

Accreditation and the use of validated/ recognised methods to analyse human semen

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SUMMARY

Accreditation of laboratories who perform diagnostic semen analysis in Australia and New Zealand is a requirement of the healthcare system. Within the accreditation process laboratories are required to set ISO standards within their policies and procedures. In order to achieve their aims, laboratories need to be able to measure a number of defined semen parameters both accurately and repetitively, especially around the lower limit of the reference intervals. The methods documented in the WHO-manual are used almost universal as the laboratory standard. Some laboratories incorporate minor method variations into their procedures. As part of the ISO requirements all variations require validation using internally approved processes that are documented and that incorporate appropriate statistical analysis and comparison of results. Validation is an ongoing process and regular review is essential. Evidence of the validation must be available for review by external auditors during accreditation. Where any validated variant method returns results that are significantly different to any method within

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the WHO-manual, the laboratory needs to develop its own, in-house reference interval for that method. *Reproductive Biology 2011 Suppl. 3: 5-15.*

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INTRODUCTION

In April 2011, Scientists in Reproductive Technology (SIRT) – the scientific group within the Fertility Society of Australia – considered the 5th edition of the “WHO laboratory manual for the examination and processing of human semen” (WHO-manual; [3]) as an appropriate standard for diagnostic semen analysis. The process of implementation was also reviewed. I have been requested to discuss some of the recommendations of the new edition in relation to laboratory accreditation. As a general rule, clinical andrology laboratories within in Australia require accreditation by the National Association of Testing Authorities (NATA) and/or the Reproductive Technology Accreditation Committee (RTAC).

For the record, I did my first Semen Analysis (SA) in 1962. I used a haemocytometer and diluting pipette to calculate sperm concentration, a wet preparation on a glass slide to determine sperm motility and an eosin/nigrosin stained smear to view the sperm vitality and morphology. There were so many sperm in the sample (over 2000×10^6 sperm/ml) that their motility could be seen without the aid of a microscope when the semen was placed in a narrow test-tube. Almost all of the sperm had uniform, “perfect” morphology. The semen had been collected by me from an overexcited bull.

My career as a reproductive biologist started as Chief Semen Collector (self described – job title was actually Laboratory Technician) in the Department of Veterinary Physiology, The University of Sydney. The semen was collected for the academic staff of this department and its numerous graduate students who were engaged in research in basic reproductive biology. They were interested in determining the metabolic requirements and molecular structure of sperm. They were also keen to study the influence of female reproductive tract secretions on sperm activity. In all these studies, various measures of sperm structure and function were standardised using data

derived from semen analysis, particularly sperm count and motility (e.g. metabolic activity/million motile sperm), in order to facilitate comparative analysis. The results of these studies were published in peer reviewed scientific journals. The quality of the research was used to support applications for funding. Consequently, it was imperative that the methods employed were accurate and repeatable and that they could be validated, if required, by other similar institutions. Every method needed scientific rigour.

I was encouraged to attend university myself. During a stint as an undergraduate between 1967 and 1970 I learnt more about scientific principles. I learned that the basic tools for scientific endeavour, whether in an experimental setting or in a more practical environment, were defined in the stepwise process as Aim, Method, Result and Conclusion. While these principles may be hidden away in more current terminology, such as policy and procedure, they continue to apply in the case of the diagnostic SA in the human.

THE AIM OF SEMEN ANALYSIS

In a purely scientific sense, the aim of a diagnostic SA should be to accurately analyse and record observations relative to a number of defined parameters associated with a semen sample. The major parameters are those already described for the bull semen; sperm concentration, motility and morphology. In order to achieve this aim, some other measurements are required such as semen volume. In addition to the basic aim of accurate scientific “measurement”, a clinical aim can be appended. The laboratory was requested to perform the SA by a medical practitioner who needs to use the results to diagnose the expectation of whether the sample of semen was produced by a male with normal, reduced or absent fertility. Furthermore, in the modern Assisted Reproductive Technology (ART) clinical laboratory, there is a further aim associated with medical treatment. In this case, the results of a SA are used to predict the type of fertility treatment best suited to the male and his female partner who are attempting to start a family.

