DNA Measurements in Disposable Cuvettes vs a Micro-volume Spectrophotometer

There are numerous micro-volume spectrophotometers on the market that measure DNA using sample volumes as low as 1µl. However the sample handling technology required to measure these small volumes also means that these instruments are inherently less accurate and less reproducible than a conventional spectrophotometer with a cuvette because of the reduced pathlength required to measure these small volumes.

Concentration of DNA in ng/µl is calculated by taking the Absorbance at 260nm and multiplying by a factor of 50. This calculation is valid when a measurement is carried out at a 10mm pathlength.

To be able to measure very small volumes the micro-volume spectrophotometers typically have pathlength of 1mm, 0.5mm, 0.2mm or less. Therefore to calculate the concentration of DNA the measured Absorbance at 260nm is normalised to a 10mm pathlength.

Pathlength	Normalisation Factor
1mm	10
0.5mm	20
0.2mm	50
0.1mm	100

It is not only the Absorbance from the sample that is multiplied by the normalisation factor but also any measurement system noise which can lead to less reproducible measurements.

The other disadvantage of shorter pathlength instruments is seen when measuring low concentration samples - at the low Absorbance being measured the noise becomes a higher proportion of the measured signal resulting in less accurate measurements

For example: if we measure a 10ng/µl DNA sample in a 10mm pathlength cuvette the Absorbance at 260nm is:

10/50 = 0.2 Absorbance

The same sample measured using a micro-volume instrument with a 1mm pathlength the Absorbance would be:

(10/50)/10 = 0.02 Absorbance

Assuming that the inherent noise of both instruments is the same, 0.002A, this accounts for a 1% error on the instrument with a 10mm pathlength cuvette but a 10% error on the 1mm pathlength micro-volume instruments. This % error increases as the concentration decreases. This noise/pathlength normalisation effect also explains why an instrument with a 10mm pathlength cuvette has a lower detection limit (0.5ng/µl) in comparison to a micro-volume instrument (reported as 2-5 ng/µl).

10mm pathlength cuvettes are available that will work with 10µl of sample however many users find these cuvettes difficult to use and prefer disposable cuvettes of a higher volume. The attached data shows the comparison of 70µl disposable UV plastic cuvettes in a Biowave II compared to a leading dedicated micro-volume instrument.

Detection limit

The detection limit of the system is determined by the underlying noise and is calculated by multiplying the Standard Deviation of a set of readings by a factor of 3. For this experiment a buffer solution was used as a blank and then 10 repeat measurements were made of the same solution. The experiment was then repeated with 10 different disposable cuvettes to determine variation between cuvettes.

Replicate	Biowave with 70µl cell (ng/µl)	Biowave with 10 different 70µl cell (ng/µl)	Micro-volume spec (ng/µl)
1	0.0	0.1	-0.3
2	-0.05	0.15	-0.5
3	-0.05	0.15	-0.7
4	-0.1	0.2	-0.3
5	-0.05	0.6	-0.6
6	-0.05	0.15	-0.6
7	-0.05	0.15	-0.7
8	-0.05	-0.05	-0.4
9	-0.1	0.3	-0.4
10	0.0	0.25	-0.4
Average	**-0.05**	**0.20**	**-0.49**
Std Dev	**0.0333**	**0.1683**	**0.1524**
%CV	**-66.67%**	**84.16%**	**-31.10%**
Detection Limit	**0.1 (ng/µl)**	**0.5 (ng/µl)**	**0.5 (ng/µl)**

Conclusion

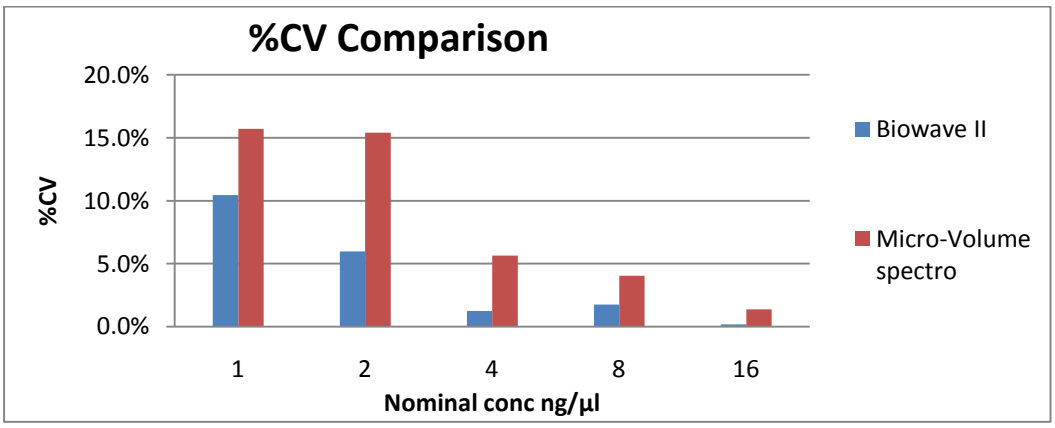
The above experimental data confirms that a 10mm pathlength cuvette provides a lower detection limit than a leading micro-volume instrument. Even when using 10 different disposable cuvettes the detection limit matches that of the micro-volume instrument.

Repeatability at low concentrations

To determine the repeatability of measuring low concentrations of DNA samples on the Biowave II and the micro-volume spectrophotometer, 5 replicates of each of the following concentrations were measured.

