Gentra Autopure LS Technology Assessment

Andrew Wallace
NGRL (Manchester)

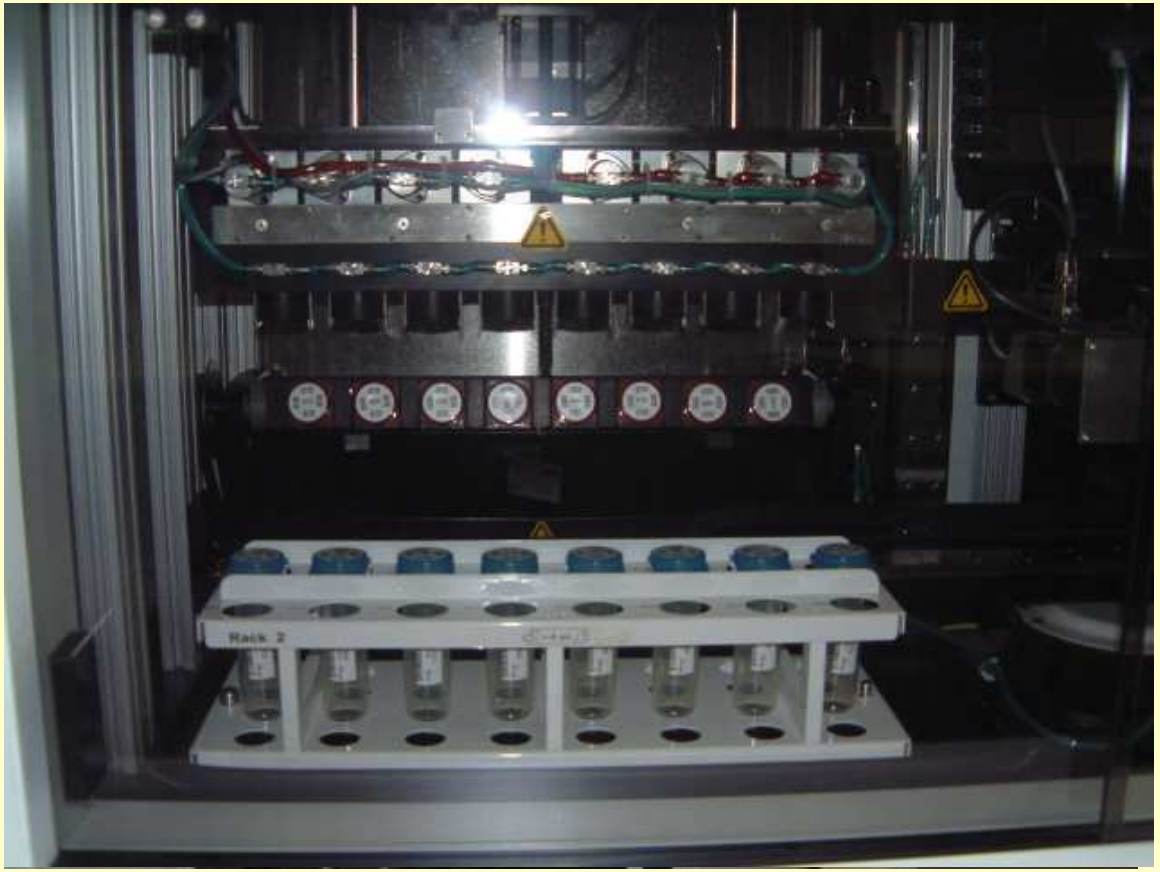
The Gentra Autopure LS

- High throughput, large scale, automated DNA extractor
- Uses PureGene® chemistry
- Accepts blood samples from 1-10mL
- Two extraction scales
  - 1 – 5mL
  - 5 – 10mL
- 8 or 16 sample batch size
- Process up to 96 samples per 8hr day (1 Technician) in batches of 16 (24000 pa!)
- 80 mins per batch of 16 samples

The Gentra Autopure LS

- Cost of instrument £135,000
- Cost of consumables ~ £10 per sample (inc. plasticware)
- Yield of DNA claimed up to 500μg from a 10mL sample
- Fully automated (blood > DNA in TE) with onboard centrifuge and robot arm
- Reagents continuously adjusted to blood volume
- Barcoded internal sample tracking

