PertussisProj

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```
library(datapasta)
```

```
Warning: package 'datapasta' was built under R version 4.1.3
```

```
cdc <- data.table::data.table(
                                  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),
          No..Reported.Pertussis.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,
```

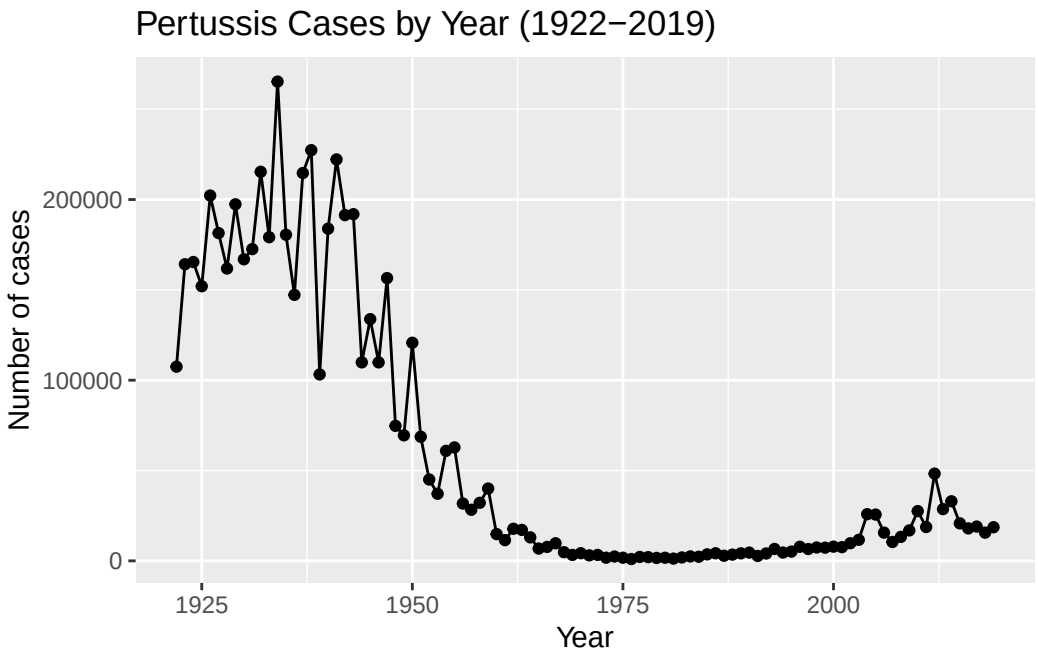
```
                                                120718,68687,45030,37129,60886,
                                                62786,31732,28295,32148,40005,
                                                14809,11468,17749,17135,13005,6799,
                                                7717,9718,4810,3285,4249,3036,
                                                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)
        )
View(cdc)
```

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)
```

```
Warning: package 'ggplot2' was built under R version 4.1.3
```

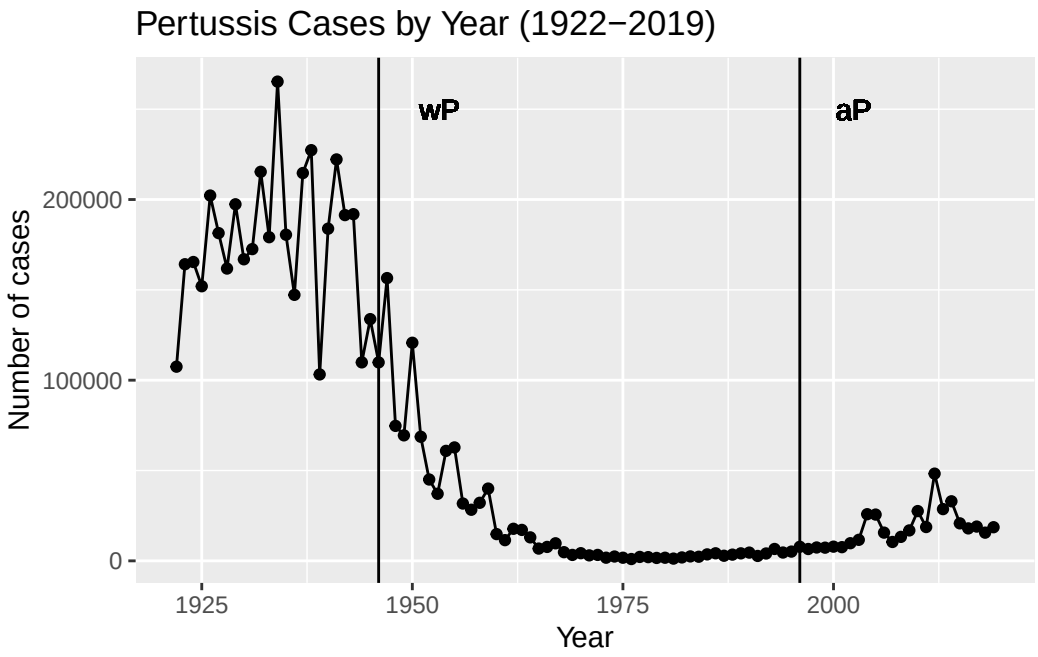
```
p <- ggplot(cdc) +
  aes(Year, No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  scale_y_continuous(labels = function(x) format(x, scientific = FALSE)) +
  labs(title = "Pertussis Cases by Year (1922-2019)", x="Year", y="Number of cases")
p
```



