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The Establishment of the 3rd National Reference Standard for Tachypleus Tridentatus Lysate

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ABSTRACT

Objective To establish the 3rd national reference standard for Tachypleus Tridentatus Lysate Reagent. **Method and Results** The candidates of reference standard were studied for the physical and chemical properties, and the stability. To determined its sensitivity by collaboration calibration. **Conclusions** The sensitivity of the 3rd national reference standard for TAL is 0.06EU / ml, lot number is 150603-201003.

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Abbreviations: TAL,Tachypleus Tridentatus Lysate; MVD, Maximum valid dilution; NIFDC, National Institutes for Food and Drug Control

Introduction

Tachypleus Tridentatus Lysate reagent (TAL) is a kind of biological extraction, the activity may has difference between batches. In China, every TAL factory set up its own internal TAL reagent reference to calibrate the sensitivity of each batch, and control the quality of the TAL reagent. Whereas the TAL reagent production process is difference in different manufacturers, TAL quality also has difference. Sometimes TAL reagent from different factory may get different results for the same sample, which had influenced the accuracy of bacterial endotoxin test.

Since 1970, FDA has established five consecutive batch of national reference standard of limulus reagent (LAL) to control the quality of the limulus reagent better, used to calibrate, confirm, arbitrate

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the sensitivity of the limulus reagent and calibrate the potency of the bacterial endotoxin standard[1].

The first batch of national reference standard of TAL reagent was set up in 1988 in China, and the second batch was in 1997. but the new reference standard had not been produced since 2000 due to various reasons[2]. There are eight manufacturers for TAL reagent in China, they all only use their own internal reagent as a standard to calibrate the sensitivity of TAL reagent.

In order to calibrate sensitivity from different manufacturer productions with a unified scale, better regulate and control the quality of the TAL reagent in our country, National Institutes for Food and Drug Control (NIFDC) established a new national reference standard of TAL reagent in 2010.

Methods and Results

1.Production of the candidate batches

For production of a new national reference standard of TAL reagent, the NIFDC presented the requirements for the preparation and quality standard in the end of 2009. There were two TAL reagent manufacturers apply for it, and they acc--omplished in April 2010. NIFDC sampled 100 samples in the two manufacturers for study the technical indicators of candidates. Information of the TAL reference standard Candidates is as follows, see table 1.

2. Quality control for the candidate standard

We refered to the TAL quality standard [WS1 - 364 (B - 123) - 91] to test the properties, moisture, 24 hours of self coagulation, buffering capacity, sterile, and stability of the Candidate samples.

The test results are listed.

2.1 Properties

Specifications: white or almost white freeze-dried block or powder, easy to dissolve in water. Results: they were all white freeze-dried powder and soluble in water. That is the two candidates all meet the requirements.

2.2 Moisture

Specifications: The residual moisture of the 2 candidate batches was determined by Coulomb method，the residual moist--ure should not exceed 5%. Results: the two candidates all meet the requirements. Candidate B has lower moisture content, see table 2.

Table 1: Information of TAL reference standard Candidates

Candidate number	Specifications	Package	Lablled sensitivity
A	2.2ml/vial	Sealing ampoule	0.06EU/ml
B	2.2ml/vial	Vacuum gland bottle	0.06EU/ml

Table 2: results of residual moisture

Candidate number	Residual Moisture%
A	3.7
B	3.2

2.3 24 hours of self coagulation,

Specifications: Dissolved, each 0.1ml packed in 10×75cm tube, a vial gave into 4 parallel tubes, and then added 0.1ml water for bacterial endotoxin test to each parallel. Incubated for 24 hours in 37±1℃. Each batch of candidates for two vials, ultimately none tube forming gel was allowed. Results: The Results are shown in table 3. The actual pyrogen contamination of the vaccines can be detected to ensure clinical drug safety. So, the two candidates all meet the requirements.

Table3: The results of 24 hours of self coagulation

Candidate number	results of 24 hours of self coagulation
A	---- ----
B	---- ----

2.4 Buffer capability

Specifications: take 5% glucose injection or 0.9% sodium chloride injection, add dilute hydrochloric acid to adjust the pH value in 2.90 ~ 3.00, mix this solution with the same volume of TAL reagent solution, the pH value of the mixture should be in 6.00 ~ 8.00. The Results are that two candidates all meet the requirements, see table 4.

Table 4: Results of Buffer capability

Candidate number	pH value of the mixture
A	6.53
B	7.14

2.5 Sterile

Specifications:According to the sterility test, the samples should meet the requirements. There were no bacterial growth, and the two batches all meet the requirements.

2.6 Stability

Specifications: Detect the sensitivity of the samples preserved at 37℃ to study the stability of each batch. Results: The Results are shown in Table 5, and The two batches are valid for more than 3 years.

3. Collaborate the sensitivity of the candidates

A national collaborative study was run by the NIFDC Seven laboratories including Institute of Drug Control Laboratories and manufacturers enrolled in the study.

We co-calibrate the sensitivity of the candidates against the 7th National Standard of Endotoxin（150600-00707）.

The study protocol was based on the “Confirmation the labelled lysate sensi--tivity” in the “Bacterial endotoxin test. Participants were requested to provide 5 results of each batch.

The log relative sensitivity from 7 participants were combined and analyzed. Table 6 provide a complete overview of the sensitivity for each candidate batches, which show the geometric mean, 95% confidence intervals, CV% for the results. The sensitivity of candidate B is closer to the lablled sensitivity. The standard deviation and coefficient of variation of candidate B are less than candidate A, which indicate that the difference between vials of the candidate B is smaller.

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Table 5: Results of Stability

Candidate number	sensitivity at 22 months （EU/ml）	sensitivity at 33months （EU/ml）	sensitivity at 44 months （EU/ml）
A	0.066	0.06	0.06
B	0.055	0.06	0.06

Table 6: The results of Collaboration

Candidate number	A	B
Labelled lysate sensitivity	0.06EU/ml	0.06EU/ml
n	35	35
The geometric mean	0.066EU/ml	0.055EU/ml
SD	0.007	0.005
95%confidence intervals	0.059~0.072	0.051~0.060
CV%	11.0	8.64

Discussion and Conclusion

Test results show that the two batches of the candidates are in line with the requirements of all the standards.

Mainly through the stability, moisture content, sensitivity calibration results and differences between vials to evaluate and select reference standard. The stability can be maintained for more than 3 years for all the two candidates; but the moisture content of candidate B is lower, which can be conducive to maintain the sensitivity better The calibration results showed that the candidate B was closer to the labelled sensitivity of 0.06EU/ ml, and the difference between vials (CV%) was smaller than candidate A.

Based on the results of the comprehensive evaluation, it is recommended to establish batch B as the 3rd national reference standard for TAL.

The lot number of 3rd national reference standard for TAL is 150603-201003, the specification is 2.2ml, and the labelled sensitivity is 0.06EU/ml. It was approved in July 15, 2011, for calibrate, confirm, arbitrate the sensitivity of the limulus reagent and calibrate the potency of the bacterial endotoxin standard, it also can be used to verify the operation and environment for bacterial endotoxin test, and to detect bacterial endotoxin in samples.

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