Semen analysis (SA) is the primary tool used in the evaluation of male fertility. The clinical laboratory has increasingly complex and relevant roles in evaluating male infertility and sterility. However, a lack of standardized SA methodology can make it difficult to interpret or establish global reference values for semen parameters such as sperm count and motility. Because of the difficulties in standardization, individual laboratories have adopted specific methods and procedures for SA that can target individual patient populations. In an attempt to enhance the standardization of SA performance and interpretation, the World Health Organization (WHO) recently published guidelines on the examination and processing of human semen.1 While the WHO provides interpretation guidelines based on reference ranges and a thorough explanation of sample collection and processing, the effects of differences in methodologies are not fully addressed by the WHO.

Methodological differences are very important, especially when evaluating a patient sample such as semen that is composed of formed heterogeneous components. The complexity of semen samples can be appreciated when considering the pathways through which its biochemical components are formed. Spermatozoa are produced in testicular seminiferous tubules via spermiogenesis. Spermatozoa are produced in testicular seminiferous tubules via spermiogenesis. Spermatozoa are produced in testicular seminiferous tubules via spermiogenesis. Spermiogenesis is the last developmental stage of spermatozoa where haploid spermatids become motile sperm. SA in the clinical laboratory includes assessment of semen components that include not only sperm, but also acidic secretions from the prostate and alkaline seminal vesicle. The chemical properties of these secretions are essential to fertility due to their effect on initial coagulation and subsequent liquefaction of semen after ejaculation.

Because of the aforementioned factors, SA reports must reflect analysis of a sample that includes all of the components in the ejaculate. Specifically, when comparing the first portion of the ejaculate vs the second portion, the first portion contains a higher concentration of sperm,
is more acidic, contains prostatic secretions (vs seminal vesicle secretions), contains enzymes for liquefaction (vs coagulation enzymes), and composes 30% vs 70% of the ejaculatory volume. The macroscopic phase of the SA is usually performed within 1 hour of collection, after the clot (coagulum) has liquefied. Reported macroscopic parameters include alkalinity, appearance, viscosity, volume, and vitality. Subsequently, the microscopic portion of the SA is performed to assess sperm concentration, motility, morphology, total sperm count, and the presence of other cells.

Optimal preparation and viability of the specimen are essential to provide accurate interpretative results and to yield the best product for evaluating and managing infertility as well as in vitro fertilization procedures. In this study, we evaluated 2 different SA wash methodologies to determine optimal testing times from the sample collection. Optimal testing times were compared between methodologies for overall motility, rapid moving, and total sperm count. Additionally, we evaluated the use of Pre-Seed using the same criteria. The goal of the study was to determine optimal analytical performance for SA with respect to time, methodology, and collection method.

Materials and Methods

For the isolation gradient selection, semen samples were obtained from the Tripler Army Medical Center (TAMC) chemistry andrology section for semen analysis. Twenty consecutive samples used in this study were submitted as part of TAMC’s standard of care testing. All samples were analyzed first with a complete SA using the Computer Assisted Semen Analysis (CASA) software (Beverly, MA) on the IVOS 1 platform by Hamilton Thorn (Beverly, MA) in accordance with our standard operating procedures. Subsequent to initial evaluation, the patient samples were split into two 2.0 mL aliquots for method comparison. A main purpose of this study was to examine analytical differences between 2 different types of motile sperm isolation gradients. To separate the motile sperm populations, the split samples were layered on top of each of the isolation gradients. Each gradient was prepared with either Isolate Sperm Separation Medium (Irvine Scientific, Santa Ana, CA Inc., 35% and 70% concentrations) or PureSperm 40/80 (Nidacon International, Moindal, Sweden AB, 40% and 80% concentrations). Volumes for the gradients were 2.0 mL for the lower layer, 2.0 mL for the upper layer, and 2.0 mL of the patient sample for a total volume of 6.0 mL. Samples in the respective 2 layered gradients were then centrifuged at 300 g for 20 minutes. The supernatant from the tube was removed without disturbing the pellet and was discarded to yield a final volume of approximately 0.5 mL. Five mL were removed from the final sample and CASA was performed; the results were recorded and subsequently evaluated using the Mini Tab statistical program, Honolulu, HI.

After separation, the isolated motile sperm from sample aliquots were washed in either Sperm Wash Medium (Irvine Scientific) or PureSperm Wash (Nidacon International AB). Wash medium was added to a total volume of 5.0 mL and the mixtures were centrifuged twice for 15 minutes; the supernatant was removed and discarded after each wash. The final aliquots were adjusted to a volume of 0.5 mL wash medium and CASA was performed to determine retention of the sperm population within each washed aliquot. Samples were subsequently incubated at 37°C in a dry incubator. After incubation for 30, 60, 120, and 180 minutes, 5 mL aliquots were removed from the incubated sample and CASA was performed and results were recorded. The purpose of the incubation study was to assess whether the medium would maintain the motility, forward progression, and count through the various incubation periods.

