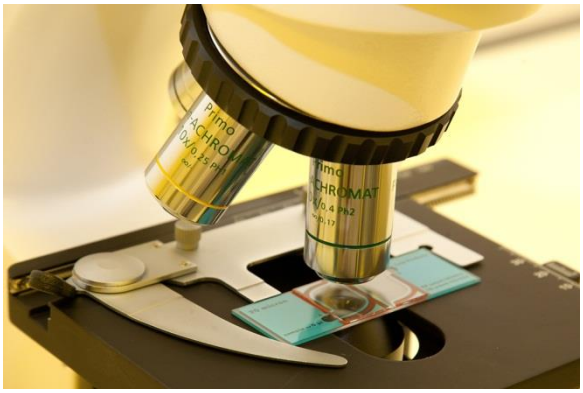
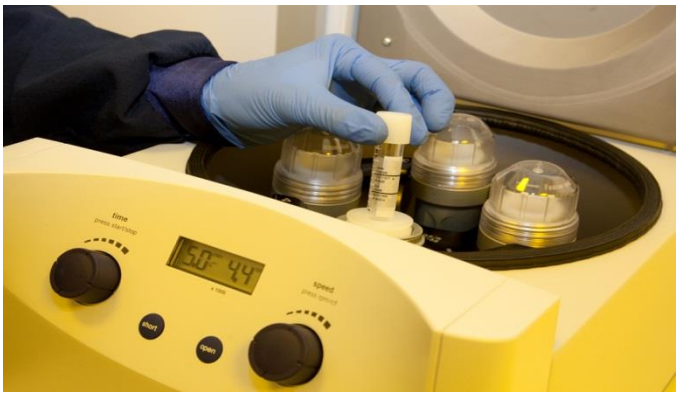
Cambridge IVF – Uncertainty of Measurement in Semen Analysis





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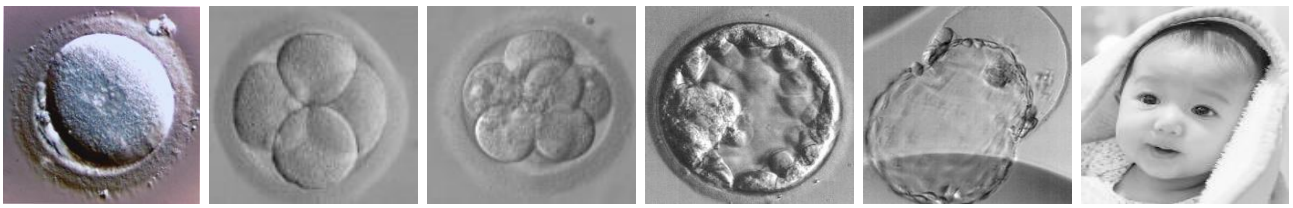

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1. What is uncertainty of measurement?

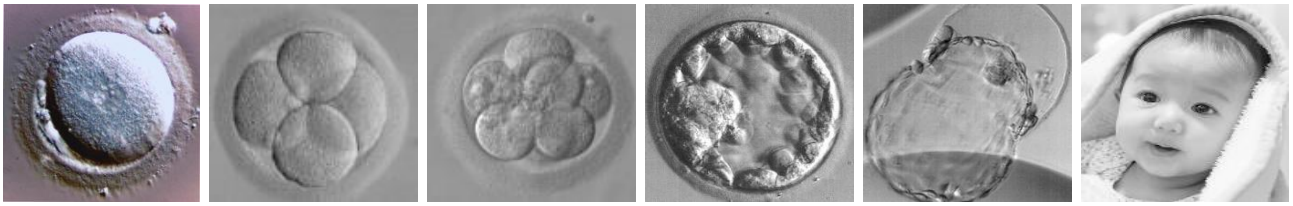
In a *measurement process* (even when all the measurement factors which can be controlled are controlled), repeated observations made under the same conditions, are rarely found to be identical. This anomaly is due to variables such as operator, reference standard(s), materials, environment, calibration and test methodology. Measurement results can never be regarded as a true value and are always accompanied with an uncertainty that in most cases can be quantified and subsequently qualified.

The '*measurement uncertainty*' is a property of a measurement. Measurement of uncertainty is a consequence of the unknown influence of a random series of effects and limits to corrections for systematic effects and is therefore expressed as a quantity, *i.e.* an interval about the result. It is evaluated by combining a number of uncertainty components. The components are quantified either by evaluation of the results, by several repeated measurements or by estimation based on data from records, previous measurements, knowledge of the equipment and practical experience of performing the measurement according to a standard operating procedure. In most cases, repeated measurement results are distributed about the average in a typical normal distribution in which there is a greater probability that the expected value lies closer to the mean than the extremes.

Evaluation from repeated measurements is performed by applying a relatively simple mathematical formula which is derived from statistical theory and the '*standard uncertainty*' for each contribution is estimated. These contributions are combined to form the 'Combined Standard Uncertainty' which is multiplied by a coverage factor (k=2) to give the 'Expanded Measurement Uncertainty' providing a coverage probability of approximately 95%.
When reporting uncertainty it is important to indicate the level of confidence.

The International Standard *ISO 15189*:2012 'Medical Laboratories – Requirements for Quality and Competence' standard 5.5.1.4 requires:

- The laboratory shall determine measurement uncertainty for each measurement procedure in the examination phase used to report measured quantity values on patients' samples.



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- The laboratory shall define the performance requirements for the measurement uncertainty of each measurement procedure and regularly review estimates of measurement uncertainty.

2. How can we express that measurement uncertainty?

Since there is always a margin of doubt about any measurement, we need ask 'How big is the margin?' and 'How bad is the doubt?' This is conventionally achieved by considering the width of the margin and the confidence level (which states how sure we are that the 'true value' is within that margin).

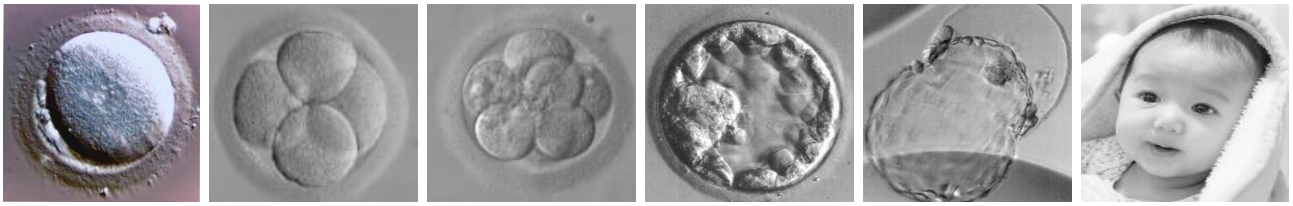
It is important to realise that uncertainty is not the same as error. Error is the difference between the measured value and the 'true value' of the thing being measured, whereas uncertainty is the quantification of the doubt about the measurement result. Any error whose value we do not know is a source of uncertainty.

It is also important to consider that a semen analysis result is often described as for example, 'normal', 'abnormal' or 'subnormal; with these categorisations being based on one or more results of tests performed within the analysis. Whilst it is possible to quantify the measurement uncertainty of some of the individual quantitative elements of a semen analysis, it is not possible to derive the uncertainty measurement of the semen analysis result as a whole due to several unavoidable and uncontrollable confounding factors described below.

3. Why is consideration of uncertainty important?

Uncertainty is a (usually quantitative) indication of the quality of the result. It gives an answer to the question, how well does the result represent the value of the quantity being measured? It allows users of the result to assess its reliability, for example for the purposes of comparison of results from different sources or with reference values or ranges.

In the case of a semen analysis, a result is often compared to a reference range. In this case, knowledge of the uncertainty shows whether the result is well within the reference range or only just makes it. Sometimes a result is so close to the limits of the reference range that the risk associated with the possibility that the measured parameter may not fall within the limit, once the uncertainty has been allowed for, must be considered. In other words, results



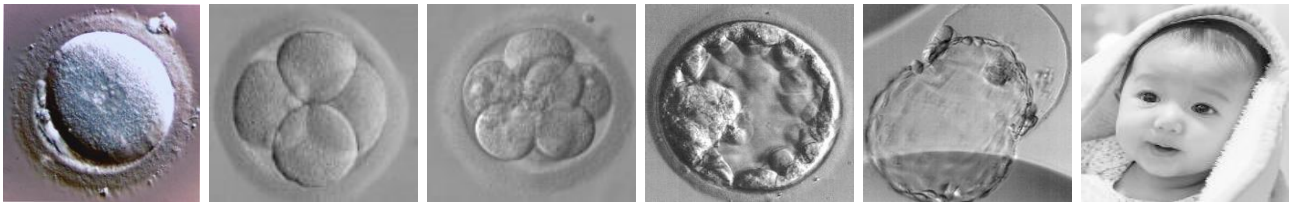
which fall just outside the normal range may in reality be within the normal range.

