

utilization of pipettes

- **liquid handling - overview**
- **pipetting large volume**
 - **glass / plastic pipette**
 - **pipetting**
- **pipetting micro volume**
 - **air cushion pipette**
 - **forward pipetting / reverse pipetting**
 - **positive displacement pipette**
 - **optimal handling**
 - **tips**
 - **problematic liquids**
 - **sources of error**
- **summary and outlook**

Overview

- dispenser
- burettes
- glass / plastic pipettes
- piston-stroke pipettes
- electronic pipettes and dispenser
- automated systems



- glass / plastic pipettes
- graduated pipette
- disposable pipette
- volumetric pipette
- accuracy classes



- using pipetting aids
- never lay down the pipettes with Peleus-ball
- three times rinsing
- aspirating vertically
- adjusting the meniscus vertically
- wipe of the liquid outside
- dispensing while holding the container at an angle
- observe the efflux time



Piston-Stroke pipettes

Positive displacement pipettes

single channel , multi channel (saving time)

dispensers

electronic pipettes with different techniques:

Multipipette, Stepper, Tacker, Repeater

(saving of time, programmable,

reduction of individual mistake

ergonomic work)



pipetting micro volume → piston stroke pipettes air cushion

fixed volumes

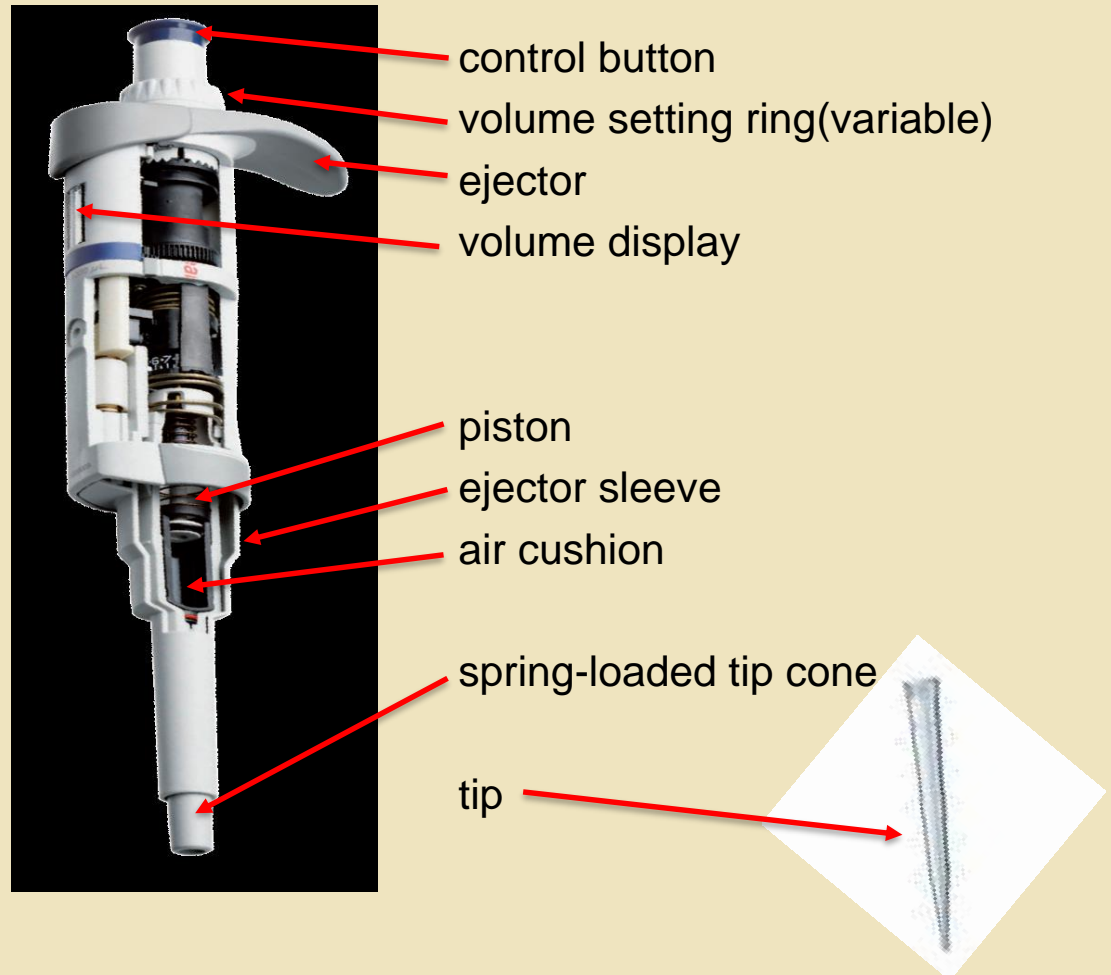
adjustable volumes

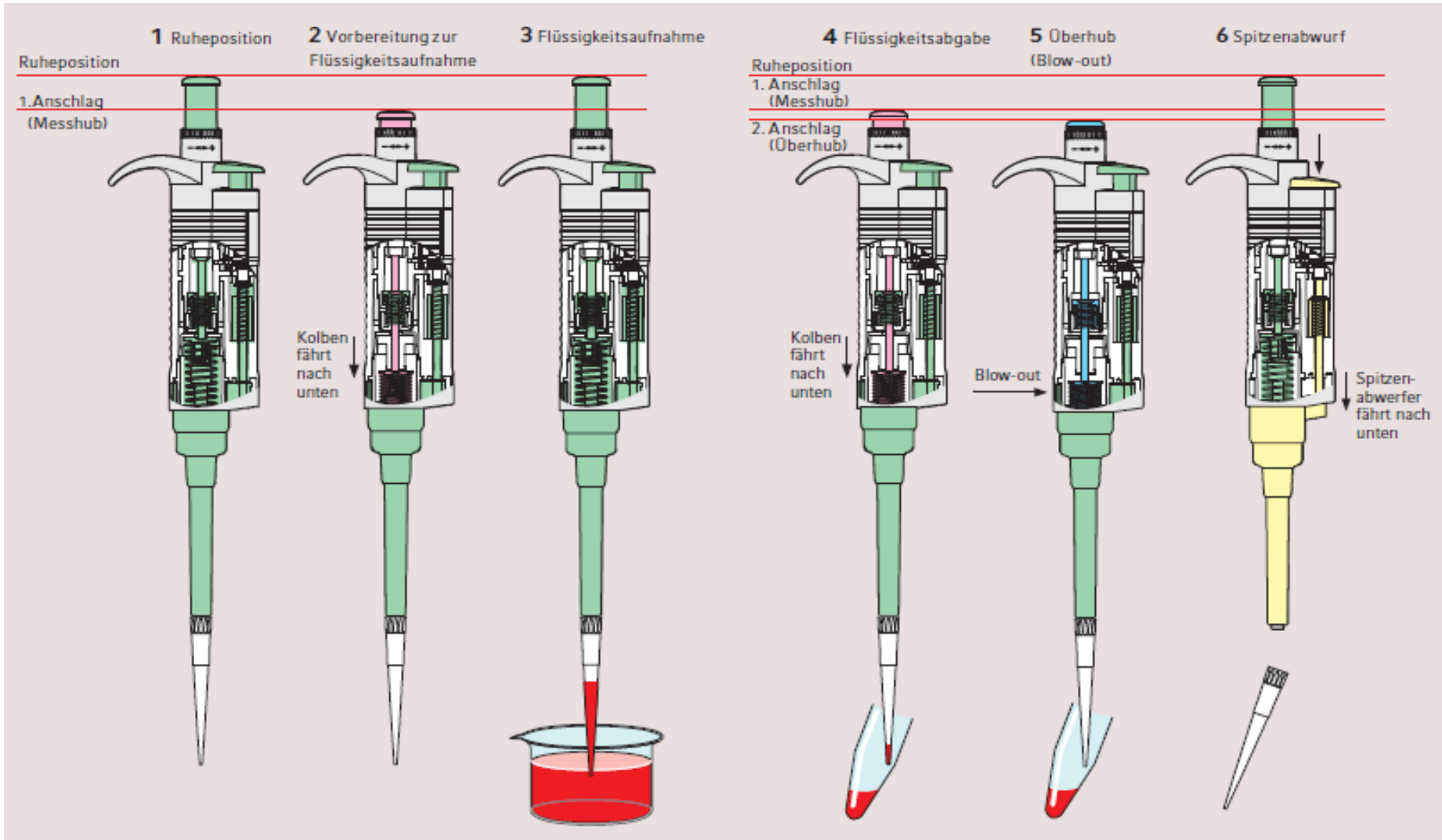
0,1µl to 10 ml

different techniques

**saturate the
air cushion**

autoclavable





pipetting micro volume → piston stroke pipettes
→ air cushion → forward and reverse

