Compounding at Scale

Protocol last updated (1/21/26)
This document is a work in progress.

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1. Introduction

Pharmaceutical compounding at scale requires stringent environmental controls, standardized protocols, and robust QC measures to ensure sterility and efficacy. With the western descent into fascism, decentralized compounding is quickly becoming critical. In this guide we (Aurelia Labs) detail a scalable compounding process for HRT, emphasizing particulate mitigation, and cost optimization. The methodology bridges gaps between (often unsafe) clandestine practices and regulated production, offering a template for reproducible, small-scale pharmaceutical manufacturing.

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2. Materials and Methods

2.1. Equipment and Materials

Personal Protective Equipment (PPE)

- **KN95 masks** – Buy a bulk case; replace after each use.
- **UVC safety glasses** – Must block 254nm UV light; never substitute with regular sunglasses.
- **100% cotton lab coats** – Wash separately using unscented detergent; no fabric softener.
- **Disposable booties (shoe covers)** – Long, elastic-cuffed preferred.
- **Splash goggles** – Wear when handling Alconox (detergent) or hydrochloric acid (HCl).
- **PVC-coated gloves** – *Critical:* Never use nitrile gloves with HCl (they degrade).
- **Respirator with acid gas cartridges** – 3M P100 cartridges recommended.
- **Sterile gloves** – For handling sterilized items; discard after single use.
- **Nitrile gloves (non-sterile, powder-**

Glassware & Tools

(All borosilicate glass unless noted)

- **Media bottles (2L)** – Substitute with Erlenmeyer flasks if needed.
- **Beakers**: 10mL, 50mL, 100mL, 250mL, 500mL.
- **Funnels**: 20mm, 50mm, 150mm, 200mm.
- **Graduated cylinders (50mL)** – Store pipettes/stir rods.
- **Stir rods (6-8”, 3mm thickness)**.
- **Serological pipettes & controller**: Four or more 10mL, two or more 50mL.
- **Vacuum filtration apparatus** – 500mL, 1L, 2L sizes; PTFE membranes (0.22µm).
- **Lab spatulas** – Assorted sizes for powders.
- **Surgical trays (3x)** – 6”x8”x2” with lids; use autoclave tape.
- **Volumetric flasks** – For precise API dilutions (e.g., 100mL, 250mL).

Key note for amateurs: Always test equipment, label all chemicals clearly, store acids separately, never reuse disposable PPE or filters, refer to MSDS (Material Safety Data Sheets) for all chemicals.

free) – Buy a case.

- **Hairnets/ties** – Prevent contamination from loose hair.

Core Equipment

- **Laminar flow hood** – Buy a Fan Filter Unit (FFU) to build yourself; horizontal airflow preferred.
- **Sonicator (30L+)** – For cleaning glassware; use with 1% Alconox solution.
- **Autoclave (20L+)** – Sterilizes equipment. *Alternative:* 15psi pressure cooker (validate cycles with autoclave tape).
- **UV sterilization cabinet** – Ensure 254nm UVC bulbs; test with fluorescent cards.
- **Mechanical vial capper** – Manual ones cost 100−200; electric versions are pricier.
- **Medical vacuum pump (-85kPa)** – For filtration; pair with **clear silicone tubing** (avoid latex).
- **Hot plate with magnetic stirrer** – 200W minimum; include Teflon-coated stir bars.
- **Infrared thermometer** – Monitor solution temperatures.
- **Microgram balance** – Accuracy ±0.0001mg for measuring APIs.
- **Milligram scale** – Accuracy ±1mg for larger quantities.
- **Acid wash bin** – Deep, chemical-

Consumables

- **Distilled water** – For rinsing and solutions.
- **APIs** – Estradiol enanthate, testosterone, etc.
- **Inactive ingredients**: MCT oil, benzyl alcohol (preservative), benzyl benzoate.
- **0.22µm PTFE filters** – For sterilizing solutions.
- **Kim wipes/paper towels** – Wipe spills; lint-free.
- **Sterilization pouches/rolls** – Autoclave-safe bags.
- **10% HCl, Alconox, 10% sodium hypochlorite** – For cleaning.
- **Vials/stoppers/caps** – Autoclave vials; sterilize stoppers with UVC.
- **Syringes (1mL, 5mL) + blunt needles** – Transfer solutions safely.
- **Labels + waterproof markers** – Track batch numbers/dates.
- **Baking soda** – Neutralize acid spills.

