

Submission ID #: 69240

Scriptwriter Name: Deepthi Das

Project Page Link: https://review.jove.com/files_upload.php?src=21118248

Title: Formulation of Vitamin A Tracer Doses for Administration to Human Subjects Using Retinol Isotope Dilution Technique

Authors and Affiliations: Georg Lietz¹, Anthony Oxley^{1,2}

¹Human Nutrition and Exercise Research Centre, Newcastle University

²Fera Science Ltd.

Corresponding Author:

Georg Lietz Georg.lietz@ncl.ac.uk

Email Addresses for All Authors:

anthony.oxley@fera.co.uk

Georg.lietz@ncl.ac.uk

Author Questionnaire

1. **Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**
3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**
If **Yes**, how far apart are the locations? **500m**
4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 13

Number of Shots: 42

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

Videographer's NOTE: For the interview audio, one channel was captured at a lower level than the other to help accommodate any sound peaking. I recommend using only the louder channel as mono.

~~**REQUIRED:** Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Georg Lietz:** The research provides guidance on preparing vitamin A isotope tracers for retinol isotope dilution studies to assess vitamin A stores.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~**Describe the research gap or limitation in existing methods and how this protocol addresses them.**~~

- 1.2. **Georg Lietz:** This study provides validated, practical food-safe tracer preparation guideline for accurate preparation, dosing and quality control of vitamin A isotopes.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~**What does this protocol allow researchers to study or measure?**~~

- 1.3. **Georg Lietz:** The protocol allows researchers to measure total body and liver vitamin A stores in humans using retinol isotope dilution.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~**Describe the most important consideration or challenge when performing this protocol.**~~

- 1.4. **Georg Lietz:** Ensuring accurate, food-safe tracer preparation while preventing degradation and delivering precise, reproducible oral doses is a key challenge.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Georg Lietz**: Subsequent analyses include plasma isotope ratio measurement by LC-MS/MS, GC-IRMS or GC-MS to calculate total body vitamin A stores.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

1.6 **Georg Lietz, Newcastle University**: (authors will present their testimonial statements live)

1.6.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

How does the video format of your article help you to share your work with the broader scientific community?

1.7 **Georg Lietz, Newcastle University** : (authors will present their testimonial statements live)

1.7.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

The study was approved by the National Research Ethics Service, North-East Sunderland Committee, the Research Ethics Board of the University of the Philippines Manila and the Institutional Review Board at the University of California

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Determination of Tracer Dose and Oil Volume for Retinol Isotope Dilution

Demonstrator: Georg Lietz

~~2.1. To begin, obtain and review the Study 1 documents [1]. Use the inclusion and exclusion criteria presented to shortlist eligible individuals [2].~~

~~2.1.1. WIDE: Talent reviewing study 1 eligibility documents.~~

~~2.1.2. TEXT ON A PLAIN BACKGROUND:~~

~~Study 1~~

~~Include healthy adults:~~

~~— both male and female~~

~~— age: 18–45 y~~

~~Exclude adults:~~

~~— who are pregnant or are smokers~~

~~— who have chronic conditions: hypertension, diabetes, liver, kidney, gastrointestinal, or lipid metabolic disorders~~

~~— whose BMI > 30~~

~~— who used supplements (multivitamins containing vitamins A, C, E, or beta-carotene) in the past 3 months~~

~~2.2. Obtain and review the Study 2 documents [1]. Use the inclusion and exclusion criteria presented to shortlist eligible individuals [2].~~

~~2.2.1. Talent reviewing study 2 eligibility documents.~~

~~2.2.2. TEXT ON A PLAIN BACKGROUND:~~

~~Study 2~~

~~Include children:~~

~~— age: 12–18 months~~

~~— reside in the study area~~

~~Exclude children:~~

~~— who did not receive high-dose vitamin A supplementation~~

~~- who live in areas not exposed to vitamin A-fortified foods in local markets~~

2.3. To prepare stable isotope tracer doses for retinol isotope dilution studies, first determine the total tracer requirement [1]. Select vitamin A stable isotopes with greater than 95 percent all-trans purity [2]. To calculate the tracer dose level in retinol

equivalents, determine the total number of subjects based on power calculations and expected attrition [3]. Multiply the dose level by the final number of subjects and account for pipetting losses to determine the total tracer requirement [4].