It is sometimes stated that some details related to ensuring the accuracy of the measurements within a SA can be unnecessary because accuracy does not always influence decision making and clinical relevance. This may be partially true where the results for each measured parameter lie well within the defined reference interval for the “normal” male. However, the achievement of quality within the laboratory is only enhanced by setting quality goals and expectations. The laboratory should have an overriding aim related to the moral and ethical treatment of the man from whom the semen was derived. Men do not want to hear that they may be responsible for fertility problems. Neither do women. If the man is suspected then the laboratory and the clinician need to be relatively sure that it is the case.

So the real aim of the SA in terms of quality care for the consumer/patient should be to produce a result from any single sample of semen that will be statistically indistinguishable from the result obtained in a different setting/laboratory anywhere. A SA on a sample produced and tested in Broome, Western Australia should give the same/similar result if it had been collected and tested in Invercargill, New Zealand. The most important area for diagnosis of expected fertility is in those samples where one or more of the crucial parameters are measured near the validated values of the “normal” range. In this range accuracy becomes very significant. If this aim is to be achieved then standardised and validated methods are essential.

ACCREDITATION

In Australia, it has long been required that any supplier of pathology tests who wishes to have its referred patients benefit from the government mandated, universal healthcare rebate system (Medicare) must be accredited. The government has a signed contract with the National Association of Testing Authorities (NATA) to undertake and complete this task for it. Diagnostic SA is a recognised pathology test with a listed Medicare item number. NATA conducts its audits on a 3 year rotation. A NATA scientific officer, with the aid of a specialized Technical Adviser, conducts the assessments against internationally recognised and documented standards (International

Organization for Standardization – ISO). In the particular case of a medical laboratory, the standard is ISO – 15189. One major requirement of ISO-15189 is for the test methods to be clearly documented and validated.

In addition, the Fertility Society of Australia (FSA) set up a Code of Practice (CoP) in 1987 for centres using ART and formed a sub-committee known as the Reproductive Technology Accreditation Committee (RTAC) to audit compliance. As a peer review system, the first step was to seek assurance that all ART units would sign an agreement to comply with the CoP. The RTAC system now has many years of successful experience and has reviewed its processes based on the experience. During the 1990s it was observed that a number of ART units were also undertaking ISO accreditation with respect to their management systems. Consequently, in the 2005 version of the CoP, it became a requirement that accredited ART units must use a documented quality management system (QMS) within the organization. As with NATA, the RTAC system has always required documented methods.

RTAC accreditation in 2011 is no longer dependent on peer review. Issuing of an RTAC licence is dependent upon the issuing of a certificate of compliance with the current RTAC CoP by an external Certification Body. The audits are conducted annually by a specialised auditor who may require the assistance of a specialist with technical expertise.

Irrespective of the accreditation system, the major role of the specialist technical adviser is to check the likelihood that the aims of the organization with respect to SA have been achieved and will continue to be achieved throughout the next accreditation period. It is noteworthy that most of the andrology laboratories within ART units are NATA accredited. Thus, when undertaking RTAC certification, the auditor will generally spend little time in assessing the semen testing. They are accustomed to accepting the SA section of the organization as being fully accredited if documentary evidence of their current NATA accreditation is presented to the auditor during the assessment.

During an accreditation assessment there are a large number of issues that potentially impinge on the laboratory's achievement of its aims. Matters such as the qualifications of the staff (particularly the responsible manager), the selection, training and ongoing competence of the testing staff, the equip-

ment in use, the maintenance of the equipment and the quality of the result-collection and reporting processes are all considered. Furthermore, there are issues to assess that are related to a variety of customers, particularly the users of the service (patients), referring doctors, outsourced services and suppliers. But for this presentation the subject is limited to the methods employed in the SA laboratory.

