

# ORIGIO Liquid Paraffin and SAGE Oil for Tissue Culture

## A rigorous assessment process for quality control

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## Manufacturing Oil for Tissue Culture for over 15 years

Closed culture under an oil overlay has been successfully applied in embryology since the 1960s<sup>1</sup>.

The oil overlay stabilizes pH, temperature, and osmolality and prevents evaporation. Therefore, when designing a closed culture system, oil quality is of fundamental importance and the product should be handled, processed and stored accordingly.

However, oils are not generally well defined and poor-quality oil can affect embryo growth and development. Therefore, it is important to understand the ways oils vary in type and quality depending upon their source (1), purification (2), testing (3), washing (4), and storage (5).

This white paper describes the process we follow to produce our high-quality oils and defines the set of tests we perform to ensure quality consistency.

### Summary

Mineral oil overlays are obligatory for successful manipulation and micro-drop culture of gametes and embryos. Therefore, CooperSurgical use very pure oils that meet the requirements of the United States Pharmacopeia (USP) and the European Pharmacopoeia (Ph Eur). Composed of saturated hydrocarbons, peroxidation is minimized by correct storage at 2–8°C, away from UV light.

CooperSurgical perform bioassay tests – a sterility test, Limulus Amebocyte Lysate (LAL) tests for endotoxins, a Sperm Survival Test (SST), Mouse Embryo Assay (MEA) and extended MEA (eMEA). In addition, CooperSurgical perform more sensitive physicochemical tests of hydrocarbon composition, Atomic Fingerprinting, and a Peroxide Value Test (POV), that are necessary to confirm that oils consist of saturated carbon chains and do not contain peroxide.

This stringent program ensures ORIGIO Liquid Paraffin and SAGE Oil for Tissue Culture are of high quality.

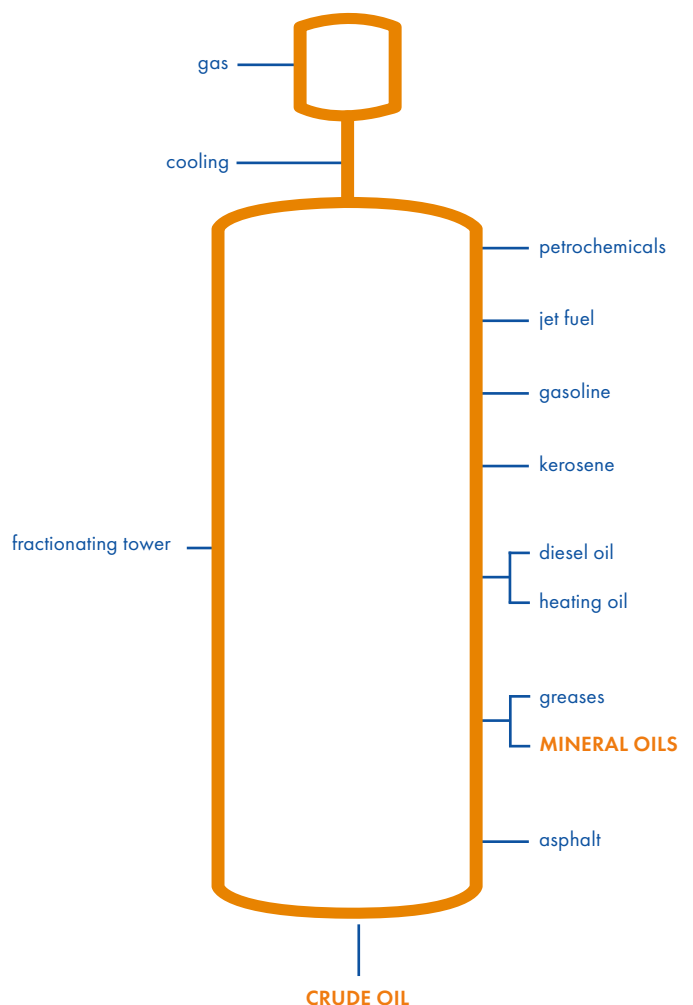
## 1. Source of oils

Mineral oils are highly refined generic petroleum crude oil distillates. The petroleum from which culture oil is ultimately derived occurs naturally as a product of plant and animal decomposition over past millennia.

