

## TWO-WAY MULTIVARIATE REPEATED MEASUREMENTS MODEL FOR BETWEEN-UNITS FACTORS WITH TWO COVARIATES

Abdul Hussein Saber AL-Mouel and Atyaf Sally Fakhir  
 Department of Mathematics-College of Education, University of Basrah  
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### Abstract

This research is devoted to study of two-way Multivariate repeated measurements analysis of covariance model (MRM ANCOVA), which contains two between-units factors (factor A and factor B), and two covariates  $(Z_1, Z_2)$ . For this model the two covariates are time-independent, that is measured only once. The test statistics of various hypotheses on between-units factors and the interaction between them are given, and includes the application of the theoretical part. It studies the effect of some internal and external factors on blood parameters, which are available in the experiment during one year through nine months. The data are taken from Department of Biology, College of Education, University of Basrah. Then, the data are analyzed according to the our model.

**Key Words:** Two-Way multivariate repeated measures model, Wilks distribution, Wishart distribution, Analysis of covariance, Covariates.

### Introduction

Repeated measurements analysis is widely used in many fields, for example, in the health and life sciences, epidemiology, biomedical, agricultural, industrial, psychological, educational research and so on, repeated measurements is a term used to describe data in which the response variable for each experimental unit is observed on multiple occasions and possibly under different experimental conditions [8]. The focus of this paper is the two-way multivariate repeated measurements analysis of covariance (MRM ANCOVA) model with two covariates. We can conclude from the applications study that the main effects for two factors into unites (one year and nine months), the effects of each of the error two covariates  $(Z_1, Z_2)$  and the interaction between factors among units are high incorporeal and we found that the effects of each of attendant factors and the factors among units will be not incorporeal. We used for extraction the results MATLAB program.

#### 1.1 Two-Way Multivariate Repeated Measurements Design

There is a variety of possibilities for the between units factors in a two-way design. In a

randomized two-way MRM experiment, the experimental units are randomized to two or more between units factors or groups. The response variables are measured on each of p occasions. We consider the case of a multivariate response variables and two between-units factors (factor A and factor B). Also we assume that we have two covariates. For convenience we use the following notation:-

where "#" means a number.

$p = \#$  of responses = td,  $t = \#$  of levels of Time,  
 $d = \#$  of levels of Day.

$q = \#$  of groups = ab,  $a = \#$  of levels of between-units factor A.

$b = \#$  of levels of between-units factor B.

$n_{jk} = \#$  of experimental units assigned to level (j,k) of (A,B);

$n = n_{11} + \dots + n_{ab}$  is total sample size.

For convenience, we define the following linear model and the parameterization for the

two-way repeated measurements design with two between-units factors incorporation two covariates:-

$$Y_{ijklm} = \mu + \alpha_j + \beta_k + (\alpha\beta)_{jk} + \tau_l + \gamma_m + (\alpha\tau)_{jl} + (\alpha\gamma)_{jm} + (\beta\tau)_{kl} + (\beta\gamma)_{km} + (\tau\gamma)_{lm} + (\alpha\beta\tau)_{jkl} + (\alpha\beta\gamma)_{jkm} + (\alpha\tau\gamma)_{jlm} + (\beta\tau\gamma)_{klm} + (\alpha\beta\tau\gamma)_{ijklm} + (Z_{1ijk} - \bar{Z}_{1...})\eta_1 + (Z_{2ijk} - \bar{Z}_{2...})\eta_2 + e_{ijklm} \quad (1.1)$$

where

$i = 1, \dots, n_{jk}$  is an index for experimental unit of level (j, k) of the treatment factors (A, B),

$j = 1, \dots, a$  is an index for levels of the between-units factor (Group A),

$k = 1, \dots, b$  is an index for levels of the between-units factor (Group B),

$l = 1, \dots, t$  is an index for levels of the within-units factor (Time),

$m = 1, \dots, d$  is an index for levels of the within-units factor (Day),

$Y_{ijklm} = [Y_{ijklm1}, \dots, Y_{ijklmr}]$  is the response measurement of within-units factors (Time, Day) for unit i within treatment factors (A, B),

$\mu = [\mu_1, \dots, \mu_r]$  is the over all mean,

$\alpha_j = [\alpha_{j1}, \dots, \alpha_{jr}]$  is the added effect of the  $j^{th}$  level of the treatment factor A,

$\beta_k = [\beta_{k1}, \dots, \beta_{kr}]$  is the added effect of the  $k^{th}$  level of the treatment factor B,

$(\alpha\beta)_{jk} = [(\alpha\beta)_{jk1}, \dots, (\alpha\beta)_{jkr}]$  is the added effect of the interaction between the factors A and B at levels j, k,

$\tau_l = [\tau_{l1}, \dots, \tau_{lr}]$  is the added effect of the  $l^{th}$  level of Time,

$\gamma_m = [\gamma_{m1}, \dots, \gamma_{mr}]$  is the added effect of the  $m^{th}$  level of Day,

$(\alpha\tau)_{jl} = [(\alpha\tau)_{jl1}, \dots, (\alpha\tau)_{jlr}]$  is the added effect of the interaction between the treatment factors A and Time at levels j, l,

$(\alpha\gamma)_{jm} = [(\alpha\gamma)_{jm1}, \dots, (\alpha\gamma)_{jmr}]$  is the added effect of the interaction between the treatment factors A and Day at levels j, m,

$(\beta\tau)_{kl} = [(\beta\tau)_{kl1}, \dots, (\beta\tau)_{klr}]$  is the added effect of the interaction between the treatment factors B and Time at levels k, l,

$(\beta\gamma)_{km} = [(\beta\gamma)_{km1}, \dots, (\beta\gamma)_{kmr}]$  is the added effect of the interaction between the treatment factors B and Day at levels k, l,

$(\tau\gamma)_{lm} = [(\tau\gamma)_{lm1}, \dots, (\tau\gamma)_{lmr}]$  is the added effect of the interaction between the Time and Day at levels l, m,

$(\alpha\beta\tau)_{jkl} = [(\alpha\beta\tau)_{jkl1}, \dots, (\alpha\beta\tau)_{jklr}]$  is the added effect of the interaction between the treatment factors A and B, and Time at their  $j^{th}$ ,  $k^{th}$  and  $l^{th}$  levels respectively,

