Journal of Modern Applied Statistical Methods

Volume 2 | Issue 2 Article 12

11-1-2003

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Journal of Modern Applied Statistical Methods
November, 2003, Vol. 2, No. 2, 389-399

1538 – 9472/03/$30.00

Test Of Homogeneity For Umbrella Alternatives In Dose-Response Relationship For Poisson Variables

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This article concerns the testing and estimation of a dose-response effect in medical studies. We study the statistical test of homogeneity against umbrella alternatives in a sequence of Poisson distributions associated with an ordered dose variable. We propose a test similar to Cochran-Armitage's trend test and study the asymptotic null distribution and the power of the test. We also propose an estimator to the vertex point when the umbrella pattern is confirmed and study the performance of the estimator. A real data set pertaining to the number of visible revertant colonies associated with different doses of test agents in an *in vitro* mutagenicity assay is used to demonstrate the test and estimation process.

Key words: $C(\alpha)$ statistic, maximum likelihood estimate, monotone trend test, Poisson distribution, vertex point

Introduction

Medical studies often evaluate treatment effects at several doses of a test drug. One usually assumes a priori, based either on past experience with the test drug or on theoretical considerations, that if there is an effect on a parameter of interest, the response is likely monotonic with dose, i.e., the effect of the drug is expected to increase or decrease monotonically with increasing dose levels. Comparing several doses with a placebo in a clinical dose study is then typically performed by one-sided many-to-one comparisons or trend tests assuming an order restriction. Monotonicity of dose-response relationship, however, is far from universal.

Instances may be found where a reversal or downturn at higher doses is likely to occur. For example, many therapies for humans become counterproductive at high doses. Similarly, in many *in vitro* mutagenicity assays, experimental organisms may succumb to toxic effects at high doses of the test agents, thereby reducing the number of organisms at risk of mutation and causing a downturn in the dose-response curve (Collings et. al., 1981; Margolin et al., 1981). These types of non-monotonic dose-response behavior may not be caused by a random effect, but may occur due to an underlying biological mechanism. Mechanistic arguments for non-monotonic dose-response shapes can be found in many medical areas, such as toxicology (Calabrese & Baldwin, 1998), carcinogenesis (Portier & Ye, 1998), and epidemiology (Thorogood et al., 1993).

One of the simplest non-monotonic dose-response is the so-called umbrella pattern in which the response increases (decreases) until certain dose level (usually unknown) and then decreases (increases). Ames, McCann and Yamasaki (1975) reported experimental data exhibiting this pattern from three replicate Ames tests in which plates containing Salmonella bacteria of strain TA98 were exposed to various doses of Acid Red 114. The number of visible revertant colonies on each plate was observed. Figure 1 is a scatter plot of the number of visible revertant colonies against dose level, which

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clearly indicates an umbrella pattern peaked between the third dose and the fourth dose. This same phenomenon is also observed and discussed by Simpson and Margolin (1986).

When the dose-response curve contains an umbrella pattern, the usual statistical trend tests become inadequate because of their power loss and inherent, and possibly erroneous decisions (Collings et al., 1981; Bretz & Hothorn, 2001). The statistical test of homogeneity in response against an umbrella alternative has been studied by many authors. Most of these discussions deal with a continuous response variable and assume the normality for the associated distributions. The typical approaches under the assumption of normality are based on the framework of one-way analysis of variance and the simultaneous confidence intervals for umbrella contrasts of mean parameters (Bretz & Hothorn, 2001; Rom et al., 1994; Shi, 1988; Marcus & Genizi, 1994; Hayter & Liu, 1999). Nonparametric approaches have also been considered by several authors (Lim & Wolfe, 1997; Mack & Wolfe, 1981 & 1982, Simpson & Margolin, 1994).

When data are based on counts such as those reported by Ames, McCann and Yamasaki (1975), however, a more reasonable distributional assumption might be the Poisson distribution. The statistical test of homogeneity against umbrella alternatives in a sequence of Poisson distributions associated with an ordered dose variable has not been addressed in the biostatistics literature to the best of our knowledge. This article studies this problem using an approach based on so-called $C(\alpha)$ statistics as proposed and studied by Neyman (1959) and Bailey (1956). The $C(\alpha)$ statistics are also discussed in more details by Moran (1970) and by Cox and Hinkley (1974) under the more general category of score statistics.

