Sydney IVF Culture Oil

Quality Assurance

COOK MEDICAL

Cook's Sydney IVF Culture Oil: Introduction

Culture oil protects gametes and embryos during ART processes by providing a barrier between the embryo culture media and air to stop evaporation and minimize fluctuations in pH, temperature, and osmolality.[1] The purity of the oil is of paramount importance in safeguarding embryo development and yielding consistent results.[2, 3] To ensure that it meets high standards of purity, Cook culture oil is rigorously tested before, during, and after manufacture. This white paper describes the extra measures Cook Medical takes to provide pure and consistent culture oil.

Mineral Oil: Sourcing and Quality

Cook Medical sources pure, highly refined mineral oil for the manufacture of Cook culture oil.

Crude oil is the base material for many petroleum products, from gaseous methane to liquid mineral oil to asphalt. Crude oil comprises a complex mixture of organic compounds, including hydrocarbons, which contain hydrogen and carbon.

The hydrocarbons can be classified into 4 categories:

- Alkanes (paraffins) – saturated structure hydrocarbon chains
- Alkenes (olefins) – unsaturated molecules
- Alicyclics – saturated (naphthenes) and unsaturated ring structures
- Aromatics – cyclic structures with conjugated double bonds

It is desirable that highly refined mineral oil (commonly referred to as liquid paraffin) consists exclusively of saturated hydrocarbons, which improve chemical stability and prevent oxidation of the oil.[4]

Fractional distillation extracts mineral oil from crude oil on the basis of the molecular weights of its constituent hydrocarbons. Many alkanes, alicyclics, and aromatic hydrocarbons have the same or similar molecular weights; therefore, during distillation they are all extracted in the same fraction of mineral oil. To create mineral oil that is suitable for use in pharmaceuticals and IVF products, further refinement is required. This additional refinement involves processes such as hydrotreating, in which hydrogen, heat, and pressure convert and remove aromatics and unsaturated bonds from the mineral oil.

Light Mineral Oil and Light Paraffin Oil

Cook culture oil is manufactured from oil that meets all the requirements of the United States Pharmacopeia (USP) and National Formulary monograph for Light Mineral Oil. Additionally, Cook culture oil is tested against the European Pharmacopoeia (Ph. Eur.) monograph for Light Liquid Paraffin.

The following suite of tests is conducted to ensure compliance with the USP and European Pharmacopoeia standards.

Test Description	Specification
Infrared absorption spectrophotometry – purified mixture of liquid-saturated hydrocarbons obtained from petroleum	Complies with USP/Ph. Eur.
Limit of polycyclic and aromatic hydrocarbons	Complies with USP/Ph. Eur.
Readily carbonized substances	Complies with USP/Ph. Eur.
Relative density	Complies with USP/Ph. Eur.
Acidity or alkalinity	Complies with USP/Ph. Eur.
Specific gravity	Complies with USP/Ph. Eur.
Solid paraffins	Complies with USP/Ph. Eur.
Viscosity	Complies with USP

Quality Assurance of Cook Culture Oil

Every precaution is taken to ensure the consistency of Cook culture oil. A specialist team identified the critical quality attributes for Cook culture oil and further defined the activities that must occur in order to confirm that the final product will always meet the most stringent quality criteria.

In addition to the controls referenced in the section above, Cook subjects its culture oil to further tests and safeguards. These tests and safeguards are discussed in the remainder of this paper and include:

1. Special handling and packaging
2. Biocompatibility testing
3. Testing for the presence of VOCs in mineral oil
4. Peroxide value determination
5. Peroxide toxicity testing

[1] Morbeck DE, Leonard PH. Culture systems: mineral oil overlay. In: Smith GD, Swain JE, Pool TB, eds. *Embryo Culture: Methods and Protocols*. Totowa, NJ; Humana Press; 2012:325-331.
[2] Otsuki J, Nagai Y, Chiba K. Damage of embryo development caused by peroxidized mineral oil and its association with albumin in culture. *Fertil Steril*. 2009;91(5):1745-1749.
[3] Otsuki J, Nagai Y, Chiba K. Peroxidation of mineral oil used in droplet culture is detrimental to fertilization and embryo development. *Fertil Steril*. 2007:88(3):741-743
[4] Prince RJ. Base oils from petroleum. In: Mortier RM, Fox MF, Orszulik ST, eds. *Chemistry and Technology of Lubricants*. 3rd ed. New York, NY; Springer Dordrecht Heidelberg; 2010:3-33.

