

believe that the temperature-control mechanism employed in the device is a robust, reproducible and reliable method of addressing the variation in ambient temperature in the home test environment.

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## Development of a novel home sperm test – What are the limitations?

Sir,

I read with great interest the article by Björndahl *et al.* entitled ‘Development of a novel home sperm test’ published in the January 2006 issue of *Human Reproduction*.

The device is based on a lateral-flow immunoassay using an anti-CD59 antibody conjugated to colloidal gold to generate a visible red line when sperm are present. Progressively motile sperm from liquefied semen are separated by a direct swim-up through hyaluronic acid, and the sperm fraction from the swim-up reacts with the anti-CD59 antibody. The appearance of a clear red line, due to the colloidal gold label on the antibody-bound sperm, indicates a concentration of  $10 \times 10^6$ /ml or greater progressively motile sperm in semen.

CD59 is an 18–20 kDa GPI-linked glycoprotein thought to play a role in protecting cells from attack by complement (Rooney *et al.*, 1993). In addition to viable sperm, CD59 is also present in white blood cells, immature and dead sperm and in seminal plasma (Rooney *et al.*, 1993). Separation of progressively motile sperm allows the detection of only progressively motile cells on the nitrocellulose strip, avoiding potentially erroneous results due to the presence of round spermatids, red blood cells or leukocytes in semen.

The study included 150 men selected from research donors ( $n = 132$ ), subfertile males ( $n = 7$ ) and post-vasectomy donors ( $n = 11$ ). The subfertile males had an abnormal semen analysis, with a concentration of  $<10 \times 10^6$  progressively motile sperm per ml of semen. The concentration of progressively motile sperm, obtained by computer-assisted sperm analysis (CASA) (Hamilton Thorne, Beverley, MA, USA), and a modified Kremer test (hyaluronate migration test [HMT]) was

performed on each semen sample. The results obtained from the device were compared with those for samples where the reference methods were concordant. Of the 150 subjects enrolled, five were excluded due to insufficient volume and a further two due to technical problems. For the comparison with the combined reference method (CASA and HMT), 14 subjects were excluded because the results of the two reference tests were not concordant.

The device gave a positive, red test line in 95 of the 129 valid experiments and no test line in the remaining 34. Of the 95 positive results, 91 (95.8%) also showed positive results with the reference tests, and of the 34 negative device results, 32 (94.1%) were negative in the reference tests. Thus, the device gave results with high sensitivity and specificity.

The authors concluded that the device provides an accurate, rapid and easily visualized estimate of the concentration of progressively motile sperm in a semen sample that can be used by a man in the comfort of his own home.

However, the authors should clarify the following points:

(i) The sample population used in this study is highly biased, since 88% of the semen samples were obtained from normal donors and only 5.3% from subfertile donors. In addition, the authors failed to mention what was the range of liquefaction times for the different semen samples tested. About 12% of semen samples produced by males undergoing infertility screening have high viscosity with liquefaction times above 60 min (Wilson and Bunge, 1975). Moreover, a significant proportion of these samples have liquefaction times ranging between 3 and 12 h post-ejaculation. Although semen viscosity could be reduced in the andrology laboratory by resorting to semen dilution or by inducing semen liquefaction with either enzymatic or non-enzymatic agents, this cannot be done at home by patients. As a result, should a representative subfertile and infertile male population be included in this study, a significant number of patients would not have been able to perform the test due to high semen viscosity. The assay time would have been 60 min or higher in 12% of the patients or more. A home sperm test with such a high rate of cancellations and high turnaround time would be obsolete.

(ii) Semen viscosity will preclude users from accurately loading the semen sample into the sample port of the device. In addition, users will not know when the semen sample has liquefied and is ready to be loaded.

(iii) A *sine qua non* condition for accuracy in semen analysis is sample homogeneity (de Ziegler *et al.*, 1987). This is especially important when dealing with viscous samples from infertility patients. Therefore, in addition to exceedingly high turnaround analysis time, lack of sample homogeneity due to semen viscosity would produce inaccurate results.