$(\alpha\beta\gamma)_{jkm} = [(\alpha\beta\gamma)_{jkm1}, \dots, (\alpha\beta\gamma)_{jkmr}]$  is the added effect of the interaction between the treatment factors A and B, and Day at their  $j^{th}$ ,  $k^{th}$  and  $m^{th}$  levels respectively,

$(\alpha\tau\gamma)_{jlm} = [(\alpha\tau\gamma)_{jlm1}, \dots, (\alpha\tau\gamma)_{jlmr}]$  is the added effect of the interaction between the treatment factor A and Time and Day at their  $j^{th}$ ,  $l^{th}$  and  $m^{th}$  levels respectively

$(\beta\tau\gamma)_{klm} = [(\beta\tau\gamma)_{klm1}, \dots, (\beta\tau\gamma)_{klmr}]$  is the added effect of the interaction between

the treatment factor B and Time and Day at their  $k^{th}$ ,  $l^{th}$  and  $m^{th}$  levels respectively

$(\alpha\beta\tau\gamma)_{jklm} = [(\alpha\beta\tau\gamma)_{jklm1}, \dots, (\alpha\beta\tau\gamma)_{jklmr}]$  is the added effect of the interaction between the treatment factors A, B and Time and Day at their  $j^{th}$ ,  $k^{th}$ ,  $l^{th}$  and  $m^{th}$  levels respectively,

$Z_{1ijk} = [Z_{1ijk1}, \dots, Z_{1ijk r}]$  is the value of covariate  $Z_1$  for unit i within groups j, k,

$\bar{Z}_{1...} = [\bar{Z}_{1...1}, \dots, \bar{Z}_{1...r}]$  is the mean of covariate  $Z_1$  over all experimental units,

$\eta_1 = [\eta_{11}, \dots, \eta_{1r}]$  is the slope corresponding to covariate  $Z_1$ ,

$Z_{2ijk} = [Z_{2ijk1}, \dots, Z_{2ijk r}]$  is the value of covariate  $Z_2$  for unit i within groups j, k,

$\bar{Z}_{2...} = [\bar{Z}_{2...1}, \dots, \bar{Z}_{2...r}]$  is the mean of covariate  $Z_2$  over all experimental units,

$\eta_2 = [\eta_{21}, \dots, \eta_{2r}]$  is the slope corresponding to covariate  $Z_2$ ,

$e_{ijklm} = [e_{ijklm1}, \dots, e_{ijklmr}]$  is the random error on of within-units factors (Time, Day) at their levels (l, m) for unit i within treatment factors (A, B) at their levels (j, k) respectively.

For the parameterization to be of full rank, we impose the following set of conditions :

$$\begin{aligned} \sum_{j=1}^a \alpha_j &= 0 ; \sum_{k=1}^b \beta_k = 0 ; \sum_{l=1}^t \tau_l = 0 ; \sum_{m=1}^d \gamma_m = 0 ; \\ \sum_{j=1}^a (\alpha\beta)_{jk} &= \sum_{k=1}^b (\alpha\beta)_{jk} = 0 ; \sum_{j=1}^a (\alpha\tau)_{jl} = \sum_{l=1}^t (\alpha\tau)_{jl} = 0 \\ \sum_{j=1}^a (\alpha\gamma)_{jm} &= 0 = \sum_{m=1}^d (\alpha\gamma)_{jm} ; \sum_{k=1}^b (\beta\tau)_{kl} = 0 = \sum_{l=1}^t (\beta\tau)_{kl} \\ \sum_{k=1}^b (\beta\gamma)_{km} &= 0 = \sum_{m=1}^d (\beta\gamma)_{km} ; \sum_{l=1}^t (\tau\gamma)_{lm} = 0 = \sum_{m=1}^d (\tau\gamma)_{lm} \\ \sum_{j=1}^a (\alpha\beta\tau)_{jkm} &= 0 = \sum_{k=1}^b (\alpha\beta\tau)_{jkm} = \sum_{m=1}^d (\alpha\beta\tau)_{jkm} \\ \sum_{j=1}^a (\alpha\tau\gamma)_{jlm} &= 0 = \sum_{l=1}^t (\alpha\tau\gamma)_{jlm} = \sum_{m=1}^d (\alpha\tau\gamma)_{jlm} \\ \sum_{k=1}^b (\beta\tau\gamma)_{klm} &= 0 = \sum_{l=1}^t (\beta\tau\gamma)_{klm} = \sum_{m=1}^d (\beta\tau\gamma)_{klm} \\ \sum_{j=1}^a (\alpha\beta\gamma)_{jkm} &= 0 = \sum_{k=1}^b (\alpha\beta\gamma)_{jkm} = \sum_{m=1}^d (\alpha\beta\gamma)_{jkm} \\ \sum_{j=1}^a (\alpha\beta\tau\gamma)_{jklm} &= 0 = \sum_{k=1}^b (\alpha\beta\tau\gamma)_{jklm} = \sum_{l=1}^t (\alpha\beta\tau\gamma)_{jklm} = \\ & \sum_{m=1}^d (\alpha\beta\tau\gamma)_{jklm} \end{aligned}$$

$$\sum_{i=1}^{n_{jk}} Z_{1ijk} = \sum_{i=1}^{n_{jk}} Z_{2ijk} = 0 \tag{1.2}$$

$$\sum_{j=1}^a Z_{1ijk} = \sum_{j=1}^a Z_{2ijk} = 0, \sum_{k=1}^b Z_{1ijk} = \sum_{k=1}^b Z_{2ijk} = 0$$

We assume that  $e_{ijklm}^s$  are independent with

$$e_{ijklm} = (e_{ijklm1}, \dots, e_{ijklmr})', i.i.d \sim N_r(0, \Sigma_e) \tag{1.3}$$

where  $N_r$  denotes to the multivariate-normal distribution, and  $\Sigma_\varepsilon$  is  $r \times r$  positive definite variance- covariance matrix.

