Analyse statistique des données de qPCR du projet ATB biofilm

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01/04/2022

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Fonctions

Fonction pour importer les données et ajouter les colonnes "ID", "[ADN]ng/µL" et "Dilution".

Fonction de remplacement des données.

```
tri_qPCR = function(tab,Y.intercept,slope,gammeNTC=FALSE,LOQ,moyLOQ,target=FALSE,range=FALSE,ref){
    #paramètre target pour sélectionner un gène en particulier pour la suite
    if (target!=FALSE){
        tab=subset(tab, Target==target)
    }

#On calcule la moyenne et le range des Tm STD
m.Tm.STD=mean(as.numeric(tab$`Melt Temperature`[which(tab$ID=="STD" & tab$`Melt Temperature`!="None")
#on attribue la même valeur au m.Tm.STD min et max pour
m.Tm.STD.min=m.Tm.STD
m.Tm.STD.max=m.Tm.STD
#si on choisit l'option range
```

```
if (range==TRUE){
 r.Tm.STD=range(as.numeric(tab$`Melt Temperature`[which(tab$ID=="STD" & tab$`Melt Temperature`!="Non
 m.Tm.STD.min=r.Tm.STD[1]
 m.Tm.STD.max=r.Tm.STD[2]
}
#On calcule le range des Cq STD
r.Cq.STD=range(as.numeric(tab$Cq[which(tab$ID=="STD" & tab$Cq!="None")]))
\#Si on a des \mathit{Tm} différents des \mathit{Tm} \mathit{Ok}
tab$Cq modif=tab$Cq #création d'une colonne pour les Cq modifiés (copie de la colonne Cq)
tab$Cq_modif[which(tab$Content!="NTC" & tab$`Melt Temperature`!="None" & (tab$`Melt Temperature`<=m.Ti
#si on a des None pour les Tm
tab$Cq_modif[which(tab$`Melt Temperature`=="None")]=40
tab$Tm_QC="PASS" #création d'une colonne indiquant si le Cq de la ligne (l'échantillon) a été modifié
tab$Tm_QC[which(tab$Content!="NTC" & (tab$`Melt Temperature`<=m.Tm.STD.min-1 | tab$`Melt Temperature`
#Si on a des NTC avec des Cq (pas NA), et des pics au même endroit que les Tm STD => on crée une list
if (gammeNTC=="NTC"){
 li=which(tab$Content=="NTC" & is.na(tab$Cq)==FALSE & tab$`Melt Temperature`>=m.Tm.STD.min-1 & tab$`
 tab$Tm QC[li]=FALSE
  if (length(li)!=0){ #si cette liste existe bien (non vide)
    μ.Cq.NTC=mean(as.numeric(tab$Cq[li]))
    r.Cq.NTC=range(as.numeric(tab$Cq[li]))
    #On visualise le range et la moyenne des Cq NTC
    print(µ.Cq.NTC)
    print(r.Cq.NTC)
    #Pour les échantillons avec Tm +/- moy des Tm STD (Tm OK)
    ech=which(tab$`Melt Temperature`>=m.Tm.STD.min-1 & tab$`Melt Temperature`<=m.Tm.STD.max+1)
    \#Si\ Cq(ech) >= LOQ\ (Cqmean\ NTC\ -\ 1)
    ech2=which(tab$Cq[ech]>=(\mu.Cq.NTC-1))
    if (length(ech2)!=0){ #si on vérifie bien cette condition
      tab$Tm_QC[ech]=FALSE
      #Si CqmeanNTC < 35
      if (\mu.Cq.NTC<35){
        tab$Cq_modif[ech]=\mu.Cq.NTC
        }
      \#Si\ CqmeanNTC > 35
      else{
        tab$Cq_modif[ech]=35-2
     }
}
if (gammeNTC=="gamme"){
  #parametre gammme => oui => Cq le moins concentré range max +2 pour non quanti
  ech.out = which(tab$Cq > max(tab$Cq[which(tab$ID=="STD")])) #si Cq > à Cq le moins concentré
```

```
tab$Cq_modif[ech.out]=moyLOQ+2
  tab$Tm_QC[ech.out]=FALSE
}
#On calcule SQ avec les nouveaux Cq
\# \ tab\$SQ\_final=10^{((as.numeric(tab\$Cq\_modif)-Y.intercept)/slope)}
tab$SQ_final=10^((as.numeric(tab$Cq_modif)-tab$"Y-Intercept")/tab$Slope)
#on garde la valeur de départ pour les STD
tab$SQ_final[which(tab$ID=="STD")]=tab$`Starting Quantity (SQ)`[which(tab$ID=="STD")]
#on rajoute col SQ/dil
tab$SQ_dil=tab$SQ_final #on copie la colonne SQ_final pour la modifier par rapport aux dilution des é
dil_ech=which(tab$Dilution!="NA" & tab$Dilution!="ND" & tab$Dilution!="24.02.20") #on sélectionne les
tab$SQ_dil[dil_ech]=tab$SQ_final[dil_ech]*as.numeric(tab$Dilution[dil_ech])
tab$rapport=tab$SQ_dil/ref #ou SQ_final
#on rajoute une colonne pour les log des rapports
tab$log_rapport=log(tab$rapport,10) #attention bien mettre la base 10 (sinon base exp(1))
return(tab)
```