2.1.1 WIDE: Talent reviewing the study document **NOTE: Use 2.1.1 here**

2.3.1. SCREEN: 69240-2.3.1 *Video editor: Crop out the buchem BV header and address, number on the top.*

2.3.2. SCREEN: JoVE-69240-2.3.2-to-2.3.5.mp4 00:02-00:12.

2.3.3. SCREEN: JoVE-69240-2.3.2-to-2.3.5.mp4 00:42-01:01.

2.4. Select the dose volume based on the planned dosing method, such as administration with a positive displacement pipette [1]. Using the selected dose volume, calculate the required tracer concentration in sunflower oil [2]. Then calculate the total volume of sunflower oil required for the study [3] and convert this volume to the corresponding oil weight using the relative density of sunflower oil [4].

2.4.1. SCREEN: JoVE-69240-2.4.1-to-2.4.3.mp4. 00:19-00:23 and 00:35-00:38

2.4.2. SCREEN: JoVE-69240-2.4.1-to-2.4.3.mp4. 00:20-01:29 .

2.4.3. SCREEN: JoVE-69240-2.4.4-to-2.4.5.mp4 00:22-00:33.

2.4.4. SCREEN: JoVE-69240-2.4.4-to-2.4.5.mp4 00:54-01:02.

3. Preparation of Stable Isotope-Labeled Tracer in Dedicated Areas for Food Preparation

3.1. Carefully open the lid of the amber bottle containing the predetermined total amount of stable isotope tracer, place it on an analytical balance, and tare it to zero [1]. Use a sterile 10-milliliter syringe to add sunflower oil dropwise until the desired weight is reached with an accuracy of plus or minus 0.1 grams [2].

3.1.1. Talent opening the amber bottle containing the stable isotope tracer, placing the bottle on an analytical balance and taring the balance to zero. *Show the added shot with different bottles using split screen when the VO says "Carefully open the lid of the amber bottle containing ' .* **Author's NOTE:** Shot 3.1.1: We have asked the videographer to take an additional shot of 2 different bottle sizes to emphasize the importance of asking the manufacturer to deliver the doses in an adequate bottle size to allow direct addition of oil to the tracer dose. **Videographer's NOTE:** The shot marked 3.1. OPT was filmed to show the size comparison of the different supplied bottles.

3.1.2. Talent adding oil dropwise into the amber bottle with a sterile 10-mL syringe.

3.2. Place the bottle in a sonicating water bath. Add water until the level is above the oil in the bottle but below the bottle lid [1]. Sonicate the bottle at 14 Watts per liter, 44 kilohertz for 15 minutes. Ensure that the temperature of the sonicating water bath does

not exceed 50 degrees Celsius [2]. Then, remove the bottle, mix carefully by hand, and re-sonicate for 15 minutes [3].

- 3.2.1. Talent placing the bottle in the sonicating water bath and filling the bath to the appropriate level.
- ~~3.2.2.~~ Talent keeping the bottle for sonication. *Videographer: Please capture the settings showing 14 W/L, 44 kHz and 50 °C. If the settings show 50 °C, crop it out or blurr it. The temperature should be below 50 °C and not 50 °C*
- 3.2.3. Talent removing the bottle, mixing it by hand, returning it to the sonicator, and switching it on.

3.3. Place the bottle in a heated water bath at 50 degrees Celsius for 30 minutes to completely dissolve the microcrystals [1]. Check a 20-microliter oil sample for microcrystals under a light microscope at 200x magnification [2]. If microcrystals remain, repeat the sonication and heating steps until the tracer fully dissolves [3]. Use a positive-displacement pipette to aliquot the total oil preparation into 2 to 4 sterile glass amber vials [4].

