Lab 19 Pertussis Resurgence (mini project)

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Background

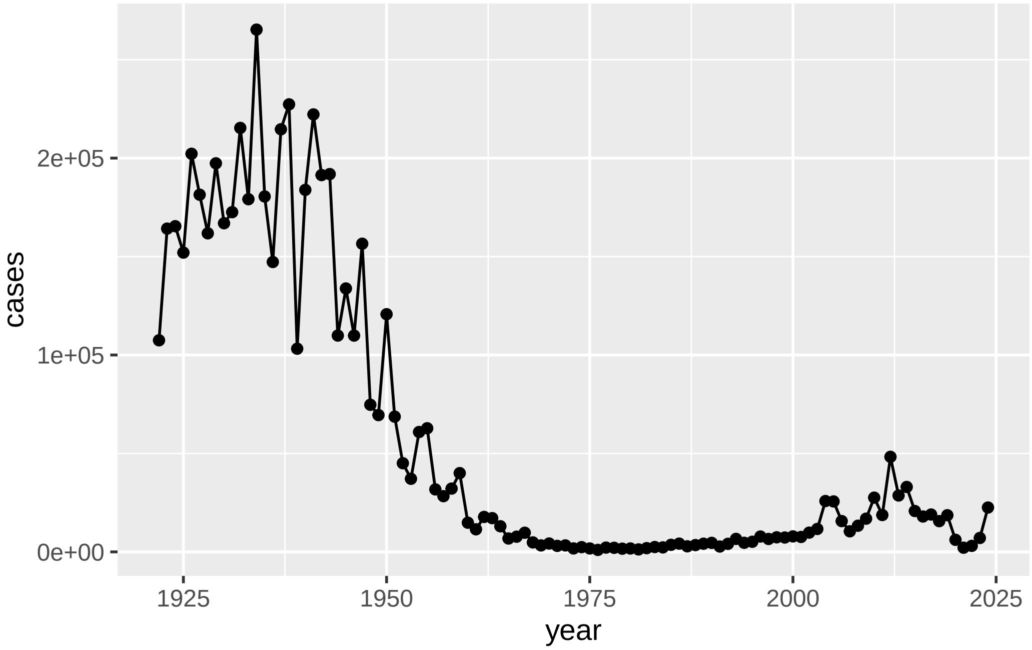
Pertussis is a bacterial lung infection also known as Whooping cough. Let's begin by examining CDC reported case numbers in the US:

```
cdc <- data.frame(
                                 year = c(1922L,1923L,1924L,1925L,
                                          1926L,1927L,1928L,1929L,1930L,1931L,
                                          1932L,1933L,1934L,1935L,1936L,
                                          1937L,1938L,1939L,1940L,1941L,1942L,
                                          1943L,1944L,1945L,1946L,1947L,
                                          1948L,1949L,1950L,1951L,1952L,
                                          1953L,1954L,1955L,1956L,1957L,1958L,
                                          1959L,1960L,1961L,1962L,1963L,
                                          1964L,1965L,1966L,1967L,1968L,1969L,
                                          1970L,1971L,1972L,1973L,1974L,
                                          1975L,1976L,1977L,1978L,1979L,1980L,
                                          1981L,1982L,1983L,1984L,1985L,
                                          1986L,1987L,1988L,1989L,1990L,
                                          1991L,1992L,1993L,1994L,1995L,1996L,
                                          1997L,1998L,1999L,2000L,2001L,
                                          2002L,2003L,2004L,2005L,2006L,2007L,
                                          2008L,2009L,2010L,2011L,2012L,
                                          2013L,2014L,2015L,2016L,2017L,2018L,
                                          2019L,2020L,2021L,2022L,2023L,2024L),
         cases = c(107473,164191,165418,152003,
                                          202210,181411,161799,197371,
                                          166914,172559,215343,179135,265269,
                                          180518,147237,214652,227319,103188,
                                          183866,222202,191383,191890,109873,
                                          133792,109860,156517,74715,69479,
                                          120718,68687,45030,37129,60886,
                                          62786,31732,28295,32148,40005,
                                          14809,11468,17749,17135,13005,6799,
                                          7717,9718,4810,3285,4249,3036,
                                          3287,1759,2402,1738,1010,2177,2063,
                                          1623,1730,1248,1895,2463,2276,
                                          3589,4195,2823,3450,4157,4570,
                                          2719,4083,6586,4617,5137,7796,6564,
                                          7405,7298,7867,7580,9771,11647,
                                          25827,25616,15632,10454,13278,
                                          16858,27550,18719,48277,28639,32971,
                                          20762,17972,18975,15609,18617,
                                          6124,2116,3044,7063, 22538)
       )
```

Plot of cases per year for Pertussis in the US

> Q1. With the help of the R "addin" package datapasta assign the CDC pertussis case number data to a data frame called cdc and use ggplot to make a plot of cases numbers over time.