We propose a test similar to the Cochran-Armitage trend test and study the asymptotic null distribution and the power of our test. We also propose an estimator of the vertex point when the umbrella pattern is confirmed and study the performance of the estimator. A real data set reported by Ames, McCann and Yamasaki (1975) pertaining to the number of visible revertant colonies associated with different doses of test agents in an *in vitro* mutagenicity assay is used to demonstrate the test and estimation process. We also present results of a simulation study about the proposed test and estimation.

Methodology

We consider an experiment in which independent random samples are taken from k distinct dose levels. Suppose that the k dose levels are meaningfully ordered. Let $d_1, d_2, ..., d_k$ be the scores associated with these dose levels and $d_1 \le d_2 \le ... \le d_k$. We assume that at dose level i, the response follows a Poisson distribution with mean $\mu_i, i = 1,2,...,k$. Let n_i be the sample size associated with dose level i and $n = \sum_{i=1}^{k} n_i$. Let x_i be the total response in the i-th dose level. For each i and p, $1 \le i, p \le k$, let $d_i^p = (d_i - d_p)^2$ and $\bar{d}^{\,p} = \sum_{i=1}^{k} n_i d_i^p / n$. Suppose that the relationship between the mean response and the score takes the form of

$$\mu_i = H\left[\alpha + \beta\left(d_i - d_p\right)^2\right],$$

where H is a monotonic function that is twice differentiable, d_p is the dose level associated with the vertex dose of the umbrella pattern. Notice that when $p = 1$ or k, this formulation reduces to the monotone trend. We consider the problem of testing $H_0: \beta = 0$ against the alternative hypothesis $H_a: \beta \ne 0$. The likelihood function as a function of α, β, and p is:

$$L(\alpha, \beta, p) \propto \Pi_{i=1}^{k} \exp\left\{-n_i H\left[\alpha + \beta\left(d_i - d_p\right)^2\right]\right\}\left\{H\left[\alpha + \beta\left(d_i - d_p\right)^2\right]\right\}^{x_i}.$$

p Is Known

When p is given, the test is the same as the trend test based on the redefined dose score $d_i^p, i = 1,2,...,k$. The test is based on the $C(\alpha)$ statistic (Moran, 1970) and is obtained by evaluating the derivative of the loglikelihood with respect to β at the maximum likelihood estimate of α under H_0:

$$C(\alpha) = \frac{\partial \log L}{\partial \beta}|_{\hat{\alpha},\beta=0} = \frac{H'(\hat{\alpha})}{H(\hat{\alpha})}\left(\sum_{i=1}^{k} x_i d_i^p - \hat{x}\sum_{i=1}^{k} n_i d_i^p\right),$$

where

$$\hat{x} = \frac{\sum_{i=1}^{k} x_i}{n},$$

and $\hat{\alpha} = H^{-1}(\hat{x})$. The test statistic for testing $H_0: \beta = 0$ against the alternative hypothesis $H_a: \beta \neq 0$ is obtained after dividing $C(\alpha)$ by its asymptotic standard deviation under H_0 computed from the information matrix of (α, β) (Tarone, 1982):

$$X_p^2 = \frac{\left[\sum_{i=1}^{k} x_i d_i^p - \hat{x}\sum_{i=1}^{k} n_i d_i^p\right]^2}{\hat{x}\sum_{i=1}^{k} n_i\left(d_i^p - \bar{d}^p\right)^2}. \qquad (1)$$

Notice that this test statistic does not depend on the choice of the function H. Under the null hypothesis, X_p^2 has an asymptotic Chi-square distribution with one degree of freedom. Notice also that this test statistic is identical in formula to the test statistic for testing monotone trend with the redefined score in binomial proportions proposed by Armirage (1955). In addition, Tarone (1982) showed that, like the binomial trend test (Tarone & Gart, 1980), this Poisson trend test is asymptotically locally optimal against any choice of smooth monotone function H that satisfies

$$\mu_i = H\left[\alpha + \beta\left(d_i - d_p\right)^2\right],\ i = 1,2,...,k.$$

p Is Unknown

When p is unknown and $H_0: \beta = 0$ is tested against the alternative hypothesis $H_a: \beta \neq 0$, we propose to reject $H_0: \beta = 0$ when $X^2 = \max_{1\le p\le k} X_p^2$ is large. Let $\lambda_i = \lim_{n\to\infty} \frac{n_i}{n}$ and assume that $0 < \lambda_i < 1$ for $1 \le i \le k$. For $1 \le p \le k$, let $d^p = \sum_{i=1}^{k} \lambda_i d_i^p$ and $\mu = \sum_{i=1}^{k} \lambda_i \mu_i$. Let Δ be the k by k matrix whose (i, p) entry is given by

$$\Delta_i^p = \frac{d_i^p - d^p}{\sqrt{\mu\sum_{i=1}^{k} \lambda_i\left(d_i^p - d^p\right)^2}}.$$

