# 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 equipment and supplies needed for the *in vitro* test method, level of personnel training, costs of the *in vitro* test method, and time to complete the method are necessary. This information provides additional information as to whether the time, personnel cost, and effort required to conduct the test method are considered reasonable.

## 11.1 Transferability of the BCOP Test Method

Test method transferability addresses the ability of a method to be accurately and reliably performed by multiple laboratories (ICCVAM 2003). Issues of transferability include laboratories experienced in the particular type of procedure, and otherwise competent laboratories with less or no experience in the particular procedure. The degree of transferability of a test method affects its interlaboratory reproducibility.

### 11.1.1 Facilities and Major Fixed Equipment

The facility requirements necessary to conduct the BCOP test method include a standard laboratory setup for nonsterile tissue culture, and proximity to an abattoir or other bovine eye supplier so that eyes can be obtained from freshly slaughtered animals. The major equipment necessary to conduct the test method is readily available and includes an opacitometer, cornea holders, a UV/VIS spectrophotometer or microplate reader, and a water bath or forced air incubator. Suppliers and estimated costs of this equipment are summarized in **Table 11-1** to the extent this information was available.

If histopathology is included as a component of the BCOP test method, the testing facility may choose to process the tissue in-house, whereby the facility would need equipment for tissue processing, sectioning, and staining. This specialized equipment would add significant cost to the major equipment required for the BCOP assay. Most likely, if a facility is not already equipped to prepare tissue slides for histological evaluation, this portion of the test method could be outsourced to an appropriate contractor as is done at IIVS (Harbell J, personal communication).

In contrast, the *in vivo* rabbit eye test requires a facility that meets the approval of applicable State and Federal regulations to house live laboratory animals. The primary expense for equipping a facility to conduct the *in vivo* rabbit test 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 remaining equipment and supplies necessary to conduct the BCOP test method (e.g., dissection equipment, micropipettors, petri dishes, volumetric flasks) are readily available in most scientific laboratories or can be obtained from any of several scientific laboratory equipment vendors.

**Table 11-1 Suppliers and Costs of Major Equipment for the BCOP Assay**

| Equipment | Supplier/Manufacturer(s) | Estimated Costs[1] |
|---|---|---|
| Opacitometer | Stag Bio (Clermont, France)<br>Spectro Design (Riom, France) | ***Not yet provided*** |
| Cornea holders | Stag Bio (Clermont, France)<br>Spectro Design (Riom, France) | ***Not yet provided*** |
| UV/VIS Spectrophotometer | Beckman, Fisher Scientific, Perkin Elmer, Thermo Spectronic | Starting at $6000 for an 8-cell holder unit with a spectral range from 200 -1100 nm |
| Microplate reader | Bio-Rad, Bio-Tek Instruments, Cambrex, Fisher Scientific, Molecular Devices, PerkinElmer | Starting at $6500 for a 96-well plate absorbance reader with a spectral range from 400 -750 nm |
| Water bath | e.g., Brinkmann Instruments, Fisher Scientific, Neslab | 2 L capacity ~ $590<br>5 L capacity ~ $750<br>10 L capacity ~ $875<br>20 L capacity ~ $1100 |
| Incubator - forced air | e.g., Fisher Scientific, Precision, Thermo Electron | 2.2 cu. ft. ~ $2100<br>3.75 cu. ft. ~ $2230<br>5.0 cu. ft. ~ $2460 |

[1]Estimated costs based on 2004 catalog prices.

Similarly, the remaining equipment and supplies necessary for conducting the *in vivo* rabbit eye test are readily available in most toxicity testing laboratories or could be readily obtained from any of a number of scientific laboratory equipment vendors.

## 11.2 BCOP Test Method 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 level of training and expertise needed to conduct the test method reliably and accurately, as well as the training requirements needed to ensure that personnel are competent in the test method, are discussed below.

### 11.2.1 Required Level of Training and Expertise Needed to Conduct the BCOP Test Method

A training period of between two to three months is usually required for a technician with general laboratory skills to conduct all aspects of the assay independently and proficiently (Harbell J, personal communication). A training video or other visual media to provide guidance on performing the assay may be considered useful. During the training period the technician would learn how to:

- dissect the cornea from the bovine eye
- identify corneas with defects
- mount the cornea in a corneal holder without damaging the epithelium or endothelium

- add assay medium and test substances to the appropriate chamber of the corneal holder
- properly time and conduct incubations
- calibrate and use the opacitometer
- rinse the cornea without damaging it
- perform quantitative opacity readings
- conduct the permeability evaluation

There are currently no known proficiency criteria used to ensure that personnel are performing the test method competently. Rather, this must be demonstrated through experience with the oversight of an experienced supervisor. All of the tasks in the BCOP assay are technically simple to perform. When a technician has mastered all aspects of the protocol, and can independently conduct the assay, such that the positive control falls within its historical range, the technician has essentially demonstrated proficiency in the assay.

In contrast, the *in vivo* test method requires training in the care and handling of laboratory animals. Possibly the most difficult aspect of the *in vivo* test method to master is the subjective assessment of corneal opacity, iritis, conjunctival chemosis, conjunctival redness, and discharge as endpoints in the evaluation of ocular irritancy. The laboratory personnel must be adequately trained to accurately and consistently identify these endpoints. It is not known what, if any, proficiency requirements are in place for the *in vivo* test.

## 11.3 Cost Considerations

The current cost for a GLP compliant BCOP assay at IIVS is $1400 per test substance (Harbell J, personal communication). This cost includes both positive and negative controls. Histology can be added to the standard BCOP assay for $650 and includes both slides and photographs. Another source reports that a typical GLP compliant BCOP study for one sample with benchmarks and histology costs about $4,500, and this includes two time courses and one benchmark (Cuellar N and Swanson J, personal communication). In comparison, a GLP-compliant EPA OPPTS Series 870 Acute Eye Irritation test (EPA 1998) in the rabbit ranges from $765 for a 3 day/3 animal study up to $1665 for a 21 day/3 animal study at MB Research Laboratories (MB Research laboratories, personal communication). While the cost of the BCOP assay includes concurrent positive controls, the *in vivo* rabbit test method does not include equivalent controls.

## 11.4 Time Considerations

Use of the BCOP test method would significantly reduce the time needed to assess the ability of a test substance to induce ocular corrosivity or severe irritancy, when compared to the currently accepted *in vivo* rabbit eye test method. The *in vivo* Draize rabbit eye test is typically carried out for a minimum of one to three days. Depending upon the severity of ocular effects produced by a test substance, the method can be extended for up to 21 days. Comparatively, the standard BCOP test method can be completed in about five hours for liquid test substances and seven hours for solid test substances, once the bovine eyes arrive at the testing facility. However, one source notes that the turnaround time from initiation of the

study to receipt of the final report is similar for the BCOP assay and the *in vivo* rabbit eye test (Cuellar N and Swanson J, personal communication).