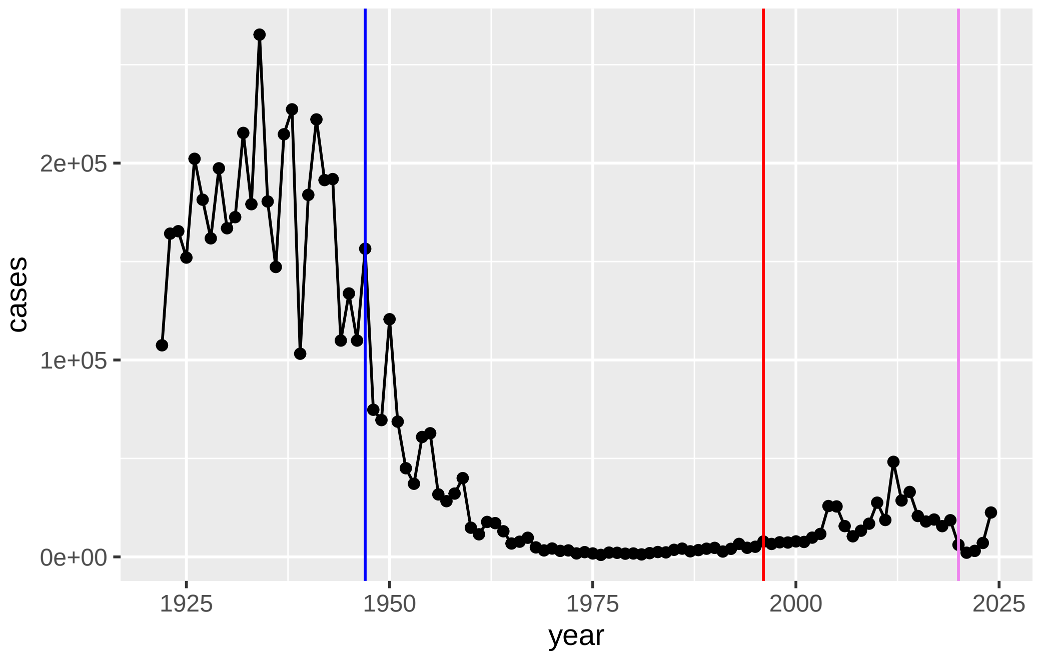
```
library(ggplot2)

ggplot(cdc) +
  aes(year, cases) +
  geom_point() +
  geom_line()
```



> Q2. Using the ggplot geom_vline() function add lines to your previous plot for the 1946 introduction of the wP vaccine and the 1996 switch to aP vaccine (see example in the hint below). What do you notice?

Add some major milestone time points to our plot:

```
ggplot(cdc) +
  aes(year, cases) +
  geom_point() +
  geom_line() +
  geom_vline(xintercept = 1947, col = "blue") +
  geom_vline(xintercept = 1996, col = "red") +
  geom_vline(xintercept = 2020, col = "violet")
```



The full introduction of the wP (whole-cell) Pertussis immunization in the mid 1940s lead ta dramatic reduction in case numbers (from over 200,000 to 100s).

> Q3. Describe what happened after the introduction of the aP vaccine? Do you have a possible explanation for the observed trend?

The switch to the aP (newer acellular formalization in the US did not result in further decline to the case number but increase of case number shown after the switch.

The 2020 lock-down and social distancing measures help mitigate the propagation of the disease, resulted in temporary decline of case number.

The CMI-PB Project

The mission of CMI-PB is to provide the scientific community with a comprehensive, high-quality and freely accessible resource of Pertussis booster vaccination.

Website: https://www.cmi-pb.org/

They make their data available via JSON format API endpoints - basically the database tables in a key:value type format like "infancy_vac":"wP". To read this we can use the `read_json()` function from the **jsonlite** package by install with `install.packages("jsonlite")`.

```r
library(jsonlite)

subject <- read_json(path = "https://www.cmi-pb.org/api/v5_1/subject",
                     simplifyVector = TRUE)

head(subject)
```

```
  subject_id infancy_vac biological_sex              ethnicity  race
1          1          wP         Female Not Hispanic or Latino White
2          2          wP         Female Not Hispanic or Latino White
3          3          wP         Female                Unknown White
4          4          wP           Male Not Hispanic or Latino Asian
5          5          wP           Male Not Hispanic or Latino Asian
6          6          wP         Female Not Hispanic or Latino White
  year_of_birth date_of_boost      dataset
1    1986-01-01    2016-09-12 2020_dataset
2    1968-01-01    2019-01-28 2020_dataset
3    1983-01-01    2016-10-10 2020_dataset
4    1988-01-01    2016-08-29 2020_dataset
5    1991-01-01    2016-08-29 2020_dataset
6    1988-01-01    2016-10-10 2020_dataset
```

Q. How many "subjects"/individuals are in this dataset?

```r
nrow(subject)
```

```
[1] 172
```

Q4. How many wP and aP subjects are there?

```r
table(subject$infancy_vac)
```

```

aP wP 
87 85 
```

