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Good practice in laboratory diagnostic andrology: Association of Reproductive and Clinical Scientists guidelines 2024

Association of Reproductive and Clinical Scientists

BIOGRAPHY

The Association of Reproductive and Clinical Scientists (ARCS) is the professional body for those working in Reproductive Science in the UK and worldwide. Founded in 2020 (from BAS, ACE and ABA), ARCS strives to promote high standards of practice and advance the reproductive science profession through training, education and community.

Meurig T. Gallagher[1,*], Emily Roxburgh[2], Gwen Bennett[3], Susan Parker[4], Jackson C. Kirkman-Brown[5], Association of Reproductive and Clinical Scientists[6]

KEY MESSAGE

The Association of Reproductive and Clinical Scientists (ARCS) suggests these guidelines are appropriate and effective for ensuring the best possible care for individuals undergoing diagnostic semen analysis. Efforts have been made to use inclusive language that is reflective of the wide range of patients who seek information and diagnosis.

ABSTRACT

These guidelines update and clarify items relating to diagnostic andrology in the 2012 Association of Biomedical Andrologists Laboratory Andrology Guidelines for Good Practice Version 3. The main change separates diagnostic and therapeutic andrology into individual documents; post-vasectomy semen analysis still references the 2016 guideline. These guidelines seek to incorporate and clarify internationally agreed methodology following the World Health Organization *Laboratory Manual for the Examination and Processing of Human Semen* 6th edition and publication of ISO 23162:2021. Significant updates include: requiring four-category grading for motility (A, rapidly progressive; B, slowly progressive; C, non-progressive; D, immotile); a four-part morphology assessment (head, midpiece, tail, cytoplasmic droplets) as essential for quality assurance (even if only the percentage of 'normal' is reported); and specifying sperm toxicity testing procedures for diagnostic andrology. These guidelines include a section on haematospermia, an observation requiring rapid onward referral. An Association of Reproductive and Clinical Scientists (ARCS) working group wrote these guidelines, with review by ARCS members. The aim is to guide good practice in laboratories but they are not intended as a tool to judge the practice of centres within the UK or beyond.

[1] Centre for Human Reproductive Science, Institute of Metabolism and Systems Research, College of Medical and Dental Science, University of Birmingham, Edgbaston, Birmingham, UK
[2] Hull and East Riding Fertility, Hesslewood Office Park, Hessle, Hull, UK
[3] Pathology Department, Betsi Cadwaladr University Health Board, Ysbyty Maelor, Croesnewydd Road, Wrexham, UK
[4] Andrology Department, Pilgrim Hospital, Northern Lincolnshire and Goole Hospitals NHS Foundation Trust, Boston, UK
[5] Birmingham Women's Fertility Centre, Birmingham Women's Hospital, and Birmingham and Centre for Human Reproductive Science, IMSR, College of Medical and Dental Science, University of Birmingham, Birmingham, UK
[6] https://www.rbmojournal.com/article/S1472-6483(24)00291-8/fulltext

KEY WORDS
Andrology
Best practice
Diagnostic andrology
Guidelines
Reproductive science

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*Corresponding author. E-mail address: m.t.gallagher@bham.ac.uk (M.T. Gallagher). https://doi.org/10.1016/j.rbmo.2024.104373 1472-6483/© 2024 The Author(s). Published by Elsevier Ltd on behalf of Reproductive Healthcare Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)
Declaration: The authors report no financial or commercial conflicts of interest.

INTRODUCTION

The following guidelines have been developed by ARCS to be used in diagnostic andrology laboratories. They aim to providing guidance on current good practice for diagnostic andrology and should be seen as the discipline-specific supplement to the United Kingdom Accreditation Service's *Medical Laboratory Accreditation* (United Kingdom Accreditation Service (UKAS)) of ISO 15189 (*International Organization for Standardization (ISO), 2022*) and (where appropriate) the Human Fertilisation and Embryology Authority (HFEA) Code of Practice for licensed centres (*Human Fertilisation & Embryology Authority, 2023*).

All laboratories are obliged to comply with other legislation such as the following: the *Health and Safety at Work Act 1974* ("*Health and Safety at Work Act 1974," 1974*); Control of Substances Hazardous to Health (COSHH) Regulations 2022 ("*Control of Substances Hazardous to Health (COSHH) regulations," 2002*); and, in cases where it applies, the *Human Fertilisation and Embryology (HFE) Act 2008* ("*Human Fertilisation and Embryology Act," 2008*), noting that the HFE Act does not itself regulate diagnostic andrology.

The overarching accreditation standard for medical laboratories is ISO 15189 (*International Organization for Standardization (ISO), 2022*), which is assessed in the UK by the United Kingdom Accreditation Service. The specialist accreditation ISO 23162 (*International Organization for Standardization (ISO), 2021*) provides standards for basic semen analysis but does not apply to post-vasectomy assessment. The development of ISO 23162 gives *de facto* references for the basic examination of human semen, with wide international consensus (*Björndahl et al., 2022*). As such, laboratories carrying out diagnostic semen analysis should at a minimum be accredited to either ISO 15189 or ISO 23162.

Guidelines for current good practice have been drawn together from current legislation, from the World Health Organization ('*WHO 2021*'; *World Health Organization, 2021*), ISO 15189 (*International Organization for Standardization (ISO), 2022*) and ISO 23162 (*International Organization for Standardization (ISO), 2021*), from experts in the field of clinical and laboratory diagnostic andrology, and from professionals in associated disciplines such as embryology and blood and tissue banking. Evidence for good practice is provided where relevant and where possible; otherwise a consensus and pragmatic view from ARCS is provided.