Test sur les données

Tri des données de qPCR

Plots

Graphique des Cq en fonction des Tm pour chaque gène qnr, permettant de vérifier les résultats obtenus avec la fonction de tri des données de qPCR.

Analyse statistique

Normalité des rapports SQgene/SQref (dilués)

Rapport SQgene/SQref

Vérification, pour chaque gène qnr, de la normalité des rapports SQ(gène)/SQ(ref) avec le test de Shapiro. Visualisation graphique de la répartition des données.

Log des rapports SQgene/SQref

Fusion des tableaux qPCR et metadata (pour avoir les facteurs dans le même tableau)

Modèle pour les campagnes AB

Analyse de la significativité des facteurs bioréacteur, matériel, inoculum, campagne et jour sur le rapport SQ(gène)/SQ(ref).

Il y a 32 échantillons pour la campagnes A et B, mais que 2 échantillons à comparer entre 2 conditions (que Inoculum sans prendre en compte les autres facteurs par exemple)

Pb avec modele AB qui n'affiche pas I ??????????

La normalité n'étant pas toujours respectée, une transformation des rapports en log est nécessaire :

Modèle pour les campagnes EF

Analyse de la significativité des facteurs dosage (forte concentration ou faible concentration) et traitement en FQ sur le rapport $SQ(g\`{e}ne)/SQ(ref)$.

La normalité n'étant pas toujours respectée, une transformation des rapports en log est nécessaire :

Comparaison des moyennes au témoin

Campagnes AB

Campagnes EF

Comparaison au témoin (None) en fonction du type d'ATB (FQ1, FQ2 ou FQ1+FQ2):

Comparaison au témoin (None) en fonction du dosage (High ou Low) :

Fonction finale résultats

Résultats

qnrA

```
results_log(PCR_19_qnrA_tri,metadata)
```

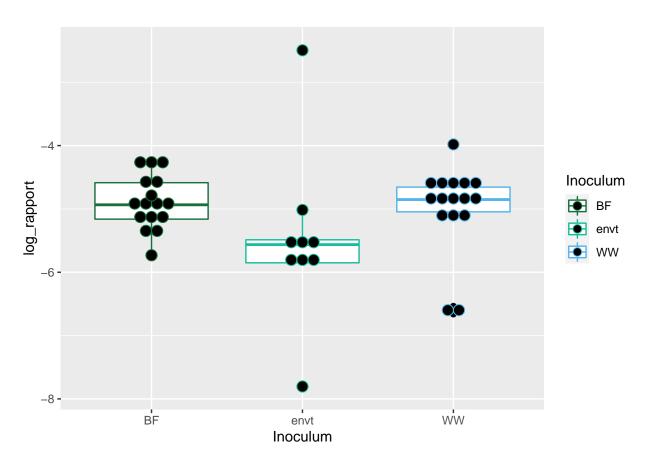
```
##
##
        **************Campagnes A et B**********
##
##
##
##
## [1] "####Summary model"
##
## Call:
## lm(formula = AB.log ~ 1 + B + M + I + M:I + C + Jour + I:Jour +
##
       Jour:M)
##
## Residuals:
##
       Min
                1Q Median
                                ЗQ
                                       Max
## -1.5234 -0.2045 0.1469 0.3643
                                    0.6021
##
## Coefficients: (1 not defined because of singularities)
##
               Estimate Std. Error t value Pr(>|t|)
                           0.34126 -14.687 7.51e-13 ***
## (Intercept) -5.01196
## BB2
               -0.08716
                           0.30523 -0.286
                                               0.778
## BB3
               -0.09564
                           0.43167
                                    -0.222
                                               0.827
## BB4
               -0.01221
                           0.43167
                                    -0.028
                                               0.978
## MP
                0.53241
                           0.37383
                                     1.424
                                               0.168
```

```
## IWW
                     NA
                                NA
                                        NA
                                                 NA
## CB
               -0.18575
                           0.21583
                                    -0.861
                                              0.399
## JourJ14
               0.21883
                           0.37383
                                     0.585
                                              0.564
## MP:IWW
               -0.02400
                           0.43167
                                    -0.056
                                              0.956
## IWW:JourJ14 -0.18061
                           0.43167
                                    -0.418
                                              0.680
## MP:JourJ14 -0.44468
                           0.43167
                                    -1.030
                                              0.314
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
##
## Residual standard error: 0.6105 on 22 degrees of freedom
## Multiple R-squared: 0.1671, Adjusted R-squared: -0.1736
## F-statistic: 0.4904 on 9 and 22 DF, p-value: 0.8653
##
##
##
##
    ******Comparaison au témoin*****
```

