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ORIGINAL ARTICLE

The Consistency Research of Two Incubation Methods in Anti-EBNA1-IgA Antibody Detection

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SUMMARY

Background: **The aim of this study was to prove the difference between 37°C water incubation method and the BEP III automated immunoassay analyzer incubation method in anti-EBNA1-IgA antibody detection and to find the best way to improve the consistency of the two incubation methods.**
Methods: **The 37°C water incubation method and BEP III analyzer incubation method were used with the same panel of samples (n = 39) in anti-EBNA1-IgA antibody detection. Except for incubation, the rest of the steps were performed by the BEP III analyzer in both groups. All the data were analyzed by SPSS 17.0 software. Line charts and bar charts were used to compare the difference between the two incubation methods in anti-EBNA1-IgA antibody detection. We planned to find the best incubation scheme for BEP III analyzer, consistent with the water incubation method, using three groups of prolonged incubation time experiments.**
Results: **A sample panel of 39 outpatients were analyzed by two incubation methods. The results showed by line charts that the water incubation method had higher S/CO values than the BEP III analyzer incubation method. Meanwhile the water incubation group had more positive results (61.5%) and less borderline positive results (35.9%) than that of the BEP III analyzer incubation group which were 43.5% and 51.2%, respectively, in the stacked bar charts. We found that by prolonging the incubation time in the BEP III analyzer for 6 minutes in the first and second incubation steps the S/CO values we increased and achieved statistically coincident results with water incubation group.**
Conclusions: **There were biases between the 37°C water incubation method and the BEP III analyzer incubation method in anti-EBNA1-IgA antibody detection. The water incubation method had higher S/CO values than the BEP III analyzer incubation method in paired groups and led partly to a difference in test results. By prolonging the BEP III analyzer incubation time properly, it can reduce the difference to some extent resulting in statistically similar results with the water incubation method.**
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KEY WORDS

anti-EBNA1-IgA, ELISA, incubation method, consistency

INTRODUCTION

Epstein-Barr infection and presence of a nasopharyngeal cancer cases in the family increases the risk of developing NPC. Anti-EBNA1-IgA may be present in the sera of non-cancer individuals and predict NPC [1]. The ELISA method is widely used in anti-EBNA1-IgA de-

tection. We found that in the ELISA incubation step using 37°C water incubation method always had higher S/CO values than BEP III analyzer incubation in the same sample. So, we thought the incubation method may be the key reason for biases. The ELISA microplate usually requires several minutes to attain the equilibrium temperature of 37°C in an automatic enzyme immunoassay analyzer, but in the water incubation it can reach 37°C rapidly. In our article, the anti-EBNA1-IgA reagent used a 37°C water incubation as the standard incubation method before delivery. Therefore, the aim of our experiments was to prove the difference of the two incubation methods and find the best way to improve the consistency of the BEP III analyzer incubation method and the water incubation method.

MATERIALS AND METHODS

Samples

We selected the samples from patients of the Traditional Chinese Medicine Hospital of Guangdong Province from January through December 2015.

Methods and analyses

Anti-EBNA1-IgA antibody reagents were provided by ZhongShan biological engineering Ltd. Instruments included the BEP III automatic enzyme immunoassay analyzer, ShangHai JingHong water bath, and the Tecan freedom evo2 pretreatment system. The BEP III analyzer prolonged experiments were designed to reduce the difference of the two incubation methods. Scheme 1: both the first and the second incubation steps were extended for 5 minutes. Scheme 2: the first incubation step was extended for 6 minutes and the second incubation step was extended for 5 minutes. Scheme 3: both the first and the second incubation steps were extended for 6 minutes.

Interpretation of results: cutoff = 0.5 + NC, sample OD_{450}/cutoff ratios of each sample were calculated. Negative: < 0.8, borderline positive: 0.8 ~ 1.2, positive: > 1.2.

Analysis variation

Intra assay CV of anti-EBNA1-IgA antibody ELISA kit was the variation of 20 repetitions of one sample and internal quality control of the current month as the inter-assay CV of the anti-EBNA1-IgA.

Statistical analysis

Data were analyzed by SPSS 17.0. Line charts were used for cases analyzed by the water incubation and BEP III automatic enzyme immunoassay analyzer. Bar charts were used to compare the S/CO values of two incubation methods. Paired chi-square tests were used to compare the difference between the water incubation method and BEP III analyzer incubation improved schemes. Regression analysis was done to estimate the correlation coefficient and regression equation of the two methods.

RESULTS

Quality control

The analysis variation was detected before the experiments; the intra-assay CV was 4.1% and the inter-assay CV was 8.8%.

A panel of 39 outpatient samples were compared using line charts (Figure 1). We can see higher S/CO values in the water incubation group than in the BEP III analyzer incubation group.

Differences in the results of the two incubation methods

The results were compared by stacked bar charts (Figure 2) showing that the consistency of the two incubation methods was poor. There were 61.5% (24/39) positive results and 35.9% (14/39) grey area results in the water incubation group while 43.5% (17/39) and 51.2% (20/39), respectively, in BEP III analyzer group.

The results between the water incubation and improved BEP III analyzer incubation methods were compared by paired *t*-test (Table 1). Since there was no statistical difference between the S/CO values of the Scheme 3 group ($p > 0.05$), this proved the results in the Scheme 3 group were consistent.