Let  $Y_{ijk} = [Y_{ijk1}, Y_{ijk2}, \dots, Y_{ijkp}]'$  i.e.

$$Y_{ijk} = \begin{bmatrix} Y_{ijk11} & Y_{ijk21} & \dots & Y_{ijkp1} \\ Y_{ijk12} & Y_{ijk22} & \dots & Y_{ijkp2} \\ \vdots & \vdots & \ddots & \vdots \\ Y_{ijk1r} & Y_{ijk2r} & \dots & Y_{ijkpr} \end{bmatrix} \quad (1.4)$$

Let the variance- covariance matrix of  $\vec{Y}_{ijk}$  be denoted by  $\Sigma$ , where

$$\vec{A} = Vec(A).$$

The  $Vec(\cdot)$  operator creates a column vector from a matrix A by simply stacking the column vectors of A below one another [8].

Where the variance- covariance matrix  $\Sigma$  of the model (1.1) satisfies the assumption of compound symmetry, i.e.

$$\Sigma = I_p \otimes \Sigma_\varepsilon = \begin{bmatrix} \Sigma_\varepsilon & 0 & \dots & 0 \\ 0 & \Sigma_\varepsilon & \dots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \dots & \Sigma_\varepsilon \end{bmatrix} \quad (1.5)$$

where

$I_p$  denotes the  $p \times p$  identity matrix.

$\otimes$  is the Kronecker product operation of two matrices.

$$\text{So } e_{ijk} = [e_{ijk1}, \dots, e_{ijkp}]' \sim i.i.d. N_{p \times r}(0, I_p \otimes \Sigma_\varepsilon) \quad (1.6)$$

### 1.2 Transforming the Two-Way Multivariate Repeated Measurements Analysis of Covariance (ANCOVA) Model

In this section, we use an orthogonal matrix to transform the observations  $Y_{ijk}$  for

$i = 1, \dots, n_{jk}, j = 1, \dots, a, k = 1, \dots, b$ .  
Let  $U^*$  be any  $p \times p$  orthogonal matrix. It is partitioned as follows:

$$U^* = \begin{bmatrix} p^{-\frac{1}{2}} j_p & U_T & U_D & U_{T \times D} \end{bmatrix}, \quad (1.7)$$

where  $j_p$  denotes the  $p \times 1$  vector of one's,  $U_T$  is  $p \times (t-1)$  matrix,  $U_D$  is  $p \times (d-1)$  matrix, and  $U_{T \times D}$  is  $p \times (t-1)(d-1)$  matrix.

$U_T^T j_p = 0, U_T^T U_T = I_{t-1}, U_D^T j_p = 0, U_D^T U_D = I_{d-1}$ , and

$$U_{T \times D} j_p = 0, U_{T \times D} U_{T \times D} = I_{(t-1)(d-1)}.$$

$$U_T = \begin{bmatrix} u_{11} & \dots & u_{1,t-1} \\ \vdots & \ddots & \vdots \\ u_{p1} & \dots & u_{p,t-1} \end{bmatrix}, U_D = \begin{bmatrix} v_{11} & \dots & v_{1,d-1} \\ \vdots & \ddots & \vdots \\ v_{p1} & \dots & v_{p,d-1} \end{bmatrix},$$

$$U_{T \times D} = \begin{bmatrix} w_{11} & \dots & w_{1,(t-1)(d-1)} \\ \vdots & \ddots & \vdots \\ w_{p1} & \dots & w_{p,(t-1)(d-1)} \end{bmatrix}$$

Let

$$Y_{ijk}^* = Y_{ijk} U^*$$

$$[Y_{ijk1}^*, Y_{ijk2}^*, \dots, Y_{ijkp}^*]^r = [Y_{ijk1}, Y_{ijk2}, \dots, Y_{ijkp}]^r U^*$$

$$\begin{bmatrix} Y_{ijk11}^* & \dots & Y_{ijkp1}^* \\ \vdots & \ddots & \vdots \\ Y_{ijk1r}^* & \dots & Y_{ijkpr}^* \end{bmatrix} = \begin{bmatrix} Y_{ijk11} & \dots & Y_{ijkp1} \\ \vdots & \ddots & \vdots \\ Y_{ijk1r} & \dots & Y_{ijkpr} \end{bmatrix} \left[ p^{\frac{-1}{2}} j_p \quad U_T \quad U_D \quad U_{T \times D} \right] \quad (1.8)$$

So

$$\begin{aligned} Cov(\vec{Y}_{ijk}^*) &= Cov(\vec{Y}_{ijk} U^*) = Cov\left(\left(U^* \otimes I_r\right) \vec{Y}_{ijk}\right) \\ &= \left(U^* \otimes I_r\right) \Sigma \left(U^* \otimes I_r\right) \end{aligned} \quad (1.9)$$

So

$$\begin{aligned} Cov(\vec{Y}_{ijk}^*) &= \left(U^* \otimes I_r\right) \left(I_p \otimes \Sigma_\varepsilon\right) \left(U^* \otimes I_r\right) \\ &= I_p \otimes \Sigma_\varepsilon = \begin{bmatrix} \Sigma_\varepsilon & 0 & \dots & 0 \\ 0 & \Sigma_\varepsilon & \dots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \dots & \Sigma_\varepsilon \end{bmatrix} \end{aligned} \quad (1.10)$$

### 1.3 Analysis of covariance (ANCOVA) for the Two-Way Multivariate Repeated Measurements Model

In this section, we study the ANCOVA for the effects of between-units factors for the two-way RM model (1.1). Also we give the null hypotheses which are concerned

with these effects and the interaction between them, and the test statistics for them.