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?

Pertussis cases dramatically fall after the introduction of the wP vaccine in 1946. The aP vaccine maintained the low case rate until after 2000, where they have been slowly rising.

```
p + geom_vline(xintercept = 1946) +
  geom_vline(xintercept = 1996) +
  geom_text(aes(x=1946, label="wP", y=250000, hjust = -1)) +
  geom_text(aes(x=1996, label="aP", y=250000, hjust = -1))
```



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

Case rates have risen after the introduction of the aP vaccine and can be due in part to reduced vaccination rates as a result of "evidence" vaccines contribute to autism (these results were fabricated), and immunity from aP vaccination may not be as long lasting as wP vaccination given the differences in immune memory we may expect based on formulation (live/attenuated generally confer longer immunity as vaccine most closley mimics natural infection and provokes strong immune response).

```
library(jsonlite)

subject <- read_json("https://www.cmi-pb.org/api/subject", simplifyVector = TRUE)
head(subject, 3)
```

```
  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
  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
```

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

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

```
aP wP
47 49
```

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

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

```
Female   Male
    66     30
```

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

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

```
        American Indian/Alaska Native Asian Black or African American
  Female                             0    18                        2
  Male                               1     9                        0

        More Than One Race Native Hawaiian or Other Pacific Islander
  Female                  8                                        1
  Male                    2                                        1

        Unknown or Not Reported White
  Female                      10    27
  Male                         4    13
```

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?

The average age of wP individuals is 35 while the average age of aP individuals is 24. There is roughly a ten year difference between these 2 groups which is consistent with the roll out of the aP vaccine in the late 90s.

```
library(lubridate)
```

```
Attaching package: 'lubridate'

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

    date, intersect, setdiff, union
```

```
library(dplyr)
```

```
Warning: package 'dplyr' was built under R version 4.1.3
```

```
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
```

```
subject$age <- today() - ymd(subject$year_of_birth)

wp <- subject %>% filter(infancy_vac == "wP")
```

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

```
 Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
   28      32      35      36      40      55 
```

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

```
 Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
   23      25      26      25      26      27 
```

