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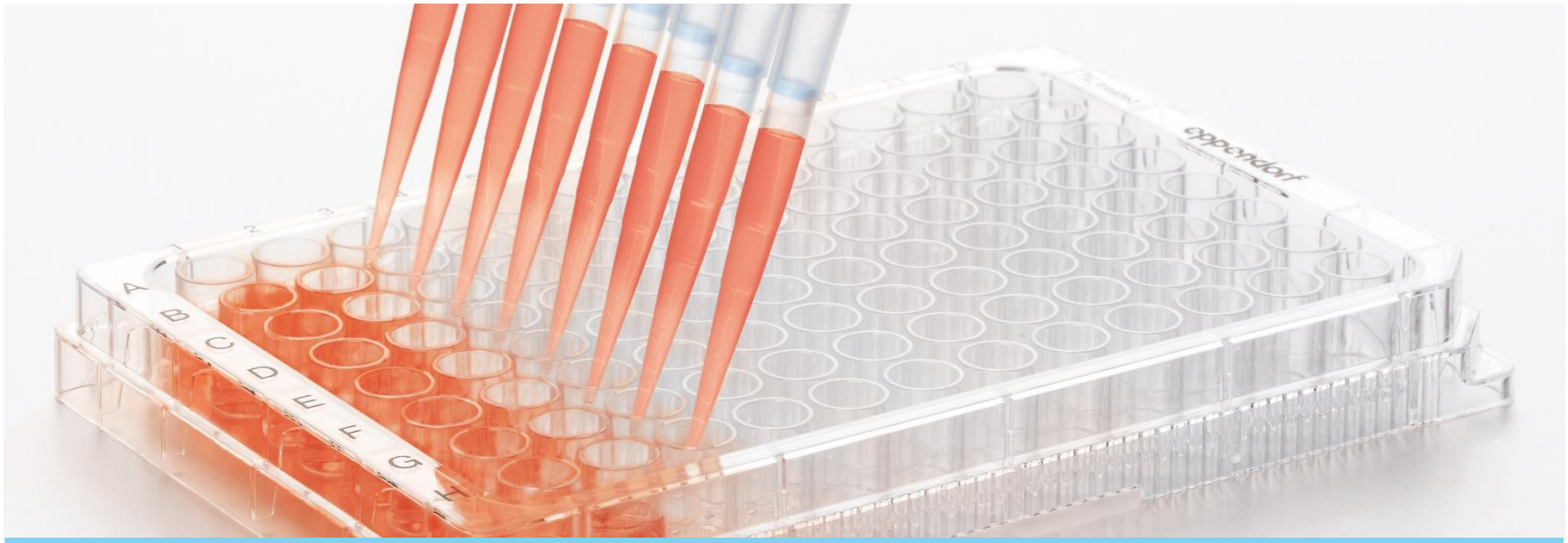

Protect Your Cells with Proper Pipetting

How liquid handling influences your cell culture work

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Hamburg, Germany

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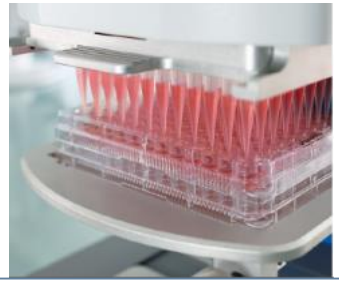
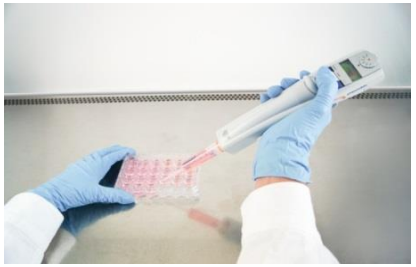
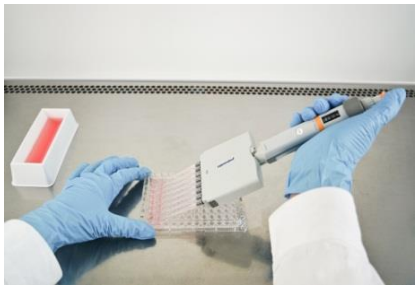
Contents

Pipetting: An Every Day Task?

- Many different types of
  - Applications
  - Liquids
- So ordinary
- **Highest impact** of all techniques

Choice of Pipetting Tool

Liquid Handling Systems

	Air-cushion		Positive displacement
Automated Technique Speed			
Electronic Speed			
Manual			