Now

$$Y_{ijk1}^* = Y_{ijk} p^{\frac{-1}{2}} J_p = \begin{bmatrix} Y_{ijk11}^* \\ Y_{ijk12}^* \\ \vdots \\ Y_{ijk1r}^* \end{bmatrix} = \begin{bmatrix} \frac{1}{\sqrt{p}} \sum_{l=1}^t \sum_{m=1}^d Y_{ijklm1} \\ \frac{1}{\sqrt{p}} \sum_{l=1}^t \sum_{m=1}^d Y_{ijklm2} \\ \vdots \\ \frac{1}{\sqrt{p}} \sum_{l=1}^t \sum_{m=1}^d Y_{ijklmr} \end{bmatrix}$$

From (1.1), we obtain

$$\begin{aligned} Y_{ijk1}^* &= p^{\frac{-1}{2}} \sum_{l=1}^t \sum_{m=1}^d Y_{ijklm} \\ &= p^{\frac{-1}{2}} \sum_{l=1}^t \sum_{m=1}^d (\mu + \alpha_j + \beta_k + (\alpha\beta)_{jk} + \tau_l + \gamma_m + (\alpha\tau)_{jl} + (\alpha\gamma)_{jm} + (\beta\tau)_{kl} + \\ &\quad (\beta\gamma)_{km} + (\tau\gamma)_{lm} + (\alpha\beta\tau)_{jkl} + (\alpha\beta\gamma)_{jkm} + (\alpha\tau\gamma)_{jlm} + (\beta\tau\gamma)_{klm} + (\alpha\beta\tau\gamma)_{jklm} + \\ &\quad (Z_{1ijk} - \bar{Z}_{1\dots})\eta_1 + (Z_{2ijk} - \bar{Z}_{2\dots})\eta_2 + e_{ijklm}) \\ &= p^{\frac{1}{2}}\mu + p^{\frac{1}{2}}\alpha_j + p^{\frac{1}{2}}\beta_k + p^{\frac{1}{2}}(\alpha\beta)_{jk} + p^{\frac{1}{2}}(Z_{1ijk} - \bar{Z}_{1\dots})\eta_1 + p^{\frac{1}{2}}(Z_{2ijk} - \bar{Z}_{2\dots})\eta_2 + \\ &\quad p^{\frac{-1}{2}} \sum_{l=1}^t \sum_{m=1}^d e_{ijklm} \\ \therefore Y_{ijk1}^* &= \mu^* + \alpha_j^* + \beta_k^* + (\alpha\beta)_{jk}^* + (Z_{1ijk}^* - \bar{Z}_{1\dots}^*)\eta_1^* + (Z_{2ijk}^* - \bar{Z}_{2\dots}^*)\eta_2^* + e_{ijk1}^* \end{aligned}$$

Then the set of vectors

$$(Y_{1111}^*, \dots, Y_{n_{11}111}^*), (Y_{1211}^*, \dots, Y_{n_{21}211}^*), \dots, (Y_{1a11}^*, \dots, Y_{n_{a1}a11}^*), \dots, (Y_{1ab1}^*, \dots, Y_{n_{ab}ab1}^*)$$

Have mean vectors

$$\begin{aligned} &p^{\frac{1}{2}}\mu + p^{\frac{1}{2}}\alpha_1 + p^{\frac{1}{2}}\beta_1 + p^{\frac{1}{2}}(\alpha_1\beta_1) + p^{\frac{1}{2}}(Z_{1i11} - \bar{Z}_{1\dots})\eta_1 \\ &+ p^{\frac{1}{2}}(Z_{2i11} - \bar{Z}_{2\dots})\eta_2, p^{\frac{1}{2}}\mu + p^{\frac{1}{2}}\alpha_2 + p^{\frac{1}{2}}\beta_1 + p^{\frac{1}{2}}(\alpha_2\beta_1) \\ &+ p^{\frac{1}{2}}(Z_{1i21} - \bar{Z}_{1\dots})\eta_1 + p^{\frac{1}{2}}(Z_{2i21} - \bar{Z}_{2\dots})\eta_2, \dots, p^{\frac{1}{2}}\mu + p^{\frac{1}{2}}\alpha_a \\ &+ p^{\frac{1}{2}}\beta_1 + p^{\frac{1}{2}}\alpha_a\beta_1 + p^{\frac{1}{2}}(Z_{1ia1} - \bar{Z}_{1\dots})\eta_1 \\ &+ p^{\frac{1}{2}}(Z_{2ia1} - \bar{Z}_{2\dots})\eta_2, p^{\frac{1}{2}}\mu + p^{\frac{1}{2}}\alpha_1 + p^{\frac{1}{2}}\beta_2 \\ &+ p^{\frac{1}{2}}(\alpha_1\beta_2) + p^{\frac{1}{2}}(Z_{1i12} - \bar{Z}_{1\dots})\eta_1 + p^{\frac{1}{2}}(Z_{2i12} - \bar{Z}_{2\dots})\eta_2, p^{\frac{1}{2}}\mu \\ &+ p^{\frac{1}{2}}\alpha_2 + p^{\frac{1}{2}}\beta_2 + p^{\frac{1}{2}}(\alpha_2\beta_2) + p^{\frac{1}{2}}(Z_{1i22} - \bar{Z}_{1\dots})\eta_1 \\ &+ p^{\frac{1}{2}}(Z_{2i22} - \bar{Z}_{2\dots})\eta_2, \dots, p^{\frac{1}{2}}\mu + p^{\frac{1}{2}}\alpha_a + p^{\frac{1}{2}}\beta_2 + p^{\frac{1}{2}}\alpha_a\beta_2 \end{aligned}$$

$$+p^{\frac{1}{2}}(Z_{1ia2} - \bar{Z}_{1...})\eta_1 + p^{\frac{1}{2}}(Z_{2ia2} - \bar{Z}_{2...})\eta_2, \dots, p^{\frac{1}{2}}\mu + p^{\frac{1}{2}}\alpha_a$$

$$+p^{\frac{1}{2}}\beta_b + p^{\frac{1}{2}}\alpha_a\beta_b + p^{\frac{1}{2}}(Z_{1iab} - \bar{Z}_{1...})\eta_1 + p^{\frac{1}{2}}(Z_{2iab} - \bar{Z}_{2...})\eta_2$$

Respectively, and each of them has covariance matrix  $\Sigma_{\epsilon}$ .

