Critical Factors Impacting Interlaboratory Transferrability of the Mouse Embryonic Stem Cell Test

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The mouse Embryonic Stem Cell Test (EST) assesses a compound's ability to inhibit differentiation of embryonic stem cells into myocardiocytes in parallel with cytoxocity endpoints in adult and embryonic stem cells. Though intralaboratory transferability was found to be acceptable among European labs during the validation process, we had difficulty consistently running the EST in the United States. The problems encountered most often were: 1) a low number of contracting myocardiocytes in the control cultures; and 2) attachment and loss of embryoid bodies that require suspension culture. We initiated a program to identify critical factors impacting the outcome of the assay, and have identified and resolved three technical issues. First, the plasticware specified in the ECVAM protocol has a different catalog number when ordering from the United States. Switching plasticware eliminated attachment of the embryoid bodies. Next, using FCS at a concentration of 15% rather than at 20% improved the reliability of differentiation. Finally, using different lot numbers of serum, even from the same supplier, significantly impacted the consistency of the differentiation assay, indicating that prequalifying lots of serum is a necessary step to successfully running the EST. We have developed a serum qualification procedure and have significantly improved the performance of the assay in our hands . We recommend establishing a public forum for researchers who are working with the EST to facilitate communicating serum lot qualification efforts, and to address additional technical difficulties laboratories may encounter while setting up the EST.