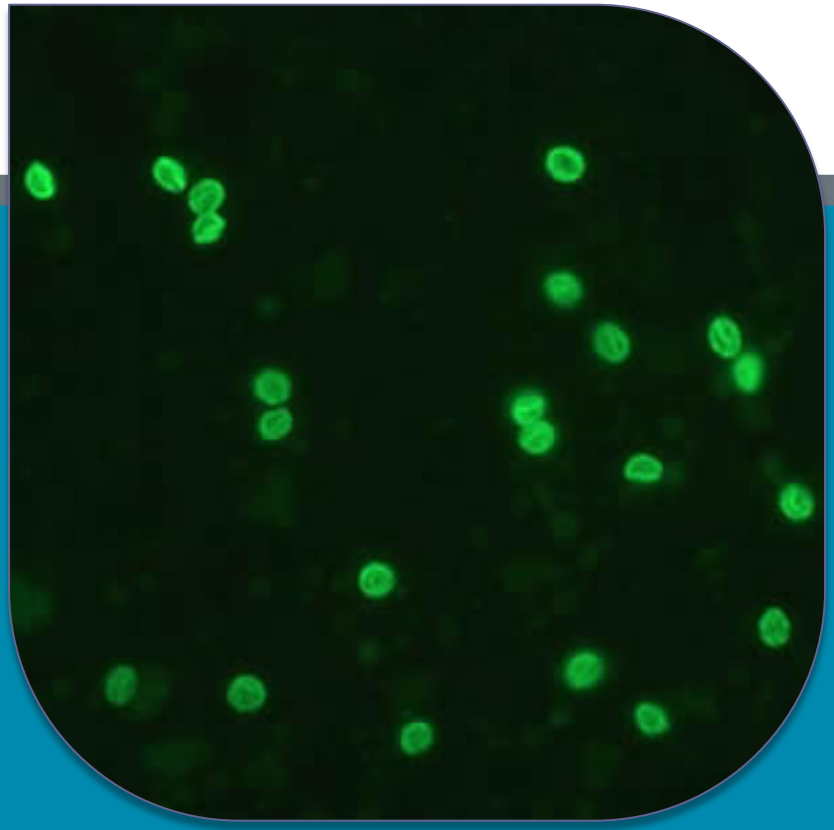


# *Cryptosporidium* Methods - Best Practices and Useful Tips

## Teaching Grandma to Suck Eggs...

Brett Brewin

IDEXX

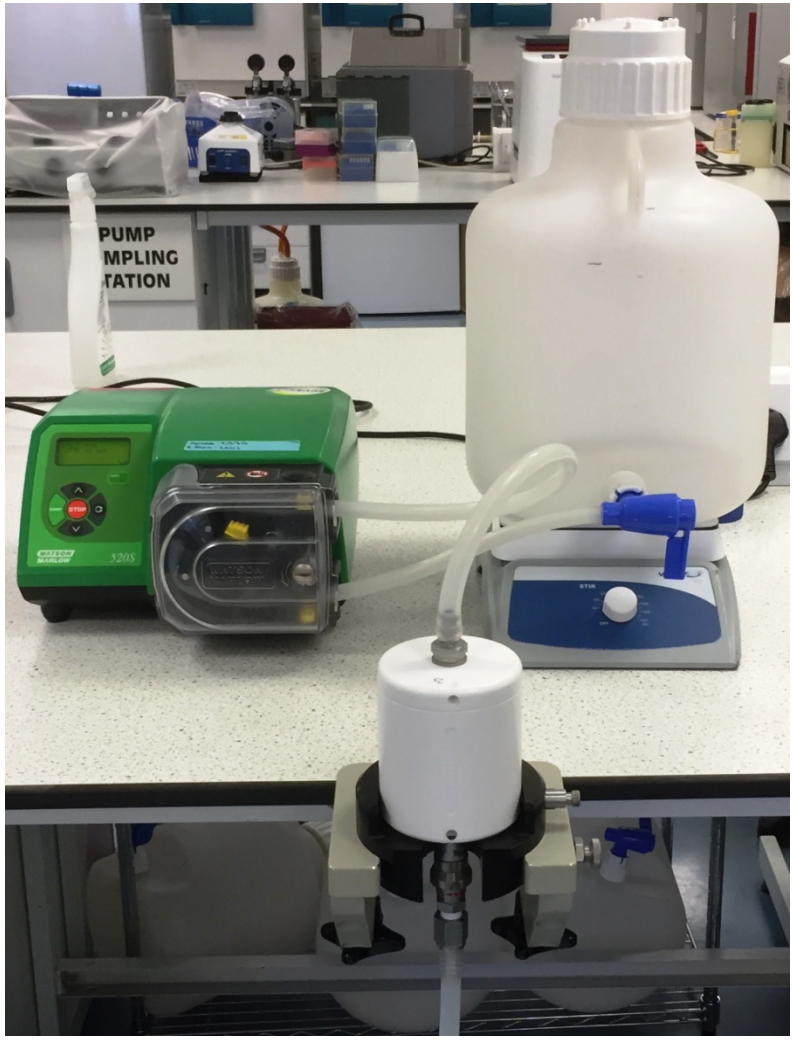
