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Determination of potency of tetanus immunoglobulin products by commercial tetanus antitoxoid test kits

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ABSTRACT

Objectives: The aims of this study were to determine the potency of tetanus immunoglobulin products imported to Thailand in 2014-2016 by commercial anti-tetanus ELISA test kit for human use and to compare the potency results of 2 different test kits. **Materials and methods:** Potency of 14 tetanus immunoglobulin products was determined by commercial anti-tetanus ELISA test kit; Sekisui Diagnostics in Thai National Control Laboratory and the test results were compared to the manufacturer's results tested by commercial anti-tetanus ELISA test kit; Scimedx Corp. **Results:** Potency results of Thai National Control Laboratory and manufacturer' results were in close agreement, were reliable and no significant differences ($P = 0.166$). And the potency values complied with European Pharmacopoeia specifications. **Conclusion:** The study demonstrated that commercial anti-tetanus ELISA test kits for human use could be used as a routine quality control test and the results from a different brand of test kits were comparable.

INTRODUCTION

Tetanus toxin is a life-threatening toxin affecting the nervous system. If it enters the circulatory system, it can cause clinical syndromes such as painful muscular contractions, spasms and other severe effects, including death [1,2]. Anti-tetanus or tetanus immunoglobulin (TIG) is a lifesaving product that provides passive immunity for patients who have never been vaccinated or have not been revaccinated for more than 10 years. Two types of anti-tetanus products are available: One produced from human plasma (TIG) and another produced from horse serum (tetanus antiserum) [3-5]. To ensure the capacity of TIG to neutralize the toxin, the potency of the product should be determined by comparing the antibody titer to a reference preparation, with the results calculated in international units. The European Pharmacopoeia monograph Human TIG (0398) gives a clear outline of the *in vivo* assay to be performed to determine the potency of human TIG during their development [6,7]. Due to cost, time, variation of test results, and ethical aspects [4], the use of experimental animals for drug testing has been discouraged. Current good scientific research principles state that if no replacement method is available, then either reduction of animal use or certain refinements are considered adequate for implementation of the three Rs principles [8]. Recently, both enzyme-linked immunosorbent assay (ELISA) and toxoid inhibitory assay (TIA) have been accepted by the European Pharmacopoeia, 4th ed. (Ph. Eur.) [9] to determine the potency of TIG products by comparing the TIG titer with a reference standard.

At present, there is no commercial ELISA kit available for TIG product potency testing; therefore, laboratories have to prepare their own in-house ELISA reagents, as well as plate coating, to perform this assay. Experience in these techniques is key to achieving accurate and reproducible results [10]. However, several commercial tetanus antitoxoid test kits for human use are available for measuring anti-tetanus antibody levels after immunization [11,12]. If these test kits could determine the potency of a TIG product, they could be a

feasible and reliable method for future use. With this idea, an attempt had been made by a TIG manufacturer to validate a commercial tetanus antitoxoid test kits for human use to MNT and this change was approved by Thailand Food and Drug Administration (FDA; data not shown as confidential information from Thailand FDA). This capacity of commercial tetanus antitoxoid test kit was confirmed by our previous work, and the results demonstrated satisfactory performance [13]. Thus, to avoid unnecessary using experimental animals according to the 3Rs principle we chose TIG products from this manufacturer for our study. Therefore, the objectives of this work were to determine TIG potency by commercial antitoxoid test kit for human use and to evaluate whether its results would be comparable to different test kit brand used by TIG manufacturer.

MATERIALS AND METHODS

ELISA Test Kit

A commercial Tetanus IgG ELISA test kit (Sekisui Diagnostics, Lexington MA, USA) was validated and selected for this study.

Samples

TIG product samples 14 lots from 1 manufacturer that imported to Thailand during 2014-2016 were used in this study. Their potency was determined using ELISA test kit from Scimedx Corp., Denville NJ, USA.

Reference Materials

The reference standard used was the World Health Organization (WHO) 1st International Standard for TIG, Human, produced by the National Institute for Biological Standards and Control (NIBSC TE-3), with labeled potency of 120 IU/mL [14].

Methods

General equipment for the assay included an ELISA reader, plate shaker, and automatic plate washer. Other ELISA reagents were supplied in the test kit.

