Enzymatic Testing in a Wine Laboratory

What to look out for and how to do it well

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Webinar Formalities

- This Webinar is being recorded and will be published on the Enartis website
- Please refrain from using the chat box during the presentation, there will be 15 minutes for questions at the end of the presentation
- If you are having technical difficulties please use chat box 2, Whitney will be there to help
- Please complete the survey at the end of the webinar

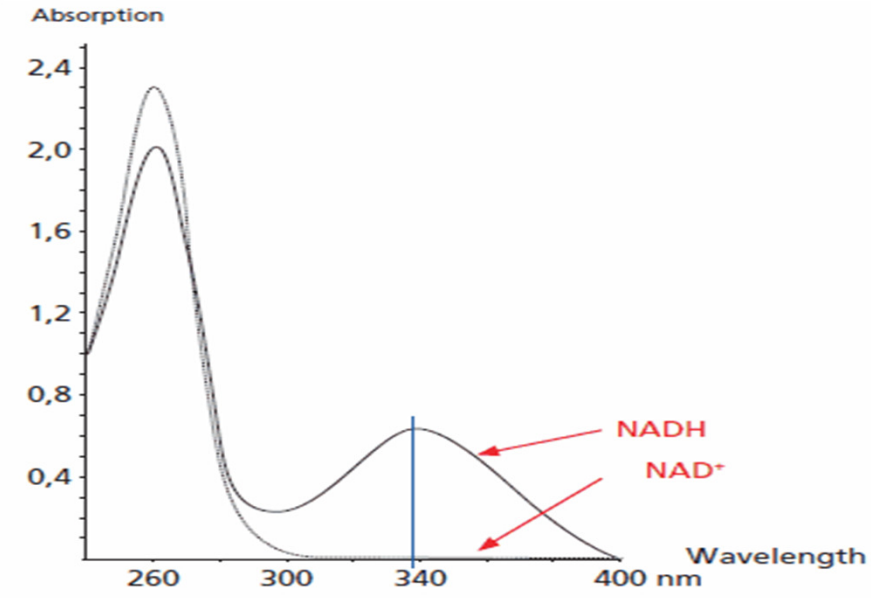
Enzymatic testing in wine

Malic Acid
Glucose and Fructose
Acetic Acid

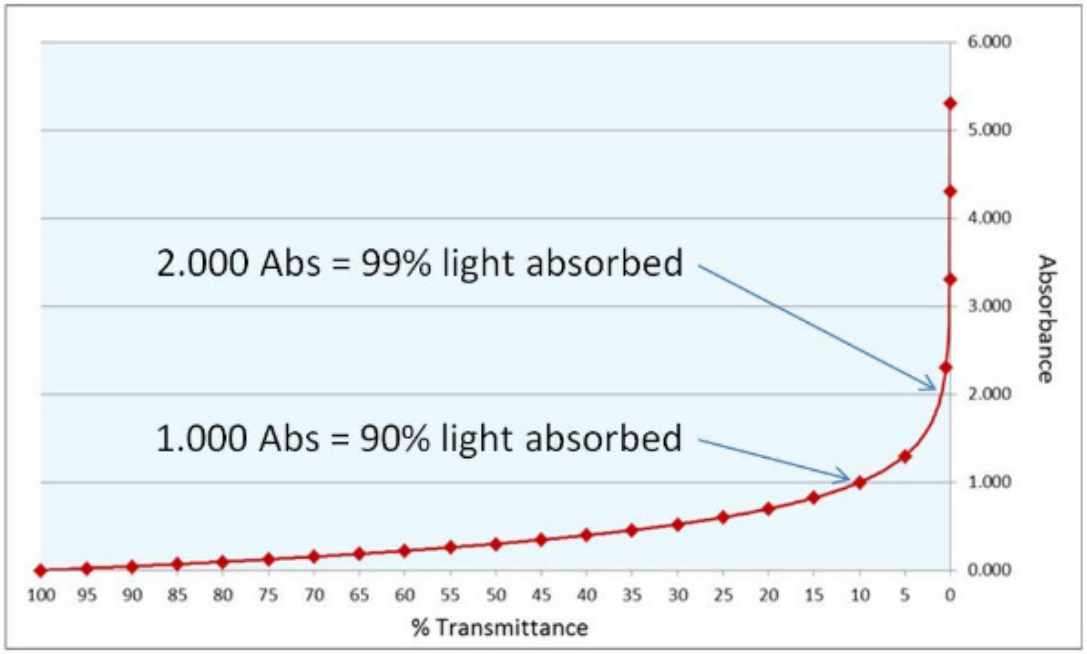
→ Reduction of NAD+ to NADH

Ammonia
Citric Acid

→ Oxidation of NADH to NAD+



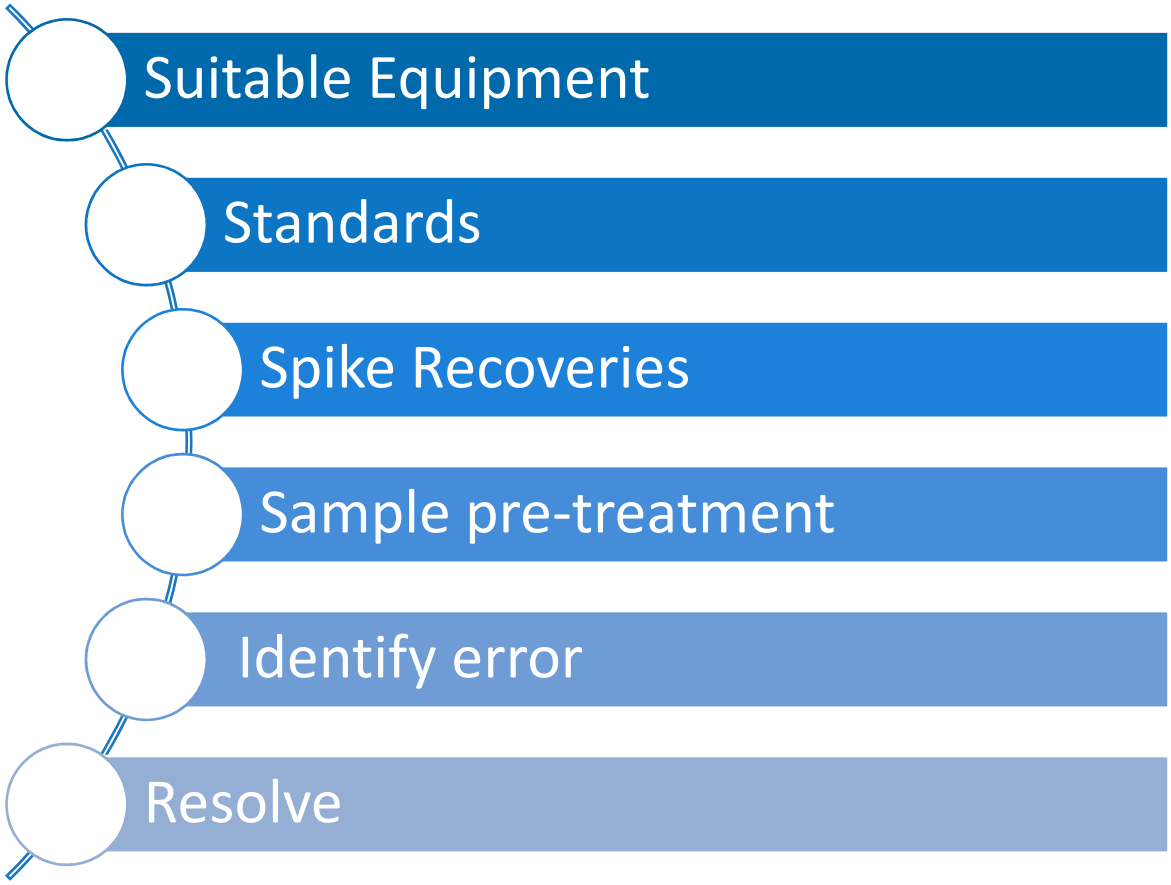
A closer look at absorbance…



Spectrophotometers measure the light that is **not** absorbed by the sample

<1.5 ABS

How to test wine *well*



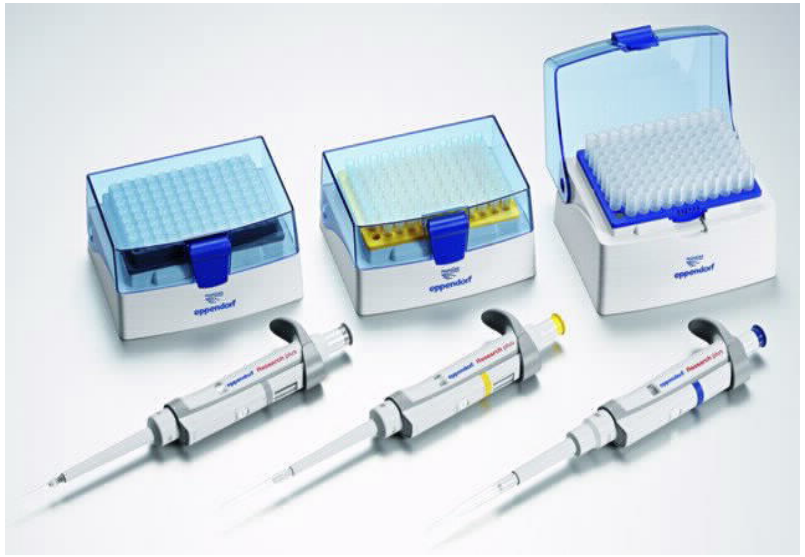
What are you trying to achieve?

Spectrophotometer

- Research thoroughly
- Calibrate as specified by manufacturer
- Follow a maintenance schedule
- Service as recommended

Pipettes

- Clean
- Calibrated and checked
- Handled appropriately
- Tips fit well and are clean



Enzymes/Kits

- Stored cold
- In date
- No mixing of batches
- Lids shut when not in use