# Overview

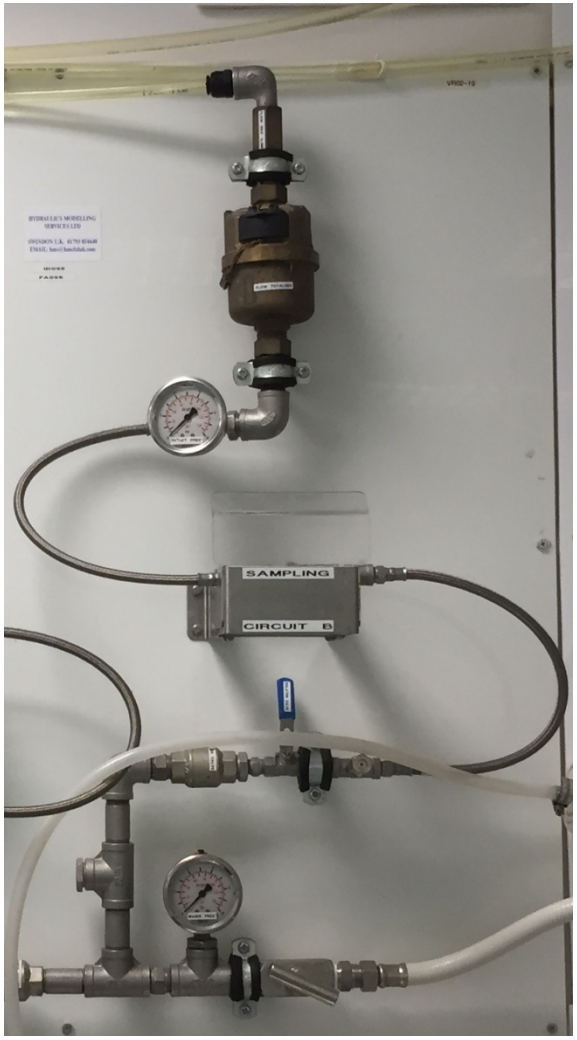
- Method Overview
- Sample Filtration
- Filter Elution
- Eluate Concentration
- Sample Purification
- Sample Staining
- A Word about Controls
- The Key to Consistency

