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Retrospective Quality Assessment of Lyophilized Rabies Vaccine by Vero Cell for Human Use

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ABSTRACT

Objective: A retrospective analysis of critical quality attributes was conducted to evaluate product quality and manufacturing process control capability of lyophilized rabies vaccine (Vero cell) for human use during 2014-2018. Methods: Statistical analysis was performed on three critical quality evaluation parameters: potency assay, residual bovine serum albumin content, and moisture content. Quality trends and batch-to-batch consistency were assessed by integrating these results with the company's annual process performance index. Results & Conclusion: All critical parameter test results, including potency, residual bovine serum albumin, and moisture content, conformed to standard specifications. Most results remained within annual mean ±3SD, indicating favorable inter-lot consistency. All process performance index values were ⩾1.33, demonstrating adequate process control capability.

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Key Words: Rabies, Rabies Vaccine, Quality Analysis

Abbreviations: HDC, Human Diploid Cell; BSA, Bovine Serum Albumin; ELISA, Enzyme-linked Immunosorbent Assay; CVS, Challenge Virus Standard; SPF, Specific Pathogen Free; Ppk, Process performance index; SPC, Statistical Process Control.

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Introduction

Rabies is a vaccine-preventable zoonotic viral disease distributed across all continents except Antarctica, causing over 59,000 deaths in more than 150 countries and territories per year. Following the onset of clinical symptoms, rabies is almost invariably fatal[1,2]. Around the word, with over 29 million individuals receiving postexposure prophylaxis annually, the substantial demand and broad target population necessitate stringent quality control of human rabies vaccines.

In 1882, Louis Pasteur pioneered the development of human rabies vaccine. The technology has since evolved from early animal-derived neural tissue vaccines and avian embryo vaccines to primary cell culture vaccine, culminating in contemporary purified vaccines cultured in primary hamster kidney cells, chick embryo cells, human diploid cells (HDCs), and Vero cells[3]. The purified Vero cell rabies vaccine, pioneered by Institut Mérieux and granted production licensure in 1985, demonstrated favorable safety and efficacy profiles comparable to HDC vaccines in clinical observations. Characterized by high viral titers, substantial production capacity, and cost-effectiveness, this vaccine platform has achieved widespread global utilization[3,4].

Test data were sourced from Guangzhou Promise Biological Co., Ltd. (hereinafter "Promise"). The rabies vaccine produced by the company is manufactured using Vero cells cultured on bioreactor microcarriers inoculated with the fixed rabies virus strain (aGV strain), followed by culture, harvest, concentration, virus inactivation, and purification, with addition of sucrose and human serum albumin prior to lyophilization (specifications: 1.0 ml/dose).

In quality control, potency is the most critical indicator of vaccine efficacy, residual bovine serum albumin (BSA) content is an important safety parameter, and moisture content is a critical stability parameter. Between 2014 and 2018, test data from 991 sub-batches were accumulated. Potency, residual BSA content, and moisture content were selected as key quality indicators for evaluation, with results calculated as mean $\pm$ 3SD. The Chinese Pharmacopoeia specifies that lyophilized rabies vaccine for human use (Vero cell) should have a potency not less than 2.5IU/dose, residual BSA content not more than 50ng/dose, and moisture content not more than 3.0% [5]. To ensure vaccine efficacy and safety, internal release specifications were enhanced to: potency not less than 4.3IU/dose and residual BSA content not more than 15 ng/dose.

Besides that, this practice also aligns with WHO guidelines, which explicitly require manufacturers and regulatory authorities to prioritize monitoring of critical quality parameters, particularly trend analysis of potency [6]. Therefore, trend analysis serves as a critical tool for controlling and ensuring

product quality and consistency.

In this research, we conducted a retrospective analysis of five consecutive years of data on potency, residual bovine serum albumin (BSA) content, and moisture content to evaluate vaccine product quality and manufacturing process control capability. These findings may provide guidance for future product quality assessment and release specifications.

Materials and Methods

1.Equipment

Thermo Scientific™ 1300 Series Class II Type A2 Biological Safety Cabinet, BioTek ELx808™ Microplate Reader, Binder KBF 240 incubator, Metrohm 831 KF Coulometer, Sartorius BT124S Analytical Balance.

2.Vaccine and Reagents

Final vaccine product: lyophilized rabies vaccine for human use (Vero cell, Working Cell Bank, Passage No.: 137) manufactured by Promise. Challenge virus: CVS strain. Rabies vaccine potency reference standard (non-human use, Ref. No.: 250009) was obtained from the National Institutes for Food and Drug Control (NIFDC, Beijing, China). Quantitative BSA enzyme-linked immunosorbent assay (ELISA) kit was purchased from Wuxi Bosheng Medical Biotechnology Development Co., Ltd. Karl Fischer reagent was supplied by Sigma.