We often encounter patients who get differing results (and therefore sometimes conflicting advice) when they have semen analyses performed in more than one laboratory. Whilst this is, more often than not, due to the inherent within-individual variability of semen samples a further complication may be arising. To illustrate this point imagine (although it is of course impossible) a patient were able to have the same sample analysed simultaneously in two Andrology labs. Would we expect the laboratories to get identical results? Only within limits, we may answer, but when the results are close to the specification limit it may be that one laboratory indicates 'normality' whereas another indicates an 'abnormality'. From time to time accreditation bodies have to investigate complaints concerning such differences. This can involve much time and effort for all parties, which in many cases could have been avoided if the uncertainty of the result had been known by the service user.

4. Where can uncertainty arise during semen analysis?

Many things can undermine a measurement of a semen analysis parameter and importantly these flaws in the measurement may be visible or invisible. Although patients and Andrologists do their best, the nature of semen analyses dictate that they are rarely performed under absolutely perfect conditions and as such, errors and uncertainties can arise from the areas detailed in the table below. However, in some areas it is possible to attempt to control for and minimise these errors and uncertainties and the ways in which we attempt to do this is also included below.

Source of error or uncertainty	Control/minimisation methods
The laboratory equipment used to perform measurements • Measuring instruments (pipettes, counting chambers etc.) can suffer from errors including bias, changes due to ageing, wear, or other kinds of	• Formal installation and validation • Regular maintenance • Calibration



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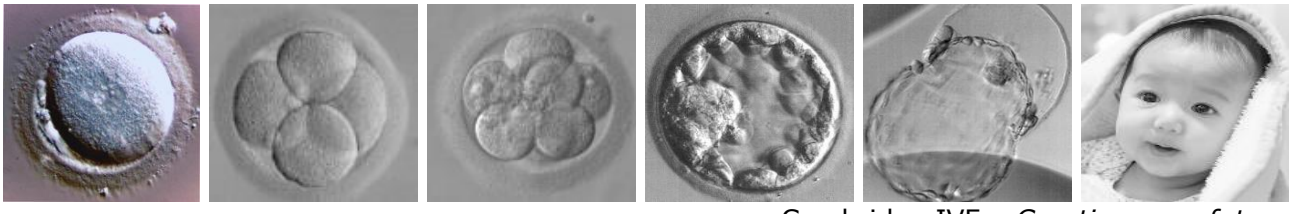
drift, poor readability, noise (for electrical instruments) and many other problems. • Please note that the uncertainty of measurement generated by the pipettes used during semen analysis pales into insignificance compared with the other sources of uncertainty described in sections below	
The patient • It is well recognised that the 'quality' of semen samples produced can vary hugely for a variety of reasons, not least of which is normal biological variation. • As such it is not considered best practice to base a diagnosis on only one semen analysis.	• Performance of repeat semen analyses to help derive a 'representative' set of indicative results upon which a robust diagnosis can be made
The semen sample • Human semen is a heterogeneous fluid which undergoes a process of liquefaction shortly after ejaculation. • The constituents of seminal plasma are not capable of sustaining sperm motility and viability over prolonged periods outside of the body	• The Andrology Laboratory examines the sample within 60 minutes of it being produced wherever possible • Semen samples are well mixed (homogenised) before aliquots are removed for assessment purposes • Awareness that sampling a non-liquefied sample may lead to an erroneous result
Semen sample collection The way in which a semen sample is collected can hugely affect its quality. • Duration of abstinence • Collection method • Collection vessel • Incomplete collection • Exposure to adverse temperature • Ejaculation to analysis interval	• Patients are advised to abstain from ejaculation for a minimum of two and a maximum of seven days. • Patients are advised to collect their samples by masturbation • Patients are advised to only use the container provided by the Andrology Laboratory • Patients are advised to inform the Andrology Laboratory if any of the



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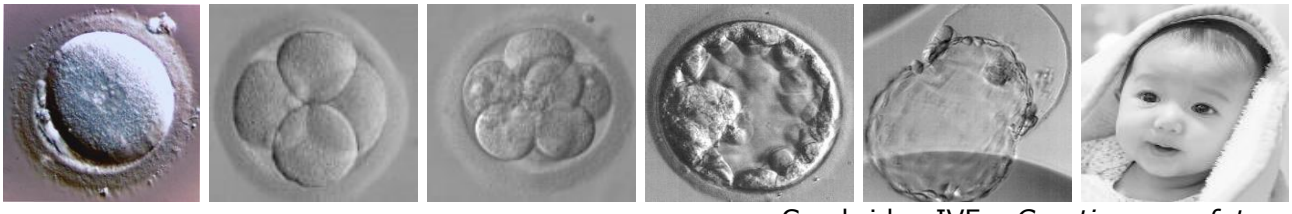
	sample was spilled. • Patients are advised to protect the sample form extremes of temperature • Patients who produce their sample off-site are advised to deliver the sample to the Andrology laboratory within 60 minutes of production
Imported uncertainties • Calibration of for example, pipettes or heated-stages will have an inherent uncertainty which is then built into the uncertainty the measurement being made. • The uncertainty due to not calibrating equipment regularly would represent a much greater concern.	• It is not possible to control for this *per se* although it is essential that equipment is regularly calibrated • Where equipment is used for UKAS accredited diagnostic purposes, calibration should be performed to a metrologically traceable standard.
Operator skill and judgment • Some measurements (e.g. assessment of sperm motility by eye) are subjective and depend upon the skill and judgment of the operator. For example a sperm is deemed to be progressing rapidly (grade A) if it is moving >25µm/sec which equates approximately to 5 x the length of a sperm head. • The human eye is unavoidably drawn to moving objects and as such is inclined to overestimate sperm motility in any population.	• Training • IQC • EQA
Sampling issues • The measurements that are made relating to a particular semen sample must be representative of the sample itself. • This is particularly relevant as	• Semen samples are well mixed before aliquots are removed for assessment purposes • Awareness that sampling a non-liquefied sample may lead to an erroneous result • Reporting failure to liquefy and



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human semen is a heterogeneous fluid which undergoes a process of liquefaction shortly after ejaculation.	hyper-viscidity where observed as part of the final report
The environment • Temperature, air pressure, humidity and many other conditions can affect the measuring instrument or indeed, the sample being measured.	• Patients are advised to protect the sample from extremes of temperature • Patients are advised to only use the container provided by the Andrology Laboratory • All motility assessments are performed at 37°C

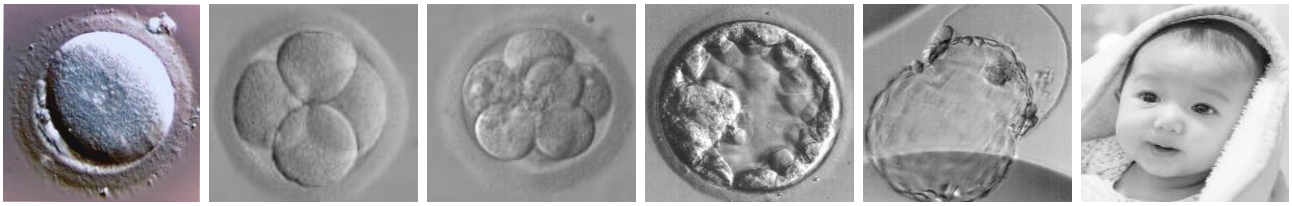
As illustrated in the table above, there are many physical and practical considerations which contribute to uncertainty of measurement in relation to semen analysis and it is not possible for all of them to be controlled by the laboratory as many of the control methods described above rely heavily on patient understanding and compliance.

5. How often should uncertainty measures and calculations be reviewed?

This document is reviewed every two years as per our document review schedule. However, this review date may be expedited if new information pertaining to uncertainty and its relevance to semen analysis becomes available – for instance due to;

a) New guidance in the published scientific literature
b) Whenever a new process is introduced
c) Whenever a significant change to an existing process or procedure is introduced
d) A new piece of equipment or consumable is implemented
e) A significant change in staffing personnel is necessitated

If there is no change to the above parameters then uncertainty of measurement limits described in this SOP will not be reviewed. Where no review is undertaken this will be recorded as part of the document review process record.



