Abstract

Post vasectomy semen analysis (PVSA) is the procedure used to establish whether sperm are present in the semen following a vasectomy. PVSA is presently carried out by a wide variety of individuals, ranging from doctors and nurses in General Practitioner (GP) surgeries to specialist scientists in andrology laboratories, with highly variable results.

Key recommendations are that: 1) PVSA should take place a minimum of 12 weeks after surgery and after a minimum of 20 ejaculations; 2) Laboratories should routinely examine samples within 4 hours of production if assessing for presence of sperm. If non-motile sperm are observed, further samples must be examined within 1 hour of production; 3) Assessment of a single sample is acceptable to confirm vasectomy success if all recommendations and laboratory methodology are met and no sperm are observed. Clearance can then be given; 4) The level for special clearance should be <100,000/ml non-motile sperm. Special clearance cannot be provided if any motile sperm are observed and should only be given after assessment of two samples in full accordance with methods contained within these guidelines.

Surgeons are responsible both pre-operatively and post-operatively for the counselling of patients and their partners regarding complications and the possibility of late recanalisation after clearance.

These 2016 guidelines replaces the 2002 British Andrology Society (BAS) laboratory guidelines and should be regarded as definitive for the UK in the provision of a quality PVSA service, accredited to ISO 15189:2012, as overseen by the United Kingdom Accreditation Service (UKAS).

KEYWORDS

Guidelines, sterilisation, male, laboratory, protocols, spermatozoa, vasectomy
INTRODUCTION

Post vasectomy semen analysis (PVSA) is the laboratory procedure used to establish whether sperm are present in the semen following a vasectomy. As such, PVSA indicates whether surgery has been successful to achieve male sterilisation.

PVSA continues to be carried out by a wide variety of individuals with different levels of training. This ranges from doctors and nurses in GP surgeries to specialist scientists in dedicated andrology laboratories. PVSA may also be performed in general pathology laboratories. Consequently, the test quality and control of the procedure is variable, which is clearly not acceptable for a “Yes/No” indicator of surgical success.

In the UK, the move towards laboratory accreditation overseen by the United Kingdom Accreditation Service (UKAS) to the international standard ISO 15189:2012, is driving the quality of the PVSA process forward. Only laboratories meeting this analytical standard should be considered as providing the necessary reliability of result to confirm successful vasectomy.

In 2002 the British Andrology Society (BAS) published a set of laboratory guidelines for PVSA [1]. Advances in technique options, observations of areas where uncertainty exists and compliance with the ISO 15189:2012 standard, now make additions to the 2002 BAS guidelines desirable. Revisions include clarifying factors around when and how to test and the number of samples that should be examined.

These 2016 guidelines have been agreed and accepted across the three major groupings and co-author professional bodies for the field as best practice providing for standardisation of seminal analysis protocols and reporting of results. Crucially it should be noted that they are not only clinical recommendations but also take into account good laboratory practice and the accuracy of diagnosis. As such they supercede any prior guidelines published for the UK. The guidelines do not deal with the counselling of patients or discuss the indications for male sterilisation, for which compliance with the Faculty of Sexual & Reproductive Healthcare (FSRH) clinical guidance for male and female sterilization is recommended[2].

It is always important for patients to be warned that a vasectomy may apparently fail at any time, though the actual chance of this is rare, at less than 1% if correctly performed [3-8], with some reports suggesting that temporary reappearance of sperm may occur a year after clear PVSA [9].

Failures can be classed into early and late recanalisation. Recanalisation is considered to have occurred where, after an initial azoospermic sample, there is a rapid increase in sperm numbers [10 11]. These are suggested to comprise the major category of vasectomy failures [12]. The potential for late recanalisation has been reported to be around 0.04% to 1% [4 13].

Measurements of vasectomy failure are complex to interpret, as many are only discovered after an adverse outcome, i.e. occurrence of a pregnancy. It should also be recognized that no data exist relating to women who may seek pregnancy terminations after this unexpected outcome, perhaps without telling their partner. As pregnancies have been confirmed to occur even after repeated azoospermic samples, [14] due caution around the meaning of a PVSA result should always be noted in reports. The patient should be provided with information from his clinician regarding the likelihood of a successful vasectomy operation and the possibility of recanalisation. It is recommended that such information should be given both verbally and in writing [12].