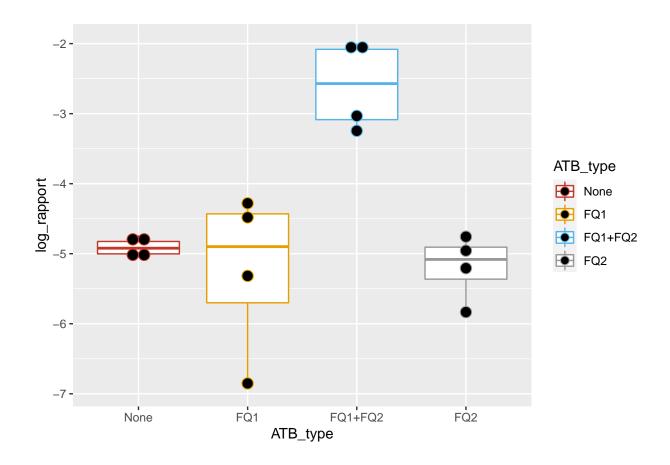
Warning: Inoculum was coerced to a factor.

Dunn (1964) Kruskal-Wallis multiple comparison

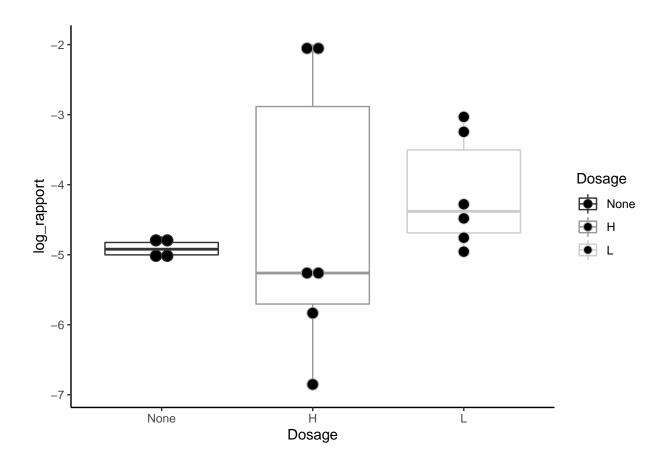
p-values adjusted with the Bonferroni method.



```
Z
    Comparison
                           P.unadj
## 1 BF - envt 2.4027862 0.01627070 0.04881209
      BF - WW -0.1180563 0.90602307 1.00000000
## 3 envt - WW -2.5029603 0.01231594 0.03694781
##
##
##
       ##
##
##
##
##
## [1] "###Summary model"
##
## Call:
## lm(formula = EF.log ~ 1 + FQ1 + FQ2 + FQ1:FQ2)
## Residuals:
       Min
                1Q
                    Median
                                 3Q
                                         Max
## -1.61967 -0.21328 0.01664 0.44489 0.95266
##
## Coefficients:
             Estimate Std. Error t value Pr(>|t|)
##
## (Intercept) -4.9058
                          0.3543 -13.848 9.66e-09 ***
## FQ11
              -0.3266
                          0.5010 -0.652 0.52673
## FQ21
              -0.2827
                          0.5010 -0.564 0.58295
## FQ11:FQ21
               2.9195
                          0.7085
                                 4.120 0.00142 **
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
## Residual standard error: 0.7085 on 12 degrees of freedom
## Multiple R-squared: 0.7612, Adjusted R-squared: 0.7015
## F-statistic: 12.75 on 3 and 12 DF, p-value: 0.0004852
##
##
##
##
  ******Comparaison au témoin*****
##
##
##
## ***ATB_type***
## Bin width defaults to 1/30 of the range of the data. Pick better value with 'binwidth'.
```



```
##
    Dunnett's test for comparing several treatments with a control :
##
##
       95% family-wise confidence level
##
## $None
##
                      diff
                              lwr.ci
                                       upr.ci
                                                pval
                -0.3266245 -1.6710077 1.017759 0.8521
## FQ1-None
## FQ1+FQ2-None 2.3101268 0.9657436 3.654510 0.0016 **
               -0.2827145 -1.6270977 1.061669 0.8960
## FQ2-None
##
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
##
##
##
## ***Dosage***
```

