Class 15: Pertussis mini-project

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Background

Pertussis, a.k.a. whooping cough, is a highly infectious respiratory disease caused by the bacteria *B. Pertussis*.

The CDC tracks pertussis cases numbers per year. Lets have a closer look at this data:

CDC data

We will use the **datapasta** R package to "Scrape" this data into R.

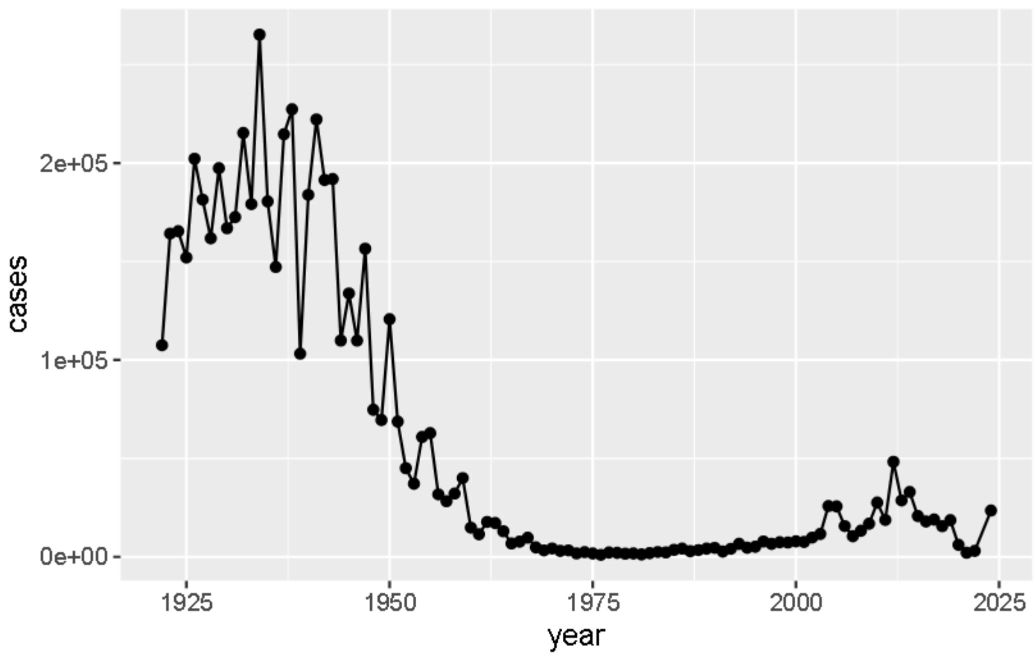
```
cdc <- data.frame(
                                 year = c(1922L,1923L,1924L,1925L,
                                          1926L,1927L,1928L,1929L,1930L,1931L,
                                          1932L,1933L,1934L,1935L,1936L,
                                          1937L,1938L,1939L,1940L,1941L,1942L,
                                          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,202
         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,
                                          20762,17972,18975,15609,18617,6124,2116,3044,23544
       )
```

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)

baseplot <- ggplot(cdc) +
  aes(year, cases) +
  geom_point() +
  geom_line()

baseplot
```

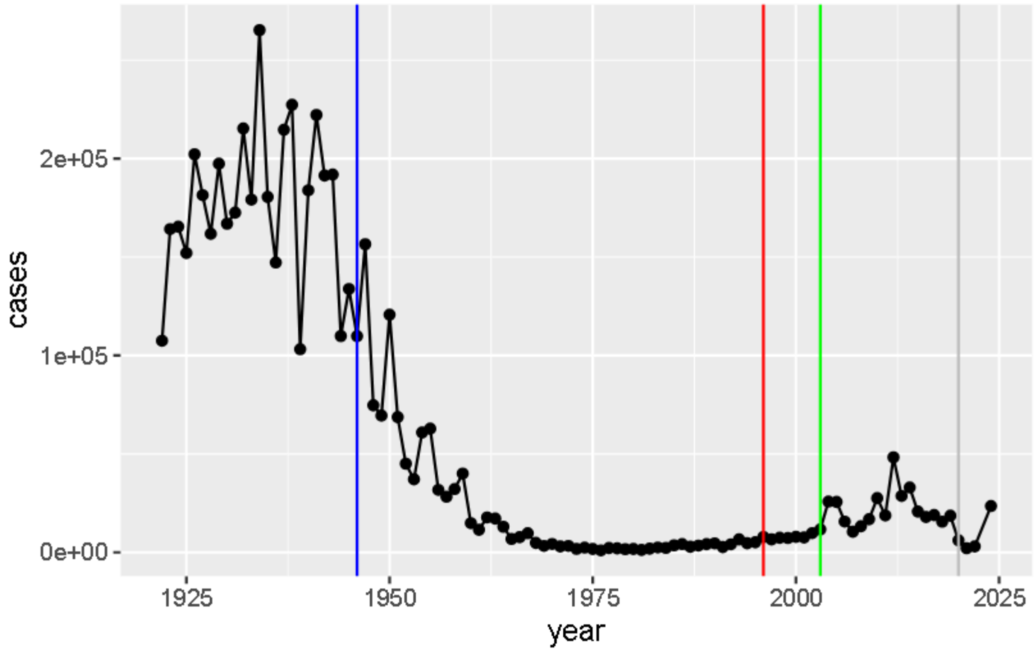


Q2. Using the ggplot geom_vline() function add lines to your previous plot for the 1946 introduction of the wP vaccine and the 1996 switch to aP vaccine (see example in the hint below). What do you notice?

Add some landmark developments as annotation to our plot. We include the first whole-cell (wP) vaccine roll-out in 1946.

Let's add the switch to aceullular vaccine (aP) in 1996.