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

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

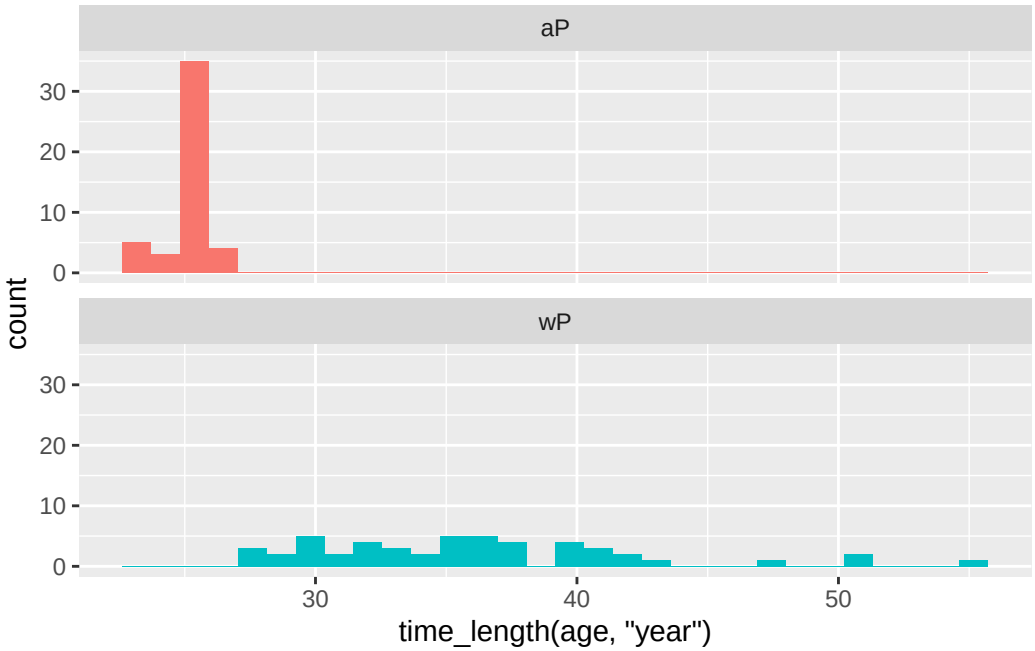
```
[1] 30.69678 51.07461 33.77413 28.65982 25.65914 28.77481
```

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

Yes, the difference in age between the aP and wP group is significantly different.

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

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



Combining multiple tables

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

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:

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

```
Joining, by = "subject_id"
```

```
dim(meta)
```

```
[1] 729  14
```

```
head(meta)
```

```
  specimen_id subject_id actual_day_relative_to_boost
1           1          1                           -3
2           2          1                          736
3           3          1                            1
4           4          1                            3
5           5          1                            7
6           6          1                           11
  planned_day_relative_to_boost specimen_type visit infancy_vac biological_sex
1                             0         Blood     1          wP         Female
2                           736         Blood    10          wP         Female
3                             1         Blood     2          wP         Female
4                             3         Blood     3          wP         Female
5                             7         Blood     4          wP         Female
6                            14         Blood     5          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
1 13481 days
2 13481 days
3 13481 days
4 13481 days
5 13481 days
6 13481 days
```

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(titer, meta)
```

```
Joining, by = "specimen_id"
```

```
dim(abdata)
```

```
[1] 32675    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 1413 6141 6141 6141 6141
```

Q12. What do you notice about the number of visit 8 specimens compared to other visits?

There are significantly fewer specimens at visit 8 relative to the 7 previous visits.

```
table(abdata$visit)
```

```
   1    2    3    4    5    6    7    8
5795 4640 4640 4640 4640 4320 3920   80
```

IgG1 Ab titers

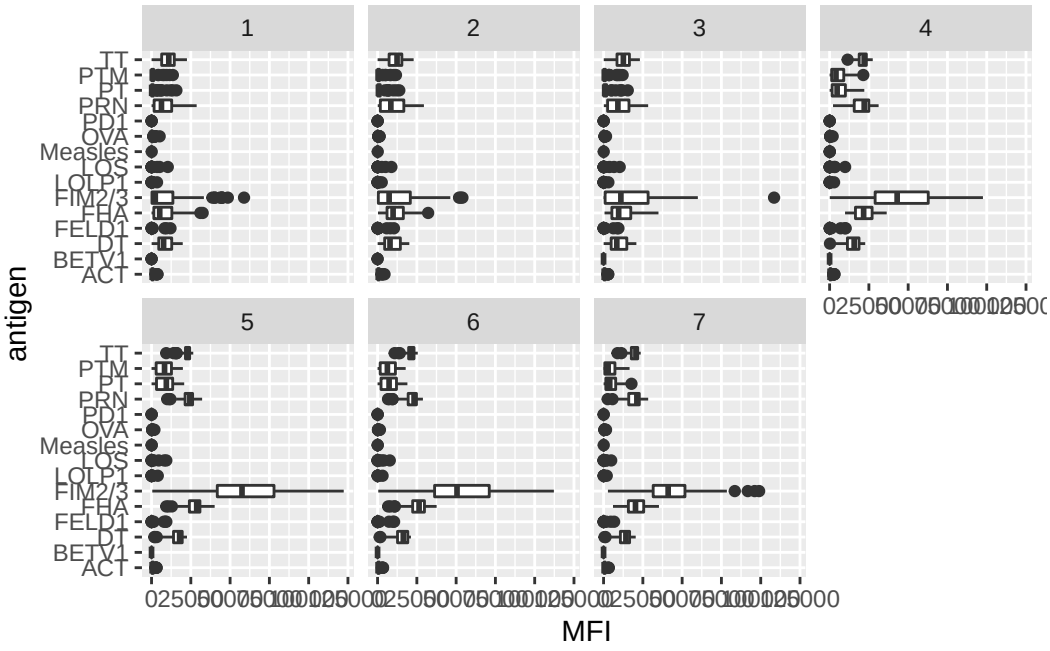
```
ig1 <- abdata %>% filter(isotype == "IgG1", visit!=8)
head(ig1)
```