SEMEN ANALYSIS METHODS

In their book “Quality and Risk Management in the IVF Laboratory”, David and Sharon Mortimer [2] summarize the requirements for the laboratory test as described in ISO – 15189. Methods should be selected preferably from authoritative sources – text books, refereed scientific papers or respected guidelines. In-house methods are acceptable but, if used, they must be appropriately validated.

From the accreditation, technical adviser’s point of view, it has been the experience that almost all andrology laboratories in Australian and New Zealand choose the WHO-manual as their standard. The option of using other quality textbooks or published articles is rarely encountered during the audit process. In-house variations covering small subsections of the WHO-manual procedures are occasionally observed.

Choosing the WHO-manual as the standard carries a number of advantages. The manual was first published in 1980. Improvements and updates have been managed by appointment of a distinguished international review panel for each edition. These panels have sought to continually achieve global consensus and consider recommendations from those actually working at the “coal face”. The new edition seems particularly impressive in its desire to provide details, explanatory notes and rationale for some of the descriptions of analytical measurement that in the past have been left to the individual to ponder and argue over. The use of worked examples, discussion of sampling and other errors and a large chapter on quality assurance is considered to have enhanced the overall strength of the document. As well as providing well defined methods, an additional advantage is the section and table

defining appropriate lower reference limits (normal range) and statistically derived confidence intervals.

Where a laboratory chooses to use the WHO-manual as its standard, it is required to regularly audit the performance of the testers to ensure they are diligently applying the approved methods – an internal audit within their QMS. As would be expected of an external audit, the auditor should enter the workplace and view the work instructions (documented methods) while simultaneously observing a SA being conducted. The check-off at the workplace ensures each step within the manual is followed exactly by the trained staff.

One disadvantage of the WHO-manual that has received some comment relates to change itself. Often the diagnostic SA laboratory is a small section of a busy clinical service laboratory. The supervisor responsible for the andrology laboratory probably hopes that diagnostic SA methods will never change. If standardized methods change then they are aware that there will be a need for effort and input from them to retrain staff, revise documentation and call in the computer systems designer to alter electronic methods, data collection and report production. Change is never popular even if it is for the better.

ACCEPTING VARIATIONS

There are always some areas of the WHO-manual that create some discussion points and lead some laboratories to consider developing their own variations. The absence of a clearly defined lower reference limit for normal sperm morphology in the 4th edition caused initial problems. However, these were generally overcome by use of appropriate statements on laboratory reports that were derived from the text within the manual. In the 5th edition, there is a notable change away from defining sperm motility in different classes depending upon the rapidity of their individual forward progression. Some laboratory managers refer to published data showing that the likelihood of sperm being transported to the site of fertilization and actual oocyte penetration and fertilization is greater if there are significant numbers of sperm

with high grades of motility. They may choose to continue to report their sperm motility using the system where motility is graded as rapid, slow, non-progressive or immotile. Their opinion as to this WHO limitation should be considered as an extra, in-house refinement as the total % motility is still determined by WHO methodology.

A variation to a part or parts of the WHO-manual may be selected and approved by the laboratory director within the QMS. These methods with non-compliance to the WHO-manual must receive appropriate implementation if they are to be accepted as appropriate procedures during accreditation. The variation needs to pass an extensive validation procedure. The actual methods of validation must be documented. Records of the initial validation procedure and the results must be kept and made available for review by accrediting bodies. Non-standard methods must also be reviewed at some defined but regular intervals and all reviews must be documented as evidence of compliance.

As examples of potential variation from the WHO-manual 5th edition, let's consider the following:

1) Measurement of semen volume

The accuracy of measuring semen volume by aspirating the semen into a pipette has been seriously questioned and not recommended as the volume will likely be underestimated. This opinion has been further investigated by SIRT in a multicentre investigation [1]. This group compared gravimetric and pipette aspiration analyses and showed a significant difference that is not resolved by use of correction factors. Laboratories that do consider the use of any alternative methods for SA to the WHO-manual are especially referred to this paper to review the type of procedures that are required to adequately validate and document their method.