- Suitable for desired outcome

Water source

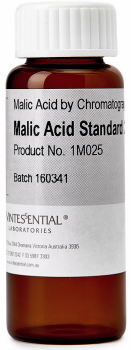
- Free of mould and metal contamination

Cuvettes

- Free of contamination
- Right path length
- No scratches/marks

Model Wine Solutions

- Model standard of the analyte
- Clear matrix
- Can include small amounts of ethanol
- Commercially available
- Easy to make your own water/analyte mix to desired concentration
- Used in spike recoveries



Secondary Wine Standards

- Ensures no matrix interference occurring
- Checks necessary pre-treatments are correct
- Choose a wine/s that represents a similar matrix and at a level of expected concentration of analyte
- Plot on a control chart to identify outliers and bias easily

Spike Recovery

Test: Malic acid determined by discrete analyser, calibration range 0 – 3g/L

Procedure: Analyse sample (**Y**)

Add 1mL of the top calibration standard (3g/L) to 4mL of sample

Analyse this spiked sample (**Z**)

Calculation: $$\% \text{ Recovery} = \frac{z}{(0.8 \times y) + (0.2 \times 3)} \times 100$$

Interpretation: For most tests, a recovery of between 95-105% is acceptable

✓**Measures trueness & identifies possible bias**

Pre-Dilution

- Review recommended dilution rates in instructions
- Dilute so sample concentration is under the upper limit of the kit/method
- **Don’t over dilute**