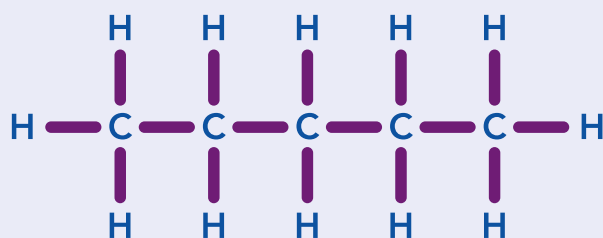
Oils are organic chemical compounds composed of saturated and unsaturated hydrocarbon chains. Saturated hydrocarbon chains are joined to each other by single bonds, this makes them quite unreactive.

Refined oil is mainly composed of saturated hydrocarbon chains (linear, branched or cyclic alkanes) but, occasionally, may also contain undesirable unsaturated alkenes (Figure 1).

Alkenes contain a carbon—carbon double bond which leaves the hydrocarbon unsaturated, making it more unstable. This should be avoided in oil to be used for tissue culture as oxidation of the hydrogen results in increasing levels of hydroperoxide, which is detrimental to embryos.

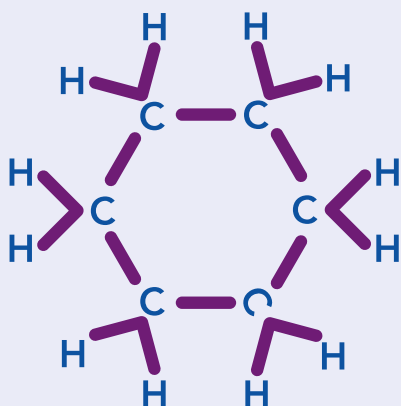


Saturated Hydrocarbon Chains



Alkanes

Cyclic Hydrocarbon



Naphthenic Hydrobonds - CycloAlkanes

Unsaturated Hydrocarbon Chains

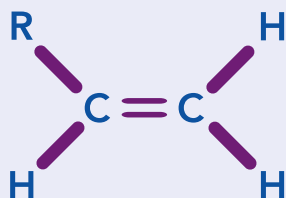


Figure 1: Hydrocarbon species comprising mineral oil

## 2. Purification of oils

During the purification process, double bonds within the alkenes present in the oil are saturated via hydrogenation to form alkanes. This renders the oil inert to oxidation.

Depending on the stringency of processing, mineral oils may be classified as either technical or food/medicinal grade; the latter meets higher specifications, as set by the USP and Ph Eur. Both our oils meet the higher specification as medical grade oils.

Medical grade mineral oils must be tasteless, odorless, inert, colorless liquids at room temperature. They should have no impact on pH and contain very low levels of any polycyclic aromatics, aromatic nitrogen, sulfur compounds, and unsaturated hydrocarbons. Therefore, medical grade mineral oils are ideally suited for use as a culture media overlay.

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ORIGIO Liquid Paraffin and SAGE Oil for Tissue Culture meet the requirements of the United States Pharmacopeia's (USP) formula for light mineral oil and the European Pharmacopoeia (Ph Eur) requirements for light paraffin. They have been widely used in ART laboratories for many years.

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## 3. Testing

It is important to test oil rigorously, and we undertake numerous tests throughout the manufacturing procedure. These include a combination of well-known quality control tests such as sperm survival, sterility, and MEA. However, we have also devised more specialized tests, including extended MEA (eMEA), and Atomic Fingerprinting tests which include Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance Spectroscopy (NMR), and Gas Chromatography coupled with Flame Ionization Detection (GC-FID). These analyses check the hydrocarbon saturation profile, double bonds and aromatic groups, and the presence of Volatile Organic Compounds (VOCs). We also test for the presence of peroxides (see Table 1).



## Tests

## Methods

## Parameters

### Atomic Fingerprinting Tests

A test for unexpected chemical features such as unsaturation, aromaticity or other functional groups based on FTIR and NMR Spectroscopy	Fourier Transform Infrared Spectroscopy (FTIR)	The FTIR spectrum shows spectral features consistent with a mixture of fully saturated hydrocarbons with a large linear alkane component
	Nuclear Magnetic Resonance (NMR) Spectroscopy	Data shows consistency with alkanes present in the oil No chemical shift attributable to double bonds, aromatic groups or other functional groups detected by NMR analysis
Identification of VOCs defined as compounds with boiling point $\leq 250^{\circ}\text{C}$	Gas Chromatography with Flame Ionization Detection (GC-FID)	No volatile components with boiling point $\leq 250^{\circ}\text{C}$