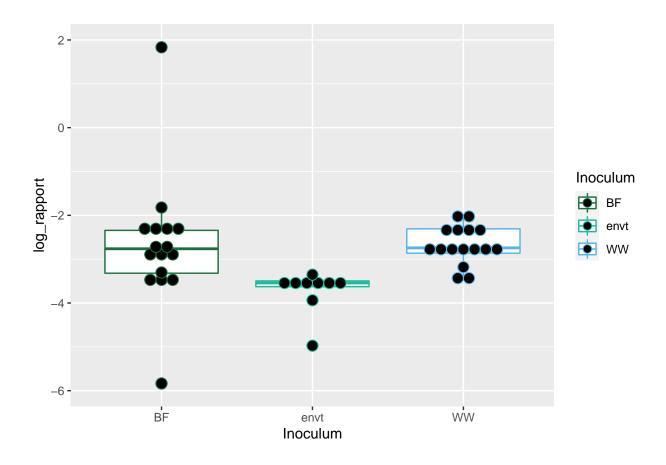


```
##
## Dunnett's test for comparing several treatments with a control :
## 95% family-wise confidence level
##
## $None
## diff lwr.ci upr.ci pval
## H-None 0.3533514 -1.790565 2.497268 0.8810
## L-None 0.7805071 -1.363409 2.924424 0.5705
##
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

qnrB

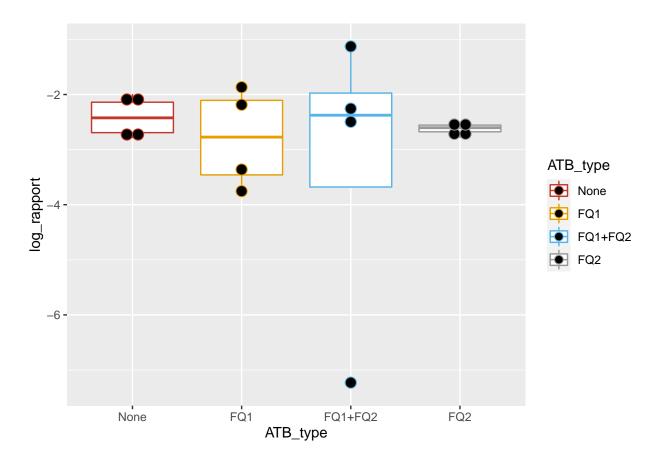
```
results_log(PCR_20_qnrB_tri,metadata)
```