- Use the lowest possible dilution to reduce error
- Check accuracy of dilutions using standards

Degassing

Bubbles can cause..

- Reduced assay transmittance
- Sensing errors in DA's
- Random error in pipettes and DA probes occurring during aspiration

Decolourisation

- Coloured wines can reduce assay transmittance
- PVPP
- *May* get away with just pre-dilution (if needed as well)
- Avoid using alternative methods without trialling

Filtering

- Highly turbid wines reduce assay transmittance
- Solids block tubing and probes in DA'S
- Filter through 0.45um

$$\text{Concentration} = \frac{(\text{Final volume} \times \text{MW})}{(\varepsilon \times \text{light path} \times \text{sample volume} \times 1000)} \times \Delta A$$

✓ **Always perform a blank and a duplication!**

Watch out for **low recoveries**

- Kit expired or stored incorrectly
- Reagents too cold
- Working outside kit testing range
- Incorrect sample prep
- Short incubation time
- Incorrect reagent prep
- High blank recovery

Watch out for **high recoveries**

- Contamination (eg. Cuvettes, pipette tips)
- Incubation time incorrect
- Incorrect reagent prep
- Poor photometric accuracy
- Too much sample added

Watch out for **fluctuating absorbance**

- Incorrect sample pre-treatment
- Instrument faults
- Cuvette lined up incorrectly
- Unstable power supply

