

Influence of Storage Conditions on Indocyanine Green Stability and Fluorescence Intensity

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Introduction

Indocyanine green (ICG) is a near-infrared fluorescent dye used in several medical applications. Once reconstituted ICG begins to degrade, as such the manufacturer's recommendation is to use within 6 hours to ensure the quality of fluorescence intensity (FI). In veterinary medicine, small quantities of ICG (0.1mL or 1/100th of a bottle) are used resulting in waste due to its short *shelf* life and limits accessibility due to the high bottle cost (~\$450 CAD).

Methods

Preparation and Storage

- ICG reconstituted with (provided) sterile water to standard 2.5mg/mL concentration
- 0.1mL of ICG transferred to 1mL syringes and capped with a 22G needle
- Aliquots stored at room temperature, 4°C, -20°C and -80°C

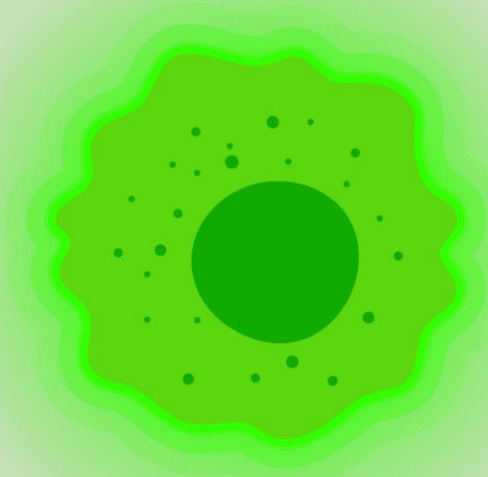
Cell Line Preparation

- Human ovarian epithelial cancer cells (Coav-3) to replicate a tissue monolayer *in vitro*
- Normal human ovarian surface epithelial as a control

Fluorescence Evaluation

- Cells treated with ICG and evaluated at 1, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120 hours and 1, 2, 3, 4, 6, 8, 14 and 29 weeks
- FI evaluated using the Odyssey DLx imager via a black 96-well microplate

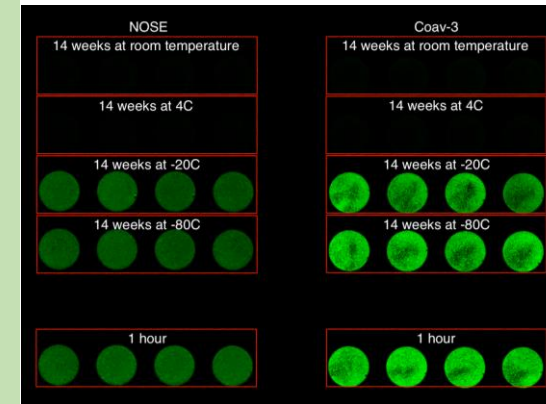
Reconstituted ICG may have an extended shelf life when stored at freezing conditions (-20°C and -80°C).



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Preliminary Results

- Increased fluorescence seen in human **cancer cells** compared to **normal cells**
- No perceivable fluorescence in the NIRF window (650-775nm) after 5 days when stored at room temperature
- When stored in the freezer (-20 and -80°C), ICG has a similar fluorescence intensity to the initial preparation
- Subjective changes in colour of the solution after 5 days when stored at room temperature



Impact

By establishing best practices for the storage of ICG for use in veterinary clinical applications, we have the potential to:

- **Reduce product waste**
- **Decrease overall cost**
- **Increase accessibility**
- **Improve patient outcomes**