Biological QC

- **Incubator** – Use a food dehydrator (adjustable to 23°C).
- **Inoculation loops** – Disposable or replaceable tips.
- **Petri dishes + agar** – Sterilized, with light malt extract/dextrose/potato flakes for microbial/fungal testing.
- **Media bottles** – Sterilize culture media before use.

resistant container for HCl rinses.

- **pH strips** – Check pH of cleaning solutions.
- **Timer** – Track sterilization/cleaning cycles.
- **Infrared sterilizer** – Stertilizes small tools

Particulate QC

- **Darkfield microscope** – 4x, 10x, 40x objectives required.
- **Ocular micrometer** – Calibrate with stage micrometer slide.
- **Microscope slides/coverslips** – Pre-cleaned.

Safety & Miscellaneous

- **Fire extinguisher** – Class B (for chemical fires).
- **First aid kit** – Include eyewash and acid burn supplies.
- **Hazardous waste containers** – Label for HCl, Alconox, etc.
- **Logbook** – Record batch details, QC results, and equipment checks.

2.2. Environmental Specifications

Cleanroom Standards

The cleanroom must meet ISO Class 6 particulate limits (Table 1) and at minimum ISO Class 6 air handling (60 air changes/hour via H14 HEPA filtration).[1] Stainless steel work surfaces and borosilicate glassware minimize contamination. A UVC sterilization cabinet (254 nm) is essential. A reverse osmosis water system & sink with hazardous waste diversion is recommended.

Table 1: ISO Class 6 Cleanroom Particulate Limits

Particle Size	Max. Particles/m^3
≥0.1 µm	1,000,000
≥0.2 µm	237,000
≥0.3 µm	102,000
≥0.5 µm	8,320
≥5 µm	293

1 Chaudhari, Gaurav A., and Dr. Suhas H. Sarje. Clean Room Classification for Pharmaceutical Industry. International Journal of Engineering and Technical Research (IJETR) 3, no. 4 (April 2015): 1. ISSN 2321-0869.

Air Handling & Filtration

The DIY cleanroom environment relies on a multi-layered approach to particulate control. Central to air quality management is the use of H14-grade HEPA filters, which achieve 99.995% efficiency at capturing particles ≥0.3 µm. Airflow is engineered to recirculate through the building, minimizing operational costs while maintaining ISO Class 6 air change standards. A heat pump positioned outside the cleanroom regulates temperature.

An airlock serves as a transitional zone for storing PPE and lab attire. A refrigerator is used for short-term preservation of oils and active pharmaceutical ingredients (APIs). We recommend an additional medical-grade freezer (-20°C) for long-term stability of the sensitive inputs. Work surfaces are constructed from NSF-certified stainless steel to resist corrosion, and all tools are restricted to steel or borosilicate glass to avoid microplastic shedding or other contaminants.

You're unlikely to achieve the ISO 6 standard uniformly across the room, but it's very achievable on the compounding side of the room (flow hood(s) + intake).

Laminar Flow Hoods

Laminar flow hoods are central to maintaining sterility during compounding. Horizontal airflow configurations are preferred, as they minimize turbulence over work surfaces and reduce the risk of particulate contamination. Vertical airflow is reserved for scenarios involving biological hazards, such as live cultures, where operator protection takes precedence. A minimum working area of 2' x 4' is required, though an 8' x 2' layout optimizes efficiency for high-volume batches. To reduce costs, a DIY hood can be constructed using a commercially available Fan Filter Unit (FFU).

Safety Systems

A 254nm UVC sterilization cabinet is employed to sterilize stoppers, caps, and small tools. Prior to use, the UVC bulb's wavelength is verified using a fluorescent test card to ensure germicidal and sporocidal efficacy. We recommend the placement of a Class B fire extinguisher (for chemical fires) within immediate reach of the compounding area. Hazardous waste containers should be clearly labeled (e.g., "Acid Waste") to prevent cross-contamination during disposal.