- 3.3.1. Talent placing the bottle in the heated water bath.
- 3.3.2. Talent checking the oil sample under a light microscope. *Video editor: Please add the image labeled 'JoVE-69240-3.3.2.tif' as an inset.*
- 3.3.3. Talent returning the bottle for repeated sonication and heating.
- 3.3.4. Talent aliquoting the oil preparation into sterile glass amber vials.

Videographer's NOTE: The shot marked 3.3 OPT was captured to display the specific pipette required for the process.

Videographer's NOTE: The shot marked 3.3 OPT 2 was captured to display the measurement number of the pipette required for the process.

Videographer's NOTE: Shot 3.3.4 was captured correctly as 3.3.4 take 4.

3.4. Take two 2-milliliter amber vials [1]. Add 200 microliters of pure vegetable oil to one vial and 200 to 350 microliters of the stable isotope dose to the second vial [2].

- 3.4.1. Talent adding 200 µL of pure vegetable oil in 2mL amber vial

Videographer's NOTE: Shot 3.4.1 was accidentally marked as 3.3.4.

Videographer's NOTE: Shot 3.4.2 was accidentally marked as 3.4.1.

- 3.4.2. Talent adding 200 - 350 µL of the stable isotope dose in 2mL amber vial

3.5. Blanket the vials with argon to minimize oxidation [1]. Close the vials tightly and store them in a cryobox labeled with isotope concentration, dose volume, preparation date, and project name at minus 20 degrees Celsius [2].

- 3.5.1. Talent purging the amber vials with argon.
- 3.5.2. Talent closing the vials and placing the labeled cryobox into a minus 20 degrees Celsius food freezer.

4. Determination of Stable Isotope Tracer Concentration in the Laboratory

- 4.1. Select the dilution based on the tracer dose concentration [1]. Take three volumetric flasks of the required volumes and fill each flask with n-hexane to approximately 1 centimeter below the calibration line [2-TXT]. Next, dispense the appropriate volume of the stable isotope tracer dose into the correct volumetric flask [3]. Then fill the flask up to the mark with additional hexane [4]. Invert the flask 10 times to mix the oil with the hexane [5].

- 4.1.1. TEXT ON A PLAIN BACKGROUND

- Dose ≤ 0.5 mg retinol equivalents

- Measure 100 μL stable isotope dose; dilute in 50 mL volumetric flask

- Dose > 0.5 to ≤ 2 mg retinol equivalents

- Measure 50 μL stable isotope dose; dilute in 50 mL volumetric flask

- Dose > 2 mg retinol equivalents

- Measure 50 μL stable isotope dose; dilute in 100 mL volumetric flask

- 4.1.2. Talent taking 3 appropriately sized volumetric flasks and filling each one with n-hexane to 1 centimeter below the calibration line. **TXT: 1mg retinol equivalent dose was chosen for demonstration**

- 4.1.3. Talent dispensing the appropriate tracer volume into the correct volumetric flask.

- 4.1.4. Talent ~~dispensing the oil into hexane and~~ making the flask up to volume with additional hexane. **NOTE: Do not show adding oil if that was shot. Oil and tracer were already mixed before this step**
Videographer's NOTE: Shot 4.1.4 required an additional shot that was captured and marked as shot 4.1.4 ADD.

- 4.1.5. Talent inverting the flask 10 times to mix.

- 4.2. Pipette the same volume of vegetable oil as the stable isotope tracer mix calculated previously [1] into the appropriate flask containing hexane to prepare the spectrophotometric blank [2]. Invert the flask 10 times to mix the oil with hexane [3].

- 4.2.1. Talent pipetting the appropriate volume of vegetable oil

- 4.2.2. Talent dispensing the vegetable oil into hexane to prepare the spectrophotometric blank.

