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Performance evaluation of reference substance for oral live rotavirus vaccines

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Abstract: *The results showed that the appearance inspection and moisture content of the reference material for oral rotavirus live vaccine met the requirements; The titration results for 12 years showed that the reference titers of oral rotavirus live vaccines stored below -20 ℃ were within the quality control range of 6.2-7.2lgCCID$_{50}$/mL, with an annual detection mean of 6.6-6.8lgCCID$_{50}$/mL and an annual detection standard deviation of 0.17-0.23lgCCID$_{50}$/mL. Trend analysis shows that the reference titer of the oral rotavirus live vaccine is stable and there is no downward trend. The reference material for oral rotavirus live vaccines currently in use has stable and effective performance, ensuring the accuracy and reliability of virus titer detection results for oral rotavirus live vaccines and oral trivalent reconstituted rotavirus attenuated live vaccines (Vero cells) in daily use. This can provide assurance for the quality control of the two types of rotavirus vaccines.*

Keywords: *Oral live rotavirus vaccine, Reference substance, Appearance, Moisture content, Virus titer, Overall trend analysis*

1. Introduction

Rotavirus is the main cause of severe dehydrated gastroenteritis in children under 5 years old worldwide [1,2]. Currently, there is no specific antiviral treatment for rotavirus. Once severe symptoms occur, the only clinically effective intervention is fluid replacement therapy. The incubation period of rotavirus infection is very short, estimated to be less than 48 hours. In emergency rooms and children who require hospitalization, the characteristics of rotavirus disease are usually watery diarrhea, vomiting, and fever, which can lead to electrolyte imbalance, shock, and in some cases even death[3]. At present, vaccination against rotavirus is the most effective method for preventing and controlling rotavirus infection. Vaccination against rotavirus has led to a significant reduction in diarrhea deaths and hospitalizations [4,5].

Oral rotavirus live vaccine and oral trivalent reconstituted rotavirus attenuated live vaccine (Vero cells) are the first domestically approved rotavirus vaccine for preventing rotavirus induced diarrhea, independently developed by Lanzhou Institute of Biological Products Co., Ltd. (referred to as Lanzhou Company). The viral titer is a key indicator for evaluating the effectiveness of rotavirus vaccines as a biological activity of the vaccine. The $CCID_{50}$ method, also known as the microcytosis assay or cell culture half infection dose, is mainly used to detect viral infection titers: the virus is diluted in multiples or gradients and inoculated into cells to observe its cytopathic effect (CPE). The $CCID_{50}$ is calculated using the Spearman Kaber method to obtain the viral infection titer. In virus titer detection experiments, unlike measles vaccines, rotavirus vaccines have relatively weaker destructive effects on host cells, and some cellular lesions cannot be presented under a microscope. Enzyme linked immune-sorbent assay is needed to more intuitively display cellular lesions.

For over 20 years, the $CCID_{50}$ combined with enzyme-linked immunosorbent assay (ELISA) has been used for the detection of viral titers in oral rotavirus live vaccines. Based on this, the total viral titer of the oral trivalent reconstituted rotavirus attenuated live vaccine (Vero cells) of Lanzhou Company has also been detected using this method. Due to the variability of biological activity testing methods for vaccines due to multiple factors, there may be differences in testing between different laboratories and personnel, which increases the difficulty of quality control. In virus titer testing, the use of reference substance can ensure the accuracy of vaccine potency testing, minimize interference between laboratories and various influencing factors, and provide a unified reference standard for related products, Enable production enterprises and quality control departments to better control product quality [6-7]. The

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reference material for oral rotavirus live vaccine was prepared by Lanzhou Company in June 2006 and recognized by legal institutions for quality control of viral titers in oral rotavirus live vaccines. It has been in use for 17 years and its stability is evaluated annually by the company. This article examines the appearance, moisture content, and viral titer of reference substance for oral rotavirus live vaccines, and summarizes and analyzes the virus titration data accumulated over 12 years to evaluate the performance of reference substance for oral rotavirus live vaccines. This is to better guide the quality control of viral titers for oral rotavirus live vaccines and oral trivalent reconstituted rotavirus attenuated live vaccines (Vero cells), ensuring the accuracy of vaccine efficacy.

2. Materials and Methods

2.1. Samples

Reference material for oral rotavirus live vaccine (batch number: 20060601), stored at -20 ℃ or below, prepared by Lanzhou company and recognized by legal institutions.

2.2. Cells

MA104 cells sensitive to rotavirus, purchased from ATCC.

2.3. Main reagents and instruments

RPMI Medium 1640 (1x) and trypsin were both purchased from Gibco Corporation in the United States; Xinsheng Bovine serum was purchased from Lanzhou Minhai Biotechnology Co., Ltd. 96 well cell culture plates were purchased from Corning Corporation in the United States; The rotavirus detection kit is produced by the Diagnostic Products Laboratory of Lanzhou Company. C30S Coulometric KF Titrator purchased from Mettler Toledo Group; The INC153 type CO2 cell culture incubator was purchased from Memmert, Germany; The 1555 constant temperature incubator was purchased from SHEL LAB Company in the United States; The HERAcell150i CO2 incubator and MK3 ELISA reader were both purchased from Thermo Scientific in the United States; SpectraMax ABS ELISA reader was purchased from MD Company in the United States; CountstarAltari cell analyzer was purchased from Shanghai Ruiyu Biotechnology Co., Ltd.

