Calculating Serostatus Thresholds

2025-05-22

Introduction

The purpose of this document is to compute a threshold of serostatus when there is not an external threshold, like there was for measles in Lab 4. This document describes how to use control samples with known prior exposure status to a pathogen (i.e., positive or negative) to establish a threshold, then describes how to calculate test performance characteristics of this threshold (i.e., sensitivity and specificity). Upon completing this document, you will be able to:

- Identify a threshold of seropositivity using well-characterized control samples (negative controls only, or positive and negative controls together).
- Characterize the sensitivity and specificity of this threshold.

General housekeeping

Before we start, let's navigate to the appropriate working directory. You can accomplish this by navigating to the "Session" tab of Rstudio, and choosing "Set Working Directory" –> "Choose Directory" and using your file browser to navigate to the `seroanalytics_workshop` folder. Alternatively, you can modify the code below as appropriate for your files to get to the `seroanalytics_workshop` folder.

```
# set my_path to be the working directory location of where
# the *seroanalytics_workshop* folder is stored on your
# computer
my_path <- "/OneDrive-JohnsHopkins/seroanalytics_workshop"

# source the functions file from the directory using the
# my_path location the functions file is saved in the
# Source/ folder within the working directory
source(paste(my_path, "Source/utils.R", sep = "/"))
```

Reading in data

Note this is the same procedure as in Lab 3 and Lab 4.

```
control_data <- read.csv(paste(my_path, "Data/simulated_control_long_training_data.csv",
    sep = "/"))

sample_data <- read.csv(paste(my_path, "Data/simulated_sample_wide_training_data.csv",
    sep = "/"))
```

```
# this converts sample_data from a wide to a long
# dataframe. edit the column names to your data.
sample_long <- reshape(sample_data, varying = setdiff(names(sample_data),
    c("id", "age", "sex")), v.names = "mfi", timevar = "antigen",
    times = setdiff(names(sample_data), c("id", "age", "sex")),
    idvar = "id", direction = "long")
rownames(sample_long) <- NULL
```

Choosing a cutoff based on negative controls.

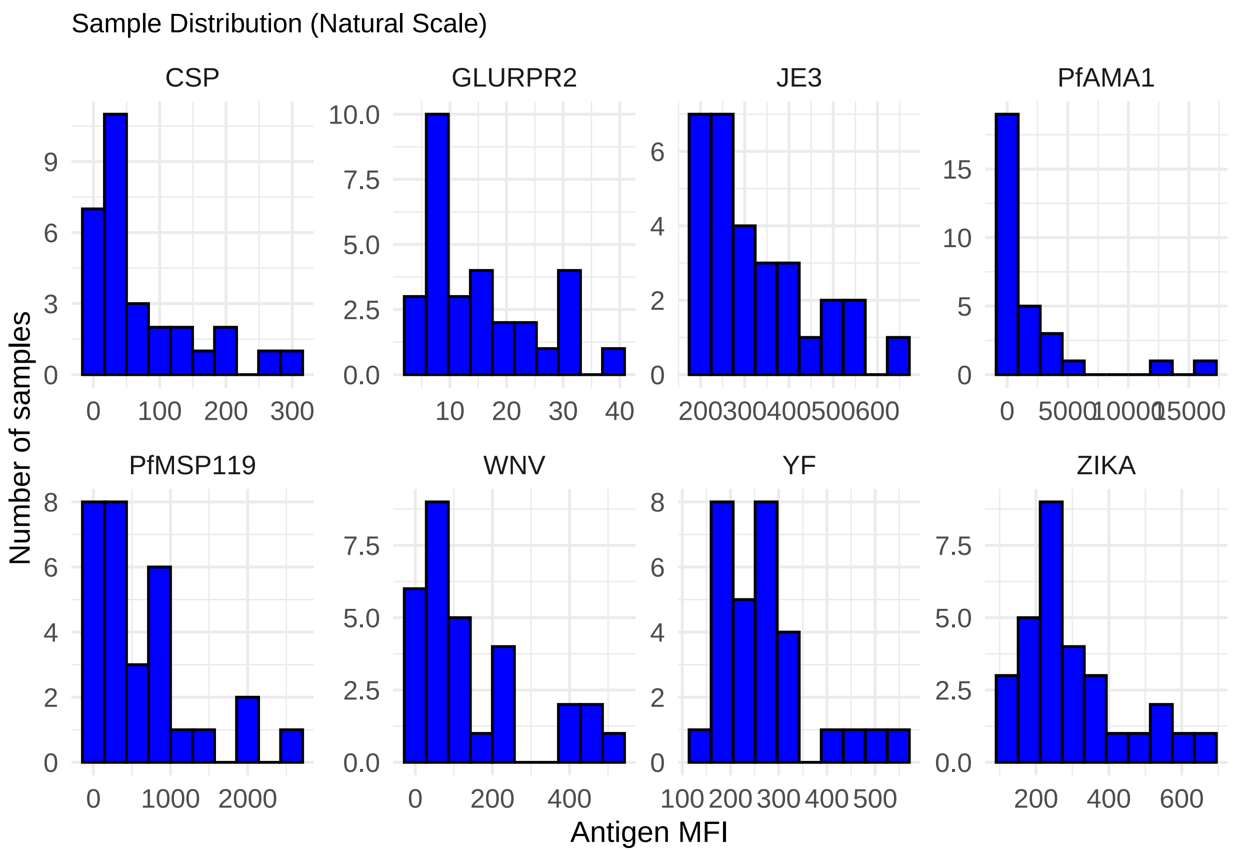
We begin by focusing on PfAMA1 responses in this section. Feel free to explore other antigens after you have completed these steps.

