HUMAN VACCINES & IMMUNOTHERAPEUTICS
2016, VOL. 12, NO. 7, 1805–1807
http://dx.doi.org/10.1080/21645515.2016.1143994

Taylor & Francis
Taylor & Francis Group

COMMENTARY

OPEN ACCESS

Establishment of the 3rd national standard for lot release testing of the Japanese encephalitis vaccine (Nakayama-NIH strain) in Korea

Sunmi Lee[a,c,#], Hyungsil Moon[b,#], Min Gyu Kim[a], Do Keun Kim[a], Hye Joo Chung[a], Yong Keun Park[c], and Ho Jung Oh[a]

[a]National Institute of Food & Drug Safety Evaluation, Ministry of Food and Drug Safety, Cheongju-si, Republic of Korea; [b]Daejeon Regional Office of Ministry of Food and Drug Safety, Daejeon, Republic of Korea; [c]School of Life Sciences and Biotechnology, Korea University, Seoul, Republic of Korea

ABSTRACT

In Korea, 2 inactivated Japanese encephalitis vaccines from Nakayama-NIH and Beijing-1 strain have been utilized to date. The 1st national standard for lot release testing of the JE vaccine was established in 2002. The 2nd national standard, established in 2007, is currently in use for JE vaccine (Nakayama-NIH strain) potency testing. However, the supply of this standard is expected to be exhausted by 2015, necessitating the establishment of a new national standard with quality equivalent to that of the existing standard. Quality control tests were performed to verify that the new standard candidate material was equivalent to that of the 2nd national standard, proving its appropriateness for potency testing of JE vaccine. In addition, based on the results of a collaborative study conducted among 4 institutions including Ministry of Food and Drug Safety, the potency of the new national standard material was determined to be 2.69 neutralizing-antibody titer ($\log_{10}$) per vial. Therefore, the newly established national standard material is expected to be used for the Japanese encephalitis vaccine lot release in Korea.

ARTICLE HISTORY
Received 18 November 2015
Revised 28 December 2015
Accepted 15 January 2016

KEYWORDS
japanese encephalitis (JE) vaccine; korean national standard; lot release testing; plaque reduction neutralization assay; potency assay

The Japanese encephalitis virus (JEV), which belongs to the genus *Flavivirus* of the family Flaviviridae, causes viral encephalitis, in the Asia-Pacific region.[1,2] Approximately 50,000 cases of viral encephalitis are reported every year, of which 10,000 result in death, of those who recover, up to 15,000 are left with severe complications such as permanent brain damage.[3,4] Three types of Japanese encephalitis (JE) vaccines for human use are currently in use: a mouse brain-derived and inactivated vaccine, a cell culture-derived inactivated vaccine, and a cell culture-derived live attenuated JE vaccine.[2] The mouse brain-derived inactivated JE vaccine is produced in several Asian countries, and also widely used within the Korean JE control program.[2,5] In 2013, the Korean Ministry of Food and Drug Safety (MFDS) commissioned the production of the 3rd national standard candidate material to a Korean JE vaccine manufacturer in order to replace the 2nd national standard to encourage national lot-release potency testing of the Nakayama-NIH strain–derived JE vaccine. The manufacturing method used in this exercise was same as that used during establishment of the 1st and the 2nd national standard material.[5] Following the manufacturing process, 3 thousand lyophilized vials were delivered to MFDS and some of them were used to qualify the material. The quality control (QC) items were selected on the basis of the testing criteria for the JE vaccine per the Minimum Requirements for Biological Products (MBP) of Korea as well as the World Health Organization requirements for JE vaccines (inactivated) for human use.[2,6] Although the potency testing was performed in accordance with the test method in the MBP, other assays except potency testing were accomplished by Korean Pharmacopoeia (KP).[7]

Table 1 shows the results of QC assessment during the manufacture of the candidate material. As shown, residual protein was detected at a concentration of 37.8 μg/mL in the final product, which was lower than the minimum criterion (80 μg/mL). The pH was 7.2 and moisture content was 0.8%, which fulfilled the specifications of the JE vaccine standard. In terms of sterility testing, no bacterial or fungal growth was observed in the final product, confirming that the candidate material was manufactured under sterile conditions. In addition, Foreign Insoluble Matter Test and Insoluble Particulate Matter Test for Injections satisfied to the respective specifications. Using storage conditions identical to those used for JE vaccine standard material, testing at $-70 \pm 10^{\circ}$C for up to 24 months resulted in no major changes in the potency of the candidate material. Additionally, the moisture content of the candidate material was similar to that of the 2nd standard material. (data not shown) The long-term stability of the standard material will

CONTACT Ho Jung Oh ohojung@korea.kr National Institute of Food & Drug Safety Evaluation, Ministry of Food and Drug Safety, Osongsaengmyung 2-ro, Osongeup, Cheongju-si, Chungchungbuk-do, 363700 Republic of Korea.