```
##
## Call:
## lm(formula = AB.log ~ 1 + B + M + I + M:I + C + Jour + I:Jour +
##
       Jour:M)
##
## Residuals:
                  10
                     Median
                                    30
## -1.99737 -0.35074 -0.03568 0.35518 2.70140
##
## Coefficients: (1 not defined because of singularities)
              Estimate Std. Error t value Pr(>|t|)
## (Intercept) -3.08238
                          0.50339 -6.123 3.66e-06 ***
                                    0.149 0.883236
## BB2
               0.06690
                           0.45025
## BB3
                           0.63675 -1.187 0.247739
              -0.75606
## BB4
               0.08996
                           0.63675
                                    0.141 0.888931
## MP
                2.12396
                           0.55144
                                     3.852 0.000865 ***
                                        NA
## IWW
                                NA
                     NA
                                                 NA
## CB
               -0.05065
                           0.31837
                                    -0.159 0.875047
              -0.16755
                           0.55144
                                    -0.304 0.764098
## JourJ14
## MP:IWW
               -1.06372
                          0.63675
                                    -1.671 0.108977
## IWW:JourJ14 0.33910
                          0.63675
                                    0.533 0.599680
## MP:JourJ14 -0.87032
                           0.63675 -1.367 0.185485
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.9005 on 22 degrees of freedom
## Multiple R-squared: 0.5173, Adjusted R-squared: 0.3198
## F-statistic: 2.62 on 9 and 22 DF, p-value: 0.03156
##
##
##
##
   ******Comparaison au témoin*****
## Bin width defaults to 1/30 of the range of the data. Pick better value with 'binwidth'.
## Warning: Inoculum was coerced to a factor.
## Dunn (1964) Kruskal-Wallis multiple comparison
    p-values adjusted with the Bonferroni method.
```

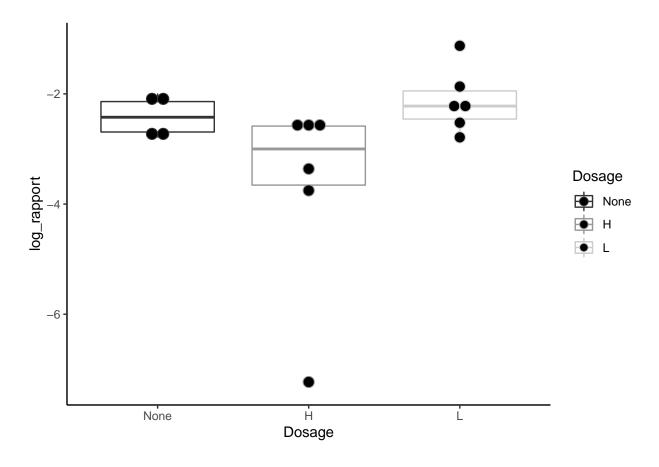


```
##
    Comparison
                      Z
                            P.unadj
                                         P.adj
## 1 BF - envt 3.3113094 0.0009286049 0.0027858147
     BF - WW -0.5312532 0.5952433298 1.0000000000
## 3 envt - WW -3.7620926 0.0001684976 0.0005054928
##
##
##
       ##
##
##
##
## [1] "####Summary model"
##
## lm(formula = EF.log ~ 1 + FQ1 + FQ2 + FQ1:FQ2)
##
## Residuals:
     Min
              1Q Median
                           3Q
                                  Max
## -3.9523 -0.2840 0.0844 0.6500 2.1496
##
## Coefficients:
##
             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -2.4081 0.7194 -3.348 0.00581 **
## FQ11
            -0.3838
                       1.0173 -0.377 0.71258
## FQ21
            -0.2212 1.0173 -0.217 0.83156
```

```
## FQ11:FQ21
               -0.2641
                        1.4387 -0.184 0.85740
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
## Residual standard error: 1.439 on 12 degrees of freedom
## Multiple R-squared: 0.06169,
                                  Adjusted R-squared: -0.1729
## F-statistic: 0.263 on 3 and 12 DF, p-value: 0.8507
##
##
##
##
   ******Comparaison au témoin*****
##
##
##
## ***ATB_type***
```



```
##
## Dunnett's test for comparing several treatments with a control :
## 95% family-wise confidence level
##
## $None
## diff lwr.ci upr.ci pval
## FQ1-None -0.3837818 -3.113659 2.346096 0.9640
```

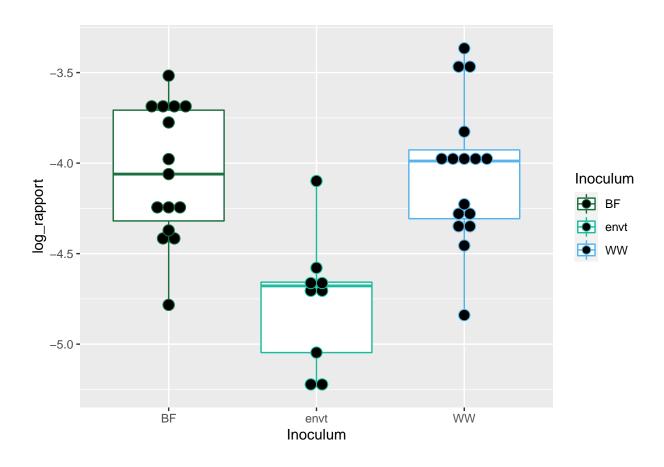