We will begin by exploring the distribution of the negative controls. One way to determine a cutoff is to consider samples above the distribution of negative controls to be positive. This can be particularly useful for long-asting markers of prior exposure that do not show signs of waning, as is the case for PfAMA1 (i.e., a long-lasting marker of prior malaria exposure). There are several ways one can consider the distribution of negative controls. The simplest way is to assign every sample that has a higher MFI value than the highest negative control to be positive. We can begin with this method.

First, we can visualise the distribution of negative controls.

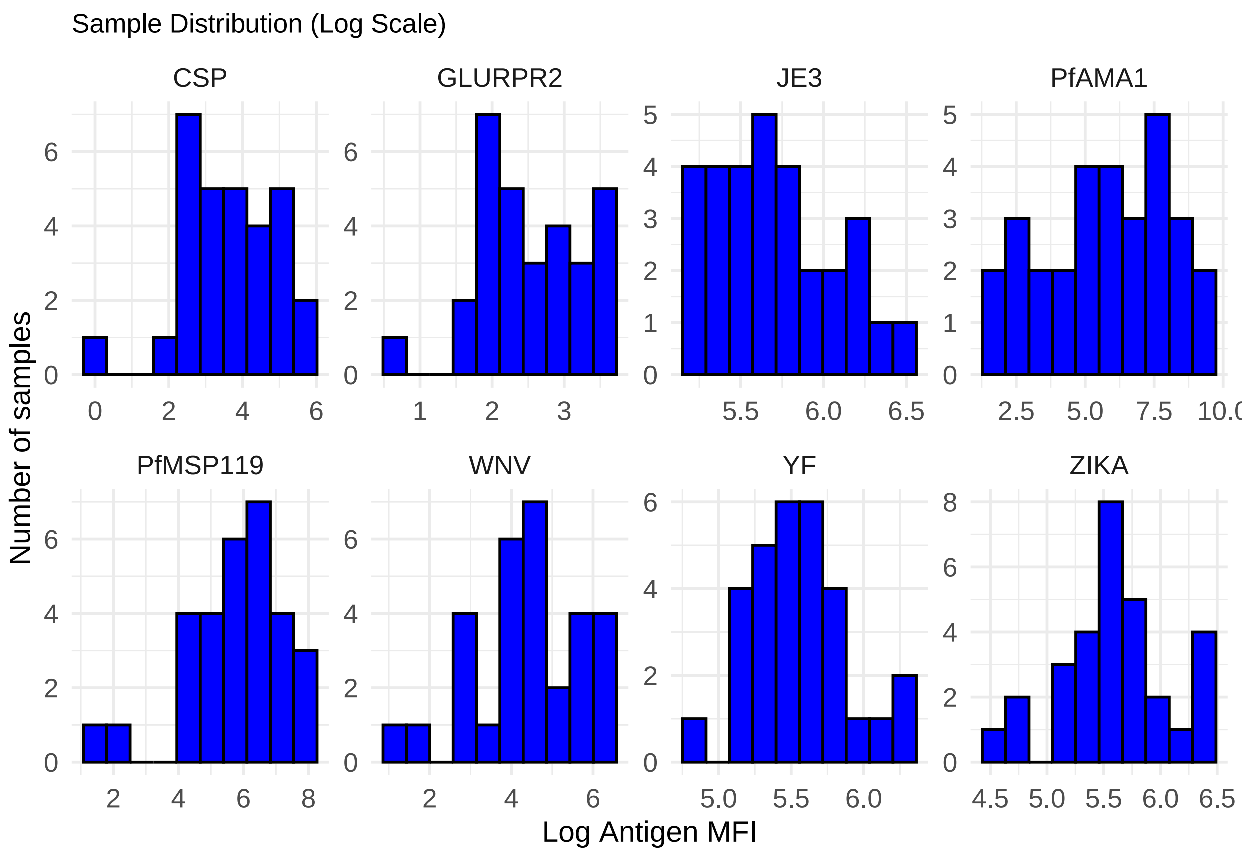
```
#restrict to negative controls
control_negs <- control_data[control_data$pos_neg=="negative",] #edit this line if needed

#check distributions of data - untransformed
neg_controls_natural_scale <- ggplot(control_negs, aes(x = mfi)) +
      geom_histogram(bins = 10, color = "black", fill = "blue") +
      facet_wrap(~ antigen, scales = "free", ncol = 4) +  # <- Set 5 columns per row
      labs(
        title = "Sample Distribution (Natural Scale)",
        x = "Antigen MFI",
        y = "Number of samples"
      ) +
      theme_minimal() +
      theme(
        strip.text = element_text(size = 10),  # Smaller font for facet labels
        axis.text = element_text(size = 10),  # Smaller font for axis text
        plot.title = element_text(size = 10)  # Smaller font for the plot title
      )
    neg_controls_natural_scale
```



```
    neg_controls_log_scale <- ggplot(control_negs, aes(x = log(mfi))) +
      geom_histogram(bins = 10, color = "black", fill = "blue") +
      facet_wrap(~ antigen, scales = "free", ncol = 4) +  # <- Set 5 columns per row
      labs(
        title = "Sample Distribution (Log Scale)",
        x = "Log Antigen MFI",
        y = "Number of samples"
      ) +
      theme_minimal() +
      theme(
        strip.text = element_text(size = 10),  # Smaller font for facet labels
        axis.text = element_text(size = 10),  # Smaller font for axis text
        plot.title = element_text(size = 10)  # Smaller font for the plot title
      )

neg_controls_log_scale
```