Color versions of one or more figures in this article can be found online at www.tandfonline.com/khvi.

[#] These authors contributed equally.

© 2016 Sunmi Lee, Hyungsil Moon, Min Gyu Kim, Do Keun Kim, Hye Joo Chung, Yong Keun Park, and Ho Jung Oh. Published with license by Taylor & Francis.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.

Table 1. QC test results for the 3rd national JE vaccine standard candidate material.

Test	Source of Test Method	Specification	Test result
Protein content[a] (μg/mL)	KP-07-35	$\leq$ 80	37.8
pH [b]	KP-07-41	6.8 – 7.4	7.2
Moisture content[c] (%)	KP-07-29	$\leq$ 3.0	0.8
Sterility [d]	KP-07-47	No growth	No growth
Foreign insoluble matter[e]	KP-07-21	None	None
Insoluble particulate matter for injection[f] (particles/vial)	KP-07-25	Particles greater than 10 μm $\leq$ 6,000 in size	44
		Particles greater than 25 μm $\leq$ 600 in size	1
Potency[g](NT-Ab titer ($\log_{10}$)/vial)	MBP-JE vaccine	Not lower than the neutralizing antibody titer of the standard	2.77 (Reference: 2.76)

Notes. All assays, except potency tests, were performed according to the general testing protocols of Korean Pharmacopoeia (KP-07). The potency test was performed according to the method for JE vaccine as described in the Minimum Requirements for Biological Products (MBP-JE vaccine). In detail, 4-week-old Institute for Cancer Research (ICR) mice were administered with the diluted solution for the test sample and standard material via intraperitoneal injection twice in a 7-day interval. Next, serum samples were collected from the mice 7 days after the 2nd injection and incubated for 90 min at 36 $\pm$ 1°C with a suspension consisting of challenge virus (JEV-Nakayama-NIH strain; 200 PFUs/0.4 mL). Subsequently, the mixed solutions were transferred to plates containing cultured chicken embryo cells and allowed to react for 90 min. The cells were then stratified and cultured for 2 days in a CO_2 incubator. The plaque reduction rate was calculated for the experimental group as well as the control group (treated with culture medium alone), and the neutralizing capacities of the antibodies of each serum were determined.

[a]Protein content: Determined by quantification of ammonia, the byproduct of distillation after decomposing the protein (precipitated by trichloroacetic acid) using sulfuric acid

[b]pH: Measured using the potentiometric instrument (pH meter) at 25 $\pm$ 2°C

[c]Moisture content: Frozen weight of test specimens that were vacuum-dried at 60°C for 3 hours

[d]Sterility: Determined by monitoring of bacterial or fungal growth for 14 days under both aerobic (at 20–25°C) and anaerobic (at 30–35°C) conditions

[e]Foreign insoluble matter: Manually observed and counted (at 3,200 Lux) with the specimen suspended in solvent

[f]Insoluble particulate matter for injection: Measured using a particle counter, with the specimen dissolved in de-ionized water

[g]Potency: Result of PRN assay using the 2nd national standard as a reference material (shown in parentheses) in the test procedure.

continue to be monitored under the assurance program for national standards by MFDS.