FERTILITY OF RESIDUAL SPERM POST SURGERY

Ejaculates may contain potentially fertile sperm immediately after vasectomy [15], and patients should continue with contraceptive precautions until successful vasectomy has been confirmed. This has been extensively discussed in the 2002 BAS guidelines [1] and by the FSRH [2].
POST VASECTOMY SEMEN SAMPLES

Scheduling of sample testing

The time between the vasectomy and the PVSA has been widely discussed without agreement, with some publications even suggesting a wait of up to 6 months before first analysis [16]. However, the majority of published literature suggests a minimum number of weeks before testing of between 12 [6 11 16 17], 14 [18 19] and 16 weeks [1 20]. Decreased patient compliance has been noted with longer intervals [6 17 20].

The earlier the PVSA testing occurs, the more the likelihood increases of a false positive result. This may be due to an inflammatory reaction and/or temporary bruising within the testis. There is also evidence that early recanalisation can occur within the first few weeks following a vasectomy, and this may be more common after certain techniques [12] and may be transient [21]. Testing too early may also cause problems with analysis due to high sample viscosity, presence of residual (usually non-motile) sperm and raised levels of round cells that may mask sperm presence. Therefore, the desire to perform prematurely early PVSA has to be balanced against the increased patient inconvenience and also the workload / cost to the laboratory (e.g. [16 17 22 23]) as repeat tests would be required for accurate confirmatory diagnosis.

There is a consensus that ‘sufficient ejaculations’ should take place between the vasectomy and the PVSA, since ejaculatory frequency will affect time to azoospermia [1 11 24]. Men with fewer than 3 ejaculations per week have been reported to reach azoospermia around 5 weeks later than those with a higher number of ejaculations [25]. It has also been reported that azoospermia may not be achieved until 60 ejaculates for some individuals [25]. However, systematic review data indicates that by 20 ejaculates, 80% of men should show azoospermia or sperm numbers beneath detectable levels [11]. It is therefore recommended that knowing the number of ejaculates is important, though some authors have debated this point [24].

The recommendation is Grade B due to robust evidence, noting that it is surprising and disappointing that no conclusive multi-centre study has yet occurred for such a prevalent procedure.

**Recommendation 1: PVSA should take place a minimum of 12 weeks after surgery and after a minimum of 20 ejaculations.**

Pre-Assessment

Sample collection instructions

The laboratory staff should ensure that the person requesting the PVSA test is provided with clear ‘user information’ in the form of written instructions for sample collection. Sample production should be arranged on an appointment basis to ensure the laboratory has sufficient time for each assessment to be completed within recommended analytical time frames.

*The specimen container*

Gamma-irradiated and mouse embryo assay (MEA)-tested specimen containers are recommended for use, together with CE-marking for containers manufactured in Europe. However, none of these tests confirm the level of sperm non-toxicity. Specimen containers should therefore additionally be confirmed as non-toxic to sperm via an in-house sperm toxicity bioassay [26]. Confirmation of sperm non-toxicity should also apply to all consumables used in the PVSA process that come in to contact with the sample, e.g. any tubes, slides, coverslips and pipette tips. Traceable sperm toxicity testing is required for each new batch and make of item, in accordance with ISO 15189:2012.

If samples are provided in unscreened specimen containers, the PVSA report will only be valid if no sperm are observed. If sperm are observed, and they are immotile, then no confirmation of sperm non-toxicity from the

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specimen container can be provided. As such, the PVSA would need to be repeated in a screened specimen container to ascertain if motile sperm are present in the ejaculate. Use of unscreened specimen containers is considered a waste of resources, not least in terms of patient and laboratory time.

Collection of the sample

Instructions to patients

Patients should be advised to produce a sample in accordance with guidelines for good practice e.g. [27] [28]. The abstinence period should be between 2 and 7 days as per the latest WHO guidance [28].

Men should be asked to collect their entire ejaculate by masturbation. Use of coitus interruptus, condoms or oral production is not recommended as this can lead to sample contamination. One study reported that 9.4% PVSA samples were not completely collected [29], which could increase the risk of misdiagnosis if analysed. Strict criteria for rejection of incomplete samples should be in place, as assessment of incomplete samples will introduce an unacceptable level of uncertainty. However, assessment of an aliquot may be used to guide future management if motile sperm are present.