6. Quantification of uncertainty in semen analysis

This section seeks, where possible, to quantify the uncertainty of measurement for individual parameters within a semen sample. This allows individual parameters within the analysis to be appropriately interpreted.

At the end of each section, we have attempted to relate the data to clinical practice by suggesting some points for consideration, which may assist with interpretation of the data in a clinical context.

6.1 Intra-patient variation

To demonstrate just how much the quality of semen samples can vary within the same individual, the table below shows data relating to 20 donors who provided a total of 754 ejaculates (minimum of 10 each) at the Hewitt Fertility Centre (HFC) in Liverpool, one of the largest andrology service providers in the UK in 2014. The parameter means for all 20 donors are shown together with the mean CV, with the latter representing the between-sample variation for each donor.

	Volume	Concentration	Progression (a+b)	Total Count
Mean (Range)	3.3 (1.1-6.5)	74.8 (43.5-136)	56.0 (44.5-68.5)	227.5 (85.3-557.1)
CV (%) (Range)	26.1 (15.3-42.3)	33.2 (13.6-49.6)	15.2 (10.1-21.5)	43.9 (20.7-68.4)

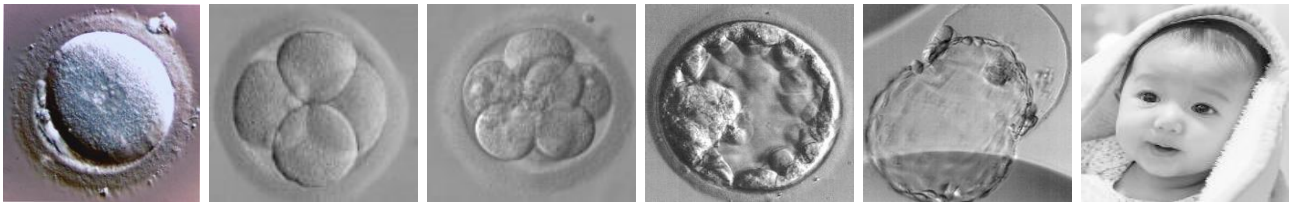
Points for consideration

- *Men inherently produce samples of very variable quality.*
- *Diagnosis of sub-fertility should not be based on a single semen sample.*

Revalidation measures

- Cambridge IVF will replicate the work performed by HFC every 10 years or sooner in response to any significant changes in demographic or laboratory methodology.

6.2 Measurement of semen sample volume



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Semen volume is measured by weight. Uncertainty related to the measurement of semen sample volume is very small as demonstrated below where the weight of the same semen sample + pot was determined 10 times. (August 2021, Cambridge IVF)

Mean weight (g)	15.37
Range	15.36 – 15.38
CV (%)	0.03

The uncertainty in measurement pertaining to assessment of sample volume is therefore **negligible (<1%)** in the context of the reliability of the results obtained.

Points for consideration

- *The reported volume of a semen sample is extremely reliable.*

Revalidation measures

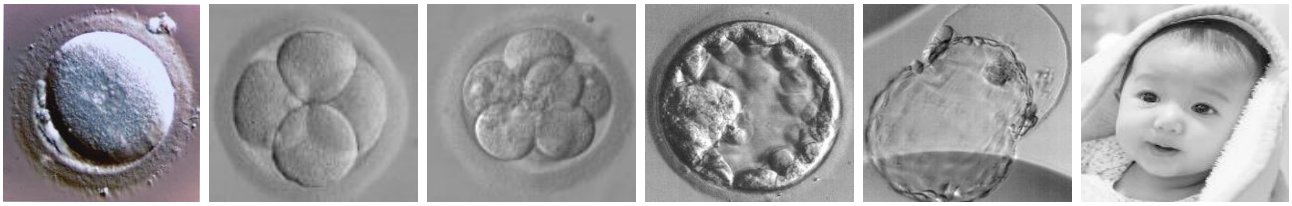
- Revalidation will only be required if the sample container type or the manufacturing process of the sample container changes. Otherwise, re-assessment is not required.

6.3 Sperm concentration

Assessment of semen concentration

Assessment of semen concentration is dependent on two key elements, the precision of the operator in performing the assessment and the accuracy of the dilution prepared for the purpose of that examination.

All of the pipettes used in the preparation of dilutions for the assessment of semen concentration by the Cambridge IVF laboratory are subject to an annual, metrologically traceable calibration process by an independent specialist engineer. The results provide uncertainty calculations which, in the case of all pipettes involved represent an error threshold certified at less than 1% of the volume aspirated (Range 0.001-1.18% at most recent calibration in September 2022). The effect of the uncertainty afforded due to the use of a pipette in the process of assessment of semen concentration is therefore negligible.



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The laboratory uses a number of independently labelled Improved Neubauer Haemocytometer devices for the manual assessment of sperm concentration. Each haemocytometer is calibrated every 6 months using the Accubead QC-Bead System (Microm, UK) which is a two part test using a 'Hi' bead suspension (range 34-46M/ml) and a 'Lo' bead suspension (range 16-24M/ml). Any haemocytometer found to fail calibration is discarded and replaced with a new device which passes calibration.

Intra-operator variation is measured and accounted for by bi-weekly IQA assessment according to Cambridge IVF standard operating procedure 'Andrology Service Quality Assurance Procedures' (C.IVF/LabSOP/Diagnostic/19).

Points for consideration

- *The preparation of a seminal dilution for assessment of semen concentration does not contribute significantly to the uncertainty of the results obtained.*

Revalidation measures

- Re-certification of pipettes is performed annually to a UKAS traceable standard. The uncertainty reported as a result of these calibration exercises will be used to review this section annually.

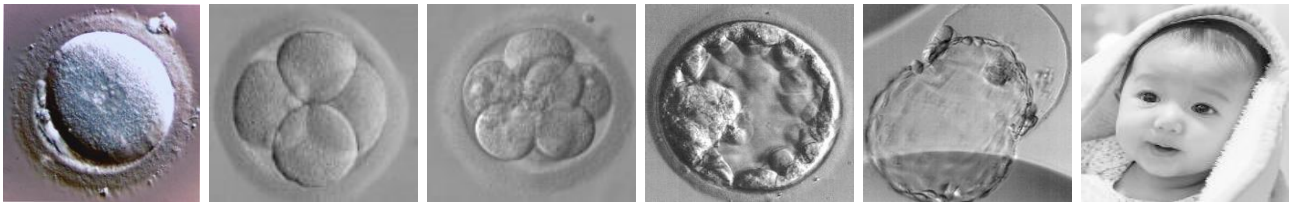
Intra-operator variability

The table below shows the results of 10 concentration measurements performed on the same sample by the same operator.

Variability observed with the same competent practitioner performing a manual concentration measurement on the same sample 10 times (August 2021)

n=10	
Mean concentration (M/ml)	13.0
Range	11.40 – 14.50
CV (%)	7.4

The CV with regard to intra-operator uncertainty is **low (<10%)**.



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Inter-operator variability

The table below shows the results of 5 operators performing manual concentration measurements on 10 different samples. Please note that the variability seen here may be a combination of true 'inter-operator' variability together with sampling error.

Variability observed with 5 competent practitioners performing a manual concentration measurement on the same sample at the same time (August 2021)

		Operator					Concentration (M/ml)			
		1	2	3	4	5	Mean	Range	SD	CV (%)
Sample Number	1	89.0	90.0	98.5	87.7	82.5	89.53	82.5 - 98.5	5.79	6.46
	2	82.7	95.3	95.0	85.2	88.3	89.30	82.7 – 95.3	5.72	6.41
	3	89.3	85.0	96.8	86.0	87.0	88.83	85.0 – 96.8	4.75	5.35
	4	91.2	90.7	94.3	86.8	89.0	90.40	86.8 – 94.3	2.78	3.07
	5	87.3	95.5	100.5	86.4	80.2	89.99	80.2 – 100.5	8.01	8.91
	6	87.8	90.8	103.6	87.2	97.5	93.38	87.2 – 103.6	7.03	7.52
	7	86.2	84.8	95.8	90.3	84.3	88.29	84.3 – 95.8	4.81	5.45
	8	83.7	92.2	96.5	86.7	82.0	88.20	82.0 – 96.5	6.04	6.85
	9	86.0	91.3	92.3	87.3	90.0	89.40	86.0 – 92.3	2.67	2.99
	10	85.0	80.3	93.3	85.2	88.2	86.40	80.3 – 93.3	4.78	5.53

The CVs in the context of inter-operator uncertainty is **low (<10%)**.