The potency of TIG samples was determined using the Sekisui test kit in a ISO/IEC 17025 certified Thailand National Control Laboratory (NCL). All steps of the assay were performed in accordance with the Sekisui kit manufacturer's instructions, using reagents that were supplied with the kit [15]. Only high and low control solutions provided by the manufacturer's kit were used for each run, but the reference standard used was from NIBSC. Tetanus toxin-coated microtiter plates (12 break-apart 8-well strips) were used. The NIBSC standard TE-3 was serially diluted into four concentrations: 0.125, 0.25, 0.5, and 1 IU/mL. All four dilutions were diluted further (1:50) to 0.0025, 0.005, 0.01, and 0.02 IU/mL. TIG samples were diluted according to the labeled potency to be the same four concentrations as described for the standard and were tested in triplicate. Purified peroxidase-labeled antibody against human IgG conjugated with horseradish peroxidase was used, and 3,3',5,5'-tetramethylbenzidine was used as substrate. The optical density was measured at 450 nm and 630 nm as reference wavelengths. The potency values were compared with those obtained from the TIG product manufacturer.

The potency of TIG products was determined by with Bioassay Assist software using a parallel-line statistical model [16] that is recommended by the WHO for bioassay of biological products [17]. The data of two test kits were analyzed by paired *t*-test for *P* value.

RESULTS

The parallel line assay was performed to determine TIG potency by the bioassay and identify the samples were TIG products. These 2 lines of reference standard and samples showed satisfactory parallelism and linearity of log dose and response (optical density) and demonstrated no significant difference in terms of preparation and homogeneity between them (Figure 1).

All potency of the 14 TIG products forms NCL by Sekisui test kit complied with Ph. Eur. specifications; the stated potency was 100 IU mL, with confidence limits not <80% and not more than 125% [6]. When compared with manufacturer' results, the range of potency of these 14 lots done by the manufacturer was 264-328 IU/mL, while the NCL results

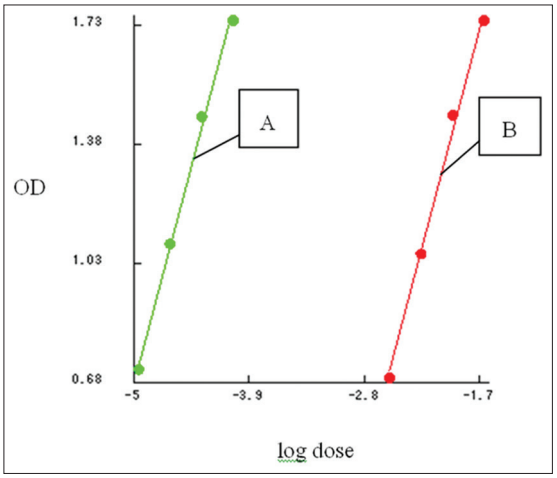

Figure 1: Parallel lines calculated from log concentrations and optical density to compare the values of the standard (A line) and tetanus immunoglobulin samples (B line); a standard unit of 1 IU/mL was used

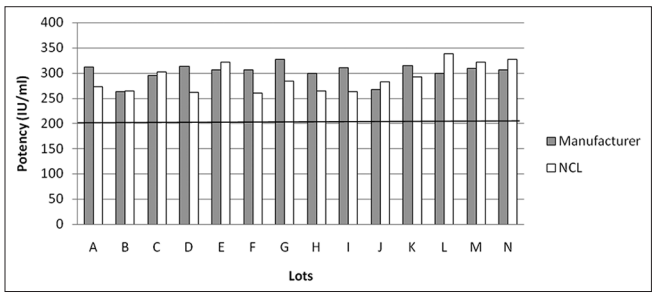

Figure 2: Correlation of potency test results from the NCL and the manufacturer's laboratory. The solid line is the European Pharmacopoeia specification of potency for tetanus immunoglobulin products (not less than 100 IU/mL)

Table 1: Potency of 14 TIG samples tested by the NCL and by a manufacturer of a commercial tetanus antitoxoid test kit for human use