Now, we can identify the cutoff as being the MFI value of the highest negative control.

```
highestNeg_cutoff <- max(control_negs$mfi[which(control_negs$antigen ==
    "PfAMA1")])
```

Now, we might want to understand how "good" our cutoff is: one way to assess this is to compute the sensitivity and specificity under this cutoff. Recall, sensitivity is the proportion of true positives that are accurately classified as positive, and specificity is the proportion of true negatives that are accurately classified as negative. Note, we are able to calculate this because we have both positive and negative control samples; this may not always be the case.

```
# compute serostatus using the highest negative value
control_data_ama1 <- control_data[which(control_data$antigen ==
    "PfAMA1"), ]
serostatus_highNeg <- ifelse(control_data_ama1$mfi > highestNeg_cutoff,
    1, 0)

# add this serostatus variable to our original control
# dataset for PfAMA1
control_data_ama1WithSerostatus <- cbind(control_data_ama1, serostatus_highNeg)

# compute sensitivity
sens_ama1_highestNeg <- length(which(control_data_ama1WithSerostatus$pos_neg ==
    "positive" & control_data_ama1WithSerostatus$serostatus_highNeg ==
    1))/length(which(control_data_ama1WithSerostatus$pos_neg ==
    "positive"))
```

```
sens_ama1_highestNeg
```

```
## [1] 1
```

```
# compute specificity
spec_ama1_highestNeg <- length(which(control_data_ama1WithSerostatus$pos_neg ==
    "negative" & control_data_ama1WithSerostatus$serostatus_highNeg ==
    0))/length(which(control_data_ama1WithSerostatus$pos_neg ==
    "negative"))

spec_ama1_highestNeg
```

```
## [1] 1
```

Another common method that only relies on negative controls is to use a fixed number of standard deviations above the mean of the negative controls to establish a cutoff. In this example, we choose 3 standard deviations above the mean of the negative controls. Note here, we will work on the log scale. We will calculate the same test performance characteristics for this cutoff.

```
# calculate the cutoff
mean3sd_cutoff <- mean(log(control_data_ama1$mfi[which(control_data_ama1$pos_neg ==
    "negative")])) + 3 * sd(log(control_data_ama1$mfi[which(control_data_ama1$pos_neg ==
    "negative")]))

# calculate serostatus
serostatus_mean3SD <- ifelse(log(control_data_ama1$mfi) > mean3sd_cutoff,
    1, 0)

# add this serostatus variable to our original control
# dataset for pfAMA1
control_data_ama1WithSerostatus <- cbind(control_data_ama1WithSerostatus,
    serostatus_mean3SD)

# compute sensitivity
sens_ama1_mean3sd <- length(which(control_data_ama1WithSerostatus$pos_neg ==
    "positive" & control_data_ama1WithSerostatus$serostatus_mean3SD ==
    1))/length(which(control_data_ama1WithSerostatus$pos_neg ==
    "positive"))

sens_ama1_mean3sd
```

```
## [1] 0
```

```
# compute specificity
spec_ama1_mean3sd <- length(which(control_data_ama1WithSerostatus$pos_neg ==
    "negative" & control_data_ama1WithSerostatus$serostatus_mean3SD ==
    0))/length(which(control_data_ama1WithSerostatus$pos_neg ==
    "negative"))

spec_ama1_mean3sd
```

```
## [1] 1
```

Exercise 1: What do you notice about the sensitivity and specificity when comparing these two approaches? Which approach would you choose?

