A study in the effects of different treatments on the growth of high fluorescence virus

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Bergen, September, 2011
Bikram Maharjan

1 Introduction

This study is carried out to see whether the different treatment effects does different impact or not in the increment and the decrement of viruses under the given time period, *i.e.,* the treatments, that have been applied, make any sense in the increment and the decrement of these viruses? or is there any influence of the treatments on the virus?. If there seem differences on the increment and the decrement of viruses for the given treatment effects in the given time period then the study is conducted to analyse whether these apparent differences are statistically significant or not. In other words, this study is carried out to get the conclusion about the statement: "Is there any significant difference between the treatments?". In our study, the experimental data is taken for 25 days [J.B. Larsen, A. Larsen, R. Thyrhaug, G. Bratbak and R.-A. Sandaa, 9 April 2008], in which three different fixed types of treatments are applied on the different batches of virus and three randomly choosen such batches are treated under the each of the treatments. So, there are nine different random groups of virus such that each of three random groups are treated under only one particular fixed treatement, *i.e.,* each one treatment has only three random groups. So, there are nine random groups for three different fixed treatment effects. This experiment has a balanced data set as each unique treatment has three replications and within each replication, the data is observed for 25 days . In this study, these random groups are known as the meso groups. Hence, the main purpose of this study is to know if those applied three treatments are statistically significant or not. It has been assumed that the experiment is conducted under the same circumstances for all the parallel mesos.

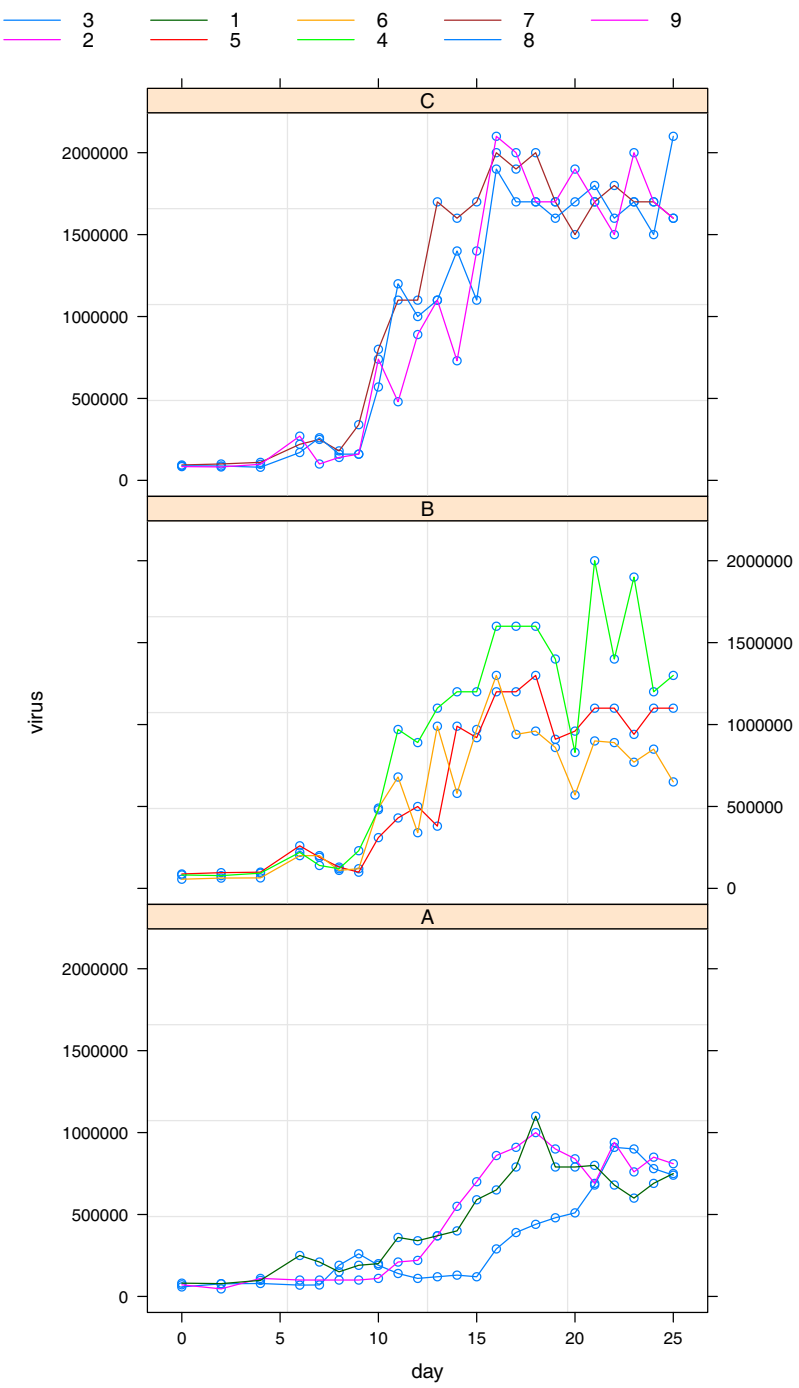

Figure 1.1: Plot of the given dataset with respect to numbers of viruses versus time period for the three different treatments

Figure 1.1 is the plot of the given dataset for three fixed treatment effects for 25 days of time period. We can see that at the begining of the experiment, there is no such a noticeable increment or decrement of viruses in all three treatments. Whereas at the middle of the experiment, there starts some rapid increment of viruses with seemingly different level of intensities for all three different treatments and, it increases gradually and reaches some upper level of increment and maintains this level approximately to the end of the time period. In the figure, it seems that the levels of increments differ by the treatment. There also seems that the rapid increment seems to start earlier in the treatment C than in the treatment A and B. And the steepness of the increment curves for all mesos in the treatment A seems to be lower than in the treatment B and C with respect to the time period.

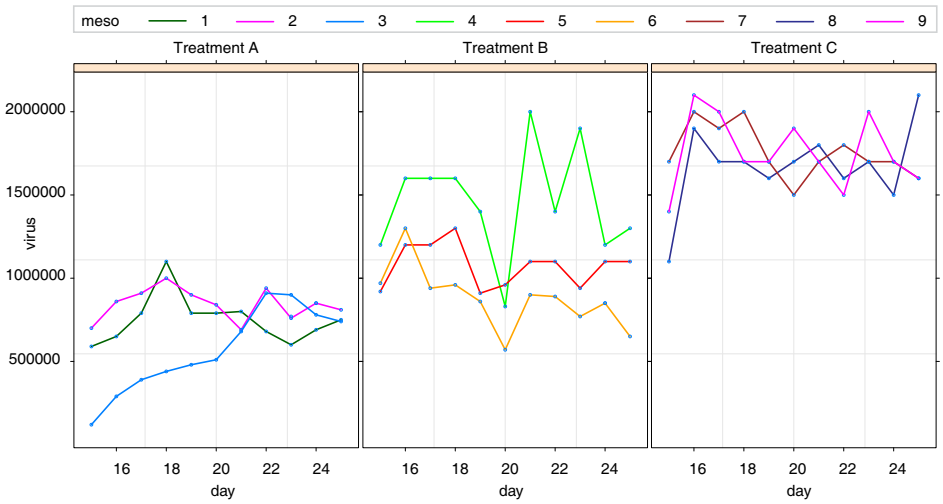

Figure 1.2: Plot of the meso groups variability within the three different treatments with respect to the time period, when $k > 15$

Our study concentrates on whether these apparent differences, that is, the increment of virus growth because of the different treatment effects, are statistically significant or not. For this purpose we begin our study by analyzing the data taken after some days of the experiment beginning. Since at the beginning of the experiment, there seem no differences in the growth of virus in all three treatments. But at the end of the experiment, there seem to be larger differences in the data points and the levels of the increment are also seemed to be different for different treatments. The Figure

1.2, plotted after day 15, shows the difference in the increment of virus in different treatments. That is, in all three treatments the variability of data points increase with the increment of the virus growth where all three meso groups in the treatment A have the lower upper level than in the treatment B and C, *i.e.,* in average, treatment A has the lowest virus increment level among these three treatments. There, also seems to be some variability in data points within all treatments and these variability of data points are comparatively higher in the treatment B than in the treatments A and C. So, the treatment B has more scattered data points at the upper right part of the curve. However, in our study, we mainly focus on the effects of treatments on the average virus increment level rather than to see the individual within variability of the meso groups within each treatment. So, we further analysis if these different levels of increment of viruses for different treatments are statistically significant or not.

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Preparation for the analysis

In our study, let us denote the three fixed treatments A, B, and C by $i = 1, 2$, and 3 respectively, the random meso groups by $j = 1, 2, \cdots, 9$ (Here, for $i = 1, j = 1, 2, 3$; for $i = 2, j = 4, 5, 6$; for $i = 3, j = 7, 8, 9$) and, the time period by $k = 1, 2, 3, \cdots, 25$. Hence, the response variable, Y_{ijk} is k^{th} day's observation of the j^{th} meso group for the i^{th} treatment, *i.e.,* each observation is nested under the particular meso group and these meso groups are nested within the particular treatment. Such type of experiment is known as *Nested design.* Since in our study, three different treatment effects appear as the fixed effect and the nine meso random groups appear as the random effect, the study is combination of both fixed and random effects. So, it is also known as *Mixed Effects Model.* In figure 1.1, we have seen that there is the highest level of average increment of the viruses in the treatment C and also, there is a lot of variation between the meso groups under the same treatment. But our main concern is to study the treatment effects on these viruses. So, when we plot the data set after a certain time period, say, $k > 15$, figure 2.1, gives an approximately the last part of data curve of our study which clearly visualizes the differences among the upper level of increment of viruses in the three different treatments. Where the treatment C has the highest level of average increment of viruses among three treatment effects which is also above the average level of the grand mean (for $k > 15$) of the data set. The treatment A has the lowest average increment of virus, *i.e.,* the average level of a treatment A is below the grand mean whereas the average of treatment B has the level approximately near the grand mean.

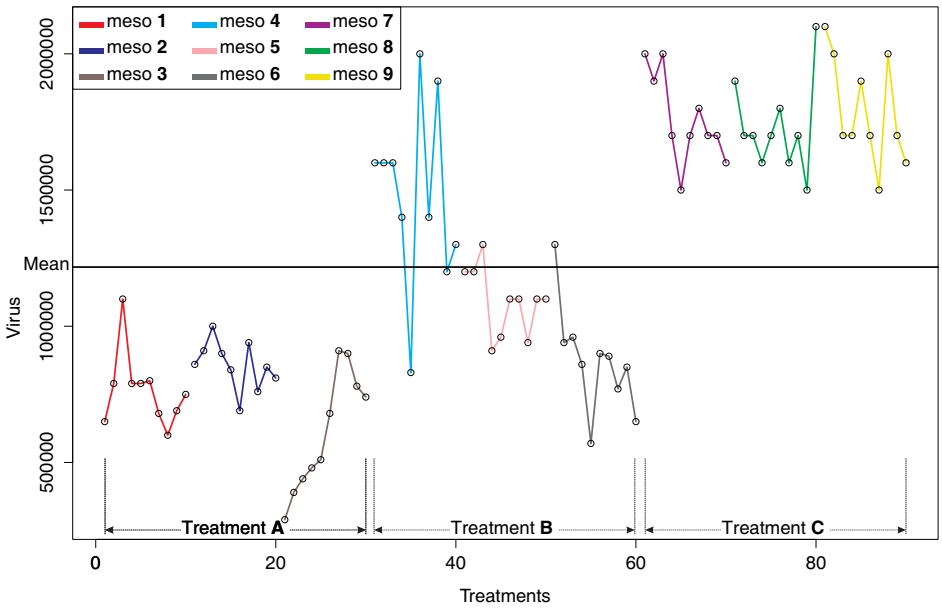

Figure 2.1: Plot of the dataset when $k > 15$ for three different treatments

There also seems that the within variability of data points is higher in the treatment B, that is, the highest scattered data points are seemed in the treatment B. These types of variability within each treatment and between treatments arise due to the cause of various factors. But, in our study, we mainly focus on only one cause factor, *i.e.,* the treatment effect. Therefore, we apply an appropriate statistical technique to analyse if these different upper part differences arise due to the different treatment effects or not. Generally, we apply *t-test* to test the significance of the difference between two group means. But in our case, there are three treatments and in such type of cases, a test is done by the analysis of variance, abbreviated by *ANOVA*, concerned with analyzing variation in means. An analysis of variance refers to an additive decomposition of data into a grand mean, main effects, possible interactions and an error term [Gelman, 2005].