Liquid Handling Systems

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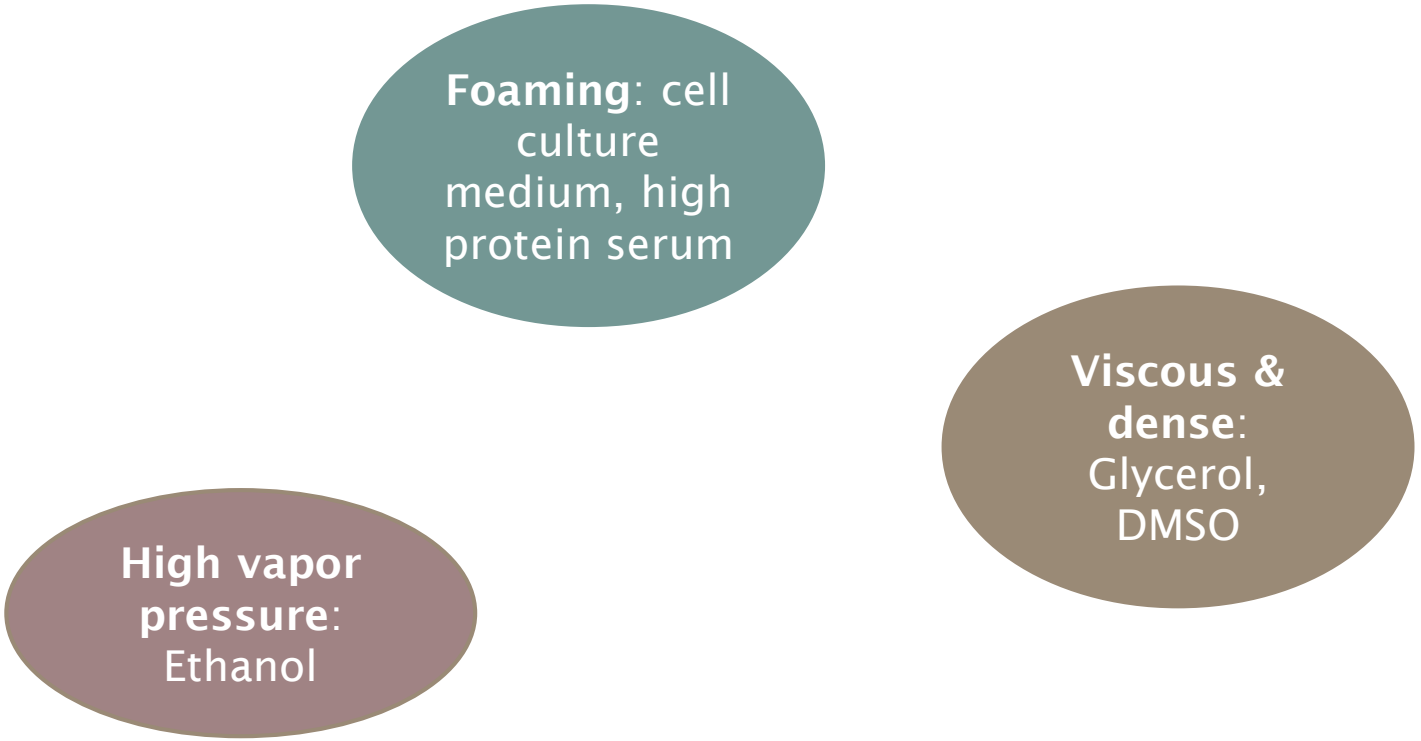
- Device + tip = System
- Tip according to application