forward pipetting

- aqueous liquid

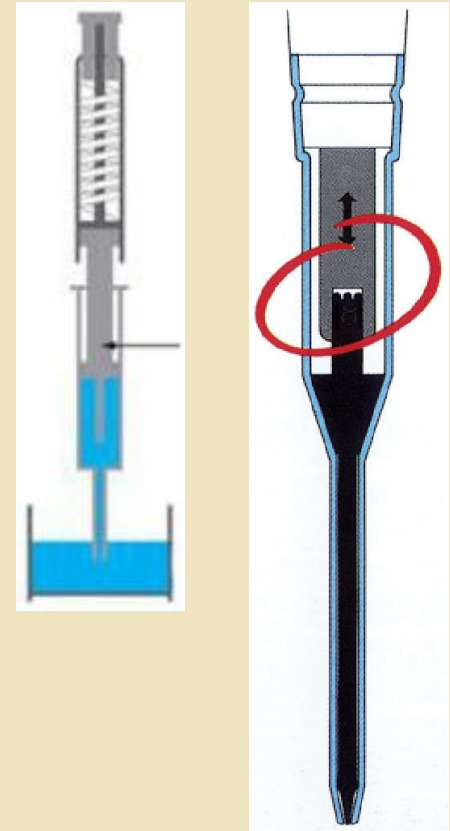


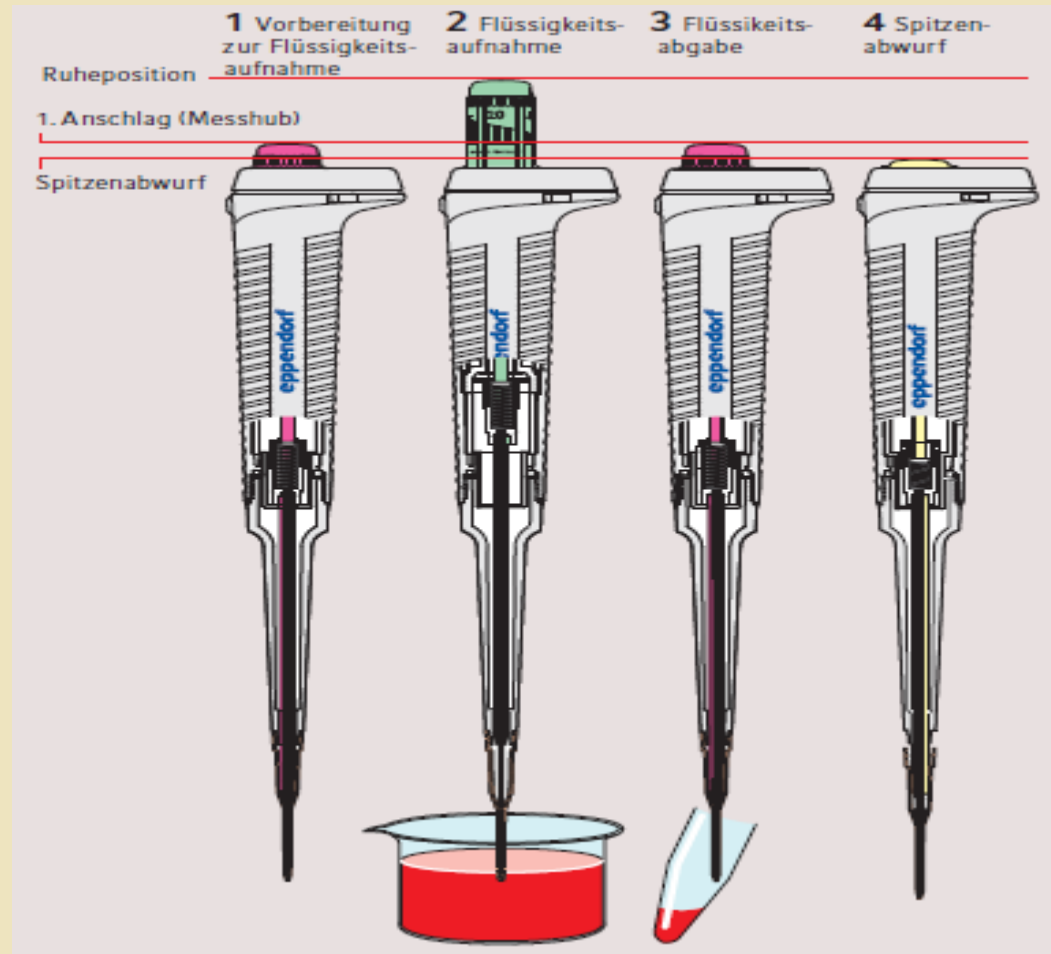
reverse pipetting

- viscous liquid
- volatile liquid

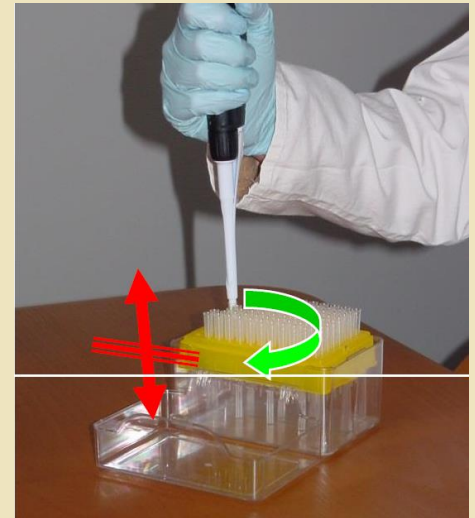


- tip with integrated piston