```
##
     Dunnett's test for comparing several treatments with a control :
##
       95% family-wise confidence level
##
##
## $None
##
                diff
                        lwr.ci
                                  upr.ci
                                           pval
## H-None -1.2659689 -3.162061 0.6301229 0.2040
## L-None 0.2833033 -1.612789 2.1793951 0.9006
##
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
```

qnrS

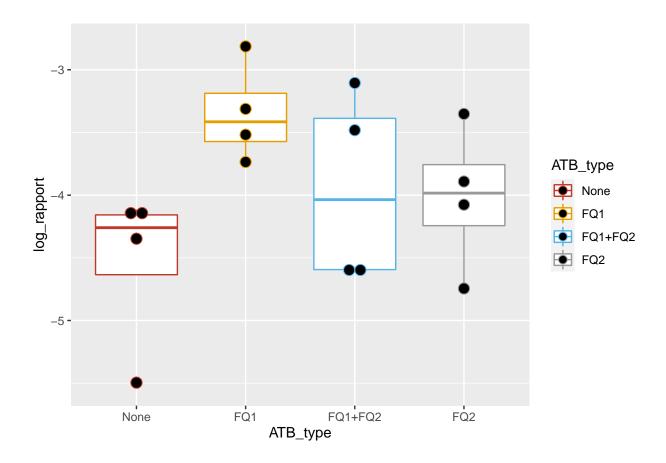
```
results_log(PCR_21_qnrS_tri,metadata)
##
##
       ##
##
##
##
## [1] "###Summary model"
##
## Call:
  lm(formula = AB.log ~ 1 + B + M + I + M:I + C + Jour + I:Jour +
##
      Jour:M)
##
## Residuals:
                 1Q
                     Median
                                  3Q
## -0.41589 -0.12013 0.04109 0.12045 0.36258
## Coefficients: (1 not defined because of singularities)
              Estimate Std. Error t value Pr(>|t|)
## (Intercept) -4.21074
                         0.13306 -31.646 < 2e-16 ***
## BB2
              0.12651
                          0.11794
                                   1.073 0.29558
## BB3
                         0.17054 -0.310 0.75963
              -0.05286
## BB4
              -0.09606
                         0.16680
                                  -0.576 0.57080
## MP
              0.62343
                          0.14554
                                   4.284 0.00033 ***
## IWW
                              NA
                                      NA
                                               NA
                    NΑ
                          0.08527
## CB
               0.11115
                                   1.303 0.20653
## JourJ14
              -0.23979
                          0.15398
                                  -1.557 0.13436
## MP:IWW
              -0.13094
                          0.17054
                                  -0.768 0.45118
## IWW:JourJ14 -0.09668
                          0.17054 -0.567 0.57678
## MP:JourJ14 -0.14367
                          0.17054 -0.842 0.40904
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 0.2359 on 21 degrees of freedom
## Multiple R-squared: 0.7252, Adjusted R-squared: 0.6074
## F-statistic: 6.158 on 9 and 21 DF, p-value: 0.0002961
##
##
##
##
   ******Comparaison au témoin*****
## Bin width defaults to 1/30 of the range of the data. Pick better value with 'binwidth'.
## Warning: Inoculum was coerced to a factor.
## Dunn (1964) Kruskal-Wallis multiple comparison
```

p-values adjusted with the Bonferroni method.

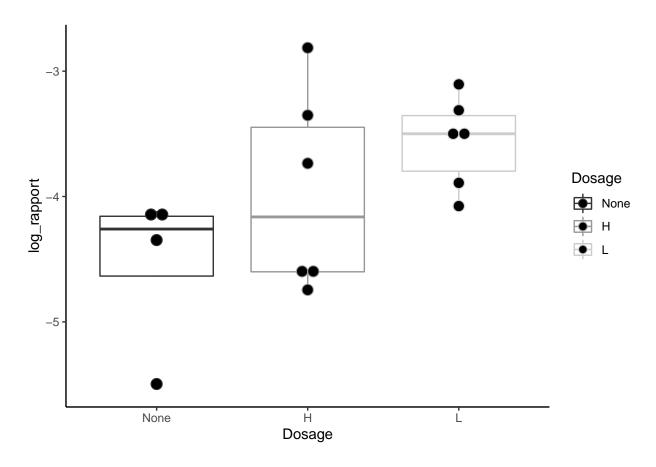