Liquid Categories in Cell Culture

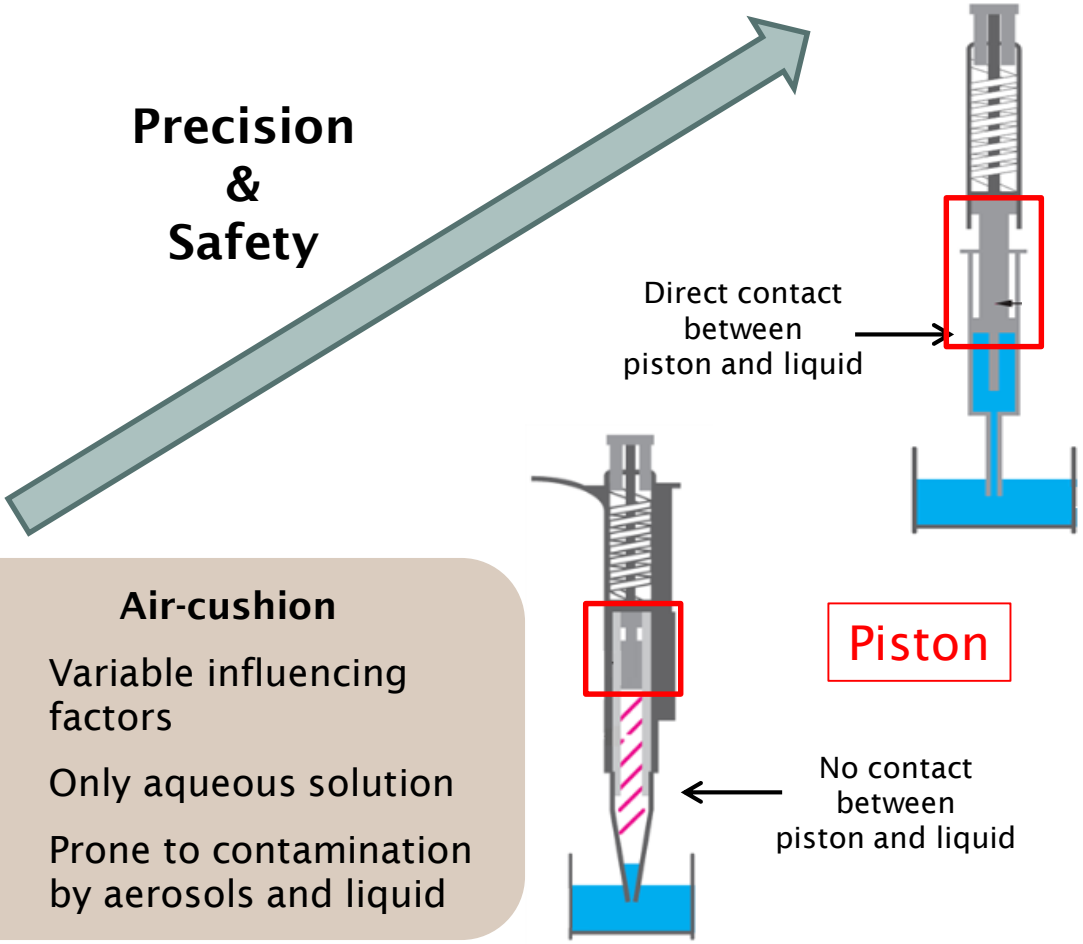


Poll Question 1:

Which pipetting instruments do you use for cell culture?
(Select any that apply)

- Manual and/or electronic dispenser
- Manual and/or electronic air-cushion pipettes
- Electronic pipette controller
- Classic pipette controller (Peleus ball)

Air-Cushion vs. Positive Displacement



Positive displacement

= Dispenser

- Unaffected by physical properties of liquid
- Suitable for "problematic" liquids
- Contamination free

Air-cushion

- Variable influencing factors
- Only aqueous solution
- Prone to contamination by aerosols and liquid

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Poll Question 2:

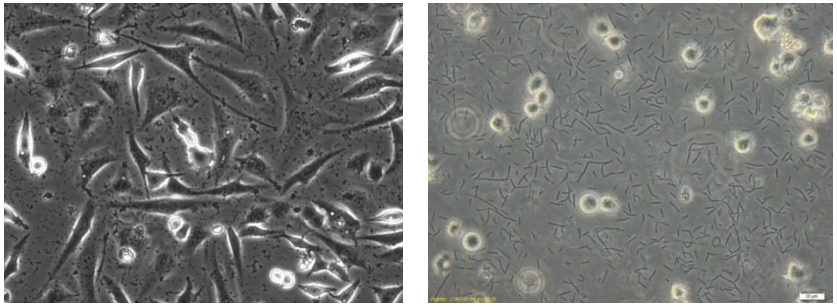
Have you ever had any problems with contamination in your cell culture lab?
(Select one answer)

- Never
- Rarely
- Sometimes
- On a regular basis

Types of Contamination

Microbial contaminants

- Fungi and yeast
- Bacteria
  - Mycoplasma

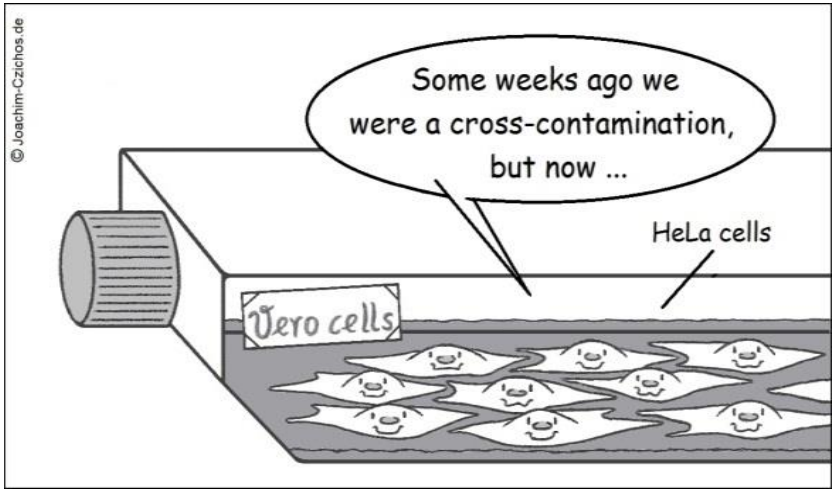


CHO cells (left) and HL60 cells (right) contaminated with bacteria

Eukaryotic contaminants

- Cross-contaminated and misidentified cell lines

15-30% worldwide



Consequences of Contamination

Loss of time

- When you have to repeat experiments

Increase in cost

- For materials, reagents, etc.

Loss of reproducibility

- Contaminated vs. non-contaminated cells

Loss of significance

- When working with misidentified or cross-contaminated cell lines

Avoiding contamination is much easier than dealing with it.

Maintaining a Sterile Environment

- Preventive hygiene
- Personal protective equipment
- Being organized inside the biosafety cabinet
- Aseptic handling
- Pipetting techniques

It´s in Your Hands

Your hands are a major source of germ-transfer

- Wash hands before you start working
  - Water + detergents remove particles
  - Disinfectants inactivate microorganisms
- Disinfect gloves sufficiently and repeatedly
  - Select a gloves model covering the wrist