```
baseplot +
  geom_vline(xintercept = 1946, col = "blue") +
  geom_vline(xintercept = 1996, col = "red") +
  geom_vline(xintercept = 2020, col = "gray") +
  geom_vline(xintercept = 2003, col = "green")
```



In the introduction of the wP vaccine, I notice a pretty dramatic decrease in the number of cases compared to previously.

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

After the introduction of the aP vaccine, pertussis cases are once again rising and increasing. An explanation could include more sensitive PCR-based testing or vaccination hesitancy or even bacterial evollution.

We went from ~200,000 cases pre wP vaccine to ~1,000 cases in 1976. The US switched to the aP vaccine in 1995. We start to see a big increase in 2004 to ~26,000 cases.

There is a ~10 year lg from aP roll out to increasing case numbers. This holds true of other countries like Japan, UK, etc.

Key Question: Why does the aP vaccine induce immunity way faster than that of the wP vaccine?

CMI-PB

The CMI-PB (Computational Models of Immunity Pertussis Boost) makes available lots of data about the immune response to Pertussis booster vaccination.

Critically, it tracks wP and aP individuals over time to see how their immune response changes.

CMI-PB make all their data freely available via JSON format tables from their database. Let's read the first one of these tables:

```r
library(jsonlite)
```

```
Warning: package 'jsonlite' was built under R version 4.4.2
```

```r
subject <- read_json("https://www.cmi-pb.org/api/v5/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
```

> Q4. How many aP and wP infancy vaccinated subjects are in the dataset?

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

```

aP wP 
87 85 
```

> Q. How many subjects/patients are in the dataset?

```
nrow(subject)
```

```
[1] 172
```

> Q5. How many Male and Female subjects/patients are in the dataset?

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

```

Female   Male 
   112     60 
```

> Q6. What is the breakdown of race and biological sex (e.g. number of Asian females, White males etc…)?

```
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
```

> Q Does this do a good job of representing the US populus?

No it is not representative.

> 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
```

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

```
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
```

```
ap <- subject %>% filter(infancy_vac == "aP")

round( summary( time_length( ap$age, "years" ) ) )
```

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
     22      26      27      27      28      34 
```

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

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
     22      32      34      36      39      57 
```

The average age of wP individuals is 27 years old. The average age of aP individuls is also 34 years old. They are significantly different.

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

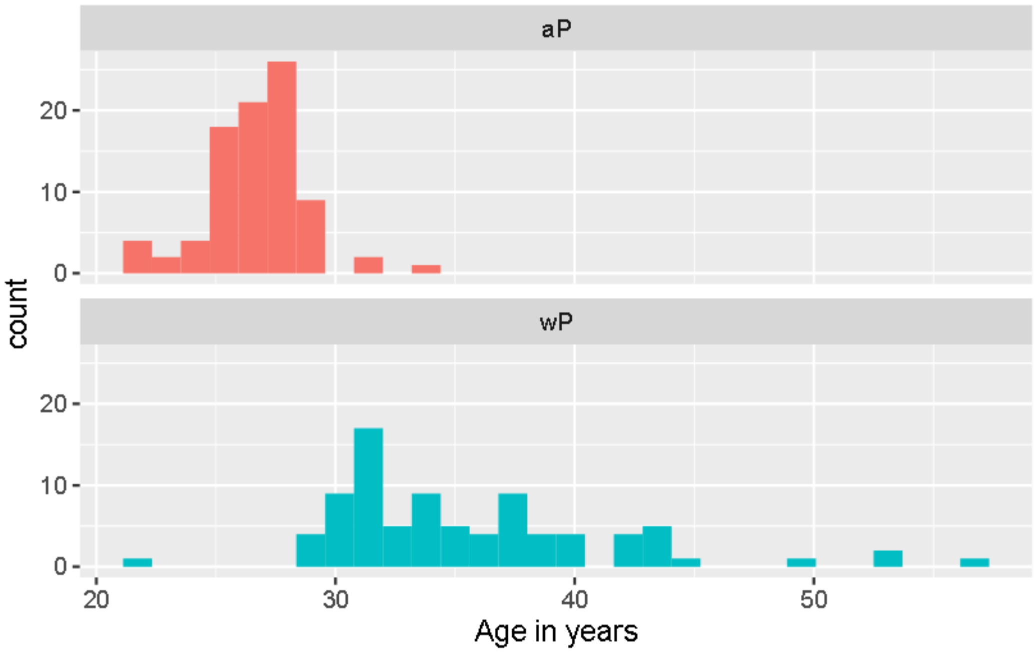
```r
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

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

> Q9. With the help of a faceted boxplot or histogram (see below), do you think these two groups are significantly different?