```
##
    Comparison
                       Z
                             P.unadj
                                         P.adj
## 1 BF - envt 3.36773609 0.0007578811 0.002273643
     BF - WW 0.01884238 0.9849668429 1.000000000
## 3 envt - WW -3.39165673 0.0006947142 0.002084143
##
##
##
       ##
##
##
##
## [1] "####Summary model"
##
## lm(formula = EF.log ~ 1 + FQ1 + FQ2 + FQ1:FQ2)
##
## Residuals:
               1Q
                   Median
                                ЗQ
## -0.96315 -0.45429 0.07875 0.42863 0.84014
##
## Coefficients:
##
             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -4.5327 0.3060 -14.813 4.49e-09 ***
## FQ11
             1.1880
                        0.4327 2.745
                                      0.0178 *
## FQ21
             0.5166
                        0.4327
                                1.194
                                      0.2556
```

```
## FQ11:FQ21
               -1.1171
                        0.6120 -1.825 0.0929 .
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
##
## Residual standard error: 0.612 on 12 degrees of freedom
## Multiple R-squared: 0.3872, Adjusted R-squared: 0.234
## F-statistic: 2.527 on 3 and 12 DF, p-value: 0.1067
##
##
##
##
   ******Comparaison au témoin*****
##
##
##
## ***ATB_type***
```



```
##
## Dunnett's test for comparing several treatments with a control :
## 95% family-wise confidence level
##
## $None
## diff lwr.ci upr.ci pval
## FQ1-None 1.1879525 0.02676133 2.349144 0.0446 *
```



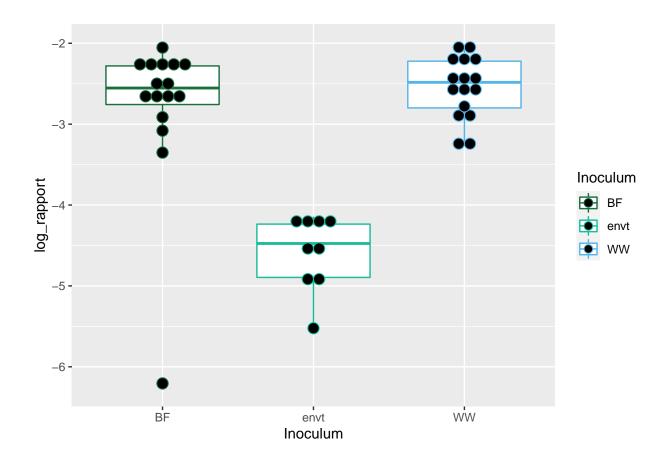
```
##
     Dunnett's test for comparing several treatments with a control :
##
       95% family-wise confidence level
##
##
## $None
##
               diff
                         lwr.ci
                                  upr.ci
                                           pval
## H-None 0.5592930 -0.43300798 1.551594 0.3010
## L-None 0.9686971 -0.02360389 1.960998 0.0556 .
##
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
```

qnrD