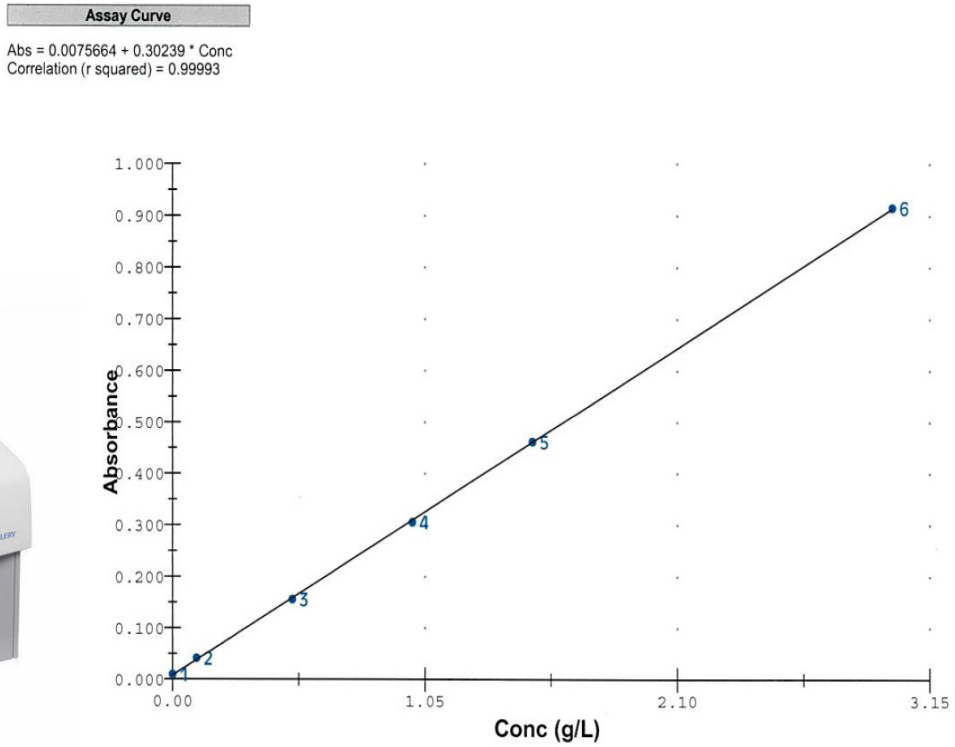
Watch out for **random error**

- Contaminated cuvettes
- Inaccurate pipetting
- Method discrepancies
- Cuvette facing wrong way
- Human error

Watch out for **bias**

- Poor kit storage
- Gradual contamination over time
- Pipettes dispensing high or low
- Pre-dilutions are inaccurate