This study was conducted over a-2 year period (approximately), which included manufacture and QC testing of the candidate material. During the 2nd year, a collaborative study was performed to confirm the potency of the candidate material. Four institutions, including MFDS and Korean JE vaccine manufacturers; Boryung Biopharma, Co., Green Cross, Corp., and Korea Vaccine, Co., participated in this collaborative study. As shown in Table 2, each of the institutions, randomly designated A to D, performed 10 iterations of plaque reduction neutralization (PRN) assays to compare the potency of the 2nd standard and the 3rd standard candidate material. Plaque reduction neutralization assays currently used for lot release testing of inactivated JE vaccine products measure the extent of plaque reduction in chicken embryo cells previously inoculated with serum from mice immunized with vaccine products or national standard, followed by exposure to a suspension of challenge virus.[6] The mean values of potency were 2.67 and 2.69 neutralizing-antibody (NT-Ab) titers ($\log_{10}$) for the 2nd and 3rd standard materials, respectively, while the intra-assay coefficient of variation (CV) values for the various institutions were 6.69 and 7.92 for the 2nd and 3rd standard materials, respectively. The proficiency of the 4 test institutions in terms of Z-scores were −0.05, 0.19, −0.25, and −0.15 for the 2nd national standard and 0.46, 0.60, −0.74, and −0.32 for the 3rd national standard candidate material, for institutions A, B, C, and D, respectively. As all values were within the range of $\pm$ 2, the proficiency between the institutions was determined to be satisfactory. As shown in Figure 1, based on the results from the collaborative study between the 4 institutions, the assigned potency of the 3rd national standard candidate material was determined to be 2.69 NT-Ab titer ($\log_{10}$) per vial. The upper and lower limits of the 95% confidence interval of the mean were 2.76 and 2.62 for the 3rd standard candidate material and 2.72 and 2.61 for the 2nd standard material. Additionally, as the *p*-value was 0.53 (which exceeded 0.05) it was concluded that there was no significant difference between the 2nd national standard and the 3rd standard candidate material.

QC tests of the 3rd national standard candidate material for the Nakayama-NIH strain-based JE vaccine revealed that the candidate material fulfilled the criteria described by the current official compendium of Korea. The collaborative study found that the potency of the 3rd national standard

Table 2. Results of the collaborative study comparing the potency of the JE vaccine standard material.

Standard Material	Laboratory[a]	Result			
		N[b]	GMT[c]	GCV[d](%)	Inter GCV[e](%)
2nd(code no. 07/022)	A	10	2.80	5.97	6.69
	B	10	2.82	1.72	
	C	10	2.48	3.45	
	D	10	2.56	1.73	
3rd (candidate)	A	10	2.90	6.52	7.92
	B	10	2.85	2.20	
	C	10	2.47	1.49	
	D	10	2.55	2.20	

Notes. [a]Laboratory: one of the participating laboratories, the National Center for Lot Release of the MFDS, Green Cross Corp. (Korea), Boryung Biopharma. Co. (Korea), and Korea Vaccine Co. (Korea) (in randomized order)

[b]N: number of tests

[c]GMT: geometric mean titer. GMT was calculated by dividing the sum of the test results by the test number (10) for each testing institution

[d]GCV: geometric coefficient of variation among tests for each test institution. GCV was calculated by dividing the standard deviation of the test results (SD of A, B, C, D = 0.17, 0.05, 0.09, 0.04 for the 2nd standard, respectively, and 0.19, 0.06, 0.04, 0.06 for the 3rd standard candidate, respectively) by the GMT for each testing institution

[e]Inter GCV: geometric coefficient of variation between the test institutions for each standard material. Inter GCV was calculated by dividing the standard deviation of the total test results (SD of 40 tests = 0.18 for 2nd standard and 0.21 for 3rd standard candidate) by GMT (GMT of 40 tests = 2.66 for 2nd standard and 2.68 for 3rd standard candidate) for each standard material.

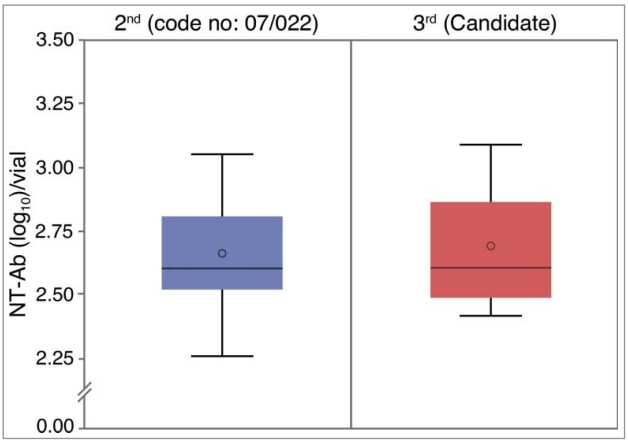

Figure 1. The assigned potency of the 3rd national JE vaccine standard candidate material The potency of the candidate material was determined to be 2.69 NT-Ab titer (log_{10}) via statistical analysis of results of 40 PRN assays performed by 4 institutions. Geometric mean titers ± SDs were 2.67 ± 0.18 and 2.69 ± 0.21 for the 2nd and the 3rd standard material, respectively.