3

Analysis of Variance

The great British statistician *R. A. Fisher* developed the analysis of variance method in the 1920s. The main objective of this technique is a significance test, using his F distribution, for detecting differences among a set of population means. *F-Statistic* is defined as the ratio of two independent chi-square variates divided by their respective degrees of freedom and it follows Snedecor's $F-$distribution with (ν_1, ν_2) degree of freedom with probability function given by

$$f(x|\nu_1,\nu_2) = \frac{\Gamma(\frac{\nu_1+\nu_2}{2})}{\Gamma(\frac{\nu_1}{2})\Gamma(\frac{\nu_2}{2})}(\frac{\nu_1}{2})^{\nu_1/2}\frac{x^{(\nu_1-2)/2}}{(1+\frac{\nu_1}{\nu_2}x)^{(\nu_1+\nu_2)/2}} \qquad 0 \leq x \leq \infty$$

The analysis of variance (ANOVA) is an *F-test* of a *null hypothesis* that there is no significant difference between all the treatments against an *alternative hypothesis* that there is at least two of the treatment means are unequal. Variation is inherent in nature. The total variation in any set of numerical data is due to a number of causes which may be classified as:
(i) Assignable causes, and (ii) Chance causes.
The variation due to assignable causes can be detected and measured whereas the variation due to chance causes is beyond the control of human hand and can not be traced separately. For the validity of the $F-$test in ANOVA, the following assumptions are made:

- The observations are independent (independence).
- The variance is the same for all observations (homogeneous variance).
- Parent population from which observations are taken is normal (nor-

mality), and

- Various treatment and environmental effects are additive in nature.

[S. C. Gupta and V. K. Kapoor, 1994]. Researchers use ANOVA in many ways. Generally, reseachers use ANOVA in three ways: one-way ANOVA, two-way ANOVA and N-way multivariate ANOVA [Dr. James, 1996-2011]. Since in our study, we are mainly concern with the treatments effects only, so we focus on *one-way anova* in this study.

3.1 One way ANOVA

Let I denotes the number of treatments (or classes) to be compared. The means of the response variable for the corresponding treatments are $\mu_1, \mu_2, \mu_3, \cdots \mu_I$. Mathematically, we write

$$H_0 : \mu_1 = \mu_2 = \cdots = \mu_I; \qquad i = 1, \cdots, I$$

against

$$H_1 : \mu_i \neq \mu_j \qquad \text{for some } i, j$$

If H_0 is false, there may be all the treatment means are differ, may be some differ, or may be merely one mean differs from the others. The test analyzes whether the differences observed among the treatment means could have reasonably occured by chance, if H_0 were true.

In our study, we are interested to see whether there is significant difference between the treatment effects or not. We try to define a simple model by decomposing the response variable into the following two components:

- The variation between the treatments (or the classes), *i.e.,* the variation due to different types of treatments.

- The variation within the treatments, *i.e.,* the inherent variation of the response variable within the treatments.

The first type of variation is due to assignable causes, also known as treatment effects, which can be detected and controlled by human endeavour and the second type of variation is due to chance causes, also known as errors or

residuals, which are beyond the control of human hand. So the sources of variation are

- Effect of the treatment
- Error ε produced by numerous causes of such magnitude that they are not detected and identified with the knowledge that we have and they together produce a variation of random nature obeying Normal law of errors.

3.2 Mathematical Model

In this case the linear mathematical model will be

$$\begin{aligned} Y_{ijk} &= \mu_i + \varepsilon_{ijk} \\ &= \mu + (\mu_i - \mu) + \varepsilon_{ijk} \\ &= \mu + \alpha_i + \varepsilon_{ijk} \end{aligned} \tag{3.1}$$

where $(i = 1, 2, \cdots, I; \quad j = 1, 2, \cdots, J; \quad k = 1, 2, \cdots, k)$

- Y_{ijk} is the response variable (*i.e.,* the number of virus in our study) from the k^{th} day of the j^{th} meso batch for the i^{th} treatment.
- μ is the general mean effect given by

$$\mu = JK \sum_{i=1}^{I} \mu_i / IJK$$

  where μ_i is the fixed effect due to the i^{th} treatment *i.e.* if there were no treatment differences and no chance causes then the output of the response will be μ.

- α_i is the effect of the i^{th} treatment given by $\alpha_i = \mu_i - \mu, (i = 1, 2, \cdots I)$, *i.e.,* the i^{th} treatment increases or decreases the output of response by an amount α_i and we get,

$$\begin{aligned} JK \sum_{i=1}^{I} \alpha_i =& JK \sum_{i=1}^{I} (\mu_i - \mu) \\ =& JK \sum_{i=1}^{I} \mu_i - IJK\mu = N\mu - N\mu \ . \end{aligned}$$

where $IJK = N$, the total number of observations.

$$JK \sum_{i=1}^{I} \alpha_i = 0.$$

- ε_{ijk} is the error effect due to chance.

3.2.1 Assumptions in the Model

- Population from which observations are taken is normal.
- The variance of the population distribution is homogenous for all treatments.
- The error terms are independent and normal *i.e.* $\varepsilon \sim N(0, \sigma^2)$

under the third assumption, the model (3.1) becomes as

$$E(Y_{ijk}) = \mu_i = \mu + \alpha_i$$

[S. C. Gupta and V. K. Kapoor, 1994].

3.3 Statistical Analysis of the Model (3.1)

When the assumptions of the ANOVA fullfills, then we use an ANOVA test. Let us write

$$\bar{Y}_{...} = \text{overall mean} = \frac{\sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} Y_{ijk}}{IJK}$$

$$\text{and} \quad \bar{Y}_{i..} = \text{mean of the } i^{th} \text{ treatment} = \frac{\sum_{j=1}^{J} \sum_{k=1}^{K} Y_{ijk}}{JK}$$

The parameters μ and α_i in model (3.1) are estimated by the principle of least squares on minimizing the error sum of squares given by

$$E = \sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} \varepsilon_{ijk}^2 = \sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} (Y_{ijk} - \mu - \alpha_i)^2 = 0$$

The normal equations for estimating μ and α_i are

$$\frac{\partial E}{\partial \mu} = -2\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(Y_{ijk} - \mu - \alpha_i) = 0 \tag{3.2}$$

and

$$\frac{\partial E}{\partial \alpha_i} = -2\sum_{j=1}^{J}\sum_{k=1}^{K}(Y_{ijk} - \mu - \alpha_i) = 0 \tag{3.3}$$

From equation (3.2), we get

$$\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} Y_{ijk} - N\mu - JK\sum_{i=1}^{I}\alpha_i = 0$$

$$\hat{\mu} = \frac{\sum_{j=1}^{J}\sum_{k=1}^{K} Y_{ijk}}{N} = \bar{Y}_{...} \tag{3.4}$$

From equation (3.3), we get

$$\sum_{j=1}^{J}\sum_{k=1}^{K} Y_{ijk} - JK\hat{\mu} - JK\hat{\alpha}_i = 0$$

$$\hat{\alpha}_i = \frac{1}{JK}\sum_{j=1}^{J}\sum_{k=1}^{K} Y_{ijk} - \hat{\mu} = \bar{Y}_{i..} - \hat{\mu}$$

$$\hat{\alpha}_i = \bar{Y}_{i..} - \bar{Y}_{...} \tag{3.5}$$

and, substituting above values in (3.1), we get

$$Y_{ijk} = \bar{Y}_{...} + \bar{Y}_{i..} - \bar{Y}_{...} + \varepsilon_{ijk}$$

$$Y_{ijk} - \bar{Y}_{...} = \bar{Y}_{i..} - \bar{Y}_{...} + \varepsilon_{ijk}$$

To balance the $L.H.S$ and $R.H.S$, we introduce $\varepsilon_{ijk} = Y_{ijk} - \bar{Y}_{i..}$ such that

$$Y_{ijk} - \bar{Y}_{...} = \bar{Y}_{i..} - \bar{Y}_{...} + Y_{ijk} - \bar{Y}_{i..}$$

$$\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(Y_{ijk} - \bar{Y}_{...})^2 = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(\bar{Y}_{i..} - \bar{Y}_{...} + Y_{ijk} - \bar{Y}_{i..})^2$$

But, we know, $\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(Y_{ijk}-\bar{Y}_{i..})=0$.

$$\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(Y_{ijk}-\bar{Y}_{...})^2=JK\sum_{i=1}^{I}(\bar{Y}_{i..}-\bar{Y}_{...})^2+\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(Y_{ijk}-\bar{Y}_{i..})^2$$

But, $\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(Y_{ijk}-\bar{Y}_{i..})=0$, since the algebric sum of the deviations of the treatments from their mean is always zero.

$$TSS=SSB+SSW$$

where,

$TSS=$ Total Sum of Squares which measures the total variability in the dataset.

$SSB=$ Between Sum of Squares which measures the variation between the different treatments

$SSW=$ Error Sum of Squares or Within Sum of Squares which meaures the variation between the response variables under the particular given treatment.

Hence, the total sum of squares (TSS) is the sum of treatment sum of squares (SSB) and error sum of squares (SSW).
Here, we have,

$$\begin{aligned}Y_{ijk}&=\mu+\alpha_i+\varepsilon_{ijk}\\ \bar{Y}_{i..}&=\mu+\alpha_i+\bar{\varepsilon}_{i..}\end{aligned}$$

then

$$\begin{aligned}Y_{ijk}-\bar{Y}_{i..}&=\varepsilon_{ijk}-\bar{\varepsilon}_{i..}\\ \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(Y_{ijk}-\bar{Y}_{i..})^2&=\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(\varepsilon_{ijk}-\bar{\varepsilon}_{i..})^2\\ SSW&=\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(\varepsilon_{ijk}-\bar{\varepsilon}_{i..})^2\\ E(SSW)&=\sum_{i=1}^{I}E[\sum_{j=1}^{J}\sum_{k=1}^{K}(\varepsilon_{ijk}-\bar{\varepsilon}_{i..})^2]\end{aligned}$$

$$
\begin{aligned}
E(SSW) &= \sum_{i=1}^{I}(JK-1)\sigma^2 \\
&= I(JK-1)\sigma^2 \\
\hat{\sigma}^2 &= \frac{SSW}{I(JK-1)}
\end{aligned}
$$

also,

$$
\begin{aligned}
Y_{ijk} &= \mu + \alpha_i + \varepsilon_{ijk} \\
\bar{Y}_{i..} &= \mu + \alpha_i + \bar{\varepsilon}_{i..} \\
\bar{Y}_{...} &= \mu + \bar{\alpha} + \bar{\varepsilon}_{...}
\end{aligned}
$$

$$
\begin{aligned}
\bar{Y}_{i..} - \bar{Y}_{...} &= \alpha_i - \bar{\alpha} + \bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...} \\
\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(\bar{Y}_{i..} - \bar{Y}_{...})^2 &= \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(\alpha_i - \bar{\alpha} + \bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...})^2 \\
SSB &= JK[\sum_{i=1}^{I}(\alpha_i - \bar{\alpha})^2 + (\bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...})^2 \\
&\quad + 2(\alpha_i - \bar{\alpha})(\bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...})] \\
E(SSB) &= JK[E[\sum_{i=1}^{I}(\alpha_i - \bar{\alpha})^2 + (\bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...})^2]] \\
&\quad \text{Since } E(\bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...}) = 0 \\
E(SSB) &= JK[E[\sum_{i=1}^{I}(\bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...})^2]] + JK[\sum_{i=1}^{I}(\alpha_i - \bar{\alpha})^2] \\
E(SSB) &= (A) + (B)
\end{aligned}
$$

while considering part (A), we have

$$\varepsilon_{ijk} \sim N(0, \sigma^2) \Rightarrow \bar{\varepsilon}_{i..} \sim N(0, \sigma^2/JK)$$

and when $X_i \sim N(0, \tau^2) \quad \Rightarrow \quad E[\sum_{i=1}^{I}(X_i - \bar{X})^2] = (I-1)\tau^2$

$$\text{So,} \quad JK[E[\sum_{i=1}^{I}(\bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...})^2]] = JK(I-1)\frac{\sigma^2}{JK}$$

$$(A) = (I-1)\sigma^2$$

Therefore,

$$E(SSB) = (I-1)\sigma^2 + JK[\sum_{i=1}^{I}(\alpha_i - \bar{\alpha})^2]$$

We know, under $H_0 : \mu_1 = \mu_2 = \cdots = \mu_I = 0 \Rightarrow H_0 : \alpha_1 = \alpha_2 = \cdots = \alpha_I = 0$. So, we have

$$\begin{aligned} E(SSB) &= (I-1)\sigma^2 \\ E[SSB/(I-1)] &= \sigma^2 \\ \hat{\sigma}^2 &= \frac{SSB}{(I-1)} \equiv \frac{SSW}{I(JK-1)} \end{aligned}$$

but, under H_1 not all α_i's are $0 \Leftrightarrow$ not all μ_i's are equal. So,

$$\begin{aligned} E[\frac{SSB}{(I-1)}] &= \sigma^2 + \frac{JK}{I-1}\sum_{i=0}^{I}(\alpha_i - \bar{\alpha})^2 \\ &= \sigma^2 + \frac{JK}{I-1}\varphi(\alpha) \\ &\quad [\text{ Since } \varphi(\alpha) = \sum_{i=1}^{I}(\alpha_i - \bar{\alpha})^2 \text{ }]. \end{aligned}$$

we know, the $F-$ ratio is defined as,

$$F = \frac{SSB/(I-1)}{SSW/I(JK-1)}$$

Since, under H_0,

$$\begin{aligned} E[SSB/(I-1)] &= \sigma^2 \text{ and,} \\ E[SSW/I(JK-1)] &= \sigma^2 \\ F &= \frac{SSB/(I-1)}{SSW/I(JK-1)} \approx 1. \end{aligned}$$

$\Rightarrow$ $F-$ ratio around 1 under the H_0.

