The Role of Prevalidation in the Development, Validation and Acceptance of Alternative Methods

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Summary — Experience has shown that the outcome of large and expensive validation studies on alternative methods can be compromised if their managers do not insist that optimised test protocols and proof of their performance are submitted *before* the start of the formal validation study. One way for the sponsors of validation studies to confirm both the likely relevance of a method for its stated purpose and its readiness for validation would be to require a prevalidation study before formal validation was contemplated. This process would involve the developers (or other proponents of the method) and selected independent laboratories in *protocol refinement* (Phase I) and *protocol transfer* (Phase II). The optimised protocol would then be assessed in a *protocol performance* phase (Phase III), which would involve the testing of a relevant set of coded test materials and an evaluation of a proposed prediction model. In certain circumstances, a successful outcome of Phase III might be sufficient for promotion of the regulatory acceptance of the method. Normally, however, the method would proceed to a formal validation study. The European Centre for the Validation of Alternative Methods, a recognised validation authority, now proposes to introduce this prevalidation scheme into its validation strategy.

Key words: alternative methods, prevalidation, protocol optimisation, protocol transfer, replacement, validation.

Introduction

Large multi-laboratory validation programmes are inherently very expensive, while the resources available for validating new test methodologies are limited. Thus, it is imperative that potential alternative methods are carefully evaluated for intralaboratory and interlaboratory reproducibility and transferability, and for their ability to predict an *in vivo* endpoint, *before* they are accepted into formal validation trials.

Problems were encountered in early ring trials on alternative methods, where the use of different protocols in the participating laboratories made the direct comparison of results very difficult (1). The lack of uniformity in the technical conduct of several assay systems during these early validation programmes caused, at the least, extensive delays in the studies themselves, and, at worst, potentially compromised the value of large amounts of data.

Despite attempts to standardise the protocols used in more-recent studies, it has become apparent that the assays do not always perform as expected in different laboratories. In many cases, even though the protocols

This is the first report of the ECVAM Task Force on Prevalidation and represents the agreed conclusions of its members as individual scientists.

were thought to have been sufficiently well-standardised, the technical details in the protocols and the accompanying standard operating procedures (SOPs) were often insufficient to ensure consistent results. Inadequately prepared protocols increase the complexity of study management, and increase the amount of variation introduced in the practical conduct of the assays. They also adversely affect the apparent performance of the assays, and jeopardise the usefulness of the results generated in the study as a whole.

The recent report of the second Amden (Amden II) workshop on validation (2) identifies five main stages in the validation process: test development, prevalidation, validation, independent assessment, and progression toward regulatory acceptance. The prevalidation stage was highlighted as an important component in the evolution of a new test. The report recommends that proper test development and protocol optimisation should receive much greater emphasis than was envisaged in the original Amden (Amden I) proposals (3).

A type of less formal prevalidation has also been described as "test optimisation" by Goldberg (4) as part of the Center for Alternatives to Animal Testing (CAAT) evaluation approach, and as "test development" by the CAAT Validation and Technology Transfer Committee (5). The more-formal methods used to attain test optimisation which are described in this manuscript are consistent with CAAT's goals of an orderly process toward test validation.

Prevalidation should focus on three main phases, namely, *protocol refinement*, *protocol transfer* and *protocol performance*. This is necessary to identify any unexpected problems of assay standardisation, design, transferability and data analysis, prior to the inclusion of a test in the formal validation process. The primary objective of prevalidation would be to define and demonstrate the robustness and reproducibility of *in vitro* test protocols. It would also establish the ability of the test to predict specific *in vivo* endpoints for specific types of test materials, and would ultimately result in maximising the amount of information derived from any subsequent large-scale, multi-laboratory validation studies.

Overall responsibility for the validation process will often rest with an official body, i.e. a recognised validation authority (RVA). In the context of this article, we refer to the European Centre for the Validation of Alternative Methods (ECVAM) as one such authority. However, it is recognised that this role could also be fulfilled by several other independent or governmental organisations, such as ZEBET, in Germany, and the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM), in the USA. The RVA could also act as the sponsor of a prevalidation study, as defined in the Amden II report (2), as could a number of other organisations, such as CAAT, the European Cosmetic, Toiletry and Perfumery Association (COLIPA) and the American Cosmetic, Toiletry and Fragrance Association (CTFA).