Sample lots	Potency (IU/mL)		Ratio
	Manufacturer	NCL	
A	312	273	1.14
B	264	265	1.00
C	296	302	0.98
D	314	262	1.20
E	306	322	0.95
F	307	261	1.18
G	328	285	1.15
H	299	265	1.13
I	311	263	1.18
J	268	283	0.95
K	315	293	1.08
L	299	339	0.88
M	309	322	0.96
N	306	327	0.93
Mean	302.43	290.14	1.05
Range	264-328	261-339	0.93-1.20

TIG: Tetanus immunoglobulin

were 261-339 IU/mL (Table 1). The average ratio of these two groups was 1.05, and the *P* value from paired *t*-test was 0.166, and both sets of data were above 200 IU/mL, which complied with the specifications of Ph. Eur. (Figure 2).

DISCUSSION AND CONCLUSION

Our study demonstrated that the selected commercial tetanus antitoxoid test kits could be used to determine the potency of TIG and the potency results from NCL and manufacturers showed close agreement.

The potency of 14 TIG products was estimated by its manufacturer using a different brand of test kit, and the results might be the difference as a discrepancy of test results among different laboratories, and test kits could be found [11]. To control variations of the study, several issues were considered. All TIG samples used in this study were produced from the same manufacturer for their homogeneity and quality consistency. The selected test kit; commercial Tetanus IgG ELISA test kit (Sekisui Diagnostics, Lexington MA, USA) and the kit used by manufacture were the same ELISA principle, and validation of selected test kit against this TIG product was performed before the study. In addition, the reference standard used by this TIG manufacturer was the European Directorate for the Quality of Medicines, or EDQM, which complied with the WHO reference standard that we used. Therefore, the results from both test kits were conformed as demonstrated by their ratio and *P* value.

To our knowledge, no previous work found regard to the determination of TIG potency by commercial tetanus antitoxoid test kits and comparison between different brands of test kits. Previous studies only evaluated the suitability of several test kits to detect IgG to tetanus toxoid in human serum [1,12], as well as a collaborative study of in-house EIA and TIA techniques for TIG products [2]. This study results provided a meaningful outcome for quality control of TIG products that commercial anti-tetanus toxoid ELISA test kit could be used. The results from both ELISA test kits were robust and showed good concordance between the two laboratories that the average potency ratio manufacturer: NCL was very closed and no significant difference between them regard to $P = 0.166$. The selected test kit was suitable to be used as a routine test regard to its availability and pricing.

There are several benefits of using the ELISA test kit compared to the MNT. It is simple because typical ELISA equipment is used. It can be performed rapidly, in 1 day, so more samples can be tested, while the MNT takes 4 days. Besides, it is also economical as it is lower in cost that the MNT which uses 24 mice to test one sample while 1 test kit for 6 samples. As it is an *in vitro* test, the variation can be well controlled; since antigen-coated microtiter plates and ELISA reagents are supplied with the test kit, these would resolve any discrepancy between laboratories due to the incompetence of the assay.

However, there were some limitations regard to the availability and pricing of test kits. Several test kits are available worldwide, but only a few are marketed in Thailand and are very expensive. From our preliminary study, 3 ELISA test kits with the same principle had been evaluated; Tetanus IgG ELISA, VaccZyme™ Anti-Tetanus Toxoid IgG Enzyme Immunoassay Kit (The Binding Site Group, Birmingham, UK) and Sekisui test kit (Sekisui Diagnostics, Lexington MA, USA) and all of which had satisfactory performance. Therefore, the Sekisui test kit was selected for this study according to its availability in Thailand in 2014 and the cheapest price.

In addition, there are five TIG products registered in Thailand: Three are IgG produced from humans, and the other two are from horses. Of the five TIG brands registered in Thailand, only three brands are currently being imported and marketed in the country: Two are human IgG, and one is antiserum from horses [18]. Therefore, there was a limitation about the amount of samples; only 14 lots of TIG product from only one manufacturer have been imported since 2014. Further study would be done if ELISA is used for other TIG brands and using a different brand of commercial anti-tetanus toxoid ELISA test kits. In conclusion, the present study suggested that the commercial anti-tetanus toxoid ELISA test kits could be used to determine TIG potency and the results from a different brand of test kits which were the same ELISA principle were comparable.

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