material (2.69 NT-Ab titer (log_{10}) per vial) was equivalent to that of the 2nd national standard. Therefore, the tested candidate material was established as the national standard for potency testing of the JE vaccine following the 2nd national standard in Korea. However, there is a recent, active, international movement to transition from *in vivo* potency testing to more sensitive and reproducible *in vitro* tests.[8,9,10] Accordingly, the application of *in vitro* methods for lot release testing will require additional studies to estimate the *in vitro* potency values for the newly established 3rd national JE vaccine standard.

Abbreviations

JEV	Japanese encephalitis virus
MFDS	Ministry of Food and Drug Safety
JE	Japanese encephalitis vaccine
MBP	Minimum Requirements for Biological products
PRN	plaque reduction neutralization
NT-Ab	neutralizing-antibody titer
QC	quality control
CV	coefficient of variation
KP	Korean Pharmacopoeia.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Boryung Biopharma Co., Green Cross Corp. and Korea Vaccine Co. for their participation in the collaborative study and assistance with experiments.

Funding

This study was supported by a grant from the scientific research program (13171Bio309) at the National Institute of Food and Drug Safety Evaluation of the Ministry of Food and Drug Safety, Republic of Korea.

References

[1] Misra UK, Kalita J. Overview: Japanese encephalitis. Prog Neurobiol 2010; 91:108-12; PMID:20132860; http://dx.doi.org/10.1016/j.pneurobio.2010.01.008

[2] World Health Organization. Requirements for Japanese Encephalitis Vaccine (inactivated) for human use. In: Technical Report Series No 771. 1988; http://www.who.int/biologicals/publications/trs/areas/vaccines/jap_encephalitis/WHO_TRS_771_(part2)_A6.pdf?ua=1

[3] World Health Organization. Guidelines for the production and control of Japanese encephalitis vaccine (live) for human use. In: Technical Report Series No 910. 2002; http://www.who.int/biologicals/publications/trs/areas/vaccines/jap_encephalitis/WHO_TRS_910_A3.pdf?ua=1

[4] Kurane I, Takasaki T. Immunogenicity and protective efficacy of the current inactivated Japanese encephalitis vaccine against different Japanese encephalitis virus stains M. Vaccine 2000; 18:33-5; PMID:10821971; http://dx.doi.org/10.1016/S0264-410X(00)00041-4

[5] Kim JO, Shin JH, Baek SY, Min KI, Min BS, Ryu SR, Kim BK, Kim DK, Ahn MJ, Park M, et al. A collaborative study on Korean standard JE vaccine for potency assay. J Microbiol Biotechnol 2004; 14:745-50.

[6] Ministry of Food and Drug Safety. Minimum requirement for Biological Products. In: MFDS 2011-74. 2011; http://law.go.kr/admRulSc.do?menuId=1&subMenu=1&query=#liBgcolor0

[7] Ministry of Food and Drug Safety. Korean Pharmacopoeia (X). In: MFDS 2012-9. 2012; http://www.mfds.go.kr/eng/index.do?nMenuCode=50

[8] Kim DK, Kim HY, Kim JY, Park KB, Han E, Kim J, Ban S, Han S, Park YK, Nam JH. Development of an in vitro antigen-detection test as an alternative method to the in vivo plaque reduction neutralization test for the quality control of Japanese encephalitis virus vaccine. Microbiol Immunol 2012; 56:463-71; PMID:22486472; http://dx.doi.org/10.1111/j.1348-0421.2012.00462.x

[9] Butrapet S, Kimura J, Zhou S, Yasui K. Neutralizing mechanism of a monoclonal antibody against Japanese encephalitis virus glycoprotein E. Am J Trop Med Hyg 1998; 58:389-98; PMID:9574781

[10] Srivastava K, Aira Y, Mori C, Kobayashi Y, Igarashi A. Antigenicity of Japanese encephalitis virus envelope glycoprotein V3 (E) and its cyanogens bromide cleaved fragment examined by monoclonal antibodies and Western blotting. Arch Virol 1998; 96:97-107; http://dx.doi.org/10.1007/BF01310993