(iv) What is the cell-to-cell variability in the density of CD59 on the surface of spermatozoa recovered after the swim-up? This would be particularly important in teratozoospermic samples and in samples with a high proportion of sperm with residual cytoplasmic retention obtained from infertility patients. Cell-to-cell variability in CD59 density would result in inaccurate results.

The shortcomings related to semen viscosity could be circumvented by developing tests that accelerate semen liquefaction before sample loading like the case of FertILMARQ, the first FDA-approved home sperm test and the only test currently available in the market that incorporates this proprietary technology. However, since the signal produced by the authors' device is based on the binding of an antibody to sperm, use of enzymatic or non-enzymatic agents that accelerate semen liquefaction would result in hydrolysis or denaturation of the antibody and CD59 epitopes on the surface of sperm, thus excluding this option.

In conclusion, although the device reported by Bjorndahl *et al.* (2006) could be used in a laboratory setting for the determination of motile sperm in washed semen samples, the application of this device to raw semen has severe limitations that make the device commercially nonviable as a home sperm test.

## References

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## Reply: Development of a novel home sperm test – What are the limitations?

Sir,

We appreciate the comments from Dr Alvarez and Dr Agarwal and welcome the opportunity to address them.

### Regarding point 1

(i) We clearly stated in the *Material and Methods* the selection criteria for the study (see Björndahl *et al.*, 2006). We stated that we intended that a target minimum of 25% of subjects should have progressively motile sperm concentrations below the test threshold (10 million progressively motile cells/ml semen). The selected research donors had a variety of semen characteristics—some were recruited specifically because they had poor semen quality—thus the authors statement '88% of semen samples were not obtained from normal donors' is incorrect.

(ii) High viscosity and incomplete liquefaction are different issues (see Mortimer, 1994; WHO, 1999), and it is inappropriate to combine them. In the article, we stated that all samples were allowed to liquefy for 30 min at 20°C. This was set to try and mimic the home environment. Increased viscosity is not necessarily associated with impaired fertility potential, but even if it did reduce the ability of sperm to migrate from the semen into the hyaluronate, then this biological effect would be detected by the device.

(iii) Incomplete liquefaction and non-liquefaction are different issues, but both are considered diagnostic findings that require medical assessment by a properly trained clinical andrologist. Should the liquefaction be so incomplete as to impair sperm penetration into hyaluronate, this is diagnostic (no red line would appear) and the patient with a problem will seek medical advice. Non-liquefaction is considered a pathological finding, and hence would be detected by the device (no red line—sufficient number of sperm unable to penetrate into the hyaluronate).

### Regarding point 2

There is no 'sample port' in the device. We clearly stated in the article how the test works (see Figure 1 in the article and associated text). In our experiments, the semen samples were ejaculated directly into containers and after 30 min loaded into the device. When the man is using this at home, he ejaculates directly into the sample collection container, which is integral to the device—no transfer of semen/sperm is necessary (see <http://www.fertell.co.uk> for patient instructions and photographs). The man is instructed that after 30 min when semen has filled the sample well, he needs to put the top on the collection container and push a button (blue one in Figure 1 in the article). When this occurs, a portion of the semen sample (600 µl) comes in direct contact with the hyaluronate (via a mesh). Only if the volume is less than the critical limit (600 µl) will the test fail, i.e. no red line will appear. Men are advised to abstain for at least 2 days (not greater than 7 days) before doing the test. If they have a semen volume of 600 µl or less, this is a diagnostic finding considered to have clinical importance.

### Regarding point 3

Once again the authors are confusing increased viscosity with incomplete liquefaction (see above). The interface between the gel and semen is deliberately large to allow more sperm to successfully penetrate the hyaluronate (see Tang *et al.*, 1999). This may compensate for potential heterogeneity in the semen sample. In our trials, we obtained a high degree of accuracy (>95% compared with the Hamilton Thorn and modified Kremer testing). Thus, we do not believe that sample heterogeneity is a significant issue when using this device.

### Regarding point 4

CD59 was used because it is well known to be present on sperm (and within the cell), as stated in our article (see review Harris *et al.*, 2006). Studies performed by other authors have shown