Let $A = (a_{ij})$ be the k by k matrix such that $a_{ij} = 0$ if $i \neq j$ and $a_{ij} = \mu_i\lambda_i$ if $i = j$. The following theorem gives the limiting distribution of the proposed test when the null hypothesis is true.

Theorem 1: If H_0 is true, then for any $x > 0$,

$$\lim_{n\to\infty} P\left(X^2 \ge x^2\right) = 1 - \int_{-x}^{x}\int_{-x}^{x}...\int_{-x}^{x} \frac{1}{\sqrt{(2\pi)^k\ |\Delta' A\Delta|}} \exp\left[-\frac{X'\left(\Delta' A\Delta\right)^{-1} X}{2}\right] dx_1 dx_2 ... dx_k, \qquad (2)$$

where $X = \left(x_1, x_2, ..., x_k\right)'$ and $|\ |$ is the matrix determinant.

The proof of Theorem 1 can be found in Appendix. Notice that the asymptotic null distribution does not depend on the unknown common mean $\mu_1 = \mu_2 = ... = \mu_k$ as the common mean μ is cancelled out in the integrand. Therefore, μ=1 can always be assumed for the computation. The evaluation of

the integration can be done by the iterative algorithm proposed by Genz (1992). This algorithm begins with a Cholesky decomposition of the covariance matrix and then uses a simple Monte-Carlo algorithm. Another possible way of evaluating the distribution of the test statistic under the null hypothesis is through a large simulation of the test statistic. We point out that the asymptotic null distribution does depend on the unknown proportion $\lambda_i, i = 1,2,...,k.$ $\frac{n_i}{n}$ can be used for λ_i in the computation based on the consistency of $\frac{n_i}{n}$ to λ_i. In addition, according to Šidák and Zbyněk (1967), under H_0,

$$\Pr\left(X^2 \le x^2\right) \ge \left[2\Phi(x) - 1\right]^k,$$

where Φ is the distribution function of the standard normal distribution. Therefore, under H_0,

$$\lim_{n\to\infty} \Pr\left(X^2 \ge x^2\right) \le 1 - \left[2\Phi(x) - 1\right]^k,$$

which then provides a conservative test of H_0 against H_a.

Estimation of the Vertex Point

If the alternative hypothesis is true, the problem of interest is then the estimation of the true vertex point. To avoid the problem of parameter identification, we assume that the umbrella pattern satisfies

$$\mu_1 \le \mu_2 \le ... \le \mu_{l-1} < \mu_l > \mu_{l+1} \ge ... \ge \mu_k$$

or

$$\mu_1 \ge \mu_2 \ge ... \ge \mu_{l-1} > \mu_l < \mu_{l+1} \le ... \le \mu_k,$$

i.e., we only consider the case where a single vertex point l exists. Notice that this formulation does not rule out the possibility that the vertex point is on the boundary of the dose interval if a monotone trend is the alternative hypothesis. We propose to estimate l by $\hat{l}$ such that $X^2_{\hat{l}} = \max_{1\le p\le k} X^2_p$, where X^2_p is given by (1). Notice that as $n \to \infty$, for any $1 \le p \le k$,

$$\lim_{n\to\infty} \frac{X_p^2}{n} = \frac{\left[\sum_{i=1}^k \lambda_i \mu_i d_i^p - \sum_{i=1}^k \lambda_i \mu_i \sum_{i=1}^k \lambda_i d_i^p\right]^2}{\sum_{i=1}^k \lambda_i \mu_i \sum_{i=1}^k \lambda_i \left(d_i^p - d^p\right)^2}$$

$$= \frac{\sum_{i=1}^k \lambda_i (\mu_i - \mu)^2}{\sum_{i=1}^k \lambda_i \mu_i} R^2_{U^p, V},$$