2) Accurate semen sampling

When a semen sample is to be taken where an accurate volume needs to be dispensed (such as during dilution for sperm concentration analysis) then a positive displacement pipette is to be used. Furthermore, there is instruction in the method regarding the use of these pipettes. Again, use of an alternative method requires validation.

3) Counting sperm in low volume chambers

The sperm counting section of the WHO-manual is exhaustive in its descriptions and worked examples for semen with normal concentrations through to semen with low sperm counts. Chambers with depths of 100 μm are recommended. However, there is clear discussion related to the use of low volume chambers (such as Makler) and testing their validity. As discussed previously, the most important consideration for accuracy relates to the area of the lower limit of the normal range – around 15×10^6 sperm/ml. One problem related to the use of the Makler counter in this important part of the normal range is the number of sperm counted to give a statistically acceptable sampling error and for comparing replicate counts. Semen is added to a Makler chamber undiluted and there is a maximum of 100 squares to count on the grid pattern. Consequently, when a sample is close to the lower limit there will be less than 200 sperm within the 100 squares of the grid. One replicate count will thus require the chamber to be filled and counted twice to obtain the goal of 200 sperm counted per replicate.

VALIDATION

Validity generally refers to the extent to which a concept, conclusion or measurement is well-founded and corresponds accurately to the real world. An excellent example of the approach to validating a method in SA has been reported [1], and important considerations are found in their analysis of the data. The number of observations required to reach a reasonable conclusion must be considered along with the types of statistical analyses and point at which statistical significance is reached.

A laboratory may choose to explore a non-standard method using this approach. After validation they may be able to provide evidence that the results when using this method are not significantly different to the standard. Accreditors should not have any issue with the implementation and use of this alternative provided that the validity is regularly audited and reviewed. On the other hand, during validation of a method against a standard procedure,

the results may be found to differ. The linear regression and correlation plots and statistical calculations used in the SIRT multi-centre trial [1] clearly show that, while the different measurements of sperm volume are closely correlated, there was an underestimation of volume when measured by aspiration of semen into a pipette. As a result, if a laboratory chose to measure volume by pipette aspiration then the normal range and lower limits defined by the WHO-manual will no longer be acceptable.

Within the accreditation system, it is considered insufficient for a laboratory attempting to validate a method variation to simply compare the results of 10 calculations/observations with 2 different methods then “eyeball” the averages/means and state that they are not significantly different. It is expected that samples used in a validation procedure will cover the expected “normal” range of the test. But it is also advisable to include a relatively rigorous examination with some samples around the lower limits of the reference interval where clinical decision making has serious repercussions for the patient.

DEFINING A REFERENCE INTERVAL

My consultations with other similar technical assessors suggest that major variations in methods away from the WHO manual are unusual. Once laboratory directors become aware of and comfortable with the requirements to conform with ISO-15189 or the RTAC CoP, they usually decide on the pathway of least stress if and when their method results stray from the standard. If they continue to feel there are shortcomings in WHO-manual methods and that they have in-house improvements, the added complication will be that the laboratory will now be required to set in-house reference intervals to be appended to their reports and used by the clinician that will be related to the method in use. This can be a turning point in their decision making process because it adds a new level of difficulty.

To achieve an in-house derived reference interval, the laboratory must define the population of men who they will need to collect samples from for analysis. Most of the men attending fertility clinics for semen analysis

are in infertile relationships and it is argued that these are unacceptable as “normal” men. It is not a simple task to find a large group of “normal” men who have become fathers in the last 12 months using sexual intercourse as the route to pregnancy and who would be prepared to each provide a single semen sample for the purpose.

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