# Method Overview

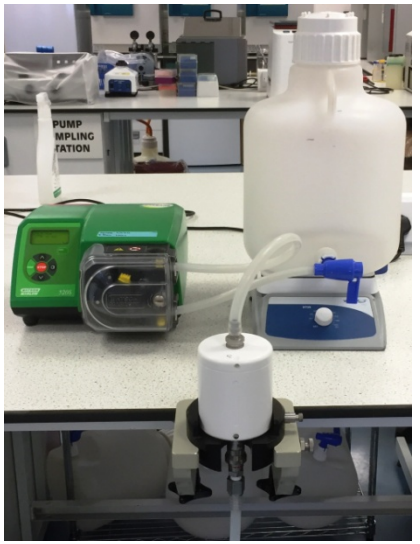
- Sample Collection
  - filtration (10L raw - 1000$^{+}$L finished)
- Filter Elution
  - volume reduced to 250-1200ml
- Eluate Concentration
  - volume reduced to ~10ml
- Target Isolation (Purification)
  - volume reduced to ~50-100µl
- Target Identification
  - microscopy (9-15mm well slide)

# Sample Filtration



# Sample Filtration



- Be consistent
  - volume, flow rate and pressure
- Keep equipment in good order
  - reusable or disposable?
- Check tubing for wear and tear (replace if required)
- Mix the sample continuously
- Rinse the sample container
  - 2 x 500ml is better than 1 x 1L (2 x 1L is even better)
  - DO NOT use rinses containing detergents

# DID YOU KNOW...

Your choice of sampling tubing can dramatically affect the performance of the method!

For example, (oo)cysts stick to peroxide cured but not platinum cured silicone tubing!

# Filter Elution

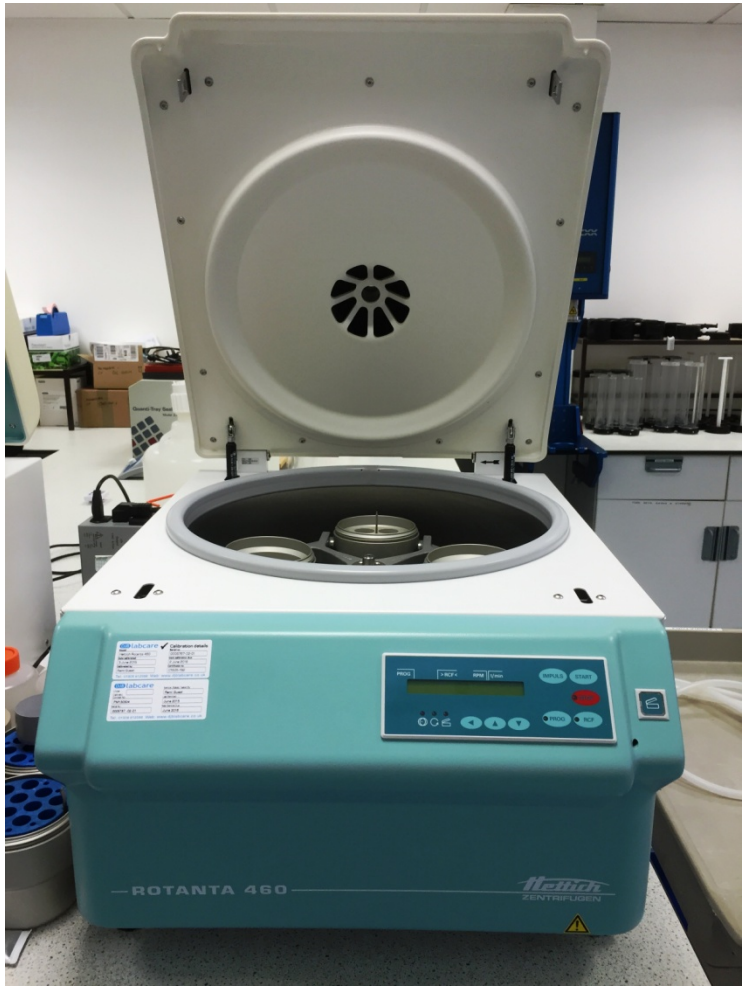
# Filter Elution

- Be consistent
  - automate where possible
  - use consistent methodology
- Buffer solutions
  - make them consistently
  - use pre-prepared reagents
  - make them regularly
  - use clean equipment (manufacture & storage)
  - keep them out of direct sunlight
- Maintain equipment appropriately