Receipt of Samples

On delivery of the semen sample to the laboratory reception, staff should ensure that the specimen container is clearly labelled with at least three patient identifiers (e.g. name, date of birth, and clinic number) and that details of sample collection are recorded, e.g. abstinence period and confirmation that the entire sample was collected. To achieve this, provision of clear understandable information to the patient is a pre-requisite alongside a checklist at the laboratory reception to confirm any factors relating to sample production. All staff dealing with patient contact should be trained appropriately in respecting privacy and dignity. Associated facilities and systems for the service should also ensure that this is recognised as an important part of diagnostic provision.

Overall Guidance on Sample Procurement

To prevent time delays or fluctuations in temperature, on-site production facilities are recommended. However, off-site production is acceptable provided samples are kept as close to body temperature as possible during transport to the laboratory. This transport should take place immediately after sample production since any change in temperature may affect sperm viability and motility. The elapsed time period must be stated on the report form.

As the period elapsed between production and examination increases, so does the risk of sperm losing motility, since seminal plasma may have deleterious effects on sperm motility [30]. As such, an exposure for more than 1 hour may confound test results. In 2012 the American Urological Association (AUA) decided that 2 hours between production and analysis was sufficient, stating that rather than precise motion quality, it is the presence or absence of motility that is important for a PVSA [31]. This 2 hour interval has been supported by Australian and Dutch workers [18](Royal Australasian College of Surgeons, 2009). However, the data to support this decision is minimal.

The ABA, BAS and BAUS consider that presence or absence of sperm can be assessed within a 4 hour period post-ejaculation. However, in cases where non-motile sperm are present in the ejaculate, it is strongly recommended that further fresh samples should be examined within 1 hour of production to confirm absence of motility and/or allow vitality testing. This requirement for a repeat sample meets the WHO recommendations for semen analysis [28].

The recommendation is Grade C due to limited evidence, but having the backing of experts and the three professional bodies.
**Recommendation 2a:** Laboratories should routinely examine samples within 4 hours of production if assessing for presence of sperm. If non-motile sperm are observed, further samples must be examined within 1 hour of production.

**Postal / courier transport of samples**

The 2002 BAS guidelines allowed for postal delivery of samples\(^1\). However, there are no published studies to demonstrate process validation for detection of sperm transported in this way. Indeed it would be very difficult to suggest that standard postal methods would meet the necessary requirements for knowing sample handling and environmental control were adequate. This may also compromise ISO 15189:2012 accreditation as the laboratory would not have control of the process and thus cannot establish uncertainty.

The time of production should be recorded on the specimen container, and samples should be no more than 4 hours old on examination. If samples are over 4 hours old as stated above, there is currently insufficient data to validate the accuracy of any results obtained, particularly with relation to uncontrolled transport situations. In this situation, an observation of no sperm present must have the caveat that no accurate validation for transport samples is available and it is suggested that a fresh sample is provided in accordance with these guidelines.

The longer the time a sample spends in transit to a laboratory, the greater the risk of sperm degradation. The professional bodies are therefore in agreement that actual ‘postal’ delivery of samples, with analysis times beyond 4 hours does not have sufficient underpinning evidence to be deemed acceptable good laboratory practice for an accurate diagnosis and should not be used. It should also be highlighted that failure to follow guidelines may have medico-legal consequences.

Laboratories accepting posted/couriered samples should ensure that patients understand the sample/request form labelling requirements and the current regulations for the transport and packaging of samples\(^32\). Patients and physicians must also be fully aware that a PVSA performed on a posted/couriered sample should only be used to detect the absence of sperm.

If non-motile sperm are observed in the posted/couriered, it is mandatory that a freshly produced sample be examined within 1 hour of production to exclude the possibility that motile sperm are present as per recommendation 2. Clear communication with the patient that sperm have been found and hence another sample is necessary under certain higher-stringency conditions should ensure they attend to clarify their personal fertility situation.

The recommendation is a Grade A recommendation as it is a requirement for acceptability of results in a diagnostic test.

**Recommendation 2b:** Where examination of a sample occurs outside recommended parameters this must be clearly noted on the report and clearance should not be given.

**LABORATORY ASSESSMENT**

**Recommended equipment**

Due to the viscous nature of semen samples, a positive displacement pipette (PDP) should be used for all aliquot sampling. A phase contrast microscope, with x200 and x400 magnification, is required.