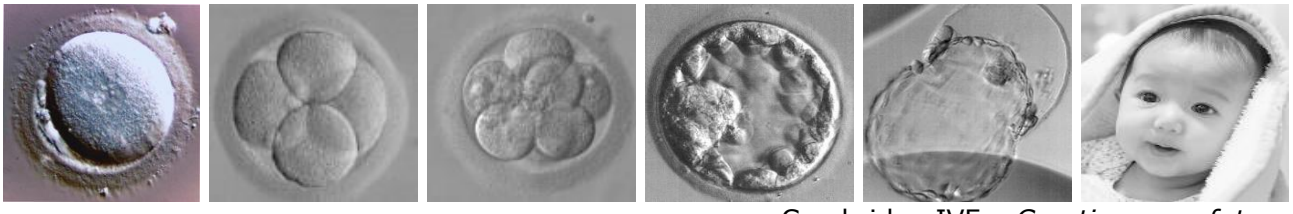
Points for consideration

- *The uncertainty associated with manually measuring sperm concentration can be large, particularly at lower sperm concentrations.*

6.4 Sperm Motility

There are principally four areas in which uncertainty of measurement can be introduced when measuring sperm motility these being

i. the time interval between ejaculation and analysis
ii. the effect of temperature
iii. the effect of the operator
iv. the difference between operators



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Time interval between ejaculation and analysis

Sperm motility in some semen samples will start to decline after approximately 60 minutes. As such, the Andrology Laboratory endeavors to perform all motility analyses within 60 minutes of ejaculation.

Points for consideration

- *Patients who produce samples off-site should be strongly advised to deliver the samples to the Andrology Lab within 60 minutes of ejaculation.*
- *The time interval between ejaculation and analysis will not be reassessed until evidence in the literature, (WHO 2021) or professional guidance suggests to time interval should be reconsidered. If these do change, the assessments will be completed prior to implementation into the laboratory.*
- *Any instance where sperm motility is reduced and the motility assessment was performed over 60 minutes after ejaculation (e.g. where the sample was produced off-site) will be highlighted on the report.*

Effect of temperature

Figure 3 below, demonstrates that more sperm swim faster at 37°C than at room temperature.

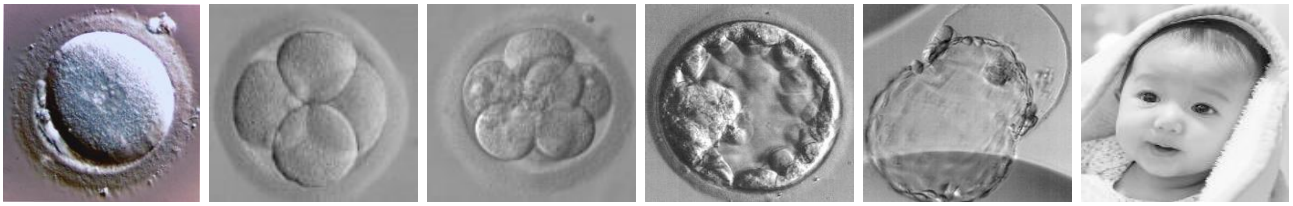

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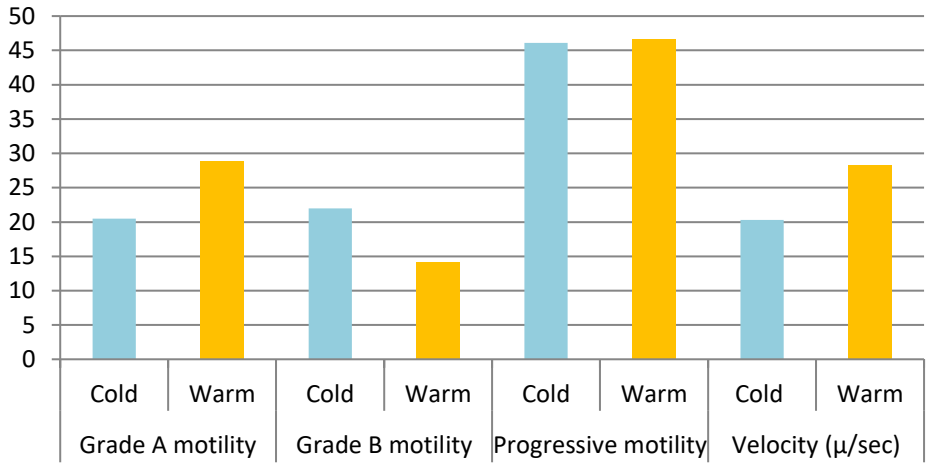
Figure 3. The effect of temperature on sperm motility
(Cold 19-23°C; warm 36-37°C, n=25)



Data from Hewitt Centre, Liverpool (October 2020)

Points for consideration

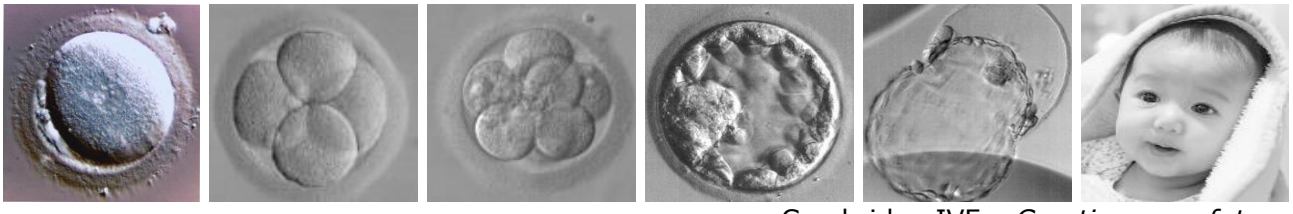
- *All motility analyses performed within the Cambridge IVF are performed at 37°C.*
- *Failure by patients to follow instructions regarding sample production and transport will be noted on the report.*
- *This has not been reassessed in this document version as evidence in the literature (WHO 2021) outlines no change in guidelines to temperature. If this does change, the assessments will be completed prior to implementation into the laboratory.*

Intra-operator variability in measurement of sperm motility

The table below shows the results of 10 manual motility measurements performed on the same sample by the same operator. Please note that:

i. some variability was observed due to natural 'deterioration' of sperm motility over the time taken to perform the 10 measurements and;
ii. The variability seen here will be a combination of true 'intra-operator' variability together with sampling error.

Variability observed with the same competent practitioner performing a manual motility assessment on the same sample 10 times (August 2021)



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Motility grade (n=10)	Mean	Range	CV (%)
Rapid Progressive (a)	38.5	35-42	7.3
Forward Progressive (b)	12.1	7-17	25.7
Non-progressive (c)	2.8	2-4	28.2
Non-motile (d)	46.6	40-51	8.8

The CV associated variability in assessment of motility is **high (>25%)**. Please not that:

- Some of this variability is associated with a deterioration of the sample over time which would not be a relevant factor in a diagnostic semen analysis.
- A significant degree of unwitting bias is inherent in this experiment as the operator may subconsciously 'adjust' their measurement as they already 'know' their previous results.

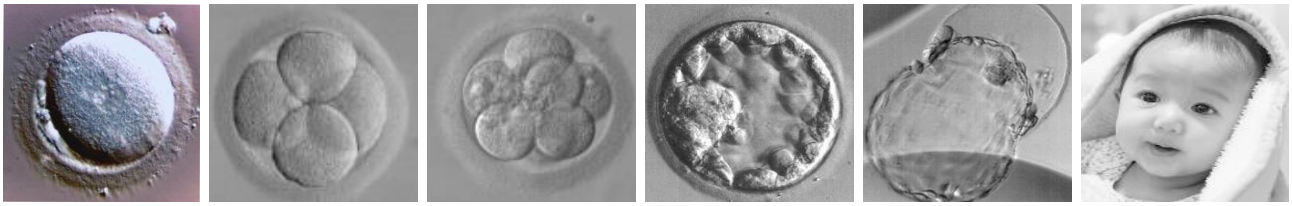
As these factors to not apply in a diagnostic semen analysis, the actual uncertainty associated with the test is likely to be **lower than stated**.

6.4.1 Inter-operator variability

The table below shows the results of 5 competent practitioners performing manual motility measurements at the same time on 10 samples. Please note that the variability seen here may be a combination of true 'inter-operator' variability together with sampling error. In addition, a degree of subjectivity is associated with the assessment of motility into 4 groups, however it is also accepted that categorisation of sperm into 4 categories of motility represents best laboratory practice.