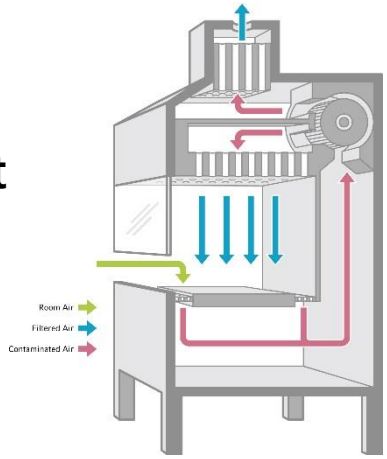
Handling Pipettes

Have a dedicated set of pipettes and tips in the cell culture lab

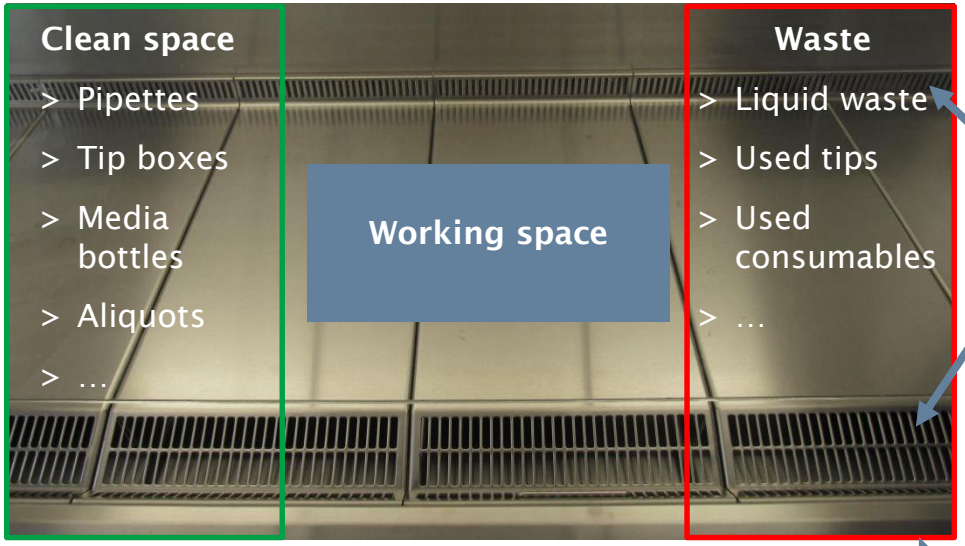
- Disinfect pipettes and tip boxes thoroughly before each use in the biosafety cabinet
  - Prefer wipe disinfection over spray disinfection
- Regularly disassemble pipettes to clean the inside
  - If possible use autoclavable pipettes
- Avoid moving your hands over open vessels

The Place of Action

Organize your working area inside the biosafety cabinet



Work in the middle third of the working space



Don´t cover the air grilles

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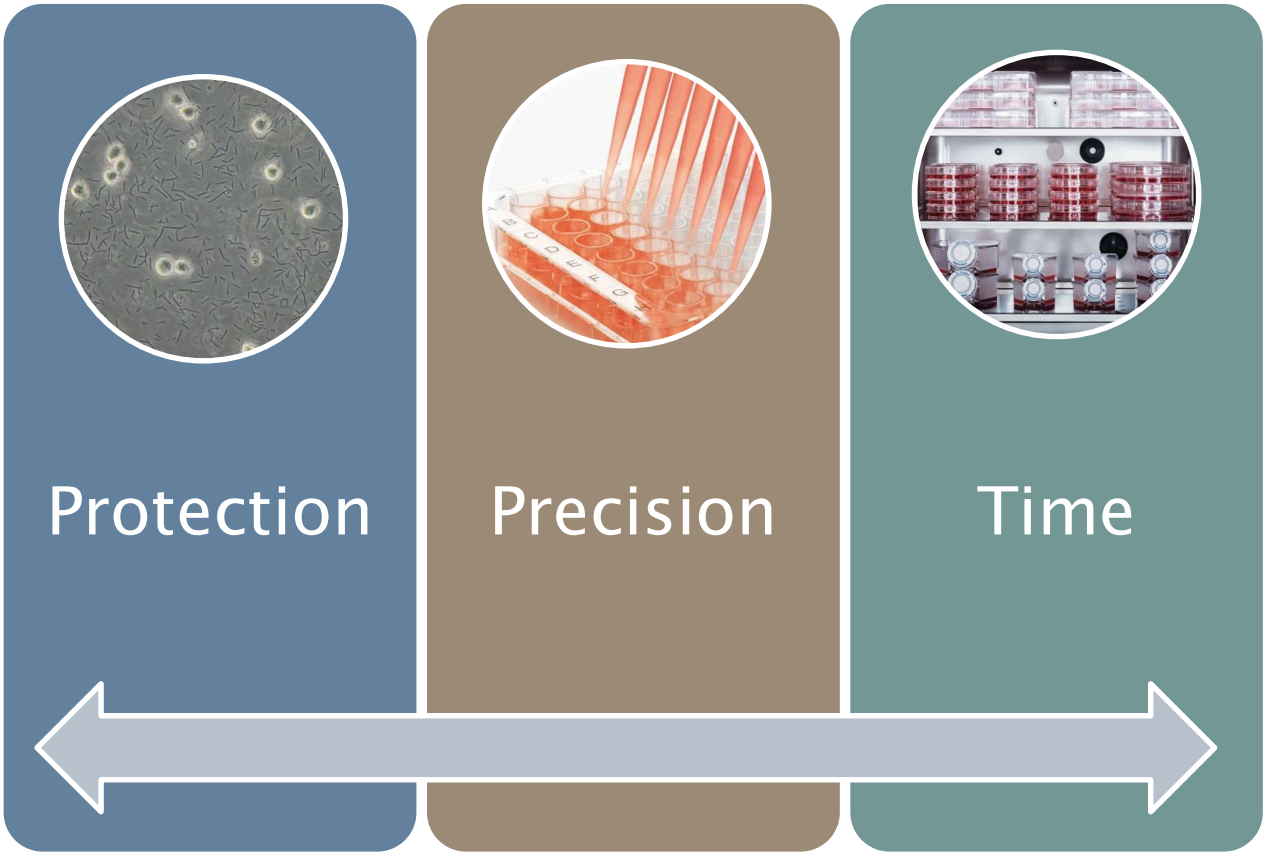
Contents