```r
ggplot(subject) +
  aes(time_length(age, "year"),
      fill=as.factor(infancy_vac)) +
  geom_histogram(show.legend=FALSE) +
  facet_wrap(vars(infancy_vac), nrow=2) +
  xlab("Age in years")
```

```
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```



Let's get more data from CMI-PB, this time about the specimens collected.

```
specimen <- read_json("https://www.cmi-pb.org/api/v5/specimen",
                      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
```

> 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:

Now we can join (merge) these two tables subject and specimen to make one new meta table with the combined data.

```
library(dplyr)

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

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

```
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 14202 days           1
2    1986-01-01    2016-09-12 2020_dataset 14202 days           2
3    1986-01-01    2016-09-12 2020_dataset 14202 days           3
4    1986-01-01    2016-09-12 2020_dataset 14202 days           4
5    1986-01-01    2016-09-12 2020_dataset 14202 days           5
6    1986-01-01    2016-09-12 2020_dataset 14202 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
```

> 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.

Now read an "experiment data" table from CMI-PB

```r
abdata <- read_json("https://www.cmi-pb.org/api/v5/plasma_ab_titer",
                    simplifyVector = TRUE)

head(abdata)
```

```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgE               FALSE   Total 1110.21154       2.493425
2           1     IgE               FALSE   Total 2708.91616       2.493425
3           1     IgG                TRUE      PT   68.56614       3.736992
4           1     IgG                TRUE     PRN  332.12718       2.602350
5           1     IgG                TRUE     FHA 1887.12263      34.050956
6           1     IgE                TRUE     ACT    0.10000       1.000000
   unit lower_limit_of_detection
1 UG/ML                 2.096133
2 IU/ML                29.170000
```

```
3 IU/ML                 0.530000
4 IU/ML                 6.205949
5 IU/ML                 4.679535
6 IU/ML                 2.816431
```

One more join to do of meta and abdata to associate all the metadata about the individual and their race, biological sex, and infincy vaccination status together with Antibody levels.....

```r
ab <- inner_join(abdata, meta)
```

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

```r
head(ab)
```

```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgE               FALSE   Total 1110.21154       2.493425
2           1     IgE               FALSE   Total 2708.91616       2.493425
3           1     IgG                TRUE      PT   68.56614       3.736992
4           1     IgG                TRUE     PRN  332.12718       2.602350
5           1     IgG                TRUE     FHA 1887.12263      34.050956
6           1     IgE                TRUE     ACT    0.10000       1.000000
   unit lower_limit_of_detection subject_id infancy_vac biological_sex
1 UG/ML                 2.096133          1          wP         Female
2 IU/ML                29.170000          1          wP         Female
3 IU/ML                 0.530000          1          wP         Female
4 IU/ML                 6.205949          1          wP         Female
5 IU/ML                 4.679535          1          wP         Female
6 IU/ML                 2.816431          1          wP         Female
               ethnicity  race year_of_birth date_of_boost      dataset
1 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
2 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
3 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
4 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
5 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
6 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
         age actual_day_relative_to_boost planned_day_relative_to_boost
1 14202 days                           -3                             0
2 14202 days                           -3                             0
3 14202 days                           -3                             0
4 14202 days                           -3                             0
5 14202 days                           -3                             0
```

```
6 14202 days                              -3                              0
  specimen_type visit
1         Blood     1
2         Blood     1
3         Blood     1
4         Blood     1
5         Blood     1
6         Blood     1
```

> Q How many Ab measurements do we have?

```
nrow(ab)
```

```
[1] 52576
```

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

How many isotypes?

```
table(ab$isotype)
```

```

  IgE    IgG   IgG1   IgG2   IgG3   IgG4 
 6698   5389  10117  10124  10124  10124 
```

How many antigens?

```
table(ab$antigen)
```

```

    ACT   BETV1      DT   FELD1     FHA  FIM2/3   LOLP1     LOS Measles     OVA 
   1970    1970    4978    1970    5372    4978    1970    1970    1970    4978 
    PD1     PRN      PT     PTM   Total      TT 
   1970    5372    5372    1970     788    4978 
```

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