```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1    IgG1                TRUE     ACT 274.355068      0.6928058
2           1    IgG1                TRUE     LOS  10.974026      2.1645083
3           1    IgG1                TRUE   FELD1   1.448796      0.8080941
4           1    IgG1                TRUE   BETV1   0.100000      1.0000000
5           1    IgG1                TRUE   LOLP1   0.100000      1.0000000
6           1    IgG1                TRUE Measles  36.277417      1.6638332
   unit lower_limit_of_detection subject_id actual_day_relative_to_boost
1 IU/ML                3.848750          1                           -3
2 IU/ML                4.357917          1                           -3
3 IU/ML                2.699944          1                           -3
4 IU/ML                1.734784          1                           -3
5 IU/ML                2.550606          1                           -3
```

```
6 IU/ML                  4.438966          1                          -3
  planned_day_relative_to_boost specimen_type visit infancy_vac biological_sex
1                             0         Blood     1          wP         Female
2                             0         Blood     1          wP         Female
3                             0         Blood     1          wP         Female
4                             0         Blood     1          wP         Female
5                             0         Blood     1          wP         Female
6                             0         Blood     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
1 13481 days
2 13481 days
3 13481 days
4 13481 days
5 13481 days
6 13481 days
```

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

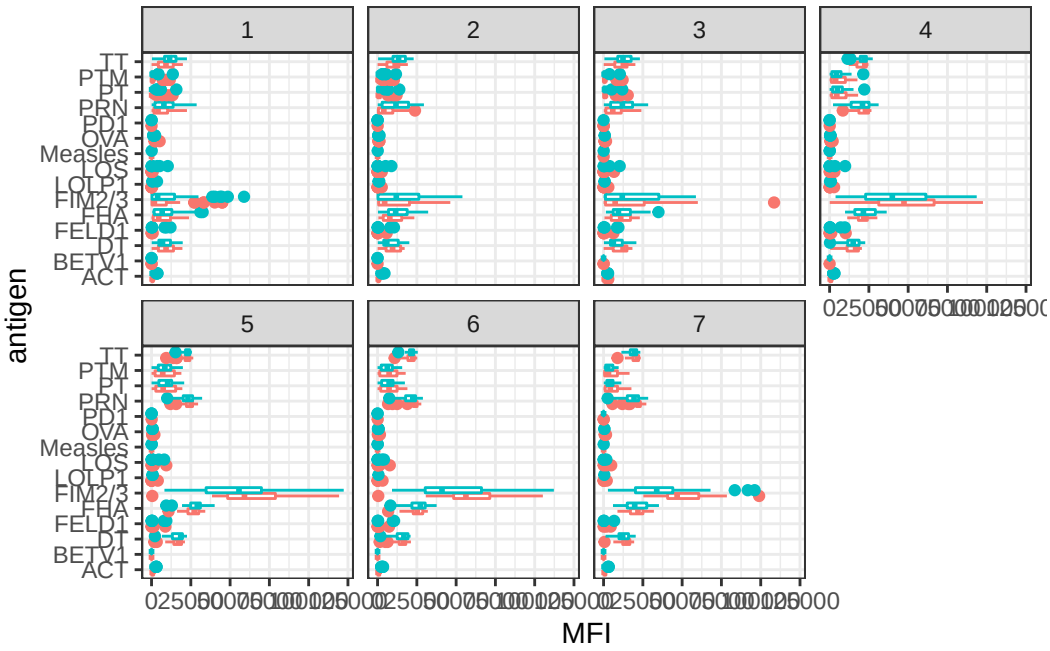
```
ggplot(ig1) +
  aes(MFI, antigen) +
  geom_boxplot() +
  facet_wrap(vars(visit), nrow=2)
```



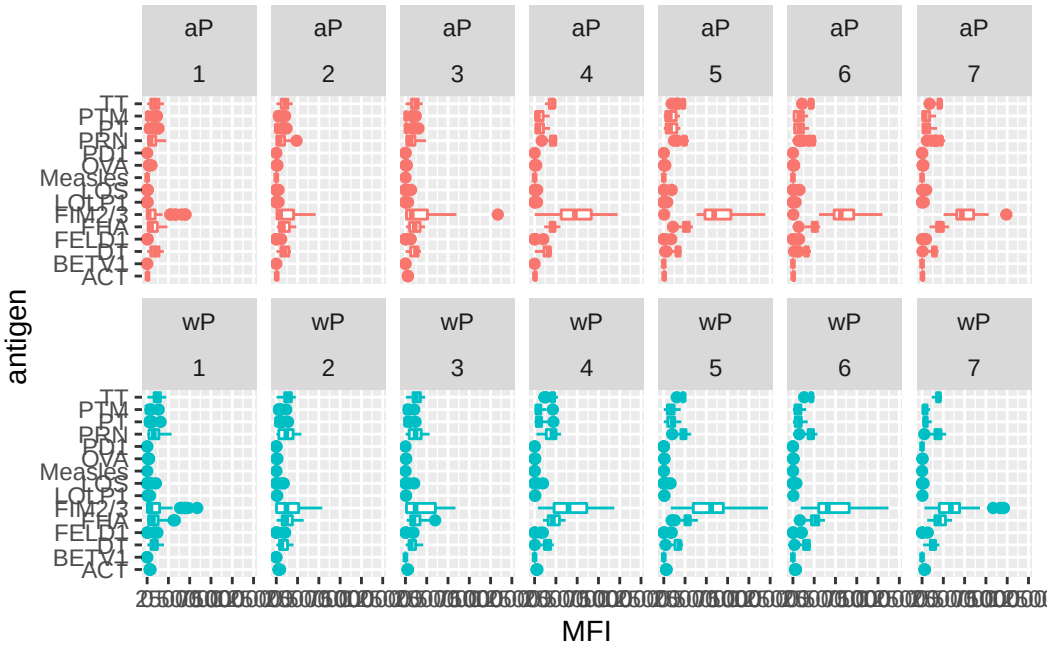
Q14. What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others?

FIM2/3 antigen shows differences in the level of IgG1 antibody targeting. This antigen is a pertussis protein and vaccination with pertussis produces antibodies to pertussis.