This document should be regarded as superseding any previous Association of Biomedical Andrologists, Association of Clinical Embryologists or British Andrology Society good practice documentation on diagnostic andrology or toxicity testing for diagnostics when it directly impinges thereon. The decision to move to *WHO 2021* standards (*World Health Organization, 2021*) for diagnostic analysis toxicity testing was taken to ensure alignment with internationally agreed standards. As clarified in the *WHO 2021* manual, toxicity testing must involve exposure to the relevant analyte (e.g. raw semen) and not a different material (e.g. prepared spermatozoa) where a different reaction and response could be observed (see section 2.5.10 in *World Health Organization, 2021*).

It should be noted that the existing guidelines on post-vasectomy semen analysis (PVSA) (*Hancock et al., 2016*) and uncertainty measurement (*Sanders et al., 2017*) are maintained. Matters relating to donor banking and cryostorage will be considered in separate documents.

Diagnostic semen analysis is undertaken at a variety of sites in the UK including dedicated laboratories and HFEA-licensed assisted reproductive technology treatment centres. For brevity throughout these guidelines, 'centres' are referred to as meaning any premises where diagnostic semen analysis is performed.

These guidelines are dedicated to ensuring that diagnostic semen analysis delivers accurate results that can be interpreted according to the *WHO 2021* manual international data. Failure to comply with these methodologies risks undermining the accuracy and relevance of any result for patient diagnosis. It should also be noted that basic semen analysis may find other observations that are critical to patient care beyond a question of fertility, as for example in the new section on haematospermia, and therefore informed, validated and accurate service provision is essential.

A. PERSONNEL AND TRAINING

A.1. Diagnostic laboratory management and professional direction should be carried out by a scientist from a relevant discipline with delegated authority. The individual should be a healthcare scientist with recent appropriate and relevant experience and should be at healthcare scientist grade 7 or above (or an equivalent).

A.2. If such an individual is not available, centres should seek direction from a scientist at another centre. This should be carried out by formal arrangement with an appropriate contract and take account of the need of the appointed individual to be named on the other centre's HFEA licence (if required) to permit access to confidential information, if appropriate.

A.3. The precise personnel requirements should be appropriate for the service. Each service should have access to a specialist consultant, principal or clinical scientist in reproductive science (andrology) for guiding practice, actions and guidance for referrers. They need not be in the same geographical location or organization but must have funded dedicated time. This may be different from local leadership and, for example, could be provided via Service Level Agreement (SLA) with another provider. The purpose is to ensure the highest standards of guidance and patient safety and care.

A.4. The relationship between the clinical lead and the laboratory lead should be defined within the centre's quality manual and in the relevant laboratory documentation.

A.5. Any diagnostic laboratory should have a designated quality manager but, depending upon laboratory size, this may be incorporated into one of the existing positions or lie within an overarching parent organization and not be a full-time role.

A.6. Staff number and skill mix should be appropriate for the workload.

A.7. As an approximate guide, it has historically been suggested that there should be a minimum of 1.0 'whole-time equivalent' staff in place per 1500

specimens per year if the individual is only engaged in examination procedures relating to those specimens (*Tomlinson et al., 2012*). With the additional requirements for fully implementing these guidelines and the changes in *WHO 2021* (*World Health Organization, 2021*) to improve the standards of diagnosis, it is suggested that 1000 specimens per annum per individual may be more reasonable. If automation is *in situ*, this may allow this figure to be increased. Sufficient administrative and support staff should be available for appointments, specimen reception and appropriate pre- and post-examination procedures. These figures are approximate and should be based on workforce calculations relating only to diagnostic semen analysis. An increased number of tests of lower complexity, for example PVSA, in large-volume chambers could thus be substituted for the above. Centres must therefore base their workforce calculations on the service mix and complexity of testing provided and whether licensed procedures resulting in treatment are offered.

A.8. All new staff should undergo a comprehensive orientation and induction programme both at an institutional level, for example the parent organization, as well as at laboratory level.

A.9. All members of the laboratory should have documented evidence of their competence for each laboratory process that they undertake. Recommendations and information on appropriate training should be offered where appropriate. Maintenance of competence should be demonstrated by ongoing continuing professional development (CPD), a continued programme of direct observation of procedural skills (DOPS) and measurable examination audits. The DOPS and competence audits should occur as defined by the quality management system but as a minimum on a 2-yearly basis. Membership of the formal CPD scheme operated by ARCS is beneficial for scientists working in andrology, and maintenance of CPD should be supported by the employer. Larger laboratories may wish to have a nominated training officer/lead to assist with this process.

A.10. All staff should undergo an annual appraisal/joint review and keep and maintain a personal development folder, documenting all relevant training, whether it is 'in-house' or external.

A.11. There should be administrative and support staff of appropriate skill level in post to meet the needs of the service and ensure subjects' and service users' satisfaction.

A.12.There should be adequate cover by fully trained and competent personnel for staff holiday and sickness.

A.13. There should be an ongoing funded training programme for all staff relevant to their job role, including the opportunity to attend relevant clinical and scientific meetings. All training should be formally recorded as part of each individual staff member's personal development portfolio.

B. PREMISES AND ENVIRONMENT

B.1. Generally, the premises and environment should be designed and constructed to a specification that suit its intended purpose. The main considerations for the facility are the well-being of staff and subjects, the maintenance of the quality of the sample, and the facility's general suitability for all required activities.

B.2. The andrology laboratory should provide adequate space for the levels of staff, equipment and activity within it.

B.3. Laboratory premises must take account of the need for environmental control, safety and confidentiality.

B.4. An emergency power supply should be provided for all critical items of equipment, including incubators, fridges/freezers and monitoring equipment.

B.5. Equipment should be used within a sufficient and safe operating space in accordance with the manufacturers' specifications.

B.6. Laboratory surfaces including work surfaces, floors and walls must have non-porous surfaces that can be cleaned easily.

B.7. There should be a clear separation between laboratory and clerical areas.

B.8. Attention should be paid to general working conditions, such as ergonomic bench and seating height, ambient temperature, air quality and lighting.