The Gentra Autopure LS



The Gentra Autopure LS

- Purchased in March 2002
- In service from April 2002 for all blood samples >1ml
- Year ending 31/12/2003 lab received 4575 samples
- 2587 were blood samples (56.5%)
- 2105 were processed on Gentra (81.3%)

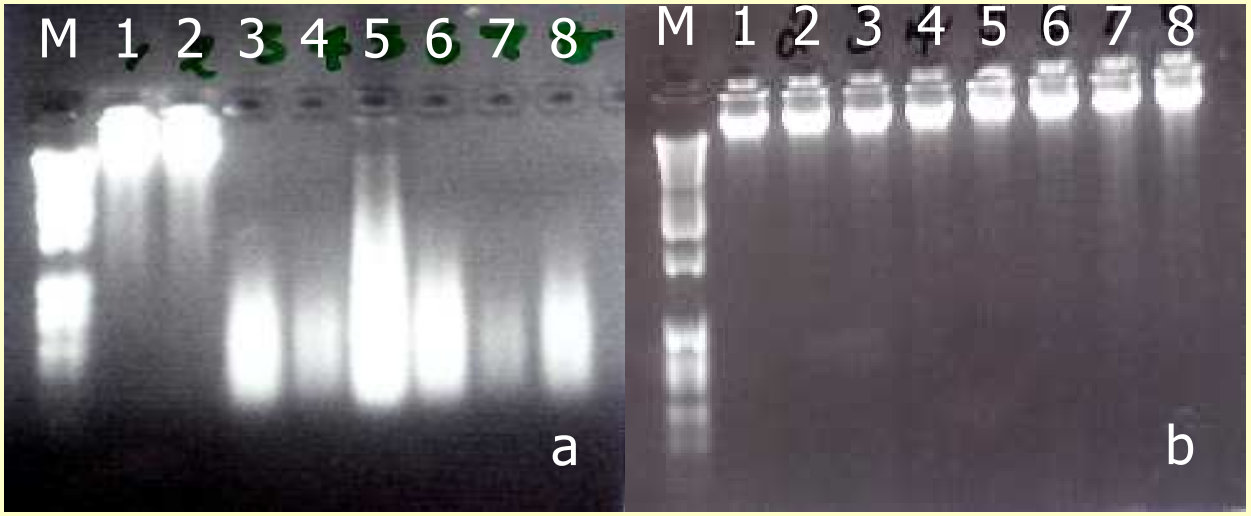
Problems (i)

Intermittent sample failure
Failure to lyse

- **Traced problem to use of NaEDTA as anticoagulant**
- **Leads to slower lysis**

Problems (ii)

Spontaneous degradation



Samples tested by Microbiology lab
Identified coliform bacteria in affected samples and reagents
Poor housing of instrument
Low turnover of reagents

Measuring Performance

Retrospectively audited performance over a 6 month period from July – Dec 2003

During this period 110 extraction runs were carried out on 863 samples with a 1.2% failure rate

We focussed in on 201 samples selected at random spread evenly throughout the survey period

Measured the following parameters:

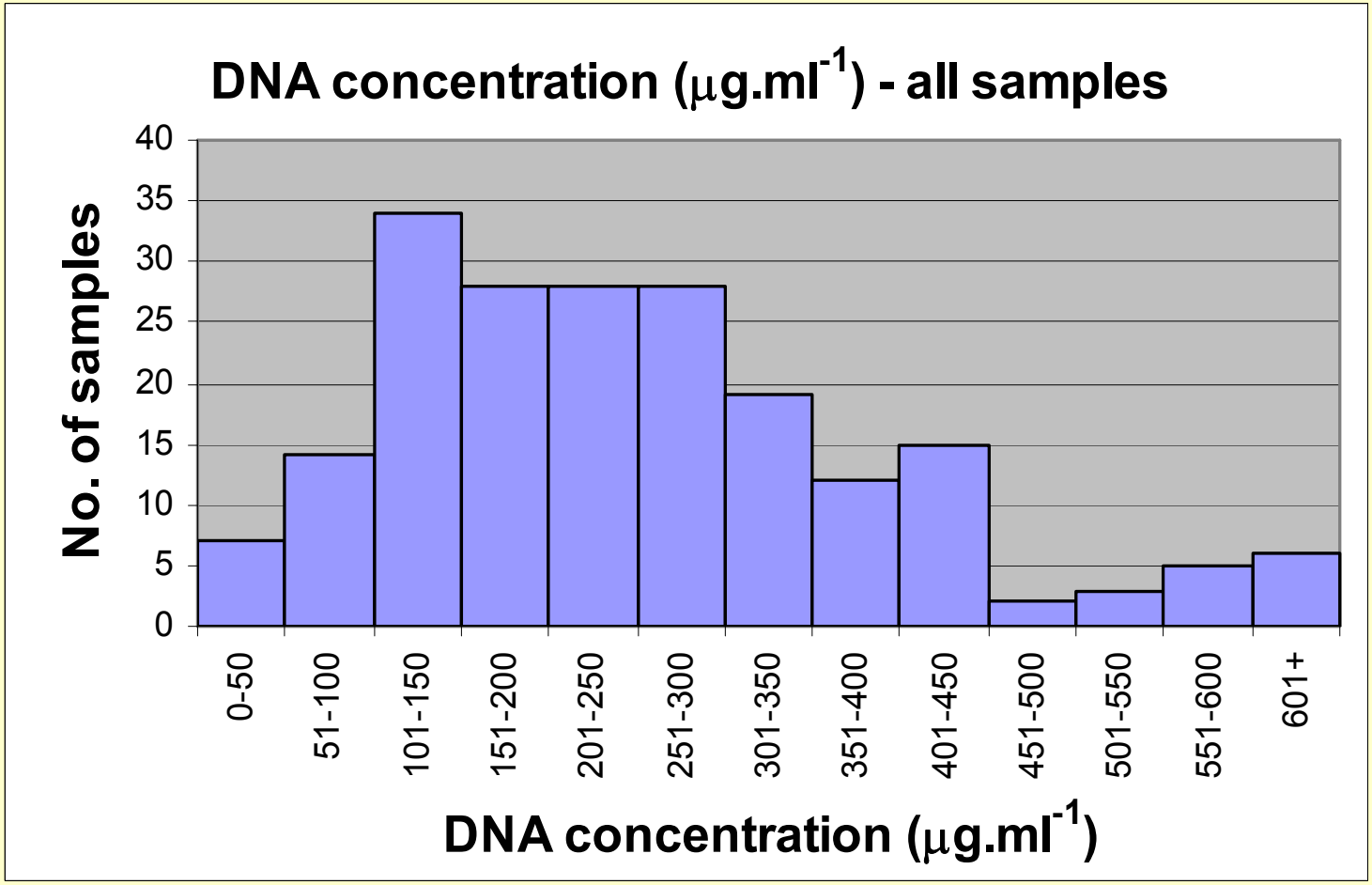
- Extraction success/failure (5μL agarose gel electrophoresis)
- DNA concentration
- DNA yield per ml of input blood
- DNA purity (260/230 & 260/280 ratio)