```
ggplot(ig1) +
  aes(MFI, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit), nrow=2) +
  theme_bw()
```

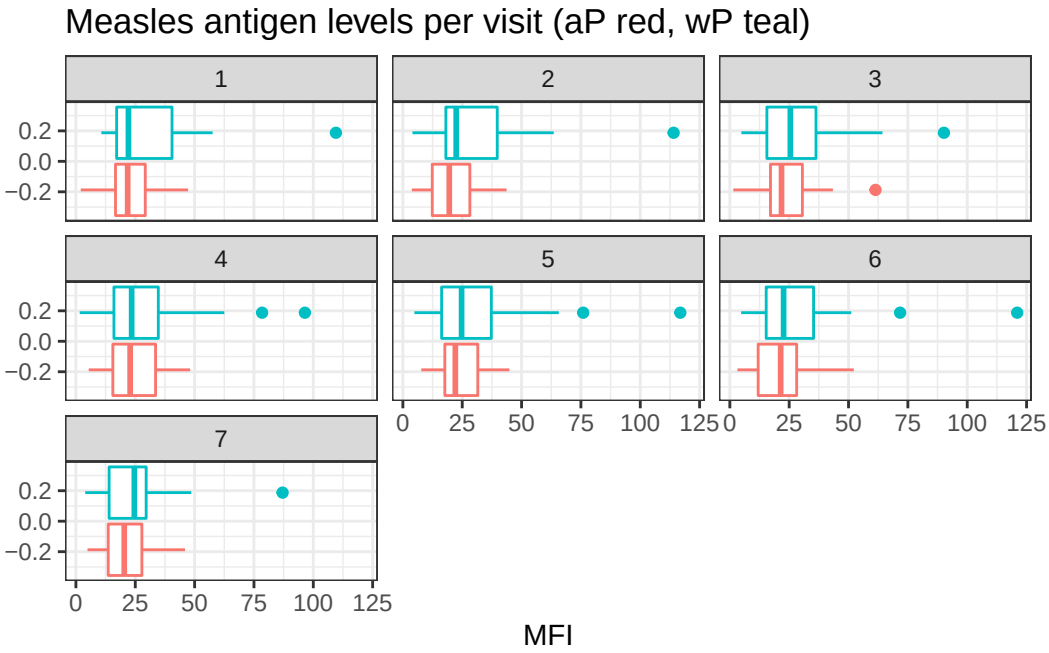



```
ggplot(ig1) +
  aes(MFI, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

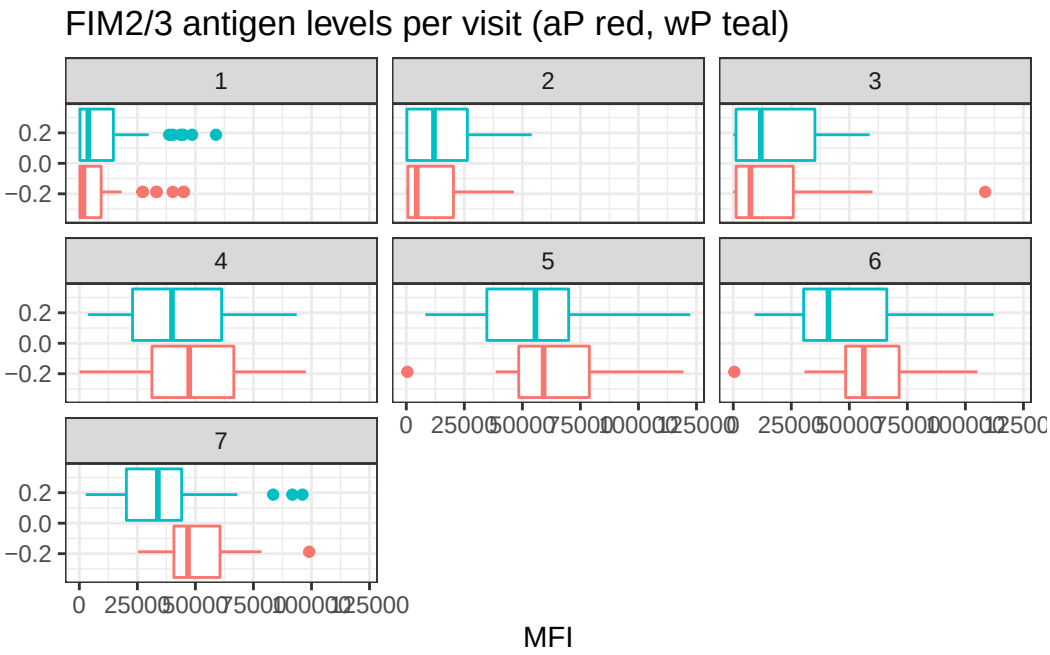


Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("Measles", that is not in our vaccines) and a clear antigen of interest ("FIM2/3", extra-cellular fimbriae proteins from B. pertussis that participate in substrate attachment).

```
filter(ig1, antigen=="Measles") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  labs(title = "Measles antigen levels per visit (aP red, wP teal)") +
  theme_bw()
```




```
filter(ig1, antigen=="FIM2/3") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  labs(title = "FIM2/3 antigen levels per visit (aP red, wP teal)") +
  theme_bw()
```



Q16. What do you notice about these two antigens time course and the FIM2/3 data in particular?