So, the null hypotheses of the same treatment effects are:

$$H_{01}: \alpha_1^* = \alpha_2^* = \dots = \alpha_a^* = 0$$

$$H_{02}: \beta_1^* = \beta_2^* = \dots = \beta_b^* = 0$$

$$H_{03}: \alpha_1^*\beta_1^* = \alpha_2^*\beta_1^* = \dots = \alpha_a^*\beta_1^* = \alpha_1^*\beta_2^* = \alpha_2^*\beta_2^* = \dots = \alpha_a^*\beta_2^* = \dots$$

$$= \alpha_1^*\beta_b^* = \alpha_2^*\beta_b^* = \dots = \alpha_a^*\beta_b^* = 0$$

$$H_{04}: (Z_{1i11}^* - \bar{Z}_{1...}^*) = (Z_{1i21}^* - \bar{Z}_{1...}^*) = \dots = (Z_{1ia1}^* - \bar{Z}_{1...}^*)$$

$$= (Z_{1i12}^* - \bar{Z}_{1...}^*) = (Z_{1i22}^* - \bar{Z}_{1...}^*) = \dots = (Z_{1ia2}^* - \bar{Z}_{1...}^*) = (Z_{1i1b}^* - \bar{Z}_{1...}^*)$$

$$= (Z_{1i2b}^* - \bar{Z}_{1...}^*) = \dots = (Z_{1iab}^* - \bar{Z}_{1...}^*) = 0$$

$$H_{05}: (Z_{2i11}^* - \bar{Z}_{2...}^*) = (Z_{2i21}^* - \bar{Z}_{2...}^*) = \dots = (Z_{2ia1}^* - \bar{Z}_{2...}^*)$$

$$= (Z_{2i12}^* - \bar{Z}_{2...}^*) = (Z_{2i22}^* - \bar{Z}_{2...}^*) = \dots = (Z_{2ia2}^* - \bar{Z}_{2...}^*) = (Z_{2i1b}^* - \bar{Z}_{2...}^*)$$

$$= (Z_{2i2b}^* - \bar{Z}_{2...}^*) = \dots = (Z_{2iab}^* - \bar{Z}_{2...}^*) = 0$$

$$H_{06}: (Z_{1i11}^* - \bar{Z}_{1...}^*) + (Z_{2i11}^* - \bar{Z}_{2...}^*) = (Z_{1i21}^* - \bar{Z}_{1...}^*) + (Z_{2i21}^* - \bar{Z}_{2...}^*) = \dots$$

$$= (Z_{1ia1}^* - \bar{Z}_{1...}^*) + (Z_{2ia1}^* - \bar{Z}_{2...}^*) = (Z_{1i12}^* - \bar{Z}_{1...}^*) + (Z_{2i12}^* - \bar{Z}_{2...}^*)$$

$$= (Z_{1i22}^* - \bar{Z}_{1...}^*) + (Z_{2i22}^* - \bar{Z}_{2...}^*) = \dots = (Z_{1ia2}^* - \bar{Z}_{1...}^*) + (Z_{2ia2}^* - \bar{Z}_{2...}^*)$$

$$= (Z_{1i1b}^* - \bar{Z}_{1...}^*) + (Z_{2i1b}^* - \bar{Z}_{2...}^*) = (Z_{1i2b}^* - \bar{Z}_{1...}^*) + (Z_{2i2b}^* - \bar{Z}_{2...}^*) = \dots =$$

$$(Z_{1iab}^* - \bar{Z}_{1...}^*) + (Z_{2iab}^* - \bar{Z}_{2...}^*) = 0$$

The ANCOVA based on the set of transformed observations above the  $Y_{ijk1}^*$

provides the ANCOVA for between-units effects. This leads to the following form for the sum squares terms:

$$SS_A, SS_B, SS_{Z_1}, SS_{Z_2}, SS_{A \times B}, SS_{u(\text{Group } Z_1 Z_2)}, SS_{u(A \times B)}$$

$$SS_A = \sum_{j=1}^a n_{jk} (\bar{Y}_{jk1}^* - \bar{Y}_1^*) (\bar{Y}_{jk1}^* - \bar{Y}_1^*) \quad k = 1, \dots, b$$

$$SS_B = \sum_{k=1}^b n_{jk} (\bar{Y}_{jk1}^* - \bar{Y}_1^*) (\bar{Y}_{jk1}^* - \bar{Y}_1^*) \quad j = 1, \dots, a$$

where

$$\bar{Y}_{jk1}^* = \sum_{j=1}^a \sum_{k=1}^b \sum_{i=1}^{n_{jk}} \frac{Y_{ijk1}^*}{n_{jk}}, \quad \bar{Y}_1^* = \frac{\sum_{j=1}^a \sum_{k=1}^b \sum_{i=1}^{n_{jk}} Y_{ijk1}^*}{n}$$

$$SS_{Z_1} = \sum_{j=1}^a \sum_{k=1}^b \sum_{i=1}^{n_{jk}} \frac{1}{n_1^*} \left[ (\bar{Y}_{ijk1}^* - \bar{Y}_{jk1}^* - \eta_2^* Z_{2ijk}^*) (\bar{Y}_{ijk1}^* - \bar{Y}_{jk1}^* - \eta_2^* Z_{2ijk}^*)' \right]$$

$$SS_{Z_2} = \sum_{j=1}^a \sum_{k=1}^b \sum_{i=1}^{n_{jk}} \frac{1}{n_2^*} \left[ (\bar{Y}_{ijk1}^* - \bar{Y}_{jk1}^* - \eta_1^* Z_{1ijk}^*) (\bar{Y}_{ijk1}^* - \bar{Y}_{jk1}^* - \eta_1^* Z_{1ijk}^*)' \right]$$

$$SS_{A \times B} = \sum_{j=1}^a \sum_{k=1}^b n_{jk} (\bar{Y}_{jk1}^* - \bar{Y}_1^*) (\bar{Y}_{jk1}^* - \bar{Y}_1^*)'$$

$$SS_{u(\text{Group } Z_1 Z_2)} = \sum_{j=1}^a \sum_{k=1}^b \sum_{i=1}^{n_{jk}} (C(C)')$$

where

$$C = (Y_{ijk1}^* - \bar{Y}_{jk1}^* - 2\bar{Y}_{ijk}^* - 2\bar{Y}_{jk}^* + \eta_1^* Z_{1ijk}^* + \eta_2^* Z_{2ijk}^*)$$

$$\bar{Y}_{ijk1}^* = \frac{\sum_{j=1}^a \sum_{k=1}^b \sum_{i=1}^{n_{jk}} Y_{ijk1}^*}{n_{jk}q}$$

$$SS_{u(A \times B)} = \sum_{j=1}^a \sum_{k=1}^b \sum_{i=1}^{n_{jk}} (\bar{Y}_{ijk1}^* - \bar{Y}_{jk1}^*) (\bar{Y}_{ijk1}^* - \bar{Y}_{jk1}^*)'$$