### Bioassay Testing

Mouse Embryo Assay (MEA)	Mouse embryo culture under ORIGIO Liquid Paraffin and SAGE Oil for Tissue Culture and blastocyst evaluation	Blastocyst formation rate at 96 h
Extended Mouse Embryo Assay (eMEA)	Mouse embryo culture under ORIGIO Liquid Paraffin and SAGE Oil for Tissue Culture and blastocyst evaluation	Blastocyst rate at 120 h
Endotoxin Test Ph Eur, USP	Limulus Amebocyte Lysate (LAL) test is recommended by international pharmacopeias as the method for detecting bacterial toxins	$<0.1$ EU/mL
Sterility Test Ph Eur, USP	Recommended test for bacterial growth	No evidence of growth after 14 days
Sperm Survival Test (SST)	Sperm survival after culture under oil	Sperm survival after incubation
Peroxide Value (POV) Test Ph Eur	Very sensitive POV measurements are required to pick up levels that are toxic to gametes and embryo	POV is expressed as milliequivalents of hydroperoxide per kilogram of oil (mEq/Kg)

Table 1

### 3.1 Atomic Fingerprinting tests

Atomic Fingerprinting is a comprehensive method that quantitatively analyzes the physicochemical properties of oil using a combination of several techniques.

It is a sensitive, direct analysis of oil composition that provides data on purity, consistency, and reliability. The tests assess possible variables in the oil; for example, batch-to-batch variability in composition and molecular weight. They also confirm that oils are comprised of saturated hydrocarbon chains with no detectable unsaturated hydrocarbon chains.

FTIR relies on the absorption and emission of infrared radiation at different wavelengths. Based on the established emission spectra of known chemical libraries, it can be used to identify and quantify various chemical functional groups, including saturated hydrocarbon species.

NMR spectroscopy relies upon the quantum mechanical magnetic properties of atomic nuclei and, similar to FTIR spectroscopy, can be used to identify and quantify saturated and unsaturated hydrocarbon species.

GC-FID relies upon the chromatographic separation of sample components, by virtue of their relative volatility, followed by ionization and further separation according to their molecular weight. Each component is identified using matching with an extensive computer database. Hence, GC-FID can detect any trace contaminants or molecular outliers and illustrates the distribution patterns of alkanes within the mineral oil.

We also undertake the testing of kinematic viscosity of our oils, which is a more relevant measure than absolute viscosity since it is determined by its specific gravity at 40°C. This is close to the temperature within an incubator when used as an oil overlay.

### 3.2 Peroxide testing

One measure of oil oxidation, the peroxide value (POV), is mainly based on the presence of hydroperoxide and is related to the level of molecular oxygen. Its measurement requires the use of very sensitive POV meters and is expressed as milliequivalents of hydroperoxide per kilogram of oil (mEq/Kg).

Peroxidation of unopened oil from some manufacturers has been observed to increase the POV to 0.12mEq/Kg and 0.21mEq/Kg 6 and 15 months after production, respectively<sup>2</sup>. Dose-dependent significant decreases in fertilization, cleavage, and blastocyst formation rates have been reported in murine gametes and embryos exposed to peroxidized oil, with POVs of 0.5mEq/Kg and 1.0mEq/Kg. This has proven detrimental to all embryos by day 3 and day 2 of culture, respectively<sup>2</sup>. Our test detects peroxide levels under 0.02 mEq/Kg and is therefore integral to reliable quality assurance.

### 3.3 Extended Mouse Embryo Assay (eMEA)

Historically, most tests of mineral oil quality have been based upon indirect assays of the oil's clinical performance, including the one-cell F1 hybrid mouse embryo assay (MEA). However, inbred mouse embryos are known to be more resilient than human embryos. Therefore, the standard MEA, based upon ≥80% blastocyst development following 96h of culture, may not be sensitive enough to detect low peroxide levels within oil that are, nevertheless, still detrimental to human embryo development<sup>3,4</sup>.

It has been suggested that sensitivity can be increased by extending the analysis, which will help determine the percentage of embryos at the expanded blastocyst stage (eMEA)<sup>4</sup>.



## 4. Oil washing

Washing oil can reduce levels of toxicity<sup>5</sup>. Oil can be washed with media, with or without HSA, or with water<sup>5</sup>. CooperSurgical manufactures both washed and unwashed oils, so you can use your preferred washing procedure.

## 5. The importance of correct storage

Once refined mineral oils undergo further processing, secure storage in isolation from sources of potentially contaminating volatile hydrocarbons is essential. Fully saturated mineral oils are relatively inert and protected from oxidation under normal conditions. Nevertheless, oxidation can occur following extended storage, under elevated temperatures or on exposure to sunlight or ultra-violet (UV) light. In such circumstances, peroxides could form in the oil and have the potential to cause damage to cell membranes via lipid peroxidation by free radicals<sup>2,5</sup>.