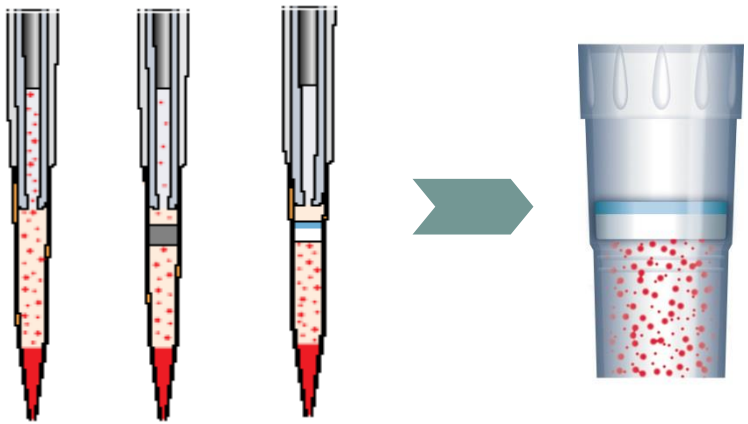
- Fundamentals of Pipetting
- Avoiding Contamination – Best Practice
- **Pipetting Techniques in Cell Culture**
- From Cell Seeding to Cell Banking

What is Important in Cell Culture.....



Aerosols

- An effective filter tip protects your pipette while working
  - Protects pipette from contamination
  - Protects culture from cross contamination



✓ **Contamination-free with positive displacement system**

Filter Tips

Binds fine aerosols & biomolecules
(e.g. RNA, DNA, bacteria, virus)

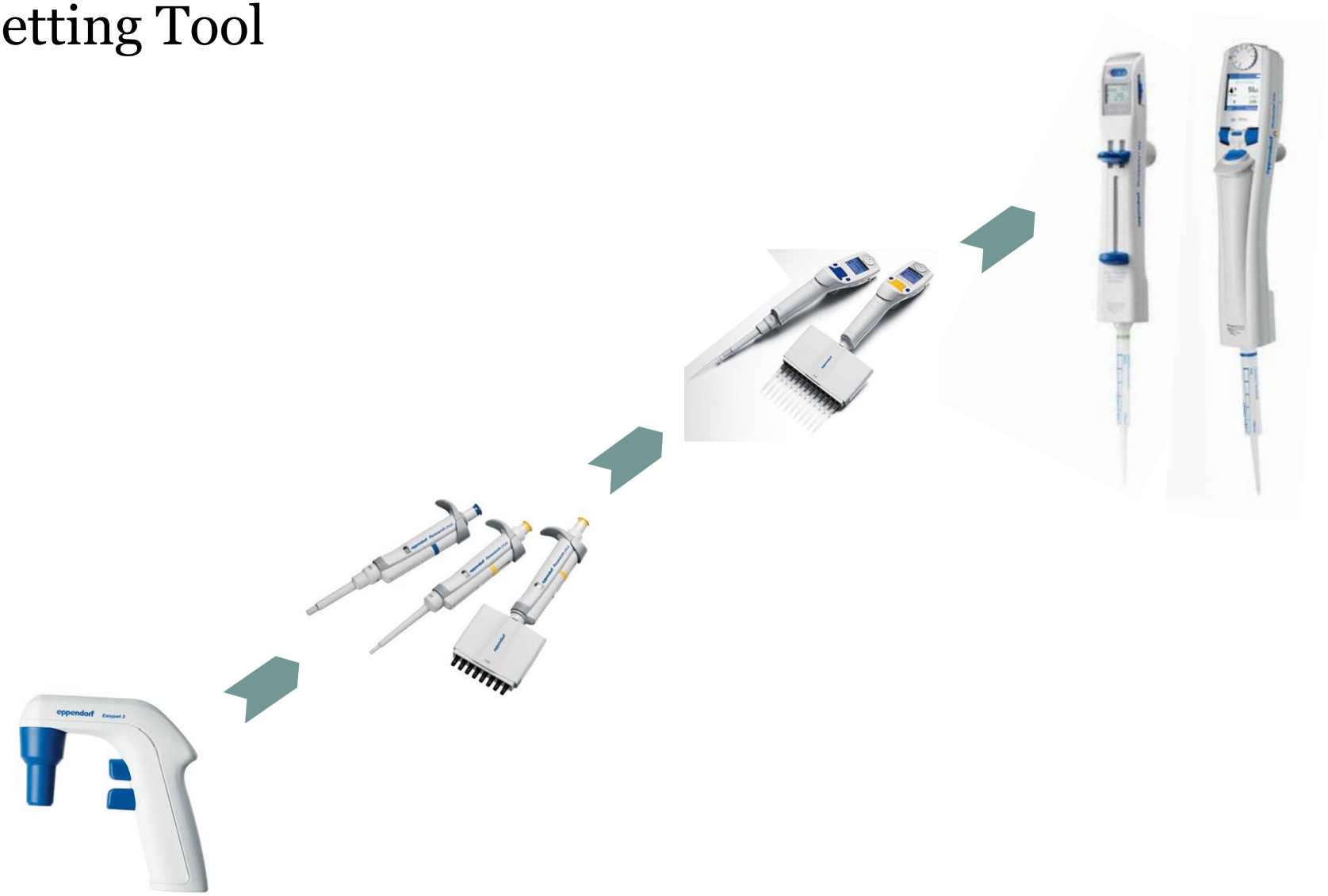
Retains drops, splashes & large aerosols