Thus

$$SS_A \sim W_r(a-1, \Sigma_e), \quad SS_B \sim W_r(b-1, \Sigma_e), \quad SS_{Z_1} \sim W_r(1, \Sigma_e), \quad SS_{Z_2} \sim W_r(1, \Sigma_e)$$

$$SS_{A \times B} \sim W_r((a-1)(b-1), \Sigma_e), \quad SS_{u(\text{Group } Z_1 Z_2)} \sim W_r(n-ab-2, \Sigma_e)$$

$$SS_{u(A \times B)} \sim W_r(n-ab, \Sigma_e)$$

where  $W_r$  denotes the multivariate-Wishart distribution.

The multivariate Wilks test [10]

$$T_{w_1} = \frac{|SS_{u(A \times B)}|}{|SS_{u(A \times B)} + SS_A|} \quad \text{when } H_{01} \text{ is true, } T_{w_1} \sim \Lambda_r(n-ab, a-1)$$

$$T_{w_2} = \frac{|SS_{u(A \times B)}|}{|SS_{u(A \times B)} + SS_B|} \quad \text{when } H_{02} \text{ is true, } T_{w_2} \sim \Lambda_r(n-ab, b-1)$$

$$T_{w_3} = \frac{|SS_{u(A \times B)}|}{|SS_{u(A \times B)} + SS_{A \times B}|} \quad \text{when } H_{03} \text{ is true, } T_{w_3} \sim \Lambda_r(n-ab, (a-1), (b-1))$$

$$T_{w_4} = \frac{|SS_{u(A \times B)}|}{|SS_{u(A \times B)} + SS_{Z_1}|} \quad \text{when } H_{04} \text{ is true, } T_{w_4} \sim \Lambda_r(n-ab, 1)$$

$$T_{w_5} = \frac{|SS_{u(A \times B)}|}{|SS_{u(A \times B)} + SS_{Z_2}|} \quad \text{when } H_{05} \text{ is true, } T_{w_5} \sim \Lambda_r(n-ab, 1)$$



$$T_{w_\epsilon} = \frac{|SS_u(A \times B)|}{|SS_u(A \times B) + SS_u(\text{Group } Z_1 Z_2)|} \text{ when } H_{06} \text{ is true, } T_{w_\epsilon} \sim \Lambda_r(n - ab, n - ab - 2)$$

where  $\Lambda_r$  denotes the Wilks distribution.

### 1.4: The Experiment

The experiment was carried out to study the effect of some internal and external factors on blood parameters, which are available in the experiment during one year through nine months. The data was taken from the Department of Biology, College of Education, University of Basrah.

The experiment included fishes infected with *Ancylo-discoides parasiluri* were categorized into three groups according to the level of infection:

Group A1: Light infection, Group B1: Mild infection, Group C1: Heavy infection

The blood samples were then placed in clean dry marked tubes provided with anticoagulants. Tubes were kept in the refrigerator in the laboratory prior to its examination and analysis [9].

1. Hemoglobin estimation (Hb)
2. Total erythrocyte counts (Red Blood Cell R.B.C.)
3. Haematocrit estimation (Packed Cell Volume P.C.V.)

The design of the experiment was done according to the two-way multivariate repeated measurements model considering the effects of two independent factors. Multivariate analysis was done by the fraction the total differences into two classes or groups:-

**The First Class:** refers to the differences of essential elements between the measurement elements (between-units factors) which are out of control.

**The Second Class:** refers to the differences in within-units factors which are related to External factors.

The experiment was classified into nine groups, the measurement was studied during one year through nine months.

According to the mathematical formula of the model study (1.1) and by applying the model on the experiment, we get : The sum squares, of the effects between-units factors, interaction between-units factors, the two covariates and the error of two covariates ( $Z_1, Z_2$ ), where Group A denoted the Packed Cell Volume ( a), Group B denoted the Packed Cell Volume (b),  $Z_1$  denoted the Hemoglobin estimation and  $Z_2$  denoted the Red Blood Cell , of the Experiment as follows:

1. The sum squares of the effect of between-units factors to test the hypotheses

$$H_{01} : \alpha_1^* = \alpha_2^* = \alpha_3^* = 0, H_{02} : \beta_1^* = \beta_2^* = \beta_3^* = 0$$

are  $SS_A, SS_B, SS_u(A \times B)$  which are  $27 \times 27$

matrices. So that the values of Wilks statistics is shown in the following :

$$T_{W_1} = 0.493, T_{W_2} = 0.493$$

As it is not easy to obtain a tabulated value by Wilks statistic so the values were estimated to the statistical test using the following relationship :

$$F = \frac{1 - \Lambda^{\frac{1}{s}}}{\Lambda^{\frac{1}{s}}} \times \frac{ks - r}{pm} \sim F(pm, ks - r)$$

$$s = \sqrt{\frac{p^2 m^2 - 4}{p^2 + m^2 - 5}}, \quad r = \frac{pm}{2} - 1, \quad k = n - \frac{p - m + 1}{2}, \quad \Lambda \sim \Lambda_{p, n, m}$$

where  $m$  refers to the degree of freedom incorporating of the effect to null hypothesis test and  $p$  means the number of measurement values for each experiment unit.  $\Lambda$  is the estimated value of Wilks and

$k, s, r$  are explained in above and  $n$  taken the degree of freedom about the error. It is clear from the above that the Wilks statistics is distributed the Wilks distribution in the following :

$$\Lambda_{01} \sim \Lambda_{9,18,2} \text{ with } F = 0.471, F \sim F(18,20), F_c(18,20,0.05) = 2.15, \text{ and}$$

$$\Lambda_{02} \sim \Lambda_{9,18,2} \text{ with } F = 0.471, F \sim F(18,20), F_c(18,20,0.05) = 2.15$$

At 0.05 level of significance, we found that the calculated F-value is less than the statistical test value, so there are no level of significance found among the nine groups.

