

# Stability of trivalent human papillomavirus (types 16, 18, 58) recombinant vaccine (*Escherichia coli*)

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*To the Editor:* There are 530,000 new cases of cervical cancer and 270,000 deaths annually in the world, 85% occur in developing countries. Among them, about 150,000 new cases of cervical cancer are in China each year, accounting for one-third of the total patients worldwide.<sup>[1]</sup> Vaccines are the most economical and effective ways of preventing infectious diseases. To stop the transmission of human papillomavirus (HPV) fundamentally, the most effective measure is to vaccinate safe and effective HPV vaccines.<sup>[2,3]</sup> Numerous studies have shown that vaccines based on HPV L1 virus-like particles (VLPs) have good safety and protective efficacy.<sup>[4]</sup> Infection of HPV16, HPV18, and HPV58 viruses is the main cause of cervical cancer in Chinese women. By preventing the infection of HPV16, HPV18, and HPV58 viruses, 85.0% to 93.7% of cervical cancers can be prevented.<sup>[5]</sup>

Beijing Health Guard Biotechnology, Inc. (Daxing District, Beijing, China) selected HPV types 16, 18, and 58 as the targeting virus strains and developed the recombinant trivalent HPV vaccine (expressed in *Escherichia coli*), adjuvanted with aluminum (hereinafter referred to as “HPV trivalent vaccine”). This product is composed of HPV16, HPV18, and HPV58 VLPs expressed by recombinant *E. coli* and aluminum adjuvant, showing a milky white suspension. It is used to prevent HPV16, HPV18, and HPV58 infections and related diseases such as cervical cancer. The HPV trivalent vaccine had obtained the clinical trial approval in October 2017 and the number was 2017L04835. The cryo-electron microscopy images of HPV16, HPV18, and HPV58 VLPs are shown in Figure 1.

Stability research is an important part of the entire vaccine development, and it is the basis for product validity. It can be used to determine the rationality of producing process, formulation of prescription, and selection of packaging materials. Vaccine stability studies generally include real-time stability studies (long-term stability studies), accelerated stability studies, high-temperature experimental studies, and light condition experimental studies. Long-term stability studies can be used as the main basis for setting vaccine preservation conditions and expiration dates. Accelerated and stress condition tests can be used to understand the stability of the vaccine in the short-term deviation from the preservation conditions and extreme conditions.

This article describes the regularity of the HPV trivalent vaccine in different storage conditions such as long-term, accelerated, high temperature, and light. The scientific basis for the long-term storage, reasonably preservation conditions, and storage life of HPV trivalent vaccine was based on the test results. In this study, we tested three batches of HPV trivalent vaccines that were prepared in the pilot plant and stored in the prefilled syringes at 2 to 8°C.

The tests of stability include identity, appearance, the volume of fill, pH, osmolarity, aluminum content, polysorbate-80 content, sterility test, bacterial endotoxin, abnormal toxicity, *in vitro* relative potency analysis, and *in vivo* potency evaluation.

The first condition is long-term stability tests. Long-term trials were conducted under actual storage conditions of vaccines, and the purpose is to provide the basis for formulating the effective period of the vaccine. Took 1000

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10.1097/CM9.0000000000001659

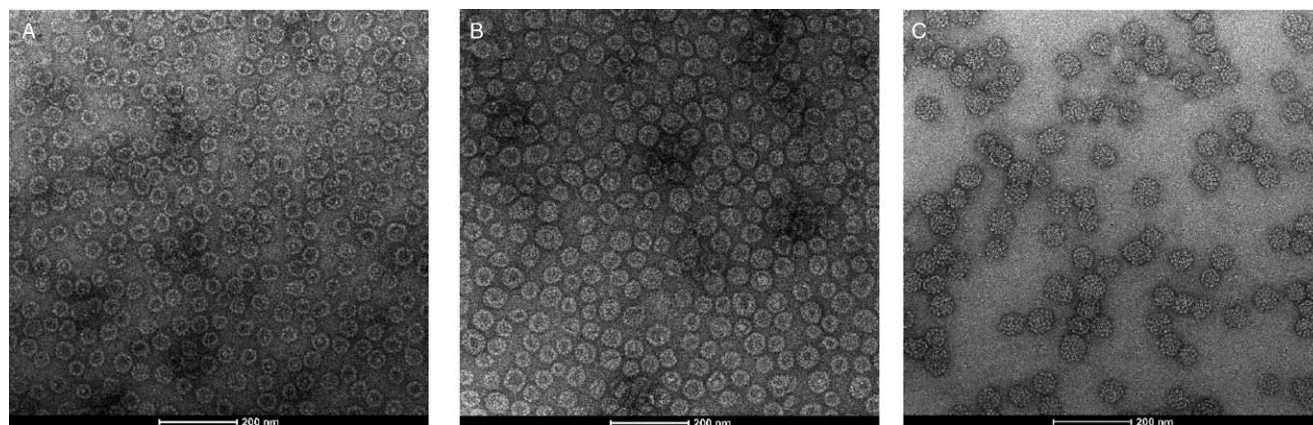
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Chinese Medical Journal 2021;134(24)

