

# Aliquoting Good Practice Points

Proper aliquoting techniques are fundamental to maintaining sample integrity and ensuring reliable experimental results in laboratory settings. This comprehensive guide covers critical quality concerns, best practices, and organizational strategies that every laboratory professional should master to minimize contamination risks, prevent sample degradation, and maintain experimental reproducibility.

 **by Fertility Guidance Technologies**



# Understanding Quality Concerns in Laboratory Aliquoting

Quality concerns when making aliquots in laboratory settings primarily revolve around three critical areas that can significantly impact experimental outcomes and research validity. Manual aliquoting presents unique challenges as a tedious, repetitive process that is highly susceptible to human error, potentially leading to unusable results and substantial material waste. The consequences of poor aliquoting practices extend beyond immediate material loss, affecting downstream applications, experimental reproducibility, and ultimately the reliability of scientific findings. Understanding these quality concerns is the first step toward implementing effective mitigation strategies.



Adhering to strict protocols and utilizing appropriate tools can effectively mitigate these

# Contamination: The Primary Quality Threat

Contamination represents one of the most critical risks during aliquoting procedures and can originate from multiple sources, each requiring specific prevention strategies. Understanding contamination pathways is essential for maintaining sample purity and experimental validity.

## Airborne Contaminants

Opening samples to ambient air introduces microbes, dust particles, and environmental pollutants that can compromise sample integrity, particularly for sensitive materials.

## Cross-Contamination

Transfer of material between different samples can skew analytical results and invalidate experimental conclusions through sample-to-sample interference.

## Equipment Contamination

Improperly cleaned tools, glassware, or work surfaces serve as reservoirs for contaminants that can be transferred to pristine samples.

## Human-Derived Contamination

Skin cells, hair, respiratory droplets, and clothing fibers represent significant sources of microbial and particulate contamination.

# Airborne Contamination Prevention

## Sterile Environment Requirements

Work must be conducted under sterile conditions, particularly in laminar flow hoods or biosafety cabinets, especially when handling sensitive materials such as cell culture media, biological samples, or sterile reagents. These controlled environments provide HEPA-filtered air that removes particles and microorganisms.

Laminar flow hoods create a unidirectional airflow pattern that sweeps contaminants away from the work surface, while biosafety cabinets provide both product and personnel protection. Regular certification and proper operation of these devices are crucial for maintaining sterile conditions.

Environmental monitoring through settle plates and air sampling can verify the effectiveness of contamination control measures and identify potential issues before they compromise sample integrity.



# Preventing Cross-Contamination

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## Use Separate Sterile Tips

Employ individual sterile pipette tips for every single aliquot to eliminate any possibility of sample-to-sample transfer. Never reuse tips between different samples or even different aliquots of the same sample.

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## Implement Buffer Zones

Maintain physical separation between different sample types and use designated areas for specific sample categories to prevent inadvertent mixing or confusion.

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## Follow Concentration Order

Prepare aliquots in order of increasing concentration to minimize the impact of potential carryover. Start with the most dilute samples and progress to more concentrated solutions.



# Equipment and Surface Decontamination

## Workspace Preparation

- Disinfect work areas with 70% ethanol before and after each aliquoting session
- Use sterile, single-use containers and laboratory utensils whenever possible
- Implement UV sterilization for work surfaces when appropriate
- Maintain dedicated equipment for specific sample types to prevent cross-contamination

## Equipment Validation

Regular calibration and cleaning validation of pipettes, balances, and other equipment ensures consistent performance and prevents contamination. Maintain detailed cleaning logs and equipment maintenance records to track compliance with decontamination protocols.



Proper decontamination extends beyond visible cleanliness to include chemical and

# Human Contamination Control

Human-derived contamination represents a significant and often underestimated source of sample compromise. Implementing comprehensive personal protective equipment (PPE) protocols and proper hygiene practices is essential for maintaining sample integrity.

## Personal Protective Equipment

Wear appropriate PPE including gloves, laboratory coats, and masks to create a barrier between human-derived contaminants and samples. Change gloves frequently and between different sample types.