```
table(ab$dataset)
```

```
2020_dataset 2021_dataset 2022_dataset 2023_dataset 
       31520         8085         7301         5670 
```

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

Let's focus in on IgG- one of the main antibody types responsive to bacteria or viral infections.

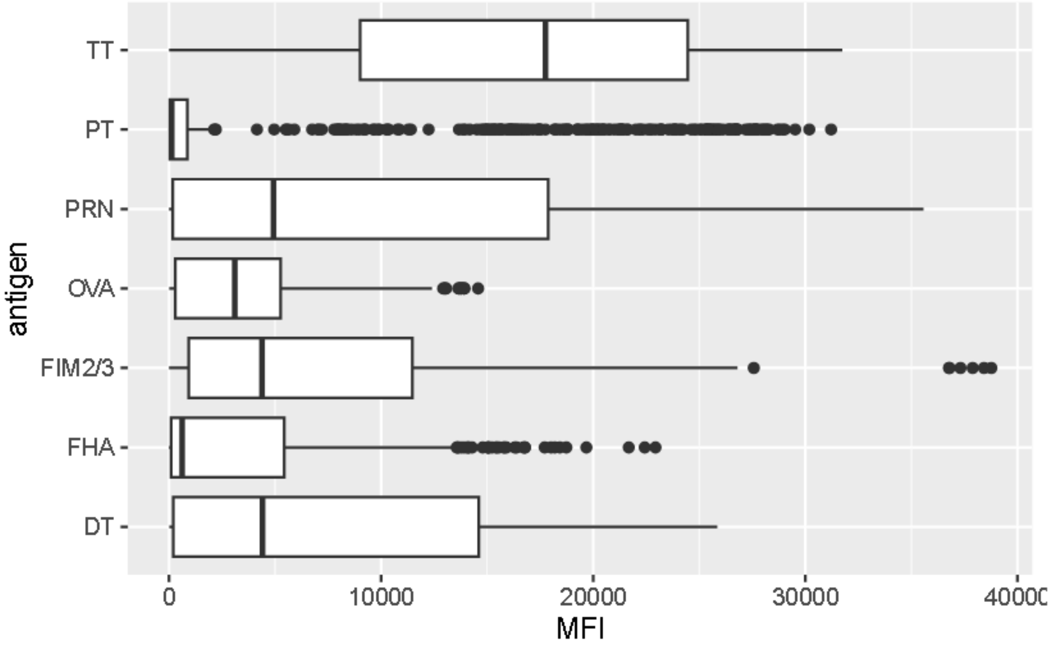
```r
igg <- filter(ab, isotype=="IgG")
head(igg)
```