Exercise 2:

Using code below, and the sample data (not control!) for PfAMA1, compute the seroprevalence and 95% confidence intervals using the highest negative cutoff, and then adapt the code to use the mean plus 3SD cutoff. Do you notice any differences?

```
# Applying cutoff:

# ifelse function, if first statement is true, then outcome
# is set to 1, and if first statement is false, then
# outcome is 0 since the first statement is a vector, then
# outcome will be a vector of 1's and 0's 1 indicates
# seropositive, and 0 indicates seronegative
seropositivity <- ifelse(sample_data$PfAMA1 > highestNeg_cutoff,
    1, 0)

# number of people seropositve and seronegative
cat("Table of number seronegative and seronegative", "\n")
```

```
## Table of number seronegative and seronegative
```

```
table(seropositivity, useNA = "always")
```

```
## seropositivity
##    0    1 <NA>
##  469  531    0
```

```
# percent of people seropositive and negative
cat("Table of percent seronegative and seronegative", "\n")
```

```
## Table of percent seronegative and seronegative
```

```
round(prop.table(table(seropositivity, useNA = "always")), 3) *
    100
```

```
## seropositivity
##    0    1 <NA>
## 46.9 53.1  0.0
```

```
# Set up to calculate the confidence interval x is the
# number seropositive and you can get the number
# seropositive in your sample from the seropositive table
# above
x <- 531

# n is the total number of samples in your data and note in
# the data we saw above there were no NA values, but if you
```

```r
# do have NA values be sure to exclude them from this round
n <- nrow(sample_data)

# conf is the confidence interval and a 95% is a standard
# CI but you can adjust this if you want
conf <- 0.95

# Estimate the exact interval using the epitools function
ci <- binom.exact(x, n, conf.level = conf)

cat("CI lower", round(ci$lower, 4) * 100, "%, CI upper", round(ci$upper,
    4) * 100, "%")
```

```
## CI lower 49.95 %, CI upper 56.23 %
```

Now we will use the positive and negative controls jointly to come up with a cutoff that seeks to maximize both sensitivity and specificity. First we will plot an ROC curve: this curve describes the relationship between sensitivity and specificity.

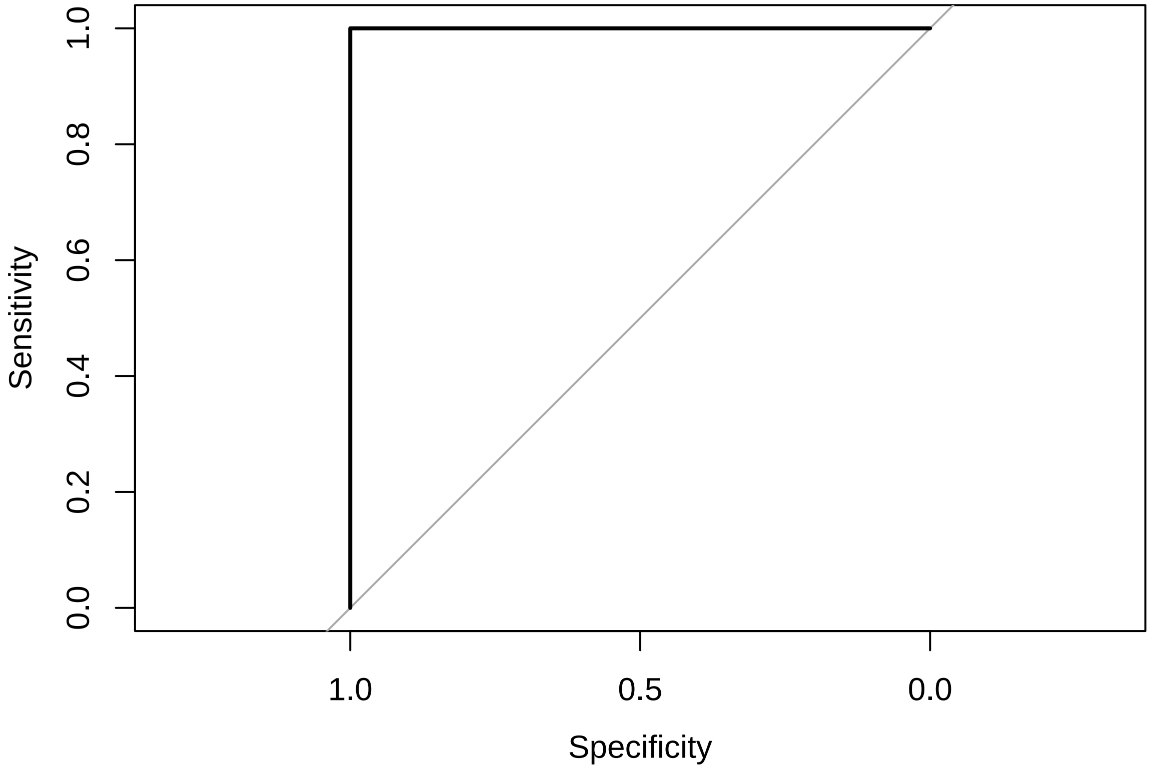
The ROC fixes a threshold for seropositivity and then computes sensitivity and specificity under that threshold. Then the method repeats this process for a different threshold, and explores the entire range of possible thresholds (in our case from -11.5 to 58767 MFI).

```r
# this fits the ROC curve
rocFit <- roc(response = control_data_ama1$pos_neg, predictor = control_data_ama1$mfi)

# look at the output
rocFit
```

```
##
## Call:
## roc.default(response = control_data_ama1$pos_neg, predictor = control_data_ama1$mfi)
##
## Data: control_data_ama1$mfi in 30 controls (control_data_ama1$pos_neg negative) < 30 cases
(control_data_ ama1$pos_neg positive).
## Area under the curve: 1
```

```r
plot(rocFit)
```