But, when H_0 is wrong, we have

$$E[SSB/(I-1)] = [\sigma^2 + \frac{JK}{I-1}\varphi(\alpha)] > \sigma^2 \quad \text{and,}$$
$$E[SSW/I(JK-1)] = \sigma^2$$

therefore,

$$F = \frac{SSB/(I-1)}{SSW/I(JK-1)} \quad \text{such that } \frac{E(SSB/I-1)}{E(SSW/I(JK-1))} > 1, \text{ under } H_1.$$

$\Rightarrow$ $F-$ ratio tending to be large under H_1.
where $(I-1)$ and $I(JK-1)$ are the *degrees of freedom* for sum of squares due to treatment effects and sum of squares due to the error term respectively.

3.4 Kruskal-Wallis Test

The Kruskal-Wallis test is a nonparametric (distribution free) test, which is used to compare three or more groups of sample data. This test is used when assumptions of ANOVA are not met. ANOVA is a statistical data analysis technique that is used when the independent variable groups are more than two. In ANOVA, we assume that distribution of each group should be normally distributed. But in the Kruskal-Wallis test, we have the following assumptions.

3.4.1 Assumptions

- The observations are not necessarily normally distributed.
- The observations in each group come from populations with the same shape of distribution but possibly shifted with respect to each other.

[McDonald, 2009]. If different groups have have different shapes (one is skewed to the right and another is skewed to the left, for example, or they have different variances), the Kruskal–Wallis test may give inaccurate results [Morten W. Fagerland, 10 May 2009]. If normality assumptions are met, then the Kruskal-Wallis Test is not as powerful as ANOVA [Dr. James, 1996-2011]. However, these distributions should be continuous and have identical form [Green and Salkind, 2008].

3.4.2 Hypothesis

Null hypothesis (H_0): Null hypothesis assumes that the distributions are from identical populations.

Alternative hypothesis (H_1): Alternative hypothesis assumes that the distributions are shifted with respect to each other.
In this test, the following assumptions are made:

- The samples drawn from the population are random.
- The treatments are independent.
- The measurement scale for should be at least ordinal.

3.4.3 Procedure

- Arrange the data of all samples in a single series in ascending order.
- Assign rank to them in ascending order. In the case of a repeated value, assign ranks to them by averaging their rank position.
- Once this is complete, ranks of the different samples are separated and summed up as $R1, R2, R3$ etc [Dr. James, 1996-2011].
- To calculate the value, apply the following formula:

$$H = \frac{12}{N(N+1)}(\sum_{i=1}^{I} \frac{R_i^2}{n_i}) - 3(N+1)$$

  Where,
  H = Kruskal-Wallis Test Statistic
  N = total number of observations in all samples
  R_i = Sum of the rank observation for group i and n_i is the number of observation in group i [Lowery, 1999-2011].

- Finally, The null hypothesis of equal population medians would then be rejected if $H \geq \chi^2_{(\alpha,I-1)}$. Appropriate multiple comparisons would then be performed on the group medians [Wikipedia/Kruskal].

In our case, we have 3 treatment groups and, for each treatment group, we have 30 observations. So, $\chi^2_{(.05,2)} = 5.991$

Treatment	R_i	n_i
A	583.5	30
B	1310	30
B	2201.5	30
		$N = 90$

Table 3.1: Rank totals for three treatments

Hence, we get, the Kruskal-Wallis test statistic $(H) = 64.15$, *i.e*, the calculated test statistics is greater than the tabulated test statistic at 5 % level of significane. Therefore, we reject the null hypothesis. That is, the distributions are not from identical populations.

3.5 Traditional one way anova table

Source of variation	Degree of freedom	Sum of Squares	Mean Squares	F-value	p-value
Treatments	$(I-1)$	SSB	$SSB/(I-1)$	$\frac{MSB}{MSE}$	
Residuals	$(N-I)$	SSE	$SSE/(N-I)$		
Total	$(N-1)$	TSS			

Table 3.2: Traditional one-way ANOVA source table

The p-value gives the statistical significance of the F-ratio with reference to the $F_{(\nu_1,\nu_2)}$, where ν_1 and ν_2 are the numerator and denominator degrees of freedom, respectively [Gelman, 2005].

Now, when we use the given dataset for $K > 15$ in a model (3.1), we have

$$Y_{ijk} = \mu + \alpha_i + \varepsilon_{ijk}$$

where,

$$i = 1, 2, 3; \quad j = 1, 2, \cdots, 9; \quad k = 16, 17, \cdots, 25$$

$\mu =$ The grand mean for all data points, after day 15.

$\alpha_1 =$ Effects of the treatment A.

$\alpha_2 =$ Effects of the treatment B.

$\alpha_3 =$ Effects of the treatment C.

Then, by using the *lm* function, we get the following estimates of the average for treatment A, B and C respectively :

	Treatment A	Treatment B	Treatment C
Estimates ($\hat{\mu_i}$)	74.40×10^4	114.76×10^4	176.60×10^4

Table 3.3: Estimates of treatments.

and the corresponding ANOVA table is given by,

Response	DF	Sum Squares	Mean Squares	F-value	p-value
Treatment	2	15.7×10^{12}	78.50×10^{11}	128.74	$< 2.2e^{-16}$
Residuals	87	53.05×10^{11}	60.98×10^9		

Table 3.4: Traditional One-way ANOVA table for $k > 15$

Residual standard error: 246900 on 87 degrees of freedom.
i.e. $\hat{\sigma}^2 = 60.98 \times 10^9$

Because of the small $p-$ value and the calculated $F_{(2,87)} = 128.74$ which is greater than the tabulated $F_{(2,87)}(.05)$, made us to conclude that there is significant difference between the treatment effects.

Here, for the model (3.1), the assumption of independence of errors is not satisfied because within each meso the observations are not independent. Their correlation is larger for observations coming from the same meso than for two observations coming from different mesos. So, we try to define another model by treating the meso group effect as a random effect which is known as the *mixed effects* model.

4

Mixed Effects Model

Now, we try to change the model (3.1) to motivate the random effects model in our dataset for $k > 15$. We write

$$Y_{ijk} = \mu + \alpha_i + b_{ij} + \varepsilon_{ijk} \tag{4.1}$$

for $i = 1, \quad j = 1, 2, 3; \qquad$ for $i = 2, \quad j = 4, 5, 6; \qquad$ for $i = 3, \quad j = 7, 8, 9$ and, $k = 16, 17, \cdots, 25$

where, μ is the overall mean for the entire treatments, *i.e.,* the grand mean, α_i is a fixed variable representing the deviation from the grand mean for the i^{th} treatment, b_{ij} is a random variable representing the effect of j^{th} meso group on the treatment i and, ε_{ijk} is a random variable representing the deviation of a response variable for k^{th} day of meso group j on treatment i from the mean effect of treatment i. To be a statistical model, the distribution of the random variables b_{ij} and ε_{ijk} must be specified. We assume both of these variables are independent and normally distributed with mean zero and variances τ^2 and σ^2 respectively. That is,

$$b_{ij} \sim \ N(0, \tau^2), \qquad \varepsilon_{ijk} \sim \ N(0, \sigma^2)$$

Where b_{ij}'s are called *random* effects because they are incorporated with the particular experimental units − mesos in our case − that are selected from the population of interest. They are *effects* because they represent a deviation from the treatment mean. That is, the "effect" of the treatment i is to shift from the treatment mean $\mu + \alpha_i$ to $\mu + \alpha_i + b_{ij}$ [Pinheiro and Bates, 2000]. Since the model (4.1) has both fixed and random effects, it is

known as *Mixed Effects* model or *Mixed* model.

4.1 Statistical Analysis of the Model (4.1)

When the above mentioned assumptions are fullfilled, then we can write

$$\bar{Y}_{...} = \text{overall mean} = \frac{\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} Y_{ijk}}{IJK}$$

$$\bar{Y}_{i..} = \text{mean of the } i^{th} \text{ treatment} = \frac{\sum_{j=1}^{J}\sum_{k=1}^{K} Y_{ijk}}{JK} \quad \text{and,}$$

$$\bar{Y}_{ij.} = \text{mean of the } j^{th} \text{ meso for the treatment } i = \frac{\sum_{k=1}^{K} Y_{ijk}}{K}$$

The parameters μ, τ^2 and σ^2 in model (4.1) are estimated by the principle of least squares on minimizing the error sum of squares given by

$$E = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} \varepsilon_{ijk}^2 = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} (Y_{ijk} - \mu - \alpha_i - b_{ij})^2 = 0$$

Proceeding the similar way as described in the model (3.1), after solving the normal equations, we get

$$\hat{\mu} = \frac{\sum_{j=1}^{J}\sum_{k=1}^{K} Y_{ijk}}{N} = \bar{Y}_{...} \tag{4.2}$$

$$\hat{\alpha}_i = \frac{1}{JK}\sum_{j=1}^{J}\sum_{k=1}^{K} Y_{ijk} - \hat{\mu} = \bar{Y}_{i..} - \bar{Y}_{...} \tag{4.3}$$

$$\hat{b}_{ij} = \frac{1}{K}\sum_{k=1}^{K} Y_{ijk} - \frac{1}{JK}\sum_{j=1}^{J}\sum_{k=1}^{K} Y_{ijk} = \bar{Y}_{ij.} - \bar{Y}_{i..} \tag{4.4}$$

and, substituting above values in (4.1), we get

$$Y_{ijk} - \bar{Y}_{...} = (\bar{Y}_{i..} - \bar{Y}_{...} + \bar{Y}_{ij.} - \bar{Y}_{i..} + Y_{ijk} - \bar{Y}_{ij.})$$

We introduce the error term ε_{ijk} so that both the sides are equal.

$$\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} (Y_{ijk} - \bar{Y}_{...})^2 = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} (\bar{Y}_{i..} - \bar{Y}_{...} + \bar{Y}_{ij.} - \bar{Y}_{i..} + Y_{ijk} - \bar{Y}_{ij.})^2$$

$$\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(Y_{ijk}-\bar{Y}_{...})^2 = JK\sum_{i=1}^{I}(\bar{Y}_{i..}-\bar{Y}_{...})^2 + K\sum_{i=1}^{I}\sum_{j=1}^{J}(\bar{Y}_{ij.}-\bar{Y}_{i..})^2 + \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(Y_{ijk}-\bar{Y}_{ij.})^2$$

Here, the product terms disappear because the set up is balanced.

$$TSS = SSB + SSR + SSW$$

Where,

$$TSS = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(Y_{ijk}-\bar{Y}_{...})^2 = \text{Total sum of squares.}$$

$$SSB = JK\sum_{i=1}^{I}(\bar{Y}_{i..}-\bar{Y}_{...})^2 = \text{Sum of squares due to the treatments}$$

$$SSR = K\sum_{i=1}^{I}\sum_{j=1}^{J}(\bar{Y}_{ij.}-\bar{Y}_{i..})^2 = \text{Sum of squares due to random meso groups.}$$

$$SSW = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(Y_{ijk}-\bar{Y}_{ij.})^2 = \text{Sum of squares due to error terms.}$$

Now, We know the model (4.1) is

$$Y_{ijk} = \mu + \alpha_i + b_{ij} + \varepsilon_{ijk} \tag{4.5}$$

$$\bar{Y}_{ij.} = \mu + \alpha_i + b_{ij} + \bar{\varepsilon}_{ij.} \tag{4.6}$$

Subtracting equation (4.6) from equation (4.5), we get

$$(Y_{ijk}-\bar{Y}_{ij.}) = (\varepsilon_{ijk}-\bar{\varepsilon}_{ij.})$$

$$\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(Y_{ijk}-\bar{Y}_{ij.})^2 = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(\varepsilon_{ijk}-\bar{\varepsilon}_{ij.})^2$$

$$SSW = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(\varepsilon_{ijk}-\bar{\varepsilon}_{ij.})^2$$

$$
\begin{aligned}
E(SSW) &= \sum_{i=1}^{I}\sum_{j=1}^{J} E\{\sum_{k=1}^{K}(\varepsilon_{ijk} - \bar{\varepsilon}_{ij\cdot})^2\} \\
&= \sum_{i=1}^{I}\sum_{j=1}^{J}(K-1)\sigma^2; \qquad \text{since, } \varepsilon_{ijk} \sim N(0,\sigma^2) \\
&= IJ(K-1)\sigma^2 \\
\hat{\sigma}^2 &= \frac{SSW}{IJ(K-1)}
\end{aligned}
$$

