11.0 Practical Considerations

Several issues are taken into account when assessing the practicality of using an *in vitro* test method in place of an *in vivo* test method. In addition to reliability and accuracy evaluations, assessments of the laboratory equipment and supplies needed to conduct the *in vitro* test method, level of personnel training, labor costs, and the time required to complete the test method relative to the *in vivo* test method are necessary. The time, personnel cost, and effort required to conduct the proposed test method(s) must be considered to be reasonable when compared to the *in vivo* test method it is intended to replace.

11.1 Transferability of the *In Vitro* Pyrogen Test Methods

Test method transferability addresses the ability of a method to be accurately and reliably performed by multiple laboratories (ICCVAM 2003), including those experienced in the particular type of procedure as well as laboratories with less or no experience in the particular procedure. The degree of transferability of a test method can be evaluated by its interlaboratory reproducibility. ECVAM measured the transferability (i.e., interlaboratory reproducibility) of each assay among experienced laboratories. The results presented in **Tables 7-3** and **7-4** provide an estimate of the minimum variability to be expected. Interlaboratory variability is anticipated to be greater (i.e., lower transferability) among laboratories that have less experience with the assays.

11.1.1 Facilities and Major Fixed Equipment

A standard laboratory facility for sterile tissue culture is necessary for performing the *in vitro* pyrogen test methods. The major equipment necessary to conduct the tests are readily available and include a laminar flow hood, tissue culture incubator, water bath, and spectrophotometric microplate reader.

In contrast, the RPT requires a facility that meets applicable State and Federal regulations for the care and housing of laboratory animals. The primary expense for equipping a facility to conduct the RPT would be the acquisition of an adequate animal room and associated housing (e.g., cages, bedding, food, water, etc.) for boarding animals during the study.

11.1.2 General Availability of Other Necessary Equipment and Supplies

The equipment and supplies necessary to conduct the *in vitro* pyrogen test methods (e.g., micropipetters, sterile tissue culture vessels, disposable plastic ware, assay reagents) are readily available in most scientific laboratories, or can be obtained from any of several scientific laboratory equipment vendors.

The RPT requires fewer general laboratory supplies. Those that are needed are readily available in most laboratories, or could be readily obtained from any of a number of scientific laboratory equipment vendors.

11.2 Personnel Training Considerations

Training considerations are defined as the level of instruction needed for personnel to conduct the test method accurately and reliably (ICCVAM 2003). Evaluation of the levels of

training and expertise needed to conduct the test method, as well as the training requirements needed to insure that personnel are competent in the test procedures, are discussed below.

11.2.1 Required Training and Expertise Needed to Conduct the In Vitro Pyrogen Test Methods

Laboratory personnel require training with the relevant ELISA procedures and the aseptic techniques associated with mammalian tissue culture. The quality criteria associated with each *in vitro* test method may be used to ensure that personnel are competent in the performance of the various procedures. When a technician has mastered all aspects of the protocol, and can independently conduct the assay such that the quality criteria have been met, the individual is considered to have demonstrated proficiency in the assay.

The RPT requires training in the care and handling of laboratory animals, and the collection of accurate rectal temperature measurements at the appropriate time intervals from each rabbit. The laboratory personnel must be adequately trained to maintain the animals, and to accurately and consistently record the proper body temperature. It is not known what, if any, proficiency requirements are in place for the RPT.

11.3 Cost Considerations

In addition to the major fixed equipment and overhead requirements, three additional factors contribute to the overall cost of the proposed *in vitro* test methods: 1) cost and licensing fees associated with the MM6 monocytoid cell line, 2) cost of the reagents for the ELISA procedure, and 3) personnel costs associated with obtaining human blood and performing the test methods. With respect to the RPT, the direct and indirect costs of operating an animal facility must be considered. The most notable expenses will likely include personnel to care for the maintenance of the rabbits, staff to perform the RPT, and veterinarians to monitor the health of the rabbits. As summarized in **Table 11-1**, cost estimates from various contract laboratories that perform the RPT or from one contract laboratory that performs an ELISA-based *in vitro* pyrogen test using human WB indicate that the *in vitro* test methods are considerably more cost effective (i.e., by about a factor of ten) than the RPT. Furthermore, the use of high throughput procedures to analyze the *in vitro* pyrogen tests may provide further reduced costs per test substance.

11.4 Time Considerations

The *in vitro* pyrogen methods require two half-days (i.e., one before and one after the overnight incubation) to complete if cryopreserved blood or MM6 cells are available. If fresh WB is used or if interference testing is needed, additional time will be required. On the first day, the test materials are prepared and incubated with the monocytoid cells. On the second day, cytokine release from the cells is determined by an ELISA procedure. The BET and RPT can both be completed within one working day. However, according to the USP30 NF25<151> (USP 2007b) procedure for the RPT, each rabbit must be conditioned prior to its first use by a sham test that includes all steps of pyrogenicity testing except for injection.

Table 11-1 Cost Estimates for the RPT and *In Vitro* Pyrogen Tests

Contract Laboratory	Test or Cell Line	GLP Compliant	Cost Estimate per Test	Additional Information
A	RPT	Yes	$2100[1]	-
B	RPT	Yes	$4050[1]	-
C	RPT	Yes	$3600[1]	-
D	IPT/HumanWB	ND	$315[2]	Cost decreases with number of test substances; $315 per 1 test substance; $210 per 2 to 10 test substances; $105 per 11 or more test substances. Note: IPT is not a licensed product and should not be used for the release of drugs.
E	MM6	NA	Negotiable	Use of MM6 cells for product testing require negotiation of a fee for provision and a royalty payment per batch of product tested with Dr. HWL Ziegler-Heitbrock at the University of Leicester, Dept of Microbiology, Leicester, U.K.

Abbreviations: GLP = Good laboratory practice; IPT = *In vitro* pyrogen test; MM6 = Mono Mac 6; NA = Not applicable; ND = Not determined; RPT = Rabbit pyrogen test; WB = Whole blood

[1]Each RPT includes one test substance, one positive, and one negative control performed in triplicate. Thus, a minimum of 9 rabbits is needed per test.

[2]Each IPT includes one test substance, one positive, and one negative control performed in triplicate.