3.Experimental Animals

Specific-pathogen-free (SPF) mice (12–14g) for the NIH test were procured from Guangdong Provincial Medical Laboratory Animal Center.

4. Methods

4.1Potency Assay

The NIH test was employed as specified in the Chinese Pharmacopoeia. Inoculate i.p. 0.5 ml of each dilution of test sample and reference vaccine into sixteen mice respectively, inoculate again at an interval of 1 week. Fourteen days after the first injection, inoculate i.c. each mouse with 0.03 ml of the challenging virus containing 5-100 LD50. Observe the animals daily for 14 days starting from the date of challenge, and record the death. The mice that died or manifested typical signs of encephalopathy on or after the 5th day following challenge should be included in the evaluation. Calculate the ED_{50} values of the test sample and reference vaccine then calculate the potency of the test sample.

4.2Residual Bovine Serum Albumin Content

Residual BSA content in test samples was quantified by enzyme-linked immunosorbent assay (ELISA) as specified in the Chinese Pharmacopoeia. Follow the instruction of the ELISA kit, each dilution should be applied in duplicate. The assay was valid only if the absorbance of standards, the result of internal control, the linear correlation coefficient of standard curve and absorbance difference between duplicates met the requirements set forth in the kit. Plot a regression curve with the absorbance of standards to their concentrations and

calculate BSA content of the test sample solution by inserting its absorbance into the regression equation and multiplying the result.

4.3Moisture Content

Moisture content was determined based on the Karl Fischer reaction. Add an appropriate volume of Karl Fischer reagent to the titration tube. Moisture was eliminated from the reagent and the system by pre-electrolysis. After the test specimen was dissolved in anhydrous solvent, an appropriate amount was quickly injected into the titration. Perform dead-stop titration to the electrometric endpoint. Then the water content of the specimen could be obtained from the instrument output.

4.4 Statistical Analysis and Quality Review

Trend analysis charts for potency, residual BSA content, and moisture content were generated annually to evaluate the historical quality control performance of the lyophilized rabies vaccine. Annual mean ± 3SD of critical quality attributes were served as primary analytical metrics for quality review. Single-batch data points falling within mean ± 3SD indicated acceptable vaccine quality. The Ppk index, a process performance metric[7], was utilized for assessment of overall process capability. Ppk values of 1.33-1.67 denoted adequate process control, whereas Ppk ≥ 1.67 indicated enhanced process capability [8-10].

Results

1. Statistical Summary

Statistical analysis was performed on three critical quality parameters — potency assay, residual bovine serum albumin (BSA) content, and moisture content—from vaccine lots manufactured between 2014 and 2018. Process capability was evaluated using Ppk values. Specific data are shown in Table 1.

2. Potency Assay

Potency was determined by the NIH test for 991 sub-batches of lyophilized rabies vaccine for human use (Vero cell). Values ranged from 4.6 IU/dose to 13.2 IU/dose, with a mean of 8.4 IU/dose, all exceeding the internal release specification of 4.3 IU/dose. In 2016 and 2017, three batches exhibited values slightly above the respective annual mean + 3SD. The relatively flat trend in annual mean values indicated stable potency and favorable inter-lot consistency (Figure 1).

3.Residual Bovine Serum Albumin Content

Over the five-year period, the maximum residual BSA content across all vaccine batches was 12 ng/dose and the minimum was 2 ng/dose, with four batches exhibiting values slightly above the respective annual mean + 3SD. All test results were below the internal release specification of 15 ng/dose, far below the 50 ng/dose pharmacopeial specification. Annual mean values for residual BSA content remained below 10 ng/dose (Figure 2).

Table 1. Statistical summary of lyophilized rabies vaccine for human use, 2014–2018

Parameter	Analysis Metric	2014 (n=261)	2015 (n=80)	2016 (n=332)	2017 (n=232)	2018 (n=86)	5 years (n=991)
Potency	Mean±3SD	8.4±5.4	7.5±5.5	7.4±5.3	7.3±3.8	8.0±1.8	7.7±5.0
	Ppk Value	1.38	1.48	2.75	2.04	3.05	1.72
BSA	Mean±3SD	5.7±4.9	8.7±3.8	6.6±4.5	7.7±3.2	7.0±3.6	6.8±5.0
	Ppk Value	4.49	10.76	5.14	4.53	11.98	5.54
Moisture content	Mean±3SD	0.7%±0.3%	0.6%±0.3%	0.5%±0.3%	0.5%±0.3%	0.5%±0.3%	0.6%±0.3%
	Ppk Value	3.21	2.47	9.35	11.82	6.92	4.15