Again, using the model (4.1), we have

$$
\bar{Y}_{ij\cdot} = \mu + \alpha_i + b_{ij} + \bar{\epsilon}_{ij\cdot} \tag{4.7}
$$

$$
\bar{Y}_{i\cdot\cdot} = \mu + \alpha_i + \bar{b}_{i\cdot} + \bar{\epsilon}_{i\cdot\cdot} \tag{4.8}
$$

Subtracting equation (4.7) from equation (4.8), we get

$$
(\bar{Y}_{ij\cdot} - \bar{Y}_{i\cdot\cdot}) = (b_{ij} - \bar{b}_{i\cdot}) + (\bar{\epsilon}_{ij\cdot} - \bar{\epsilon}_{i\cdot\cdot})
$$

$$
\begin{aligned}
\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(\bar{Y}_{ij\cdot} - \bar{Y}_{i\cdot\cdot})^2 &= \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}\{(b_{ij} - \bar{b}_{i\cdot}) + (\bar{\epsilon}_{ij\cdot} - \bar{\epsilon}_{i\cdot\cdot})\}^2 \\
SSR &= \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}\{(b_{ij} - \bar{b}_{i\cdot}) + (\bar{\epsilon}_{ij\cdot} - \bar{\epsilon}_{i\cdot\cdot})\}^2 \\
E(SSR) &= K\ E\sum_{i=1}^{I}\sum_{j=1}^{J}\{(b_{ij} - \bar{b}_{i\cdot})^2 + (\bar{\epsilon}_{ij\cdot} - \bar{\epsilon}_{i\cdot\cdot})^2 \qquad (4.9) \\
&\quad + 2\ (b_{ij} - \bar{b}_{i\cdot})(\bar{\epsilon}_{ij\cdot} - \bar{\epsilon}_{i\cdot\cdot})\}
\end{aligned}
$$

the expectation of the cross product term vanishes since they are independent of each other. Since $b_{ij} \sim N(0,\tau^2)$ and $\bar{\epsilon}_{ij\cdot} \sim N(0, \frac{\sigma^2}{K})$, we get

$$
\begin{aligned}
E(SSR) &= K\ I\ E[\sum_{j=1}^{J}(b_{ij} - \bar{b}_{i\cdot})^2] + \ K\ I\ E[\sum_{j=1}^{J}(\bar{\epsilon}_{ij\cdot} - \bar{\epsilon}_{i\cdot\cdot})^2] \\
E(SSR) &= K\ I(J-1)\tau^2 + \ K\ I(J-1)\frac{\sigma^2}{K} \\
K\ I(J-1)\hat{\tau}^2 &= SSR - \ I(J-1)\hat{\sigma}^2
\end{aligned}
$$

$$\hat{\tau}^2 = \frac{SSR}{K\ I(J-1)} - \frac{\hat{\sigma}^2}{K}$$

Again, using the model (4.1), we get

$$\bar{Y}_{i\cdot\cdot} = \mu + \alpha_i + \bar{b}_{i\cdot} + \bar{\epsilon}_{i\cdot\cdot} \tag{4.10}$$

$$\bar{Y}_{\cdots} = \mu + \bar{\alpha} + \bar{b} + \bar{\epsilon}_{\cdots} \tag{4.11}$$

Subtracting equation (4.10) from equation (4.11), we get

$$\bar{Y}_{i\cdot\cdot} - \bar{Y}_{\cdots} = (\alpha_i - \bar{\alpha}) + (\bar{b}_{i\cdot} - \bar{b}) + (\bar{\varepsilon}_{i\cdot\cdot} - \bar{\varepsilon}_{\cdots})$$

$$\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(\bar{Y}_{i\cdot\cdot} - \bar{Y}_{\cdots})^2 = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}\{(\alpha_i - \bar{\alpha}) + (\bar{b}_{i\cdot} - \bar{b}) + (\bar{\varepsilon}_{i\cdot\cdot} - \bar{\varepsilon}_{\cdots})\}^2$$

$$SSB = J\ K\sum_{i=1}^{I}\{(\alpha_i - \bar{\alpha}) + (\bar{b}_{i\cdot} - \bar{b}) + (\bar{\varepsilon}_{i\cdot\cdot} - \bar{\varepsilon}_{\cdots})\}^2.$$

Since the $b_{ij} \sim N(0, \tau^2)$ and $\bar{\epsilon}_{ij\cdot} \sim N(0, \frac{\sigma^2}{K})$, and, b_{ij} and ϵ_{ijk} are independent, we get

$$E(SSB) = J\ K\{\ E\sum_{i=1}^{I}(\alpha_i - \bar{\alpha})^2 + \ E\sum_{i=1}^{I}(\bar{b}_{i\cdot} - \bar{b})^2 + \ E\sum_{i=1}^{I}(\bar{\varepsilon}_{i\cdot\cdot} - \bar{\varepsilon}_{\cdots})^2\}$$

$$= J\ K\sum_{i=1}^{I}(\alpha_i - \bar{\alpha})^2 + \ J\ K(I-1)\frac{\tau^2}{J} + \ J\ K(I-1)\frac{\sigma^2}{JK}$$

$$E(SSB/(I-1)) = \frac{J\ K}{(I-1)}\sum_{i=1}^{I}(\alpha_i - \bar{\alpha})^2 + \ K\tau^2 + \sigma^2$$

$$\frac{SSB}{(I-1)} = \hat{\sigma}^2 + K\hat{\tau}^2 + \frac{J\ K}{(I-1)}\sum_{i=1}^{I}(\alpha_i - \bar{\alpha})^2$$

$$\frac{SSB}{(I-1)} = \hat{\sigma}^2 + K\hat{\tau}^2 + \frac{J\ K}{(I-1)}\sum_{i=1}^{I}\varphi(\alpha); \quad \text{where } \varphi(\alpha) = \sum_{i=1}^{I}(\alpha_i - \bar{\alpha})^2$$

Under the H_0, the sum of squares due to the treatments is,

$$\frac{SSB}{(I-1)} = \hat{\sigma}^2 + K\hat{\tau}^2$$

The above statistical analysis is very elegantly presented in the following anova table.

Source of variation	Degree of freedom	Sum of Squares	Mean Squares	F-value	p-value
Treatments	$(I-1)$	SSB	$SSB/(I-1)$	$\frac{MSB}{MSE}$	
Meso groups	$I(J-1)$	SSR	$SSR/I(J-1)$	$\frac{MSR}{MSE}$	
Residuals	$(N-I)$	SSE	$SSE/(N-I)$		
Total	$(N-1)$	TSS			

Table 4.1: ANOVA table for the *Mixed Model*.

When we apply the *lme* function in the dataset for $k > 15$, we get the following estimations for the treatments A, B and C:

Treatments	Estimates	$p-$ value
A	74.40×10^4	6×10^{-4}
B	114.76×10^4	1×10^{-4}
C	176.60×10^4	0

Table 4.2: Estimates of treatments.

and, the standard deviation of random effects due to the meso groups and error term are:

	Standard deviation due to meso groups ($\hat{\tau}$)	Standard deviation due to error term ($\hat{\sigma}$)
Estimates	18.39×10^4	19.40×10^4

Table 4.3: Estimates of random effects.

and the corresponding ANOVA table is given by,

Source	Numerator degree of freedom	Denominator degree of freedom	$F-$ ratio	$p-$ value
Intercept	1	81	354.83	$<.0001$
Treatment	2	6	20.896	< 0.002

Table 4.4: Mixed model ANOVA table.

If we look at the Table 3.3 and the Table 4.2 given by the model (3.1) and model (4.1) respectively, we found that there are no differences in the estimates of the treatments. Whereas, the residual standard error is significantly smaller in the model (4.1) than in the model (3.1). Because, in the model (3.1), there is no effect of meso, and the ε_{ijk} have to account for all variability within each treatment. Therefore variance of ε_{ijk} is large in this model. In this model, the assumption of independence is violated because the correlation is larger for observations coming from the same meso than for two observations coming from different mesos. Therefore, we do not prefer this model.

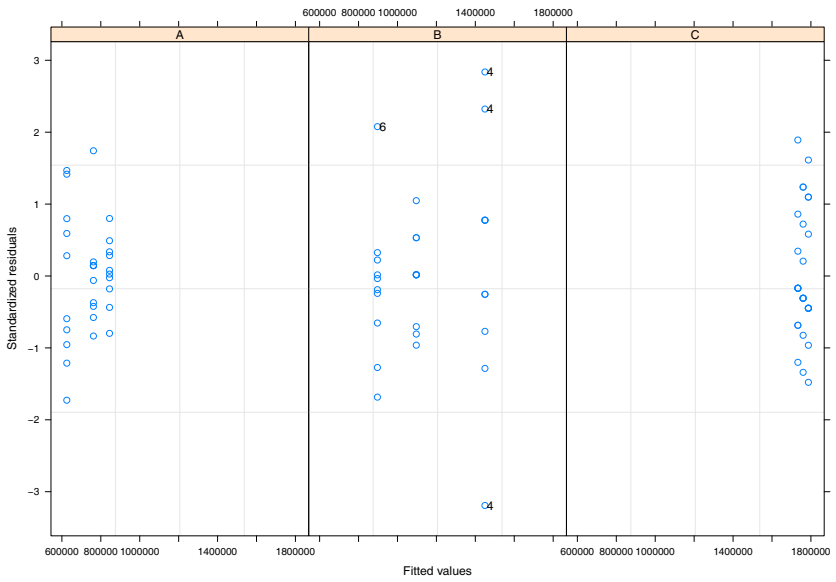

Figure 4.1: Scatter plots of standardized residuals versus fitted values for the model (4.1) by the treatment

But, in the model (4.1), there accounts the meso effects and hence the variance of ε_{ijk} becomes smaller because it splits into the meso effects variance also. If we plot the standardized residuals versus fitted values by treatments for the model (4.1), as shown in the Figure 4.1, we find that the variability in the virus growth data is greater among the treatment B than among the treatment A and C. Within each treatment, the variability seems to be constant [Pinheiro and Bates, 2000].

To analysis different variances by treatments for the within-group error, we use the *varIdent* function to model the heteroscedascity of the within-group error [Pinheiro and Bates, 2000] and we get the following results.

Treatment A	Treatment B	Treatment C
1	1.523146	1.125218

Table 4.5: Parameter estimates of variance by treatments.

Figure 4.2 shows the heteroscedastic fit of model (4.1), that is, the re-examining plots of standardized residuals versus fitted values by treatments.

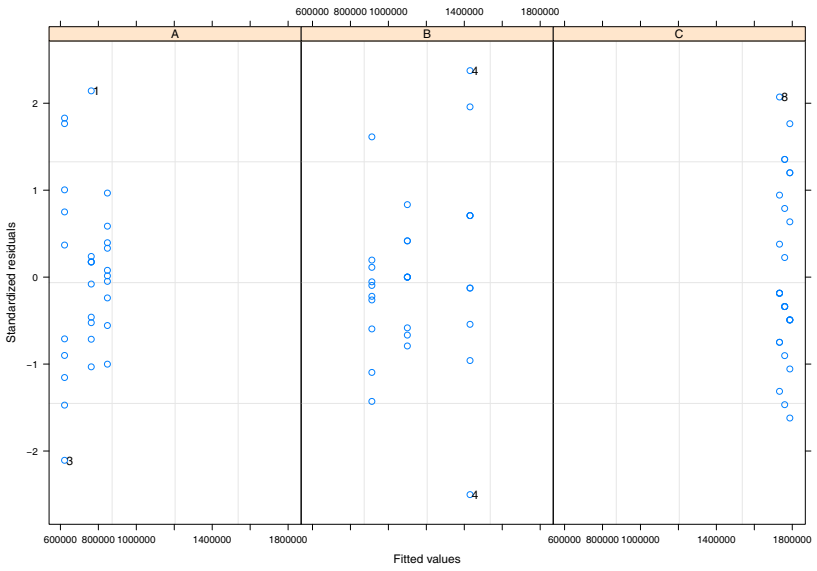

Figure 4.2: Scatter plots of standardized residuals versus fitted values for the heteroscedastic fit of model (4.1) by treatments

The standardized residuals in each treatment seem to have the same variability [Pinheiro and Bates, 2000]. Table 4.1 explains that the standard error for the treatment B and treatment C is about respectively 152% and 113% of that for the treatment A. If we apply an ANOVA test between the model (4.1) and the heteroscedastic fit of model (4.1), we find there is no significance between these two models. The output is given in the following table.