1. Special Handling and Packaging

Cook ensures that the quality of the oil is maintained throughout the life of the product by stringently controlling environmental factors that may promote degradation of the oil.

(a) Heat and light have been shown to promote oxidation of mineral oil, so Cook carefully monitors its supply chain to protect the oil from heat and light during transportation. The oil is manufactured in temperature-controlled cleanrooms, and the finished product is stored in specially designed lightproof containers in temperature-monitored cold rooms.

(b) Extreme care is taken to avoid exposing the oil to volatile organic compounds (VOCs) during transportation, storage, and manufacture. Mineral oil can absorb VOCs from the environment, and these VOCs can negatively affect the health of the embryo.[5, 6] Cook conducted uniformity studies to confirm that each new container is VOC free. Cook has performed studies to demonstrate that the raw material and finished product remain VOC free for the shelf life of the product. All new batches of raw material are screened through quality control testing for VOC.

(c) Cook culture oil is now supplied in a 2 x 50 mL multipack designed to protect the oil until the time of use. The 50 mL borosilicate glass vial has a smaller headspace than the previously supplied 200 mL vials, and the headspace is further reduced by the provision of an extra 5 mL of oil to every vial. Decreasing the headspace protects the oil by limiting the amount of oxygen that is available for the oil to absorb. The rate of oxidation correlates the amount of oxygen that the oil absorbs.[7] Photostability testing has demonstrated that the packaging protects the product from light.

2. Biocompatibility and Toxicity Testing

Cook culture oil passes biocompatibility testing for genotoxicity, cytotoxicity, human sperm survival, and MEA toxicity.

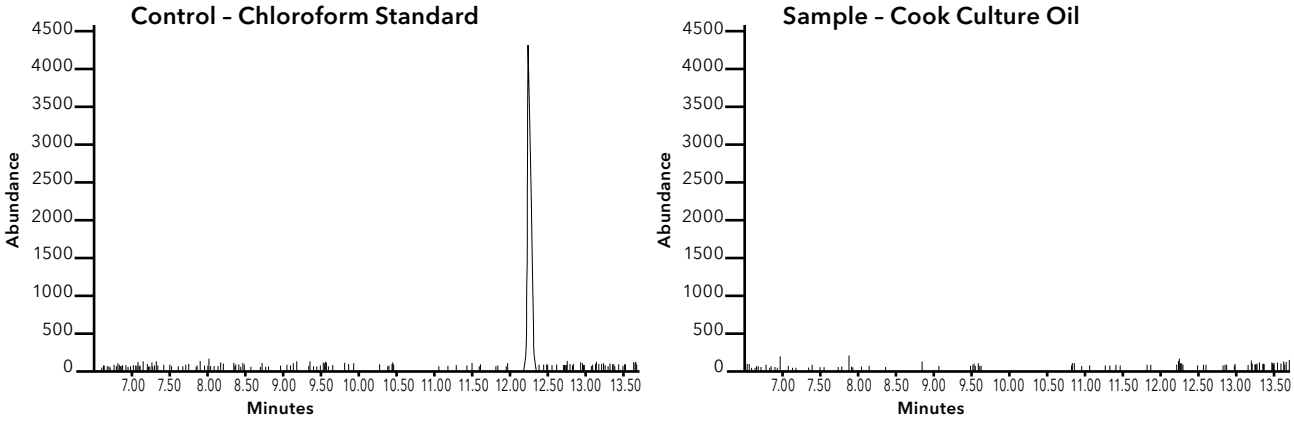
Biocompatibility testing for genotoxicity and cytotoxicity are conducted in accordance with ISO 10993, "Biological Evaluation of Medical Devices." Genotoxicity testing is an in vitro test to confirm that the culture oil does not induce genetic changes that may be transmitted via germ cells to future generations. Cytotoxicity testing uses cell culture techniques to determine that culture oil does not cause lysis of cells (cell death), inhibition of cell growth, or colony formation.