B.9. Appropriate personal protective equipment (PPE) must be provided to all staff handling biological specimens.

B.10. The diagnostic laboratory must have facilities for sample production except in rare cases where a laboratory only assesses samples produced off-site. These facilities should be private and comfortable, and provide basic washing facilities. They should not be a dedicated staff or public toilet (as carrying out a sexual act in a public toilet is a criminal offence in England and Wales; "*Sexual Offences Act," 2003*).

B.11. Sample reception areas should be designed and equipped with careful consideration of the following:

- Clutter-free and safe decontamination, ensuring adherence to local policies on infection prevention and control
- The safe delivery of samples
- The effective and efficient receipt of samples
- The comfort, privacy, security, hygiene and safety of subjects. Notably, the sample production area should be away from subject and/or staff traffic to reduce noise and maintain dignity
- The security and safety of staff
- Access for disabled persons.

B.12. Adequate provision should be made for sample disposal.

B.13. There must be secure designated controlled access areas for the storage of laboratory records (Appendix 1).

C. EQUIPMENT, INFORMATION SYSTEMS AND MATERIALS

Documented procedures must be in place to manage equipment, consumables and reagents that have a direct impact on the quality of the laboratory output.

C1. General

C1.1. There must be clear and unambiguous traceability between any piece of equipment, consumable or reagent and any subject's sample.

C1.2. Any piece of equipment or reagent that comes into contact with subjects or their biological material must comply with the regulations for medical devices ("*Medical Devices (In Vitro Diagnostic Devices etc.) (Amendment) Regulations 2024," 2024*).

C1.3. Media to be used for the preparation and culture of spermatozoa must be manufactured under conditions observing good manufacturing practice. Any additional reagents or media should be of a purity appropriate for the intended purpose.

C1.4. Any item of laboratory equipment or an accessory that is used as part of a diagnostic process should be fully validated and, where relevant, UK Conformity Assessed (UKCA) or Conformité Européene (CE) marked, or equivalent, in line with regulations.

C1.5. Any pieces of equipment or reagents that have been modified in any way and used not in accordance with manufacturers' recommendations must have documented evidence of compliance with regulation for in-vitro manufacture and be validated for that use. This must comply with the Medical Devices (In Vitro Diagnostic Devices etc.) (Amendment) Regulations 2024 ("*Medical Devices (In Vitro Diagnostic Devices etc.) (Amendment) Regulations 2024," 2024*).

C1.6. Third-party agreements should be established with suppliers of media and consumables to ensure continual quality of the product, its specification and its delivery. These should be reviewed annually.

C2. Equipment

C2.1. The service and calibration schedule for all equipment should be in line with manufacturers' recommendations and supported by relevant documentation.

C2.2. Laboratory equipment must be fit for its purpose and suitable for cleaning and decontamination. There should be a procedure in place for regular documented cleaning and maintenance as per laboratory policy.

C2.3. There should be a procedure in place for, and documented evidence of, the decontamination of all items of equipment prior to service and maintenance.

C2.4. Critical pieces of equipment such as incubators, refrigerators and monitoring equipment must be connected to emergency power supplies and linked to a suitable early warning system in the event of failure.

C2.5. New equipment should be fully validated prior to use. Laboratories should not view UKCA/CE marking as a substitute for independent validation.

C2.6. Specific procedures should be in place for ensuring the calibration of thermometers due to the impact that temperature can have on motility assessment. This calibration should include uncertainty and bias when adjusting ranges. Daily checks are recommended of items such as heated stages that encompass thermal stability over prolonged times. This checks that, for example, switching the heater on and off does not increase temperatures beyond 37°C.

C3. Media and reagents

C3.1. The media and reagents must be stored according to manufacturers' instructions. Where storage at a defined temperature is specified, ongoing records of the storage temperature should be documented and kept.

C3.2. Where possible, temperature critical consumables should be split between two temperature-controlled storage units to prevent total loss in the event of equipment failure.

C3.3. All seals and packaging on commercial products should be checked on arrival.

C3.4. Certificates of analysis and details of quality control measures should be supplied by manufacturers and it should be checked that they correspond with the batch delivered.

C3.5. Media, consumables and reagents should be logged into a stock and batch control system and used in date order or as appropriate. There should be procedures in place for batch verification and toxicity testing (see section C4) of relevant consumables and recorded as per laboratory policy.

C4. Toxicity testing of sperm collection containers

C4.1. Prior to their usage, all items used for diagnostic semen analysis that come into contact with spermatozoa, such as sperm collection containers and pipette tips, should be assessed to ensure they are non-toxic to spermatozoa using relevant exposure times. 'Relevant exposure times' can be assumed to be twice the usual duration of exposure (as stated in *WHO 2021*; *World Health Organization, 2021*).

C4.2. It is essential that any toxicity testing involves the precise combination of container/consumable and analyte. For example, sperm collection containers must be tested with raw semen and not prepared spermatozoa, where the media could alter or buffer any observed effects (as stated in *WHO 2021*; *World Health Organization, 2021*).

C4.3. Testing should occur whenever new batches of items are purchased, if concerns around storage of items emerge or if laboratories wish to check their items against those used by another diagnostic laboratory.

C4.4. Toxicity testing should be in accordance with *WHO 2021* procedures (*World Health Organization, 2021*); for clarity the following explains how to toxicity test sperm collection containers (as an example):

- An ejaculate should be collected in a known safe container
- The ejaculate should then be split in equal portions into a fresh known safe container (control) and the test container (test)
- The motility of spermatozoa in both the control and test containers should be assessed directly and then once again after 4 h
- This process should be repeated on five separate ejaculates of sufficiently high concentration and motility
- For the initial and 4 h time points, the proportion of progressively motile spermatozoa in both the control and test assessments (at that time point) should be compared using a paired statistical test such as a Wilcoxon signed-rank test (non-parametric) or paired t-test (parametric). There should not be a comparison between the initial and 4 h time points.
- If there is no statistically significant difference between the control and test samples ($P > 0.05$), the test containers can be considered non-toxic to spermatozoa.