Coefficient of Variance calculations across 5 competent practitioners performing a manual motility assessment 10 times on the same sample at the same time (August 2021)

Motility grade (n=50)	Mean CV (%)	Range CV (%)
Rapid Progressive (a)	14.75	(8.27 - 20.81)
Forward Progressive (b)	22.43	(6.98 – 39.25)
Non-progressive (c)	28.62	(13.56 – 35.95)
Non-motile (d)	8.92	(4.24 – 13.67)



Again, the CV values are **high (>25%)** indicating a level of subjectivity. The deterioration of the sample over time also has to be considered as a variable we cannot control in this analysis although all assessments were performed within 1 hour of sample production in accordance with best practice guidance.

Points for consideration

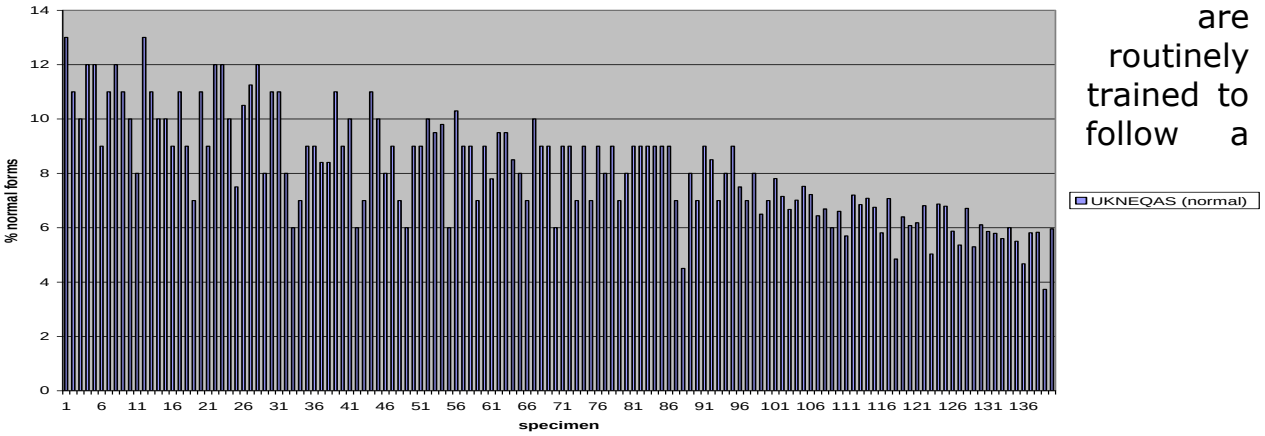
- *The uncertainty associated with measurement of sperm motility is high.*

6.5 Sperm Morphology

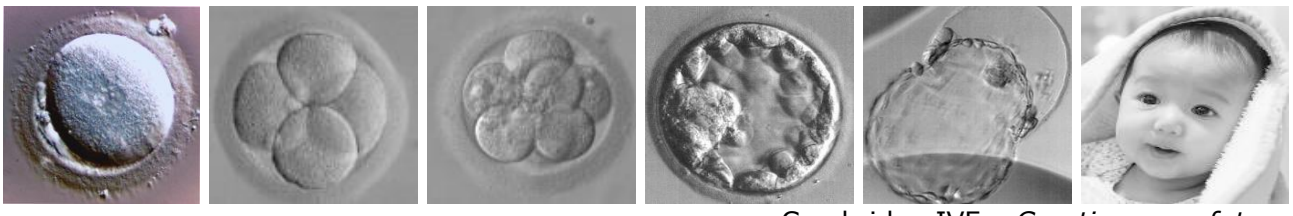
The assessment of sperm morphology is fraught with difficulty for many reasons and significant measurement uncertainty exists. Some examples of these difficulties are given below.

The figure below would suggest that a laboratories' perception of 'a normal sperm' is slowly changing to meet the needs of the new reference ranges, despite using the same sperm shape and size criteria to work to. The figure below shows the target values for % normal forms from EQA samples over the past 8 years. There is a clear relationship showing generally stricter scoring with time in response to a gradual adoption of a lower reference range:

EQA target values for % normal forms from 2005-2011 in the UK NEQAS scheme



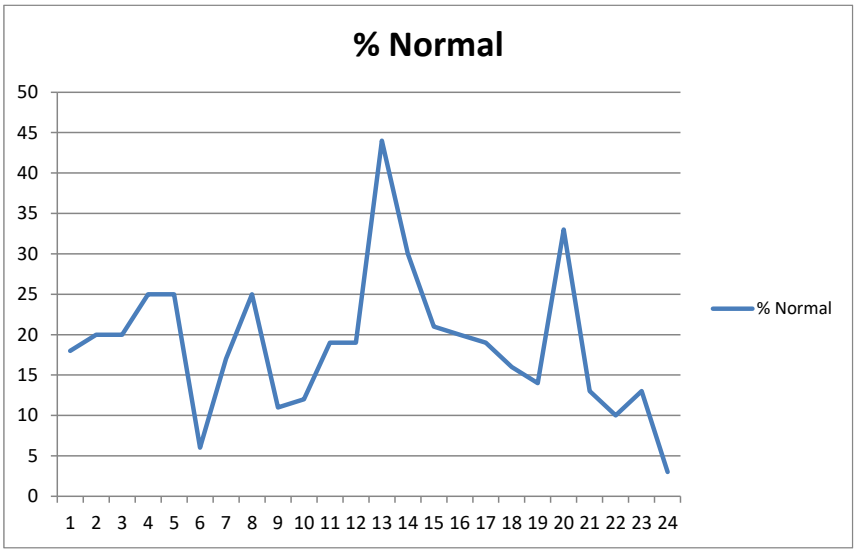
Secondly, Andrologists are routinely trained to follow a philosophy which labels any



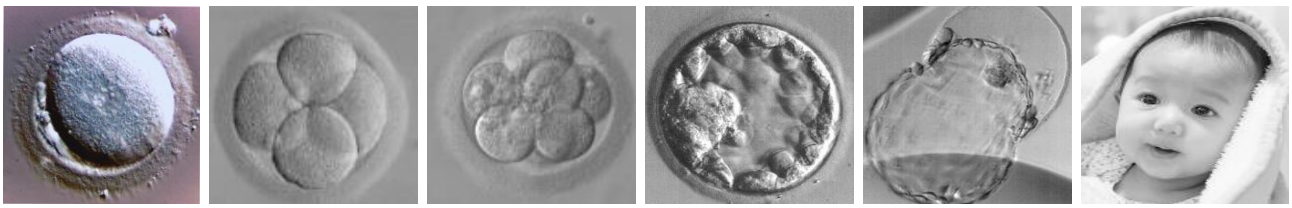
sperm which does not meet pre-defined size and shape definitions as being abnormal. By definition the group of abnormal forms then includes a significant number of 'unknowns' which could include: borderline forms; artefacts created by slide preparation; or indeed those which become adhered to artefacts such as debris or non-sperm cells. The consequence of adopting this strategy is that the uncertainty surrounding those 'unknowns' (and therefore for the entire measurement) cannot be assessed.

Thirdly, and to compound the difficulties yet further, not only can differences in fixation and staining make a difference to the overall result but individual interpretation of exactly the same sperm images show a remarkable lack of consistency across a range of operators.

A recent small study using a series of clear images sent out by the laboratory at Nottingham University Hospital to a number of centres showed that even in experienced hands, agreement on whether a sperm is normal or abnormal varied considerably. The figure below shows the % normal forms as reported by 24 individuals (fully trained to perform semen analysis) based in six different laboratories. The mean from 160 sperm images was 18.9% normal with a range from 3% to 44%!



Identification of normal forms from the same set of micrographs assessed by 24 staff in six different centres (Tomlinson 2014)



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Clearly there is a very large and unquantifiable uncertainty associated with sperm morphology assessment as currently performed, and it seems that estimating sperm morphology in terms of percentage 'normal forms' is difficult (if not impossible) with subjectivity remaining a significant problem.