where $R^2_{U^p,V}$ is the correlation coefficient between random variables U^p and V defined on the sample space $\{1,2,...,k\}$ with the multinomial probability distribution $\{\lambda_1, \lambda_2, ..., \lambda_k\}$, and $U^p(i) = d_i^p$, $V(i) = \mu_i$. Since

$$-U^p(1) \le -U^p(2) \le ... \le -U^p(p-1) < -U^p(p)$$
$$= 0 > -U^p(p+1) \ge ... \ge -U^p(k)$$

and either

$$\mu_1 \le \mu_2 \le ... \le \mu_{l-1} < \mu_l > \mu_{l+1} \ge ... \ge \mu_k$$

or

$$\mu_1 \ge \mu_2 \ge ... \ge \mu_{l-1} > \mu_l < \mu_{l+1} \le ... \le \mu_k$$

holds, the proposed estimator to the true vertex point l asymptotically maximizes the square of the correlation coefficient between U^p and V over $p = 1,2,...,k.$

A Real Example

In *in vitro* mutagenicity assays, experimental organisms may succumb to toxic effects at high doses of test agents, thereby reducing the number of organisms at risk of

mutation and causing a downturn in the dose-response curve (Collings et al., 1981; Margolin et al., 1981).

Ames, McCann and Yamasaki (1975) reported experimental data exhibiting this pattern from three replicate Ames tests in which plates containing Salmonella bacteria of strain TA98 were exposed to various doses of Acid Red 114. The number of visible revertant colonies on each plate was observed. We assume a Poisson distribution for the number of visible revertant colonies and test whether an umbrella pattern in the mean exists.

Figure 1 is a scatter plot of the number of visible revertant colonies against dose level, which clearly indicates an umbrella pattern peaked between the third dose and the fourth dose. The test statistic is

$$X^2 = \max\left(75.71, 75.76, 75.90, 76.20, 69.78, 55.96\right) = 76.20.$$

The conservative test gives a p-value less than 0.00001, indicating a strong evidence that an umbrella pattern exists. Since $\max_{1\le p\le 6} X_p^2$ is obtained when p=4, i.e., when dose $d_4 = 10000$ (μg/ml) of Acid Red 114 is used, the estimated peak dose is d_4=10000 (μg/ml).

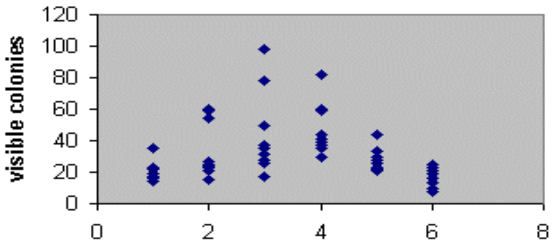

Figure 1. Visible colonies count against dose

Simulation Studies

To understand the performance of the proposed test and the estimator for the vertex point when the alternative hypothesis is true, we have carried out a simulation study to evaluate the statistical power of the proposed test and the probability that the vertex point estimator correctly estimates the true vertex point for a set of selected parameters.

In our simulation, we assume that a total of five independent Poisson distributions associated with five different dose levels $d_i = i, i = 0,1,2,3,4.$ We also assume that the sample size of all 5 groups is the same, i.e., $n_1 = n_2 = n_3 = n_4 = n_5$. Theorem 1 is used to determine the x^2 which achieves the upper 5% percentile of the test statistic under the null hypothesis.

The empirical power of the proposed test is computed as the proportion of rejections of the null hypothesis over repeated independent tests with a selected set of umbrella patterns. The performance of the proposed estimator to the vertex point is assessed by computing the empirical probability that the proposed estimator correctly estimates the true vertex point.

Table 1 presents the empirical power of the test and the empirical probability of correct estimation of the vertex point for three different choices of the true umbrella pattern and various sample sizes. Each entry in Table 1 is computed from 10000 independent hypotheses tests and estimations. All the tests assume a significance level of 5%.

The first column in Table 1 is the true mean vector $(\mu_1, \mu_2, \mu_3, \mu_4, \mu_5)$. Notice that these umbrella patterns are chosen so that each possible interior vertex point (i.e., l=2,3,4) within the boundary of the dose interval is considered. Because the monotone trend is included in the alternative hypothesis when the vertex point falls on the boundary of the dose interval, it is of interest to see how our proposed test performs in these alternatives.