```
results_log(PCR_22_qnrD_tri,metadata)
##
##
       ##
##
##
##
## [1] "###Summary model"
##
## Call:
##
  lm(formula = AB.log ~ 1 + B + M + I + M:I + C + Jour + I:Jour +
##
      Jour:M)
##
## Residuals:
                 1Q
                     Median
                                   3Q
## -2.28018 -0.21367 0.07452 0.29932 1.02433
## Coefficients: (1 not defined because of singularities)
               Estimate Std. Error t value Pr(>|t|)
                         0.376409 -5.891 6.28e-06 ***
## (Intercept) -2.217508
## BB2
              0.041856
                          0.336671
                                   0.124
                                            0.9022
## BB3
                          0.476124 -1.921
              -0.914790
                                            0.0677 .
## BB4
              -0.204552
                          0.476124 -0.430
                                            0.6717
## MP
              0.600530
                          0.412336
                                   1.456
                                            0.1594
## IWW
                               NA
                                       NA
                                                NA
                     NΑ
                          0.238062
## CB
              -0.196490
                                   -0.825
                                            0.4180
## JourJ14
              -0.597122
                          0.412336
                                   -1.448
                                            0.1617
## MP:IWW
              -0.684717
                          0.476124 -1.438
                                            0.1645
## IWW:JourJ14 -0.002761
                          0.476124 -0.006
                                            0.9954
## MP:JourJ14
              0.369856
                          0.476124
                                   0.777
                                            0.4455
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 0.6733 on 22 degrees of freedom
## Multiple R-squared: 0.41, Adjusted R-squared: 0.1687
## F-statistic: 1.699 on 9 and 22 DF, p-value: 0.1491
##
##
##
##
   ******Comparaison au témoin*****
## Bin width defaults to 1/30 of the range of the data. Pick better value with 'binwidth'.
## Warning: Inoculum was coerced to a factor.
```

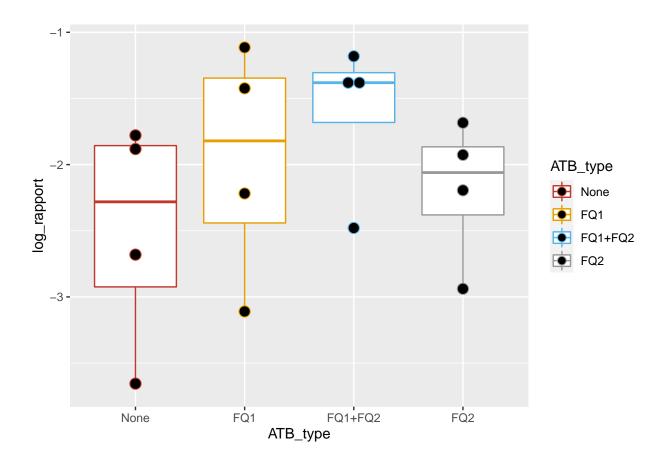
Dunn (1964) Kruskal-Wallis multiple comparison

p-values adjusted with the Bonferroni method.

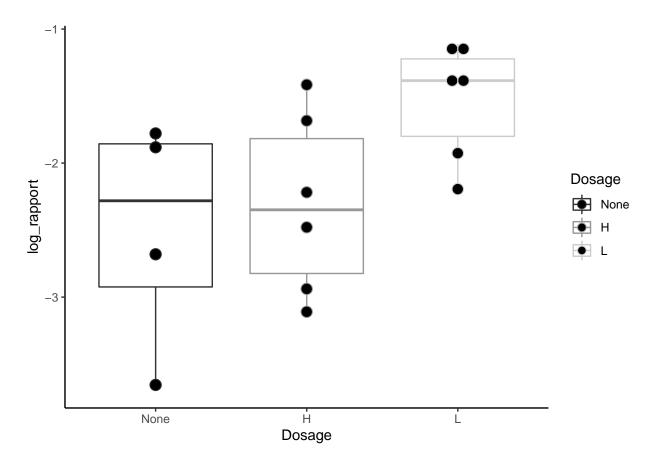


```
## Comparison
                     Z
                           P.unadj
                                        P.adj
## 1 BF - envt 3.6062663 3.106343e-04 0.0009319030
## 2 BF - WW -0.5755243 5.649368e-01 1.0000000000
## 3 envt - WW -4.0946149 4.228707e-05 0.0001268612
##
##
##
##
      ##
##
##
## [1] "####Summary model"
##
## lm(formula = EF.log ~ 1 + FQ1 + FQ2 + FQ1:FQ2)
##
## Residuals:
                       3Q
  Min
             1Q Median
## -1.1562 -0.3770 0.2246 0.5123 0.8519
##
## Coefficients:
            Estimate Std. Error t value Pr(>|t|)
##
## (Intercept) -2.49925 0.37063 -6.743 2.06e-05 ***
## FQ11 0.53279
                     0.52415 1.016
                                      0.329
## FQ21
            0.31357 0.52415 0.598
                                      0.561
```

```
## FQ11:FQ21
               0.04735
                          0.74126
                                    0.064
                                             0.950
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.7413 on 12 degrees of freedom
## Multiple R-squared: 0.2046, Adjusted R-squared: 0.005718
## F-statistic: 1.029 on 3 and 12 DF, p-value: 0.4145
##
##
##
##
   ******Comparaison au témoin*****
##
##
##
## ***ATB_type***
```



```
##
## Dunnett's test for comparing several treatments with a control :
## 95% family-wise confidence level
##
## $None
## diff lwr.ci upr.ci pval
## FQ1-None 0.5327941 -0.8736837 1.939272 0.6270
```



```
##
     Dunnett's test for comparing several treatments with a control :
##
       95% family-wise confidence level
##
##
## $None
##
               diff
                         lwr.ci
                                  upr.ci
                                           pval
## H-None 0.1917514 -0.83803922 1.221542 0.8518
## L-None 0.9682955 -0.06149509 1.998086 0.0652 .
##
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
```