Results Suggested the Cells Were Contaminated With: Lactate Dehydrogenase-elevating Virus (LDEV; togavirus)

MAP Test: LDEV assay was done on Hsd:ICR mice inoculated with a thick suspension of minced cells and assayed for serum LDH (lactate dehydrogenase) levels after 4 and 6 days. The convalescent LDH values were 1800 U/L or greater and are highly suggestive of LDEV infection.

Lactate Dehydrogenase (LDH). LDH catalyzes the reversible oxidation of lactic to pyruvic acid. It is widely distributed in mammalian tissues and isoenzymes are found in heart (1,2), erythrocytes (1,2), kidney (1,2), brain (1,2), lung (3), pancreas (3), adrenals (3), spleen (3), thymus (3), thyroid (3), lymph nodes (3), leukocytes (3), skeletal muscle (4), and liver (4).

Measured LDH values: 2120 +/- 500 U/L (day 4: 1791; day 6: 1890, 2700).

Control LDH values: Only convalescent, rather than also acute (control), assays are done in order to control costs (12% cost savings). Fifty percent (50%) of the cost of a MAP test is underwritten by DAR. The mean (+/- standard deviation) of 15 previous convalescent assays of LDH in Hsd:ICR mice inoculated with various cell lines was 530 (+/- 300) U/L (range: 159-1113 U/L). This value was significantly different (two-tailed p < 0.0001; Student t test) compared to that measured in mice given EHS cells.

Lactate Dehydrogenase-Elevating Virus (LDEV). LDEV is the most common murine tumor contaminant and mice are the only known host species. Transmission occurs primarily through passage of contaminated tumors, cells or other biological materials. The virus replicates in macrophages and is shed in urine, feces, saliva, milk and transplacentally. Natural infections are usually subclinical, but there is lifelong viremia and elevation of specific liver enzymes. Consequently, the use of EHS cells in G24 or any other mouse colony presents the risk of infection and experimental complication to other studies, particularly those of the immune system.

The diagnosis of LDEV is confounded by the fact that humoral antibody is consumed in immune complex formation with virus and is rendered an unreliable indicator of LDEV. Molecular biology techniques have not been adapted to the diagnosis of this virus. Consequently, the diagnosis is made by detecting increased levels of LDH in mice inoculated with biological materials. LDEV infection causes a 3-10 fold increase in serum LDH levels and concentrations above 1,800 U/L are considered diagnostic for infection (Lab Anim Sci 37: 356, 1987). SJL mice, due to a recessive trait, are particularly sensitive to LDEV infection and show dramatic (15-20X baseline) elevations in LDH. Aged C58 mice infected with LDEV experience clinical polioencephalomyelitis.
Recommendation: LDEV infection can be eliminated from cells by passage through a rodent species other than the mouse (i.e., nude rats) or through multiple passages in tissue culture. Rather than repeat Lactate Dehydrogenase-elevating Virus (LDEV) assessments using more sensitive mouse strains (SJL; C58), I recommend that an aliquot of EHS cells be passaged through a nude rat. The passaged cells can then be assayed to confirm elimination of LDEV and used for continued experiments. Without additional testing, the cell line cannot be verified to be pathogen-free and continued experiments should be done with inoculated mice kept isolated by the veterinary staff in G08.