Q5/6. What is the breakdown by "biological_sex" and "race"?

```r
table(subject$biological_sex)
```

```
Female   Male
   112     60
```

```
table(subject$race)
```

```
            American Indian/Alaska Native
                                        1
                                    Asian
                                       44
                Black or African American
                                        5
                      More Than One Race
                                       19
Native Hawaiian or Other Pacific Islander
                                        2
                   Unknown or Not Reported
                                       21
                                    White
                                       80
```

```
table(subject$race, subject$biological_sex)
```

```
                                            Female Male
  American Indian/Alaska Native                  0    1
  Asian                                         32   12
  Black or African American                      2    3
  More Than One Race                            15    4
  Native Hawaiian or Other Pacific Islander      1    1
  Unknown or Not Reported                       14    7
  White                                         48   32
```

This breakdown is not particulary representative of the US population - this is a serious caveat for this study. However, it is still the largest sample of it's type every assembled.

```
specimen <- read_json("https://www.cmi-pb.org/api/v5_1/specimen",
                      simplifyVector = TRUE)
ab_titer <- read_json("https://www.cmi-pb.org/api/v5_1/plasma_ab_titer",
                          simplifyVector = TRUE)

head(specimen)
```

```
  specimen_id subject_id actual_day_relative_to_boost
1           1          1                           -3
2           2          1                            1
3           3          1                            3
4           4          1                            7
5           5          1                           11
6           6          1                           32
  planned_day_relative_to_boost specimen_type visit
1                             0         Blood     1
2                             1         Blood     2
3                             3         Blood     3
4                             7         Blood     4
5                            14         Blood     5
6                            30         Blood     6
```

> Q7. Using this approach determine (i) the average age of wP individuals, (ii) the average age of aP individuals; and (iii) are they significantly different?

```
library(lubridate)
```

```
Attaching package: 'lubridate'

The following objects are masked from 'package:base':

    date, intersect, setdiff, union
```

```
library(dplyr)
```

```
Attaching package: 'dplyr'

The following objects are masked from 'package:stats':

    filter, lag
```

```
The following objects are masked from 'package:base':

    intersect, setdiff, setequal, union
```

```r
# Use todays date to calculate age in days
subject$age <- today() - ymd(subject$year_of_birth)

# aP group
ap <- subject %>% filter(infancy_vac == "aP")
round( summary( time_length( ap$age, "years" ) ) )
```

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
     23      27      28      28      29      35 
```

```r
# wP group
wp <- subject %>% filter(infancy_vac == "wP")
round( summary( time_length( wp$age, "years" ) ) )
```

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
     23      33      35      37      40      58 
```

wP group: mean age mid-30s aP group: mean age mid-20s p-value: extremely small (0.001) So, statistically, the aP and wP subjects' ages are very significantly different; wP-primed participants are substantially older than aP-primed ones.

> Q8. Determine the age of all individuals at time of boost?

```r
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

```
[1] 30.69678 51.07461 33.77413 28.65982 25.65914 28.77481
```

> Q9. Complete the code to join specimen and subject tables to make a new merged data frame containing all specimen records along with their associated subject details:

We need to "join" or link these tables with the `subject` table so we can begin to analyze this data and know who a given Ab sample was collected for and when.

```r
library(tidyverse)
```

```
-- Attaching core tidyverse packages ------------------------ tidyverse 2.0.0 --
v forcats 1.0.1     v stringr 1.5.2
v purrr   1.1.0     v tibble  3.3.0
v readr   2.1.5     v tidyr   1.3.1
-- Conflicts ------------------------------------------ tidyverse_conflicts() --
x dplyr::filter()  masks stats::filter()
x purrr::flatten() masks jsonlite::flatten()
x dplyr::lag()     masks stats::lag()
i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to becom
```

```r
meta <- inner_join(subject, specimen)
```

```
Joining with `by = join_by(subject_id)`
```

```r
head(meta)
```

```
  subject_id infancy_vac biological_sex              ethnicity  race
1          1          wP         Female Not Hispanic or Latino White
2          1          wP         Female Not Hispanic or Latino White
3          1          wP         Female Not Hispanic or Latino White
4          1          wP         Female Not Hispanic or Latino White
5          1          wP         Female Not Hispanic or Latino White
6          1          wP         Female Not Hispanic or Latino White
  year_of_birth date_of_boost      dataset        age specimen_id
1    1986-01-01    2016-09-12 2020_dataset 14582 days           1
2    1986-01-01    2016-09-12 2020_dataset 14582 days           2
3    1986-01-01    2016-09-12 2020_dataset 14582 days           3
4    1986-01-01    2016-09-12 2020_dataset 14582 days           4
5    1986-01-01    2016-09-12 2020_dataset 14582 days           5
6    1986-01-01    2016-09-12 2020_dataset 14582 days           6
  actual_day_relative_to_boost planned_day_relative_to_boost specimen_type
1                           -3                             0         Blood
2                            1                             1         Blood
3                            3                             3         Blood
4                            7                             7         Blood
5                           11                            14         Blood
6                           32                            30         Blood
  visit
```

