WHO Laboratory Manual for the examination of human semen and sperm–cervical mucus interaction

This definitive and essential source of reference has been thoroughly up-dated and revised to meet the requirements of all laboratories involved in the analysis of human semen. The book sets out the fundamental laboratory techniques that should be employed in the diagnosis of male infertility. The text includes descriptions of how to construct a conventional semen profile and provides standardized protocols for performing several optional diagnostic procedures. Such techniques are essential in the evaluation of infertile couples and in assessing fertility in men whose sperm production is suppressed by potential anti-fertility compounds or by toxic agents: they are also of interest in forensic medicine and in connection with artificial insemination. Previous editions of this volume have established themselves as the gold standard in the area of fertility investigation and treatment: this new edition continues that tradition and will be the benchmark for setting more rigorous standards for future years.

WHO LABORATORY MANUAL

for the examination of human semen and sperm–cervical mucus interaction

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Abbreviations

BSA	bovine serum albumin (Cohn fraction V)
CASA	computer-aided sperm analysis
DMSO	dimethyl sulfoxide
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
EQA	external quality assessment
HIV	human immunodeficiency virus
HOPT	hamster oocyte penetration test
HOS	hypo-osmotic swelling test
HPF	high-power field
HSA	human serum albumin (Cohn fraction V)
IBT	immunobead test
IQC	internal quality control
IU	international unit
IVF	in vitro fertilization
MAR	mixed antiglobulin reaction
MAI	multiple anomalies index
PAS	periodic acid Schiff
PBS	phosphate buffered saline
PCT	post-coital test
QC	quality control
ROS	reactive oxygen species
SCMC	sperm–cervical mucus contact
SDI	sperm deformity index
SPA	sperm penetration assay (synonymous with HOPT)
TZI	teratozoospermia index
U	(international) unit
WBC	white blood cell (leukocyte)

1 Introduction

In response to a growing need for the standardization of procedures for the examination of human semen, the World Health Organization (WHO) first published a *Laboratory Manual for the Examination of Human Semen and Semen–Cervical Mucus Interaction* in 1980. The second and third editions of the manual followed in 1987 and 1992. These publications have been used extensively by clinicians and researchers worldwide. Indeed, the third edition was translated into the following languages: Arabic, Chinese, French, German, Indonesian, Italian, Portuguese and Spanish. However, the field of andrology has continued to advance rapidly, and this, together with increased awareness of the need for standardized measurements of all semen variables, has prompted the present revision.

This fourth edition comes at a time of new developments and concerns. The landmark demonstration of contraceptive efficacy with hormonally induced suppression of spermatogenesis (World Health Organization Task Force on Methods for the Regulation of Male Fertility, 1990, 1996) should lead to the development of practical, efficient and safer hormonal methods of male fertility regulation. Advances in understanding the genetic basis of male infertility, together with the successes of assisted reproductive technology, including intracytoplasmic sperm injection, have regenerated interest in the field and brought hope for men previously considered infertile. These advances in male subfertility treatment have also led to a resurgence of interest in the assessment of sperm function. Reports of declining sperm counts and increasing incidence of urogenital abnormalities and testicular cancer in some regions of the world have aroused public concern. The deterioration of semen quality is not geographically uniform as shown by studies in Denmark, Finland, France, the United Kingdom and the United States of America. Government environmental agencies and national and international scientific societies, as well as WHO, have conducted discussions on this topic without a clear consensus emerging. Further studies, perhaps on varied populations in areas with and without known environmental pollutants or toxins, are in progress. The advances in male contraceptive development and in the treatment of male subfertility, together with increasing concerns about the environment and

putative consequences on male reproductive function, add fresh urgency to the search for new methods to achieve better standardization and improvement of semen analysis.

For these reasons, the UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction, after seeking advice from experts throughout the world via letters, journals and national andrology societies, formed a working group (see Acknowledgements) to revise the manual. The present edition is a companion to the *WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple* published in 1993 by the Cambridge University Press.

Chapter 2 of the present manual, which deals with the examination of human semen, is divided into three major parts. The first part describes procedures that are considered to be essential in semen evaluation (Section 2A, 2.1 to 2.6). The second part comprises procedures considered by most laboratories to be optional but which may be of clinical diagnostic value (Section 2B, 2.7 to 2.12). The third part (Section 2C, 2.13 and 2.14) includes methods that assess sperm functional capacity and developments in computer-aided analyses for sperm morphology. These techniques may not be available in all laboratories but may be of value in the assessment of male subfertility, in reproductive toxicology studies or as research tools. In the standard procedure section describing sperm morphology, this manual recommends the Papanicolaou stain as the preferred method and the use of a simplified classification of sperm morphology according to the so-called 'strict criteria'. Computer-aided sperm analysis (CASA) for sperm motion and the zona-free hamster oocyte test have been included in the section of optional tests, as these tests may have some diagnostic applications. The section on research tests has been rewritten and updated to reflect the current consensus on the assessment of sperm functional capacity. The statistical basis of counting errors involved in semen analysis has been added and the chapter on quality control has been expanded to include discussions on practical methods of implementing quality control in any andrology laboratory.

In Appendix I, reference values of semen variables are listed. It should be noted that it is not the purpose of the manual to establish the minimum or lowest semen values compatible with achieving a pregnancy, in vivo or in vitro. It proved difficult to reach agreement on some aspects of the assessment of sperm morphology and on the provision of reference ranges since morphology assessment remains subjective. Reference ranges for human semen present some conceptual difficulties. The relationship of semen quality to fertility is complicated by many other factors, including female fertility. Thus men with abnormal semen may still be fertile while men with better than

average semen quality produce pregnancies at higher than average rates. This manual is not designed only for laboratories that deal with subfertile couples. It also addresses the needs of laboratories investigating methods for male contraception and studies of reproductive toxicology. In this context, it is important for this manual to give reference values based on multicentre population studies of normal men and not the minimum requirements for fertilization. In addition, this manual suggests that it is preferable for each laboratory to determine its own reference ranges for each variable (e.g., with samples evaluated for men who have recently achieved a pregnancy).

Finally, it should be emphasized that the major purpose of this manual is to encourage the use of standard procedures to establish reference values (previously called 'normal' values) for semen analysis. This will permit improved comparability of results between laboratories and the amalgamation of data from different sources for analysis. Attention to the details of standard procedures should also sharpen the precision of results and their reproducibility. Above all, the prime objective of the earlier editions has remained: to provide a laboratory manual that will serve the needs of researchers and clinicians in developing countries.