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Validation studies on an alternative endpoint for the local lymph node assay (LLNA-DA): Importance of study management

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Abstract
We conducted 2 validation studies for a modified version of the local lymph node assay (LLNA), which was designated as the LLNA-DA. A total of 17 laboratories tested the validity of the assay by using 14 chemicals. Here, in addition to the experimental protocol, we prepared the study protocols describing the study purpose, the role of the participants, etc. Technology transfer was conducted by the developer of the assay. Prior to the studies, preliminary tests using only a positive control chemical were conducted to determine whether the experimental protocol prescribed for the assay was appropriate. A formatted data file was developed for data management. Fortunately, the results of these studies revealed small interlaboratory variations, and we believe that one of the factors that contributed to the successful results was the development of strategies and tools for study management at the planning stage itself. However, issues related to the management of validation studies have rarely been discussed. Strategies or tools developed for study management should be easily accessible and should be shared with researchers intending to conduct validation studies in the future.

Keywords: interlaboratory validation study, study management, protocol, technical transfer, data quality

Introduction

An interlaboratory validation study examines the reliability and relevance of a particular test method (Organization of Economic Co-operation and Development (OECD), 2005). It differs from a single laboratory study in that it involves many persons having different backgrounds and levels of experience. To minimize interlaboratory variations, it is necessary that all the participating researchers from each laboratory understand how to operate the test method and perform it accurately, according to the procedure specified for the study rather than the customary procedure used in their respective laboratories. Therefore, appropriate management is one of the challenges encountered in the success of an interlaboratory validation study.

The murine local lymph node assay (LLNA) has developed as an alternative to the guinea pig test for assessing skin sensitization. In this method, lymphocyte proliferation in the draining auricular lymph nodes is measured by the incorporation of radioactive molecules (OECD, 2002). Recently, several nonradioactive methods have been proposed. Daicel Chemical Industries Ltd. has developed a modified nonradioactive version of the LLNA that is based on the ATP content (Yamashita, 2005). Since this method was originated by Daicel Chemical Industries Ltd. and is based on the ATP content,

it is designated as the LLNA-DA. To evaluate the LLNA-DA, 2 validation studies were conducted by 23 researchers from 22 organizations. The first study examined the reliability and relevance of the method using 12 chemicals in 10 experimental laboratories. The second study examined the reliability of the method using 5 chemicals in 7 experimental laboratories.

Since these validation studies were conducted on a large scale, appropriate management was essential. Therefore, strategies and tools were developed for their management. Fortunately, the results of these studies on the LLNA-DA successfully revealed small interlaboratory variations and good relevance. We believe that one of the factors that contributed to the good results was the strategies and tools employed for managing the study. However, issues related to the management of validation studies have rarely been reported.

In this article, we report the strategies and tools that we developed for managing the LLNA-DA validation studies. First, we describe the 2 protocols used. Next, we discuss the seminar for technology transfer and the preliminary tests that were conducted. Further, we introduce the web folder that was developed for use, and we subsequently describe the formatted data file. Finally, we discuss the management of the validation studies and present our conclusion.

Two types of protocols

In commonly used dictionaries, the word "protocol" is defined as the plan for a medical treatment course or for a scientific experiment or as a predefined written procedure for designing and implementing experiments. In the context of clinical studies, its meaning is more specific. The word protocol describes a method to be used in a clinical trial or a medical research study. With regard to the purpose of a protocol in clinical studies, Collins (2001) states that "It describes in a clear and detailed manner how the trial is performed so that all investigators know the procedures. This is particularly important in multicenter trials where it can be difficult to ensure that all centers and investigators conduct the study properly." The difficulty encountered in multicenter trials that he states here is identical to that encountered in an interlaboratory validation study. Therefore, this type of a protocol that describes the method to be followed for performing various steps in a validation study should be required. On the other hand, the OECD guidance document 34 (OECD, 2005) defines a protocol as "the detailed, unambiguous step-by-step description of a test method that directs the laboratory as to how to perform the test method." In this case, the protocol pertains to the implementation of a test method but not to a validation study for the test method. Most biologists appear to be familiar with this definition, and without doubt, this type of protocol is also required in a validation study.