A centrifuge that can take 15ml tubes is recommended, with a rotor that has been calibrated to provide a 3000g force for 15 minutes as per the original BAS 2002 guidelines. This is the maximum speed recommended by WHO for the assessment of azoospermia as higher speeds may risk damage to the sperm, such as decapitation, which makes their detection less likely \(^28\). Whilst use of 15ml conical tubes is recommended,
smaller volume tubes, e.g. 1.5–2.0ml tubes may be used, according to the size of the centrifuge available. The centrifuge should be calibrated to metrologically accepted standard, in accordance with ISO 15189:2012.

**Sample examination**

It is recommended that samples are warmed to 37°C in an incubator, to assist the liquefaction process. Samples should ideally be examined once liquefaction is complete, accepting that this may not be possible, since PVSA samples may continue to be highly viscous.

Two methods of examination are possible:

1. **The original 2002 BAS guidelines method.**
   The sample should be mixed well and 10μl pipetted on to a clean non-toxic glass slide using a PDP and covered with a 22 x 22mm coverslip. This gives a suitable depth (10-20μm) to allow any motile sperm present to swim freely.
   
   Using phase contrast microscopy, the entire coverslip area should be examined in a systematic grid search pattern for the presence of sperm.
   
   If no sperm are observed, the entire semen sample should be transferred using a sterile pipette into an appropriate centrifuge tube and centrifuged at 3000g for 15 minutes. The entire pellet should be resuspended in a minimum volume of autologous seminal plasma (< 100μl) and the entire sample examined systematically for the presence of motile and non-motile sperm. An estimation of sperm concentration or number of sperm observed in each high power field (>400 magnification) should be reported. After centrifugation, the pellet may contain a large number of cells including germ cells, epithelial cells and leucocytes. This debris may make the detection of any sperm difficult and the report should state that these cells might have obscured observation of any sperm present.

2. **Large Volume Fixed Depth Slides**

   Use of the fixed 100µm depth slide method avoids the need for centrifugation and has been shown to give a similar sperm detection level [33]. A larger volume of 25μl is transferred, using a PDP, to fixed depth slides. Each slide should be filled with a single continuous flow and any excess removed once the fill is complete. The filled slides should be stored in a 37°C, humid atmosphere for at least 15 minutes prior to examination, to allow non-motile sperm to reach the basal glass plane. The chamber depth of 100µm has been specifically designed to quantify low numbers of cells in suspension and allows any motile sperm present to swim freely.

   Use of slides with a quality assurance of dimensions, e.g. CE-mark, is required to ensure consistent sample volumes and fill effects. All slides and PDP tips must have passed traceable sperm toxicity testing to that specific batch.

   The slides should be examined using phase contrast microscopy at either x200 or x400 magnification with the entire slide area being examined in a systematic grid search pattern for the presence of sperm.

**Observations of sperm via either method**

If motile sperm are observed by either method, then a full sperm concentration and motility assessment should be performed according to standard WHO procedures, using an Improved Neubauer chamber count performed according to ‘accurate’ methods (see Section 2.11 of the WHO manual for guidance about counting low sperm numbers)[28].

Other methods have recently been suggested and discussed for PVSA. [34-36]. Whilst some of the suggestions for QA/QC are useful, methods other than those specified above are not recommended.

Should a sample be centrifuged?

Microscopic examination of uncentrifuged specimens has been shown to be a reliable method for PVSA, provided >100,000 sperm per ml are present [37]. However, whilst this laboratory technique has been reported to detect high numbers of sperm present in PVSA samples, establishing the accuracy of lower sperm numbers has not been validated. This highlights the need for further research to determine the reliability of this method. Therefore, it is recommended to follow the guidelines outlined in the 2002 BAS guidelines method, as this has been shown to be a reliable method for PVSA.
numbers would not be sufficient for medico-legal consideration. An additional factor to consider is the heterogeneous nature of PVSA semen samples, which may not allow for a reliable sampling of the whole sample. If the 100µm large volume fixed depth slide method is not used, it is the consensus view of the Professional Bodies that samples should be centrifuged. The pellet can then be assessed using small volume disposable slides (10µl) or standard slides as per the 2002 BAS guidelines method.