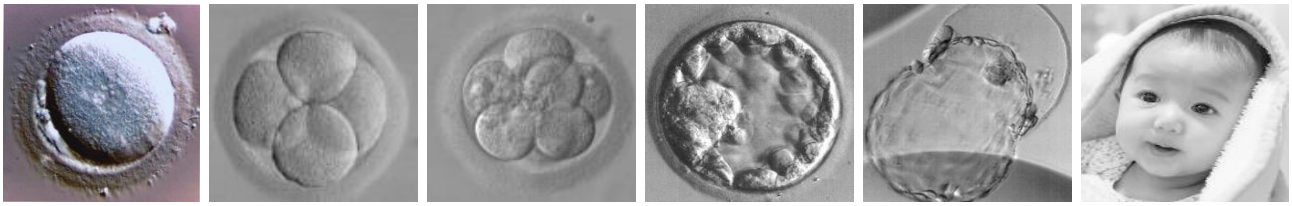
However, there are certain situations where the performance of sperm morphology is associated with an extremely low (if not zero) level of uncertainty and these are where the morphological defect applies to every sperm and such conditions are easily recognisable. This might include conditions such as globozoospermia (where the head size is increased and no acrosome is present), pin-head sperm (where the sperm heads are missing) or gross tail defects. Such conditions are often 'sterilising'.

Points for consideration

- *The high degree of uncertainty around assessing % normal forms raises questions about its clinical value.*
- *Sperm morphology assessment is of considerable value in identifying gross morphological abnormalities (e.g. globozoospermia).*

7 Uncertainty Budget

Uncertainty Budget	
Type A: *carried out by calculations from a series of repeated observations using statistical methods*	Type B: *measurements derived from other sources e.g. information from past experience of the measurements or calibration certificates*
• Calculate SD of Intra- precision	• Temperature • Humidity • Inter-operator variability • Pipette uncertainty values • Reagent variability • Calibration lot number variability. • Inter-precision variability



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7.1 How to calculate Uncertainty when using an Uncertainty Budget

- Calculate the SD of the intra-precision (A)
- Calculate the SD of the inter-precision (B)

Add the 2 uncertainties and in order to get rid of a - or **+** Square them, add them and calculate the Square Root (u)

$$u=\sqrt{A^2+B^2}$$

Expanded uncertainty
Uncertainty is calculated as 1 SD, 1 SD gives 68% confidence on the Gaussian Curve, it is reasonable to multiply the uncertainty by 2 to attain a confidence level of 95%. This is referred to the coverage factor and is represented by K

7.2 Calculating Expanded Uncertainty

Uncertainty (u) is expressed as: $u=\sqrt{A^2+B^2}$

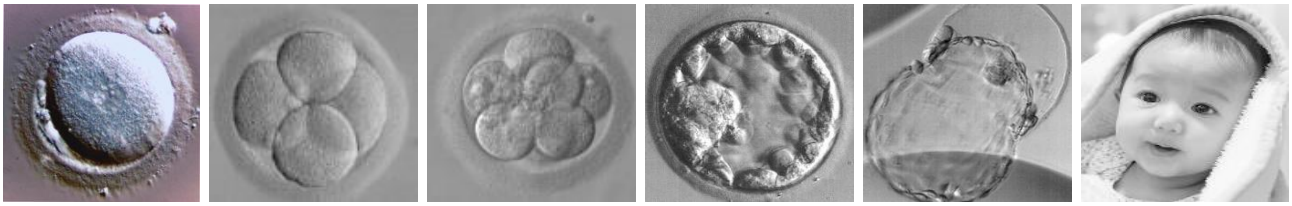
Expanded uncertainty (U) is expressed as: $U=2 \times u$

7.3 Introduction to 'Type A' & 'Type B' uncertainties and considerations

This uncertainty budget seeks to systematically examine and document all sources of measurement uncertainty associated with performing a routine semen analysis. This is done by examining;

i. readily measurable and statistically describable variability – so called 'Type A' uncertainties, and
ii. 'Type B' uncertainties, which are less easily quantified but are known to effect the outcome of the test.

Routine semen analysis is made up of several components (ejaculate volume, sperm concentration, sperm motility and sperm morphology) and such it is prudent to list Type A and Type B uncertainties for each.



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Ideally, it should then be possible to mathematically combine all the measurement uncertainties associated with measuring a particular parameter to derive an overall level of uncertainty for measuring that parameter. This document also seeks to examine whether such an approach is applicable to routine semen analysis.

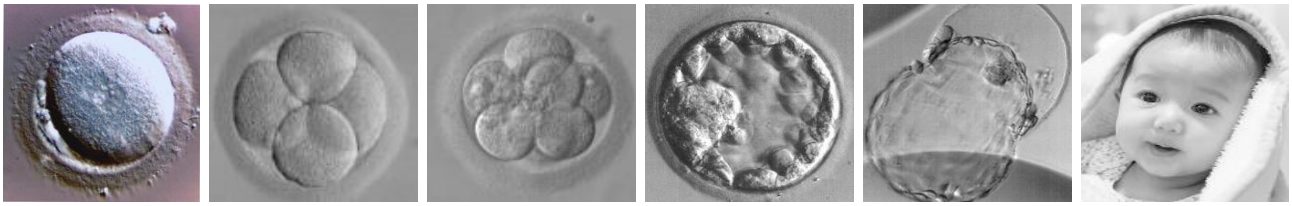
Please note that a fundamental assumption is made at the outset when performing a semen analysis in that the sample being analysed has indeed been provided by the patient. Unlike taking a venous blood sample, the requirement (in all but rare exceptions e.g. electro-ejaculation or surgical sperm retrieval) for the sample to be produced by masturbation means that due to obvious privacy and dignity restrictions, the provenance of a semen sample cannot be guaranteed with certainty.

7.3.1 Ejaculate volume (measured by weight)

Type A uncertainties	Type B uncertainties
• Volume measurement by weight • Imported uncertainty (e.g. calibration)	• Incomplete sample collection (reliance on patient honesty) • Abstinence period (reliance on patient honesty) • Intra-patient variability (a patient will naturally produce samples of varying volume) • Retrograde ejaculation (a patient may produce an incomplete sample due to partial retrograde ejaculation)

7.3.2 Sperm concentration

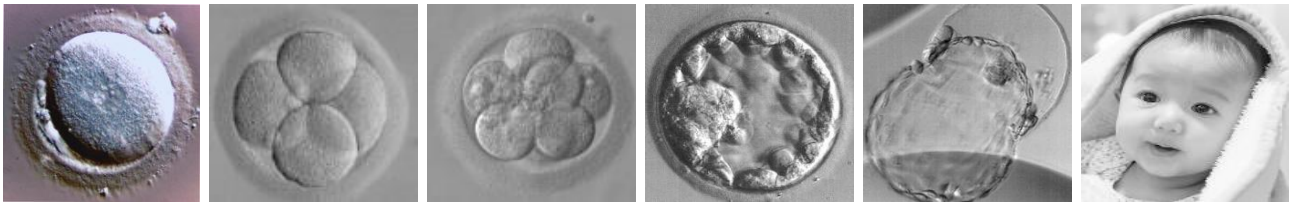
Type A uncertainties	Type B uncertainties
• Intra-sample variability • Inter- & Intra-operator variability • Inter- & Intra-chamber variability • Imported uncertainty (e.g. calibration)	• Incomplete sample collection (reliance on patient honesty) • Abstinence period (reliance on patient honesty) • Intra-patient variability (a patient will naturally produce samples of varying volume)



	• Retrograde ejaculation (a patient may produce an incomplete sample due to partial retrograde ejaculation) • Sample heterogeneity (sperm are suspended in viscous and heterogeneous seminal fluid. Vigorous sample mixing is not recommended. Semen undergoes an enzymatically driven process of liquefaction which significantly affects viscosity and consistency)

7.3.3 Sperm motility

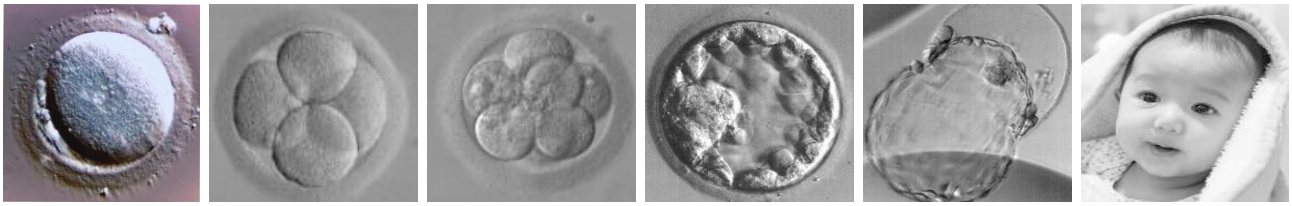
Type A uncertainties	Type B uncertainties
• Intra-sample variability • Inter- & Intra-operator variability • Imported uncertainty (e.g. calibration)	• Incomplete sample collection (reliance on patient honesty) • Abstinence period (reliance on patient honesty) • Intra-patient variability (a patient will naturally produce samples of varying volume) • Retrograde ejaculation (a patient may produce an incomplete sample due to partial retrograde ejaculation) • Sample heterogeneity (sperm are suspended in viscous and heterogeneous seminal fluid. Vigorous sample mixing is not recommended. Semen undergoes an enzymatically driven process of liquefaction which significantly affects viscosity and consistency) • Sample handling (reliance on patient to protect sample from extremes of temperature from production to delivery to the laboratory)