Commonly, we want to choose a threshold that jointly maximizes sensitivity and specificity. This is a quantity known as Youden's J. Formally, we want to choose the threshold for which the equation `sensitivity+specificity-1` has the largest possible value. We will identify the optimal threshold under these conditions and compute sensitivity and specificity.

```
# first we compute all possible values of this under the
# different thresholds of the ROC
indices <- rocFit$sensitivities + rocFit$specificities - 1

ROCThreshold <- rocFit$thresholds[which(indices == max(indices))][1]

serostatus_ROC <- ifelse(control_data_ama1$mfi > ROCThreshold,
    1, 0)

# add this serostatus variable to our original control
# dataset for PfAMA1
control_data_ama1WithSerostatus <- cbind(control_data_ama1WithSerostatus,
    serostatus_ROC)

# compute sensitivity
sens_ama1_ROC <- length(which(control_data_ama1WithSerostatus$pos_neg ==
    "positive" & control_data_ama1WithSerostatus$serostatus_ROC ==
    1))/length(which(control_data_ama1WithSerostatus$pos_neg ==
    "positive"))

sens_ama1_ROC
```

```
## [1] 1
```

```
# compute specificity
spec_ama1_ROC <- length(which(control_data_ama1WithSerostatus$pos_neg ==
    "negative" & control_data_ama1WithSerostatus$serostatus_ROC ==
    0))/length(which(control_data_ama1WithSerostatus$pos_neg ==
    "negative"))

spec_ama1_ROC
```

```
## [1] 1
```

How do these outputs compare to those from the previous cutoffs?

Exercise 3:

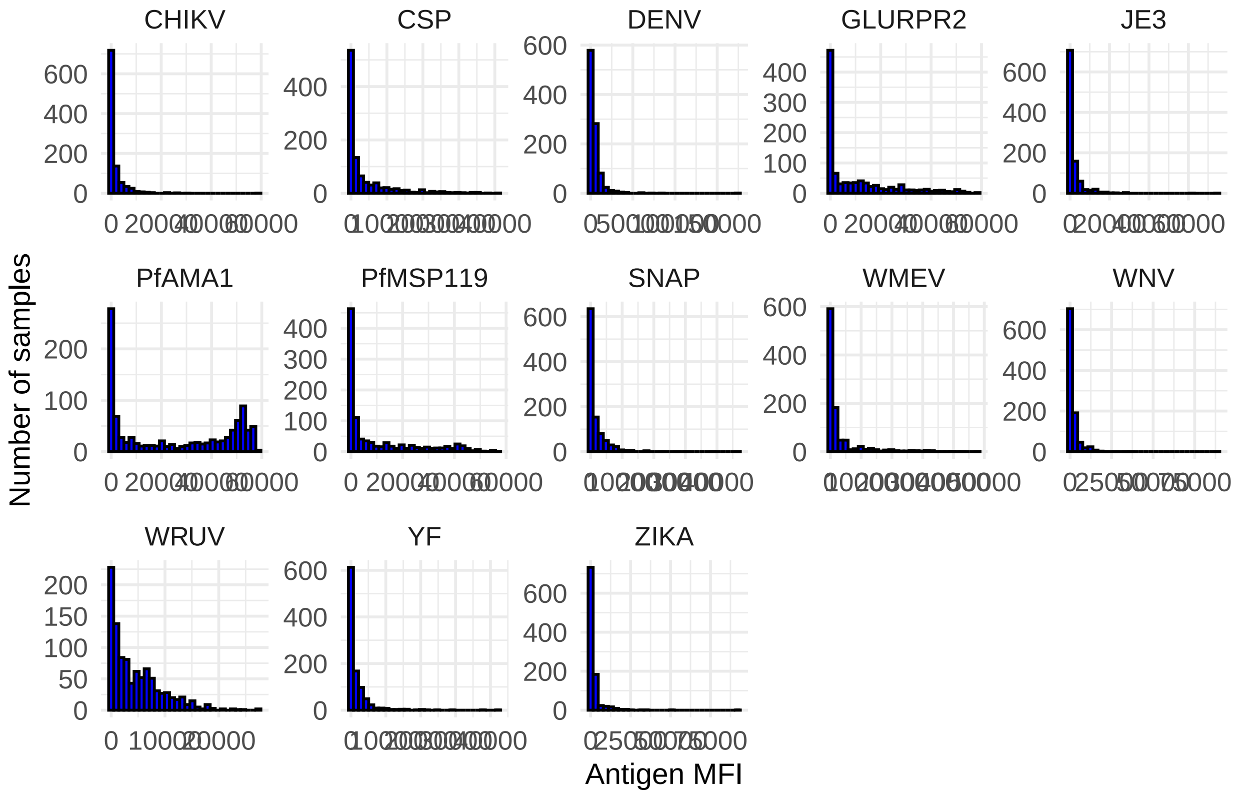
Using code from earlier in the lab, and the sample data (not control!) for PfAMA1, compute the seroprevalence and 95% confidence intervals using ROC cutoff. Do you notice any differences?