**High-viscosity Samples**

For both PVSA methods, in cases of persistent seminal viscosity, the entire ejaculate may be incubated for up to 1 hour with a protease such as alpha chymotrypsin as per standard protocols [38]. However, incubation with a protease may immobilise motile sperm present and the laboratory should highlight this on the report accordingly.

The recommendation is Grade B being supported by robust evidence and also having the backing of experts across the three professional bodies.

**Recommendation 3:** Laboratories should examine samples using either the 2002 BAS Guideline ‘coverslip and centrifugation’, or large volume fixed depth slide methods. If samples are treated with proteases, this should be highlighted on the report accordingly.

**INTERNAL QUALITY CONTROL**

In order to ensure precise and accurate results from the PVSA testing laboratory, a process of internal quality control (IQC) is required. Day-to-day monitoring of precision and accuracy reassures users of the service that there is uniformity within the laboratory.

IQC requires a central reference sample to enable direct comparisons between different laboratory staff performing PVSA. Depending on the number of PVSA samples that a laboratory receives, the simplest way to assess IQC is to use one of these samples and perform the assessment in parallel with other members of laboratory staff. The results can then be compared. Given that the primary objective is the detection of sperm, use of Shewhart charts is not necessary.

As far as the authors are aware, there is presently only one external quality assurance (EQA) scheme operating for PVSA, but this is not accredited to an ISO standard. An EQA scheme for PVSA should provide independent consensus for the presence/absence of sperm. The impracticality of providing such EQA, which should involve sending out sperm-free samples and samples with a very low numbers of sperm, has deterred other schemes being set-up nationally or internationally to date. In accordance with ISO 15189:2012 requirements, participation in an EQA scheme is required where both standard semen analyses and PVSA are performed by the same laboratory. Participation in EQA is the ideal situation for all diagnostic laboratories, however if the laboratory only performs PVSA it may be acceptable to perform testing if participating in an adequate Inter-Laboratory Comparison (ILC) scheme alongside inter-operator variability assessment, so long as at least one participating laboratory maintains results deemed acceptable in an EQA. For the specific scenario of PVSA all laboratories should establish their level of detection, via appropriate dilutions of semen, which will also help address uncertainty. Routine practice audit should also assist in provision of a timely and appropriate service.

The recommendation is a Grade A recommendation as it is a requirement for accurate results in a diagnostic test.

**Recommendation 4:** As a minimum, laboratories should participate in IQC and ILC with a laboratory which has known EQA standards. Ideally laboratories should directly participate in an accredited andrology EQA scheme for sperm count and motility assessment.

**POST ASSESSMENT - THE UNCERTAINTY FACTOR**
There are many areas where uncertainty can lead to questions about the robustness of PVSA.

The recommendations in these guidelines aim to reduce the uncertainty. However, unless strict sample rejection criteria are enforced, a higher than necessary uncertainty will impact on the PVSA. Even with strict adherence to guidelines, an uncertainty factor will remain, which should be reported to all users of the service. For example, it has been reported that a large proportion of patients (up to 25%) fail to comply with instructions regarding sample collection, making reliable laboratory assessment impossible, or fail to submit a sample in the first place[16 39 40].

Each PVSA laboratory should consider all areas that can impact on the uncertainty, including those within the laboratory. Whilst the measurement and reporting of sample volume is not routinely required, laboratories should report samples that exhibit either high (>10ml) or low volumes (<1 ml) as this may indicate non-compliance in collection procedures. Such volumes should be included and highlighted in the final report to draw attention to this possible uncertainty.

**HOW MANY SAMPLES SHOULD BE ASSESSED?**

The 2002 BAS guidelines recommended examination of two samples per patient to ensure correct evaluation[1]. A second PVSA was recommended to counter uncertainty associated with the pre-examination phase, e.g. men not following pre-examination instructions properly, insufficient collection of the whole sample and non-compliance with instructions for transport to the laboratory.

A number of publications have since considered that a single sample is sufficient [4 18 20 41-43]. After assessing the supporting evidence, the Professional Bodies agree that assessment of a single sample is appropriate provided there is strict adherence to instructions for production, delivery and time to examination, to minimise any adverse effects on the sample. Optimally, sample production on-site provides the best control over delivery and time, although this still relies on the man abiding by pre-assessment and actual collection instructions, which introduces uncertainty.

