THE DOCTORS LABORATORY

Andrology

The single most important factor determining a man's fertility potential is the production of healthy sperm. A semen analysis has classically been used as the marker of this potential, by providing information about the sperm count, motility and morphology. However, there are other parameters given in a semen analysis that are often neglected or overlooked, which may indicate important pathologies – such as infection, prostatic disease, immunological infertility, retrograde ejaculation, malformation or obstruction of the genital tract, tumour, and congenital or endocrine disorders.

Andrology booking can now be done online at **www.tdlpathology.com/andrologybooking**

Early diagnosis of the male factor is important in order to detect any underlying pathology, determine the extent of infertility and ensure appropriate treatment. It may also avoid unnecessary investigations for the female partner, particularly if her age is a limiting factor.

For men who have had a vasectomy, clearance should only be given when there is no evidence of presence of sperm in two consecutive semen samples. It is therefore vital to ensure that results are reported according to best practice guidelines. Special clearance may be given at the doctor's discretion when there are persistent non-motile sperm present.

Guidelines for Producing Samples

Ideally semen samples should be produced on-site at TDL's Patient Reception at 76 Wimpole Street. Ideally patients must abstain from ejaculation for 2-3 days prior to the test, but no less than 2 days and no longer than 5 days before the test. This requirement is important for semen analyses and post vasectomy analyses to ensure reliability of results. It is possible that samples that do not comply with guidelines for abstinence and collection may not be able to be processed. All semen samples must be produced directly into the sterile containers provided by The Doctors Laboratory.

All containers are weighed and batch tested for sperm cytotoxicity. In exceptional circumstances when semen samples are produced off-site, they can only be accepted by the Andrology Department in sample containers provided by TDL.

WHO 2010 guidelines state that two semen analyses should be performed before any diagnosis is confirmed. This may require requests for two (separate) semen analyses.

Appointments

It is important to make an appointment for all semen samples (on or off site) whether for a comprehensive semen analysis or post vasectomy analysis. It may be necessary to give patients who attend without an appointment a specific time to re-attend. The first appointments for post vasectomy samples should usually be 12 weeks and 20 ejaculations after surgery.

TDL Andrology

Appointments can be made by calling **020 7025 7940**. There is an attendance fee of £45.00 in addition to pathology charges.

Please complete a Pathology Request Form for your patient. If you would like to request other pathology, you can use the same form or complete a second additional form. Results will usually be reported to you within 48 hours.

If you would like to discuss these tests, or any aspect of this service, please contact TDL Andrology on 020 7025 7940 or email andrology@tdlpathology.com for further information.

SEMEN

TEST	CODE	SAMPLE REQS	TAT
Oxidative Stress in Semen (ROS + MIOXSYS)	SROS	**Semen**[1]	1 day
Retrograde Ejaculation	RTRO	Contact Lab	2 days
Semen Analysis, Comprehensive*	SPER	**Semen**[1]	2 days*
Semen Analysis, Post-Vasectomy**	PVAS	**Semen**[1]	2 days
Semen Analysis, Vasectomy Reversal*	SPER	**Semen**[1]	2 days*
Semen Culture	SPCU	**Semen**	2-4 days
Semen Fructose	SPCF	**Semen**	2 days
Semen Leucocytes	PMNS	**Semen**	2 days
Semen Parameters	SPOD	**Semen**[1]	1 day
Semen Zinc	SPCZ	**Semen**	up to 10 days
Sperm Aneuploidy	SPPL	**Semen**[1]	4 weeks
Sperm Antibodies (Serum)	ASAB	B	5 days
Sperm Antibodies/MAR Test (Semen)†	ASPA	**Semen**	1 day
Sperm Comet®	CMET	**Semen**	1-2 weeks
Sperm Count (Post-Vasectomy)	PVAS	**Semen**[1]	2 days
Sperm DNA Fragmentation (SCSA)	SEXT	**Semen**[1]	1-2 weeks
Sperm Morphology (Kruger strict criteria)	MRPH	**Semen**[1]	2 days

Semen parameters may be requested INDIVIDUALLY (eg count only, vitality only, etc).
Please request as SPOD and indicate on the request form which parameter is required.

