ARAB JOURNAL OF UROLOGY
2024, VOL. 22, NO. 2, 71–74
https://doi.org/10.1080/20905998.2023.2298048

Sixth edition of the World Health Organization laboratory manual of semen analysis: Updates and essential take away for busy clinicians

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ABSTRACT

Background: Semen analysis is the cornerstone for male fertility evaluation. In 2021, the World Health Organization (WHO) released its 6th edition of semen analysis manual.

Methods: We highlight the main changes in the latest 6th edition of the WHO manual of semen analysis and their possible interpretations.

Results: The manual is highly comprehensive, offering detailed information, and is widely regarded as an excellent technical reference for laboratory staff. Nevertheless, several aspects of the manual require further elucidation for infertility practitioners.

Conclusion: The recently published 6th edition of the WHO manual provides a strong framework for the assessment and processing of human semen. Grasping the modifications introduced in this updated edition and their clinical significance can enhance the quality of patient care.

ARTICLE HISTORY
Received 6 November 2023
Accepted 12 December 2023

KEYWORDS
Male infertility; semen analysis; sperm; WHO; World Health Organization

Introduction

Semen analysis provides important information regarding the contribution of the male factor to an infertile couple. The World Health Organization (WHO) laboratory manual is established as an important document to standardize the procedures for the examination of human semen. Over the past 4 decades, there have been 6 editions of the WHO manual produced. Each successive edition aimed to improve the examination of various semen parameters and incorporate new data based on scientific advances in terms of clinical understanding and technological innovations [1]. The most recent edition of the WHO guidelines on male semen preparation and analysis is the 6th edition of the WHO manual, published in 2021, which provides critical information on semen examination, sperm preparation and cryopreservation, and quality control and assurance [2,3]. Indeed, this new 6th edition of the semen analysis manual facilitates laboratory excellence by providing detailed instructions to improve the overall calculations and interpretations of the semen parameters [2,4]. The incorporation of new datasets of semen samples from various countries, in the 6th edition, helped overcome some limitations of previous editions due to the underrepresentation of some geographical areas. In this regard, the 6th edition included additional data on semen obtained from 1700 fertile men from 5 countries (China, Egypt, the Islamic Republic of Iran, Italy, and Greece). However, despite this increase in the reference population, no change was observed in the 5th percentile values of basic semen parameters from the previous 5th edition, showing that the data is robust and may not change even if more data is included (Table 1). Additionally, the 6th edition emphasizes that the use of the 5th centile values of basic semen parameters alone is not sufficient to diagnose male infertility and that further clinical and/or laboratory evaluation of the patient is needed based on the judgement of the treating physician. This article aims to highlight the main changes in the latest WHO 6th edition of semen analysis and their clinical interpretations.

The 6th edition of the WHO manual of semen analysis: general considerations

Semen examinations in the 6th edition of the WHO manual are grouped into three sections, including basic, extended, and advanced examinations [5]. These terms replace the ones used in the previous 5th edition: standard tests, optional tests, and research tests of semen [6]. The basic examinations deal with the routine examination that both clinician and laboratory can follow to determine semen variables; extended examination is used in certain conditions or

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This article has been corrected with minor changes. These changes do not impact the academic content of the article.

Table 1. WHO 2010 (5th edition) and WHO 2021 (6th edition) lower fifth percentile (with 95% confidence interval) of semen parameters from men in couples starting a pregnancy within one year of unprotected sexual intercourse leading to a natural conception (adopted from Boitrelle et al. [5]).

Parameter	WHO 2010	WHO 2021
Semen volume (ml)	1.5 (1.4–1.7)	1.4 (1.3–1.5)
Total sperm number (10^6 per ejaculate)	39 (33–46)	39 (35–40)
Total motility (%)	40 (38–42)	42 (40–43)
Progressive motility (%)	32 (31–34)	30 (29–31)
Non-progressive motility (%)	1	1 (1–1)
Immotile sperm (%)	22	20 (19–20)
Vitality (%)	58 (55–63)	54 (50–56)
Normal forms (%)	4 (3–4)	4 (3.9–4)

as needed; whereas the advanced examinations are not currently regarded routine for initial fertility evaluation but are more of research interest. Aside from these chapters, the manual also includes the topics of sperm preparation, cryopreservation, quality assessment, and control, which are all beneficial for the laboratory aspect.