```
1     1
2     2
3     3
4     4
5     5
6     6
```

Now let's join the ab_titer table with our meta table so we have all information about a given Ab measurement

```
ab_data <- inner_join(meta, ab_titer)
```

```
Joining with `by = join_by(specimen_id)`
```

```
head(ab_data)
```

```
  subject_id infancy_vac biological_sex              ethnicity  race
1          1          wP         Female Not Hispanic or Latino White
2          1          wP         Female Not Hispanic or Latino White
3          1          wP         Female Not Hispanic or Latino White
4          1          wP         Female Not Hispanic or Latino White
5          1          wP         Female Not Hispanic or Latino White
6          1          wP         Female Not Hispanic or Latino White
  year_of_birth date_of_boost      dataset        age specimen_id
1    1986-01-01    2016-09-12 2020_dataset 14582 days           1
2    1986-01-01    2016-09-12 2020_dataset 14582 days           1
3    1986-01-01    2016-09-12 2020_dataset 14582 days           1
4    1986-01-01    2016-09-12 2020_dataset 14582 days           1
5    1986-01-01    2016-09-12 2020_dataset 14582 days           1
6    1986-01-01    2016-09-12 2020_dataset 14582 days           1
  actual_day_relative_to_boost planned_day_relative_to_boost specimen_type
1                           -3                             0         Blood
2                           -3                             0         Blood
3                           -3                             0         Blood
4                           -3                             0         Blood
5                           -3                             0         Blood
6                           -3                             0         Blood
  visit isotype is_antigen_specific antigen        MFI MFI_normalised  unit
1     1     IgE               FALSE   Total 1110.21154       2.493425 UG/ML
2     1     IgE               FALSE   Total 2708.91616       2.493425 IU/ML
3     1     IgG                TRUE      PT   68.56614       3.736992 IU/ML
```

```
4     1     IgG                TRUE     PRN  332.12718       2.602350 IU/ML
5     1     IgG                TRUE     FHA 1887.12263      34.050956 IU/ML
6     1     IgE                TRUE     ACT    0.10000       1.000000 IU/ML
  lower_limit_of_detection
1                 2.096133
2                29.170000
3                 0.530000
4                 6.205949
5                 4.679535
6                 2.816431
```

> Q. How many Ab measurements do we have in total

```
nrow(ab_data)
```

```
[1] 61956
```

> Q. How many different isotypes (types of antibody(Ab))?

```
unique(ab_data$isotype)
```

```
[1] "IgE"  "IgG"  "IgG1" "IgG2" "IgG3" "IgG4"
```

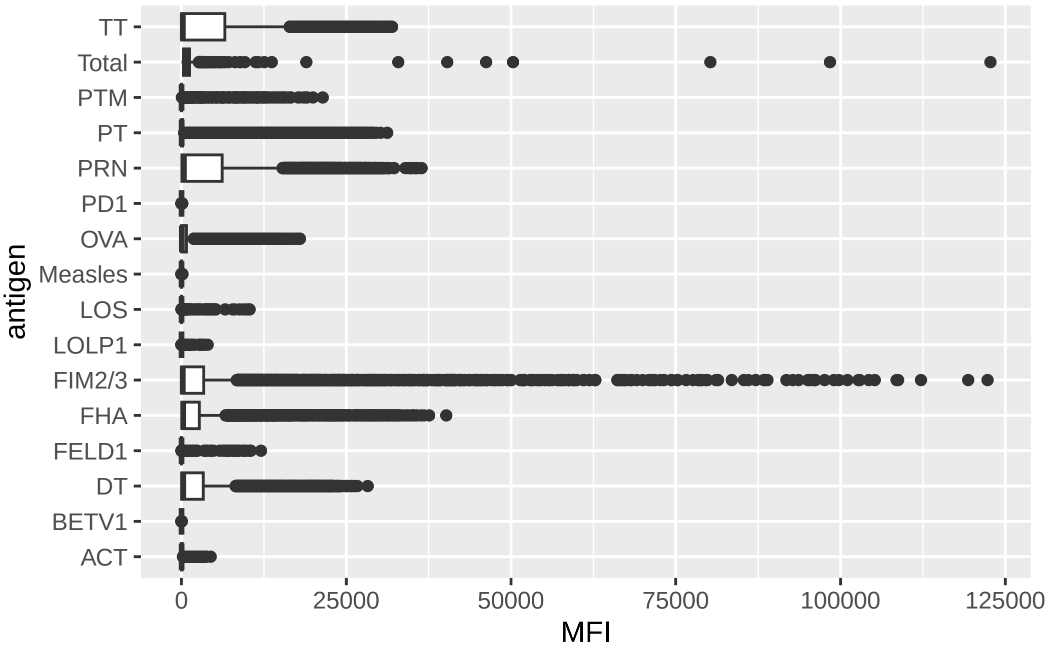
> Q. How many different antigens?