Semen Parameters	SPOD	**Semen**[1]	1 day

* If required, comprehensive semen analysis can be reported within 4 hours, with morphology to follow.

** For men who have had a vasectomy, clearance should only be given when there is no evidence of presence of sperm in a single ejaculate when recommendations are met. It is rare that a 'diagnosis' is made without confirmation, therefore patients/clinicians should be able to freely request a second confirmatory sample. Special clearance may be given at the doctor's discretion, when there are <100 000/ml non-motile sperm present after the assessment of two specimens in full accordance with recommendations. Recommendations, as given by the Association of Biomedical Andrologists, the British Andrology Society and the British Association of Urological Surgeons 2016, are as follows:

1 Analysis of post vasectomy semen samples should not occur until 12 weeks post-surgery and after a minimum of 20 ejaculates
2 Semen samples must be analysed within 4 hours of production, and in cases where sperm is found a repeat analysis must be performed within 1 hour of production
3 Semen should be provided in weighed specimen containers provided by TDL Andrology
4 Sexual abstinence should be between 2 and 7 days

† Sperm antibodies in semen are measured as part of the routine semen analysis.

TDL Andrology

BY SPECIAL ARRANGEMENT

Sperm swim test
Sperm preparation for overnight survival
Sperm motility and vitality testing for epididymal toxicity
Sperm retrieval procedures (biopsy, PESA, MESA)

Sperm cryopreservation and storage (undertaken by Andrology Solutions – HFEA licensed)

All men who store sperm must be screened for HIV 1&2, Hepatitis B, Hepatitis C and HTLV. Under HFEA regulations, sperm can be stored for an initial period of 10 years with formal consent. All patients are offered counselling prior to sperm cryopreservation.

These arrangements, and details for other specialist semen tests, are available on request. Please contact TDL Andrology on 020 7025 7940 or email sheryl.homa@tdlpathology.com for further information.

Sperm DNA fragmentation

High sperm DNA fragmentation is associated with reduced natural pregnancy rates and assisted conception pregnancy rates as well as live birth rates. In addition, DNA fragmentation leads to higher miscarriage rates as published in the ESHRE Recurrent Pregnancy Loss 2017 Guideline. High levels of DNA fragmentation may be reduced by considering varicocele repair, treatment of underlying infections or inflammation, changes in lifestyle or with antioxidant supplements.

When requesting Sperm DNA Fragmentation there are two options. Please specify whether the request is for sperm DNA fragmentation by **SCSA** or **COMET**.

- **Sperm Chromatin Structure Assay (SCSA®) [SEXT]**

  This test has the ability to measure large numbers of cells (between 5,000 and 10,000 sperm), rapidly in an ejaculate. The SCSA® test monitors the changes in fluorescence of a probe, acridine orange, to detect both single and double DNA strand breaks using flow cytometry. It has been developed using human and animal models over the last 35 years and is one of the most statistically robust tests available for sperm DNA fragmentation. It is a standardised, validated CLIA approved test with high reproducibility and low variability. The test requires a minimum sperm count of approximately 1 million/ml.

- **Sperm COMET® Assay [CMET]**

  When sperm counts are limited, DNA fragmentation can be effectively assessed using the Comet® assay as only ~5,000 sperm are required. The Comet® assay uses electrophoresis to determine abnormal sperm, and can measure both single and double strand breaks. Unlike the SCSA® test, the comet assay may be subject to inter-observer variability and may be less statistically robust as it measures low counts of 50 to 100 sperm cells from each sample.

Sperm Aneuploidy

Chromosomal abnormalities may be somatic cell in origin, in which case they can be detected by a simple blood karyotype analysis. However, most sperm chromosome anomalies arise as a result of errors during meiosis, which cannot be detected by a blood karyotype analysis. These anomalies can only be detected by looking at the sperm chromosomes directly. Studies have shown that sperm with a high rate of aneuploidy have a negative impact on pregnancy rate and are associated with recurrent pregnancy loss.