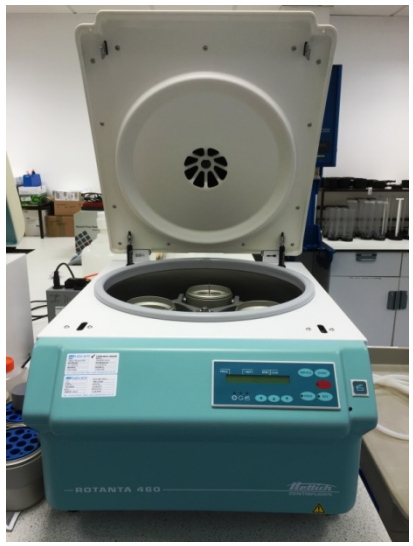
# DID YOU KNOW...

Buffer solutions can easily become contaminated with bacteria which can use surfactants as a carbon source!

# Eluate Concentration




# Eluate Concentration - Centrifugation




- Be consistent
- Location, location, location
  - solid ground and floor standing are best
- Maintenance
  - servicing but also general maintenance
- Balancing
  - balance tubes, supports & buckets & run a full rotor
  - don’t add liquid to the buckets
- Speed & time are critical (rpm ≠ rcf)
- Temperature affects centrifugation efficiency
  - for multiple runs consider refrigeration or run two units
- Don’t leave samples sitting after centrifugation

# DID YOU KNOW...

The zero brake setting on some centrifuges doesn't mean no braking!

With some models, the brake will engage initially and then cut out at a certain speed!

# Eluate Concentration - Aspiration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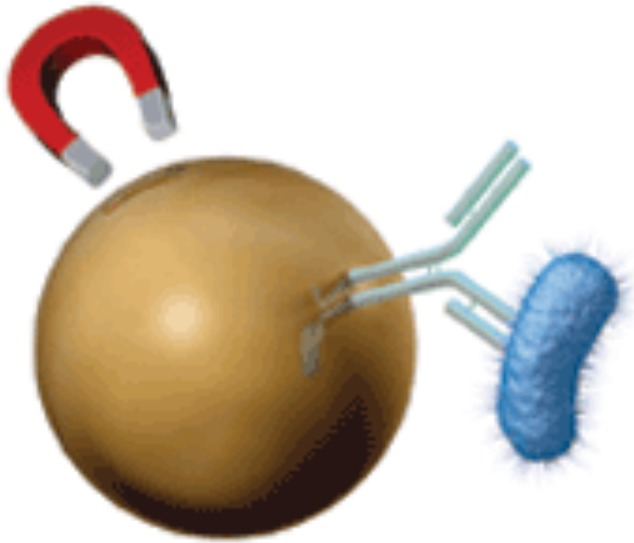
# Eluate Concentration - Aspiration


- Be consistent
  - maintain a consistent vacuum strength
  - limit aspiration rate
    - 200ml/min
- Keep it stable
  - don’t move too far
- Use wide bore pipette tips
- Keep the pipette at the surface of the liquid
- Keep your eye on the ball
- Don’t rush it

# DID YOU KNOW...

The use of wide-bore pipette tips can dramatically reduce the shear force during aspiration reducing the risk of pellet loss!

# Sample Purification - IMS

# Sample Purification - IMS


- Be Consistent
  - incubation time
  - rotation speed
  - rotation angle (it does matter)

- Leighton tubes
  - the flat side should be flat
  - don’t use tubes with a short window
  - don’t use them if they are cracked or crazed
  - replace caps regularly (make them disposable)

# Sample Purification – IMS cont...


- During transfer from the Leighton tube
  - use plastic Pasteur pipettes (not glass) for transfers
  - 3 transfer rinses are better than 2
  - be careful with the final volume
  - use low-adhesion micro-centrifuge tubes

- During magnetic separation
  - use the recommended magnets
  - ensure the magnets are in good condition
  - ensure the magnetic strip is in the correct position
  - keep things moving
  - aspirate quickly and consistently