## Hygiene Protocols

Implement strict hand hygiene before and after handling samples, avoid touching face or hair while working, and tie back long hair to prevent contamination from loose strands.

## Behavioral Controls

Minimize talking, coughing, or sneezing over open samples, work in a methodical manner to reduce unnecessary movement, and maintain proper posture to prevent clothing contact with samples.

# Sample Degradation: Environmental Factors

Many biological and chemical reagents demonstrate sensitivity to environmental changes including temperature fluctuations, light exposure, and atmospheric oxygen. Understanding degradation mechanisms enables implementation of targeted preservation strategies.



## Temperature Sensitivity

Proteins, enzymes, and many organic compounds are susceptible to thermal degradation. Maintaining consistent storage temperatures and minimizing temperature excursions during handling is crucial for preserving biological activity and chemical stability.



## Light-Induced Degradation

Photosensitive compounds can undergo photobleaching, photoisomerization, or other light-catalyzed reactions that alter their properties and reduce their effectiveness in experimental applications.



## Oxidative Damage

Exposure to atmospheric oxygen can cause oxidation of sensitive molecules, leading to structural changes, loss of activity, and formation of degradation products that may interfere with downstream applications.



# Freeze-Thaw Cycle Management



## Impact on Sample Integrity

Repeated freezing and thawing cycles can severely degrade sensitive components like proteins and enzymes, diminishing their quality and performance through ice crystal formation, osmotic stress, and conformational changes.

## Single-Use Aliquot Strategy

Create single-use aliquots from larger stock solutions to eliminate the need for repeated thawing. This approach preserves the integrity of the original stock while providing convenient portions for individual experiments or analyses.

Store aliquots at consistent, appropriate temperatures and maintain detailed records of freeze-thaw history for each sample to track degradation potential over time.

# Light Protection Strategies

Light-sensitive reagents require special handling and storage considerations to prevent photodegradation and maintain their effectiveness throughout their shelf life.

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## Low-Light Handling

Work with photosensitive materials under reduced lighting conditions, using amber lighting or dimmed laboratory lights when possible.

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## Opaque Storage

Store light-sensitive reagents in opaque containers, amber glassware, or aluminum foil-wrapped vessels to block harmful wavelengths.

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## Minimize Exposure Time

Reduce the duration of light exposure during handling and aliquoting procedures through efficient workflow design and preparation.



# Oxidation Prevention Techniques

## Minimizing Air Exposure

Aliquoting into smaller portions that can be consumed in single use minimizes repeated exposure to atmospheric oxygen. This approach is particularly important for oxygen-sensitive compounds such as reducing agents, antioxidants, and certain enzyme preparations. Consider using inert gas atmospheres (nitrogen or argon) during aliquoting procedures for highly oxygen-sensitive materials. This technique involves purging containers with inert gas before sample addition and maintaining an inert atmosphere during storage.



Vacuum sealing or using oxygen-scavenging packets in storage containers can further

# Pipetting Accuracy and Precision

Variations in aliquot volume and composition can significantly affect experimental reproducibility and data quality. Manual pipetting introduces multiple sources of error that must be systematically addressed.

- 1 Proper Pipette Selection**  
Use pipettes with volume ranges close to the target volume for optimal accuracy. Pipetting in the middle third of a pipette's range typically provides the best precision and accuracy.
- 2 Technique Standardization**  
Follow consistent pipetting practices including tip pre-wetting, controlled plunger depression, and standardized pause times at each step of the pipetting process.
- 3 Technology Enhancement**  
Consider electronic or automated pipetting systems for improved precision, consistency, and reduced operator fatigue during high-volume aliquoting procedures.

# Ensuring Sample Homogeneity

Non-homogeneous stock solutions can lead to uneven distribution of key components across aliquots, resulting in inconsistent experimental conditions and unreliable results.