- no air between piston and sample
- viscous liquid
- cold or warm
- volatile liquid → no drooping
- toxic liquid
- infectious materials → no cross-contamination
- better reproducibility

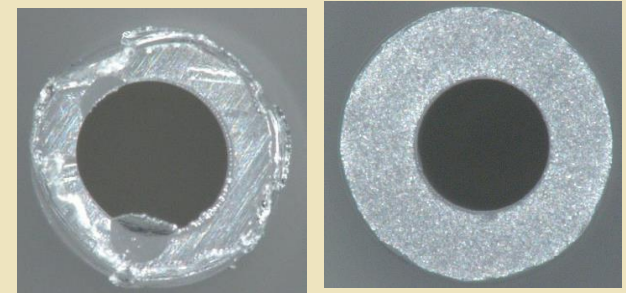
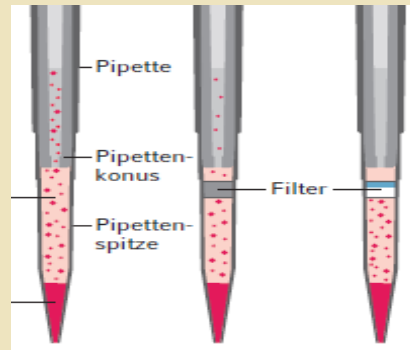