- ~~4.2.3. Talent adjusting the blank flask to the matching dilution volume.~~

- 4.2.4. Talent inverting the volumetric flask multiple times to mix the content

- 4.3. Use quartz cuvettes cleaned with hexane to measure the value of vegetable oil as the control at 325 nanometers with a spectrophotometer [1]. Add n-hexane to rinse the quartz cuvette 3 times between each measurement [2]. Then measure the stable-isotope tracer concentration [3]. After measuring all the samples, clean the cuvettes carefully with pure n-hexane [4].
 - 4.3.1. Talent measuring the value of vegetable oil as the control in a quartz cuvette.
 - 4.3.2. Talent rinsing the quartz cuvette 3 times with n-hexane between measurements.
 - 4.3.3. Talent measuring the stable isotope tracer concentration in a quartz cuvette with a spectrophotometer ~~using vegetable oil in hexane as the blank.~~
Videographer: Please take a shot of the screen showing the measurement along with this.
 - 4.3.4. Talent cleaning the cuvettes carefully with pure n-hexane after completing all sample measurements.
- 4.4. Use the isotope-specific molar extinction coefficient with the Beer-Lambert law to calculate the stable isotope tracer concentration from the average absorbance unit readings [1]. Calculate the volume of the prepared stable-isotope tracer required per dose [2].
 - 4.4.1. SCREEN: JoVE-69240-4.4.1.mp4 00:25-00:26 and 00:59-01:02 and 01:17-01:19 and 01:40-01:41.
 - 4.4.2. SCREEN: JoVE-69240-4.4.2.mp4 00:37-00:38 and 01:03-01:06.

Results

5. Results

- 5.1. The obtained total amount of retinol equivalents needed was 55.2 milligram [1], which corresponded to 63 milligram [$^{13}\text{C}_{10}$] (*Carbon-thirteen C-ten*) retinyl acetate [2]
- 5.1.1. LAB MEDIA: Table 2. *Video editor: Highlight the full section that reads "Total amount of RE required: = 55.2 mg RE"*
- 5.1.2. LAB MEDIA: Table 2. *Video editor: Highlight the full section that reads "Total amount of [$^{13}\text{C}_{10}$] retinyl acetate required: = 63.0 mg [$^{13}\text{C}_{10}$] retinyl acetate"*
- 5.2. The concentration of [$^{13}\text{C}_{10}$] retinyl acetate in 1 milliliter of sunflower oil was calculated to be 3.04 milligrams per milliliter [1] The total volume of sunflower oil needed [2] and the weight of sunflower oil needed were determined [3].
- 5.2.1. LAB MEDIA: Table 2. *Video editor: Highlight the full section that reads "Dose concentration of [$^{13}\text{C}_{10}$] retinyl acetate in oil: = 3.04 mg/ml"*
- 5.2.2. LAB MEDIA: Table 2. *Video editor: Highlight the full section that reads " Total volume of sunflower oil required: = 20.7 ml oil"*
- 5.2.3. LAB MEDIA: Table 2. *Video editor: Highlight the full section that reads "Weight of sunflower oil needed: = 19.0 g sunflower oil"*