D. PRE-ANALYTICAL PROCESSES

D1. Information for service users

Referring clinicians

D1.1. All centres must provide comprehensive service user information. This may be in written or, for example, video forms. Irrespective of how the

information is provided a record of the offer of the information and its content must exist. Where information is provided verbally, this could for example be a signed checklist. Information should include:

- An introduction to the service
- Location and relevant contact details
- Key personnel
- Normal working hours
- Scope of services
- Where relevant, how to refer a subject for:
  - Diagnostic semen analysis
  - PVSA, following the 2016 guidelines (*Hancock et al., 2016*)
- Provision of information for the subject, including how to provide user satisfaction feedback
- Instruction for the collection and delivery of the sample
- Sample acceptance criteria
- Procedures for repeating the semen analysis (where applicable)
- Results and interpretation of diagnostic tests.

Subject information

D1.2. Subject information for *semen analysis* should cover the following:

- A brief outline of the tests carried out and why they are necessary
- How the samples are collected and delivered for both samples produced 'on' and 'off' site, including:
  - The required period of sexual abstinence
  - Instructions for the collection of the sample
  - The importance of collecting the entire sample
  - The need for personal hygiene
  - The need to use the sperm collection container provided
  - Transportation to the laboratory
  - The need for accurate labelling
  - Alternative sample collection mechanisms where required
- Where the andrology laboratory is located
- How the subject obtains an appointment
- Why repeat tests are sometimes requested
- How the result is obtained, and information regarding the measurement of uncertainty of the result
- Andrology laboratory contact details
- Additional information for subjects with (or with suspected) retrograde ejaculation
- Information taking account of subjects with disabilities
- How to provide user-service feedback.

D1.3. Additional information for those having *post-vasectomy testing* should cover the following:

- How long and how many ejaculations after surgery, and that testing should take place
- Definition of clearance
- Definition of special clearance
- The need to use other forms of contraception until advised otherwise by the referring clinician or specialist.

D2. Semen analysis request/referral forms

D2.1. These are usually from a clinician and should be explicit and unambiguous.

D2.2. Request forms for semen analysis should include the following:

- Sufficient information about the subject to permit unequivocal identification
- Details of the referring clinician and practice
- The unique subject number
- Details of the investigations required
- Details of previous investigations/tests including screening for infectious diseases (as these may influence what is observed or expected, for example a previous azoospermic result)
- Relevant clinical details and history (e.g. a retrograde sample being expected, or a history of radio- or chemotherapy).

D2.3. If testing takes place as part of the investigation of a couple, the laboratory must emphasize to the requesting clinicians the need for testing to be carried out for the sperm provider and under their name and unique identifying reference.

D3. Sample collection

D3.1. Standard sperm collection containers should be UKCA/CE marked, have a secure airtight lid and be wide mouthed. Centres should be aware of the limitations of CE marking of low-risk products, as this does not indicate that sperm toxicity testing has been carried out. As such, sperm toxicity testing of batches of sperm collection containers and labelling with the weight must be performed.

D3.2. Alternative methods of sample collection should be available, such as use of non-spermicidal condoms.

D3.3. Samples should not be collected using the withdrawal method (coitus interruptus).

D3.4. Where there is the need to establish a chain of custody, for example in legal/forensic cases, samples should be produced 'on-site'.

D4. Sample reception

D4.1. There should be adequate facilities and procedures for sample reception, and the following should be considered:

- Where samples are delivered by third parties a chain of custody must be established
- Subjects producing samples 'off-site' should follow the 'instructions to subjects' and attend the andrology centre ideally within 30 min of sample production, but no longer than 50 min after it. The identity of the subject should be established by written confirmation and positively verified
- Sperm collection containers must be labelled with at least three identifiers. Where identifying information is incomplete laboratories should risk assess the processing or disposal of the sample on a case-by-case basis.

D4.2. For each subject, laboratories should confirm the information with the sperm provider:

- The practitioner who requested the test or procedure
- Whether the sample was complete
- Whether the sample was produced on- or off-site
- Whether the sample production procedure was followed
- The duration of sexual abstinence
- Any recent illness or relevant medication (examples may include recent febrile illness; this is not an absolute requirement, but if no history is taken additional care must be observed around any interpretation, and it should be recorded that no history was taken)
- The option to consent to training
- Verification of the specimen provenance should be included once it is known by the testing laboratory
- Unlabelled samples arriving at the laboratory should be discarded if identity cannot be confirmed beyond reasonable doubt.

D4.3. Centres should never take information that they are not going to include on the report form as this

confounds clinical interpretation and it may be the only time the subject volunteers this information.

D4.4. If the data are not being reported, due consideration of the UK Data Protection Act 2018 and information governance means the data should not be taken.

D4.5. Additionally, the processes in place should record the following:

- There should be a checking procedure in place to verify that the details on the sperm collection container, request and report forms correspond
- All samples and corresponding consumables should be given a unique accession number
- The time of collection, time of sample receipt and time of sample analysis must be recorded.

D5. Sample rejection

D5.1. Centres should develop their own 'sample rejection' criteria. These may be applied differently from samples used for diagnosis (semen analysis or PVSA) and consider the risk associated with partial or non-compliance with the sample acceptance criteria listed above. ARCS recommends that samples are rejected from accredited analysis reporting if:

- unequivocal identification of the subject's sample is not possible
- samples provided for PVSA are incomplete
- samples are analysed using non-standard procedures (e.g. after more than 1 h).

D5.2. Centres should give due thought to the stress to a subject if their diagnostic sample is not analysed. If the centre's sample rejection criteria are met, it may still be appropriate to analyse as much as is possible and provide a reporting statement in the form of narrative text rather than being displayed against the WHO decision limits. Importantly, in such cases it is not appropriate to issue an accredited report, and the reason for the sample rejection must be specified, as well as a cautionary statement about the interpretation of the narrative report.