## Thorough Mixing Protocols

Implement comprehensive mixing procedures before aliquoting, including:

- Gentle inversion mixing for solutions prone to foaming
- Magnetic stirring for larger volumes
- Vortex mixing for small volumes in appropriate containers
- Temperature equilibration to ensure uniform viscosity

## Special Considerations

Pay particular attention to samples containing precipitates, multiple phases, or components with different densities. These materials may require extended mixing times or specialized techniques to achieve homogeneity. Verify mixing effectiveness through visual inspection and consider analytical verification for critical applications.



# Master Mix Strategy

Creating master mixes represents an efficient approach to reducing pipetting steps and ensuring uniformity across multiple aliquots while minimizing the potential for individual component variation.

## Component Calculation

Calculate total volumes needed for all aliquots plus excess to account for pipetting losses and dead volumes.

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## Quality Verification

Verify master mix composition through analytical testing before aliquoting to confirm proper preparation.

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## Sequential Addition

Add components in order of increasing volume and mix thoroughly after each addition to ensure homogeneity.

## Efficient Distribution

Distribute master mix quickly to minimize degradation and maintain consistency across all aliquots.

# Organizational Excellence in Sample Management

Poor organization can result in wasted time, compromised samples, and misidentified materials that can invalidate entire experimental series.

Implementing systematic organizational approaches is crucial for laboratory efficiency and data integrity.

## Labeling Consistency

Inconsistent or illegible labels lead to sample misidentification, particularly problematic in large freezer storage systems where visual identification is challenging.

## Inventory Control

Poor tracking systems can result in using expired samples, inability to locate specific aliquots, and inefficient use of laboratory resources.

## Storage Optimization

Inadequate storage organization leads to temperature fluctuations, difficulty accessing samples, and increased risk of sample loss or damage.



# Advanced Labeling Systems



## Unique Identification Strategies

Implement unique alphanumeric codes or barcode systems for each aliquot to eliminate confusion and enable efficient tracking. These identifiers should be permanently associated with sample information in laboratory information management systems (LIMS).

## Label Durability

Select labels that can withstand various storage conditions including extreme temperatures, chemical exposure, and moisture. Cryogenic labels, waterproof materials, and chemical-resistant adhesives are essential for long-term sample integrity.