Measles antigen remains low over time while FIM2/3 antigen levels rise consistently till visit 5 and slightly decline on visits 6 and 7.

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

Antigen levels are similar between the aP and wP vaccine recipients.

Obtaining CMI-PB RNASeq Data

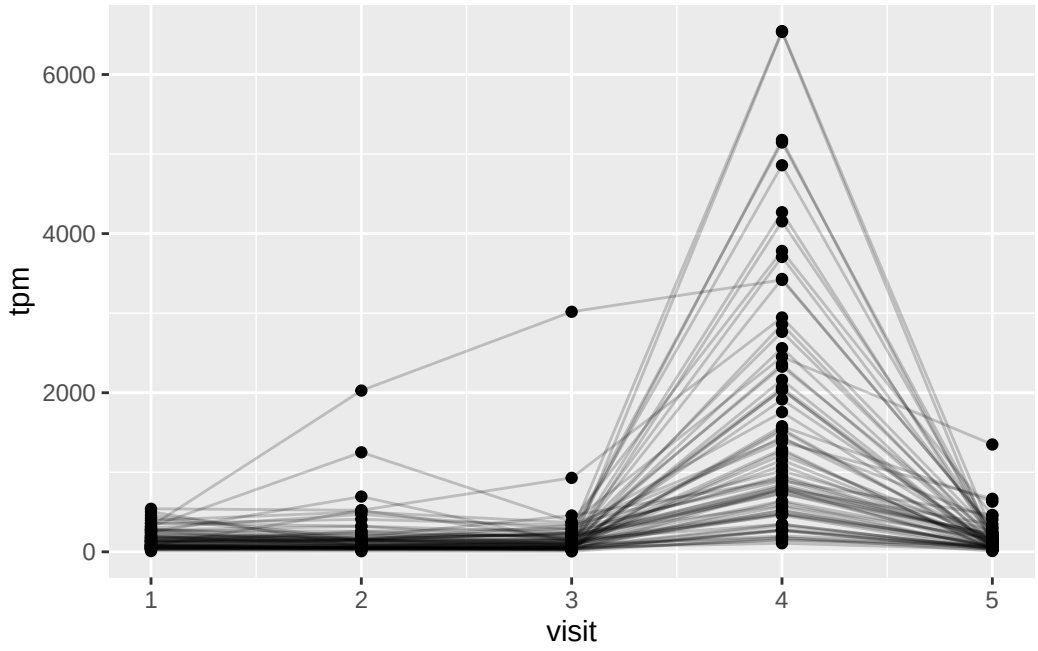
```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.
rna <- read_json(url, simplifyVector = TRUE)

ssrna <- inner_join(rna, meta)
```

```
Joining, by = "specimen_id"
```

Q18. Make a plot of the time course of gene expression for IGHG1 gene (i.e. a plot of visit vs. tpm).

```
ggplot(ssrna) +
  aes(visit, tpm, group=subject_id) +
  geom_point() +
  geom_line(alpha=0.2)
```



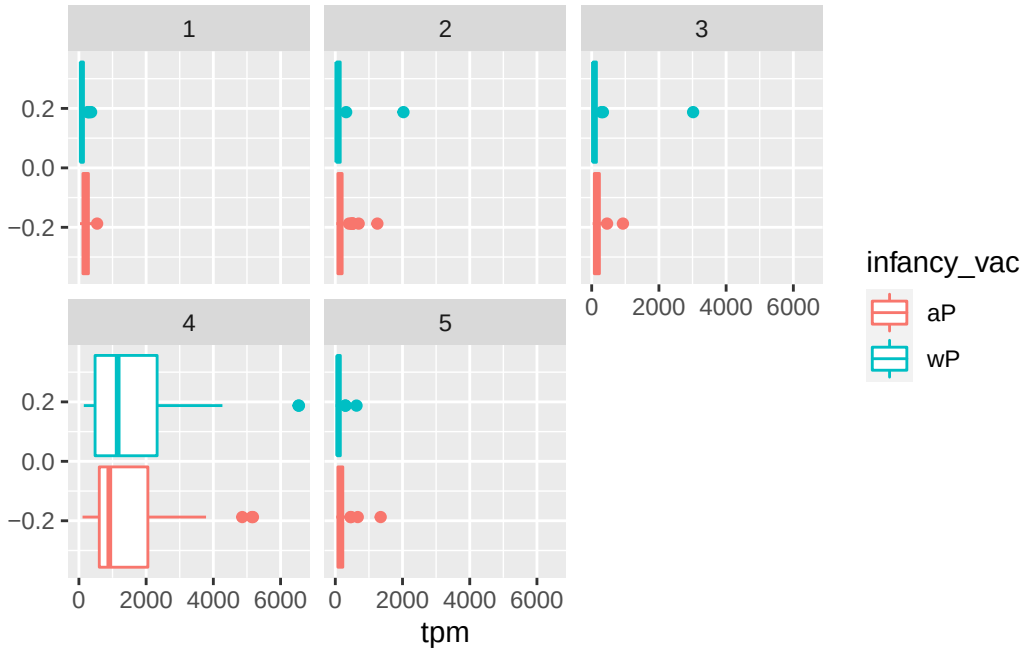
Q19.: What do you notice about the expression of this gene (i.e. when is it at it's maximum level)?