The Professional Bodies support the move towards examination of a single PVSA to establish operative success when the process is tightly controlled, complying with all recommendations and methodology guidance listed in this document. It must be noted that this is entirely dependent on ensuring that specimen acceptance criteria are fully complied with and that this is limited by the patient’s own interpretation of what constitutes complete sample collection. In many areas of medicine it is rare that a ‘diagnosis’ is made without confirmation. As such, people should be able to freely request a second confirmatory sample and this right should not be fettered.

The recommendation is Grade C due to limited evidence but having the backing of experts and the three professional bodies.

**Recommendation 5: Assessment of a single sample is acceptable to confirm vasectomy success if all recommendations and laboratory methodology are met and no sperm are observed. Clearance can then be given.**

**“SPECIAL CLEARANCE”**

Persistent non-motile sperm in initial PVSA samples is not uncommon, with studies reporting up to 33% non-azoospermic samples at 3 months, and 10% of ejaculates containing non-motile sperm at 6 months[16 44]. In one prospective study, the median time to azoospermia was 10 weeks, with most men (93%) being azoospermic by 20 weeks[25]. Similarly, sperm have been reported to temporarily reappear in ejaculates 12 months after surgery, despite previous sperm-free ejaculates[9].
In another prospective long-term follow up study, over 1000 men submitted semen samples at one, two, and three years PV[3]. Twenty of these men had non-motile sperm detected in their samples, but there were no reports of unwanted pregnancies in the partners of these men. Discussion in the literature has suggested that the risk of pregnancy occurring from men whose previous samples show non-motile sperm present is small[39] and probably no more than the risk of pregnancy after two azoospermic semen samples, as a result of spontaneous recanalisation [3 45]. The risk of fertility for a PVSA sample showing azoospermia has been estimated at about 1 in 2,000[13]. It has been suggested that repeat ejaculates 7 months after surgery are probably unnecessary because pregnancy is very unlikely to occur [39 46].

For pathology tests in general, whenever a test is equivocal, in this case when sperm are observed, then a repeat test is requested which must comply with all Recommendations. A vitality test can also be performed on the sperm to assess whether they are alive or not, but a non-motile sperm will not be able to fertilise an oocyte \textit{in-vivo}.

If a physician wishes to grant ‘special clearance’ to a man who repeatedly has reports of sperm in his PVSA, then he should be aware of the medico-legal consequences should a motile sperm be produced.

Current international reviews show a variance in the maximum number of non-motile sperm that are allowed to be detected before special clearance may be given, with the American Urological Association (AUA) in the USA and FSRH in the UK stating <100,000 sperm/ml [2 31]. In practice, accuracy demands that this is the limit, as it is not possible to reach the required levels of accuracy for a lower value by anything other than the fixed-depth slide method [47].

Laboratories must be able to validate & verify that their reported counts are accurate and what their detection accuracy or range may be, accepting there will be a measure of uncertainty. Figures beneath 100,000/ml lack robust evidence that they are necessary as in general the counting methods employed in studies suggesting them have not had the low-count stringent accuracy required. The Professional Bodies therefore believe, that with recognition of the true accuracy of counting methods in the published literature, <100,000 sperm/ml is a prudent figure to base clearance upon, with due note that this is at the limit of count accuracy of these methods.

It should be noted that no result can guarantee sterility, with a number of rare reported cases of men with no detectable sperm still going on to have proven paternity [14].

The recommendation is Grade B due being based on evidence from a number of robust though limited studies, but no ability to ever be absolute. It has the backing of experts and the three Societies.

\textbf{Recommendation 6:} The level for special clearance should be <100,000/ml non-motile sperm. Special clearance cannot be provided if any motile sperm are observed and should only be given after assessment of two samples in full accordance with these guidelines.

\textbf{CONCLUSIONS & IMPLEMENTATION}

The Professional Bodies expect that implementation of these guidelines can occur from the date of publication and should be completed within 12 months.

For all situations, where there is a deviation from compliance with these guidelines, for example if a test has been performed before 12 weeks and 20 ejaculations, this should be clearly noted on any report provided, with appropriate reference suggestion for requesting a fresh samples to test correctly within the guidelines. If departing from the guidelines, individuals and laboratories should be aware of the standards of best practice and potential medico-legal consequences.
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Competing Interest

Competing Interest: None declared.
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