ep Dualfilter T.I.P.S.®

Aerosols

- Mixing by gentle rolling rather than vigorous shaking
- Pipetting directly into a liquid or onto a surface to avoid bubbling and splashing
- Placing containers very close to each other when transferring liquids between them
- Slowly removing tube caps or stoppers

Pipetting Tool

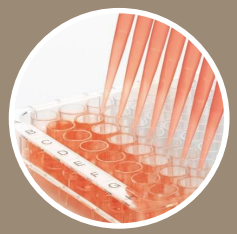


Pipetting Tool

Large volumes

- Feeding
- Subcultivation

Protection



Precision

„Fast“ solution

„Precise“ solution

- Make your choice between precision and protection!

Pipettin

2 – 5 r

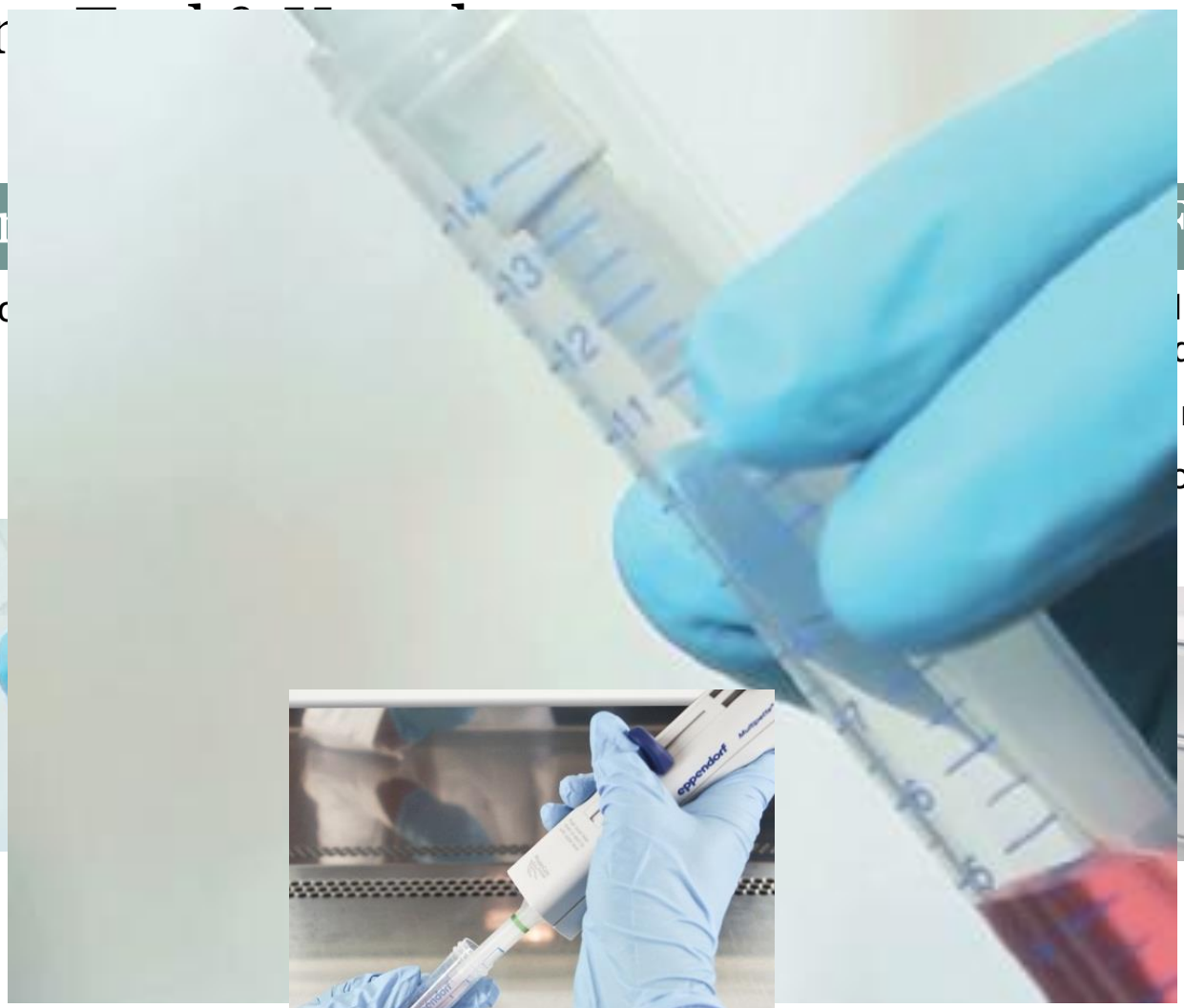
Flasks

- Pipette c

l 0 mL pipette +
d tips

r with elongated tip

ontroller



Large Volumes

Do

> 45° angle

Don't

> Over flask or dish

Small Volumes

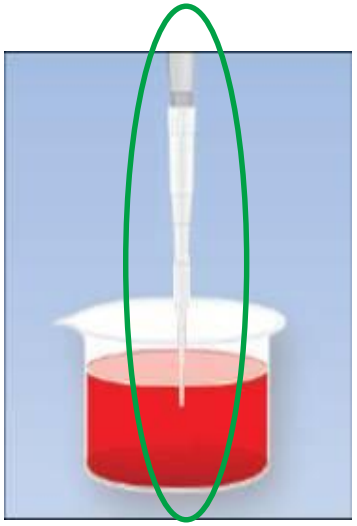
Small volumes

- Assays
- Freezing
- Compound adding

Small Volumes

Aspirating ↑

Upright!



Holding angle	0°	0°	30°- 40°

Pipette	< 20 µL	> 20 µL
	low immersion depth	a little deeper

Poll Question 3:

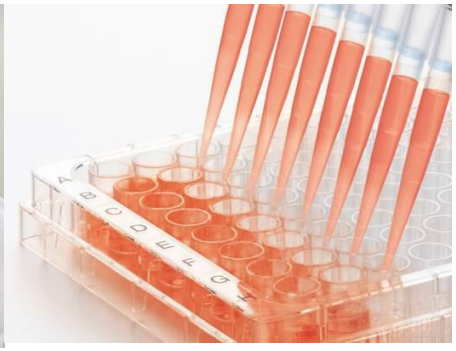
Do you use a Dispenser for small volumes?
(Select one answer)

- Yes
- No
- I am not sure how to work with these devices

Small Volumes