2.4. Reference performance test

Evaluate the performance of the reference substance for oral rotavirus live vaccine by testing its appearance, moisture content, and viral titer.

2.4.1. Appearance inspection

Before each use of the reference substance for oral rotavirus live vaccine, a visual inspection should be conducted. This reference substance is a freeze-dried formulation and appears loose in cheese color. After re dissolution, it should be a clear pink liquid with no foreign objects.

2.4.2. Moisture content detection

Randomly select 4 reference substance for oral rotavirus live vaccines and use the Fischer Coulomb titration method to test their moisture content. The test results are determined according to the standard of biological product standard substances with a moisture content of ≤ 3.0% specified in the 2020 edition of the Pharmacopoeia of the People's Republic of China (Part III) (referred to as the Chinese Pharmacopoeia).

2.4.3. Virus titer

The titer of the reference substance for oral live rotavirus vaccine should be between 6.2-7.2 $lgCCID_{50}$/mL. The reference substance titer was detected using the $CCID_{50}$ assay combined with enzyme-linked immunosorbent assay (ELISA). Take one reference sample and add 1mL RPMI Medium 1640 (1x) according to the indicated amount for re dissolution. After the final concentration is 20 μ Treat with trypsin of g/mL at 37 ℃ for 50 minutes. With a final concentration of 1 μ RPMI Medium 1640 (1x) with g/mL trypsin was used as virus culture medium. The treated reference material was diluted 10 times in a series of dilutions. The virus suspension with appropriate dilution was inoculated into a 96 well cell culture plate already filled with MA104 cells. Each dilution was inoculated into 4 wells, with 100 cells

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per well μ L. Incubate in a 37 ℃, 5% CO_2 incubator for 6 days, freeze-thaw 1-2 times, and take 50 samples from each well μ Add L to the enzyme-linked immunosorbent assay (ELISA) plate, perform ELISA detection according to the instructions of the rotavirus detection kit (enzyme-linked immunosorbent assay), determine the positivity of each well, and calculate the viral titer of the reference sample using the Spearman karber method formula. Summarize the accumulated virus titer test data of reference products for oral rotavirus live vaccines since 2012 by year, and calculate the average value ($\bar{x}$)、Standard deviation (S) and coefficient of variation (CV).

2.5. Statistical analysis

Use Excel 2016 software for data statistics and plotting.

3. Results

3.1. Appearance inspection

The results showed that the reference substance for oral rotavirus live vaccine appeared as a loose cheese colored substance, and after re dissolving, it was a clear pink liquid with no foreign objects.

3.2. Determination of moisture content

The results showed that after storing the reference material for oral rotavirus live vaccine at -20 ℃ for 17 years, four samples were randomly selected, and the moisture content test results were 2.11%~2.46%, with a mean of 2.30%, as shown in Table 1.

Table 1: Moisture content test results of reference substance for oral rotavirus live vaccine

Detection times	Moisture content(%)
1	2.32
2	2.31
3	2.11
4	2.46
$\bar{x}$	2.30

3.3. Analysis of virus titer data accumulated over 12 years

Table 2: Test results of viral titer of reference substance for oral rotavirus live vaccine

Year	Titration times	Titer range ($lgCCID_{50}$/mL)	$\bar{x}$ ($lgCCID_{50}$/mL)	S ($lgCCID_{50}$/mL)	CV (%)
2012	139	6.2~7.0	6.6	0.23	3.46
2013	129	6.2~7.0	6.6	0.21	3.18
2014	173	6.2~7.0	6.7	0.23	3.39
2015	139	6.2~7.0	6.7	0.22	3.28
2016	69	6.5~7.0	6.7	0.19	2.89
2017	96	6.5~7.0	6.8	0.19	2.74
2018	96	6.5~7.0	6.7	0.21	3.05
2019	123	6.2~7.0	6.7	0.20	2.94
2020	111	6.5~7.0	6.7	0.17	2.51
2021	97	6.2~7.0	6.7	0.18	2.67
2022	85	6.2~7.2	6.8	0.19	2.75
2023	54	6.5~7.0	6.7	0.18	2.65

The results showed that the viral titers of the reference substance for oral rotavirus live vaccine were all within its quality control range of 6.2-7.2$lgCCID_{50}$/mL; The mean value of annual testing ranges from 6.6 to 6.8$lgCCID_{50}$/mL, and the standard deviation of annual testing ranges from 0.17 to 0.23$lgCCID_{50}$/mL. The coefficient of variation of annual testing results ranges from 2.51% to 3.46%, all<10%. The viral titer test results of the reference substance for oral rotavirus live vaccine are shown in Table 2.