Used a calibrated UV spectrophotometer

Developed an SOP – deposited on website

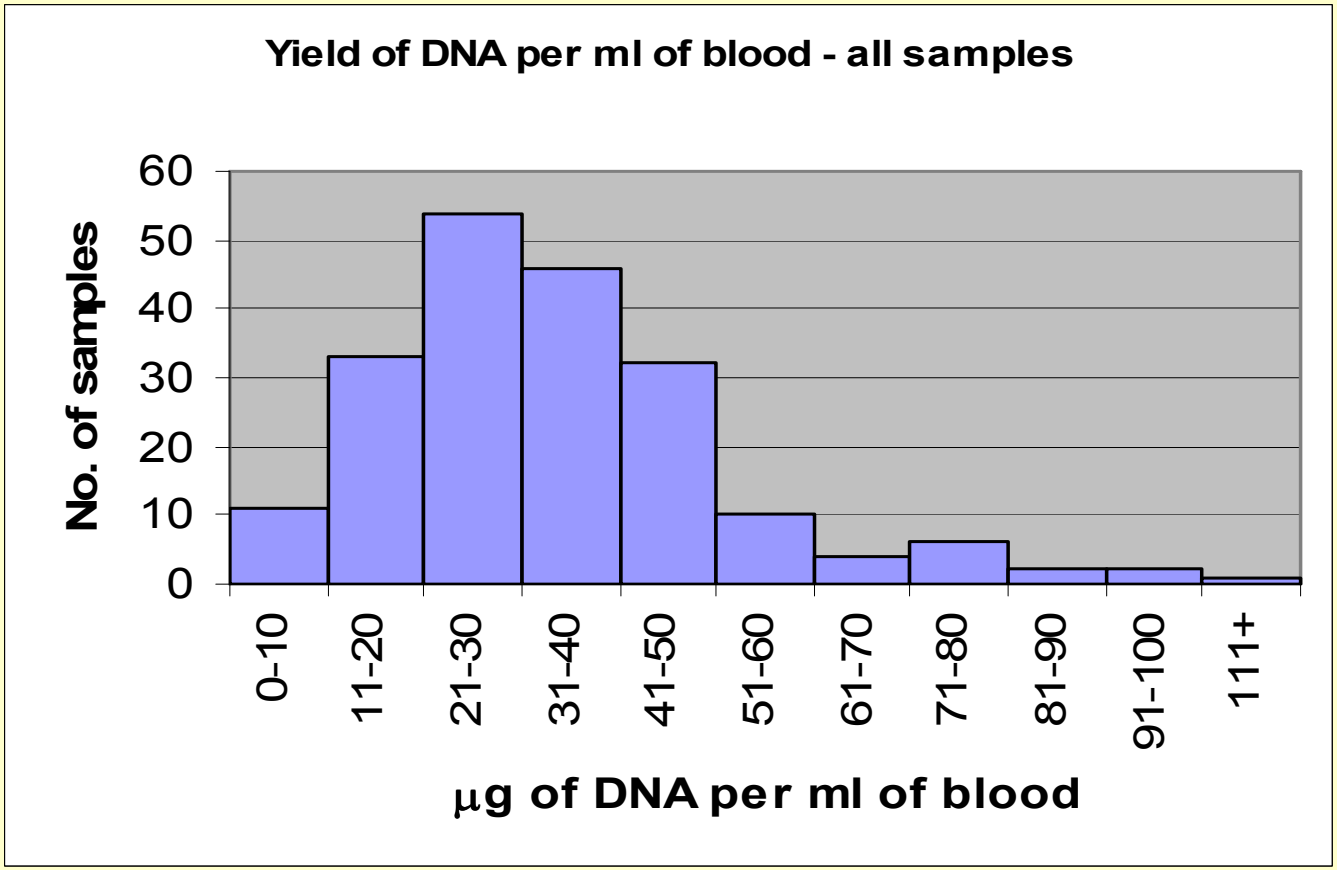
All measurements corrected for scattered light at 320nm

Variation in Output DNA Concn



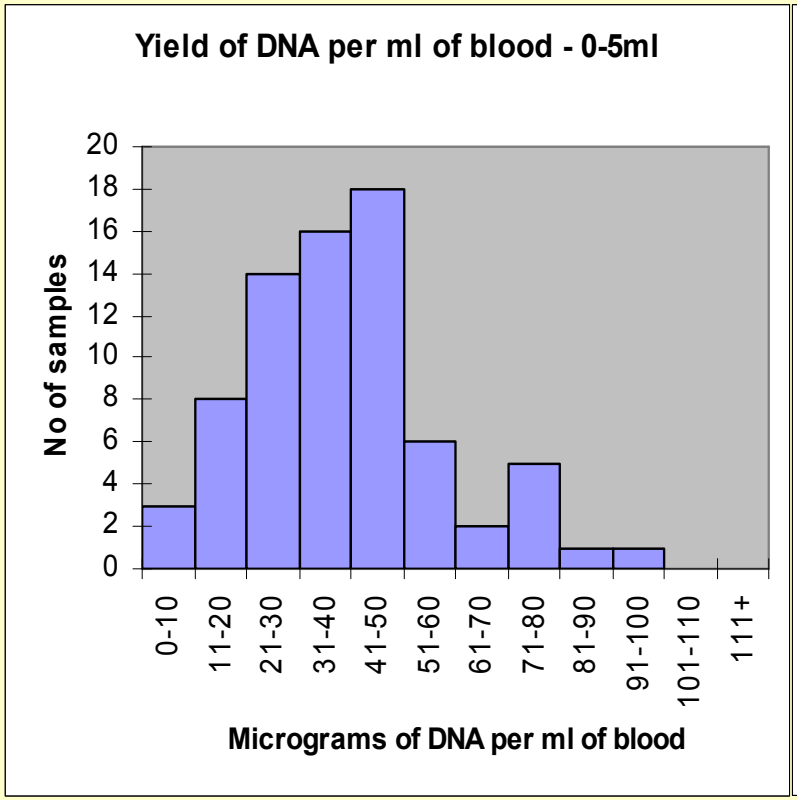
Mean 257μg.ml^{-1}; Median 239μg.ml^{-1}

