



## QwikCheck Liquefaction Kit Technical Blog

SQA-V users have asked MES a number of questions concerning the QwikCheck™ Liquefaction kit. We have compiled a list of those questions and our tech support answers to assist with the use of this product.

**Question:** When should more than 1 Vial of QwikCheck™ Liquefaction kit powder be used to treat a sample?

**Answer:** For samples with a volume of 5ml or less, 1 Vial should be used. For samples over 5ml total volume, 2 Vials may be used for treatment.

**Question:** Does the use of the QwikCheck™ Liquefaction kit affect the pH of the sample? If so, should we perform the pH testing before and/or after the liquefaction powder has been added?

**Answer:** Use of the QwikCheck™ Liquefaction kit does not affect pH. However, it might affect the testing outcome when using Urine Test Strips or MES QwikCheck™ Test Strips (adverse chemical reaction). Therefore, if using Urine Test Strips or the QwikCheck™ Test strips for pH and WBC, use the strips **before** the Liquefaction kit has been implemented.

**Question:** How soon after treating the sample with the QwikCheck™ Liquefaction kit (time-frame) should we run Motility testing? If we use more than one vial, should we wait any additional time?

**Answer:** No, you should not wait additional time if multiple vials are used. Sample analysis should begin immediately after Liquefaction per the QwikCheck Liquefaction Kit Product Insert.

**Question:** If the Viscosity is normal, but gel clumps remain, should we still use a vial of Liquefaction?

**Answer:** Per WHO 5<sup>th</sup> manual (1) Normal, liquefied semen samples may contain jelly-like granules (gelatinous bodies) which do not liquefy. However, these gelatinous bodies do not appear to have any clinical significance. The presence of mucus strands, however, may interfere with semen analysis. So to make the semen sample more uniform and eliminate sample preparation variables, it is recommended that samples of this kind be treated with the QwikCheck™ Liquefaction kit.

**Question:** When a highly viscous sample is treated with the QwikCheck™ Liquefaction kit, will motility increase slightly due to the drop in viscosity and, as a result will the % normal morphology also increase slightly?

**Question:** I believe that the liquefaction kit contains an enzyme. If that is true, doesn't it have the potential to break down the spermatozoa? If so, what may be affected? The head? The neck? The tail? Lastly, will it change the morphology?

**Answers to both questions above:** Even though "slightly" is used to describe the impact to motility, it is very difficult to characterize the exact impact to motility for all samples due to the fact that each semen sample is different in terms of viscosity level and reaction to sample treatment.

The active ingredient in the QwikCheck™ Liquefaction Kit is chymotrypsin. This is an enzyme with "proteolytic" activity (<http://en.wikipedia.org/wiki/Proteolysis>). Chymotrypsin performs limited proteolysis. As semen viscosity is associated with glycoproteins, limited proteolysis for the treatment of viscous semen samples is optimal. The limited proteolysis caused by chymotrypsin cannot destroy the spermatozoa, because the cells are protected by an external phospholipid membrane that cannot be cleaved by the protease.



There are a number of publications which address the use of limited proteolysis for the treatment of viscous and incompletely liquefied semen samples for both analytical and sperm preparation purposes. The WHO 5<sup>th</sup> edition manual recommends treating highly viscous or samples with delayed liquefaction with a proteolytic enzyme (1). Further, it was demonstrated that chymotrypsin had no effect on the detection of sperm parameters and biochemistry markers, and could be used to treat non-liquefied samples before semen analysis in the andrology laboratory (2).

Initiating limited proteolysis on high viscosity semen specimens with  $\alpha$ -chymotrypsin was shown to be an effective treatment. Treating viscous samples with  $\alpha$ -chymotrypsin can significantly improve the sample handling and preparation process. Furthermore, limited proteolysis of high viscosity semen samples using  $\alpha$ -chymotrypsin resulted in the recovery of both a better quality and a higher number of spermatozoa which can be used for assisted reproduction.(3).

**Other information of note:** Anti-sperm antibodies in semen have been associated with a decrease in fertility potential. Treatment of these sperm samples with chymotrypsin/galactose resulted in increased pregnancy rates by IUI insemination (4). As anti-sperm antibodies are proteins, the positive effect of chymotrypsin treatment may be explained by its proteolytic activity which destroys the anti-sperm antibodies.

It was also shown that the outcome of a sperm penetration assay was improved by treating the semen samples with chymotrypsin (5).

The conclusions based on the findings described in the cited publications are summarized below.

Treatment of highly viscous and incompletely liquefied semen samples with chymotrypsin results in:

- A more homogenous spread of spermatozoa in the semen volume. This results in a decreased statistical error when assessing semen (the testing sample is more representative of the overall sample).
- More reliable sample handling and semen analysis
- No or VERY minimal effect on semen parameters (test results)
- Improved outcome for assisted reproductive techniques
- Increased pregnancy rates when used to treat semen that contains anti-sperm antibodies

#### References:

1. WHO, *Laboratory Manual for the Examination and Processing of Human Semen*. Fifth edition ed. 2010, Geneva: World Health Organization.
2. Chen F, Lu JC, Xu HR, Huang YF, Lu NQ. Chymotrypsin effects on the determination of sperm parameters and seminal biochemistry markers. *Clin Chem Lab Med.*, 2006;44(11):1335-9.
3. P.M. Zavos, P.N. Zannakoupis-Zavos, J.K. Correa. Effect of treatment of seminal viscosity difficulties with  $\alpha$ -chymotrypsin on the recovery of spermatozoa for assisted reproductive technologies: comparison between the SpemPrep filtration and Percoll gradient centrifugation methods. *Middle East Fertility Society Journal*, 1997;21(3):223-9.
4. A. Bollendorf, J.H. Check, D. Katsoff, A. Fedele. The use of chymotrypsin/galactose to treat spermatozoa bound with anti-sperm antibodies prior to intra-uterine insemination. *Hum. Reprod.*, 1994; 9(3):484-8.
5. K.L. Honea, V.L. Houserman, D.C. Merryman, D.A. Free, S.E. Stringfellow. Effect of limited proteolysis with  $\alpha$ -chymotrypsin on semen with an abnormal sperm penetration assay and possible application for in vitro fertilization or intrauterine insemination. *Journal of Assisted Reproduction and Genetics*, 1993;10(4):255-60..