This is relevant given the fact that, when an umbrella pattern is likely in the dose-response relationship, the traditional statistical monotone trend tests become inadequate because of their power loss and inherent, and possibly erroneous decisions (Collings et al., 1981; Bretz and Hothorn, 2001). We simulated the statistical power of the proposed test for detecting the monotone trend and compared that to the traditional trend test as discussed by Cochran (1954) and Tarone (1982).

Table 1: Empirical Power and Probability with an Interior Vertex Point.

Umbrella Pattern	Sample Size Per Dose	Power (%)	Correct Vertex Estimation (%)
(2,2.5,3,2.5,1.5)	10	51.98	68.81
	20	84.66	77.90
	30	96.56	82.85
	40	99.22	87.18
	50	99.79	89.71
	80	100	93.59
(1.5,2,2.5,3,2.5)	10	53.31	47.84
	20	85.97	57.63
	30	96.54	62.92
	40	99.34	66.64
	50	99.86	69.15
	80	100	74.32
(2.5,3,2.5,2,1.5)	10	53.23	46.85
	20	85.47	58.08
	30	96.60	63.88
	40	99.24	66.64
	50	99.79	68.66
	80	100	74.09

Table 2 provides the empirical power and the comparison along with the empirical probability of the correct estimation of the vertex point. The second column in Table 2 is the empirical power based on our proposed test. The third column in Table 2 is the empirical power based on the test by Cochran (1954) and Tarone (1982). Because the vertex point for a monotone trend could be either l=1 or l=5, the empirical probability of the correct estimation to the true vertex points reported in Table 2 refers to the proportion over repeated estimates that either l=1 or l=5 is correctly estimated. Each entry in Table 2 is also computed from 10000 independent hypotheses tests and estimations.

Another different type of alternative hypothesis is when a flat segment appears in the Poisson mean vector $(\mu_1, \mu_2, \mu_3, \mu_4, \mu_5)$.

Table 3 presents the empirical power and the empirical probability of the correct estimation of the vertex points for several different choices of such patterns. Since the vertex point in some of these situations is not unique, the empirical probability of the correct estimation reported in Table 3 refers to the proportion that one of the possible vertex points is correctly identified over 10000 independent estimates. Data simulations are done using the random number generating function RANPOI

Table 2: Empirical Power and Probability with a Boundary Vertex Point.

Umbrella Pattern	Sample Size Per Dose	Power[1] (%)	Power[2] (%)	Correct Vertex Estimation (%)
(1.5,1.8,2.0,2.3,2.5)	10	33.69	41.85	52.86
	20	62.45	70.45	68.05
	30	80.55	86.65	78.25
	40	90.20	94.18	84.46
	50	95.58	97.45	88.14
	80	99.71	99.89	94.91
(3.5,3.4,3.0,2.8,2.6)	10	22.81	28.27	44.66
	20	40.99	49.29	57.09
	30	56.80	65.14	66.32
	40	70.90	78.63	73.20
	50	80.05	86.83	78.59
	80	94.85	97.14	88.04

[1]Proposed test, [2]Cochran & Tarone's test.

Table 3: Empirical Power and Probability with a Flat Segment in the Pattern

Umbrella Pattern	Sample Size Per Dose	Power (%)	Correct Vertex Estimation (%)
(2.5,3.0,3.0,2.5,2.0)	10	26.18	77.21
	20	50.48	87.36
	30	69.76	92.19
	40	82.76	95.12
	50	90.61	96.64
	80	98.95	98.91
(2.5,3.0,3.0,3.0,2.5)	10	11.95	83.36
	20	20.81	90.08
	30	30.39	93.72
	40	40.36	95.98
	50	49.78	97.37
	80	71.38	99.36
(2.5,2.5,3.0,3.0,2.5)	10	9.71	63.19
	20	15.07	70.92
	30	21.47	78.14
	40	28.10	81.78
	50	34.82	85.59
	80	52.70	92.83

from Statistical Analysis System (SAS Institute, Inc. 1999).

Conclusion

When an umbrella pattern is likely in the dose-response relationship, the usual statistical trend tests become inadequate because of their power loss and inherent, and possibly erroneous decisions (Collings et al., 1981; Bretz & Hothorn, 2001). We proposed in this paper a test of homogeneity against umbrella alternatives in a sequence of Poisson distributions associated with an ordered dose variable and studied the limiting null distribution and the statistical power.