2. The sum squares of the effect to interaction between-units factors to test the hypothesis

$$H_{03}: \alpha_1^* \beta_1^* = \alpha_2^* \beta_1^* = \alpha_3^* \beta_1^* = \alpha_1^* \beta_2^* = \alpha_2^* \beta_2^* = \alpha_3^* \beta_2^* = \alpha_1^* \beta_3^* = \alpha_2^* \beta_3^* = \alpha_3^* \beta_3^* = 0 \text{ is } SS_{A \times B} \text{ which is } 27 \times 27 \text{ matrix as } SS_A \text{ with the value of Wilks statistics is } T_{W_3} = 0.493 \text{ and } \Lambda_{03} \sim \Lambda_{9,18,4} \text{ with } F = 4.171, F \sim F(36,43), F_c(36,43,0.05) = 1.69$$

At 0.05 level of significance, we found that the calculated F-value is greater than the statistical test value, this means that there are level of significance of blood parameters among the nine groups.

3. The sum squares of the two covariates ( $Z_1, Z_2$ ) to test the hypotheses :

$$H_{04}: (Z_{1i11}^* - \bar{Z}_{1...}^*) = (Z_{1i21}^* - \bar{Z}_{1...}^*) = (Z_{1i31}^* - \bar{Z}_{1...}^*) \\ = (Z_{1i12}^* - \bar{Z}_{1...}^*) = (Z_{1i22}^* - \bar{Z}_{1...}^*) = (Z_{1i32}^* - \bar{Z}_{1...}^*) = (Z_{1i13}^* - \bar{Z}_{1...}^*) \\ = (Z_{1i23}^* - \bar{Z}_{1...}^*) = (Z_{1i33}^* - \bar{Z}_{1...}^*) = 0$$

$$H_{05}: (Z_{2i11}^* - \bar{Z}_{2...}^*) = (Z_{2i21}^* - \bar{Z}_{2...}^*) = (Z_{2i31}^* - \bar{Z}_{2...}^*) \\ = (Z_{2i12}^* - \bar{Z}_{2...}^*) = (Z_{2i22}^* - \bar{Z}_{2...}^*) = (Z_{2i32}^* - \bar{Z}_{2...}^*) = (Z_{2i13}^* - \bar{Z}_{2...}^*) \\ = (Z_{2i23}^* - \bar{Z}_{2...}^*) = (Z_{2i33}^* - \bar{Z}_{2...}^*) = 0 \text{ are } SS_{Z_1}, SS_{Z_2} \text{ which are } 27 \times 27 \text{ matrices.}$$

So that the values of Wilks statistics are  $T_{W_4} = 0.756, T_{W_5} = 0.756$  and  $\Lambda_{04} \sim \Lambda_{9,18,1}, \Lambda_{05} \sim \Lambda_{9,18,1}$  with  $F = 0.357, F \sim F(9,10), F_c(9,10,0.05) = 2.07$

At 0.05 level of significance, we found that the calculated F-value is less than the statistical test value, this means that there are no level of significance of blood parameters among the nine groups i.e., there are no level of significance of the two covariates on blood parameters.

4. The sum squares of the error two covariates ( $Z_1, Z_2$ ) to test the hypothesis:

$$H_{06}: (Z_{1i11}^* - \bar{Z}_{1...}^*) + (Z_{2i11}^* - \bar{Z}_{2...}^*) = (Z_{1i21}^* - \bar{Z}_{1...}^*) + (Z_{2i21}^* - \bar{Z}_{2...}^*)$$

$$\begin{aligned}
 &= (Z_{1i31}^* - \bar{Z}_{1...}^*) + (Z_{2i31}^* - \bar{Z}_{2...}^*) = (Z_{1i12}^* - \bar{Z}_{1...}^*) + (Z_{2i12}^* - \bar{Z}_{2...}^*) \\
 &= (Z_{1i22}^* - \bar{Z}_{1...}^*) + (Z_{2i22}^* - \bar{Z}_{2...}^*) = (Z_{1i32}^* - \bar{Z}_{1...}^*) + (Z_{2i32}^* - \bar{Z}_{2...}^*) \\
 &= (Z_{1i13}^* - \bar{Z}_{1...}^*) + (Z_{2i13}^* - \bar{Z}_{2...}^*) = (Z_{1i23}^* - \bar{Z}_{1...}^*) + (Z_{2i23}^* - \bar{Z}_{2...}^*) = (Z_{1i33}^* - \bar{Z}_{1...}^*) + (Z_{2i33}^* - \bar{Z}_{2...}^*) = 0
 \end{aligned}$$

is

$SS_{u(\text{Group } Z_1, Z_2)}$  which is  $27 \times 27$  matrix .

So the values of Wilks Statistics is  $T_{w_e} = 0.940$  and  $\Lambda_{06} \sim \Lambda_{9,18,16}$  with

$$F = 5.427, F \sim F(144,97), F_c(144,97,0.05) = 1$$

At 0.05 level of significance, we found that the calculated F-value is grater than the statistical test value , this means that there are level of significance of blood parameters

among the nine groups i.e., there are level of significance of the two covariates on blood parameters.