The human sperm survival assay is a toxicity test that examines the survival of human sperm after culture with an oil overlay, and the mouse embryo assay assesses the embryotoxicity of Cook culture oil.

3. Identification of Volatile Organic Compounds in Mineral Oil by Gas Chromatography/Mass Spectrometry

Volatile organic compounds (VOCs) adversely impact preimplantation embryo development and correlate with poor IVF outcome.[8] The USP stipulates that pharmaceutical-grade oils must contain minimal concentrations of VOCs. Cook screens the culture oil for a range of volatiles that are commonly found in ambient air throughout Europe, China, and the United States of America. These volatiles include ketones, toluene, chloroform, isopropanol, and xylene.[9, 10, 11, 12]

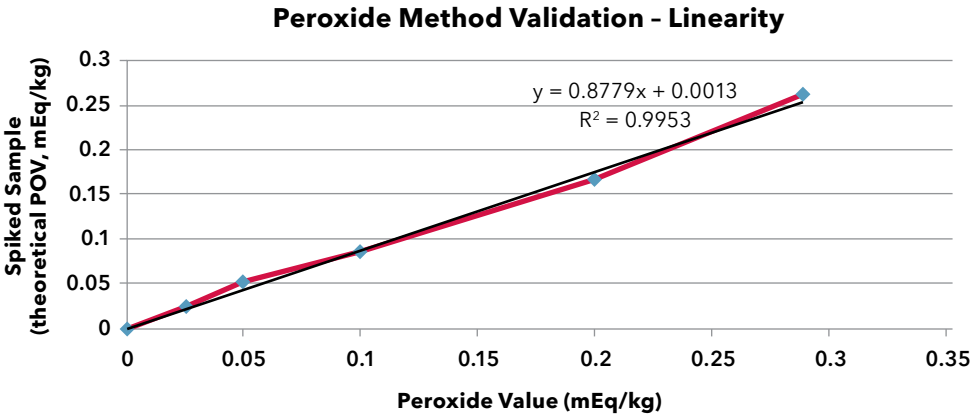
The test method for VOC analysis is headspace-gas chromatography separation coupled with electron ionization (EI) mass spectrometry. In this method, a sample of Cook culture oil is aliquoted into a vial where contaminant VOCs will diffuse into the gas phase or headspace. A sample of the headspace gas is then injected into a gas chromatography system for component analysis.



[5] Poddar TK, Sirkar KK. Henry's law constant for selected volatile organic compounds in high boiling oils. *J Chem Eng Data*. 1996;41(6):1329-1332.
[6] Cohen J, Gilligan A, Esposito W, et al. Ambient air and its potential effects on conception in vitro. *Hum Reprod*. 1997;12(8):1742-1749.
[7] Dornte RW. Oxidation of white oils. *Indust Eng Chem*. 1936;28(1):26-30.
[8] Khoudja RY, Xu Y, Li T, et al. Better IVF outcomes following improvements in laboratory air quality. *J Assist Reprod Genet*. 2013;30(1):69-76.
[9] Cohen J, Gilligan A, Esposito W, et al. Ambient air and its potential effects on conception in vitro. *Hum Reprod*. 1997;12(8):1742-1749.
[10] Hippelein M. Background concentrations of individual and total volatile organic compounds in residential indoor air of Schleswig-Holstein, Germany. *J Environ Monit*. 2004;6(9):745-752.
[11] Hodgson AT, Levin H. Volatile organic compounds in indoor air: a review of concentrations measured in North America since 1990. Berkeley, CA; Lawrence Berkeley National Laboratory; 2003. Report LBNL-51715.
[12] Liu Y, Shao M, Fu L, et al. Source profiles of volatile organic compounds (VOCs) measured in China: part I. *Atmos Environ*. 2008;42(25):6247-6260.