Model	df	AIC	BIC	Loglik	L.Ratio	$p-$value
mod2	5	2399.51	2411.84	-1194.759		
mod2.var	7	2398.31	2415.57	-1192.15	5.20	0.0741

Table 4.6: ANOVA table of model (4.1) and it's heteroscedastic fitted model.

5

Nonlinear Mixed Effects Model

In our previous chapters, we analysed the dataset for $k > 15$ and found significant differences between the treatment effects.

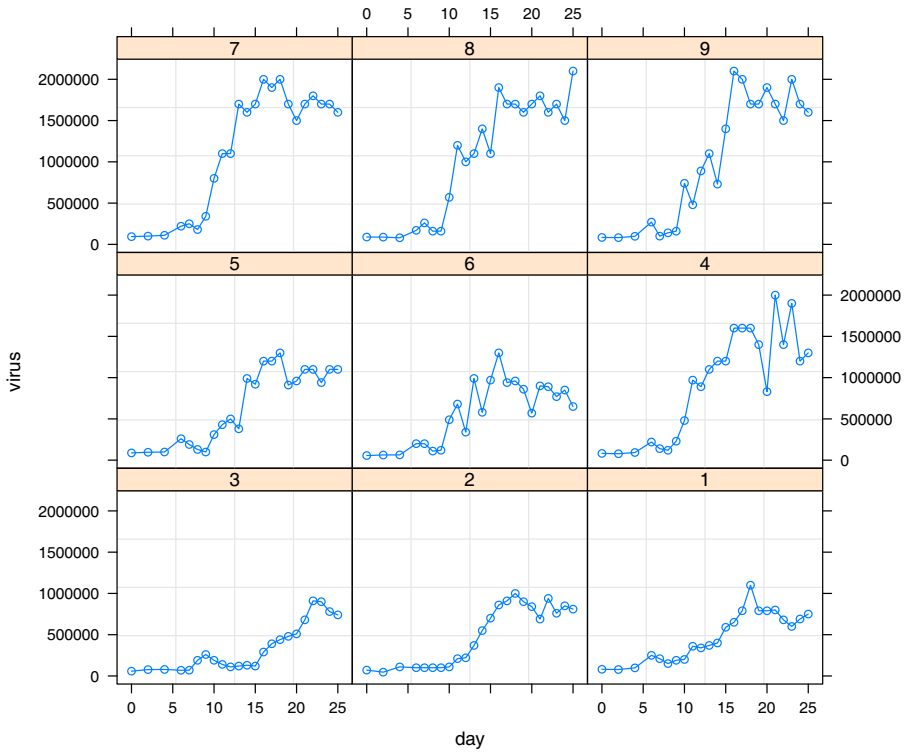

Figure 5.1: Plot for individual random meso groups of three different treatments

In this chapter, we look the whole dataset, that is for $k = 1, 2, \cdots, 25$ and try to find out the best fitted model for the dataset. As shown in Figure 5.1, if we plot a graph of the response variable (number of virus) versus the time period (day) for the random individual meso groups, we find a individual growth curve for each meso group. Where we can see for each meso group, the curve is approximately stationary for some begining days of experiment and gradually starts increasing and accelerates over the time period. Finally, it reaches at its maximum height and keeps this level approximately stationary to the end of time period. Generally, such a type of the curve nature is shown by the *sigmoid* curve which exhibit a progression from small beginnings that accelerates and approaches a climax over time. There are many functions that displays a *sigmoid* form [Wikipedia/Sigmoid]. A *logistic* curve is a common *sigmoid* curve and in such a curve the initial stage of growth is approximately exponential; then, as saturation begins, the growth slows, and at maturity, growth stops [Wikipedia/Logistic].

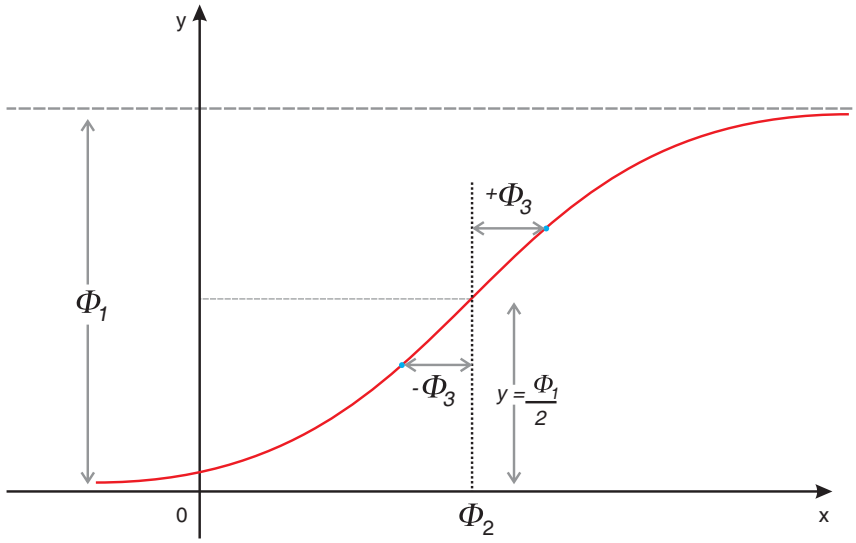

Figure 5.2: The simple logistic model showing the parameters ϕ_1, the horizontal asymptote as $x \to \infty$; ϕ_2, the value of x for which $y = \phi_1/2$; and ϕ_3, a scale parameter on the x-axis.

Figure 5.2 shows a *logistic* curve with three parameters ϕ_1, ϕ_2 and, ϕ_3 where ϕ_1 represents the maximum growth when the curve is at maturity; ϕ_2 represents the point when the slow down begins or the point where the

growth reaches half of its maximum and; ϕ_3 represents the rate of the growth at $x = \phi_2$. Which is approximately equivalent to the nature of the meso curve given by our dataset where at first phase, there is slow increasing of virus, then at second phase it gradually starts increasing, and accelarates and at third phase it becomes mature and keeps approximately the similar phase level over the time period. Such a *logistic* curve is well explained by the *logistic growth* model and is given by

$$y_{jk} = f(\boldsymbol{\phi}, x) + \epsilon_{jk} = \frac{\phi_1}{1 + e^{-(x-\phi_2)/\phi_3}} + \epsilon_{jk} \tag{5.1}$$

[Pinheiro and Bates, 2000] for $j = 1, 2, \cdots, J$ and $k = 1, 2, \cdots, K$ with

ϕ_1 :	the asymptote, gives the maximum height of the curve that reaches over the time. When $\phi_3 > 0$ then ϕ_1 is the horizontal asymptote as $x \to \infty$ and 0 is the horizontal asymptote as $x \to -\infty$.
ϕ_2 :	the inflex, explains time when the curve reaches half of its maximum level, that is, when $y = \phi_1/2$.
ϕ_3 :	the scale, gives the rate of change at $x = \phi_2$
y_{jk} :	The growth pattern at time x.
ϵ_{jk} :	within-group error associated with y_{jk} or the additive noise of the model and is normal.
$f(\phi, x)$:	average response for group j at time x, conditional on ϕ_1, ϕ_2, ϕ_3.

The above model (5.1) is linear in parameter ϕ_1 but nonlinear in parameters ϕ_2 and ϕ_3. So it is a nonlinear model.

where $y = y_{jk} = \frac{1}{2}\phi_1$ when $x = \phi_2$ such that $y_{jk} = f(\boldsymbol{\phi}, x) = \frac{\phi_1}{1 + e^{-(\phi_2-\phi_2)/\phi_3}} = \frac{\phi_1}{1 + e^{-0}} = \frac{1}{2}\phi_1$, which is half of the asymptote height and is numerically stable point for study of the curve nature. Similarly, if this point shifts from ϕ_2 to $\phi_2 \pm \phi_3$, *i.e.,* when $x = \phi_2 \pm \phi_3$, we get $f(\boldsymbol{\phi}, x) = \frac{\phi_1}{1 + e^{-1}} \simeq \frac{3}{4}\phi_1$ and $f(\boldsymbol{\phi}, x) = \frac{\phi_1}{1 + e^{1}} \simeq \frac{1}{4}\phi_1$. Now, the rate of change (slope) of the asymptote with respect to the $X-$axis is given by $\frac{dy}{dx}$ such that

$$\frac{dy}{dx} = f'(\boldsymbol{\phi}, x) = \frac{d}{dx}\left[\frac{\phi_1}{1 + e^{-(x-\phi_2)/\phi_3}}\right]$$

$$
\begin{aligned}
&= \phi_1 \left[\frac{d(1+e^{-(x-\phi_2)/\phi_3})^{-1}}{d(1+e^{-(x-\phi_2)/\phi_3})} \cdot \frac{d(1+e^{-(x-\phi_2)/\phi_3})}{dx} \right] \\
&= \phi_1 \left[-1\ (1+e^{-(x-\phi_2)/\phi_3})^{-2} \cdot \frac{-1}{\phi_3}\ e^{-(x-\phi_2)/\phi_3} \right] \\
&= \frac{1}{\phi_3} \cdot \frac{\phi_1}{1+e^{-(x-\phi_2)/\phi_3}} \cdot \frac{e^{-(x-\phi_2)/\phi_3}}{1+e^{-(x-\phi_2)/\phi_3}} \\
&= \frac{1}{\phi_3} \cdot y(x) \cdot \frac{e^{-(x-\phi_2)/\phi_3}}{1+e^{-(x-\phi_2)/\phi_3}}
\end{aligned}
$$

when $x = \phi_2$,

$$
\left[\frac{dy}{dx}\right]_{x=\phi_2} = f'(\boldsymbol{\phi}, x=\phi_2) = \frac{1}{\phi_3} \cdot \frac{\phi_1}{2} \cdot \frac{e^{-0}}{1+e^{-0}} = \frac{1}{4}\frac{\phi_1}{\phi_3}
$$

That is, the slope of curve at $x = \phi_2$ is $\dfrac{\phi_1}{4\ \phi_3}$ and, it depends on ϕ_3. Therefore the slope decreases as ϕ_3 increases or vice versa.

In our case, since there are both fixed and random effects, it becomes a nonlinear mixed effects models with $j = 1, 2, \cdots, 9$ and $k = 1, 2, \cdots, 25$. So for the given dataset, we define the parameters as

$$
\boldsymbol{\phi} = \begin{bmatrix} \phi_{1j} \\ \phi_{2j} \\ \phi_{3j} \end{bmatrix} \qquad \text{for } j = 1, 2, \cdots, 9.
$$

$$
\text{such that} \qquad \phi_{1j} = \beta_1^{(i)} + b_{1j} \qquad \phi_{1j} = \beta_2^{(i)} + b_{2j} \qquad \phi_{1j} = \beta_3^{(i)} + b_{3j}
$$

where $\beta_1^{(i)}, \beta_2^{(i)}, \beta_3^{(i)}$ are the fixed growth parameters for three treatments, $i = A, B, C$. That is, for treatments A, B and C, the fixed growth parameter $\boldsymbol{\beta} = \begin{bmatrix} \beta_1^{(A)} & \beta_2^{(A)} & \beta_3^{(A)} & \beta_1^{(B)} & \beta_2^{(B)} & \beta_3^{(B)} & \beta_1^{(C)} & \beta_2^{(C)} & \beta_3^{(C)} \end{bmatrix}^T$ and, $\boldsymbol{b} = \begin{bmatrix} b_{1j} \\ b_{2j} \\ b_{3j} \end{bmatrix}$ is random parameter due to the meso group for $j = 1, 2, \cdots, 9$. It can be summarized as

$$
\boldsymbol{\phi_j} = \boldsymbol{D_j}\boldsymbol{\beta} + \ \boldsymbol{b_j} \tag{5.2}
$$

where $[\boldsymbol{D_j}]_{(3\times 9)}$ is a design matrix [Pinheiro and Bates, February 25, 2005] such that

when $j = 1, 2, 3$ then

$$\boldsymbol{D_j} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

when $j = 4, 5, 6$ then

$$\boldsymbol{D_j} = \begin{bmatrix} 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \end{bmatrix}$$

when $j = 7, 8, 9$ then

$$\boldsymbol{D_j} = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

5.1 Parameter Estimation and Selection

Treatment	Meso	Estimation of Parameters $\phi_1,\ \phi_2,\ \phi_3$					
(i)	(j)	Fixed effects (β)			Random effects (b_j)		
	1	$\beta_1^{(A)}$	$\beta_2^{(A)}$	$\beta_3^{(A)}$	b_{11}	b_{21}	b_{31}
A	2	"	"	"	b_{12}	b_{22}	b_{32}
	3	"	"	"	b_{13}	b_{23}	b_{33}
	4	$\beta_1^{(B)}$	$\beta_2^{(B)}$	$\beta_3^{(B)}$	b_{14}	b_{24}	b_{34}
B	5	"	"	"	b_{15}	b_{25}	b_{35}
	6	"	"	"	b_{16}	b_{26}	b_{36}
	7	$\beta_1^{(C)}$	$\beta_2^{(C)}$	$\beta_3^{(C)}$	b_{17}	b_{27}	b_{37}
C	8	"	"	"	b_{18}	b_{28}	b_{38}
	9	"	"	"	b_{19}	b_{29}	b_{39}

Table 5.1: Estimations of Parmameters $\phi_1,\ \phi_2,\ \phi_3$ for *Nonlinear Mixed Model.*

When we begin to estimate the parameters for each meso by using the relation (5.2), we get three estimates of fixed effects and three estimates of random effects for the parameters ϕ_1, ϕ_2, ϕ_3 of an an individual meso. From the table 5.1, it is clearly seen that the fixed effects are similar for mesos in each treatment, that is, the fixed effects are different by treatment and, the random effects are totally different for different mesos. Hence, the model (5.1) gives both fixed effects and random effects for our dataset. We build up several models to find out a best fitted model by determining the characterstics of both fixed and the random effects of a given model (5.1). We describe procedures based on information statistics for comparing different structures of random effects component. These procedures are determining which parameters in the model should be mixed effects and which should be purely fixed effects. Most of the methods applied in this study, are based on nonlinear mixed effects methods and classes for S-plus [Pinheiro and Bates, 2000] in **R**. Table 5.2 gives an individual estimation of the fixed and the random effects for parameters ϕ_1, ϕ_2, ϕ_3 for each meso where we can see that all three estimates seem to vary with individual.