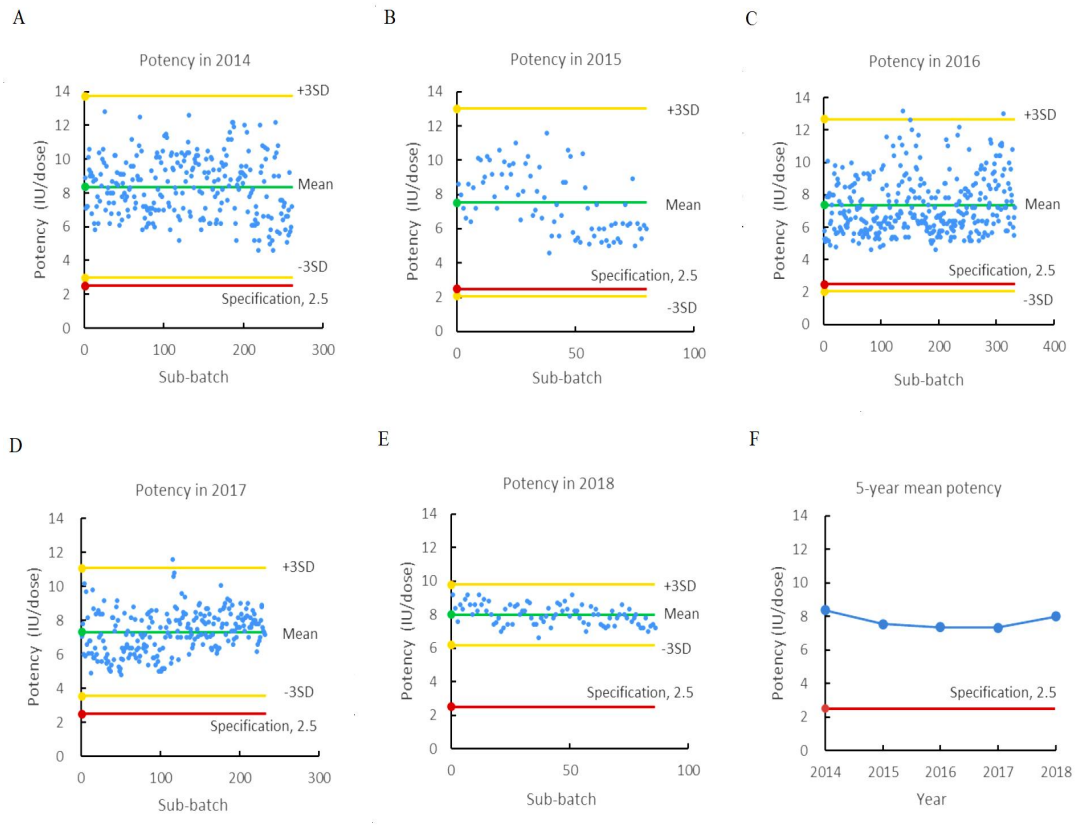

Figure1: Trend analysis of potency assay (A – F).
A: Potency in 2014; B: Potency in 2015; C: Potency in 2016; D: Potency in 2017; E: Potency in 2018; F: 5-year mean potency.