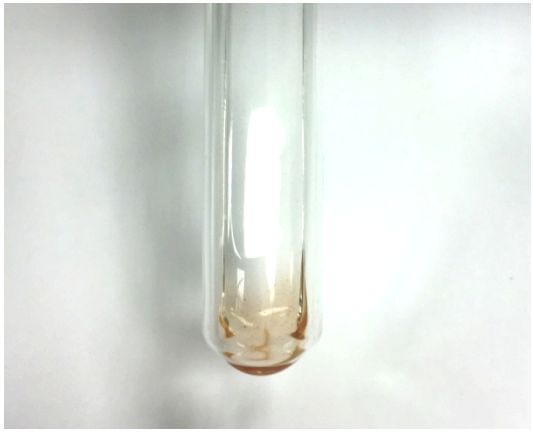
# Sample Purification – IMS cont...


- During acid dissociation
  - use standard solutions of acid and alkali
  - replace the solutions often
  - use reverse pipetting for small volumes
  - take care vortexing tubes (keep the beads at the base)

- Avoid a pH imbalance during double acid dissociation
  - there are several options
  - apply 5µl alkali to 50µl acid twice
  - apply the 10µl of alkali to 100µl of acid at the end
  - use two slides

# DID YOU KNOW...

When transferring a sample from a Leighton tube, a light coloured background can help you visualise the remaining beads!