Gene expression of the IgG1 gene peaks at visit 4.

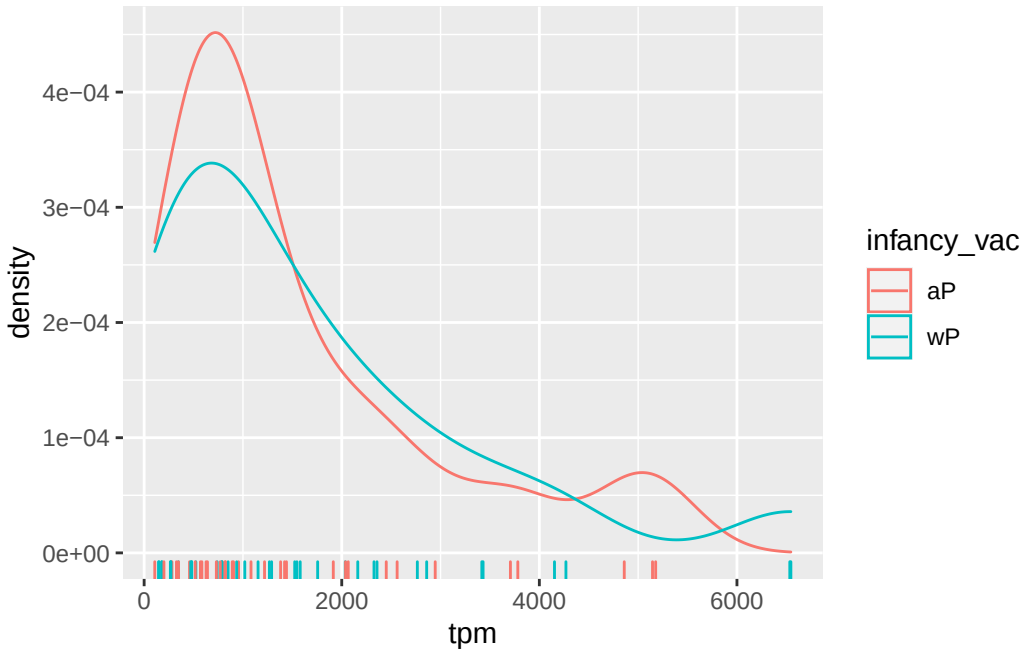
Q20. Does this pattern in time match the trend of antibody titer data? If not, why not?

This data roughly matches the maximum of the Ab titer data, as Ab titers peak at visit 5. Gene expression peaks at visit 4 and then rapidly tappers, however Ab titers remain high from visit 4 onward.

```
ggplot(ssrna) +
  aes(tpm, col=infancy_vac) +
  geom_boxplot() +
  facet_wrap(vars(visit))
```




```
ssrna %>%
  filter(visit==4) %>%
  ggplot() +
    aes(tpm, col=infancy_vac) + geom_density() +
    geom_rug()
```


density
4e−04
3e−04
2e−04
1e−04
0e+00
0
2000
4000
6000
tpm
infancy_vac
aP
wP