Attire & Cleaning

Operators wear 100% cotton lab coats to avoid shedding microplastics, which is common in synthetic cleanroom suits. Lab coats are laundered separately using unscented detergent, with washing machines first run empty to purge residual particles. Fabric softeners—which leave plastic residues—are strictly avoided. Cleaned coats are transported in airtight bags to prevent environmental contamination. Nitrile gloves, hairnets, booties, and KN95 masks are worn continuously.

2.3. Compounding Protocol

Pre-Compounding Setup

The cleanroom environment is maintained at 18–24°C and <60% humidity. Surfaces are sanitized with 10% sodium hypochlorite and 70% isopropyl alcohol, followed by particle count verification.

Equipment Prep

Ensure that milligram and gram scales are calibrated. Stage verified APIs, inactives, and glassware (cross-check lot numbers against GCMS records). Stoppers and caps should be staged in surgical trays. Stage equipment toward the flow hood in order of sterility and then priority. Non-sterile items must never enter the sterile field.

PPE Prep

Ensure that operators are wearing appropriate PPE including sterile gloves. Sterile gloves *never* leave the laminar flow.

Glassware & Equipment Sterilization

1. **Sonicator Cleaning**:
    - Fill sonicator with distilled water heated to 50°C. Add 10g/L Alconox (1% w/v solution).
    - Sonicate for 30 minutes:
        - Vacuum filtration apparatus, vial capper, graduated cylinders, beakers, funnels, pipettes, media bottles, surgical trays, hemostats, magnetic stir bars, glass stir bars (for pouring), vials, stoppers, caps.
    - Rinse thoroughly with distilled water.
2. **Filtered Water Prep**:
    - Filter 2L distilled water through a 0.22µm hydrophilic membrane under vacuum.
    - Decant into media bottles.
2. **Acid Wash**:
    - For new glassware and hard plastic labware and periodically in between batches. *Do NOT clean metal lab ware in HCl or items with metal components.*

- Transfer 10% HCl to all glassware; seal vessel or cover with parafilm to contain fumes. Agitate to remove organic residue. Incubate in glassware overnight.
- Rinse again 2-3x with distilled water; check pH to ensure neutrality.
- *Do NOT acid wash vials.*

3. **Second Sonication**:
    - Repeat sonication for 15 minutes.
    - Rinse sonicated items with filtered (0.22µm) water.

4. **Autoclaving**:
    - Clean autoclave interior/exterior with isopropyl alcohol and 10% NaOCl.
    - Autoclave glassware, instruments, and vials at 15 psi for 45 minutes.
    - *Do NOT autoclave stoppers/caps* – place on sterile trays under the hood.
5. **UVC Sterilization for Stoppers/Caps**:
    - Ensure caps and stoppers are completely dry.
    - Expose stoppers and caps (inside-up) to 200J/m3 in a surgical tray, cover, and then hold under ozone for 10 minutes with 3,000~4,000J/m3 of 254nm UVC.[2]
    - If UVC cabinet is airtight:
        - Hold stoppers and caps under ozone until needed.[3]
    - Otherwise:
        - Immediately stage (inside-down) under laminar flow.

Sterile Field Preparation

1. Clean the work surface with 70% isopropyl alcohol and 10% NaOCl.
2. Lay down the field.
    - *We prefer sterile pads, but a sterile field comes with every pair of sterile gloves.*

2 Xue, W., Macleod, J., & Blaxland, J. (2023). The use of ozone technology to control microorganism growth, enhance food safety and extend shelf life: A promising food decontamination technology. *Foods, 12*(4), 814. https://doi.org/10.3390/foods12040814.

3 *Note:* 50J/m3 is a sufficient dose to disintegrate the cell wall of nearly all microorganisms. 3,000~4,000J/m3 produces sufficient ozone to kill spores.

Solution Preparation

1. **Flush Formulation:**
    - Prepare ~250mL flush solution (prepared without API) to prime glassware and dissolve remaining hydrophobic contaminants. For 250mL filter and receiving vessel flush:
        - Add 4g/3.84mL benzyl alcohol to a beaker under laminar flow.
        - Add112g/100.2mL benzyl benzoate.
        - Measuring volumetrically, add ~134.4g/146mL MCT oil.
        - Use a magnetic stirrer (prefer at least 200W) for uniform mixing.
2. **Flush Vacuum Filtration**:
    - Filter flush solution through 0.22µm PTFE membrane under vacuum.
    - Swirl solution in receiving vessel to contact and coat glassware.
    - Discard the flush solution after glassware is primed.
2. **API Formulation**:
    - For 500mL of 20mg/mL estradiol enanthate (EEn):
        - Add 10 grams EEn to 112g/100.2mL benzyl benzoate under laminar flow.
        - Add 10g/9.6mL benzyl alcohol (preservative).
        - Measuring volumetrically, add ~354g/390mL MCT oil.
        - Use a magnetic stirrer (prefer at least 200W) for uniform mixing.
3. **API Vacuum Filtration**:
    - Filter solution through 0.22µm PTFE membrane under vacuum.