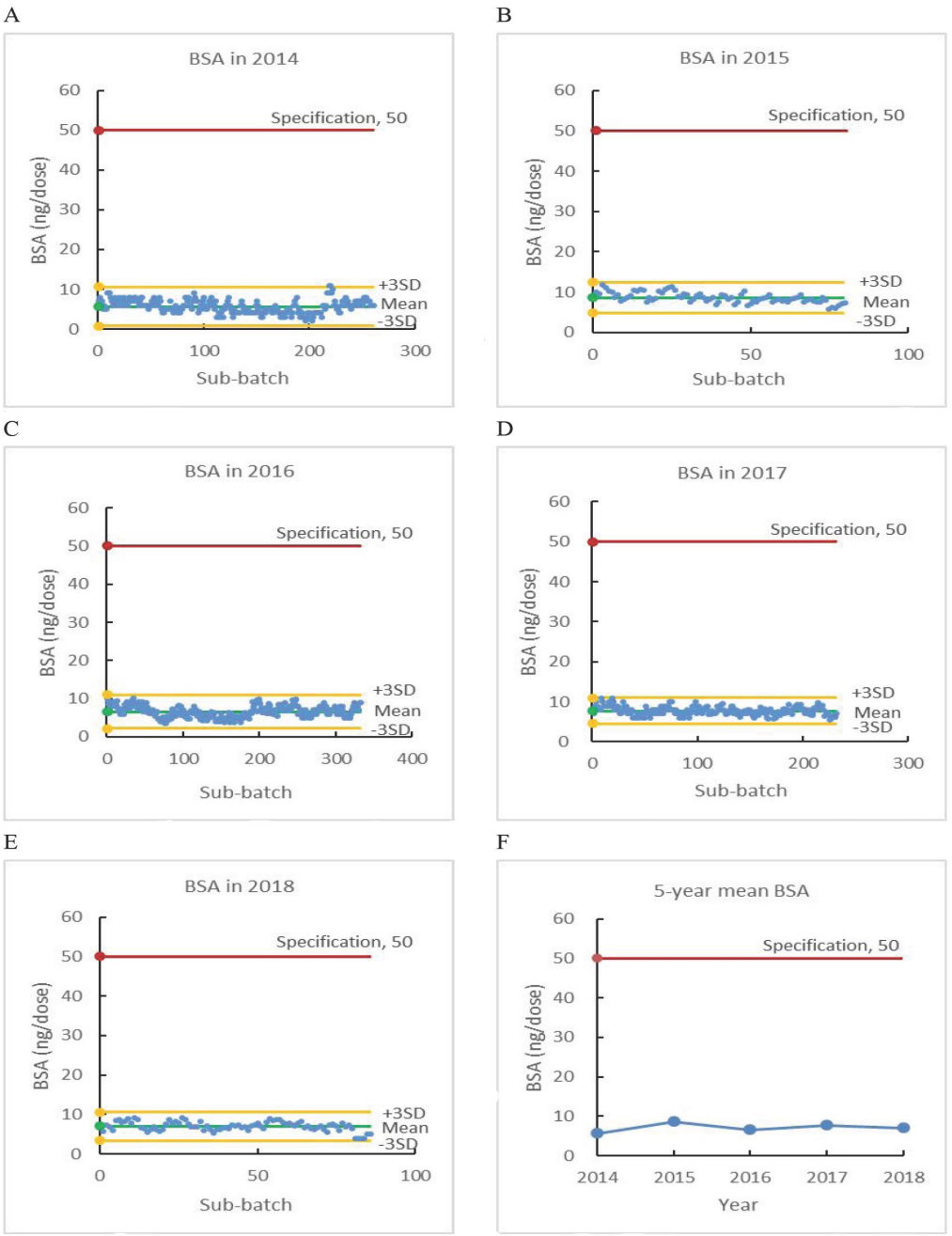

Figure2: Trend analysis of residual bovine serum albumin (BSA) content (A – F). A: BSA in 2014; B: BSA in 2015; C: BSA in 2016; D: BSA in 2017; E: BSA in 2018; F: 5-year mean BSA.

4. Moisture Content

Over the five-year period, the maximum moisture content across all vaccine batches was 1.0% and the minimum was 0.3%, with two batches exhibiting values slightly above the respective annual mean + 3SD. All test results were no higher than 1.0%, far below the 3.0% pharmacopeial specification (Figure 3).

Discussion

For Promise's lyophilized rabies vaccine for human use (Vero cell) manufactured from 2014 to 2018, the tested parameters, including potency, residual bovine serum albumin (BSA) content, and moisture content, all conformed to the release specifications. The Chinese Pharmacopoeia requires potency not less than 2.5 IU/dose, residual BSA content not more than 50 ng/dose, and moisture content not more than 3.0%. Analysis of 991 sub-batches over five consecutive years demonstrated potency not less than 4.3 IU/dose, residual BSA content not exceeding 15 ng/dose, and moisture content not exceeding 1.0%. Furthermore, annual mean values for each parameter remained close year-over-year, test results were generally stable, and inter-lot consistency was favorable.

Ppk is a Statistical Process Control (SPC) index that measures process performance and reflects overall production process capability. Analysis of historical data indicated that Ppk values for potency assay during the first two years ranged from 1.33 to 1.67, denoting adequate process control. For the subsequent three years of potency assay, as well as for residual bovine serum albumin and moisture content, Ppk values exceeded 1.67 across all years, indicating superior process control.

These findings demonstrate that the rabies vaccine manufactured by Promise from 2014 to 2018 was produced under stable process conditions, with good process control capability.

Competing interests

Authors are employees of Guangzhou Promise Biological Co., Ltd, which manufactures and commercializes the human rabies vaccines analyzed in this study. The authors declare that despite this employment relationship, the collection, analysis, and interpretation of data were conducted objectively."