	• Time to delivery (reliance on patient honesty to ensure sample is analysed within 60 minutes of ejaculation) • Procedural temperature control (consistent use of heated stages and maintaining a consistent ambient laboratory temperature at 22.5°C) • Presence of antisperm-antibodies and associated effects on motility pattern

7.3.4 Sperm morphology

Type A uncertainties	Type B uncertainties
• Intra- and Inter-sample variability • Intra- and Inter-operator variability	• Incomplete sample collection (reliance on patient honesty) • Abstinence period (reliance on patient honesty) • Intra-patient variability (a patient will naturally produce samples of varying volume) • Retrograde ejaculation (a patient may produce an incomplete sample due to partial retrograde ejaculation) • Sample heterogeneity (sperm are suspended in viscous and heterogeneous seminal fluid. Vigorous sample mixing is not recommended. Semen undergoes an enzymatically driven process of liquefaction which significantly affects viscosity and consistency) • Changing perception of a morphologically 'normal' sperm to meet defined reference ranges. • Accepted approach that 'unknown' = 'abnormal' • Fixation artefacts.



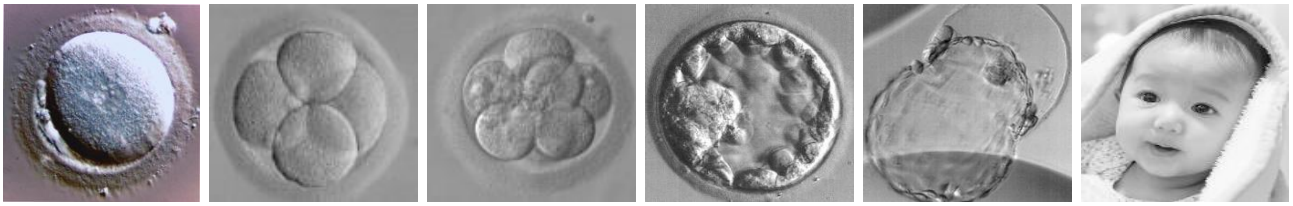
It is clear when all of these factors are considered and we accept that the vast majority of uncertainty applied to semen analysis is type B in nature that it is not possible to mathematically calculate uncertainty of measurement in any meaningful or beneficial way.

8. Post vasectomy analysis (PVSA)

Further considerations must be made with regards to PVSA. The numbers of sperm that are detectable in these particular samples are extremely low. Critical levels for the clinician are 100,000 sperm/ ml and no sperm in the ejaculate. Therefore the accuracy of numbers of sperm present should be shown with respect to measurement uncertainty.

The Neubauer haemocytometers are shown to have accuracy above the number of 56,000 sperm per ml and limitations regarding this are discussed above in section 6.3.

With regards to the limitations of technicians stating 'no sperm seen in ejaculate', tests were performed to determine actual detection limits. Serial dilutions of Accubeads were formulated. The table below shows the levels whereby Accubeads have been detected on a 10ul wet prep and with large volume fixed depth slides, and the corresponding concentration for a number of staff in the department. This data allows the calculation of limits of detection (if we say there is no sperm in the ejaculate; it actually means that there are less than 500 sperm /ml, which is acceptable).



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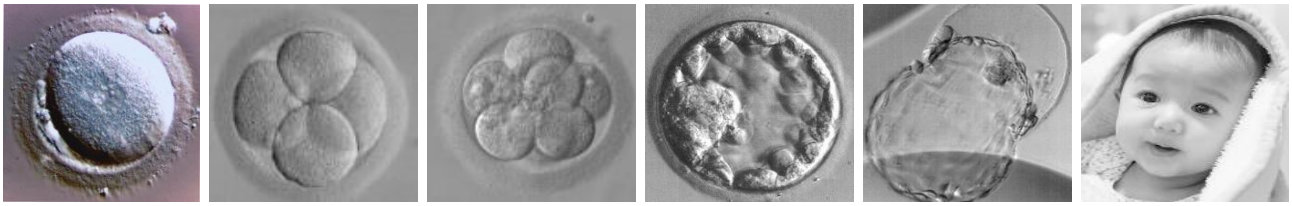
Fixed Volume Slides		Andrology Team Member					
Test Reference	Concentration (Sperm/ml)	1	2	3	4	5	6
A	≈ 62						
B	≈ 125						
C	≈ 250	✓				✓	
D	≈ 500	✓	✓	✓	✓	✓	✓
E	≈ 1,000	✓	✓	✓	✓	✓	✓
F	≈ 2,000	✓	✓	✓	✓	✓	✓
G	≈ 4,000	✓	✓	✓	✓	✓	✓
H	≈ 8,000	✓	✓	✓	✓	✓	✓
I	≈15,625	✓	✓	✓	✓	✓	✓
J	≈ 31,250	✓	✓	✓	✓	✓	✓
K	≈62,500	✓	✓	✓	✓	✓	✓
L	≈125,000	✓	✓	✓	✓	✓	✓
M	≈ 250,000	✓	✓	✓	✓	✓	✓
N	≈ 500,000	✓	✓	✓	✓	✓	✓
O	≈1M	✓	✓	✓	✓	✓	✓

*Variability observed with 6 competent practitioners determining lower detection limits of sperm per ml on a 10ul wet preparation using a fixed volume slide. (*Hewitt Centre, Liverpool, 2020*)*

✓ Indicates sperm were identified



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Direct Centrifugation		Andrology Team Member					
Test Reference	Concentration (Sperm/ml)	1	2	3	4	5	6
A	≈ 62						
B	≈ 125						
C	≈ 250	✓	✓	✓			
D	≈ 500	✓	✓	✓	✓	✓	✓
E	≈ 1,000	✓	✓	✓	✓	✓	✓
F	≈ 2,000	✓	✓	✓	✓	✓	✓
G	≈ 4,000	✓	✓	✓	✓	✓	✓
H	≈ 8,000	✓	✓	✓	✓	✓	✓
I	≈15,625	✓	✓	✓	✓	✓	✓
J	≈ 31,250	✓	✓	✓	✓	✓	✓
K	≈62,500	✓	✓	✓	✓	✓	✓
L	≈125,000	✓	✓	✓	✓	✓	✓
M	≈ 250,000	✓	✓	✓	✓	✓	✓
N	≈ 500,000	✓	✓	✓	✓	✓	✓
O	≈1M	✓	✓	✓	✓	✓	✓

*Variability observed with 6 competent practitioners determining lower detection limits of sperm per ml on a 10ul wet preparation, using direct centrifugation. (*Hewitt Centre, Liverpool, 2020*)*

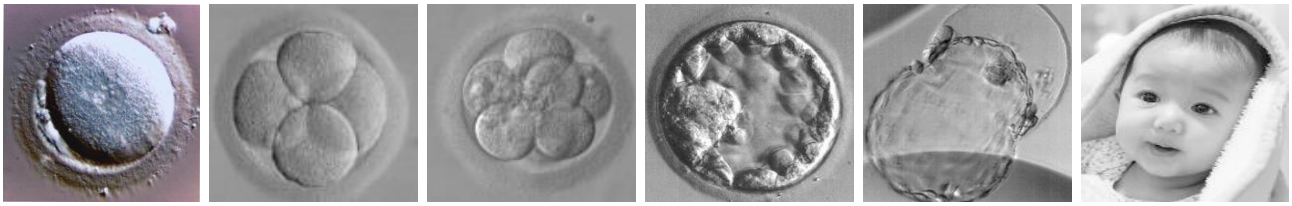
✓ Indicates sperm identified

The limits of detection for post vasectomy analysis can therefore be set at less than 500 sperm /ml when assessing samples using either the direct centrifugation or the large volume fixed depth slides method.

9. CONCLUSIONS

The information presented in this document is intended to demonstrate that it is possible to derive some statistical descriptors of some of the parameters routinely measured as part of a semen analysis (i.e. the '*Type A'* uncertainties).