Received: 12-05-2021 Edited by: Peng Lyu



**Figure 1:** The cryo-EM images of HPV VLPs. The cryo-EM images were taken by FEI Tecnai Spirit (120 kV). The samples were negatively stained and then observed. The bars on each picture represent 200 nm. The negative staining method was as follows. A 3- $\mu$ L sample was dropped on the coated copper net (Cu-200CN) and stood for about 1 min. Then, a drop of pure water was dropped on the copper net and the excess liquid was absorbed with a filter paper and repeated three times. 1% uranyl formate was used for negative staining, and excess liquid was absorbed with filter paper. After repeating the washing step, the sample was dried to be used for electron microscope observation. (A) The cryo-EM images of HPV16 VLPs. (B) The cryo-EM images of HPV18 VLPs. (C) The cryo-EM images of HPV58 VLPs. cryo-EM: Cryo-electron microscopy; HPV: Human papillomavirus; VLPs: Virus-like particles.

samples of three batches of HPV trivalent vaccines and placed them at 2 to 8°C, avoiding light. The batch numbers were 20140501, 20140502, and 20140601. Samples were tested at 3, 6, 9, 12, 18, 24, 36, 42, 48, and 60 months. The vaccine results of day 0 were taken as the initial data for the stability test. There was no significant change in appearance, the volume of fill, pH, osmolarity, aluminum content, polysorbate-80 content, and *in vivo* efficacy. The identity tests have shown HPV16, HPV18, and HPV58 VLP antigens were contained in the vaccines. Sterility tests, bacterial endotoxins, and abnormal toxicities were all qualified. But *in vitro* relative potency analysis was qualified at 48 months and out of limit at 60 months, indicating that HPV trivalent vaccine products can be stably stored for at least 48 months under these conditions.

The second condition is accelerated tests. Five hundred samples of three batches of HPV trivalent vaccine were taken and placed at 23 to 27°C. The batch numbers were the same as long-term tests. Samples were tested at 1, 2, 3, 6, and 12 months. At 23 to 27°C, the aluminum content of the HPV trivalent vaccine (batch number: 20140501) decreased slightly but was still within the limit. HPV trivalent vaccine (batch number: 20140601) showed a slight decrease in aluminum content and polysorbate-80 content at 12 months. Among the three batches of HPV trivalent vaccines, median effective dose ( $ED_{50}$ ) values of HPV16, HPV18, and HPV58 types were slightly elevated compared with day 0. However, all the three batches of the HPV trivalent vaccine were qualified at various time points at 23 to 27°C, indicating that the HPV trivalent vaccine was stable at 23 to 27°C for 12 months. The purpose of the accelerated test was to explore the stability of the vaccine during chemical or physical changed. It provided the necessary information for prescription design, process improvement, quality research, packaging improvement, transportation, and storage.

The third condition is high-temperature tests. Two hundred samples of HPV trivalent vaccine (batch number:

20140501) were placed at 37°C for 7 and 14 days, respectively, and then taken for testing. According to the results, the pH was slightly increased, the polysorbate-80 content was decreased slowly, and  $ED_{50}$  values of HPV16, HPV18, and HPV58 were slightly elevated compared with day 0. But HPV trivalent vaccines were all qualified after stored at 37°C for 7 or 14 days. So, it was confirmed that HPV trivalent vaccine was stable at high temperatures for at least 14 days.

The fourth condition is light tests. Samples were placed at 2 to 8°C and 4000 to 5000 lux, other information was the same as high-temperature tests. The purpose of high temperature and light tests was to examine the rationality of the prescription, the process of production, and packaging conditions. HPV trivalent vaccine was removed from the outer package and placed in a suitable open container. The results showed that compared with the results measured on day 0, pH was increased slowly, but still within the limit. All other inspection items were qualified and have no significant changes during the temperature of 2 to 8°C and 4000 to 5000 lux light for 14 days.

According to the tests above, the storage condition of the HPV trivalent vaccine is determined to be 2 to 8°C avoiding light. The vaccines were qualified at 48 months under that condition. It can be up to 14 days under high temperature and light conditions, and up to 12 months at 23 to 27°C. However, high-temperature light is avoided as much as possible. The period of validity is 36 months conservatively.

The good quality and stability of HPV trivalent vaccines are of great importance for Chinese women. In China, HPV vaccines are not covered by Social Medical Insurance System currently. It costs RMB 2000 to 4000 Yuan to complete the immunization procedure for three imported HPV vaccines on the market, which are namely Cervarix<sup>TM</sup>, Gardasil<sup>®</sup>, and Gardasil<sup>®</sup>9. That means a large number of people who belong to the low-income family

would not be able to afford the cost of vaccination. It reduces the production cost of the HPV vaccine by using *E. coli* as the expression host, making HPV trivalent vaccines a strong competitor to the three imported HPV vaccines.

### Conflicts of interest

None.

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**How to cite this article:** Liu YY, Zhang HJ, Shen EC, Chen D, Wang Y, Fu SJ, Yin F, Zhang GF, Shen YG, Liu YJ. Stability of trivalent human papillomavirus (types 16, 18, 58) recombinant vaccine (*Escherichia coli*). *Chin Med J* 2021;134:3020–3022. doi: 10.1097/CM9.0000000000001659