Meso	$\hat{\boldsymbol{\phi}}_1$	$\hat{\boldsymbol{\phi}}_2$	$\hat{\boldsymbol{\phi}}_3$
1	79.49×10^4	11.94	2.79
2	86.61×10^4	13.06	1.31
3	116.92×10^4	20.15	4.27
4	147.83×10^4	11.00	1.66
5	110.54×10^4	12.07	1.74
6	86.81×10^4	10.19	1.50
7	176.34×10^4	10.48	1.35
8	173.93×10^4	11.32	2.03
9	180.17×10^4	12.28	1.90
Residual standard error $= 17.11 \times 10^4$			

Table 5.2: Estimations of parmameters ϕ_1, ϕ_2, ϕ_3 for individual meso.

The plot of the individual confidence intervals for the estimates given in the Table 5.2 is shown in Figure 5.3.

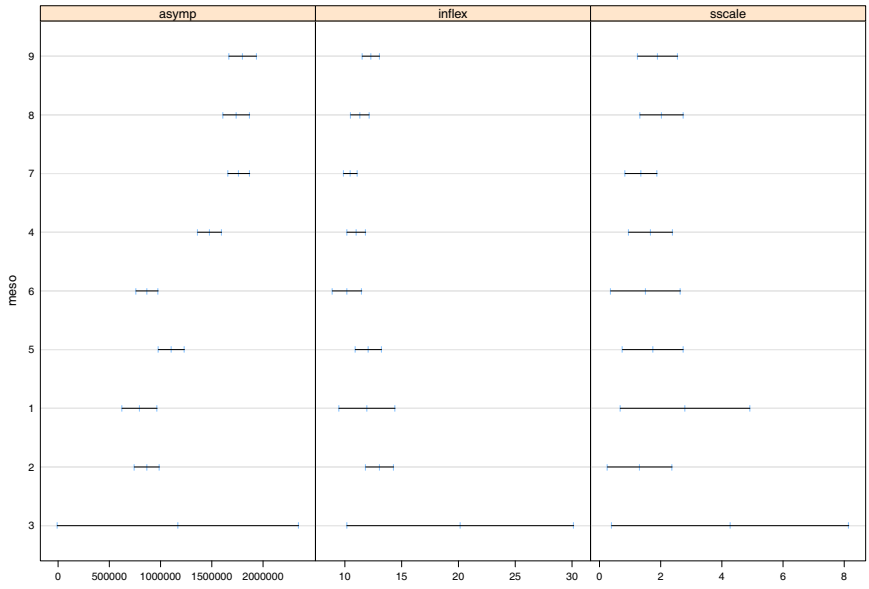

Figure 5.3: Ninety-five percent confidence intervals for an individual meso for the three different parameters of given model

These confidence intervals, as shown in the Figure 5.3, indicate that the parameter asymptote ϕ_1 has the greatest inter-individual variability, followed by the parameter inflex ϕ_2 whereas the parameter scale ϕ_3 has not such a variability with individual, as all the associated confidence intervals overlap. These results suggest that ϕ_1 and ϕ_2 can be treated as mixed effects and ϕ_3 as a purely fixed effect [Pinheiro and Bates, 2000]. We test it again by the *nlme* method also.

We now consider the *nlme* function to find more adequate result about the random effect and the fixed effect parameters for the model 5.1. For this purpose, we start a model with random effects for all parameters and then examine the fitted object to decide which, if any, of the random effects can be eliminated from the model. Since b_j are assumed to be independent and normal with mean 0 and variance-covariance matrix Ψ, the number of parameters to estimate will increase with the square of the number of random effects. In such cases where the number of random effects is large

relative to the number of individuals, it is generally recommended to use a diagonal variance-covariance matrix Ψ initially, to prevent convergence problems with an overparametrized model [Pinheiro and Bates, 2000]. We apply this approch in our dataset and we get following information.

Standard deviation of Random effects				Estimation of Fixed effects ($\hat{\beta}$)		
ϕ_1	ϕ_2	ϕ_3	Residual	$\hat{\phi_1}$	$\hat{\phi_2}$	$\hat{\phi_3}$
42.48×10^4	1.74	0.003	17.08×10^4	123.90×10^4	12.1	1.8
			Standard Error	14.429×10^4	0.62	0.14

Table 5.3: Estimation of fixed effects and the standard deviation of random effects for parameters ϕ_1, ϕ_2, ϕ_3 given by the model 4.

The small estimated value of the standard deviation of the parameter scale ϕ_3 suggests us that this term could not be implementated as a random effect parameter in our model. Whereas the remaining estimated standard deviation suggest that other radom effects must be kept in our model. To assure these facts, we use ANOVA test between the model that contains the parameter inflex ϕ_3 random effect (model 4) and the model that does not contain the parameter scale ϕ_3 random effect (model 5) [Pinheiro and Bates, 2000]. We get the following outputs.

Model	DF	AIC	BIC	LogLik	Test	L.Ratio	$p-$value
Model 4	7	5650.18	5673.51	-2818.09			
Model 5	6	5648.18	5668.58	-2818.09	4 Vs 5	0.0002	0.99

Table 5.4: Comparison of model 4 with model 5 using an ANOVA

The two fitted models, in the table 5.4, give nearly identical log-likelihoods with higher $p-$ value, confirming us that scale ϕ_3 can be treated as a purely fixed effect [Pinheiro and Bates, 2000]. Generally, if the fixed effects are unknown for the treatments then it is calculated by providing the starting values for parameters ϕ_1, ϕ_2, ϕ_3 [Pinheiro and Bates, 2000]. We remove unnecessary fixed effect parameters by using an ANOVA test of different models with different number of fixed effects estimates. The Following Ta-

ble 5.5 gives the different models with their corresponding number of fixed effects and random effects parameters.

Model	No. of parameters	Random parameters	Fixed parameters	AIC	BIC	Log-Lik.
4	7	b_1, b_2, b_3	$\beta_1, \beta_2, \beta_3$	5650.183	5673.512	-2818.091
5	6	b_1, b_2	$\beta_1, \beta_2, \beta_3$	5648.183	5668.179	-2818.091
6	5	b_1	$\beta_1, \beta_2, \beta_3$	5657.688	5674.351	-2823.844
7	13	b_1, b_2, b_3	$\beta_1^{(A)}, \beta_1^{(B)}, \beta_1^{(C)}$ $\beta_2^{(A)}, \beta_2^{(B)}, \beta_2^{(C)}$ $\beta_3^{(A)}, \beta_3^{(B)}, \beta_3^{(C)}$	5634.924	5678.249	-2804.462
8	12	b_1, b_2	$\beta_1^{(A)}, \beta_1^{(B)}, \beta_1^{(C)}$ $\beta_2^{(A)}, \beta_2^{(B)}, \beta_2^{(C)}$ $\beta_3^{(A)}, \beta_3^{(B)}, \beta_3^{(C)}$	5632.924	5672.917	-2804.462
9	10	b_1, b_2	$\beta_1^{(A)}, \beta_1^{(B)}, \beta_1^{(C)}$ $\beta_2^{(A)}, \beta_2^{(B)}, \beta_2^{(C)}$ β_3	5630.621	5663.948	-2805.310
10	8	b_1, b_2	$\beta_1^{(A)}, \beta_1^{(B)}, \beta_1^{(C)}$ β_2, β_3	5633.427	**5660.088**	-2808.713
11	11	b_1	$\beta_1^{(A)}, \beta_1^{(B)}, \beta_1^{(C)}$ $\beta_2^{(A)}, \beta_2^{(B)}, \beta_2^{(C)}$ $\beta_3^{(A)}, \beta_3^{(B)}, \beta_3^{(C)}$	5637.303	5673.963	-2807.651
12	9	b_1	$\beta_1^{(A)}, \beta_1^{(B)}, \beta_1^{(C)}$ $\beta_2^{(A)}, \beta_2^{(B)}, \beta_2^{(C)}$ β_3	5635.613	5665.608	-2808.807
13	7	b_1	$\beta_1^{(A)}, \beta_1^{(B)}, \beta_1^{(C)}$ β_2, β_3	5642.773	5666.102	-2814.386
14	6	b_1, b_3	$\beta_1, \beta_2, \beta_3$	5659.688	5679.685	-2823.844

Table 5.5: Different models with different number of estimates of parameters with AIC, BIC and log-likelihood.

Here, the values of Akaike Information Criterian (AIC) and Baysian Information Criterion (BIC) are the model comparison criteria, and evaluated as

$$AIC = -2logLik + 2n_{par}$$
$$BIC = -2logLik + n_{par}log(N)$$

where n_{par} denotes the number of parameters in the model and N the total number of observations used to fit the model [Pinheiro and Bates, 2000].

5.2 Model Selection

The best fitted model is selected with the lowest *Bayesian Information Criterion* abbreviated by BIC , under the criterion "smaller is better" [Pinheiro and Bates, 2000]. In our case, when we analysis the output given by the Table 5.5, we can see that the model 10 has the lowest **BIC** value with the least numbers of estimates. So, in our study we prefer the model 10 as a best fitted model and it has mixed effects in the parameter asymptote ϕ_1 and the parameter inflex ϕ_2. But the parameter scale ϕ_3 has purely a fixed effect. All three parameters details for three treatments are presented in the Table 5.6 below.

Treatments (i)	Parameters ϕ_1	ϕ_2	ϕ_3
For $i = A$; $j = 1, 2, 3.$	$\beta_1^{(A)} + b_{1j}$	$\beta_2 + b_{2j}$	β_3
For $i = B$; $j = 4, 5, 6.$	$\beta_1^{(B)} + b_{1j}$	$\beta_2 + b_{2j}$	β_3
For $i = C$; $j = 7, 8, 9.$	$\beta_1^{(C)} + b_{1j}$	$\beta_2 + b_{2j}$	β_3
Residual $= 17.09 \times 10^4$			

Table 5.6: Parameter detail information for all treatments

From above Table 5.6, we can say that the selected model has

- significance in the parameter asymptote ϕ_1 for all three treatments due to the variability in the fixed effects and the random effects and hence

the parameter asymptote has a different mixed effects for different treatments.

- significance in the parameter inflex ϕ_2 for all three treatments due to the variability in the random effects. But it has a common fixed effect for all three treatments. Hence it has also different mixed effects for all three treatments.
- an uniform fixed effect parameter ϕ_3 for all three treatments and hence it has a purely fixed effect.
- a lower Residual standard error.

For further detail information, a summary of model 10 is presented below.

```
Nonlinear mixed-effects model fit by maximum likelihood
   Model: virus ~ SSlogis(day, asymp, inflex, scale)
Data: hfvgr
        AIC       BIC     logLik
   5633.427  5660.088  -2808.713

Random effects:
  Formula: list(asymp ~ 1, inflex ~ 1)
  Level: meso
  Structure: Diagonal

        asymp.(Intercept)    inflex  Residual
StdDev:          141837.4  1.709932  170922.8

Fixed effects: list(asymp ~ treatment, inflex + scale ~ 1)

                     Value Std.Error  DF   t-value p-value
asymp.(Intercept) 790672.1  91029.62 194  8.685877  0.0000
asymp.treatmentB  367716.9 126780.81 194  2.900414  0.0042
asymp.treatmentC  974723.5 127015.21 194  7.674069  0.0000
inflex                12.1      0.61 194 19.725391  0.0000
scale                  1.8      0.14 194 12.144918  0.0000

Correlation:
```

```
                  as.(I) asym.B asym.C inflex
asymp.treatmentB -0.711
asymp.treatmentC -0.707  0.510
inflex            0.057 -0.016 -0.020
scale             0.084  0.006  0.042  0.067

Standardized Within-Group Residuals:
       Min         Q1        Med         Q3        Max
-3.5625885 -0.4235803  0.1741952  0.5227076  3.2616369

Number of Observations: 207
Number of Groups: 9
```

5.3 Heteroscedascity Model

If we plot a graph of standardized residuals versus fitted values by the time period, as shown in Figure 5.4, we can see that there is lower variability at the beginning of the experiment.