The recommendations for semen collection in the new manual do not principally differ from the previous 5th edition. Semen collection is conducted by masturbation after abstinence of 2 to 7 days [5]. The semen should be fully collected, and the laboratory assessment needs to be within 30 to 60 minutes. To ensure the quality, the temperature during the transport of semen samples to the laboratory is recommended to be around 20°C to 27°C. A patient should be informed of the correct requirements of semen collection to ensure accurate evaluation of semen and interpretation of the results. The editors of the 6th edition acknowledge that semen test can result in variability and uncertain cut-off to differentiate normal and infertile men. Therefore, the European Association of Urology (EAU) guidelines recommend repeating semen analysis in case of abnormal results [7].

Basic examination of semen

During the macroscopic evaluation of a semen sample, the 6th edition states that 'Information of a strong odor of urine or putrefaction can be of clinical importance; it is, therefore, important to note this in the report'. However, the assessment of semen odor could be very subjective. Additionally, the addition of this parameter is not consistent with recommendations for the safety of laboratory personnel against emerging viruses [3]. In the 6th edition, the classification of sperm motility is reverted to the previous 5th edition by distinguishing progressive motile sperm into rapid and slow. The rationale for this change is that the presence of rapidly progressive motile sperm can affect the outcome of fertility. Nevertheless, this assumption is primarily based on older articles that need more support from recent research [3].

The manual also provides a laboratory guide to give a more accurate assessment of sperm in low concentrations and emphasizes the importance of total sperm numbers per ejaculate in diagnosis rather than a mere sperm concentration. Therefore, an accurate assessment of semen volume is pivotal [3,5]. Additionally, the 6th edition suggests total sperm motility below 40% as an indication for sperm vitality assessment [3]. Sperm vitality staining provides a useful adjunctive test if the semen sample shows reduced motility since it distinguishes between 'live-immotile' and 'dead-immotile' spermatozoa and can be critical in determining whether ejaculated spermatozoa could be used for intracytoplasmic sperm injection [8].

On the other hand, there are no changes in sperm morphology assessment or interpretation in the 6th edition. However, the manual recommends a systematic approach to sperm morphology evaluation while also providing numerous photographs of normal sperm morphology as well as a wide range of morphological abnormalities [3]. Assessment of leukocytes in semen and anti-sperm antibodies, previously considered as standard tests of semen in the 5th edition, is now moved to the chapter 'extended examinations' in the 6th edition [5]. Therefore, clinicians should ask the laboratory for these tests whenever needed. For instance, clinicians can order leukocyte assessment in semen to support the presumptive diagnosis of male accessory gland infection [5].

Extended examination of semen

The main changes in the extended or optional examination of semen are the addition of sperm genetics, including chromosomal abnormalities detection, gene mutations, chromatin evaluation, sperm DNA fragmentation (SDF) testing aside from the testing of leukocyte, antibodies, immature germ cells, indices of multiple sperm defects, and biochemical assessment of accessory organ function [5]. The addition of genetic and chromatin assessment also included the discussion of how these tests can relate to male reproductive function, although more precise guidance on the indications and result interpretation is lacking. Additionally, the manual acknowledges that the SDF test represents one of the most critical additions to male infertility

evaluation and has become one of the most promising and frequently discussed topics in andrology [5]. A scientometric study in 2019 showed an increasing number of publications in SDF-related male infertility studies [9].

There are different methods that can be used to evaluate SDF [3,5]. The manual stated that terminal deoxynucleotidyl transferase (dUTP) nick end labeling (TUNEL), sperm chromatin dispersion (SCD) test, and acridine orange flow cytometry are useful tools in clinical practice. However, due to the heterogeneity and variability, the manual does not provide the exact cut-off to differentiate the normal and elevated SDF. Instead, each laboratory may have its own cut-off value. As a result, the clinical use of SDF may still face some challenges and barriers to be applied in clinical practice, as depicted in recent global surveys on the use of SDF [10,11]. Genetic and genomic tests provided in the manual also lack indication; therefore, clinicians should refer to the professional guidelines to apply these tests [5]. Karyotyping and Y chromosome microdeletion testing are indicated in the case of non-obstructive azoospermia according to the EAU and American Urological Association/American Society of Reproductive Medicine guidelines [7,12].