Analytical balance

Pronunciation link: https://www.merriam-webster.com/dictionary/analytical

IPA: /ˌænəˈlɪtɪkəl/

Phonetic Spelling: an·uh·lit·ih·kuhl

Argon

Pronunciation link: https://www.merriam-webster.com/dictionary/argon

IPA: /ˈär-ˌgän/

Phonetic Spelling: ar·gon

Attrition

Pronunciation link: https://www.merriam-webster.com/dictionary/attrition

IPA: /əˈtrɪʃən/

Phonetic Spelling: uh·trish·uhn

Beer-Lambert

Pronunciation link: No confirmed link found

IPA: /bɪr 'læmbərt/

Phonetic Spelling: beer·lam·bert

Calibration

Pronunciation link: https://www.merriam-webster.com/dictionary/calibration

IPA: /ˌkæl.əˈbreɪ.ʃən/

Phonetic Spelling: kal·uh·bray·shuhn

Cryobox

Pronunciation link: No confirmed link found

IPA: /ˈkraɪ.ɒʊ̯.bɑːks/

Phonetic Spelling: kry·oh·boks

Cuvette

Pronunciation link: https://www.merriam-webster.com/dictionary/cuvette

IPA: /kjuːˈvet/

Phonetic Spelling: kyoo·vet

Extinction coefficient

Pronunciation link: https://www.merriam-webster.com/dictionary/extinction

IPA: /ɪkˈstɪŋkʃən/

Phonetic Spelling: ik·sting·shuhn

Gastrointestinal

Pronunciation link: https://www.merriam-webster.com/dictionary/gastrointestinal

IPA: /ˌgæs.troʊ.ɪnˈtes.tə.nəl/

Phonetic Spelling: gas·troh·in·tes·tuh·nuhl

Hexane

Pronunciation link: https://www.merriam-webster.com/dictionary/hexane

IPA: /ˈhekˌseɪn/

Phonetic Spelling: hek·sayn

Hypertension

Pronunciation link: https://www.merriam-webster.com/dictionary/hypertension
IPA: /ˌhaɪ.pəˈten.ʃən/
Phonetic Spelling: hy·per·ten·shuhn

Isotope

Pronunciation link: https://www.merriam-webster.com/dictionary/isotope
IPA: /ˈaɪ.səˌtoʊp/
Phonetic Spelling: eye·suh·tohp

Kilohertz

Pronunciation link: https://www.merriam-webster.com/dictionary/kilohertz
IPA: /ˈkɪl.əˌhɜːts/
Phonetic Spelling: kil·uh·hurts

Magnification

Pronunciation link: https://www.merriam-webster.com/dictionary/magnification
IPA: /ˌmæɡ.nə.fəˈkeɪ.ʃən/
Phonetic Spelling: mag·nuh·fuh·kay·shuhn

Microcrystals

Pronunciation link: https://www.merriam-webster.com/dictionary/microcrystal
IPA: /ˌmaɪ.kroʊˈkrɪs.təl/
Phonetic Spelling: my·kroh·kris·tuhl

Microliter

Pronunciation link: https://www.merriam-webster.com/dictionary/microliter
IPA: /ˌmaɪ.kroʊˌliː.tər/
Phonetic Spelling: my·kroh·lee·ter

Molar

Pronunciation link: https://www.merriam-webster.com/dictionary/molar

IPA: /'moʊ.lə/

Phonetic Spelling: moh·ler

Oxidation

Pronunciation link: https://www.merriam-webster.com/dictionary/oxidation

IPA: /ˌɑːk.sə'deɪ.ʃən/

Phonetic Spelling: ok·suh·day·shuhn

Pipetting

Pronunciation link: https://www.merriam-webster.com/dictionary/pipette

IPA: /paɪ'petɪŋ/

Phonetic Spelling: pie·pet·ing

Retinol

Pronunciation link: https://www.merriam-webster.com/dictionary/retinol

IPA: /'ret.ən.ɔːl/

Phonetic Spelling: ret·ih·nawl

Retinyl

Pronunciation link: https://www.merriam-webster.com/dictionary/retinyl

IPA: /'ret.ən.ɪl/

Phonetic Spelling: ret·ih·nil

Sonicate

Pronunciation link: https://www.merriam-webster.com/dictionary/sonicate

IPA: /'sɑː.nə.keɪt/

Phonetic Spelling: sah·nuh·kayt

Spectrophotometer

Pronunciation link: https://www.merriam-webster.com/dictionary/spectrophotometer

IPA: /ˌspek.troʊ.fə'tɑː.mə.tə/

Phonetic Spelling: spek·troh·fuh·tom·uh·ter

Spectrophotometric

Pronunciation link: https://www.merriam-webster.com/dictionary/spectrophotometric

IPA: /ˌspek.troʊˈfoʊ.tə.məˈtrɪk/

Phonetic Spelling: spek·troh·foh·tuh·met·rik

Supplementation

Pronunciation link: https://www.merriam-webster.com/dictionary/supplementation

IPA: /ˌsʌp.lə.mənˈteɪ.jən/

Phonetic Spelling: sup·luh·men·tay·shuhn

Volumetric

Pronunciation link: https://www.merriam-webster.com/dictionary/volumetric

IPA: /ˌvɒː.ljəˈmet.rɪk/

Phonetic Spelling: vol·yuh·met·rik

Quartz

Pronunciation link: https://www.merriam-webster.com/dictionary/quartz

IPA: /ˈkwɔrts/

Phonetic Spelling: kworts