ECVAM was established by the European Commission to coordinate the validation of alternative methods at the European Union level (6). The proposals contained in this paper were warmly received at a meeting of the ECVAM Scientific Advisory Committee held on 7–8 February 1995, and they will now be implemented as a major part of ECVAM's strategy.

Responsibilities of the RVA

The role of the RVA would be to receive proposals for prevalidation studies from the developers or other proponents of methods, or from other bodies wishing the method to be evaluated (for example, other sponsors). It would then consult with its own expert advisers concerning the acceptability of these proposals in the light of the state of development of the methods concerned and their compliance with the priorities of the RVA itself.

If the advice were favourable, the RVA, acting as the sponsor, or with the other sponsors, of the prevalidation study, as envisaged in the Amden II report (2), would consider commissioning a study, appointing a steering committee and, in the case of ECVAM, awarding a contract for the performance of the work.

The RVA would receive a report from the steering committee and the management team for the study at the end of the protocol transfer phase (Phase II), and would then approve the aims and design of the protocol performance phase (Phase III). At the end of the study, the RVA would again consult with all concerned, and with its own expert advisers, in order to decide what subsequent

action would be appropriate in the specific circumstances.

Proposals for Prevalidation Studies

The developers or other proponents of a new or modified method (here designated *Laboratory 1*) would submit to the RVA, or to other potential sponsors, the case in support of their proposal that a prevalidation study should be conducted. The proposal would be accompanied by sufficient documentation concerning the purpose of the method and the need for it, evidence of acceptable test performance, and a potential prediction model. The criteria for acceptance of the proposal would include the availability of the following:

1. A clear indication of the purpose of the test.
2. Evidence of the need for the test in comparison with *in vivo* tests and other *in vitro* tests.
3. A summary of how the method had been derived and the biological basis for its relevance.
4. Details of the endpoint measured, how the data produced would be summarised (as a score or index), and how the result would be applied with respect to the stated purpose of the test (the preliminary prediction model).
5. Data derived from the test using an appropriate set of test materials.
6. A written procedure which would be sufficiently detailed to allow the test to be conducted by another laboratory.
7. Additional documentary evidence to support the submission of the test, such as in-house reports, published papers and meetings presentations, etc.

The case for taking the test further would then be considered by the RVA and its advisers.

Phases of the Prevalidation Process

The prevalidation process would involve collaboration between established and competent laboratories registered with the RVA, and would include three main phases:

Phase I: *protocol refinement*, involving interaction between Laboratory 1 (the test development laboratory or other proponent of the method), and the protocol refinement laboratory (designated *Laboratory 2*);

Phase II: *protocol transfer*, involving collaboration between Laboratory 1, Laboratory 2 and the protocol transfer laboratory (designated *Laboratory 3*); and

Phase III: *protocol performance*, comprising a blind study involving two or more laboratories, including Laboratory 2 and Laboratory 3.

The steering committee would nominate a management team to design the study, which would include representatives of the steering committee and of the three designated laboratories.

Phase I: protocol refinement

A laboratory with sufficient experience in the relevant area would be contracted to act as Laboratory 2, to modify the procedure proposed by Laboratory 1 into a workable, Good Laboratory Practice (GLP)-compliant protocol or to confirm that such standardisation had already been carried out. Laboratory 2 would also create SOPs for the test system and establish the intralaboratory reproducibility of the protocol, by using a small number of known materials of the type(s) appropriate for the method. It would be helpful if these chemicals were backed by reliable *in vivo* data, but this would not be an absolute requirement at this stage.

Discussions, on-site demonstrations and collaboration with Laboratory 1 would be permitted. In addition, liaison with the RVA and other experts, especially for statistical or technical advice, would be encouraged. Any modifications necessary to make the protocol more widely applicable or reproducible would also be appropriate at this stage.

The exact level of intralaboratory reproducibility required of an assay is a function of several variables, such as the range of the *in vivo* measurement scale, the precision of the *in vivo* scores, and the range of *in vitro* scores expected. Appropriate statistical procedures should be applied, to determine the optimal reproducibility for each specific circumstance and procedure.

Summary of Phase I

1. Creation of a workable, GLP-compliant protocol for the procedure.
2. Production of accompanying SOPs.
3. Determination of the intralaboratory reproducibility of the method.
4. Evaluation of its suitability for progression to Phase II.