E. ANALYTICAL PROCESSES

E1. Diagnostic semen analysis

E1.1. A diagnostic semen analysis must comprise an assessment of concentration, motility and morphology, with vitality testing if the motility result is low (see section E1.10).

E1.2. All examinations of live spermatozoa must be carried out using phase contrast or other appropriate contrast microscopy (e.g. differential interface contrast microscopy or Hoffman microscopy).

E1.3. Macroscopic measures including volume (measured via weight), liquefaction, odour and viscosity must be routinely recorded.

E1.4. Time-dependent measures – pH, sperm motility, vitality and agglutination assessment – should all be completed within 60 min of sample production.

E1.5. Sperm concentration dilutions should be made up within 60 min of sample production, and assessment ideally performed within 3 h.

E1.6. Sperm concentration must be assessed on immotile spermatozoa and should be assessed using a haemocytometer with improved Neubauer ruling (*World Health Organization, 2021*). Any alternative to this should be validated against a haemocytometer with improved Neubauer ruling for samples at both high and low concentration.

E1.7. Sperm motility analysis must always be performed at 37°C using a heated microscope stage.

E1.8. Four grades of motility must be assessed (A, rapidly progressive; B, slowly progressive; C, non-progressive; D, immotile). There is clinical evidence supporting sperm progressive motility being a strong predictor of fertility outcome (*Barratt et al., 2011*).

E1.9. Vitality testing using either eosin–nigrosin staining or the hypo-osmotic swelling test must be carried out for samples with low total motility (<40%).

E1.10. Sperm morphology analysis should be examined and reported on within a predefined timeframe that meets the needs of the users of the service. Morphology slides should be prepared following the procedure set out by *WHO 2021* (*World Health Organization, 2021*). Briefly, smears should be prepared, air-dried, fixed and then stained, ideally with sperm Papanicolaou staining (other staining methods can be used if validated in comparison with sperm Papanicolaou staining following WHO guidance).

E1.11. Sperm morphology analysis should be performed at 1000 × magnification following *WHO 2021* (*World Health Organization, 2021*) and ISO 15189 guidelines.

E1.12. Sperm morphology scoring must be performed as a four-category measure according to WHO guidance (*World Health Organization, 2021*). This ensures minimization of inter-operator variability in the laboratory and is essential for correct quality assurance.

E1.13. If no spermatozoa are observed in the replicate wet preparations, refer to section 2.4.88 *Low Sperm Numbers* in *WHO 2021* for details of how to process and assess these (*World Health Organization, 2021*).

E1.14. If laboratories propose methods other than those described above or recommended by *WHO 2021* (*World Health Organization, 2021*), written justification and full laboratory validation should be provided comparing the alternative method against the recommended standard. When such validations occur, they should be performed using the relevant clinical samples and timeframes as discussed in *WHO 2021* (*World Health Organization, 2021*); for example, for concentration or motility this must be with samples taken for examination within the clinical timeframe set and at or around the clinical thresholds – waste samples with different liquefaction properties therefore cannot be utilized.

E1.15. ARCS does not recommend the use of methods for sperm quality analysis that do not involve the direct microscopic visualization of the sample. This includes those that instead employ mathematical algorithms to calculate semen quality, for example spectrophotometric methods.

E1.16. ARCS highlights that methods where the semen sample cannot be assessed within 60 min of ejaculation cannot be used to make accurate diagnostic presumptions allied to the *WHO 2021* reference ranges (*World Health Organization, 2021*). If diagnostic laboratories have limitations within their service and are unable to assess samples within 1 h, samples with motility or morphology results that fall beneath the

normal reference range should not be communicated alongside these ranges as the necessary diagnostic requirements have not been met. Failure to do this may result in clinical misinterpretation of evidence.

E1.17. The *WHO 2021* manual (*World Health Organization, 2021*) does not suggest anti-sperm antibody testing or identification of leukocytes as part of a basic semen analysis. If laboratories wish to run these or other extended examinations, they should follow the guidance provided by the WHO (*World Health Organization, 2021*).

Uncertainty of measurements

E1.18. There is always a degree of error or uncertainty associated with any laboratory measurement of biological processes; see the best practice guideline on uncertainty (*Sanders et al., 2017*). All centres should report results with an associated measurement uncertainty statement.

E1.19. Below is a summary of recommendations that should be considered to reduce the level of uncertainty associated with measurements made in the andrology laboratory:

- Standardizing sample collection procedures, which will reduce the inherent biological variation in semen quality
- Complying with sample acceptance criteria
- Ensuring samples are homogeneous
- Training and ongoing assessment of competence
- Following standardized operating procedures
- Counting, at a minimum, 200 spermatozoa per analysis assessed in duplicate to give an acceptably low sampling error
- Multiple sampling of the same sperm sample
- Implementing internal quality control (IQC) procedures
- Participating in relevant external quality assurance (EQA).

E2. Retrograde ejaculates

E2.1. Section 5.9 of *WHO 2021* (*World Health Organization, 2021*) should be followed where retrograde ejaculation is suspected. It is not necessary to use density gradients if they are not available routinely in the diagnostic laboratory.

E3. Post-vasectomy semen analysis

E3.1. PVSA should be carried out in accordance with the existing guideline (*Hancock et al., 2016*) or a specific ARCS-endorsed successor should one be published.