The Automated Way



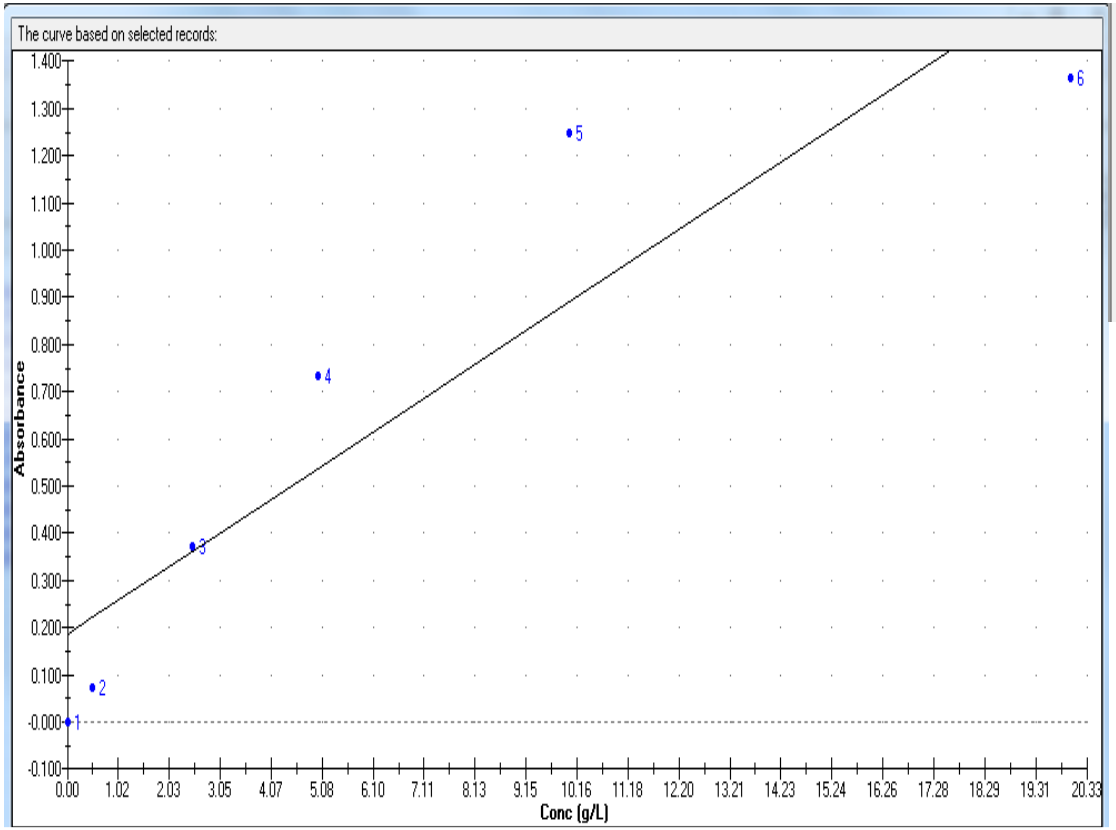
How to test well

- ✓Trial method first
- ✓Responses that range from 0 to 1.
- ✓Keep enzyme VS sample ratios the same
- ✓Calibrate before every run
- ✓Analyse your data
- ✓Clean routinely

Watch out for…

- ✗ Mixing old reagents with new
- ✗ Evaporation of reagents
- ✗ Contamination
- ✗ Poor calibrations