Optional

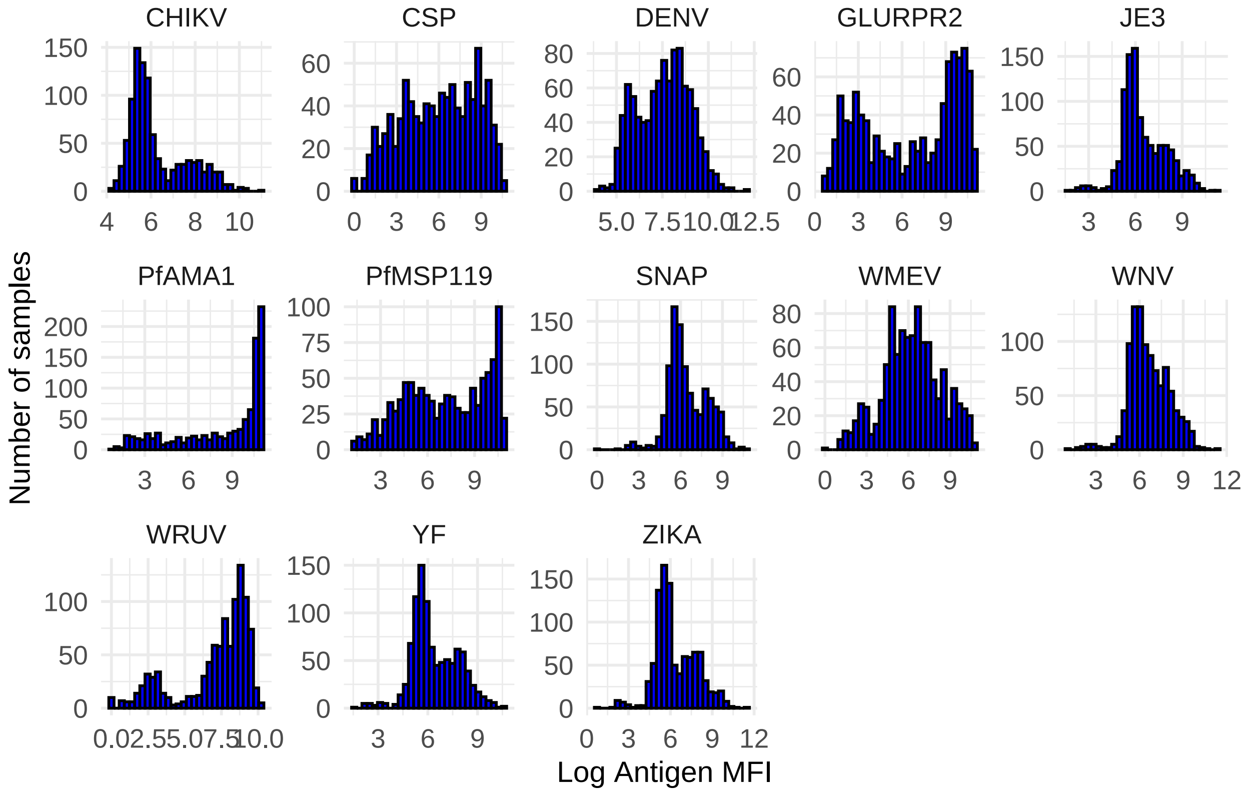
If you want to apply a finite mixture model (FMM), answer the following questions:

a. Plot your sample values and consider if they visually have a "good" distribution to fit a mixture model.

```
  faceted_natural_scale <- ggplot(sample_long, aes(x = mfi)) +
  geom_histogram(bins = 30, color = "black", fill = "blue") +
  facet_wrap(~ antigen, scales = "free", ncol = 5) +
  labs(
    title = "",
    x = "Antigen MFI",
    y = "Number of samples"
  ) +
  theme_minimal() +
  theme(
    strip.text = element_text(size = 10),  # Smaller font for facet labels
    axis.text = element_text(size = 10),  # Smaller font for axis text
    plot.title = element_text(size = 10)  # Smaller font for the plot title
  )
faceted_natural_scale
```



```
#log scale
faceted_log_scale <- ggplot(sample_long, aes(x = log(mfi))) +
  geom_histogram(bins = 30, color = "black", fill = "blue") +
  facet_wrap(~ antigen, scales = "free", ncol = 5) +  # <- Set 5 columns per row
  labs(
    title = "",
    x = "Log Antigen MFI",
    y = "Number of samples"
  ) +
  theme_minimal() +
  theme(
    strip.text = element_text(size = 10),  # Smaller font for facet labels
    axis.text = element_text(size = 10),  # Smaller font for axis text
    plot.title = element_text(size = 10)  # Smaller font for the plot title
  )
faceted_log_scale
```



b. Decide which antigen you want to fit the FMM to. Make a vector of your antigen values. If you want to do a log transformation, do so before putting data into the FMM. Use the following code to run the FMM model with 2 components:

```
mfi_values <- sample_data$JE3  #fill in your own data here
log_antigen_vector <- log(mfi_values)

k <- 2  #number of components
fmm_model <- Mclust(log_antigen_vector, G = k, modelNames = "V")
```