E4. Sperm preparation

E4.1. Sperm preparation methods are designed to take spermatozoa from the seminal fluid and place them in an artificial medium, which will support sperm function. While sperm preparation methods are not necessary for basic semen analysis, there are specific diagnostic tests that may benefit from preparation. One example is gradient preparation to assess whether, for example, any morphologically normal sperm exist in a globozoospermic sample, to inform later treatment planning. If preparing spermatozoa for a diagnostic assessment, the following should be implemented following *WHO 2021* guidance (*World Health Organization, 2021*):

- All media used for sperm preparation (e.g. density gradient media or sperm buffer) must be validated and batch numbers recorded. 'Home-made' media should not be used
- The sperm preparation method, whether density gradient centrifugation or swim-up, should be assessed as appropriate for the judged outcome. This usually requires consensus around treatment plans with a likely treatment centre and their scientific team
- All semen characteristics pre- and post-sperm preparation should be recorded using the methods described above
- Diagnostic sperm preparation should state the starting volume and re-suspension volume of spermatozoa. Sperm concentration should be reported as millions/ml
- Diagnostic thresholds should be agreed upon with clinical colleagues using clinical data and evidence in the literature
- Prepared sperm should be protected from extreme temperature and/or pH fluctuations
- There should be suitable decontamination procedures between subjects
- Laboratory staff should avoid processing more than one sample at any one time.

F. POST-ANALYTICAL PROCESS

F1. Reporting results

F1.1. Most centres issue several report types. Reporting of results must be sympathetic to the category of subject under examination and indeed the needs of the clinical user who will receive the report. For example, a semen analysis result that falls below the accepted normal threshold may be a concern to patients with infertility but may be more than satisfactory if the patient has received chemotherapy. Insightful interpretative comments and summaries are extremely important in such cases, notwithstanding a sufficient clinical history being available. When teams are not party to full clinical datasets, they should refrain from drawing conclusions based upon incomplete information. Instead, results should be provided without comment, leaving the interpretation to teams with access to the full information.

F1.2. Expert fertility clinicians may require a low level of comment, summary and interpretation of the report, yet a much higher level may be required by others (e.g. a general practitioner, an oncologist, a urologist or a subject). As discussed earlier, any key data provided by or obtained from a subject should be incorporated into the report.

F1.3. Report types may include:

- Diagnostic semen analysis reports – for fertility patients
- Diagnostic semen analysis reports – post-vasectomy
- Diagnostic semen analysis reports – post-fertility affecting conditions or treatments (e.g. chemo- or radiotherapy)
- Diagnostic semen analysis reports – for clinical trial subjects
- Diagnostic semen analysis reports – for research studies.

F2. The written report or hospital intranet report

F2.1. All centres must have a standard reporting format for all examinations. The report should include the following:

- Unequivocal identification of the subject
- Identification of and contact details for the referring doctor
- Date and time of sample production
- Date and time of the analysis and report
- Unique identification of sample
- Summary of the results alongside units of measurement including reasons if no examination is performed
- Interpretive comment highlighting abnormal results and/or inclusion of critical limits if applicable

- The reference ranges used
- Laboratory name and contact details
- Status of the report as appropriate, for example copy, interim or supplementary
- Where possible, identification of person (s) verifying results and authorizing the release of the report.

F2.2. The report format must be clear and concise, and it must contain results on all the tests performed.

F2.3. A clear and concise comment using appropriate terminology should be used to summarize the findings. All unusual findings should be reported. *WHO 2021* (*World Health Organization, 2021*) terminology can be used provided it is appropriate for the recipient of the report, noting that the WHO suggests that '-zoospermia' terminology, other than azoospermia, should no longer be used.

F2.4. Reference ranges should be provided on the report. Centres should provide validation for any reference range that is not current and published in *WHO 2021* (*World Health Organization, 2021*). Centres choosing not to use *WHO 2021* methodologies should not report the WHO reference ranges but instead provide their reference values together with appropriate validation:

- End users should be made aware of the current reference ranges, the clinical value of the test and its limitations. They should be informed of any changes in laboratory methodology and/or output
- There should be a written procedure for verifying the results and checking them prior to despatch.

F3. Alternative reporting methods

F3.1. Centres should define a written procedure(s) and perform a risk assessment(s) before reporting results through any other mechanism other than a written report. Such mechanisms could include:

- telephone
- e-mail
- a website secure login.

F3.2. Alternative reporting should only be made available to the appropriate medical practitioner (usually whoever is responsible for the care of the subject) or their delegated staff, and only in exceptional circumstances.

F3.3. Exceptional circumstances should be defined and documented by standard operating procedures and should be authorized by the medical director of the referring service.

F3.4. Any reports issued using alternative methods should be documented with a record of the date, time, name of the person contacted and name of the andrologist communicating the report

F4. Clinical interpretation

F4.1. Interpretation of the semen analysis should provide advice on the prognosis in terms of chance of conception and, where relevant, assisted conception treatment. Clinical interpretation may include the following:

- Highlighting individual semen characteristics that indicate the need for further diagnostic testing or analysis
- Interpretation of PVSA reports and advice on clearance (which must be in accordance with the guidelines for PVSA; *Hancock et al., 2016*).

F4.2. Prior knowledge of the subject and/or couple's medical and reproductive history should be gained before offering advice and recommendations for treatment. All the test results must be considered before making conclusions on the couple's fertility status.

F4.3. Centres should distinguish between clinical interpretation to clinical users of the service and to subjects receiving test results.

F4.4.Clinical interpretation should be carried out by an appropriately trained consultant scientist/clinician or equivalent who maintains their CPD in the area. This responsibility may be formally delegated to a clinical scientist. There may be additional responsibilities that need to be taken into consideration in accordance with the *Human Fertilisation and Embryology Act 2008* ("*Human Fertilisation and Embryology Act," 2008*):

- If such clinical interpretation is not available, centres should seek advice from a consultant scientist/clinician or equivalent at another centre. This should be carried out by formal arrangement and/or contract
- If interpretation is required prior to assisted conception treatment, this should be carried out in conjunction with an appropriately experienced clinical scientist.