However, what is overwhelmingly clear is that the '*Type B'* uncertainties are predominant and of such scale and relevance that the determination of an



uncertainty budget for a routine semen analysis (or indeed its individual components) is statistically meaningless.

Nevertheless, a semen analysis remains the only effective first-line test available to assess male fertility. As such it is incumbent upon those performing semen analyses to provide information to users about the overall reliability of the test result and to assist them in their interpretation particularly in relation to the uncertainties that might be associated with the result.

Whilst this document shows that it is not possible to quantify (in conventional terms) the measurement uncertainty associated with a semen analysis it does not lessen the importance of drawing to the attention of service users to where uncertainties in semen analysis arise, and perhaps most importantly, how these might be reduced.

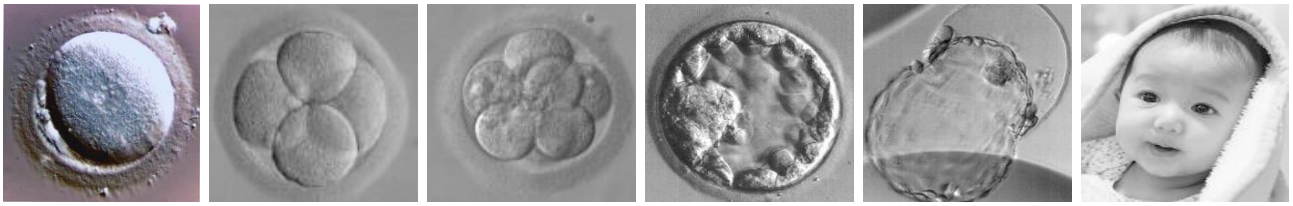
As a user of the diagnostic Andrology service – what do I need to do?
The simple answer to this question is nothing. The way in which semen analysis testing is performed and the inherent problems and difficulties therein remain unchanged. Similarly, the uncertainty of measurement associated with performing a routine semen analysis has, and will always be present to a greater or lesser degree.

As the provider of your semen analysis testing we would simply ask, having taken the time to read this document, that you consider its content when interpreting a semen analysis result within the clinical environment.

Please also remember that Cambridge IVF provide a clinical interpretation service to you and your patients and that appointments with our Consultant Clinical Scientist are available weekly and at no charge to answer any questions from you or your patients. Please call the centre on 01223 349010 for further information pertaining to this service.

10. Summary of Recommendations

a) It is essential that patients be strongly advised to follow instructions regarding sample collection and abstinence to reduce the uncertainty that this can introduce.



b) An interval of more than an hour between ejaculation and analysis may lead to a reduction in sperm motility – this will be highlighted on the report.

c) Results from samples which are not fully liquefied may not be truly representative of the sample's quality – this will be highlighted on the report.

d) Men will produce samples of very variable quality due to normal biological variation. As such, a diagnosis of sub-normality should not be made on a single semen sample and a repeat sample should be requested.

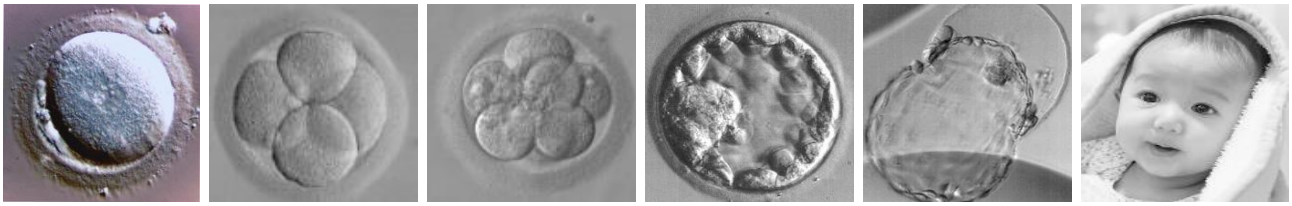
e) The measurement of sperm concentration is associated with a low degree of measurement uncertainty and this should be taken into account when interpreting semen analysis results, particularly at the limits of normality.

f) The measurement of sperm motility is associated with a high degree of measurement uncertainty and this should be taken into account when interpreting semen analysis results, particularly at the limits of normality.

g) The measurement of sperm morphology is associated with a very high degree of measurement uncertainty and this should be taken into account when interpreting semen analysis results. Measurement of sperm morphology is of considerable value in identifying gross morphological abnormalities.

11. Acknowledgements

Cambridge IVF are grateful to the Andrology Team at the Hewitt Fertility Centre, Liverpool, Dr. Mathew Tomlinson (Consultant Reproductive Scientist, Queens Medical Centre, Nottingham) and Mr David Sanders, (Andrology Laboratory Manager, Wales Fertility Institute, Cardiff) and former Chair of the Association of Biomedical Andrologists for kindly providing some of the data and referenced information used within this document.



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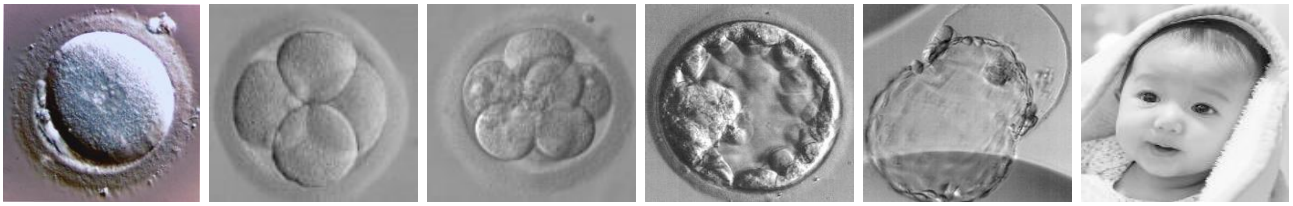
13. Definitions

Measurement: *as a process, in which a set of operations are performed to determine the value of quantity.*

Process: *an integral set of activities that use resources to transform inputs to outputs.*



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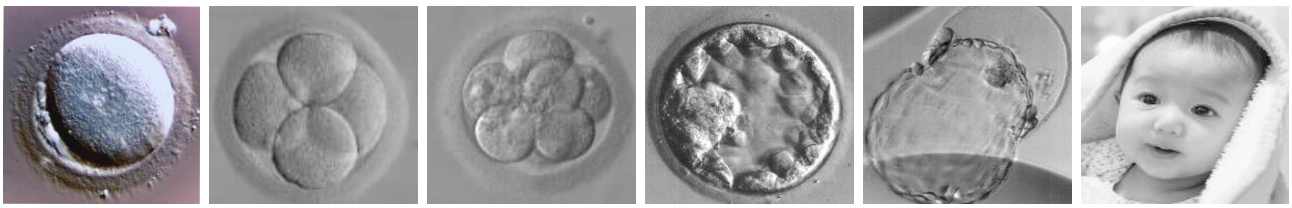
FINDING CAMBRIDGE IVF IN TRUMPINGTON

Please note that Cambridge IVF is not located on the main CUH Campus.
We are very easy to access by car, public transport and bicycle. When approaching from Trumpington Road, look out for Bidwells Estate Agent (next to the Shell garage) on the corner of Maris Lane. Kefford House is immediately behind this complex of buildings and shares a car park with them.

Getting here by car
The clinic is just off Trumpington Road, easily accessible from the M11 and A11 if coming from outside Cambridge. There is plenty of free parking at the centre indicated by yellow parking bays marked with a **'K'**. It is important that you use these parking spaces and not the others which are allocated to other buildings. You can also park at the Trumpington Park and Ride and take a bus. There is a stop close to the centre on Trumpington Road (see below).

Cycling to Cambridge IVF
There is ample covered cycle racking provided directly outside of Cambridge IVF for those wishing to cycle.

Public transport – Getting to Cambridge IVF by bus
Please visit https://www.stagecoachbus.com/plan-a-journey to plan your journey.



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FINDING CAMBRIDGE IVF AT IPSWICH HOSPITAL

The Cambridge IVF Andrology Service is located in the Central Zone, in the Gynaecology Clinic on the 1st floor. This is clearly signposted. A map of the hospital is available online, please see https://www.esneft.nhs.uk/your-visit/getting-here/ipswich-hospital/.

Getting here by car
The address for Ipswich Hospital is: Heath Road Ipswich Suffolk IP4 5PD.
Please park in car park G. Please note that parking charges apply (pay and display).
Your appointment should not last longer than one hour.

Public transport
Please visit https://www.esneft.nhs.uk/your-visit/information-for-visitors/getting-here/ipswich-hospital/ for more information on finding your way as well as information about how to get the hospital by public transport.

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