Vial Filling & Capping

1. **Aseptic Technique**:
    - Hold stoppers parallel to vials to minimize airborne contamination.
2. **Filling Process**:
    - Use a 10mL serological pipette controller to dispense solution into vials.
    - Immediately seal each vial with a sterilized stopper.
3. **Mechanical Capping**:
    - Secure caps using a mechanical vial capper.

- Inspect seals under back-illumination for particulates/defects.

Terminal Sterilization

1. **Autoclave Vials:**
   - 121C, 15psi for 30 minutes
2. **Leak Test**:
   - Invert vials for 1 hour; discard any showing leakage.

2.4 Quality Control
Equipment & Materials
Hemocytometer (HCM):

- **Grid specifications**: 3mm x 3mm grid, depth 0.1mm (total volume 0.9 µL) with metallic grid lines (avoid low-cost models).

Particulate Counting Protocol

Preparation

1. **Sanitize Work Area**:
   - Wipe laminar flow hood surfaces with 70% isopropyl alcohol.
   - Ensure no airflow disruptions (e.g., fans, open doors).
2. **Clean Hemocytometer**:
   - Use lens wipes to clean HCM and cover slip. Inspect at 100x and 400x for residual particles. Repeat cleaning until grid is spotless.

Sample Handling

3. **Prepare Sample**:
   - Invert vial **20 times slowly** to resuspend particles.
   - Draw **1mL** into a syringe; discard the first 0.2mL to avoid air bubbles.
4. **Load HCM**:
   - Place **one drop** on each grid chamber.
   - Gently lower cover slip to avoid air bubbles.

Counting Procedure

5. **Count Particles**:
    - View at 100x magnification (10x eyepiece + 10x objective).
    - Focus on quadrants 1–4 (see *Figure 1*).
    - Tally particles in three size categories: ≥5µm, ≥10µm, ≥25µm.
6. **Repeat for Accuracy**:
    - If zero particles are found:
        - Clean HCM, reload sample, and repeat twice (total 3 tests).
    - If particles exceed limits: Proceed to root cause analysis.

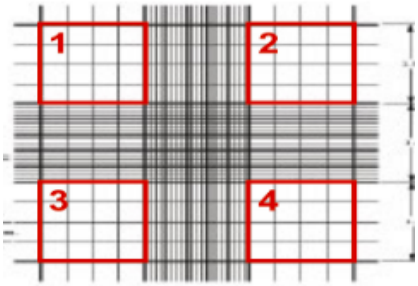

Figure 1. Hemocytometer Grid Layout (Quadrants 1-4, 64 Cells Total)

Data Analysis & USP Compliance

Calculations

1. **Particles/mL Formula**:

$$\text{Particles/mL} = \left(\frac{\text{Total Particles Counted}}{\text{Number of Cells Counted}} \right) \times 10,000$$

Example: 25 particles in 64 cells → (25/64)×10,000=3,906 particles/mL.

2. **Acceptance Limits (USP <788> for Injectables)**:

Particle Size	Max. Count in 64 Cells	Max. Particles/mL
≥10 µm	19	2,968
≥25 µm	1	156

- *Note*: USP allows ≤3,000 particles ≥10 µm/mL and ≤300 particles ≥25 µm/mL.[4]

Culture Using Agar Plates

(For microbial sterility testing of compounded solutions)

Materials Needed:

- **Agar plates** (pre-poured or DIY using: agar powder, dextrose, instant mashed potato flakes, light malt extract).
- **Inoculation loops** (disposable or flame-sterilized metal).
- **Autoclave/pressure cooker** (for sterilization).
- **Incubator** (or DIY setup: food dehydrator set to 23°C).
- **70% isopropyl alcohol** (for surface sanitization).