F5. Presentation of haematospermia

F5.1. Haematospermia is the presence of blood in the semen, which appears bright red when bleeding has occurred recently and red/brown when it is old (National Institute for Health and Care Excellence (NICE), 2022). This condition always requires further investigation, and men older than 40 years are at particularly increased risk of a serious underlying cause (*Haematospermia 2022*).

F5.2. Reporting of haematospermia should be expedited – reports should be referred to the respective general practitioner/primary care doctor within 2 working days, irrespective of whether they were the original referrer. This is to allow for rapid medically led referral through the 2-week suspected cancer pathway where appropriate in accordance with the National Institute for Health and Care Excellence (NICE) pathway (National Institute for Health and Care Excellence (NICE), 2022). The referral responsibility sits with the general practitioner as they can integrate care for the couple and have the relevant histories, but laboratories can be expected to highlight the NICE guidance to the general practitioner to guide decision making.

G. EVALUATION AND QUALITY ASSURANCE

G.1. Evaluation equates to 'measurement and analysis of performance' and is synonymous with quality assurance. In general, the requirements of evaluation are to ensure that centres are able to assess quality and can continue to provide a service that meets the needs and expectations of service users. Laboratories should define procedures under their quality management system, which includes the following:

- Audit
- Assessment of user satisfaction
- Key performance indicators (KPIs)
- IQC
- EQA
- Process validation
- Assessment of the clinical value of the service.

G1. Audit

G1.1. Audit is central to the evaluation process and is defined as the

'systematic, independent and documented process for obtaining and evaluating evidence and objectively evaluating it to determine the extent to which the pre-defined criteria are fulfilled' (ISO 19011 (*International Organization for Standardization (ISO), 2018*)). The purpose of an audit is to check compliance with above criteria. An appropriate plan of action should normally be implemented over a defined time period to meet the standards defined within the quality policy.

G1.2. Documented procedures should be established for conducting an internal audit of all applicable laboratory processes according to a predefined schedule and should be scheduled in advance to include three categories of analysis:

- *Vertical audit* — examination of all elements associated with a testing or treatment procedure to check that these elements conform to the pre-examination, examination and post-examination procedures. As a minimum, these should be carried out for:
  - Diagnostic semen analysis — infertility
  - Diagnostic semen analysis — PVSA.
- *Examination audit* — examination/witnessing of an individual performing a test procedure, for example semen analysis, to ensure that the procedure is followed correctly and that the individual appears to understand the requirements of that procedure. This could be used as part of a training exercise for new members of staff or for those learning a modified procedure. This can also be used as a regular measure of staff competence.
- *Horizontal audit* — examinations across processes to determine whether the elements are in place or indeed comply with predetermined standards at any specified moment in time (i.e. a snapshot). For example, take a part of the vertical audit one step further and determine whether, on a given day, all pieces of equipment and materials used have been through the appropriate procurement, servicing, checking and batch-testing procedures.

G1.3. For each audit there should be:

- a description of each step of the audit process
- a record of compliance where identified
- a record of any deficiencies identified
- a description of proposed remedial, corrective and preventive action.

G1.4. The results of audits should be discussed within an appropriate forum and summarized within the management review to complete the quality cycle.

G2. Assessment of user satisfaction and complaints

G2.1. The purpose of assessing user satisfaction and monitoring complaints is to establish that the service provided by the laboratory meets the needs and requirements of users (clinicians and subjects). This may include the use of subject and referring clinician questionnaires as part of the evaluation process (user satisfaction surveys). Centres should ensure that any review is fully inclusive and involves general practitioners and obstetricians/gynaecologists. The number of complaints received could be used as a KPI if required.

G3. Key performance indicators

G3.1. Centres must have procedures in place for the continuous evaluation of service quality. Key performance or quality indicators should be identified, regularly monitored and reviewed, and any deficiencies acted upon as part of the improvement cycle. Service users should be kept informed of performance via the annual management review. The chosen KPIs should be easily measurable and give an overall view of the quality of both the laboratory management and the laboratory product. The quality manager would be responsible for collating KPIs on a regular basis and presenting them to the management of the parent organization:

- Management indicators should be reviewed regularly and could include the following:
  - Staff absences
  - Staff satisfaction/turnover
  - Training and appraisal targets
  - Waiting times
  - Turnaround of reports
  - Referral rates
  - Activity/workload.
- Laboratory performance indicators could include the following:
  - Review of data logs from laboratory equipment
  - IQC
  - EQA.

G4. Internal quality control

G4.1. IQC is a set of procedures undertaken by laboratory staff for the continuous monitoring of operations and the results of measurements in order to decide whether results are reliable enough to be released within a short interval of time. In andrology this period of time is generally a working day but could be an analytical session. The methods in place should be appropriate to monitor the analytical output of the laboratory.

G4.2. In andrology, IQC should also include the assessment between operators unless a particular method has been demonstrated to be operator independent. The between-operator assessments may need to be performed continuously or at regular intervals, such as quarterly.

G4.3. It is important to note that statistically discerning between results of 3% and 5% typical forms in a morphology analysis requires approximately 1500 spermatozoa to be assessed. For this reason it is particularly important for IQC of morphology to be conducted on the four-component assessment.

G4.4. Comparison between samples and between operators is integral to diagnostic andrology quality testing. IQC should be conducted by inserting one or more control materials into every run or batch of analysis. The control materials or processes are treated in an identical, or as close as possible, manner to that performed on the test materials. The results examined must satisfy the operator that the system is in control, and diagnostic andrology is no different in principle from any other analytical procedure. As analysis uses live biological samples there are additional challenges so surrogate measures are sometimes needed; nonetheless, understanding the measurement of uncertainty is critical if the information is to be used in any decision-making process.