Phase II: protocol transfer

When the protocol had been refined to the satisfaction of Laboratory 2 and the management team responsible to the RVA, Laboratory 2 would work with Laboratory 3 to establish the transferability of the protocol. Laboratory 3 would be invited to use the protocol with the same test materials as were used in Phase I.

Discussions among the three laboratories would be permissible during this phase. Any necessary refinements of the amended protocol would be made, and, if necessary, the transferability of the amended protocol would have to be established in further testing by both Laboratory 2 and Laboratory 3. In the case of a toxicity test method, if it was agreed that the protocol had been optimised, it would be submitted to the *INVITTOX* data bank (7) at this stage.

Summary of Phase II

1. Transfer of the method to Laboratory 3 using the protocol and SOPs defined by Laboratory 2.
2. Determination of interlaboratory transferability (using the materials tested in Phase I).
3. Further refinement of the protocol, as necessary.
4. Evaluation of the suitability of the method for progression to Phase III.
5. Submission of an optimised protocol to *INVITTOX* (if a toxicity test method).

Phase III: protocol performance

If the results of the protocol transfer stage were acceptable to all three laboratories, to the management team, and to the steering committee responsible to the RVA, the method would proceed to the protocol performance phase. If not, further method development might be recommended. In the case of insuperable difficulties, it might be agreed that the method should be abandoned.

The precise aim of Phase III would be defined in consultation with the RVA, as appropriate for each study and the stated purpose of the method. For example, it could involve the investigation of a narrowly-defined class of test materials, or of a wider range than had originally been envisaged at the development stage.

Phase III would involve a blind study with an appropriate number of test materials, selected, coded and supplied independently, on behalf of the RVA. Materials from appropriate chemical/product classes would be tested blind and according to the principles of GLP. The preliminary prediction model for this set of test materials would have been agreed in advance.

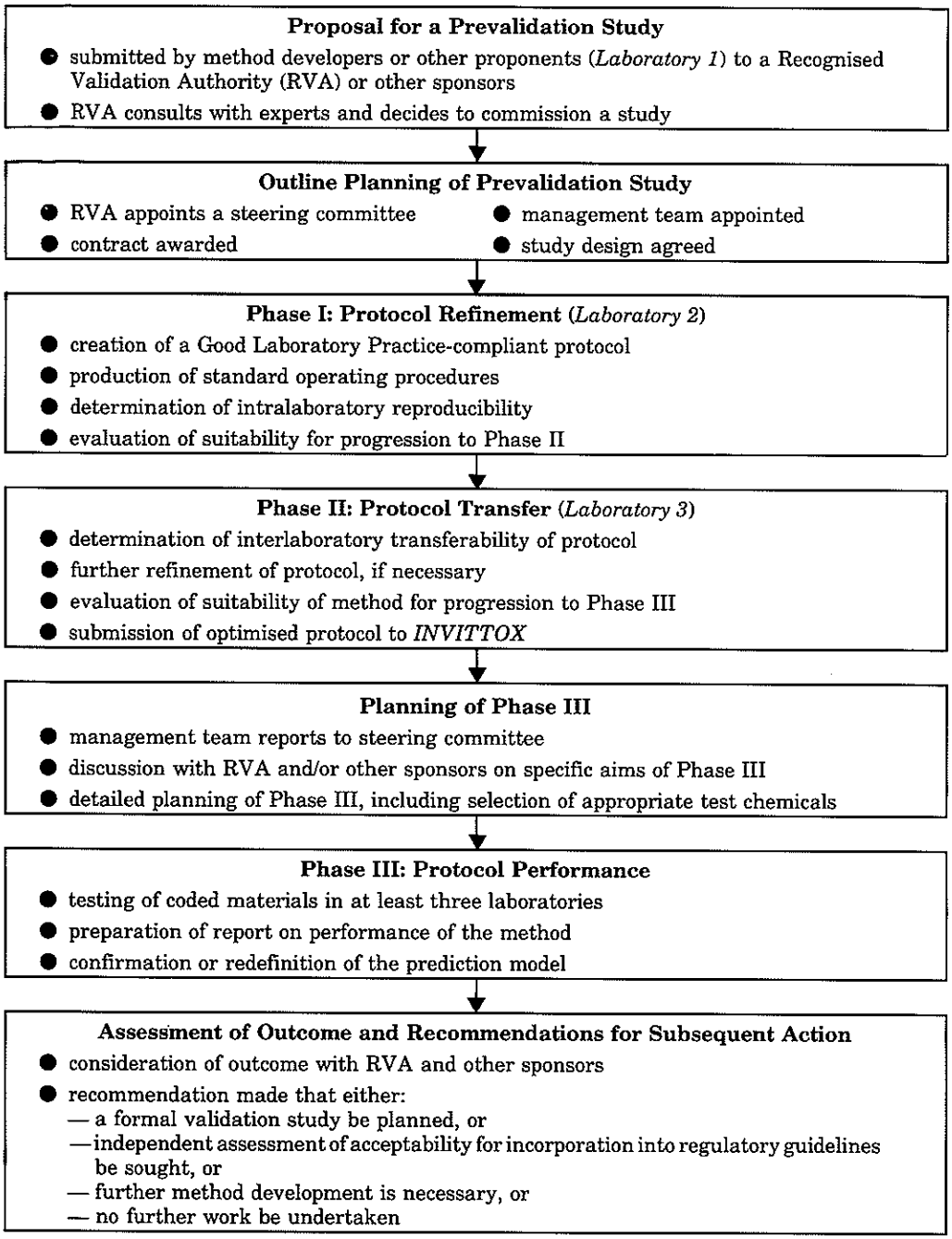
Good *in vivo* data would need to be available for the test materials, and the capacity of the method to predict the *in vivo* effects would be the basis for making a decision on whether to include the assay in a future validation study.