4. Peroxide Value Determination – A Quantitative Assessment of Hydroperoxide Content in Mineral Oil

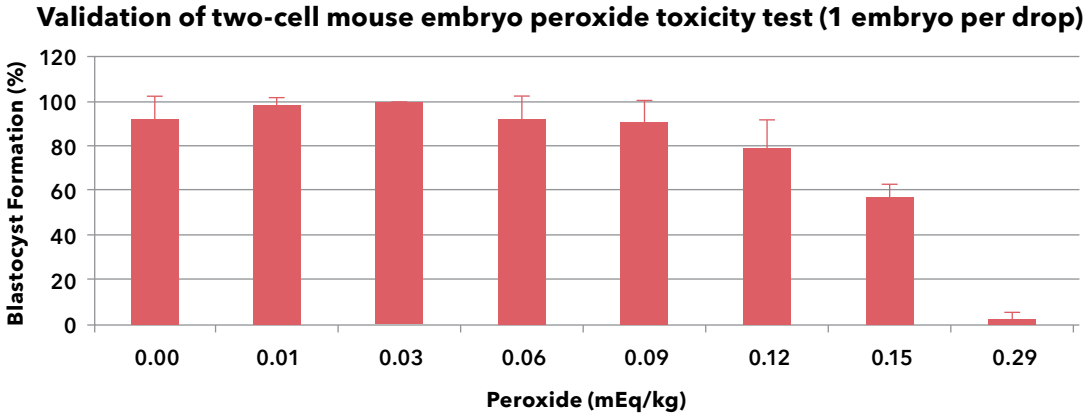
Cook has developed and validated a modified compendial test method (British Pharmacopoeia) for detecting trace levels of peroxide in oil. A peroxide value specification of ≤ 0.01 mEq/kg has been implemented as a lot-release limit for Cook culture oil. This specification is based on previous studies that correlate increases in levels of hydroperoxides with poor embryo outcomes.[13, 14]



5. Mouse Embryo Peroxide Toxicity Testing

In addition to analytical test methods, a specialized mouse embryo assay has been developed to specifically screen for peroxides in Cook culture oil. Both one-cell and two-cell mouse embryo peroxide toxicity assays were developed and validated, but the two-cell mouse embryo assay proved to be a more robust and sensitive assay. Consequently, this assay is used for peroxide toxicity release testing of all batches of finished product. This assay includes human serum albumin, and individual two-cell embryos are cultured in 10 µL drops of Cook's Sydney IVF Blastocyst Medium (K-SIBM).

The two-cell mouse embryo peroxide toxicity assay can discriminate, with 95% confidence, between samples with 0 mEq/kg peroxide and 0.12 mEq/kg peroxide. To ensure that peroxides are detected well below the level at which they would harm an embryo, the peroxide value specification is set at ≤ 0.01 mEq/kg. This specification is tenfold lower than the peroxide concentration that the mouse embryo peroxide toxicity assay can detect , but the peroxide toxicity MEA is performed for added quality assurance.



6. Conclusion

Embryo health can be critically affected by the quality of the culture oil that is used for IVF procedures. For this reason, Cook takes extra precautions to ensure that Cook's culture oil has been rigorously tested and proven to be appropriate for use in IVF procedures.

Cook implements a range of quality controls to create a high-quality finished product. This attention to detail begins with sourcing high-purity pharmaceutical-grade light mineral oil and continues through the handling of the raw material, the extensive testing of the product, and the strict controls over the manufacturing process in a cGMP facility.

In addition, Cook developed customized methodology to assess the peroxide value in the raw material and finished product, including a mouse embryo assay that is specifically designed to detect peroxide toxicity in mouse embryos. These tests are used to evaluate every lot of finished product prior to sale, and the results are reported on the certificate of analysis.

On the basis of extensive research into the complexities of light mineral oil and knowledge of the specific requirements of embryo culture systems, Cook is confident that Cook culture oil will meet the high quality standards that human IVF laboratories expect.

[13] Otsuki J, Nagai Y, Chiba K. Damage of embryo development caused by peroxidized mineral oil and its association with albumin in culture. *Fertil Steril*. 2009;91(5):1745-1749.

[14] Otsuki J, Nagai Y, Chiba K. Peroxidation of mineral oil used in droplet culture is detrimental to fertilization and embryo development. *Fertil Steril.* 2007:88(3):743.