TDL Andrology

This test uses fluorescent in situ hybridisation (FISH) to label individual chromosomes with specific probes. Hundreds of sperm are assessed from one ejaculate. There are limitations to the test as only 5 probes are currently used routinely for analysis (three of the 22 autosomes: chromosomes 13, 18 and 21, and the sex chromosomes, X and Y), although others are available upon specific request. The results are reported showing incidence of disomy or nullisomy for each of the autosomes and for both sex chromosomes. A sex chromosome ratio is also reported. It is CE marked.

Instructions for collection of Sperm DNA and Aneuploidy specimens

Sperm DNA Fragmentation or Sperm Aneuploidy testing are not part of the Comprehensive Semen Analysis and need to be requested as a separate test, test code SEXT and SPPL, respectively. Semen samples ideally need to be frozen as soon as possible after liquefaction, but not longer than 60 minutes post ejaculation. Samples must be snap-frozen for Sperm DNA Fragmentation and cryopreserved in TYB for Sperm Aneuploidy. If samples are prepared by another laboratory. Two cryovials containing not less than 0.25 mls of semen is required. Frozen samples can be sent to, or collected by TDL, by arrangement, and must be accompanied with relevant patient details, the sperm count and GDPR consent form. A count of a minimum 1 million/ml is required for accurate DNA and aneuploidy reporting.

Oxidative Stress in Semen (ROS + MIOXSYS) and Male infertility

There is now growing evidence to support a link between oxidative stress and male infertility. It is the underlying cause of sperm DNA damage and impairs semen parameters and fertilisation, adversely affects embryo development and is associated with reduced pregnancy rates. It may also increase the risk of miscarriage. High levels of ROS may be reduced by considering varicocele repair, treatment of underlying infections or inflammation, changes in lifestyle or with antioxidant supplements.

TDL provides a comprehensive assessment of oxidative stress by **combined measurement of Reactive Oxygen Species and Redox Potential**. Please request as oxidative stress test (code **ROS**).

The test includes combined testing for:

- **Chemiluminescence Assay for Reactive Oxygen Species**

  Reactive Oxidative stress may be measured by a simple chemiluminescence test in semen, which measures the level of reactive oxygen species.

- **MIOXSYS Electrochemical Assay for Redox Potential**

  Oxidative stress may be determined by an electrochemical assay which measures the redox potential in semen. This test measures the overall difference between total oxidants and antioxidants in the system.

References

Homa ST, Vessey W, Perez-Miranda A, Riyait T, Agarwal A (2015). Reactive oxygen species (ROS) in human semen: determination of a reference range. J Assist Reprod Genet 32(5):757-64.

Vessey W, Perez-Miranda A, Macfarquhar R, Agarwal A, Homa S. (2014). Reactive oxygen species (ROS) in human semen: validation and qualification of a chemiluminescence assay. Fertil Steril. 102:1576-1583.

If you would like to discuss these tests, or any aspect of this service, please contact TDL Andrology on 020 7025 7940 or 020 7307 7373, or email andrology@tdlpathology.com.

TDL Andrology

Effects of ROS-induced Oxidative Stress on Sperm

- Lipid peroxidation which damages the sperm surface causing an abnormal morphology and impaired motility.
- Damage to proteins on cell surface responsible for cell signalling and may affect enzyme function inside the cell.
- Increased semen viscosity.
- Peroxidation of DNA and subsequent unravelling or fragmentation.
- Possible mutagenic effects.
- Damage to seminiferous epithelium, damage to tubules, testicular atrophy, reduced spermatogenesis.
- Decrease in sperm vitality, motility.
- Impaired fertilization by affecting sperm capacitation and the acrosome reaction.

Causes of Elevated ROS Levels

- Genito-urinary tract infection
- Prostatitis
- Vasectomy reversal
- Varicocoele
- Cryptorchidism
- Chronic disease
- Xenobiotics
- Chemical pollutants and occupational hazards
- Heavy metal exposure
- Removal of seminal plasma during sperm preparation for assisted conception
- Drugs – cyclophosphamide, aspirin, paracetamol
- Smoking
- Excessive exercise
- Heat exposure
- Obesity
- Age

Semen samples need specialist handling – for this reason all requests for semen analyses should be made by appointment. Practices or patients should contact TDL Andrology on 020 7025 7940 to make appointments and to confirm instructions for sample collection.