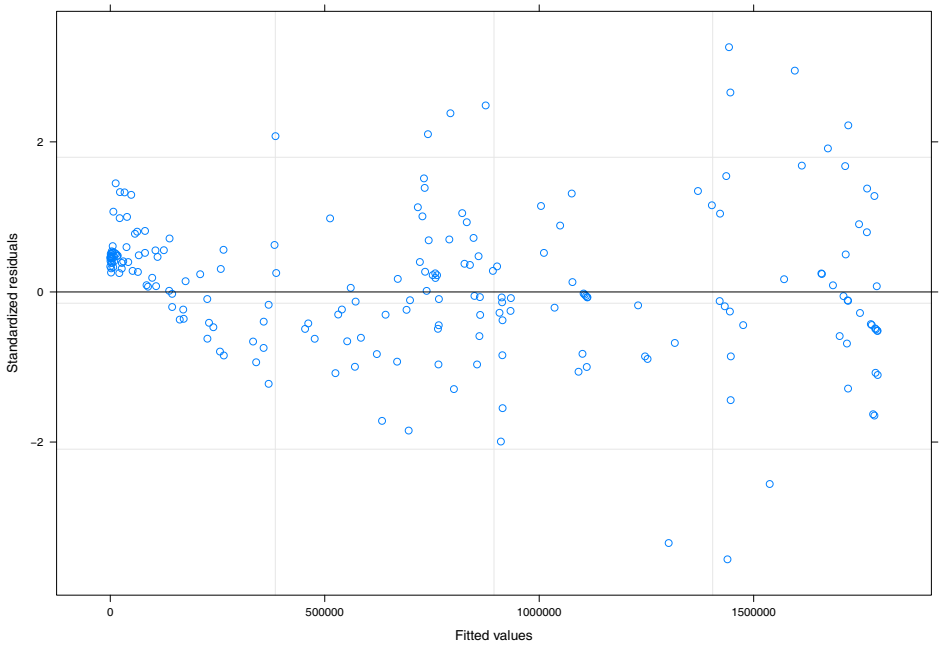

Figure 5.4: Scatter plot of Standardized residuals versus fitted values for model 10

But as time increases the variability also increases, and before ending the experiment, there exists a greater variability. Standardized residual is the residual divided by its standard deviation that is

$$\text{Standardized residual } k = \frac{\text{Residual } k}{\text{Standard Deviation of Residual } k}$$

and the residual data of the simple linear regression model is the difference between the observed data of the dependent variable Y and the fitted values $\hat{Y}$ such that

$$\text{Residual } = Y - \hat{Y}$$

[Yau, 2009 - 2011].

Such type of residual variability that increases with increment of time period, explains a heteroscedascity in a given model and such kinds of variance structure of the within-group errors are modelled by variance functions using covariates. The general variance function model for the within-group errors in the model is defined as

$$Var(\epsilon_{ijk}|b_j) = \sigma^2 g^2(\mu_{ijk}, \boldsymbol{\upsilon}_{ijk}, \boldsymbol{\gamma}) \tag{5.3}$$

where, $i = 1 \cdots I \quad j = 1, \cdots, J, \quad k = 1, \cdots, K$. The above variance function model (5.3) is applicable only when the within-group errors and random effects are assumed to be dependent. But in our case, we assume that the within-group errors are independent of the random effects, and the variance function model becomes as

$$Var(\epsilon_{ijk}) \simeq \sigma^2 g^2(\hat{\mu}_{ijk}, \boldsymbol{\upsilon}_{ijk}, \boldsymbol{\gamma}) \tag{5.4}$$

Where, the expected values $\mu_{ijk} = E[y_{ijk}|b_j]$ are replaced by their BLUPs $\hat{\mu}_{ijk} = x_{ijk}^T \boldsymbol{\beta} + z_{ijk}^T \hat{\boldsymbol{b}}_j$ such that x_{ijk} and z_{ijk} are denoting the j^{th} rows of $\boldsymbol{X}_i$ and $\boldsymbol{Z}_i$. $\boldsymbol{\upsilon}$ is a vector of variance covariates, $\boldsymbol{\gamma}$ is a vector of *variance parameters* and $g(.)$ is the variance function, assumed continuous in γ [Pinheiro and Bates, 2000]. But in our case, from the Figure 5.4, it seems that the within-group variability are increasing as growth of virus increasing. Therefore, we assume that the within-group variability are increasing with some power of the absolute value of covariate, *i.e.* with some power of the fitted values. So, we can write,

$$Var(\epsilon_{ijk}) = \sigma^2 |\hat{y}_{ijk}|^{2\gamma}$$

where $\epsilon \sim N(0, \sigma^2)$, $\hat{y}_{ijk}$ = variance covariate (the fitted value) and γ is the variance parameter. The *nlme* library provides a several *varFunc* classes [Pinheiro and Bates, 2000]. In our model, we prefer *varPower* function to model the heteroscedascity. Following table gives the different heteroscedascity models with different forms.

Model	Variance parameters	AIC	BIC	Log -likelihood	Residual
10.var	γ	5593.03	5623.025	-2787.515	5.169×10^3
10.var1	$\gamma_1, \gamma_2, \gamma_3$	5596.244	5632.904	-2787.122	25.764×10^3
10.var2	$\gamma_1, \gamma_2, \cdots, \gamma_9$	5598.687	5655.343	-2782.344	25.982×10^3

Table 5.7: Different heteroscedascity models for the best homogenous model with variance parameters, AIC, BIC and log-likelihood.

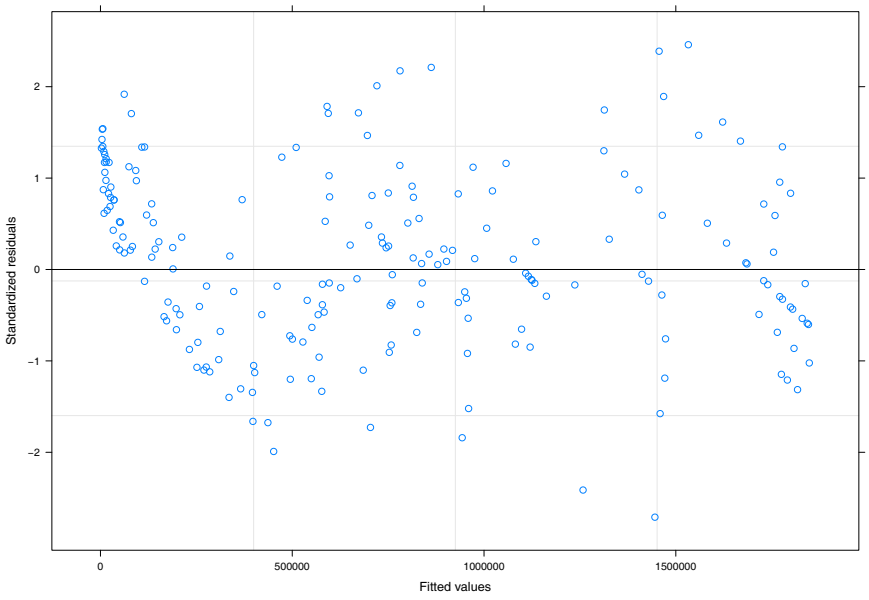

Figure 5.5: Scatter plot of Standardized residuals versus fitted values for the heteroscedascity model.

If we analyze the above Table 5.7 under the criterion of smaller BIC, we prefer the model "10.var" as the best heteroscedascity model with single variance parameter for our dataset. That is, we have, $\sigma(\epsilon_{ijk}) = \hat{\sigma}(\hat{y}_{ijk})^{0.267}$. The corresponding residual plot of the heteroscedascity model is shown above in the Figure 5.5.

Model	DF	BIC	LogLik	Test	L.Ratio	$p-$value
10	8	5660.08	-2808.71			
10.var	9	5623.025	-2787.51	10 Vs 10.var	42.40	$<.0001$

Table 5.8: Comparison of the model 10 and the model 10.var using an ANOVA

The very small $p-$value of the likelihood ratio statistic in the above ANOVA Table 5.8 confirms that the heteroscedascity model explains the data significantly better than the homoscedastic model [Pinheiro and Bates, 2000]. Figure 5.5 shows the standardized residuals have approximately the same variability. A plot of heteroscedascity fit model for the treatment effect predictions (fixed effect), meso group predictions (random effect) and the observed values, is shown in figure 5.6 (in the next page.)

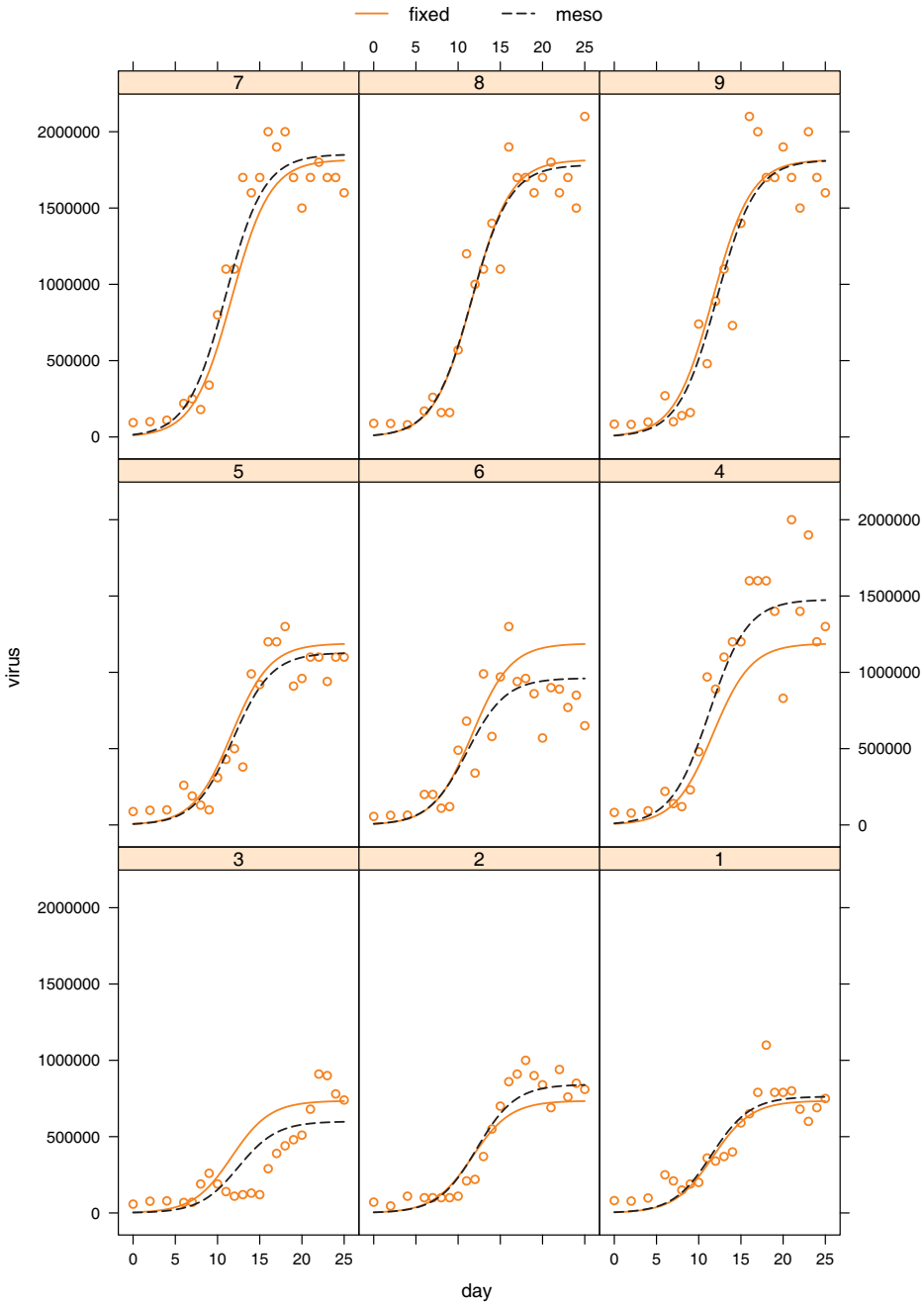

Figure 5.6: The treatment effect predictions (fixed), meso group predictions (random), and observed values for the heteroscedascity fit model.