Editor's Comments

While we note a conflict of interest regarding this article, the Editorial Board has decided to publish it due to its unique data significance. It provides a retrospective quality analysis of 991 batches of human rabies vaccines produced in China over a five-year period. Following a review by three experts, we concluded that the paper offers substantial academic value and serves as an important reference for the industry.

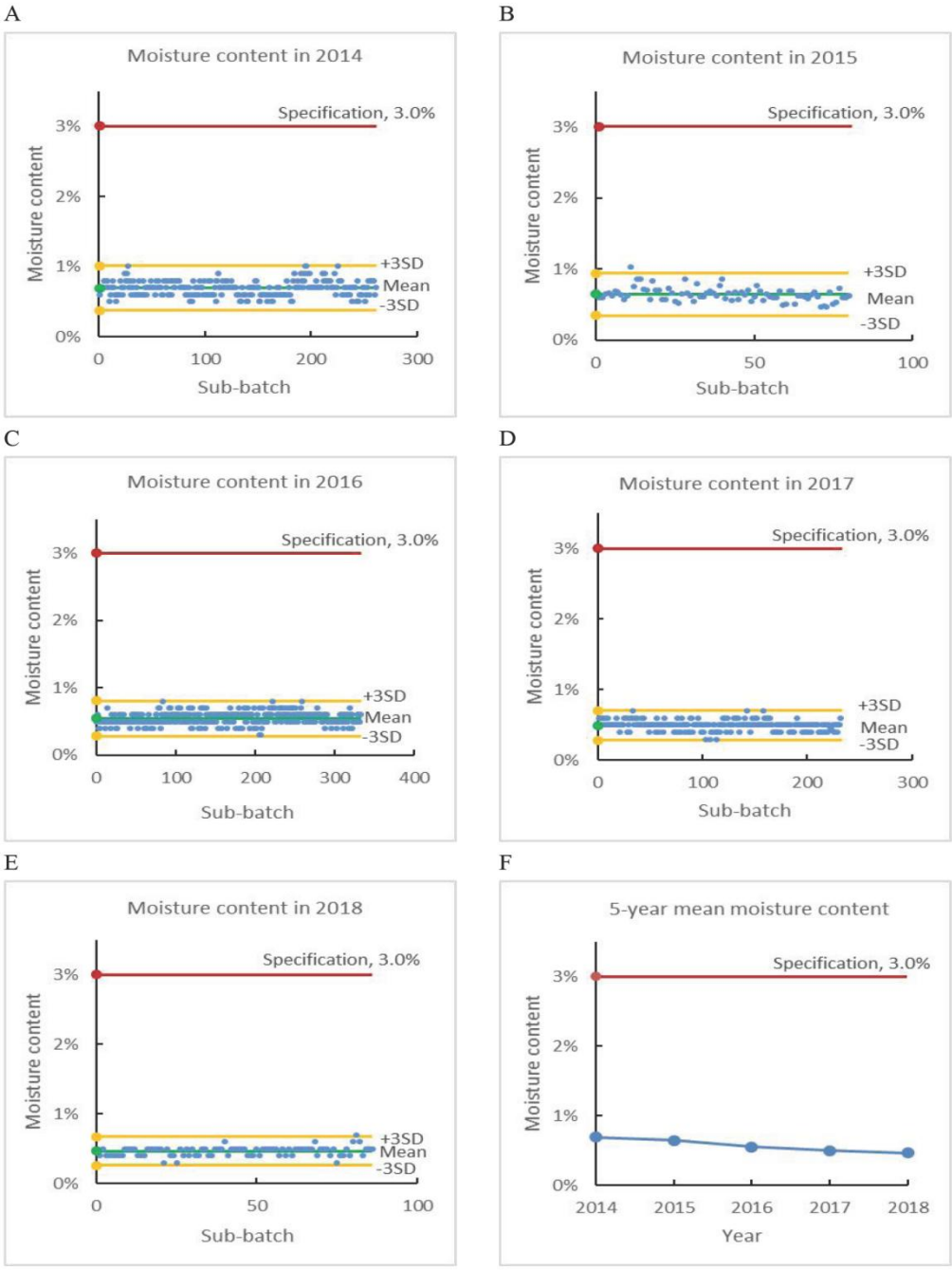

Figure3: Trend analysis of moisture content (A – F).
A: Moisture in 2014; B: Moisture in 2015; C: Moisture in 2016; D: Moisture in 2017; E: Moisture in 2018; F: 5-year mean moisture content.

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