DISCUSSION

There are several key steps such as washing, dispensing, and coloring, etc in ELISA experiments [2-5]. In order to exclude the possible influence factors, our experimental groups use the BEP III analyzer to accomplish all but the incubation steps.

We found that the BEP III incubation method always had lower S/CO values than the water incubation method in the same sample (Figure 1). That partly resulted in different evaluation of the samples (Figure 2). After the ELISA microplate was transferred from room temperature to the BEP III analyzer incubation unit, it would cause the temperature to drop 2 - 3°C. It took about 5 minutes for the ELISA microplate temperature to rise to 37°C. The best reaction temperature for sample, recombinant antigen, and enzyme was 37 ~ 40°C. In the water bath, the ELISA plate can rise rapidly to 37°C which is the key reason for the difference in the two incubation methods. Our experiments have proven that by prolonging the anti-EBNA1-IgA antibody incubation time in the first and second incubation steps (Table 1 Scheme 3), we can get more consistent results in both of the BEP III incubation method and the water incubation method.

Table 1. Comparison of the results between the water incubation method and the improved BEP III analyzer incubation method (n = 39).

Paired groups	Paired Differences		p
	Mean	Std Deviation	
Water incubation – BEP III incubation	0.310	0.118	0.000 *
BEP III incubation - Scheme 1	0.293	0.213	0.000 *
BEP III incubation - Scheme 2	0.097	0.173	0.001 *
BEP III incubation - Scheme 3	0.022	0.144	0.352

* **p < 0.05 was statistically significant in the paired group.**
BEP III incubation - Scheme 1: both the first and the second incubation steps were extended for 5 minutes.
BEP III incubation - Scheme 2: the first incubation step was extended for 6 minutes and the second extended for 5 minutes.
BEP III incubation - Scheme 3: both the first and the second incubation steps were extended for 6 minutes.
BEP III incubation - Scheme 3: regression equation was

$$y_{scheme\ 3} = 0.049 + 0.934x_{water\ bath},\ R^2 = 0.956.$$

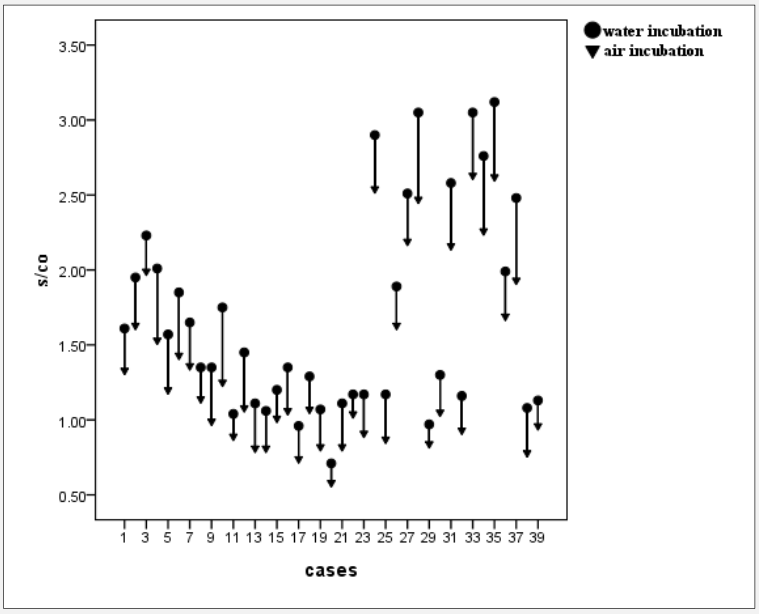

Figure 1. The S/CO value of the water incubation and BEP III incubation methods (n = 39).

CONCLUSION

Our study pointed out some ELISA experiments can be influenced by different incubation methods and cause differences in results. By prolonging the incubation time, we can reduce the difference to some extent and obtain the same results statistically in different incubation methods.

Declaration of Interest:
The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

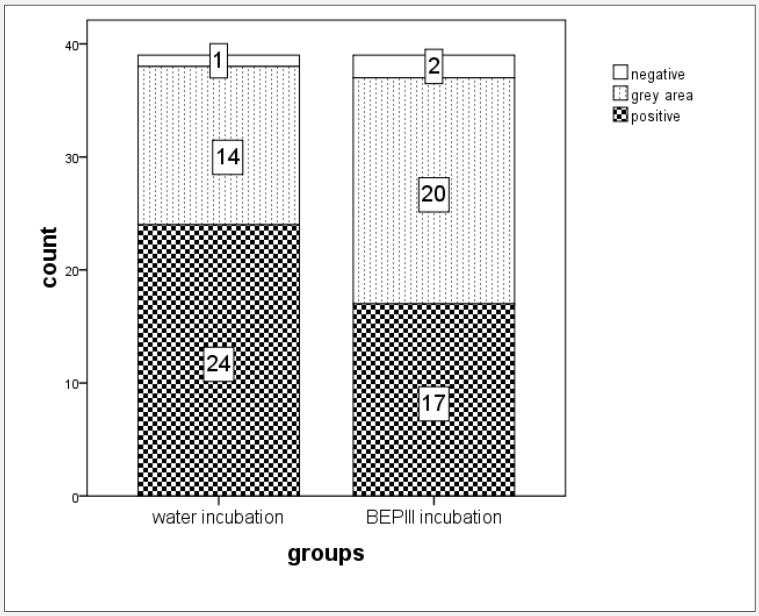

Figure 2. The results of the water incubation and BEP III incubation method (n = 39).

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