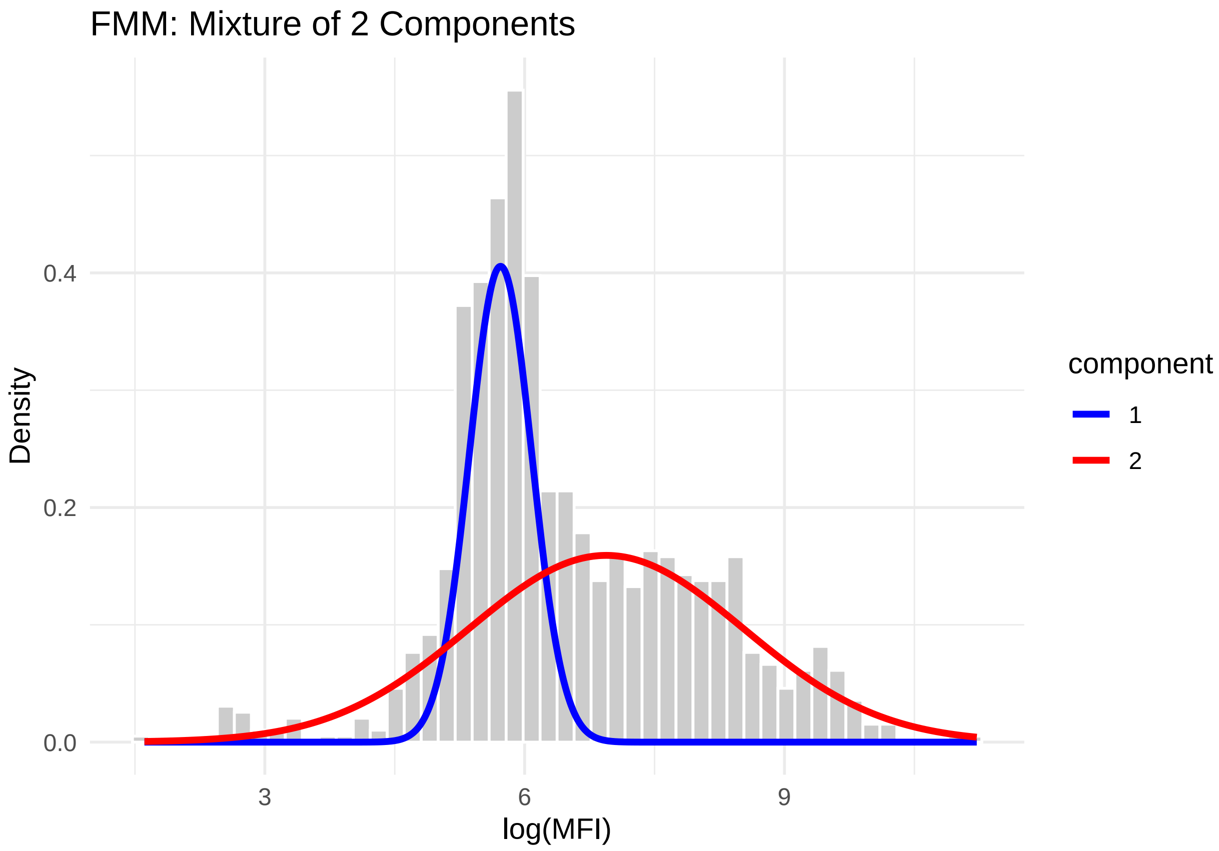
c. Make a plot of your data with the 2 predicted components mapped over them.

```
# Extract parameters
means <- fmm_model$parameters$mean
sds <- sqrt(fmm_model$parameters$variance$sigmasq)
props <- fmm_model$parameters$pro

# Create density curves for each component
x_vals <- seq(min(log_antigen_vector), max(log_antigen_vector),
    length.out = 1000)

dens_df <- data.frame(x = rep(x_vals, 2), density = c(dnorm(x_vals,
    mean = means[1], sd = sds[1]) * props[1], dnorm(x_vals, mean = means[2],
    sd = sds[2]) * props[2]), component = factor(rep(1:2, each = length(x_vals))))
```

```
# Plot histogram + density curves
ggplot() + geom_histogram(aes(x = log_antigen_vector, y = after_stat(density)),
    bins = 50, fill = "gray80", color = "white") + geom_line(data = dens_df,
    aes(x = x, y = density, color = component), size = 1.2) +
    labs(title = "FMM: Mixture of 2 Components", x = "log(MFI)",
        y = "Density") + scale_color_manual(values = c("blue",
    "red")) + theme_minimal()
```