- always hang them into a pipette holder
- only use racked tips - to refill the rack use gloves
- don't 'hack' - just turn!
- vertically holding while slowly raising liquid
- wetting and wiping
- pay attention, when dipping the tip:
 - 1- 10 μl → 1 - 2 mm
 - 10 – 200 μl → 2 - 3 mm
 - 200 – 2000 μl → 3 - 6 mm
 - 5 – 10 ml → 6 - 10 mm
- to remove the used tip, press the remove button
- cleaning the pipette, when liquid inside (air cushion)










- best results by filling 50 % of the volume
- tips with filters to avoid contamination
 - and aerosol
- tip fit on cone
- tip orifice
- material
 - water retention
 - leachables
- special tips with displacements pipettes
- tip changing during calibration yes or no?



microscopic pictures of 10 µl tip orifices

micro volume → problematic liquids

| liquid | air cushion | direct displacement |
|-----------|--|---|
| volatile | evaporation in tip and pipette - dropping pre-wetting - reverse pipetting |  |
| viscous | less flow - risk of bubbles - left over in tip slowly reverse pipetting |  |
| dense | Adjustment |  |
| cold | equilibrate to room temperature reverse pipetting |  |
| warm | evaporation into tip and pipette - contamination - corrosion - inaccurate volume – use filter tips |  |
| foaming | detergents stick to tip wall contamination risk – reverse pipetting |  |
| infective | contamination risk |  |

comparison

| | air cushion pipette | positive displacement pipette |
|----------------------|------------------------------------|--------------------------------------|
| advantages | cheaper tips | no cross-contamination |
| | | better reproducibility |
| | | no drooping |
| disadvantages | cross-contamination | single-use pipette tips |
| | drooping | |
| | aerosol formation | |
| scope of application | aqueous solution | cold/warm liquid |
| | viscous liquid (reverse pipetting) | volatile liquid |
| | | viscous liquid |
| | | infective liquid |
| | | liquid with other density than water |

| Parameter | effect /mistake | influenceable by | realize |
|---|-----------------|---|----------------|
| varying of the interval of pipette | ~1,5% | eletronic pipette | |
| depth of immersion/ hold time | ~1,0% | hold the pipette according to the recommendation for the volume | visual control |
| variation of the temperature of the pipette/air/liquid | ~0,3% | constant temperature | measuring temp |
| variation of relative humidity in the lab | ~3,0% | prewetting of the pipette tip | hygrometer |
| no slough of the last drop on the wall | ~3,0% | wipe of the last drop on the wall | visual control |
| dropping or leaky pipette tip | 0,5% - 50% | leaky seal, damaged cone, loose untighten tip | original tips |
| multiple use of pipette tips | ~4,0% | use pipette tips one time only | |
| straightness of pipette tips | ~10,0% | use pipette tips one time only,choice of the tips | visual control |
| uptaking liquid at an angel,unbalanced force and rhythem | 1-5 % | vertically holding, regulary force and rhythem | visual control |
| different vapor pressure of the pipetted liquid and the adjustment with water | 2% | adjustment with the pipetted liquid or positive displacement | |

- **choosing the correct liquid handling is essential for optimal results**
- **problematic liquids need special techniques and tools**
- **plastic consumables with high purity**
 - **mechanical and thermal load capacity,**
 - **high resistance to chemicals**
- **positive displacement system is suitable for most problematic liquids**
- **electronic pipettes offer**
 - **increased reproducibility**
 - **additional functions**
 - **saving time**
- **agreement relating to unique liquid handling to get more precision**

- *Thank you for your attention!*
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