



Oil for Embryo Culture:

Mouse Embryo Assay is the Ultimate Test for Toxicity

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Introduction: It is known that detection of toxicity by the mouse embryo assay is varied depending upon the mouse strain and methodology used. A preliminary study was conducted with adulterated medium using two MEA methods (micro-drop versus micro-well) in parallel to distinguish the threshold of tolerance to toxicity by pre-implantation mouse embryos. Additional studies were conducted to investigate the detection of toxic oil by analytical and biological methods and the results show that the biological assay is ultimately the most sensitive test for detection of toxicity.

Materials and Methods: Fresh one-cell mouse embryos (B6D2F1 x B6C3F1) were used for each assay. A minimum of 33 embryos (n=33) were used for each condition tested. Culture medium used was Continuous Single Culture-Complete (CSCM-C) that was a) adulterated with addition of formaldehyde (0-7X, where 1X = 9.3×10^{-5} %) or b) overlaid with oil containing a percentage of toxic oil (0-25%). Culture dishes (Falcon Petri dishes, Cat. # 35-1007) and wells (Greiner Bio-one 72 micro-well plate, VWR catalog # 82050-710) were set up in parallel using a **micro-drop assay** (3 embryos/20 μ L) overlaid with 7 mL oil or **micro-well assay** (10 embryos/ 10 μ L) overlaid with 9 mL of oil, respectively. Culture dishes/plates were equilibrated overnight in the incubator with 5.0-5.5% CO₂ at 37°C prior to addition of freshly harvested and washed one-cell mouse embryos. Upon retrieval, fresh mouse embryos were pooled, washed three times in culture medium and randomly distributed into the respective test conditions. The culture dishes/plates were returned to the incubator with 5.0-5.5% CO₂ at 37°C for 96 hours continuous culture. Blastocyst development (\geq expanded blastocysts) was observed and recorded at 96 hours. During screening of new lots of raw material oil, a toxic lot was identified by MEA testing. This lot was submitted, along with a good quality lot for several analytical tests including: FTIR (Fourier Transform Infrared Spectroscopy), NMR (Nuclear Magnetic Resonance Spectroscopy) and HPLC (High Performance Liquid Chromatography) to characterize the purity and variation present in these raw materials.

Results:

Biological assays for the adulterated medium study showed that the micro-drop method could detect toxicity at lower concentrations than the micro-well assay. Similarly, the threshold for toxic oil detection was lower with the micro-drop assay, demonstrating a higher level of sensitivity than the micro-well assay. Results of FTIR analysis on good and bad (toxic) oil are showed no differences between the two oils. Additional analytical methods that were conducted also did not reveal any differences between good quality and toxic oil, when each oil was tested at full strength (data not shown).

Conclusions:

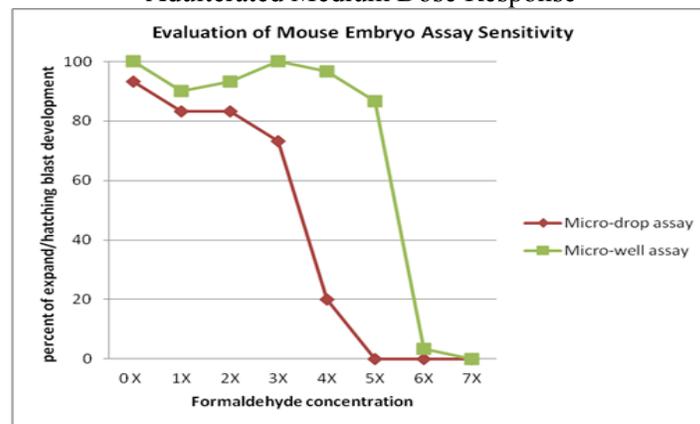
This study demonstrates that the bioassay proved to be the ultimate test for detecting toxic oil compared to analytical methods, which did not reveal any differences between good and toxic oil. Additionally, the method used for the mouse embryo assay can impact the sensitivity of detecting toxic substances. The blastocyst development data show that the threshold for detection of toxic components is lower (more sensitive) when using the micro-drop assay compared to the micro-well assay. Possible explanations for enhanced sensitivity of the micro-drop assay include: 1) availability of more autocrine/paracrine factors in the culture microenvironment in micro-wells as a result of a higher ratio of #embryos/culture volume which may benefit embryo development and consequently mask environmental toxicity thereby altering the sensitivity of this MEA, 2) the conical shape of the micro-wells may be beneficial for embryo development by allowing embryos to stay in close proximity to each other during culture, as compared to resting on the flat surface in



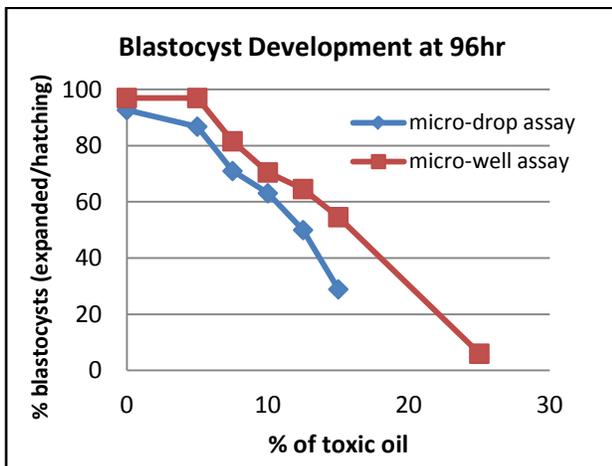
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micro-drop cultures where embryos tend to be separated, 3) the relative surface area exposure of oil to the embryo culture medium is greater for the micro-drop cultures compared to the micro-well cultures, and thus the micro-drop cultures may have greater exposure to toxins in the oil. In addition to screening incoming raw material mineral oil by the methods indicated above, Irvine Scientific initially selects the highest quality of light mineral oil based on the grade and purity from other tests including: kinematic viscosity, composition (paraffins) by High Resolution Mass Spectrometry (HRMS), aromatic content (by UV) and peroxide value (POV).

Adulterated Medium Dose Response



Toxic Oil Dose Response



% of toxic oil in overlay	% of Blastocysts at 96hr (Acceptance specification: ≥ 80%)	
	Micro-drop assay	Micro-well assay
0 %	92.7	97
5 %	86.8	97
7.5 %	71	81.6
10 %	63.1	70.5
12.5 %	50	64.6
15 %	28.9	54.6
25 %	N/A	6



FTIR Spectrum of Oil

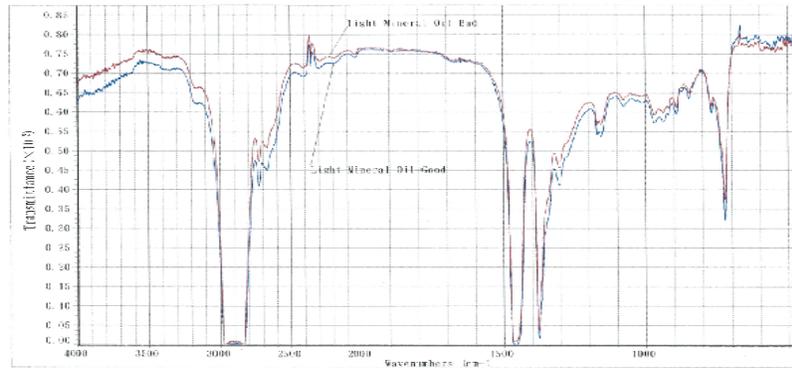


Fig.1 FT-IR Spectrum of Good and Bad Oil
Red:Bad Oil Blue:Good Oil

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