

## **11.0 PRACTICAL CONSIDERATIONS**

### **11.1 Test Method Transferability**

Test method transferability addresses the ability of a method to be accurately and reliably performed by multiple laboratories (ICCVAM, 1997). This definition includes laboratories experienced in the particular type of procedure, and otherwise competent laboratories with less or no experience in the particular procedure. It also addresses whether the necessary facilities, equipment, and trained staff to perform the method can be readily obtained, and whether the cost of the assay and the level of expertise or training needed are considered reasonable. The degree of transferability of a test method affects its interlaboratory reproducibility.

The ICCVAM Submission Guidelines (ICCVAM, 1999) request that an assessment of test method transferability be conducted with respect to the following factors that influence transferability:

- Availability of the facilities and the fixed major equipment needed to perform the test method;
- The training requirements for laboratory personnel to demonstrate proficiency with the test method;
- Costs involved in conducting the test; and
- Time needed to conduct the test.

#### **11.1.1 Facilities and Major Fixed Equipment**

The facilities needed to conduct *in vitro* ER TA assays are widely available, and the necessary laboratory equipment is readily available from suppliers. To ensure personnel and community safety, facilities should adhere to pertinent State or Federal regulations for the handling of hazardous substances and wastes.

The specific needs as related to the various *in vitro* ER TA procedures utilizing reporter genes, whether transiently or stably transfected, are essentially the same. These are described briefly below.

*Facilities:* Standard cellular or molecular biology laboratory with cell culture capabilities.

*Fixed major equipment:* Luminometer for assays requiring luciferase detection; cell incubator with temperature, CO<sub>2</sub>, and humidity controls; sterile biohazard/safety hoods; and freezer.

Cell proliferation assays have the same facility and equipment requirements as reporter gene assays, except that cell counting equipment would be an additional requirement.

## **11.2 Training Considerations**

*Assays using stably transfected/transduced cell lines:* Currently, most of the mammalian and yeast cell lines containing a stably transfected ER and a reporter are not available commercially. A high level of technical expertise would be required to establish such cell lines. However, once established in a laboratory, the cell lines could be readily used in a reporter gene assay that requires staff with basic laboratory skills and training in cell culture techniques.

*Assays using transiently transfected cell lines:* These assays require staff with basic laboratory skills and training in cell culture techniques and transient transfections.

*Cell proliferation assays:* Performing these assays requires staff with basic laboratory skills and training in cell culture and cell counting techniques.

## **11.3 Estimated Cost and Time Considerations**

**Table 11-1** provides information on the expected time needed to perform a study, special equipment needed, and other considerations. Cost information was not available for all of the assays.

**Table 11-1 Comparison of Estimated Costs, Time, and Special Equipment Needs for Different *In Vitro* ER Reporter Gene and Cell Proliferation Assays**

Assay	Cost/Test substance	Duration	Special Equipment	Other Considerations
BG1Luc4E2 Reporter Gene Assay – Stable	\$250 - \$600	3 days	Luminescence counter (luminometer) for luciferase detection (~\$20,000)	Commercially available
HeLa Reporter Gene Assay – Transient or Stable	n.a.	3-4 days	Luminescence counter (luminometer) for luciferase detection (~\$20,000)	
HepG2 Reporter Gene Assay – Transient	\$1950	3-4 days	Luminescence counter (luminometer) for luciferase detection (~\$20,000)	
MCF-7 Focus Assay	n.a.	14 days	Cell colony counter (automated)	
MCF-7 E-SCREEN Assay	n.a.	5-7 days		Estrogenic response of different MCF-7 cell subclones varies; the most sensitive subclones may not be commercially available.
MCF-7 Reporter Gene Assay – Transient	n.a.	3-4 days	Luminescence counter (luminometer) for luciferase detection (~\$20,000)	
Yeast Reporter Gene Assay – Stable	\$1600	2-3 days		

n.a. = Cost estimates not available in the literature or from laboratories conducting the assay.

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