Biowave II with 70µl disposable cell						
Nominal Concentration (ng/µl)	**0.5**	**1**	**2**	**4**	**8**	**16**
1	0.25	0.8	2.25	4.6	9.3	19.8
2	0.2	0.75	2.05	4.5	9.15	19.75
3	0.25	0.7	2.05	4.65	9.05	19.75
4	0.2	0.7	1.95	4.55	8.95	19.75
5	0.2	0.6	1.95	4.55	8.9	19.7
Average	**0.22**	**0.71**	**2.05**	**4.57**	**9.07**	**19.75**
Std Dev	**0.027386**	**0.0742**	**0.1225**	**0.057**	**0.16047**	**0.035**
%CV	**12.45%**	**10.45%**	**5.97%**	**1.25%**	**1.77%**	**0.18%**

Micro-volume spectrophotometer						
Nominal Concentration (ng/µl)	**0.5**	**1**	**2**	**4**	**8**	**16**
1	0.2	0.8	1.8	4.2	9.3	20.4
2	0.6	0.8	1.8	4	9.6	20
3	0.8	1	2.1	4.4	9.2	20.1
4	0	0.8	2.5	4.1	8.8	19.8
5	0.3	1.1	1.8	4.6	8.7	19.7
Average	**0.38**	**0.9**	**2**	**4.26**	**9.12**	**20**
Std Dev	**0.319374**	**0.1414**	**0.3082**	**0.2408**	**0.37014**	**0.274**
%CV	**84.05%**	**15.71%**	**15.41%**	**5.65%**	**4.06%**	**1.37%**

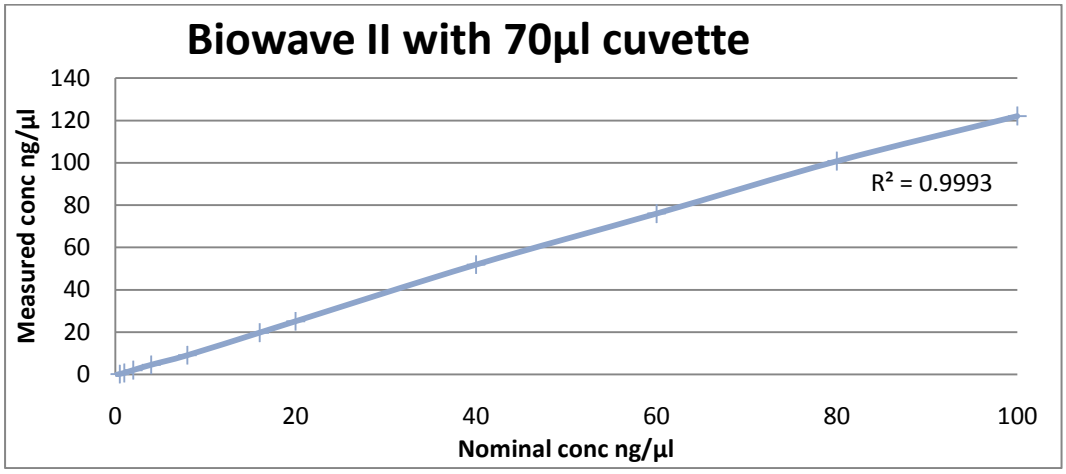


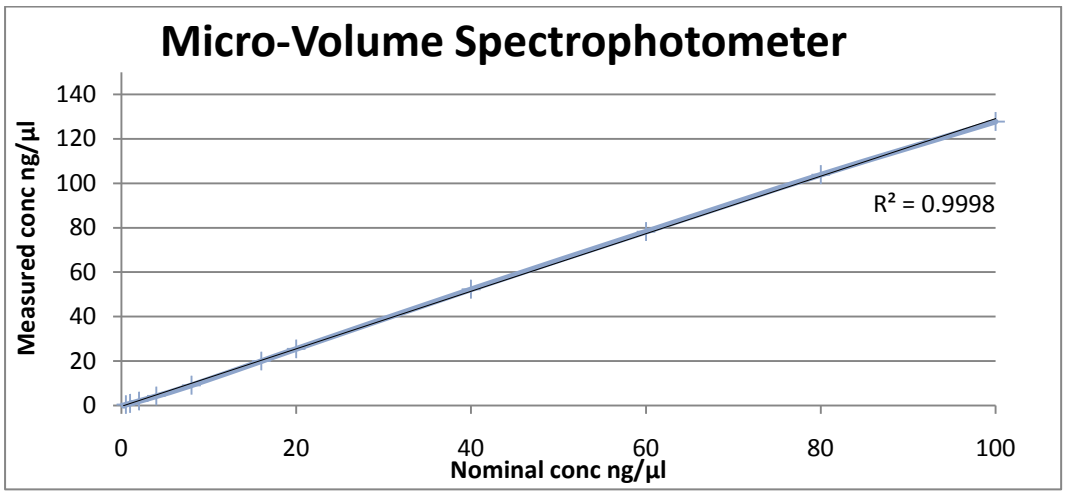
Conclusion

The Biowave II performs more reproducibly than the micro-volume spectrophotometer when measuring samples with low DNA concentrations.

Linearity

To determine the linearity of the Biowave II and the micro-volume spectrophotometer, serial dilutions of DNA were measured, 5 discrete replicates on each instrument





Conclusion

The Biowave II and the micro-volume spectrophotometer performed linearly over the measured range with excellent R^2 values.

Overall Conclusion

Whilst a Biowave with a 70µl disposable cuvette cannot carry out all of the applications of a dedicated micro-volume instrument there are some areas where it will offer a higher level of performance plus the flexibility to carry out other applications such as kinetics and Bradford assays. In the current tough financial times, the fact that the Biowave II is typically less than half the price of a typical dedicated micro-volume instrument should not be overlooked.