```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgG                TRUE      PT   68.56614       3.736992
2           1     IgG                TRUE     PRN  332.12718       2.602350
3           1     IgG                TRUE     FHA 1887.12263      34.050956
4          19     IgG                TRUE      PT   20.11607       1.096366
5          19     IgG                TRUE     PRN  976.67419       7.652635
6          19     IgG                TRUE     FHA   60.76626       1.096457
   unit lower_limit_of_detection subject_id infancy_vac biological_sex
1 IU/ML                 0.530000          1          wP         Female
2 IU/ML                 6.205949          1          wP         Female
3 IU/ML                 4.679535          1          wP         Female
4 IU/ML                 0.530000          3          wP         Female
5 IU/ML                 6.205949          3          wP         Female
6 IU/ML                 4.679535          3          wP         Female
               ethnicity  race year_of_birth date_of_boost      dataset
1 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
2 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
3 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
4                Unknown White    1983-01-01    2016-10-10 2020_dataset
5                Unknown White    1983-01-01    2016-10-10 2020_dataset
6                Unknown White    1983-01-01    2016-10-10 2020_dataset
         age actual_day_relative_to_boost planned_day_relative_to_boost
1 14202 days                           -3                             0
2 14202 days                           -3                             0
3 14202 days                           -3                             0
4 15298 days                           -3                             0
5 15298 days                           -3                             0
6 15298 days                           -3                             0
  specimen_type visit
1         Blood     1
```

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

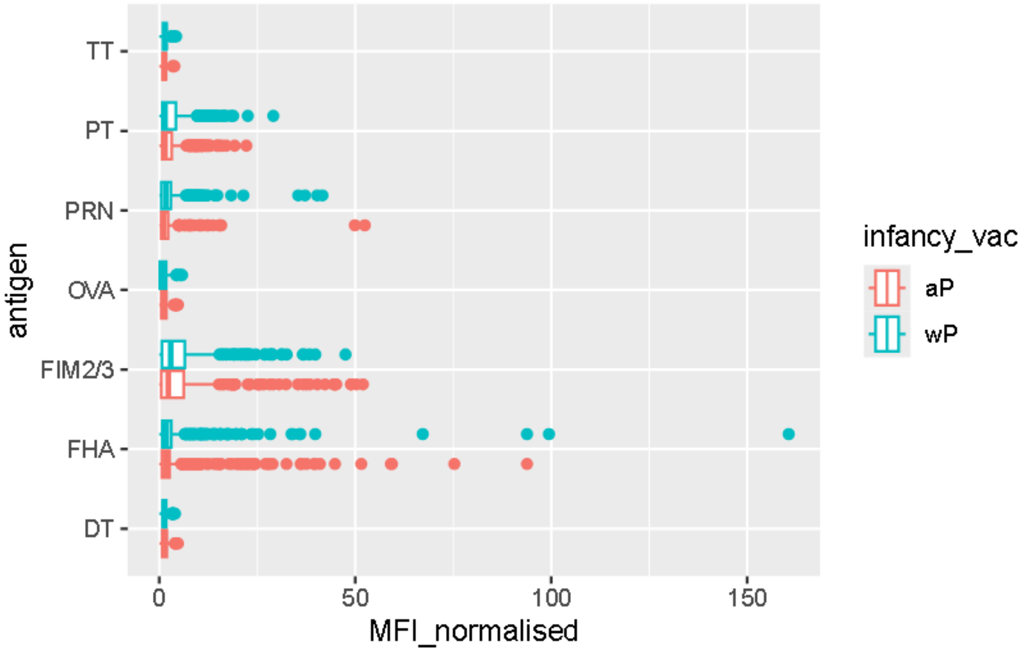
Make a first plot of MFI (Mean Flurosence Intensity- a measure of how much is detected) for each antigen.

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

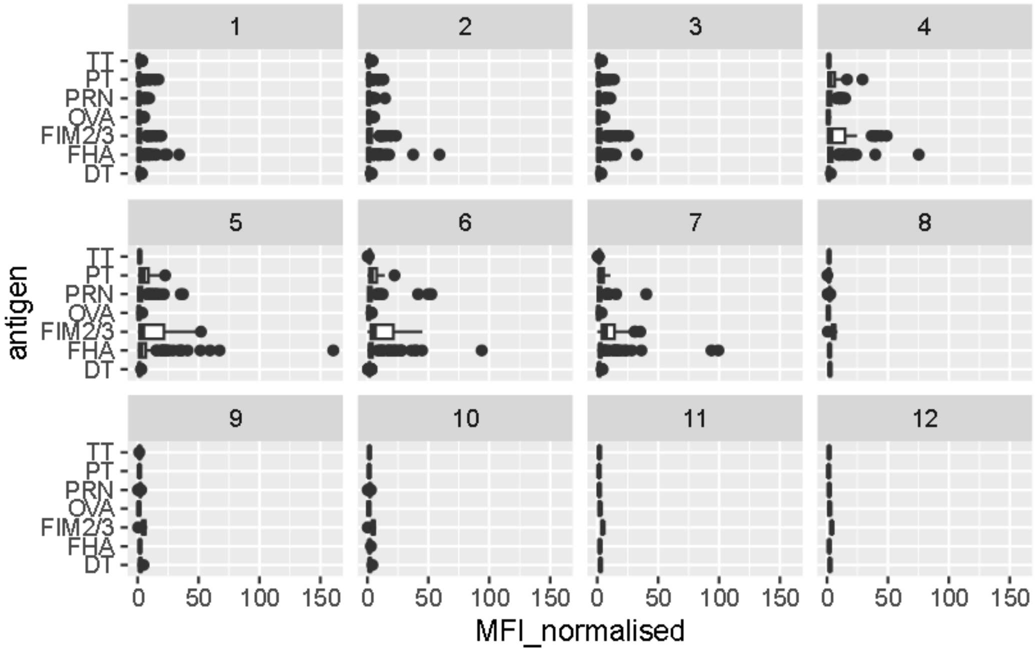


Let's color by aP/wP infancy_vac

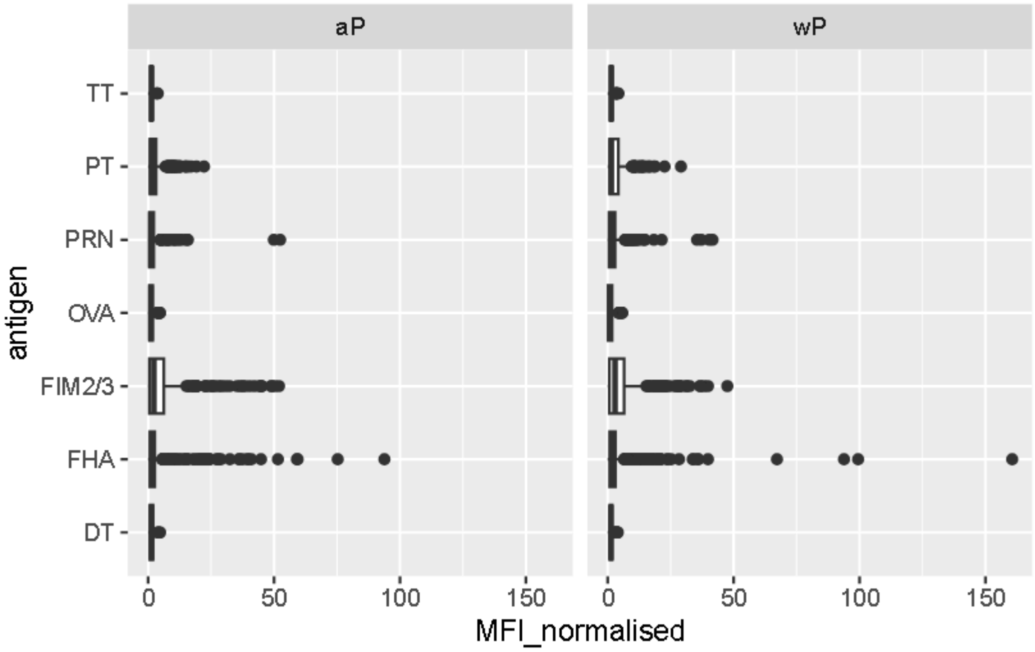
```
ggplot(igg) +
  aes(MFI_normalised, antigen, col=infancy_vac) +
  geom_boxplot()
```