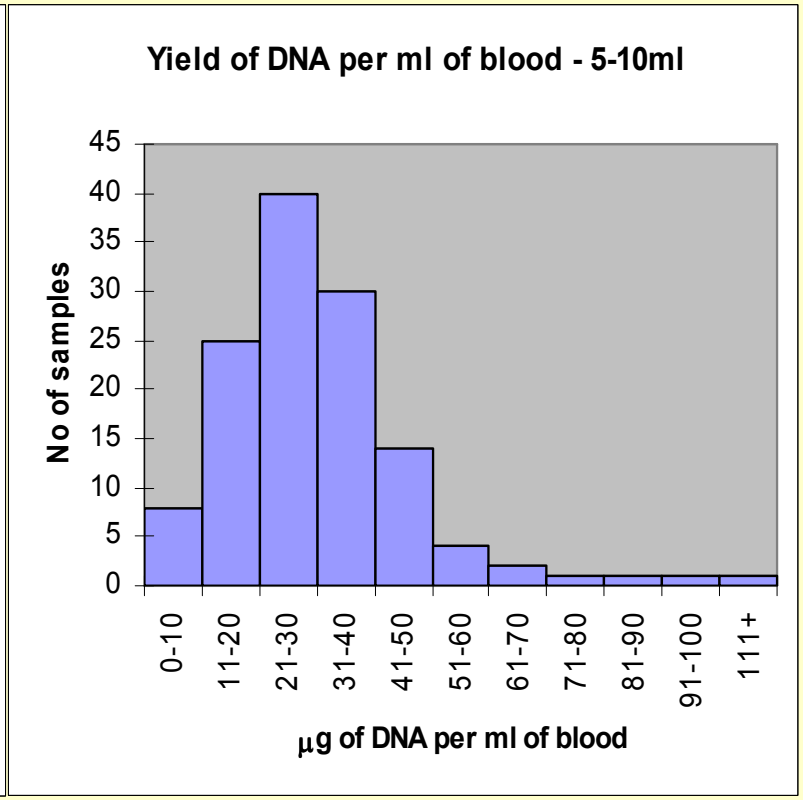
Variation in Yield of DNA



Mean 33.6µg; Median 31.2µg

Difference Between Extraction Scales