Control materials

G4.5. Material used for the purposes of IQC should be subject to the same measurement procedure as that used for subject samples. These include the following:

- Prepared 'pools' derived from clinical material (stored in formalin or cryopreserved)
- Artificial substitutes such a 'commercially available beads similar in size to spermatozoa'
- Commercial control slides
- Longitudinal comparisons of data or analytical run sets
- If EQA materials are used for IQC, the limitations, for example awareness of a

'target' value (should such a value exist) or possible degradation of materials, being taken into account
- The option to use duplicate or repeat measures on the same clinical samples both within and between operators.

G4.6. For monitoring IQC performance ARCS recommends that laboratories follow the latest *WHO 2021* guidance (*World Health Organization, 2021*).

G5. External quality assurance

G5.1. EQA is a system of objectively checking laboratory results using an external agency. The main aim of EQA is to bring about inter-laboratory comparability. If results are not consistent with required targets, a retrospective investigation should be carried out:

The laboratory should be a member of an accredited EQA scheme for sperm concentration, motility and morphology. For other measures appropriate inter-laboratory comparison processes should be in place

- The EQA scheme must be relevant and report results of the particular methods used within the laboratory relative to external markers
- The laboratory should make regular returns, all of which should be available for assessment
- The laboratory should have a procedure for the review of EQA with both staff and management. Any decisions taken for corrective action should be recorded, monitored and acted upon. Evidence of EQA review should be available for inspection by interested parties
- EQA records should be kept according to current Royal College of Pathologists guidelines (*EQA Quality Improvement Workstream One et al., 2023*).

DATA AVAILABILITY

No data was used for the research described in the article.

ACKNOWLEDGEMENTS

The authors and ARCS would like to thank David Mortimer for his significant contributions in reviewing this guideline, as well as everyone who contributed to the review process, including Andrew Allen, Stephanie Brooks, Rachel Gregoire, Sheryl Homa, Amber Joyner, Paul Knaggs, Charnpreet Marshall, Shirley Moses, David Sanders, George Southall-Brown, Olivia Vilis, Jemma Walker and Bryan Woodward.

APPENDIX 1

Guidelines for the retention of specimens and records of specimens

Permanent storage is without limit but refers to no longer than 30 years. In general stored records and specimens should be appropriately organized and so that retrieval is straightforward.

a Stained morphology slides should be kept until the signed report has been dispatched.
b Fresh semen samples should be kept until a full record of the test has been recorded.
c Request forms should be kept for at least as long as it takes for the user to receive the authorized report. Ordinarily this period does not need to be longer than 1 month after the final checked report has been sent.
d Log books (Day books) and other specimen records: At least 2 calendar years.
e Protocols (of Standard Operating Procedures): Current and outdated protocols should be dated and kept permanently on file.
f Worksheets: Should be kept for the same length of time as the related permanent (or semi permanent) specimens or preparations.
g Records of telephoned reports: Should be logged on the subject's file or other working records.
h Report copies: At least 6 months for operational purposes.
i Treatment related reports for at least 30 years.
j Internal Quality Control records: At least 10 years.
k External Quality Assurance records: 5 calendar years for subscribing laboratories.
l Accreditation documentation/ Records of inspection: Ten years or until superseded.
m Equipment maintenance logs Lifetime of the instrument (minimum of 10 years).

REFERENCES

Barratt, C., Björndahl, L., Menkveld, R., Mortimer, D., 2011. ESHRE special interest group for andrology basic semen analysis course: a continued focus on accuracy, quality, efficiency and clinical relevance. Human Reproduction 26 (12), 3207–3212.
Björndahl, L., Barratt, C.L., Mortimer, D., Agarwal, A., Aitken, R.J., Alvarez, J.G., Aneck-Hahn, N., Arver, S., Baldi, E., Bassas, L., 2022. Standards in semen examination: publishing reproducible and reliable data based on high-quality methodology. Human Reproduction 37 (11), 2497–2502.
Control of Substances Hazardous to Health (COSHH) regulations, (2002). Legislation.gov.uk
EQA Quality Improvement Workstream One, Thomas, A., Buckland, M., Woodward, B., Pritchard, D., Whitby, L., & Hodgson, C. (2023). *EQA Governance and Assurance Framework WS10401*.
Hancock, P., Woodward, B., Muneer, A., Kirkman-Brown, J., 2016. 2016 laboratory guidelines for postvasectomy semen analysis: Association of Biomedical Andrologists, the British Andrology Society and the British Association of Urological Surgeons. Journal of clinical pathology 69 (7), 655–660.
Health and Safety at Work Act 1974, (1974).
Human Fertilisation & Embryology Authority. (2023). *Code of practice*.
Human Fertilisation and Embryology Act, (2008).
International Organization for Standardization (ISO). (2018). ISO 19011:2018 (en), Guidelines for auditing management systems. In.
International Organization for Standardization (ISO). (2021). ISO 23162:2021 - Basic semen examination — Specification and test methods. In.
International Organization for Standardization (ISO). (2022). ISO 15189:2012 (en), Medical laboratories — Requirements for quality and competence. In.
Medical Devices (In Vitro Diagnostic Devices etc.) (Amendment) Regulations 2024, (2024).
Haematospermia, (2022). https://cks.nice.org.uk/topics/haematospermia/
Sanders, D., Fensome-Rimmer, S., Woodward, B., 2017. Uncertainty of measurement in andrology: UK best practice guideline from the Association of Biomedical Andrologists. British journal of biomedical science 74 (4), 157–162.
Sexual Offences Act, (2003).
Tomlinson, M., Harbottle, S., Woodward, B., Lindsay, K., 2012. Association of biomedical andrologists-laboratory andrology guidelines for good practice version 3-2012. Human Fertility (Cambridge, England) 15 (4), 156–173.
Medical Laboratory Accreditation. https://www.ukas.com/accreditation/standards/medical-laboratory-accreditation/
World Health Organization, 2021. In: Bjorndahl, L. (Ed.), WHO laboratory manual for the examination and processing of human semen sixth edition. World Health Organization.

Received 18 June 2024; accepted 27 June 2024.