```
unique(ab_data$antigen)
```

```
 [1] "Total"   "PT"      "PRN"     "FHA"     "ACT"     "LOS"     "FELD1"  
 [8] "BETV1"   "LOLP1"   "Measles" "PTM"     "FIM2/3"  "TT"      "DT"     
[15] "OVA"     "PD1"    
```

```
ggplot(ab_data) +
  aes(MFI, antigen) +
  geom_boxplot()
```

```
Warning: Removed 1 row containing non-finite outside the scale range
(`stat_boxplot()`).
```



Q10. Now using the same procedure join meta with titer data so we can further analyze this data in terms of time of visit aP/wP, male/female etc.

```
abdata <- inner_join(ab_titer, meta)
```

```
Joining with `by = join_by(specimen_id)`
```

```
dim(abdata)
```

```
[1] 61956    21
```

Q11. How many specimens (i.e. entries in abdata) do we have for each isotype?

```
table(abdata$isotype)
```

```

  IgE   IgG  IgG1  IgG2  IgG3  IgG4 
 6698  7265 11993 12000 12000 12000 
```

Q12. What are the different $dataset values in abdata and what do you notice about the number of rows for the most "recent" dataset?

```r
table(abdata$dataset)
```

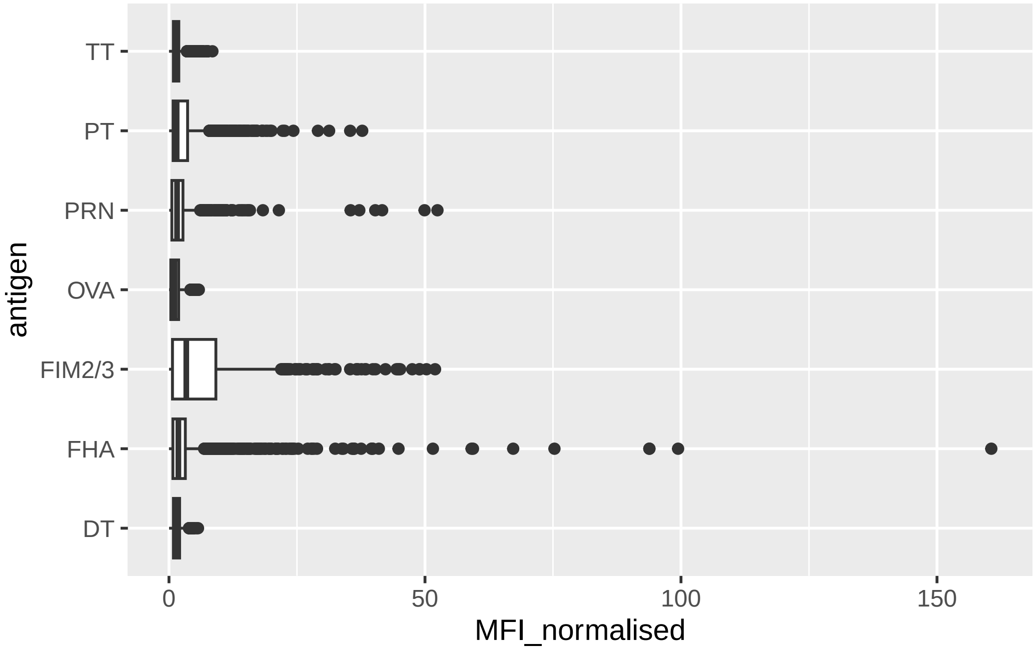
```
2020_dataset 2021_dataset 2022_dataset 2023_dataset 
       31520         8085         7301        15050 
```

Examine IgG Ab titer levels

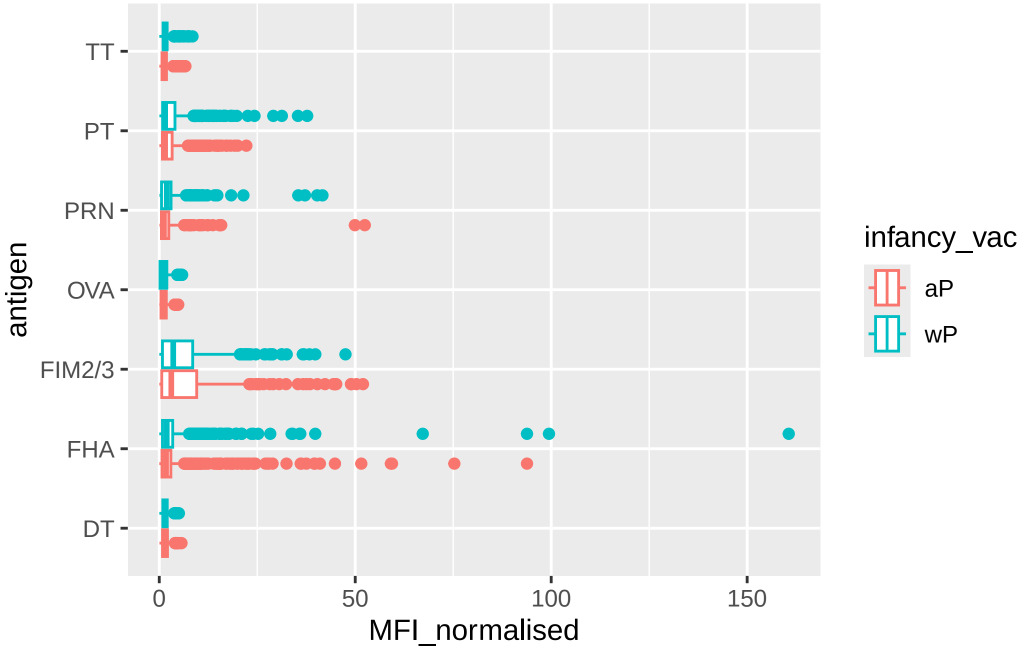
IgG is crucial for long-term immunity and responding to bacterial & viral infections