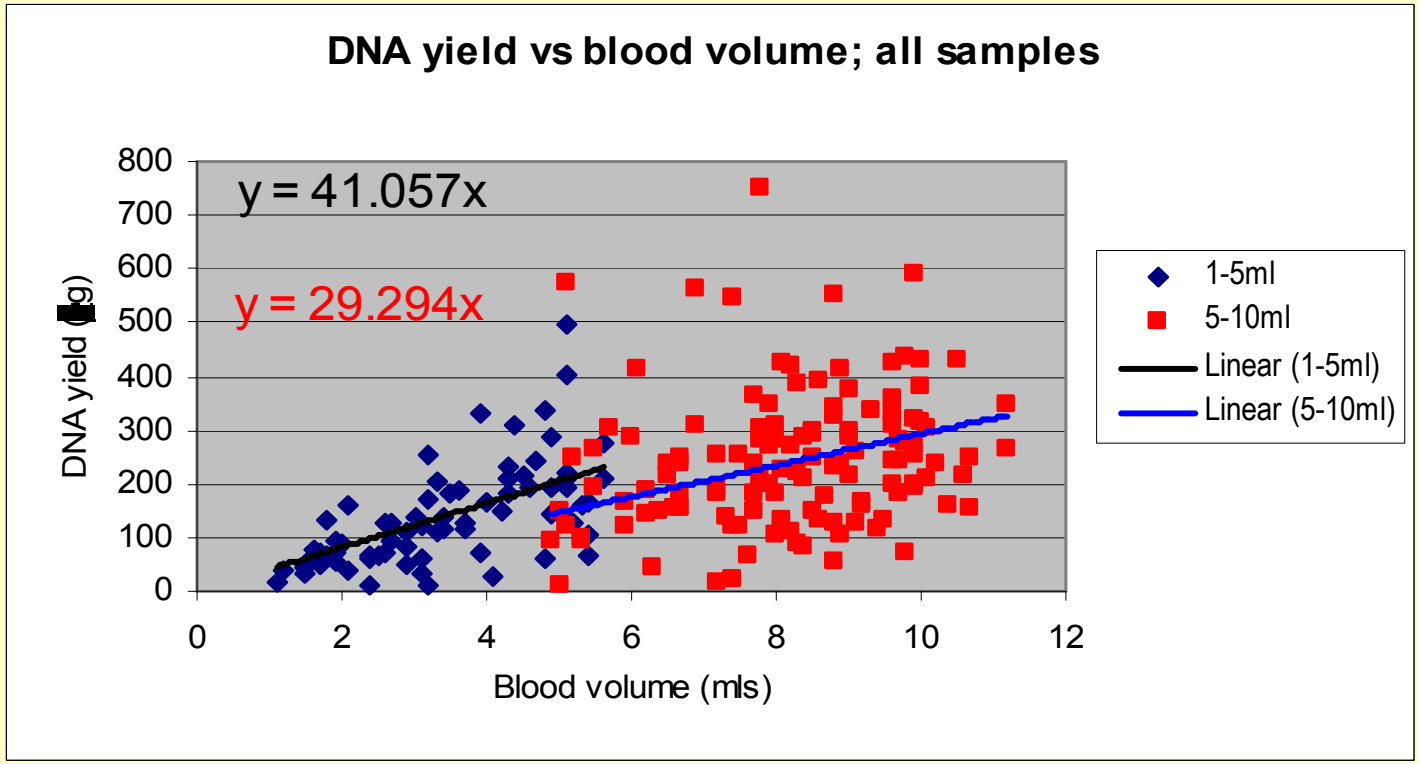




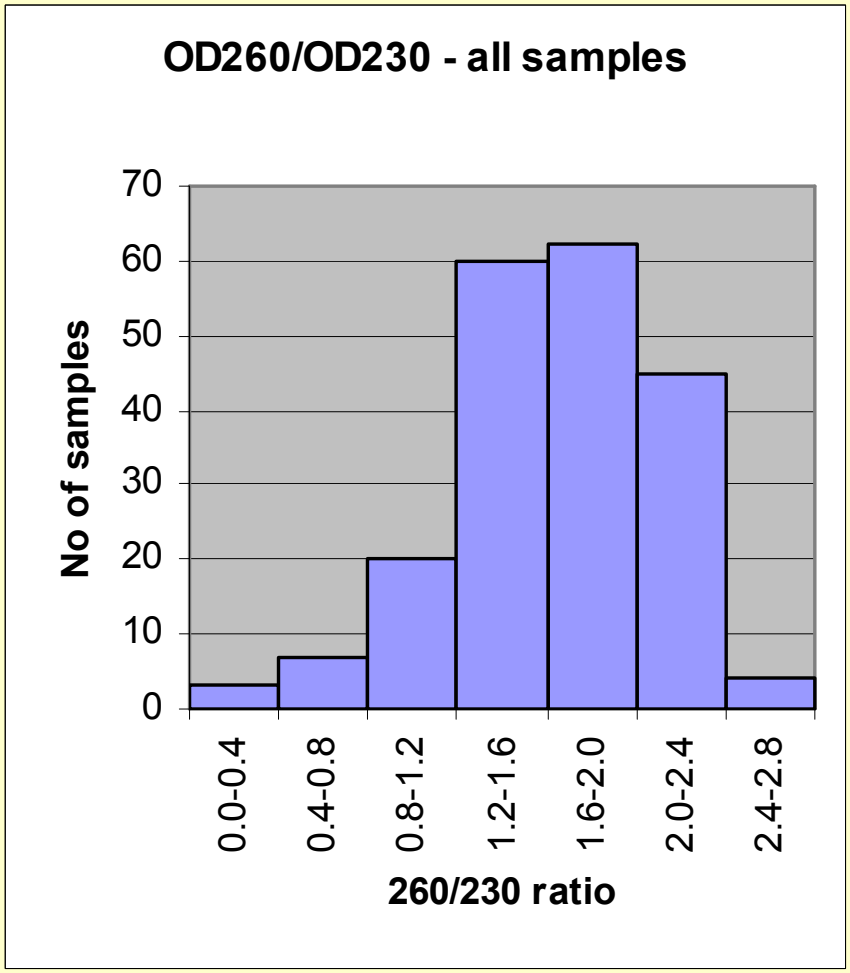
Mean 39.4µg; Median 38.1µg Mean 30.2µg; Median 27.9µg

