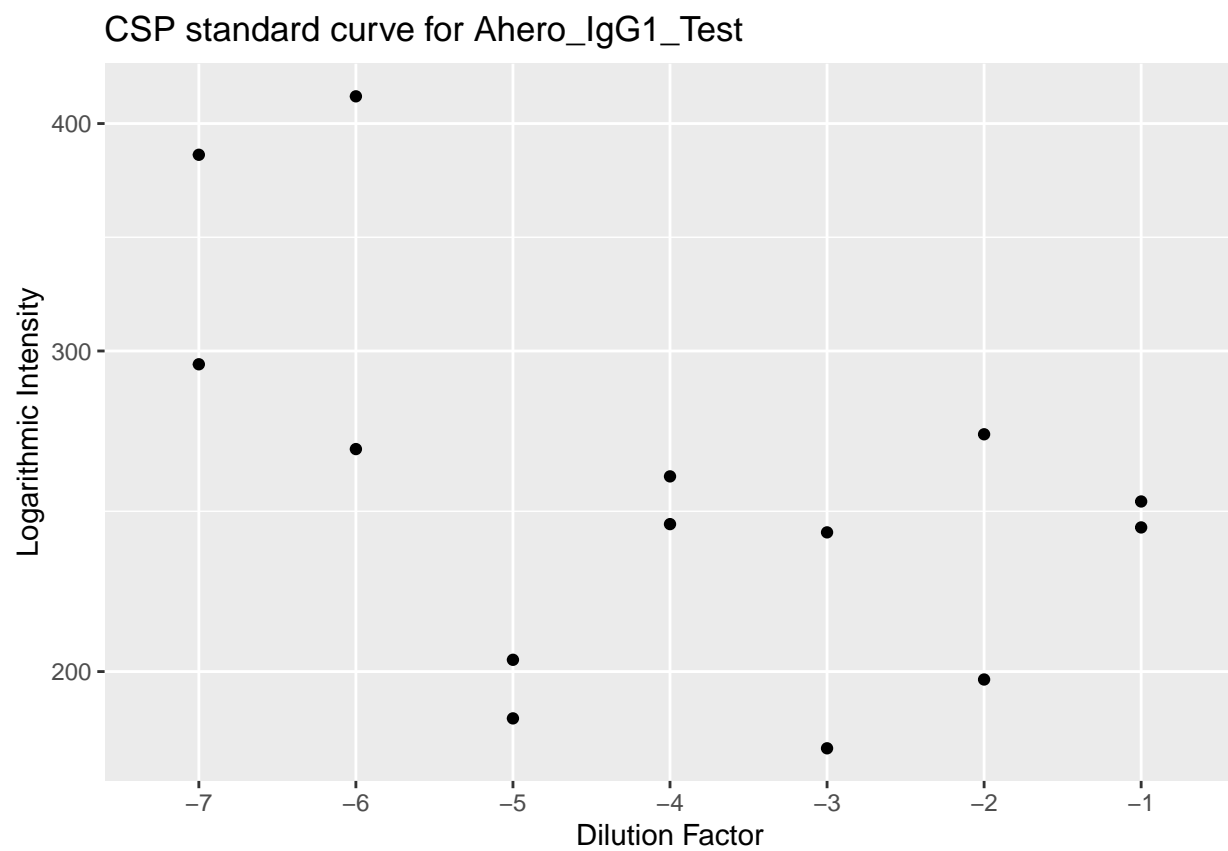
HRPII standard curve for Ahero_IgG1_Test


```
##
## [[8]]
```

CelTos standard curve for Ahero_IgG1_Test


```
##
## [[9]]
```

CSP standard curve for Ahero_IgG1_Test


```
##
## [[10]]
```

PfGarp standard curve for Ahero_IgG1_Test