Dispensing ↓

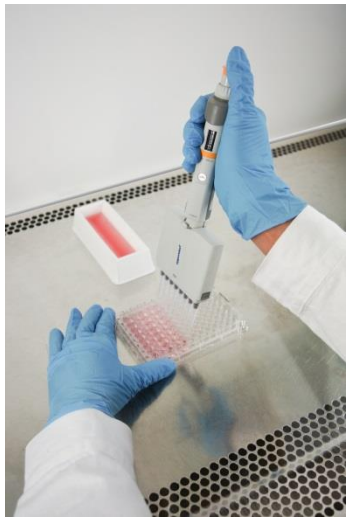
Do

- 45° angle
- Tilt the filled tip against the vessel wall
- Perform blow-out



Don't

- Upright
- Avoid moving hands over plate



Small Volumes with a Dispenser

Do: Aspiration ↑

- Any angle (precision unaffected)
- Aspirate at any depth

Do: Dispensing ↓

- 45˚ angle
- Rest tip on wall of the vessel , or free jet

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- Fundamentals of Pipetting
- Avoiding Contamination – Best Practice
- Pipetting Techniques in Cell Culture
- **From Cell Seeding to Cell Banking**

Contents

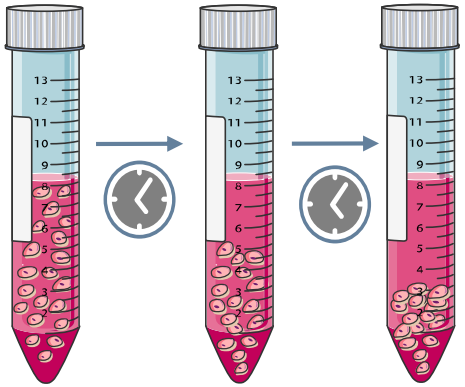
Cell Seeding is the 1st Step of an Experiment

Challenge	Getting equal starting conditions in experimental replicates
Detailed Problem	• Variations in seeding cell numbers • Formation of air bubbles during the seeding process
Possible Solutions	• Resuspend/mix the cells repeatedly during seeding • Pipette slowly and thoroughly to avoid air bubbles

Seeding Cells

Filling a plate or multiple dishes is time consuming

- Cell sedimentation in the tube is quite fast
- The longer the seeding process, the higher the decrease in cell number