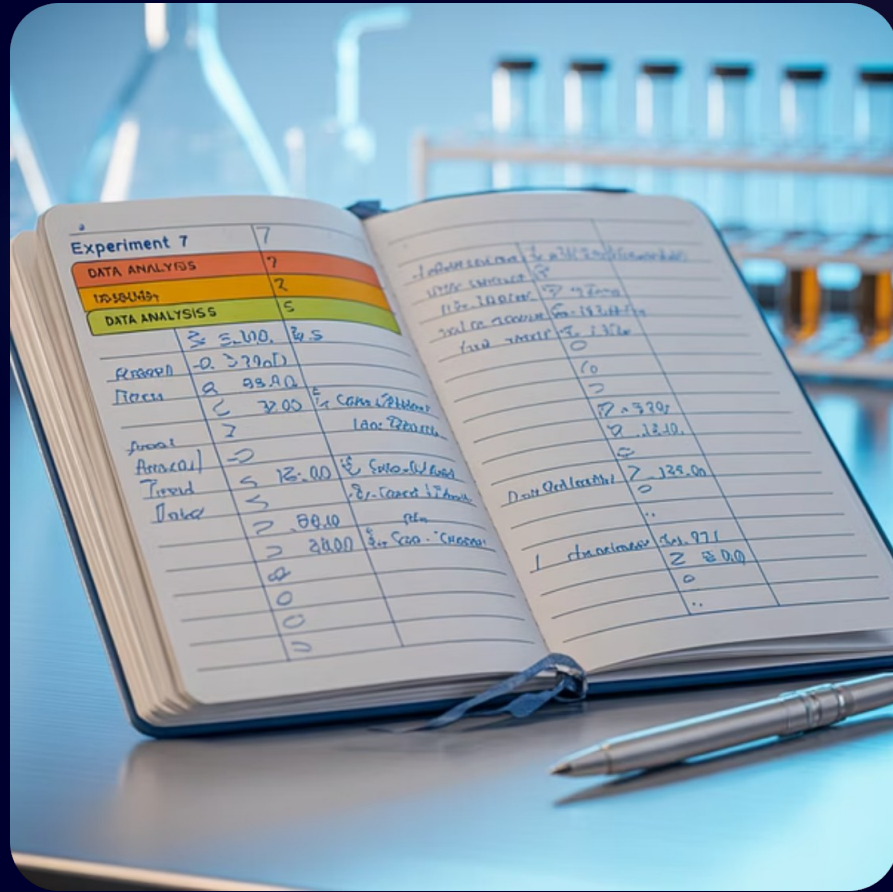
## Information Content

Include essential information such as sample name, preparation date, expiration date, lot number, concentration, and storage requirements. Use standardized abbreviations and formats for consistency across the laboratory.



# Comprehensive Documentation Practices

Maintaining detailed records of aliquoting procedures ensures traceability, reproducibility, and quality control throughout the sample lifecycle.



## Procedure Documentation

Record detailed aliquoting procedures including source material information, volumes transferred, environmental conditions, and any deviations from standard protocols.



## Digital Tracking

Utilize electronic systems for real-time tracking of sample location, usage history, and remaining quantities to optimize resource utilization and prevent stock depletion.



## Inventory Management

Implement first-in, first-out (FIFO) systems and maintain comprehensive inventories that include expiration monitoring and automated alerts for sample replacement needs.

# Standard Operating Procedures: Step-by-Step Protocol

Following standardized protocols ensures consistency and quality across all aliquoting operations while minimizing risks and optimizing efficiency.

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## Inventory Assessment

Check all available stock materials, prioritize those approaching expiration dates, and use first-in, first-out (FIFO) principles to optimize resource utilization.

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## Documentation Requirements

Mark opening dates and any additions on stock bottles with your initials. Maintain detailed records of all modifications or observations.

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## Single-Solution Focus

Aliquot one solution at a time to prevent confusion and cross-contamination. Complete all aliquots from one source before proceeding to the next material.

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## Complete Utilization

Use entire stock volumes for aliquots when possible to avoid re-capping and potential contamination issues while minimizing waste.

# Resource Management and Communication

## Proactive Supply Management

Alert the person responsible for ordering when only two units remain of any critical reagent or supply. Never use the second-to-last item without notifying appropriate personnel or initiating replacement orders. This prevents research disruptions due to unexpected material shortages.

## Single Expiration Date Policy

Process reagents with identical expiration dates together to maintain traceability and prevent confusion in storage systems. This practice reduces the risk of using expired materials and facilitates efficient inventory rotation.

## Workflow Completion

Complete each aliquoting session entirely, including proper storage and workspace cleanup, before beginning work with different materials. This systematic approach prevents cross-contamination and maintains laboratory organization.

Implement standardized cleanup procedures that include equipment decontamination, waste disposal, and documentation completion to ensure consistent laboratory practices.

# Excellence in Laboratory Aliquoting: Key Takeaways

Mastering aliquoting techniques is essential for maintaining laboratory standards and ensuring reliable experimental outcomes. These practices form the foundation of quality laboratory operations.



## Contamination Control

Implement comprehensive contamination prevention strategies including sterile techniques, proper PPE usage, and environmental controls to maintain sample purity.



## Sample Preservation

Protect samples from degradation through proper storage conditions, minimal freeze-thaw cycles, and protection from light and oxidation.



## Precision and Accuracy

Maintain consistent pipetting techniques, ensure sample homogeneity, and utilize appropriate equipment to achieve reliable volume accuracy.



## Systematic Organization

Implement robust labeling systems, comprehensive documentation practices, and efficient inventory management to optimize laboratory operations.

By adhering to these evidence-based practices, laboratory professionals can ensure sample integrity, minimize experimental variability, and contribute to the overall success of scientific research endeavors. Continuous improvement and adherence to established protocols are essential for maintaining the highest standards of laboratory excellence.