### 1.5: Conclusions

The conclusions which are obtained through out this work are given as follows when we use:

$$H_{01}: \alpha_1^* = \alpha_2^* = \dots = \alpha_a^* = 0$$

$$H_{02}: \beta_1^* = \beta_2^* = \dots = \beta_b^* = 0$$

1. the null hypotheses which are tested for the ANCOVA for between-units effects are :

$$H_{03}: \alpha_1^* \beta_1^* = \alpha_2^* \beta_1^* = \dots = \alpha_a^* \beta_1^* = \alpha_1^* \beta_2^* = \alpha_2^* \beta_2^* = \dots = \alpha_a^* \beta_2^* = \dots$$

$$= \alpha_1^* \beta_b^* = \alpha_2^* \beta_b^* = \dots = \alpha_a^* \beta_b^* = 0$$

$$H_{04}: (Z_{1i11}^* - \bar{Z}_{1...}^*) = (Z_{1i21}^* - \bar{Z}_{1...}^*) = \dots = (Z_{1ia1}^* - \bar{Z}_{1...}^*)$$

$$= (Z_{1i12}^* - \bar{Z}_{1...}^*) = (Z_{1i22}^* - \bar{Z}_{1...}^*) = \dots = (Z_{1ia2}^* - \bar{Z}_{1...}^*) = (Z_{1i1b}^* - \bar{Z}_{1...}^*)$$

$$= (Z_{1i2b}^* - \bar{Z}_{1...}^*) = \dots = (Z_{1iab}^* - \bar{Z}_{1...}^*) = 0$$

$$H_{05}: (Z_{2i11}^* - \bar{Z}_{2...}^*) = (Z_{2i21}^* - \bar{Z}_{2...}^*) = \dots = (Z_{2ia1}^* - \bar{Z}_{2...}^*)$$

$$= (Z_{2i12}^* - \bar{Z}_{2...}^*) = (Z_{2i22}^* - \bar{Z}_{2...}^*) = \dots = (Z_{2ia2}^* - \bar{Z}_{2...}^*) = (Z_{2i1b}^* - \bar{Z}_{2...}^*)$$

$$= (Z_{2i2b}^* - \bar{Z}_{2...}^*) = \dots = (Z_{2iab}^* - \bar{Z}_{2...}^*) = 0$$

$$H_{06}: (Z_{1i11}^* - \bar{Z}_{1...}^*) + (Z_{2i11}^* - \bar{Z}_{2...}^*) = (Z_{1i21}^* - \bar{Z}_{1...}^*) + (Z_{2i21}^* - \bar{Z}_{2...}^*) = \dots$$

$$= (Z_{1ia1}^* - \bar{Z}_{1...}^*) + (Z_{2ia1}^* - \bar{Z}_{2...}^*) = (Z_{1i12}^* - \bar{Z}_{1...}^*) + (Z_{2i12}^* - \bar{Z}_{2...}^*)$$

$$= (Z_{1i22}^* - \bar{Z}_{1...}^*) + (Z_{2i22}^* - \bar{Z}_{2...}^*) = \dots = (Z_{1ia2}^* - \bar{Z}_{1...}^*) + (Z_{2ia2}^* - \bar{Z}_{2...}^*)$$

$$\begin{aligned}
 &= (Z_{1i1b}^* - \bar{Z}_{1\dots}^*) + (Z_{2i1b}^* - \bar{Z}_{2\dots}^*) = (Z_{1i2b}^* - \bar{Z}_{1\dots}^*) + (Z_{2i2b}^* - \bar{Z}_{2\dots}^*) = \dots \\
 &= (Z_{1iab}^* - \bar{Z}_{1\dots}^*) + (Z_{2iab}^* - \bar{Z}_{2\dots}^*) = 0
 \end{aligned}$$

Then the multivariate Wilks test statistic for the null hypotheses for between-units effects are :

- a)  $T_{w_1} = \frac{|SS_{u(A \times B)}|}{|SS_{u(A \times B)} + SS_A|}$  when  $H_{01}$  is true ,  
 $T_{w_1} \sim \Lambda_r(n - ab, a - 1)$
- b)  $T_{w_2} = \frac{|SS_{u(A \times B)}|}{|SS_{u(A \times B)} + SS_B|}$  when  $H_{02}$  is true ,  
 $T_{w_2} \sim \Lambda_r(n - ab, b - 1)$
- c)  $T_{w_3} = \frac{|SS_{u(A \times B)}|}{|SS_{u(A \times B)} + SS_{A \times B}|}$  when  $H_{03}$  is true ,  
 $T_{w_3} \sim \Lambda_r(n - ab, (a - 1), (b - 1))$
- d)  $T_{w_4} = \frac{|SS_{u(A \times B)}|}{|SS_{u(A \times B)} + SS_{Z_1}|}$  when  $H_{04}$  is true ,  
 $T_{w_4} \sim \Lambda_r(n - ab, 1)$
- e)  $T_{w_5} = \frac{|SS_{u(A \times B)}|}{|SS_{u(A \times B)} + SS_{Z_2}|}$  when  $H_{05}$  is true ,  
 $T_{w_5} \sim \Lambda_r(n - ab, 1)$
- f)  $T_{w_6} = \frac{|SS_{u(A \times B)}|}{|SS_{u(A \times B)} + SS_{u(\text{Group } Z_1 Z_2)}|}$  when  $H_{06}$  is true ,  
 $T_{w_6} \sim \Lambda_r(n - ab, n - ab - 2)$

2. We can conclude from the applications study that the main effects for two factors into unites (one year and nine months), the effects of each of the error two covariates ( $Z_1, Z_2$ ) and the interaction between factors among units are high incorporeal and we found that the effects of each of attendant factors and the factors among units will be not incorporeal.

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## نموذج القياسات المتكررة المتعددة المتغيرات ذات الاتجاهين للعوامل بين الوحدات مع عاملين

### مرافقين

#### الخلاصة

يتناول هذا البحث دراسة تحليل التباين المشترك لنموذج القياسات المتكررة بالاتجاهين (MRM ANCOVA) حيث يحتوي على عاملين بين الوحدات (العامل  $A$  والعامل  $B$ ) وعاملين مرافقين هما  $Z_1$  و  $Z_2$ . في هذا النموذج يكون العامل المرافق مستقلاً زمنياً (أي يقاس مره واحده في كل مستوى من مستويات التجربة). وكذلك ندرس إحصائيات اختبار الفرضيات المختلفة على عوامل بين الوحدات. أما الجانب التطبيقي يتضمن دراسة تأثير بعض العوامل (الداخلية والخارجية) على عوامل الدم التي توفرت ضمن التجربة لفترة سنة واحدة وعلى تسعة أشهر وقد أخذت هذه التجربة من قسم الأحياء في كلية التربية جامعة البصرة. حيث تم تحليل هذه البيانات وفقاً لنموذجنا.