Protocol:

1. **Prepare Agar Medium**:
    - Mix:
        - 10g agar powder
        - 2g dextrose
        - 2g instant mashed potato flakes
        - 1g light malt extract
        - 500mL distilled water
    - Heat until dissolved, then sterilize in an autoclave/pressure cooker (15 psi, 20 min).
2. **Pour Plates**:
    - Cool agar to ~50°C (test with infrared thermometer).
    - Pour into sterile petri dishes (~20mL per plate).
    - Let solidify (1 hour).
3. **Inoculate with Samples and Control:**

4 United States Pharmacopeia, *General Chapter 788: Particulate Matter in Injections*, Method 2, 4–8, accessed May 2025, https://inject.soy/GeneralChapter788.pdf

- Sterilize inoculation loop (flame until red-hot, cool 10 sec).
- Dip loop into test solution (HRT oil from a vial).
- Streak agar surface in a zig-zag pattern (see *Figure 2*).
- Prepare two control plates (+/-) without using API vial.

4. **Incubate**:
    - Seal plates with Parafilm.
    - Store inverted in incubator at 32°C for 120-168 hours.

5. **Interpret Results**:
    - **No growth**: Solution is sterile.
    - **Colonies present**: Contamination detected; discard batch.

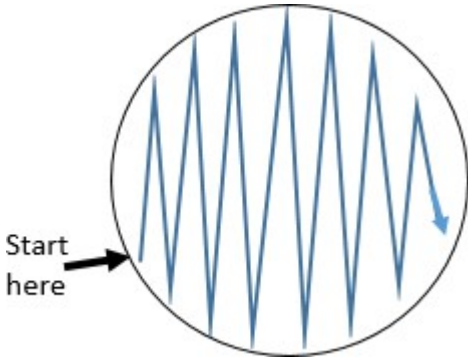

Figure 2: Zig-zag streaking method

USP PTFE Filter Examination (Manual Microscopic Method)

(For particulate matter testing per USP <788> Method 2)[5]

Materials Needed:

- **Darkfield microscope** (100x–400x magnification, mechanical stage).
- **PTFE membrane filters** (0.22µm pore, 47mm diameter).
- **Filter assembly** (Teflon filter holder or apparatus + vacuum pump).

5 United States Pharmacopeia, *General Chapter 788: Particulate Matter in Injections*, Method 2, 4–8, accessed May 2025, https://inject.soy/GeneralChapter788.pdf

- **Ocular micrometer** (calibrated with stage micrometer).
- **Particle-free water** (filtered through 0.22µm membrane).
- **Syringes** (10mL) + blunt needles.

Calibration Checklist:

- Verify ocular micrometer accuracy with a stage micrometer.
- Test particle-free water: ≤20 particles ≥10µm per 50mL.

Protocol:

1. **Prepare Filter Assembly**:
    - Rinse filter holder and funnel with particle-free water.
    - Place PTFE filter on holder (black/dark gray side up).
    - Rinse filter with 10mL particle-free water.
    - Air-dry filter in a Petri dish under flow hood (cover slightly open).
        - *The filter must be completely dry.*
2. **Filter Sample**:
    - Draw 10mL of test solution into a syringe.
    - Slowly pass through filter under vacuum.
    - Rinse filter with 10mL particle-free water.
3. **Microscopic Analysis**:
    1. Place filter on microscope stage.
    2. Scan entire surface under 100x and 400x magnification via darkfield.
    3. Roll d20 to determine grid coordinates x,y.
    4. Average particles ≥10µm and ≥25µm using the ocular micrometer:
        - Use graticule’s 10µm/25µm reference circles at coordinates x,y.
    5. Do NOT count stains, bubbles, or amorphous debris.
    6. Repeat from step 3 four times.
4. **Calculate Results**:

- Acceptance Criteria (USP <788> Test 2.B)[6]:
  - ≤3,000 particles ≥10µm per vial.
  - ≤300 particles ≥25µm per vial.

6 United States Pharmacopeia, *General Chapter 788: Particulate Matter in Injections*, accessed May 2025, https://inject.soy/GeneralChapter788.pdf sec. "Evaluation" (p. 7). For preparations supplied in containers with a nominal volume of less than 100 mL, apply the criteria of test 2.B.