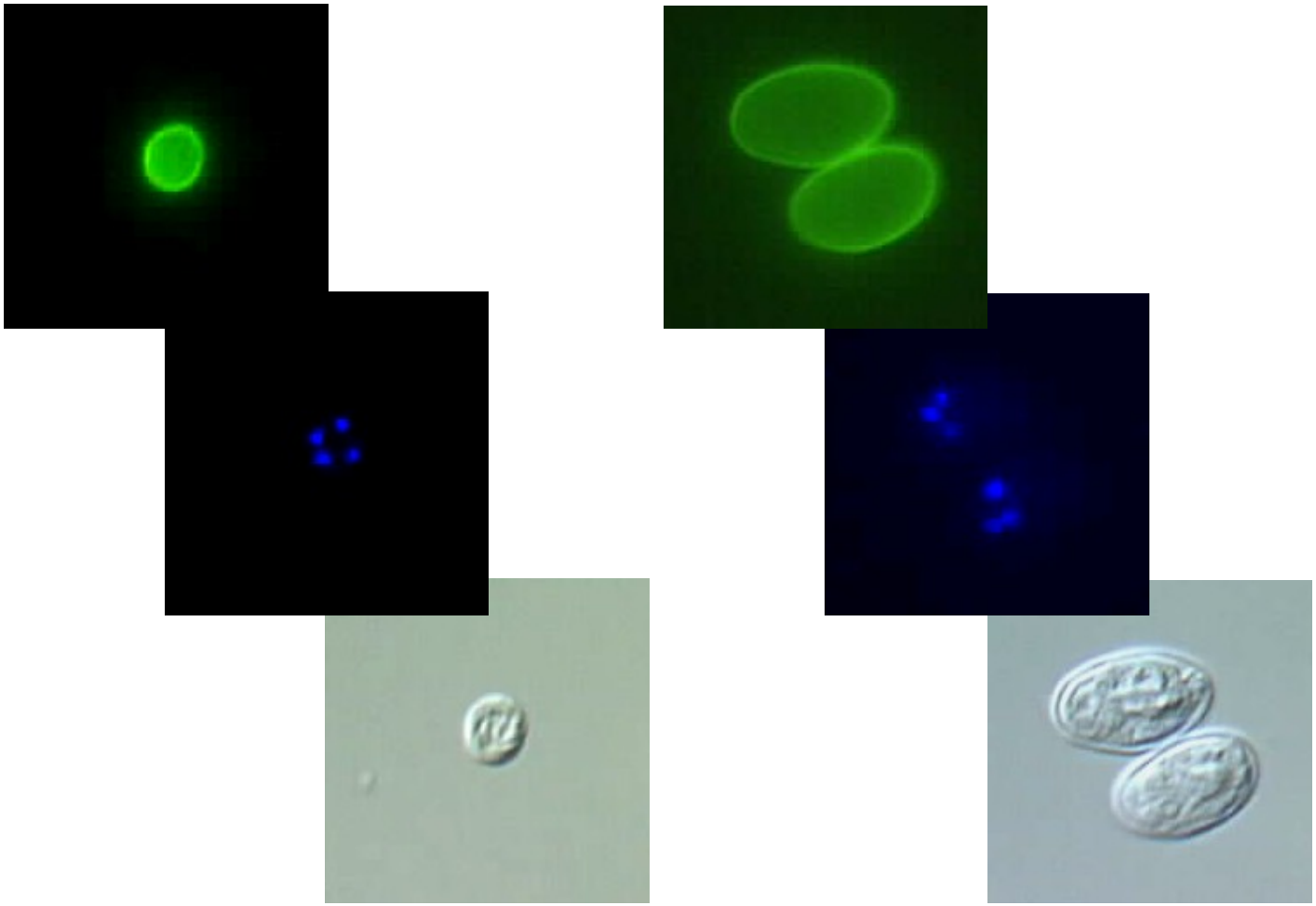


# DID YOU KNOW...

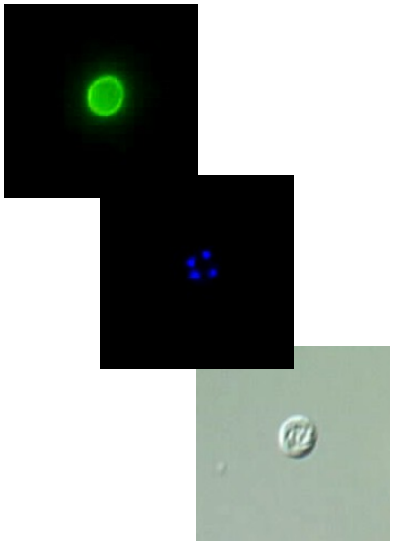
The acid is not responsible for acid dissociation!

The vortexing breaks the bond between the bead and the (oo)cyst, the acid just prevents the bond reforming!

# Sample Staining



# Sample Staining



- Be consistent
  - time and temperature affect staining and background
- Not all stains are created equal
  - always follow the manufacturer's instructions
- Angle slides to aid liquid removal
- Always use a pre-warmed humid chamber
- Consider using PBS rinses rather than reagent water rinses
  - particularly if it's in the instructions
- Dry slides down slowly*
  - be careful with slide warmers (some have hot-spots)
- Take care when mounting and sealing slides