```
ggplot(igg) +
  aes(MFI_normalised, antigen) +
  geom_boxplot() +
  facet_wrap(~visit)
```

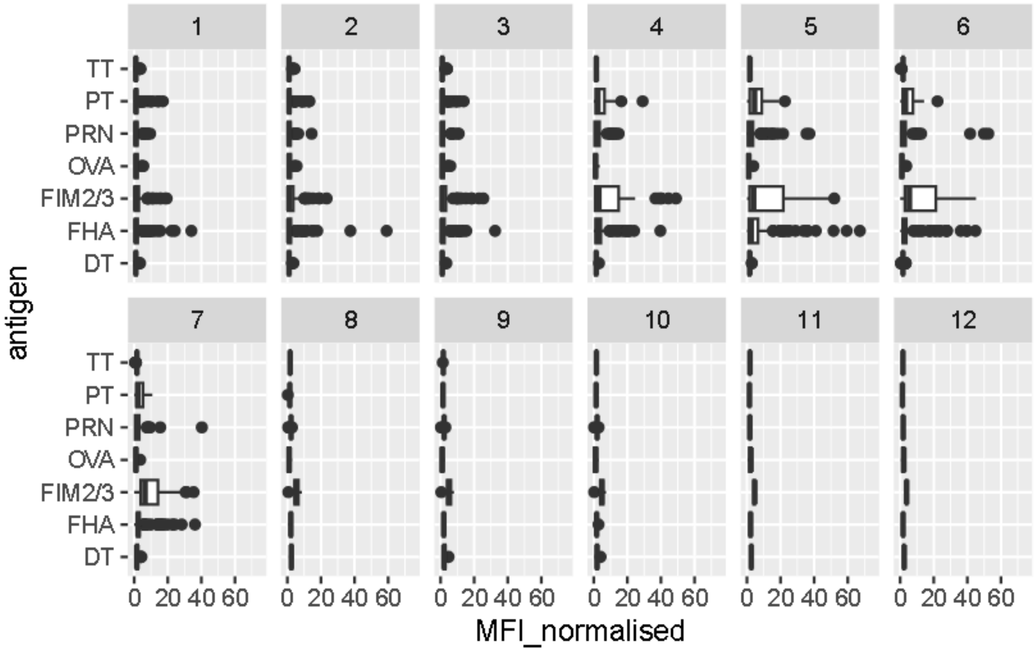


```
ggplot(igg) +
  aes(MFI_normalised, antigen) +
  geom_boxplot() +
  facet_wrap(~infancy_vac)
```



```
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()`).
```



```
table(igg$visit)
```

```
   1    2    3    4    5    6    7    8    9   10   11   12
 902  902  930  559  559  540  525  150  147  133   21   21
```

Looks like we don't have data yet for all subjects in terms of visits 8 onwards. It is happening currently and we do not have all the data for all the patients yet. So lets exclude these.

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

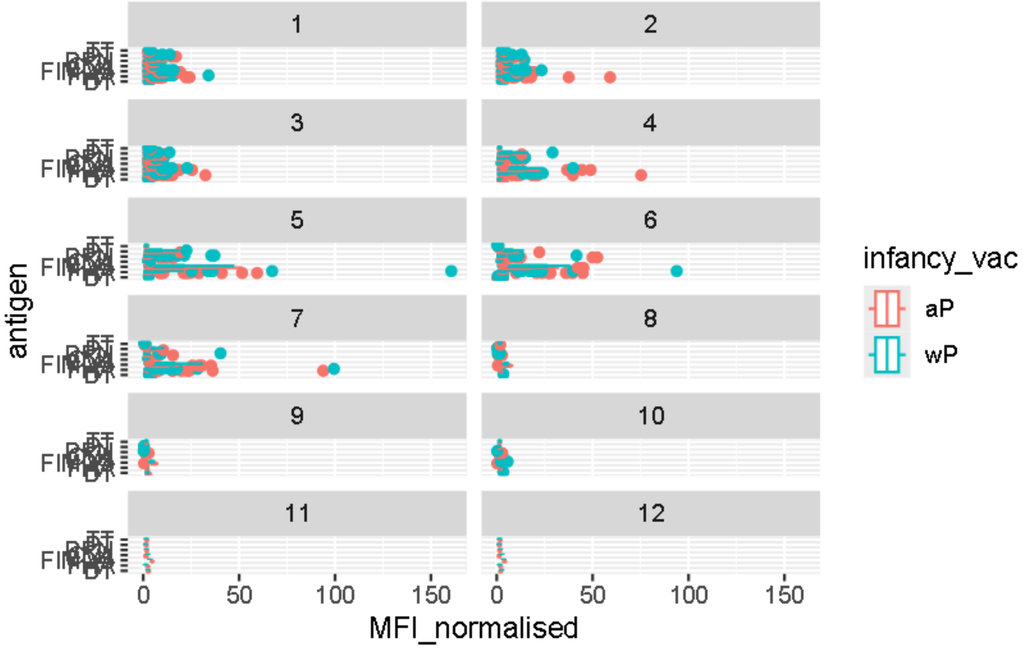
As seen in the last plot, antigen PT shows differences in the level of IgG antibody titers recognizing them over time. Moreover, FIM 2/3 peaks around 5. This is due to factors such the nature of the antigen and dynamics of immune memory.