Therefore, 2 types of protocols were prepared for the validation study of the LLNA-DA. We designated the first document as the study protocol and the second one, as the experimental protocol. Fig. 1 shows the table of contents of the study protocol used for our study.

1. Introduction
2. Purpose of the study
3. Role of the researchers
4. Standard operating procedure for LLNA-DA
5. Time schedule
6. Participant organization
7. Chemicals tested
8. Chemical allocation
9. Preparation of animals, equipment, and materials
10. Expenditure
11. Technology transfer and preliminary test
12. Data management
13. Data analysis
14. Meeting held to discuss the results
15. Announcement of the results
16. Inquiries

Fig. 1. Table of contents of the protocol employed for the first study on the LLNA-DA.

Seminar for technology transfer and preliminary tests

Even if a well-documented experimental protocol is prepared, toxicologists from different laboratories may interpret the document differently. In order to determine their understanding of the experimental protocol and to explain the execution of the test method, a 1-day seminar for technology transfer was held by the LLNA-DA developer prior to each study. It was required to be attended by at least 1 toxicologist from each experimental laboratory.

To confirm that the experimental protocol was being adequately documented, a preliminary test employing only a positive control chemical, namely, hexylcinnamic aldehyde, was conducted prior to each study.

Fig. 2 (a) and (b) shows the results of the preliminary tests performed for each study. The plot illustrates the stimulation index (SI) value, which is the endpoint of interest in the LLNA-DA and is defined as an increase in the ATP content in the chemical-treated group relative to the vehicle control group, along with its 95% confidence intervals for all the laboratories. Only one experimental dose was used in the preliminary test for the first study; however, in order to assess the dose-response relationships, 2 different doses were used in the preliminary test for the second study. Based on these plots and the historical data obtained from Daicel

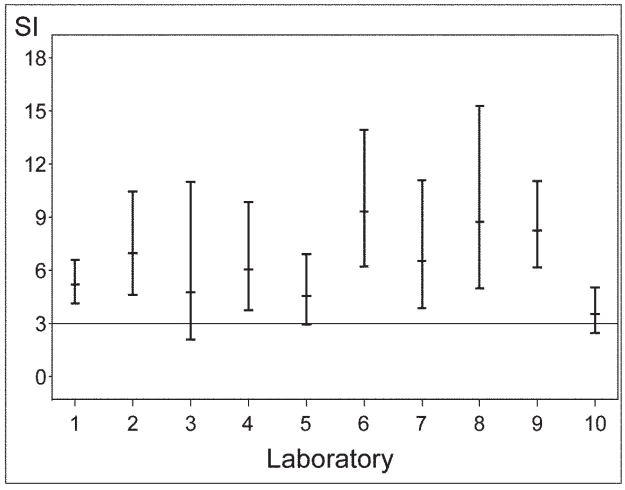

Fig. 2. (a) SI value with 95% confidence intervals obtained for the positive control chemical (25% hexylcinnamic aldehyde) in the preliminary test performed during the first study.

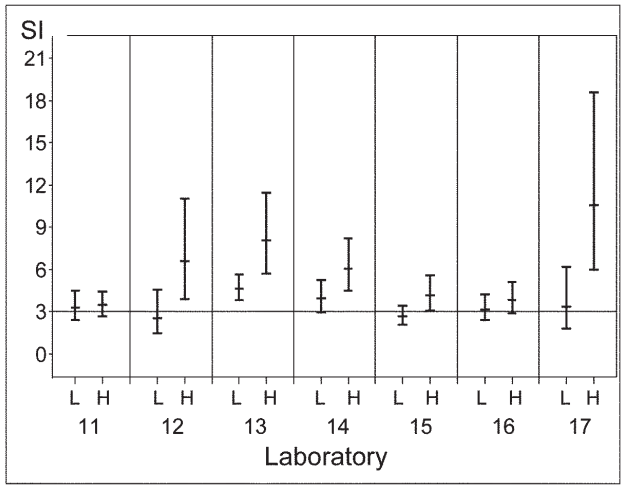

Fig. 2. (b) SI value with 95% confidence intervals obtained for the positive control chemical (10% (L) and 25% (H) hexylcinnamic aldehyde) in the preliminary test performed during the second study.

Chemical Industries Ltd., we discussed whether revisions were needed in the experimental protocol.