Although the POV of stored mineral oil should be negligible, it has been discovered to significantly increase within just six months of storage from some manufacturers<sup>2</sup>. Therefore, it is important to choose a mineral oil with a validated shelf-life, which is strictly adhered to, and store it in a refrigerator at 2–8°C away from sunlight and UV light.

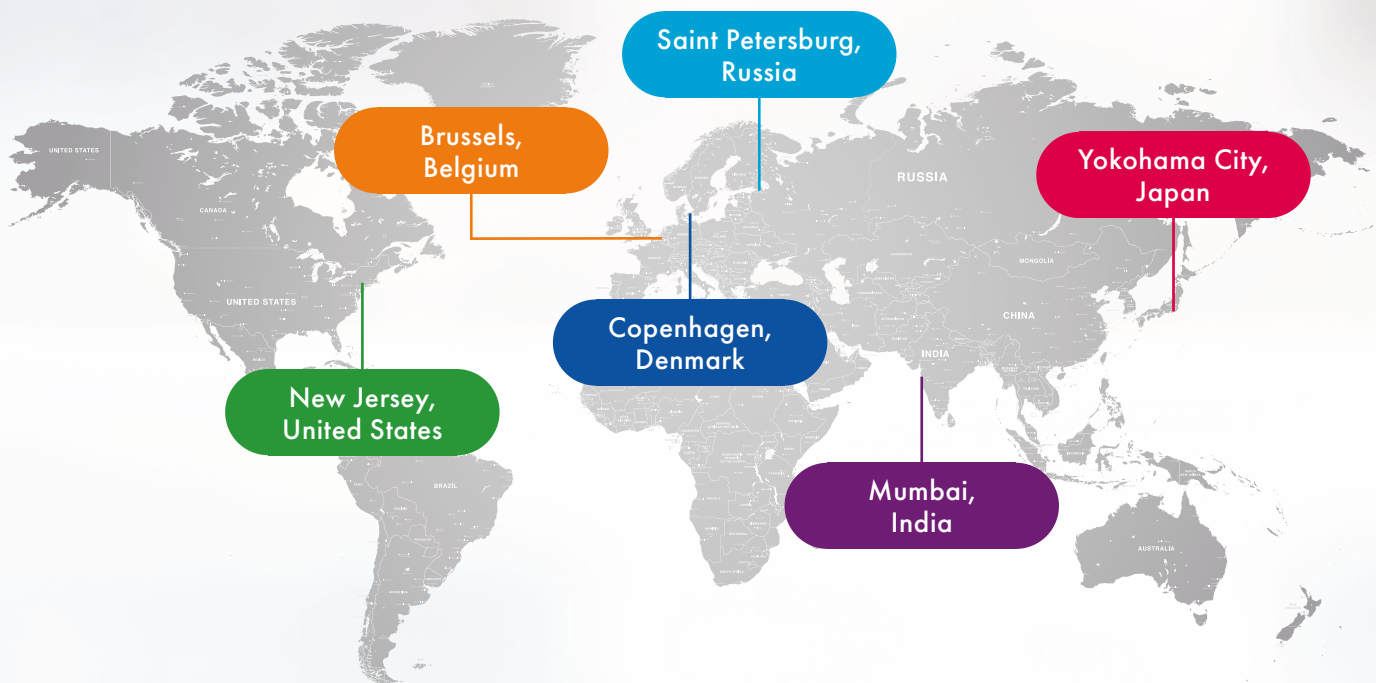
ORIGIO Liquid Paraffin and SAGE Oil for Tissue Culture are stored in non-reactive and sealed containers to avoid oxidation and contamination.



Train with CooperSurgical and optimize your performance,  
learn new skills and network with international peers

We invite customers and partners to learn new techniques and  
share best practices in our fully equipped laboratory.

At the ORIGIO Training Labs we provide evidence-based  
training by skilled, experienced embryologists which includes  
demonstrations and hands-on training in a comprehensive  
range of ART techniques and procedures.



## References

1. Brinster, RL (1963) A method for in vitro cultivation of mouse ova from two-cell to blastocyst. *Exp Cell Res* 32: 205-208.
2. Otsuki, J et al. (2007) Peroxidation of mineral oil used in droplet culture is detrimental to fertilization and embryo development. *Fertil Steril* 88: 741-743.
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4. Ainsworth, AJ et al. (2017) Improved detection of mineral oil toxicity using an extended mouse embryo assay. *J Assist Reprod Genet* 34: 391-397.
5. Morbeck, DE et al. (2010) Washing mineral oil reduces contaminants and embryotoxicity. *Fertil Steril* 94: 2747-2752.