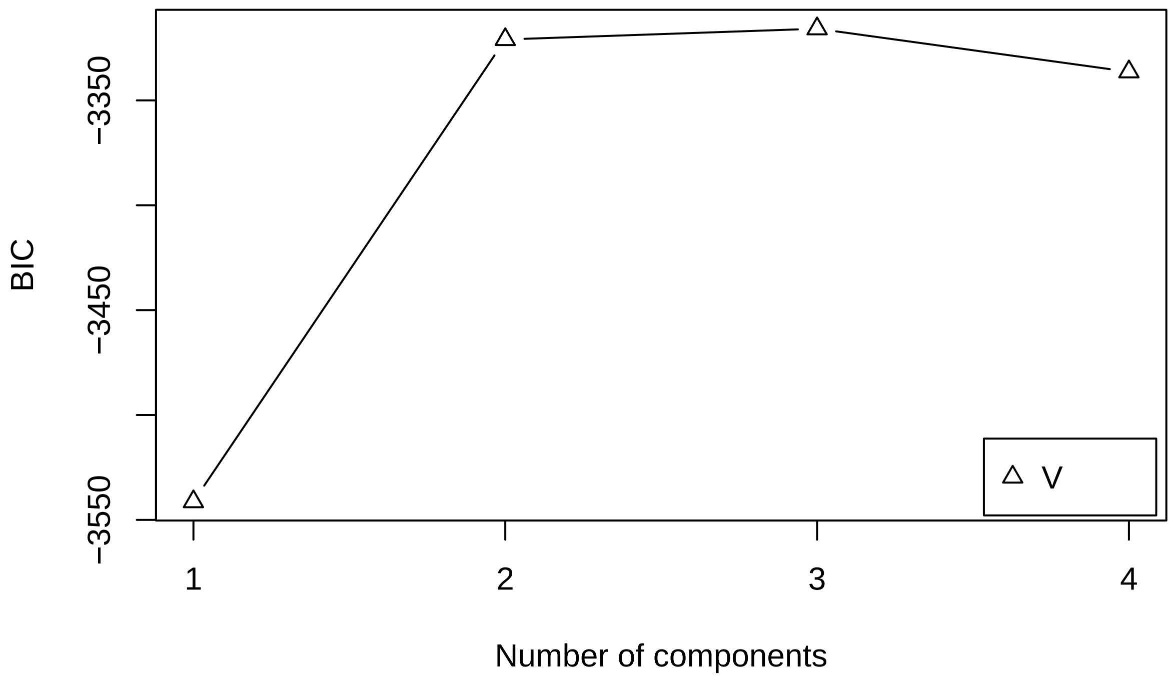
d. Test to see what number of components is the best fit for your data. Remember, FMM might not be an appropriate approach if 2 components is not the best fit for your data.

```
# Step 1: Estimate BIC values for G = 1 to 4 components
bic_values <- mclustBIC(log_antigen_vector, modelNames = "V",
    G = 1:4)

# Step 2: Extract BIC values into a data frame
bic_df <- as.data.frame(bic_values[, 1])
bic_df$G <- as.numeric(rownames(bic_df))  # G is the column for number of components
names(bic_df) <- c("BIC", "G")  # Label column names

# Step 3: Find best model based on max BIC value as derived
# from the mclust package Note that this package calculates
# a transformed BIC value; typically a model of best fit
# would have the lowest BIC value (see paper:
```

```
# https://pmc.ncbi.nlm.nih.gov/articles/PMC5096736/)
plot(bic_values)
```



```
best_index <- which.max(bic_df$BIC)

cat("Best number of components based on BIC:", best_index, "\n")
```

```
## Best number of components based on BIC: 3
```

e. If in part d, the model indicates that 2 components is not the best fit for your model, remake the plot showing your sample data fitted with what was identified to be the optimal number of components. Copy the code from parts b and c below, and edit to run with this optimal number of components.

f. Based on 2 components, calculate seroprevalence using the code below. Remember, if 2 components were not identified to be the optimal fit for your data, then the interpretation of this seroprevalence estimate is challenging.

```
# this uses the fmm_model results you generated in b.

# Step 1: Out of the 2 components, we identify which
# distribution has a higher mean, and assume that is the
# seropositive component
component_means <- fmm_model$parameters$mean
seropositive_component <- which.max(component_means)
```

```
# Step 4: Get the predicted probabilities for each sample
# For each sample, fmm_model$z is the probability of the
# component being in the first component or second
# component
probabilities <- fmm_model$z
# Below pulls out just the probability of each sample being
# seropositive
seropositive_probs <- probabilities[, seropositive_component]

# Step 5: Estimate seroprevalence Note that the mean of all
# the probabilities will be equal to the overall population
# seroprevalence
estimated_seroprevalence <- mean(seropositive_probs)

# Step 6: Report as percentage
cat("Estimated seroprevalence:", round(estimated_seroprevalence *
    100, 1), "%\n")
```

```
## Estimated seroprevalence: 63.4 %
```

g. Compare the seroprevalence you calculated in part f. to the seroprevalence calculated from the other cutoff methods earlier in the lab.