We also proposed an estimator of the vertex point when the umbrella pattern is confirmed and studied the performance of the estimator. Although the simulation study verifies that the increase of the sample size always increases the statistical power of the test and the probability of the correct estimation to the vertex point, Table 1 seems to indicate that for the selected set of parameters, the proposed estimator to the true vertex point performs better when the vertex point (l=3) is in the middle of the dose interval than when it is away from the middle of the dose interval (l=2,4). The statistical power of the proposed test, however, seems to be very comparable wherever the interior vertex is.

Our proposed test not only detects an umbrella pattern effectively based on the simulation results in Table 1, but also possesses reasonable statistical power to detect a monotone trend which is a subset of the alternative hypothesis considered in this paper. In fact, the simulation in Table 2 shows that, although our proposed test does not have as much the statistical power for detecting the monotone trend as the trend test of Cochran (1954), the difference in power between these two tests is relatively small. This is especially promising given the fact that the trend test of Cochran (1954) is asymptotically locally optimal against any choice of smooth monotone function H (Tarone, 1982).

On the other hand, the simulation results reported in Table 3 seem to indicate that the statistical power of the proposed test deteriorates when a substantial flat segment exists in the mean vector of the Poisson distributions, although the proposed vertex estimator still shows a high probability of correctly identifying one of these multiple vertex points.

Like the similarity on the test statistic for testing a monotone trend between a sequence of binomial distributions and a sequence of Poisson distributions (Armitage 1955; Cochran 1954), the proposed test and estimation techniques can be readily extended to the situation for detecting an umbrella pattern in a sequence of binomial distributions.

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Appendix

We give the proof of Theorem 1, which gives the null distribution of X^2. Let

$$Y_p = \frac{\left[\sum_{i=1}^{k} \frac{x_i d_i^p}{n} - \hat{x}\sum_{i=1}^{k} \lambda_i d_i^p\right]}{\sqrt{\mu \sum_{i=1}^{k} \lambda_i \left(d_i^p - d^p\right)^2}} = \sum_{i=1}^{k} \frac{x_i \Delta_i^p}{n}$$

where

$$d^p = \sum_{i=1}^{k} \lambda_i d_i^p$$

$$\mu = \sum_{i=1}^{k} \lambda_i \mu_i$$

$$\Delta_i^p = \frac{d_i^p - \sum_{i=1}^{k} \lambda_i d_i^p}{\sqrt{\mu \sum_{i=1}^{k} \lambda_i \left(d_i^p - d^p\right)^2}}.$$

Let $\hat{X}_i = \frac{x_i}{n}$. Note that

$$\begin{pmatrix} Y_1 \\ Y_2 \\ \cdots \\ Y_k \end{pmatrix} = \Delta' \begin{pmatrix} \hat{X}_1 \\ \hat{X}_2 \\ \cdots \\ \hat{X}_k \end{pmatrix}$$

where $\Delta = \left(\Delta_i^p\right)$ is the k by k matrix whose (i, p) entry is Δ_i^p. Since

$$\sqrt{n}\left[\begin{pmatrix} \hat{X}_1 \\ \hat{X}_2 \\ \cdots \\ \hat{X}_k \end{pmatrix} - \begin{pmatrix} \lambda_1 \mu_1 \\ \lambda_2 \mu_2 \\ \cdots \\ \lambda_k \mu_k \end{pmatrix}\right] \to N(0, A)$$

where $A = (a_{ij})$ is the k by k matrix such that $a_{ij} = 0$ if $i \neq j$ and $a_{ij} = \mu_i \lambda_i$ if $i = j$, and the limit is in distribution. Therefore,

$$\sqrt{n}\left[\begin{pmatrix} Y_1 \\ Y_2 \\ \cdots \\ Y_k \end{pmatrix} - \Delta' \begin{pmatrix} \lambda_1 \mu_1 \\ \lambda_2 \mu_2 \\ \cdots \\ \lambda_k \mu_k \end{pmatrix}\right] \to N(0, \Delta' A \Delta).$$

Theorem 1 follows from the fact that $\sqrt{n}(Y_1, Y_2, ..., Y_k)'$ and $(X_1, X_2, ..., X_k)'$ are stochastically equivalent under H_0.