6

Conclusions

The fixed-effects linear model, the linear mixed-effects model and the nonlinear mixed-effects, discussed in above chapters give us the uniform conclusion that there is significant difference in the treatment effects. But the nonlinear mixed-effects model, the logistic growth model, explained by the homogenous "model 10" not only describes the significant difference of treatments but it also tells us that the growth levels of virus under these treatments are affected by the mixed-effects and fixed effects *i.e.,* the best fitted homogenous model 10 defines the non-linear model (5.1), *i.e.,*

$$y_{jk} = f(\boldsymbol{\phi}, x) + \epsilon_{jk} = \frac{\phi_1}{1 + e^{-(x-\phi_2)/\phi_3}} + \epsilon_{jk}$$

[Pinheiro and Bates, 2000] as a mixed-effects non-linear model where the parameter asymptote ϕ_1 has the mixed-effects such that it has three different fixed effects for three different treatments and different random effects for different mesos. Similarly, the parameter inflex ϕ_2 also has the mixed-effect such that it has uniform fixed effects for all treatments and different random effects for the different mesos. Whereas the parameter scale ϕ_3 has the fixed effects only such that it has uniform fixed effects for all three treatments. Therefore, we write, for $j = 1, 2, 3.$,

$$\boldsymbol{\phi}^A = \begin{bmatrix} \phi_{1j}^{(A)} \\ \phi_{2j}^{(A)} \\ \phi_{3j}^{(A)} \end{bmatrix} = \begin{bmatrix} \beta_1^{(A)} \\ \beta_2^{(A)} \\ \beta_3^{(A)} \end{bmatrix} + \begin{bmatrix} b_{1j}^{(A)} \\ b_{2j}^{(A)} \\ 0 \end{bmatrix}$$

for $j = 4, 5, 6.$,

$$\boldsymbol{\phi}^{(B)} = \begin{bmatrix} \phi_{1j}^{(B)} \\ \phi_{2j}^{(B)} \\ \phi_{3j}^{(B)} \end{bmatrix} = \begin{bmatrix} \beta_1^{(B)} \\ \beta_2^{(B)} \\ \beta_3^{(B)} \end{bmatrix} + \begin{bmatrix} b_{1j}^{(B)} \\ b_{2j}^{(B)} \\ 0 \end{bmatrix}$$

and, for $j = 7, 8, 9.$,

$$\boldsymbol{\phi}^{(C)} = \begin{bmatrix} \phi_{1j}^{(C)} \\ \phi_{2j}^{(C)} \\ \phi_{3j}^{(C)} \end{bmatrix} = \begin{bmatrix} \beta_1^{(C)} \\ \beta_2^{(C)} \\ \beta_3^{(C)} \end{bmatrix} + \begin{bmatrix} b_{1j}^{(C)} \\ b_{2j}^{(C)} \\ 0 \end{bmatrix}$$

Where

$$\beta_2^{(A)} = \beta_2^{(B)} = \beta_2^{(C)},$$
$$\beta_3^{(A)} = \beta_3^{(B)} = \beta_3^{(C)}$$

and,

$$b_{3j}^{(A)} = b_{3j}^{(B)} = b_{3j}^{(C)} = 0$$

Following Table 6.1 gives the detail information about the three parameters for the model 10.

Treatment (i)	Meso (j)	Estimated value of Parameters				
		Asymptote ($\hat{\phi}_1$)		Inflex ($\hat{\phi}_2$)		Scale ($\hat{\phi}_3$)
		(β_1)	(b_1)	(β_2)	(b_2)	(β_3)
A (intercept)	1	79.06×10^4	-2.40×10^4	1.2	0.041	1.75
	2	79.06×10^4	7.24×10^4	1.2	0.072	1.75
	3	79.06×10^4	-4.84×10^4	1.2	4.039	1.75
B	4	36.77×10^4	28.89×10^4	1.2	-1.133	1.75
	5	36.77×10^4	-4.53×10^4	1.2	-1.017	1.75
	6	36.77×10^4	-24.35×10^4	1.2	-1.511	1.75
C	7	97.47×10^4	2.41×10^4	1.2	-1.545	1.75
	8	97.47×10^4	-4.41×10^4	1.2	-0.811	1.75
	9	97.47×10^4	1.99×10^4	1.2	0.175	1.75

Table 6.1: Estimated values of Parmameters ϕ_1, ϕ_2, ϕ_3 for the model 10.

We also get the following tables for the homogenous model 10.

	Estimates for the fixed effects		
for ϕ_1 :	$\hat{\beta}_1^{(A)}$	$\hat{\beta}_1^{(B)}$	$\hat{\beta}_1^{(C)}$
	79.06×10^4	36.77×10^4	97.47×10^4
for ϕ_2 :	$\hat{\beta}_2^{(A)} = \hat{\beta}_2^{(B)} = \hat{\beta}_2^{(C)} = 12.1$		
for ϕ_3 :	$\hat{\beta}_3^{(A)} = \hat{\beta}_3^{(B)} = \hat{\beta}_3^{(C)} = 1.8$		

Table 6.2: The estimated values of the fixed effects and their corresponding standard errors for treatments A,B and, C in the model 10

	Standard error for the estimates of the fixed effects		
for ϕ_1 :	$\hat{\beta}_1^{(A)}$	$\hat{\beta}_1^{(B)}$	$\hat{\beta}_1^{(C)}$
	9.10×10^4	12.68×10^4	12.70×10^4
for ϕ_2 :	$\hat{\beta}_2^{(A)} = \hat{\beta}_2^{(B)} = \hat{\beta}_2^{(C)} = 0.61$		
for ϕ_3 :	$\hat{\beta}_3^{(A)} = \hat{\beta}_3^{(B)} = \hat{\beta}_3^{(C)} = 0.14$		

Table 6.3: The standard error of the estimates of the fixed effects for treatments A,B and, C in the model 10

	Standard deviation of the random effects		
for ϕ_1 :	$SD\ (b_{1j}^{(A)}) =$	$SD\ (b_{1j}^{(B)}) =$	$SD\ (b_{1j}^{(C)}) = 14.18 \times 10^4$
for ϕ_2 :	$SD\ (b_{2j}^{(A)}) =$	$SD\ (b_{2j}^{(B)}) =$	$SD\ (b_{2j}^{(C)}) = 1.7$
for ϕ_3 :	$SD\ (b_{3j}^{(A)}) =$	$SD\ (b_{3j}^{(B)}) =$	$SD\ (b_{3j}^{(C)}) = 0$
for Residual:	17.09×10^4		

Table 6.4: The standard deviation of the random effects for treatments A,B and, C in the model 10

The best fitted model for our dataset is selected on the basis of the information criterion of BIC and loglikelihood values. The heteroscedasticity

fit model is also applied to minimize the with-in group variability in the best fitted model. The summary of heteroscedascity fit model is given below

```
Nonlinear mixed-effects model fit by maximum likelihood
Model: virus ~ SSlogis(day, asymp, inflex, scale)
Data: hfvgr

       AIC       BIC     logLik
   5593.03  5623.025  -2787.515

Random effects:
  Formula: list(asymp ~ 1, inflex ~ 1)
  Level: meso
  Structure: Diagonal

        asymp.(Intercept)     inflex  Residual
StdDev:            151059  0.6768236  5169.563

Variance function:
  Structure: Power of variance covariate
  Formula: ~fitted(.)

  Parameter estimates:
     power: 0.2666935

Fixed effects: list(asymp ~ treatment, inflex + scale ~ 1)
                     Value Std.Error  DF  t-value p-value
asymp.(Intercept)  734895.8  95534.02 194  7.69250   0.000
asymp.treatmentB   454212.7 135327.07 194  3.35641   0.001
asymp.treatmentC  1081529.3 137781.29 194  7.84961   0.000
inflex                 11.7      0.34 194 34.29696   0.000
scale                   2.3      0.17 194 13.60285   0.000

Correlation:
                 as.(I) asym.B asym.C inflex
asymp.treatmentB -0.690
asymp.treatmentC -0.672  0.485
```

```
inflex            0.116  0.019  0.045
scale             0.097  0.036  0.092  0.369

Standardized Within-Group Residuals:
       Min         Q1        Med         Q3        Max
-2.7097857 -0.5248593  0.1187200  0.8288833  2.4591550

Number of Observations: 207
Number of Groups: 9
```

we can see that the residual error is the least in the heteroscedascity model, indicating us that it fits the best for our dataset.

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A

R code

A.1 Grouping and selecting data after day 15

```
hfvgr=groupedData(virus~day|meso, data=hfv)
hfvgr=subset(hfvgr, day>15)
```

A.2 Applying linear model and linear mixed effect model for the dataset when K>15

```
mod1=lm(virus~treatment, data=hfvgr)
summary(mod1)
anova(mod1)

mod2=lme(virus~treatment, random=~1|meso, data=hfvgr)
summary(mod2)
anova(mod2)
```

A.3 Applying nonlinear mixed effect model (nlme) for the whole dataset

```
library(nlme)
hfv=read.table("hfv.dat",header=T)
hfvgr=groupedData(virus~day|meso, data=hfv)

form=virus~SSlogis(day,asymp,inflex,scale)
```

A.4 Selecting the model by applying different functions

```
model 1=nls(form,data=hfvgr)
summary(model 1)

model 2=nlsList(virus~SSlogis(day,asymp,inflex,scale),
data=hfvgr)
summary(model 2)
plot(intervals(model 2),layout=c(3,1),main="Model 2 :
Intervals(nlsList)")

model 3=nlsList(virus~SSlogis(I(day-12),asymp,inflex,scale),
data=hfvgr)
summary(model 3)

model 4=nlme(model 2,random=pdDiag(asymp+inflex+scale~1))
summary(model 4)
anova(model 4)

model 5=nlme(model 2,random=pdDiag(asymp+inflex~1))
summary(model 5)
anova(model 4,model 5)

model 6=nlme(model 2,random=pdDiag(asymp~1))
summary(model 6)
anova(model 4,model 5,model 6)

options(contrasts=c("contr.treatment","contr.poly"))
hfvfix=fixef(model 4)
hfvfix

model 7=update(model 4, fixed=list(asymp+inflex+scale
~treatment),start=c(hfvfix[1],0,0,hfvfix[2],0,0,
hfvfix[3],0,0))
summary(model 7)
```

```
anova(model 7)

model 8=update(model 5, fixed=list(asymp+inflex+scale
~treatment),start=c(hfvfix[1],0,0,hfvfix[2],0,0,
hfvfix[3],0,0))
summary(model 8)

model 9=update(model 8, fixed=list(asymp+inflex~treatment,
scale~1),start=c(hfvfix[1],0,0,hfvfix[2],0,0,hfvfix[3]))
summary(model 9)

model 10=update(model 9, fixed=list(asymp~treatment,
inflex+scale~1),start=c(hfvfix[1],0,0,hfvfix[2],
hfvfix[3]))
summary(model 10)
plot(augPred(model 10,level=0:1),main="Model 10 :
(R~A+I, T~A)",layout=c(3,3))

model 11=update(model 6, fixed=list(asymp+inflex+scale
~treatment),start=c(hfvfix[1],0,0,hfvfix[2],0,0,
hfvfix[3],0,0))
summary(model 11)

model 12=update(model 11, fixed=list(asymp+inflex~
treatment,scale~1),start=c(hfvfix[1],0,0,hfvfix[2],0,0,
hfvfix[3]))
summary(model 12)

model 13=update(model 12, fixed=list(asymp~treatment,
inflex+scale~1),start=c(hfvfix[1],0,0,hfvfix[2],hfvfix[3]))
summary(model 13)

model 14=nlme(model 2,random=pdDiag(asymp+scale~1),
fixed=list(asymp+inflex+scale~treatment),start=c(hfvfix
[1],0,0,hfvfix[2],0,0,hfvfix[3],0,0))
summary(model 14)
```

A.5 Selecting and ploting the best homogenous model

```
data.frame(model,aic,bic,loglik)
min(aic)
min(bic)
min(loglik)

plot(augPred(model 10,level=0:1),main="Model 10 :
(R~A+I, T~A)",layout=c(3,3))

plot(model 10,resid(.,type="p")~fitted(.),
main="Model 10 : Fitted Vs Residuals wrt days")
```

A.6 Selecting and plotting for a heteroscedascity model

```
model 10.var=update(model 10,weights=varPower(form=~fitted(.)))
summary(model 10.var)#gives lower bic value.


model 10.var1=update(model 10,weights=varPower(form=~fitted(.)
|treatment))
summary(model 10.var1)


model 10.var2=update(model 10,weights=varPower(form=~fitted(.)
|meso*treatment))
summary(model 10.var2)

data.frame(model,aic.var,bic.var,loglik.var)
min(aic.var)
min(bic.var)
min(loglik.var)

plot(model 10.var)
```