```r
igg <- ab_data |>
  filter(isotype == "IgG")
```

```r
ggplot(igg) +
  aes(MFI_normalised, antigen) +
  geom_boxplot()
```

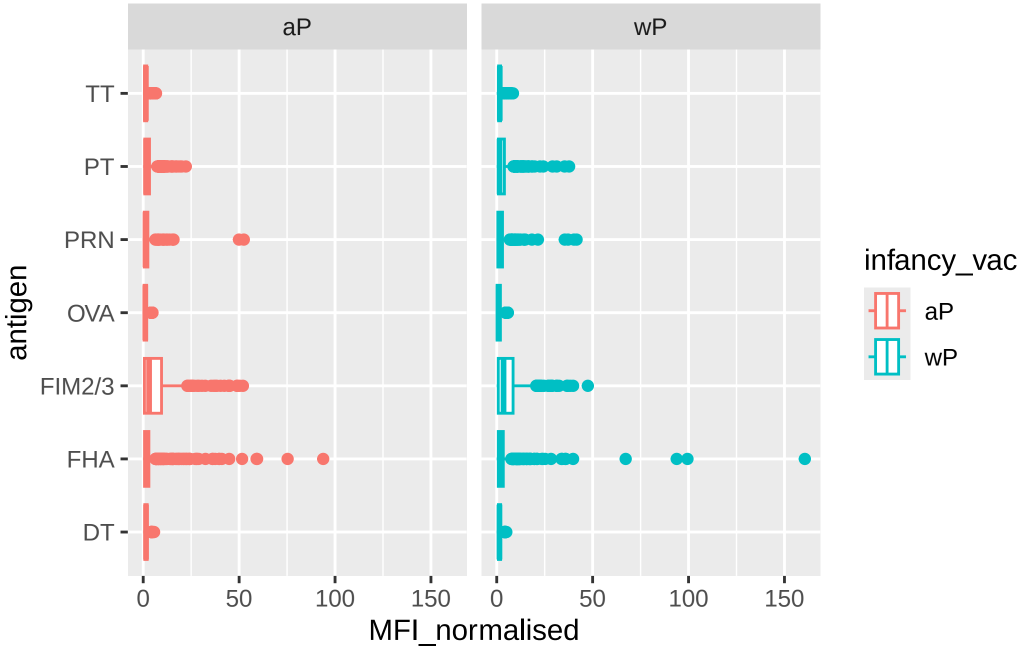


```r
ggplot(igg) +
  aes(MFI_normalised, antigen, col = infancy_vac) +
  geom_boxplot()
```



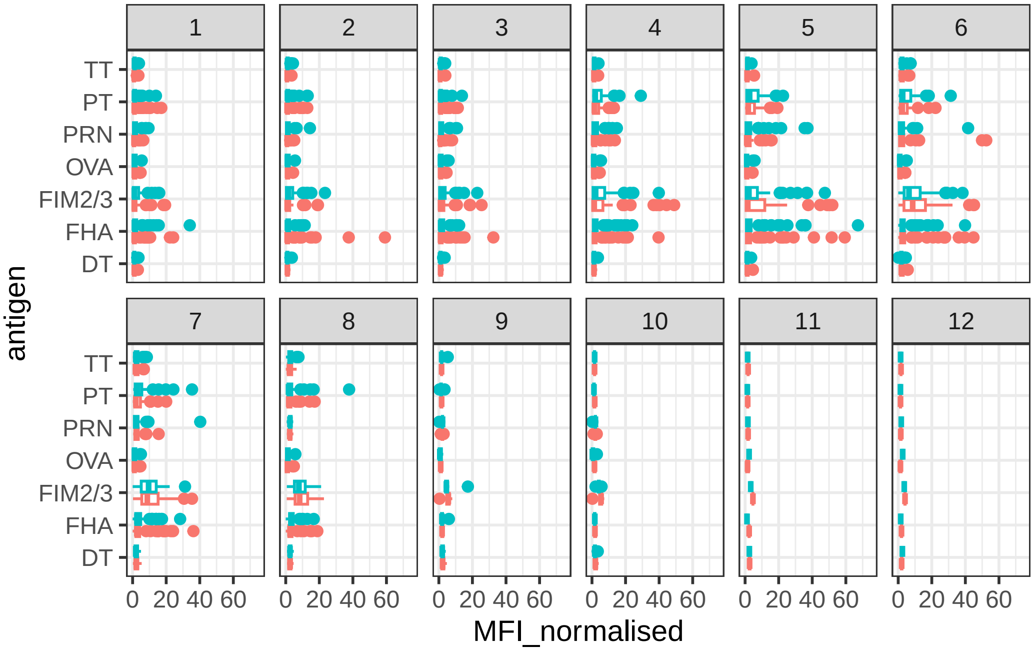
We can “facet” our plot by wP vs aP