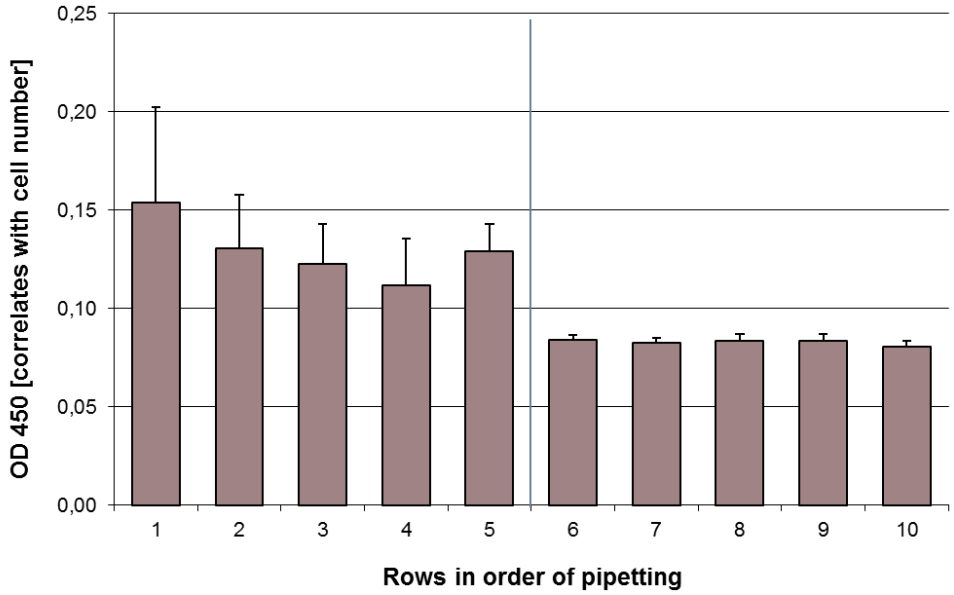
Subsequent pipetting increases the risk of air bubbles

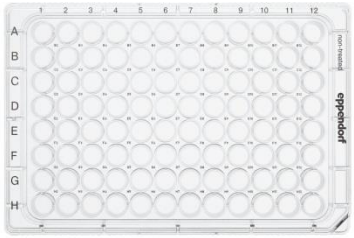
- Culture medium tends to foam
- Air bubbles can hinder cell attachment
- In addition: harsh and fast pipetting can create shear stress

Pipetting can affect Assay Results

- No mixing between rows
- Different aspiration angles and heights

- Mixing before pipetting each row
- Upright and constant aspiration height





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Source: InCelligence

Applying Assay Reagents

Multichannel

Dispenser

Cryopreservation of Cells

Challenge

Establishing frozen cell stocks of high quality by minimizing stress factors during freezing and thawing

Detailed Problem

- Cryoprotectants (e.g. DMSO) in liquid media are harmful to the cells
- Freshly thawed cells are very sensitive to shear forces

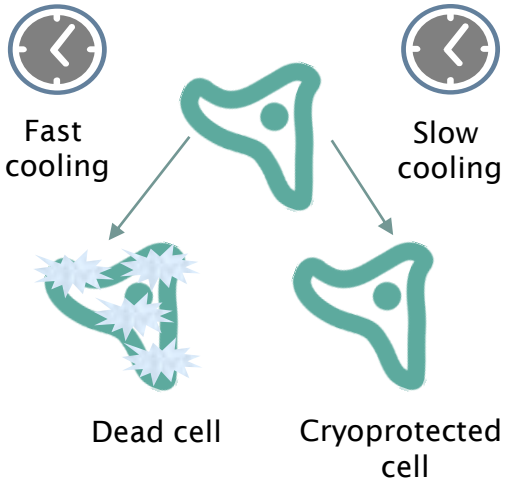
Possible Solutions

- Reduce the time that the cells are exposed to DMSO in liquid media
- Avoid harsh pipetting and don´t pipette against vessel walls

Time is a Critical Factor

Freezing of cells

- From resuspension in freeze medium to cooling
  - Work quick but don´t get hectic
- Cooling down
  - Slow cooling rate (1 °C/min) to avoid the formation of ice crystals



Thawing of cells

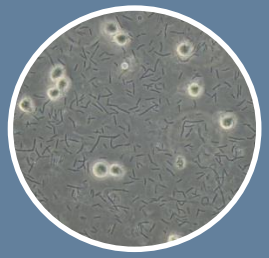
- From thawing to dilution of cryopretectant
  - As **rapidly** as possible (minimized exposure to cryprotectant)
- Freshly thawed cells are very sensitive to shear force
  - Avoid harsh and fast pipetting

Cell Banking is your Backup System

Some general remarks…

- Make sure that you don´t freeze contaminated cells
  - Test for mycoplasma
  - Authenticate cell lines
  - Filter tips for enhanced safety
  - Antibiotics can mask a contamination
- Work with a two-stage cell banking system
  - Master stock and working stock from early passaged cells
- Keep detailed and current records of your cell stocks

Pipetting in Cell Culture



Protection

- 45° for aspiration and release
- Regular disinfection and autoclaving of tools
- Dedicated set of pipetting tools
- Filter tips
- Organized working space

Precision

- Upright aspiration
- Constant immersion depth
- Empty tip with contact to vessel wall
- Perform blow-out for pipettes
- Repeated mixing of cells
- Calibrate regularly

Time

- Multichannel pipettes or dispenser for plates
- Dispenser for problematic liquids
- Don´t work with too many samples at a time

Poll Question 4:

How do you rate the content of this webinar?
(Select one answer)

- Very good
- Good
- Okay
- Poor

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Thank you