The overall trend analysis was conducted on the viral titer test results of the reference sample of oral

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rotavirus live vaccine accumulated for 12 years, with a mean of 6.7lgCCID$_{50}$/mL and a standard deviation of 0.21lgCCID$_{50}$/mL, which is basically consistent with the test analysis results of each year. The overall trend analysis chart shows that over the past 12 years, the viral titer values of the reference material for oral rotavirus live vaccines have mostly been concentrated between 6.5 and 7.0 lgCCID$_{50}$/mL, with the marked value as the center and fluctuating within the range of ± 0.5 lgCCID$_{50}$/mL of the marked value, and the trend is stable without significant decline. See Figure 1.

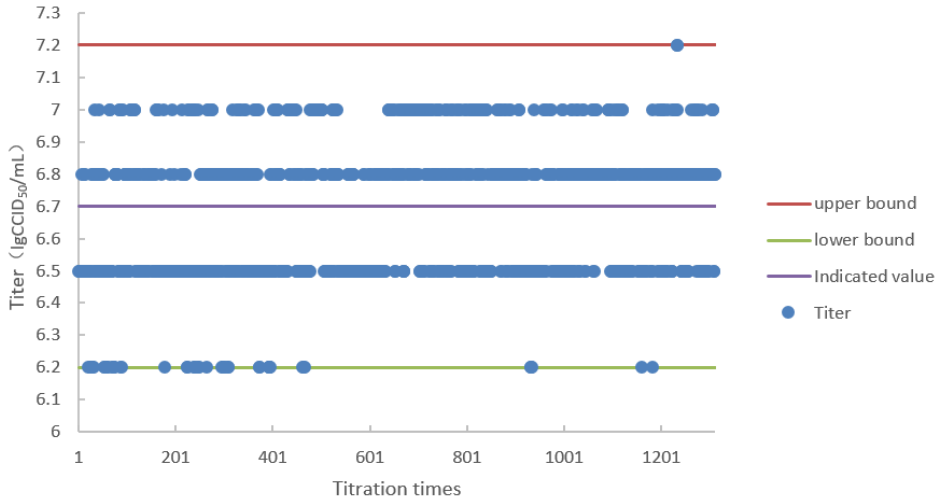

Figure 1: Analysis of titer trend of reference substance for oral rotavirus live vaccine

4. Conclusions

The reference material for oral rotavirus live vaccine was prepared by Lanzhou Company in June 2006, packaged in ampoules, hot melt sealed, and approved by legal institutions for quality control of viral titers in oral rotavirus live vaccines. Ampoules have good sealing properties, and after being hot melt sealed, they can completely block external air and maintain the stable state of the reference substance. This reference substance is usually stored below -20 ℃ and has been in use for more than 17 years. The trend of the reference material is analyzed every year. This article evaluates the appearance, moisture content, and viral titer of reference substance for oral rotavirus live vaccines. Appearance inspection is the most intuitive indicator for assessing the characteristics of reference substance, and its status and color changes can directly reflect the changes in the characteristics of reference substance. The appearance inspection of reference substance for oral rotavirus live vaccines has been in compliance with regulations for many years of use. The moisture content is one of the important indicators for evaluating the stability of freeze-dried reference substance. In this article, four reference substance for oral rotavirus live vaccines were randomly selected for moisture content testing, and the results all met the requirement of moisture content ≤ 3% in the current version of the Chinese Pharmacopoeia. The viral titer is the most important indicator for evaluating the effectiveness of reference substance for oral rotavirus live vaccines. This article analyzes the viral titer of reference substance for oral rotavirus live vaccines from 2012 to 2023, and the titer values are all within the required quality control range of 6.2-7.2lgCCID$_{50}$/mL, with a stable trend and no decrease observed; The average of each year is within ± 0.1lgCCID$_{50}$/mL of the overall mean of 6.7, and the standard deviation of each year's testing is within 0.20 ± 0.03lgCCID$_{50}$/mL. The coefficient of variation of each year's testing results is less than 10%, indicating that the performance of the current reference material for oral rotavirus live vaccines is still good and stable. It is not only for oral rotavirus live vaccines and oral trivalent reconstituted rotavirus attenuated live vaccines (Vero cells) .The accuracy of the virus titration results has played an effective reference role. In the future, we can continue to guide the quality control of the total titers of oral rotavirus live vaccines and oral trivalent reconstituted rotavirus attenuated live vaccines (Vero cells) to ensure the accuracy of rotavirus vaccine efficacy.

In the quality control of oral rotavirus live vaccine and oral trivalent reconstituted rotavirus attenuated live vaccine (Vero cell) virus (total) titers, the same batch of reference substance needs to be used for a long time, so the stable performance of the reference substance is very important. At present, the national drug standard substances do not have an expiration date, and regular stability assessments are needed in daily use to ensure their titer stability. When the titer of the reference substance for oral rotavirus live

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vaccines shows a downward trend, a new batch of reference substance should be replaced in a timely manner to ensure the accuracy and reliability of the rotavirus vaccine titer test results.

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