```
ggplot(igg) +
  aes(MFI_normalised, antigen, col = infancy_vac) +
  geom_boxplot() +
  facet_wrap(~infancy_vac)
```



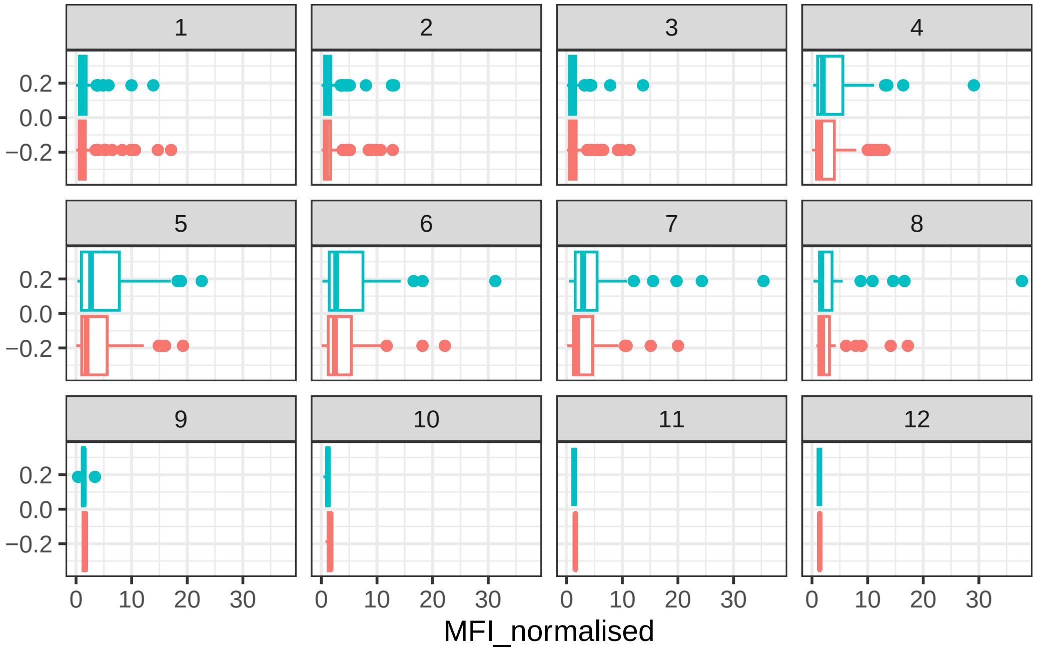
```r
ggplot(igg) +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit), nrow=2) +
  xlim(0,75) +
  theme_bw()
```

```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```



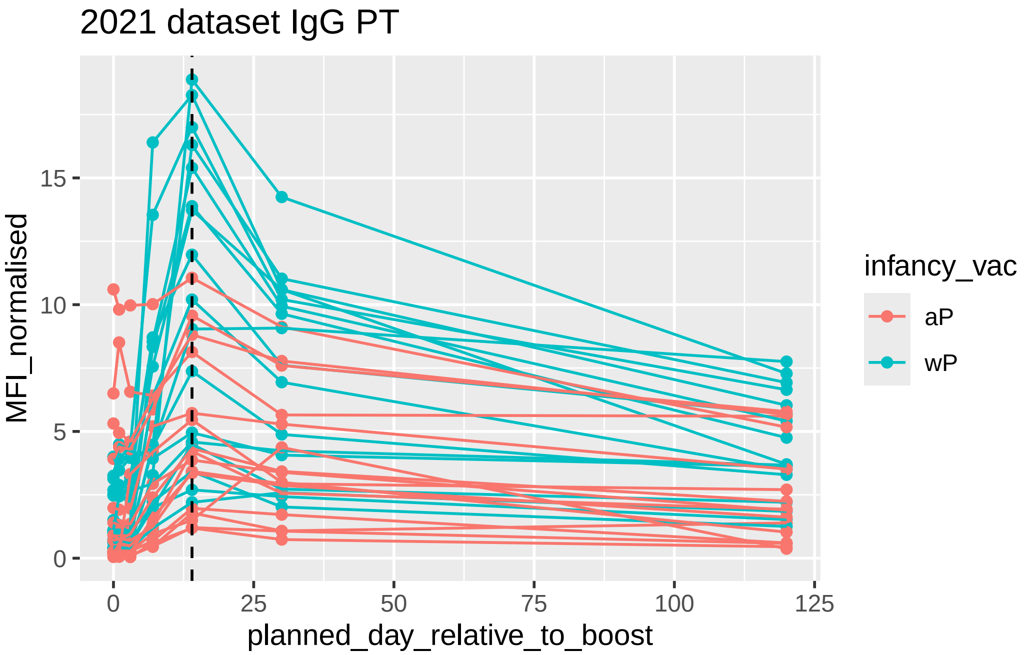
More advanced analysis digging into individual antigen responses over time:

```
filter(igg, antigen=="PT") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



Lets finish this section by looking at the 2021 dataset IgG PT antigen levels time-course:

```
filter(igg,  antigen == "PT", dataset == "2021_dataset") %>%
  ggplot() +
    aes(x=planned_day_relative_to_boost,
        y=MFI_normalised,
        col=infancy_vac,
        group=subject_id) +
    geom_point() +
    geom_line() +
    geom_vline(xintercept=14, linetype="dashed") +
    labs(title="2021 dataset IgG PT")
```



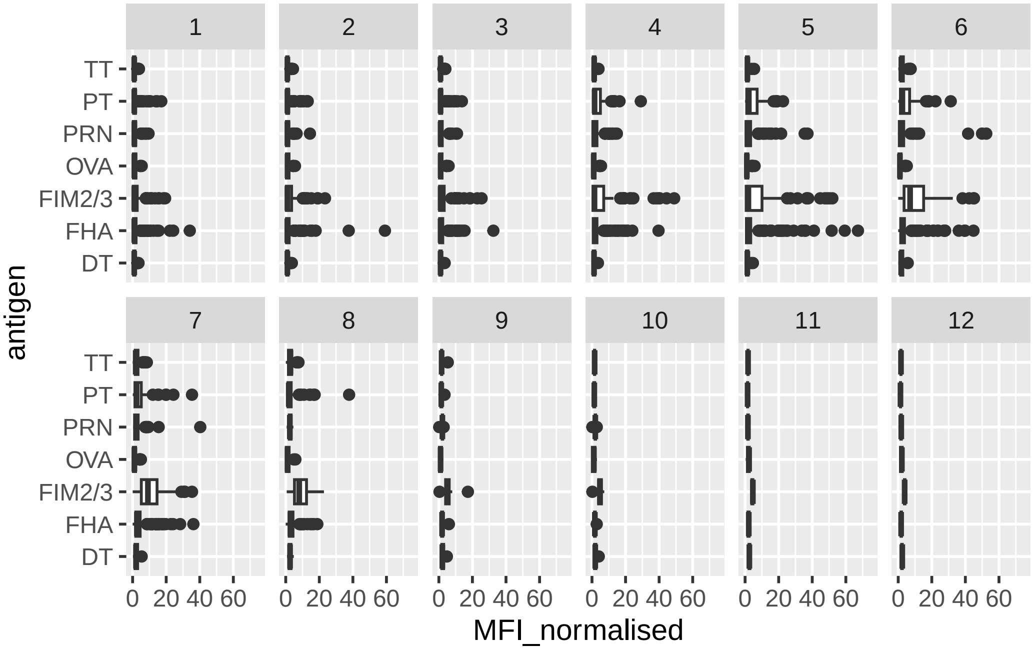
This plot shows the time course of Pertussis toxin (PT) antibody responses for a large set of wP (teal color) and aP (red color) individuals. Levels peak at day 14 and are larger in magnitude for wP than aP individuals.

There are lots of cool things to explore in this dataset and we need coding and biology knowledge to do it effectively - i.e. us!

> Q13. Complete the following code to make a summary boxplot of Ab titer levels (MFI) for all antigens:

```
ggplot(igg) +
  aes(MFI_normalised, antigen) +
  geom_boxplot() +
  xlim(0, 75) +
  facet_wrap(vars(visit), nrow = 2)
```

```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```



Q14. What antigens show differences in the level of IgG antibody titers recognizing them over time? Why these and not others?

Antigens that are part of the pertussis-containing vaccines (e.g. PT, PRN, FHA, FIM2/3, TT, DT) show clear changes in IgG titers over time: low at baseline, a strong increase after the booster, then gradual waning. In contrast, the control antigen OVA (not in the vaccine) stays low and essentially unchanged across visits. The vaccine antigens change because the booster specifically restimulates memory B cells against those proteins, whereas there is no reason for IgG against non-vaccine antigens like OVA to increase.