Use of a folder on a website

During a project, many documents related to a validation study are repeatedly revised to ensure that they reflect the opinions of each researcher. One of problems is that often important documents are lost or may fail to be updated. Therefore, all the researchers involved in the study are required to be well versed with the latest version of the documents.

To enable easy access to the latest version of the necessary documents pertaining to the validation studies, we used a commercial web tool, i.e., a folder on the website. By using this tool, all researchers could download the document via the internet onto any personal computer at their respective workplaces as and when required. Once a document was uploaded onto the web folder, it could be downloaded at any time. The web folder was set such that only the study manager was able to update the documents. When the study manager decided to upload or update a document, he accessed the web folder and uploaded the latest version of the document and then deleted the older version from the folder. Subsequently the study manager would then inform all the researches that the document had been updated. This rule was strictly followed throughout the study.

Formatted data file

To directly collect the raw data obtained from the experimental laboratories and to construct a database, an MS-Excel formatted file was prepared for entering the experimental data. One of the advantages of MS-EXCEL is that it is widely available, and many researchers can use it at their respective workplaces. Another advantage is that it has several useful functions. For example, it is possible to protect the data from being entered into an unintentional cell on the formatted file.

The empty formatted data file along with a document describing how it was to be used was distributed to the experimental laboratories prior to commencement of the experiment. Following data entry into the formatted data file, the file and the record that was maintained for the values observed during the experiment were collected from all the experimental laboratories. A biostatistician examined the values in both the file and the record. When needed, the toxicologist who carried out the LLNA-DA in the experimental laboratory was inquired about it. After resolving this issue, the biostatistician constructed a database on which all the data analysis was carried out. The purpose of constructing such a database is to ensure that the quality of the data is maintained.

Discussion

The OECD guidance document 34 (OECD, 2002) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) guidelines for the nomination and submission of new, revised, and alternative test methods (ICCVAM, 2003) are excellent documents that provide very useful information for conducting validation studies. However, both these documents focus on broad issues and are written from a more general viewpoint. On the other hand, here, we describe the management strategies and tools for a validation study from a more practical viewpoint, arising from discussions regarding some validation studies that have been conducted in Japan. In particular, some of the authors who were involved in the interlaboratory validation study for alternatives to the Draize eye irritation test, organized by the Japanese Society of Alternatives to

Animal Experiments (Ohno et al., 1998), participated. This study was conducted on a large scale and evaluated 16 cytotoxicity tests as alternative tests. The total number of experimental laboratories participating in the study was 16–24 per cytotoxicity test. Large interlaboratory variations were obtained for all the cytotoxicity tests, and it was very difficult to interpret the data and evaluate the cytotoxicity tests based on the study results because there were many instances of violation of rules that had been finalized prior to the study and misinterpretation of the experimental protocols (Omori, 1998). To clarify the purpose of the validation study, i.e., evaluating the interlaboratory variations under the experimental protocol, to transfer the experimental operations for the tests correctly, and to try to ensure data quality should have been considered from planning stage of the study. The study demonstrated that an interlaboratory validation study is a joint venture by researchers having different backgrounds and levels of experience. In other words, study management of the validation studies became a challenging issue.

We admit that the strategies and tools described here do not cover all the aspects of study management and that the strategy and tools for other validation studies should be developed by considering individual cases and various viewpoints. However, our strategies and tools proved to be efficient for at least 2 validation studies, and we believe that they could serve as a reference for researchers conducting validation studies for a test method in the future.

It is important to note that in addition to the processes described in the experimental protocol adopted for a test method, there are many factors that can contribute to the occurrence of large interlaboratory variations. In other words, it is possible that variations could arise in an established test method even if the experimental protocol is well defined and the test is conducted under Good Laboratory Practice conditions. To exclusively evaluate the test method described in the experimental protocol, attempts should be made to eliminate additional factors that could cause interlaboratory variations. Large interlaboratory variations would lead to unclear results from the study and would delay the development of a test method even in case of a well-defined method.

In conclusion, management implies all the activities that are necessary to achieve objectives continually and efficiently. To obtain scientifically valid and distinct results from a validation study, appropriate study management from the planning stage is critical. The knowledge base on the management of validation studies should be expanded and shared.

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