Phase III would involve Laboratory 2 and Laboratory 3, but Laboratory 1 might also take part, for example, if the developers or other proponents of the method had sufficient experience of working to the principles of GLP. If not, and also if the method were judged to have reached the point where progression toward regulatory acceptance could be possible at the end of Phase III, without a further formal validation study, one or more additional laboratories with the appropriate experience would be contracted to join the study for Phase III.

The data from the protocol performance stage would be submitted to the independent statistician appointed for the study, who would prepare a report for the RVA according to pre-defined test performance criteria which had been agreed in advance in discussions involving the RVA, its advisers, any other sponsors, and Laboratories 1–3. The preliminary prediction model would be confirmed or redefined at this stage.

It is conceivable that some methods could be independently judged to be acceptable for incorporation into test guidelines as a result of a satisfactory outcome of the protocol performance stage. Normally, however, it is foreseen that a more formal, and more expensive, multi-laboratory study would be needed,

Figure 1: The prevalidation process



perhaps including other methods and/or test protocols.

Summary of Phase III

1. Definition of precise aim of Phase III of the study.
2. Restatement or adjustment of the preliminary prediction model.
3. Testing of a set of coded materials using the final, optimised, protocol.
4. Preparation of a statistical report on the performance of the method.
5. Review of the performance of the method and of the outcome of the study.
6. Confirmation or redefinition of the prediction model.
7. Recommendations on options for subsequent action.

Subsequent Action

At the end of Phase III, the steering committee, the management team, and the participating laboratories, would discuss the outcome of the prevalidation study with the RVA and any other sponsors. Various subsequent actions would then be possible, for example:

1. Commissioning a formal validation study, perhaps in collaboration with one or more other RVAs or other appropriate sponsors.
2. Seeking an independent assessment with a view to the incorporation of the method into regulatory guidelines and regulatory practice.
3. Advising that further method development would be necessary, for example, in the light of the wider spectrum of materials that would need to be tested or the need for improvement of the prediction model.
4. Recommending that no further work on the method be undertaken.

Concluding Remarks

The objective of the prevalidation process is to ensure that any method included in a formal validation study is adequately prepared and ready for validation. Often in the past, it has become apparent that test protocols have been inadequately prepared, and they have subsequently been found to be insufficiently robust to endure the rigours of today's stringent and well-defined validation challenge.

We are convinced that prevalidation studies, such as those we have outlined here (Figure 1), would lead to a marked improvement in formal validation studies, resulting in a much more efficient use of financial and human resources and a greater likelihood that the expectations of those in the scientific, regulatory and animal welfare communities, who seek the replacement of current animal tests by relevant and reliable alternative methods, will be met. However, the importance of another problem identified in the Amden II report (2), namely, the availability of sufficient numbers of appropriate test materials, backed by good *in vivo* data, for use as standards in prevalidation and validation studies, cannot be overemphasised. Until this problem has been overcome, progress will be limited, despite our best endeavours.

ECVAM now intends to put this prevalidation scheme into practice, and a further report will be made in due course on the performance of this prevalidation scheme itself.

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