```
igg_7 <- filter(igg, visit %in% 1:7)

table(igg_7$visit)
```

```
   1    2    3    4    5    6    7
 902  902  930  559  559  540  525
```

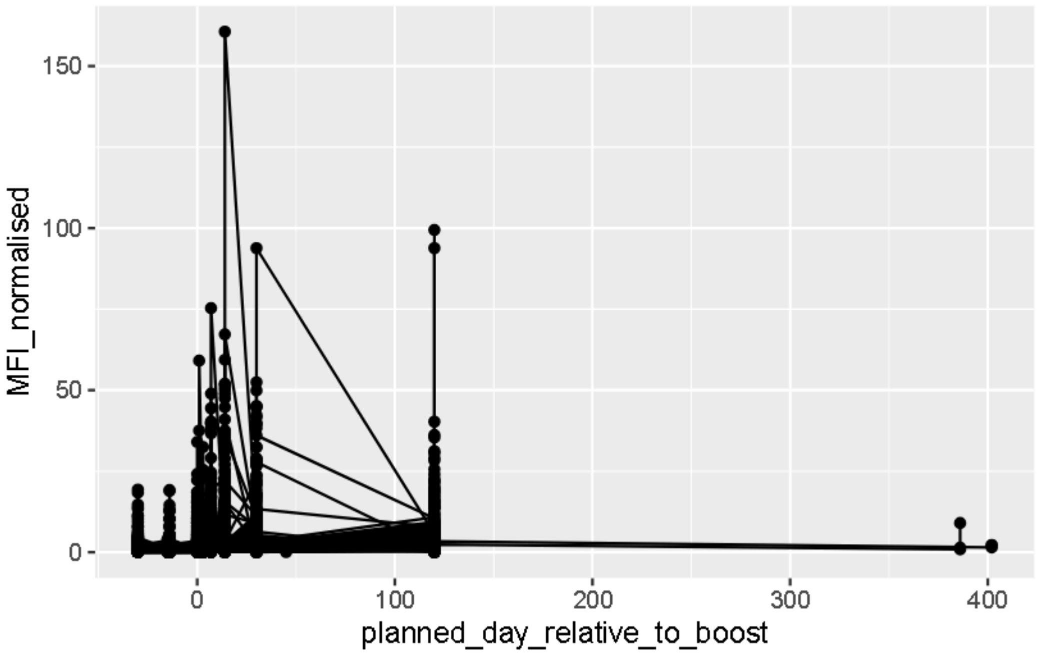
```
ggplot(igg) +
  aes(MFI_normalised, antigen, col=infancy_vac) +
  geom_boxplot() +
  facet_wrap(~visit, ncol=2)
```



Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("OVA", that is not in our vaccines) and a clear antigen of interest ("PT", Pertussis Toxin, one of the key virulence factors produced by the bacterium B. pertussis).

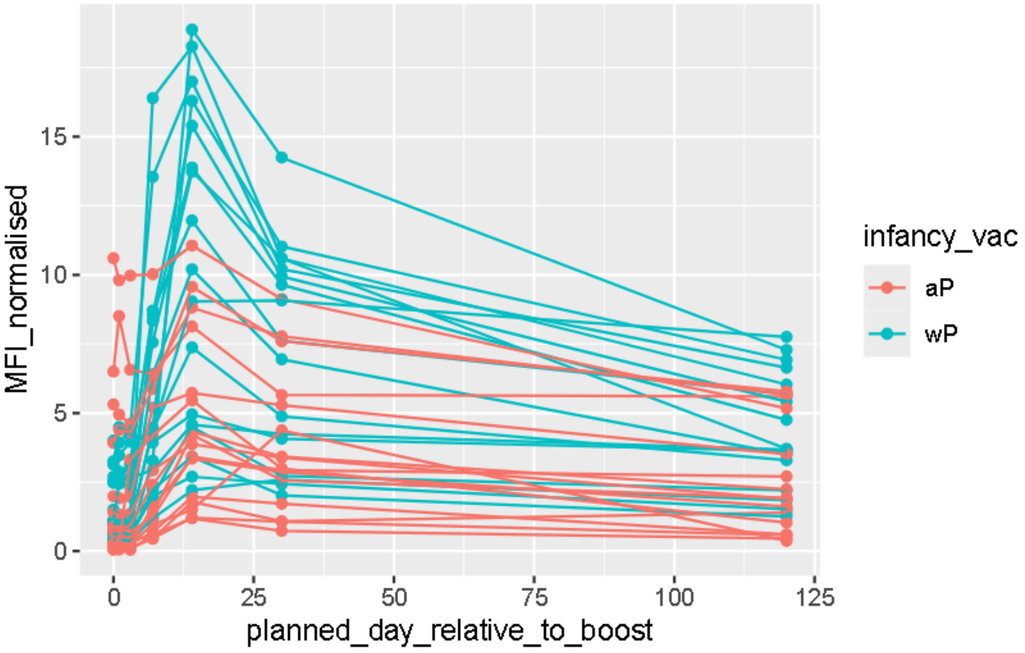
Let's try a different plot. First focus on one antigen, start with PT (Pertussis Toxin) and plot visit or time on the x-axis and MFI_normalized on the y-axis.