Z-test P<0.0006

DNA Yield vs Blood Volume

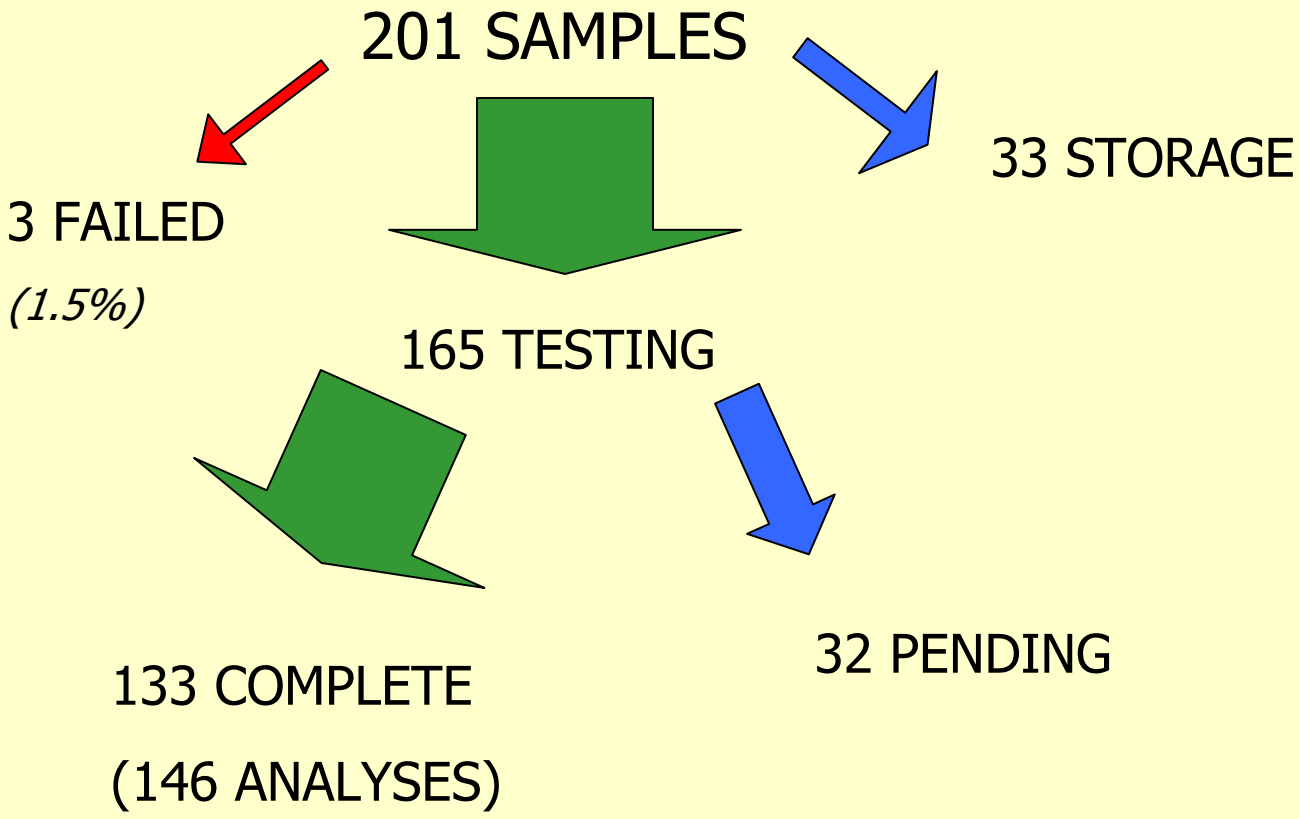


OD260/OD230 Ratio

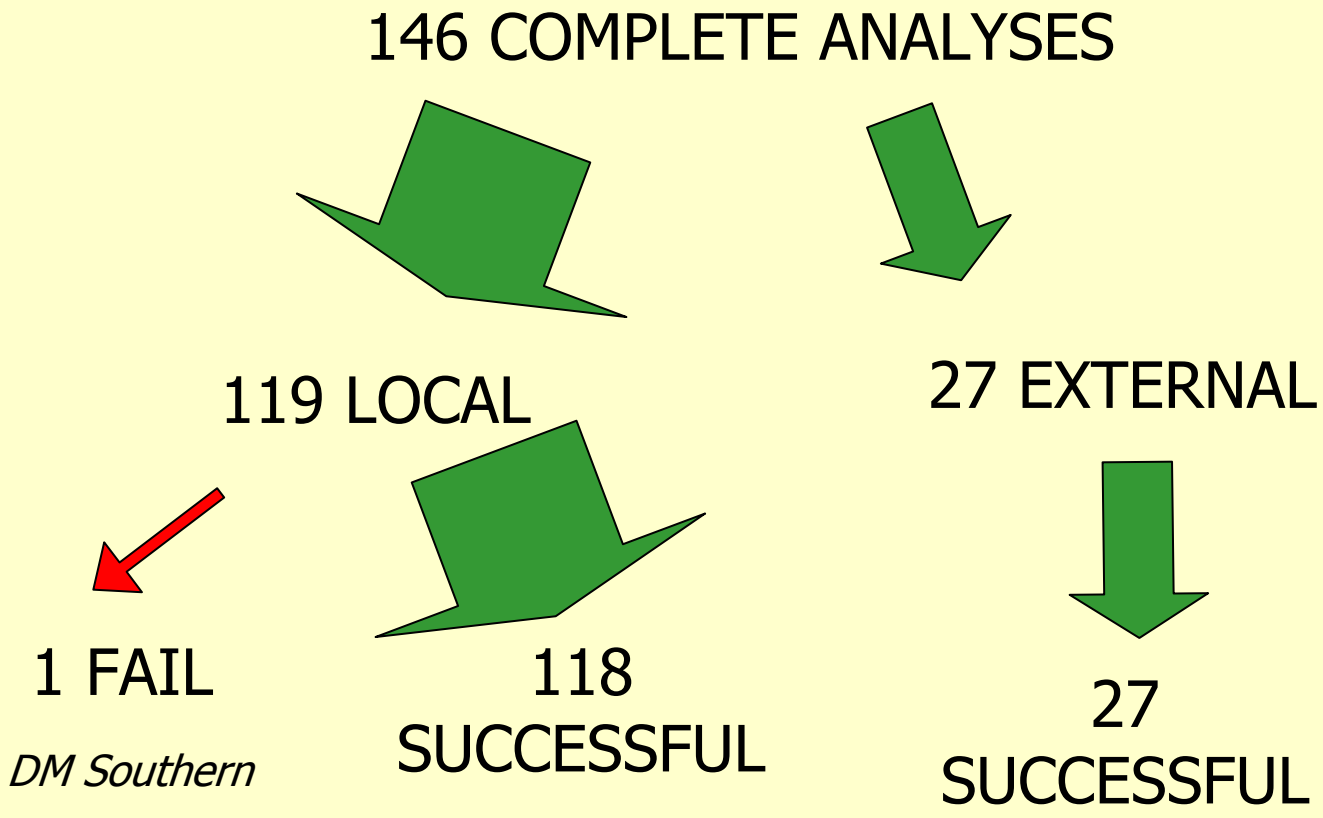


- Pure Nucleic Acids 2.0-3.0
- Mean 1.64
- 24.4% > 2.0
- Some protein present in most samples although not severe

Performance in DNA Testing



Performance in DNA Testing



Types of Successful Tests

Disease	Test	Local/Export	No Tests
FraX	PCR	Local	40
HD	PCR	Local	13
CMT	Dosage PCR	Local	12
DM	PCR	Local	5
FraX	Southern	Local	5
SCA	PCR	Local	5
AS/PWS	Bisulphite PCR	Local	4
DMD	Dosage PCR	Export	3
Friedreich's	PCR	Export	2
SMA	Dosage PCR	Export	2
BRCA1/2	MLPA	Local	1
DM	Southern	Local	1
Others	Various	Local/Export	53

Conclusions

- Instrument integrates well
- Acceptable failure rate ~2%
- High capital & consumable cost
- Some issues e.g. variable output DNA concentration unsuitable for some analyses
- DNA may be unsuitable for assays needing v. high mol wt DNA e.g. mtDNA, FSHD

Acknowledgements

- Simon Ramsden
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