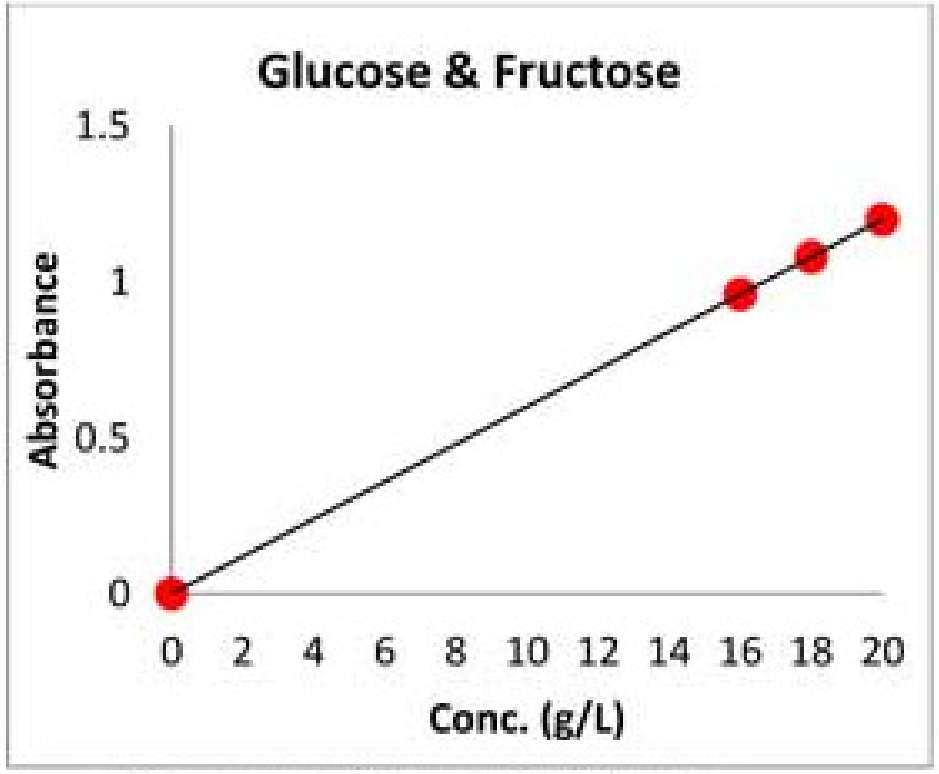
Watch out for…



Name	Absorbance	Concentration	Interpretation	Note	Referen
22/05/2018 2:55:40 PM					
Vint Comb STD 1	0.000 Abs => 0.000 Abs	0.00 g/L => 0.00 g/L			0.00
Vint Comb STD 1	-0.003 Abs => -0.003 Ab	0.00 g/L => 0.00 g/L			0.00
Vint Comb STD 2	0.073 Abs => 0.073 Abs	0.00 g/L => 0.00 g/L			0.50
Vint Comb STD 2	0.076 Abs => 0.076 Abs	0.00 g/L => 0.00 g/L			0.50
Vint Comb STD 3	0.381 Abs => 0.381 Abs	2.76 g/L => 2.76 g/L			2.50
Vint Comb STD 3	0.360 Abs => 0.360 Abs	2.46 g/L => 2.46 g/L			2.50
Vint Comb STD 4	0.758 Abs => 0.758 Abs	8.13 g/L => 8.13 g/L			5.00
Vint Comb STD 4	0.709 Abs => 0.709 Abs	7.44 g/L => 7.44 g/L			5.00
Vint Comb STD 5	1.250 Abs => 1.250 Abs	15.15 g/L => 15.15 g/L			10.00
Vint Comb STD 5	1.245 Abs => 1.245 Abs	15.08 g/L => 15.08 g/L			10.00
Vint Comb STD 6	1.363 Abs => 1.363 Abs	16.76 g/L => 16.76 g/L			20.00
Vint Comb STD 6	1.368 Abs => 1.368 Abs	16.83 g/L => 16.83 g/L			20.00

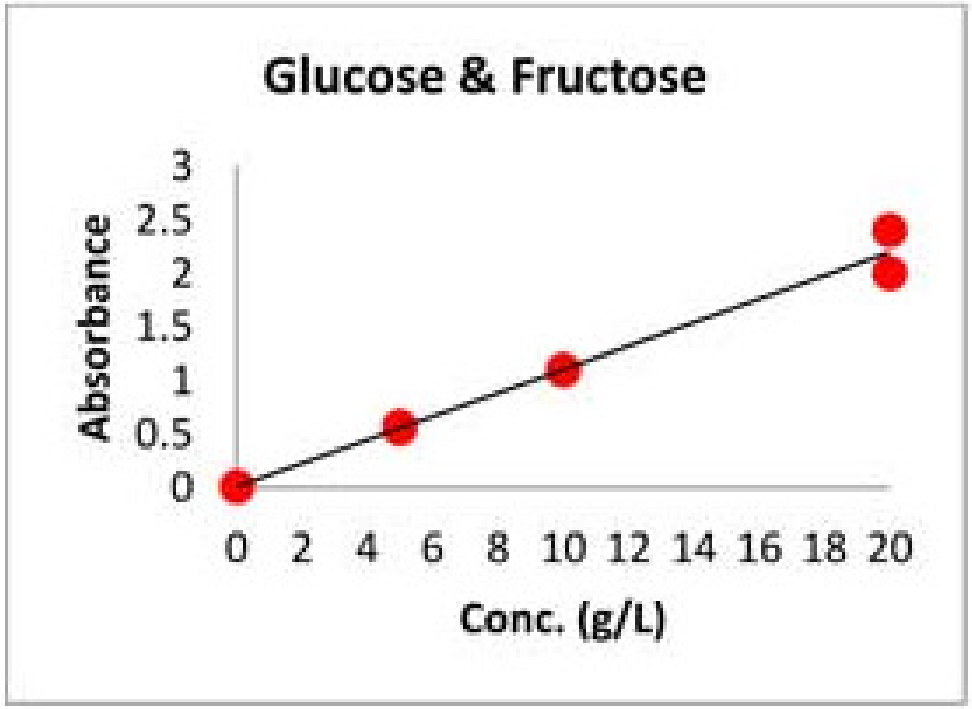
✓ **See solutions for low recoveries**

Watch out for…



✓ **Trial any new methods**

Watch out for…



✓ **Remember, <1.5 ABS**

What should you do if you get a poor calibration?

- Re calibrate!
- Omit large single outliers
- Ensure reagents are loaded into the right position
- Check you're using the right method
- Investigate enzymes/kit

Conclusion

The right results the first time!

- Suitable Equipment
- Standards
- Spike Recoveries
- Sample pre-treatment
- Identify error
- Resolve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