```
ggplot(igg_7) +
  aes(planned_day_relative_to_boost, MFI_normalised, group=subject_id) +
  geom_point() +
  geom_line()
```



```
abdata.21 <- ab %>% filter(dataset == "2021_dataset")

abdata.21 %>%
  filter(isotype == "IgG",  antigen == "PT") %>%
  ggplot() +
    aes(x=planned_day_relative_to_boost,
        y=MFI_normalised,
        col=infancy_vac,
        group=subject_id) +
    geom_point() +
    geom_line()
```



> Q16. What do you notice about these two antigens time courses and the PT data in particular?

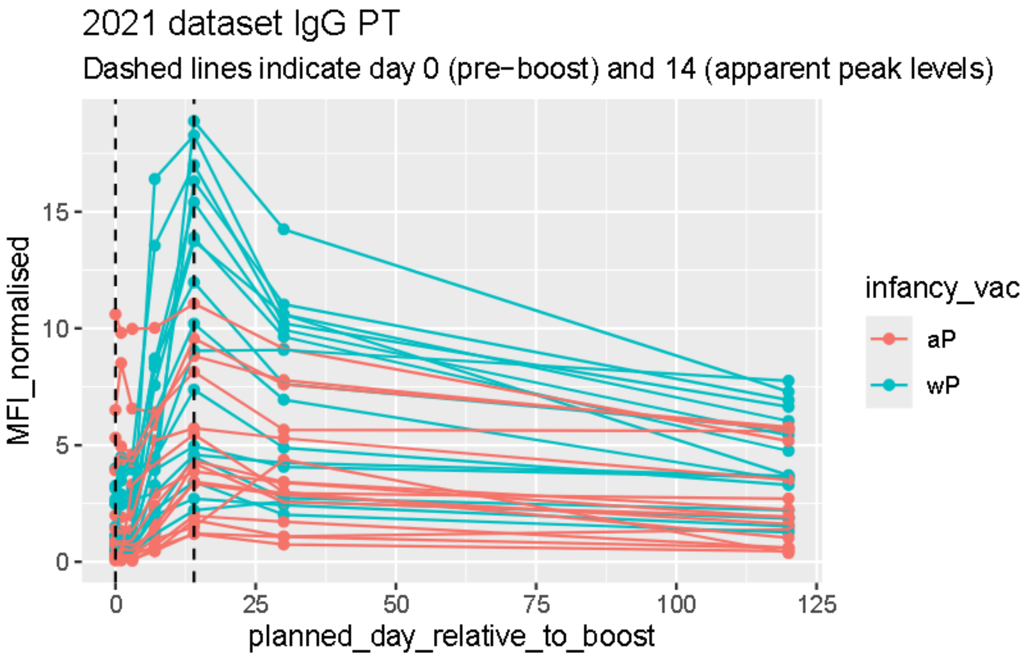
PT levels clearly rise over time and far exceed those of OVA. They also appear to peak at visit 5 and then decline. This trend appears similar for wP and aP subjects.

> Q17. Do you see any clear difference in aP vs. wP responses?

There is not a clear difference in aP vs. wP responses.

```r
abdata.21 <- ab %>% filter(dataset == "2021_dataset")

abdata.21 %>%
  filter(isotype == "IgG",  antigen == "PT") %>%
  ggplot() +
    aes(x=planned_day_relative_to_boost,
        y=MFI_normalised,
        col=infancy_vac,
        group=subject_id) +
    geom_point() +
    geom_line() +
    geom_vline(xintercept=0, linetype="dashed") +
    geom_vline(xintercept=14, linetype="dashed") +
  labs(title="2021 dataset IgG PT",
      subtitle = "Dashed lines indicate day 0 (pre-boost) and 14 (apparent peak levels)")
```



Let's finish here for today. We are beginning to see some interesting differences between aP and wP individuals. There is likely lots of other interesting things to find in this dataset.