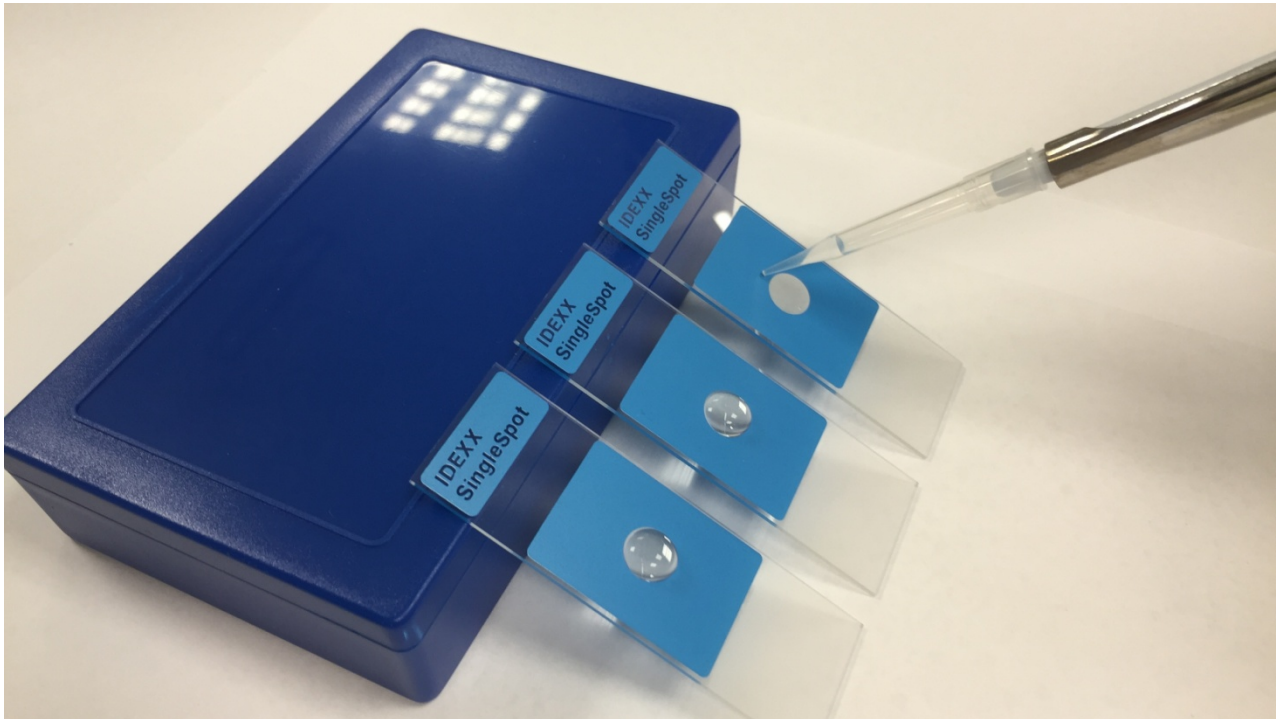
* personal preference is overnight at ambient temperature

# Sample Staining – Liquid removal



- Remove liquid as gently as possible
  - gentle aspiration
  - wicking method

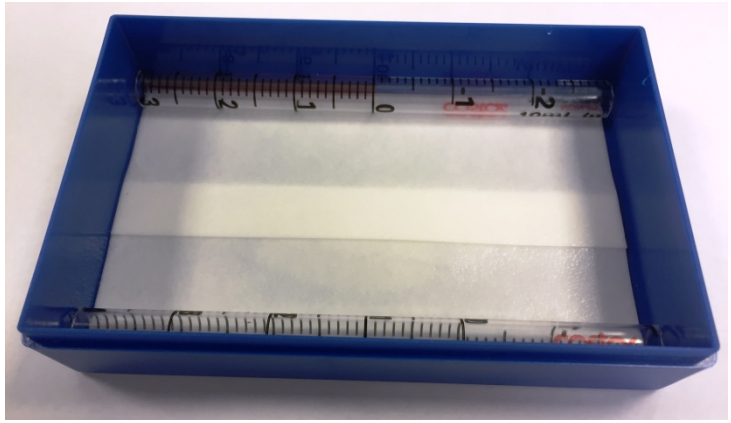
# Sample Staining – Liquid application




- Apply liquid as gently as possible
  - application from above the well lets gravity do the work and you don't need to change tips

# DID YOU KNOW...

Slide boxes make great humidity chambers and heat up quickly in an incubator!



# A Word about Controls

- Controls are important but they don’t tell you everything
- Reagent controls are important, but so are whole method controls
- Whatever you do with real samples, you should do with controls
- Spiking is key
  - if you don’t introduce all of the spike dose, you’re on a losing streak from the off
  - not all spikes are created equal – test them out

# The Key to Consistency

- If you want to have consistent results
  - YOU HAVE TO HAVE A CONSISTENT METHOD

- If you want analysts to perform consistently, give them the ability to do so:
  - have them concentrate on the right things
  - keep the controls & checks to an essential minimum
  - cut the paperwork & bureaucracy
  - don’t put quantity before quality

# Thank You!!!

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