Advanced examination of semen

Advanced examinations include specialized evaluations, mainly research-based or using an emerging technology. The main objective of the advanced examination is to understand sperm quality and function deeply at the cellular or molecular level. While some tests, such as human oocyte and zona pellucida binding, as well as oocyte penetration, were removed from the 6th edition, as they were considered obsolete, other tests, including seminal oxidative stress (OS), chromatin integrity, acrosome reactions, and computer-assisted semen analysis were included [5]. In recent years, there has been growing interest in the clinical utility of seminal OS testing for predicting sperm fertilizing potential; therefore, its categorization may be upgraded in future updates of the manual [3]. Additionally, seminal OS testing in a clinical setting may help guide clinicians as to which patients are candidates for antioxidant therapy. This would help personalize treatment for many cases diagnosed with idiopathic or unexplained infertility. The main changes in the 6th edition of the WHO manual of human semen examination are summarized in Table 2.

Case scenario

Case

A young couple in their 30s has been trying to conceive naturally for more than 2 years without success. The male patient is otherwise fit and healthy with no identifiable male risk factors in his medical history or clinical examination. His semen analysis showed a semen volume of 3 mL, sperm concentration of 36 million/mL, total motility of 15%, progressive motility of 0%, and normal sperm forms of 6%.

Management

The current semen analysis reveals a moderate reduction of sperm total motility. The patient should be asked to repeat the semen analysis within two to three weeks of the first examination and should be given clear instructions for correct sample collection. If the result of the second semen analysis indicated the persistence of sperm total motility below 40%, then a vitality test should be recommended.

Key points

- The latest 6th edition of the WHO manual entitles robust evaluation and processing of human semen and is considered a very useful guide for laboratory technicians.

Table 2. Summary of changes in the 6th edition of the World Health Organization manual of human semen analysis compared to the previous 5th edition.

	Change
Term	• Standard test → Basic examination • Optional test → Extended examination • Research test → Advanced examination
Basic Examination	• Addition of semen odor examination • Categorization of progressive sperm motility into rapid and slow • A systematic approach to sperm morphology assessment • Sperm vitality is indicated if sperm total motility is less than 40% • Removal of terms such as normozoospermia, oligozoospermia, asthenozoospermia, teratozoospermia, and necrozoospermia
Extended Examination	• Assessment of leukocyte and antibodies are considered an extended examination. • Additional discussion on sperm DNA damage and genetic testing, although no clear indications are defined for both tests. • TUNEL, SCD, and acridine orange flow cytometry for SDF are listed as useful tests in clinical practice
Advanced Examination	• Removal of human oocyte and zona pellucida binding, as well as oocyte penetration tests • Addition of seminal oxidative stress testing • Better explanation of the principle of CASA

CASA: computer-assisted semen analysis. SCD: sperm chromatin dispersion. SDF: sperm DNA fragmentation. TUNEL: terminal deoxynucleotidyl transferase (dUTP) nick end labelling.

- The 6th edition is composed of several chapters including basic, extended, and advanced examination of semen, sperm preparation, and cryopreservation techniques as well as quality assurance and quality control procedures within the laboratory.
- The 6th edition indicates that the 5th centile values of basic semen parameters are only one way to interpret the results of semen analysis, and the finding of abnormal values could be suggestive of possible male factor infertility.
- When abnormal semen parameters are detected, the patient should be referred to a male reproductive specialist for a comprehensive clinical evaluation and to receive the proper therapeutic intervention.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Abbreviation

CASA	computer-assisted semen analysis
EAU	European Association of Urology
SCD	sperm chromatin dispersion
SDF	sperm DNA fragmentation
TUNEL	terminal deoxynucleotidyl transferase (dUTP) nick end labelling
WHO	World Health Organization

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