The Pre-Seed (Valley Ford, WA) evaluation portion of this study determined the effects on SA parameters such as sperm motility and count when patients used a lubricant. Pre-Seed aids the collection of a semen sample. Twenty semen samples were obtained. Fresh 2.0 mL semen samples were washed 2 times in 5.0 mL of the Irvine Sperm Wash Medium. After each wash, the supernatant was discarded without disturbing the pellet. After the final wash, the sample was adjusted to a total volume of 0.5 mL. Ten mL of Pre-Seed was added to each sample and the mixture was incubated at 37°C in a dry incubator. Aliquots of 5 mL were removed for CASA (IVOS 1, Hamilton Thorn) after 15, 30, 60, 120, and 180 minutes. The results were evaluated using Mini Tab software.

Results

Of the 20 specimens evaluated with or without the addition of Pre-Seed, 8 of the donors were diagnosed with male factor infertility. The male factor infertility in these cases was based upon decreased serum production as defined
by WHO criteria: a semen specimen containing 1.5 million spermatozoa per mL with a 95% CI of 12-16 million sperm/mL. As illustrated in Table 1, there were no significant differences observed in motility, forward progression, or morphology without Pre-Seed compared to 1 hour post Pre-Seed treatment. All participants indicated that they would prefer using a lubricant during the collection process.

We compared the differences between 2 gradient types used for the isolation of motile sperm populations. The PureSperm 40/80 group was evaluated with respect to the Isolate gradient in 20 patients whose partners required sperm washing for intrauterine insemination. PureSperm 40/80 (38.3×10^6 sperm/mL) isolated significantly more of the motile sperm population than the Isolate (21.8×10^6 sperm/mL). The difference in motile sperm recovery between the PureSperm group vs. the Isolate group was statistically significant (P <0.001).

Data from subsequent culture monitoring, listed in Table 2, showed that sperm motility and count were not significantly different at the 0 time point, PureSperm wash (count: 39.9×10^6 sperm/mL and 83.80% motile) vs. Sperm Wash Medium (count: 37.45×10^6 sperm/mL and 77.57% motile). However, at the 2 hour time point both motility and count significantly decreased over time after incubation with the Sperm Wash Medium (count: 15.17×10^6 sperm/mL and 75.40% motile) compared with the PureSperm wash medium (count: 24.9×10^6 sperm/mL and 83.6% motile) (P <0.05).

**Discussion**

Fertility assessment of the male partner can be frustrating for both the patient and provider because a specific etiology or treatment is not often identified. Specific disorders, when identified, are characterized by an abnormal SA. Our laboratory performs 5 SA tests per day for Hawaii’s military and dependent population who require SA for post-vasectomy evaluations as well as those who are being evaluated for in vitro fertilization. SA appointments are usually scheduled up to 60 days in advance. This study evaluated preanalytic factors that may affect SA results, including the use of a lubricant and differences in the wash solutions.

The results of our investigation suggest that PreSeed may be used as a lubricant during sample collection without compromising SA results. The benefits of using PreSeed are 2-fold: First, it does not contribute to preanalytical variation (eg, alter motility, forward progression, or morphology) when up to 10 mL are mixed with the sample 1 hour after collection. Second, it may make the sample collection process easier and less stressful for patients. Moreover, PreSeed is non-toxic to sperm. The use of a lubricant that does not cause preanalytical variation is especially useful not only in intra-uterine insemination, but also in SA when marked individual variability necessitates at least 2 collections 1 to 2 weeks apart.1,5

We also evaluated the effect of 2 different wash gradients used in the isolation of motile sperm for IUI procedures. Optimal separation procedures should result in the highest

### Table 1. Pre-Seed Study Table

<table>
<thead>
<tr>
<th>Without Pre-Seed</th>
<th>Pre-Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility</td>
<td>45.6%</td>
</tr>
<tr>
<td>Forward progression</td>
<td>47.6%</td>
</tr>
<tr>
<td>Morphology (&gt;12%)</td>
<td>17.5%</td>
</tr>
</tbody>
</table>

*n = 20 patients, 8 male factor*  
Motility, forward progression, and morphology with and without the use of a lubricant during sample collection.

### Table 2. Composite Table Showing Differences Between Count and Motility Over Time

<table>
<thead>
<tr>
<th>Type of Medium</th>
<th>Time: 0</th>
<th></th>
<th>Time: 2h</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count (1×10^6 sperm/mL)</td>
<td>Motility (%)</td>
<td>Count (1×10^6 sperm/mL)</td>
<td>Motility (%)</td>
</tr>
<tr>
<td>PureSperm Wash</td>
<td>37.9</td>
<td>83.8</td>
<td>24.9</td>
<td>83.6</td>
</tr>
<tr>
<td>Sperm Wash Medium (Irvine)</td>
<td>37.4</td>
<td>77.6</td>
<td>15.2</td>
<td>75.4</td>
</tr>
</tbody>
</table>

*Isolation gradient counts and motilities of the samples prior to incubation time (time = 0) and the changes in count and motility after incubation time (time = 2h).*
possible sperm concentration in a sample that is mostly free from cellular contamination such as dead sperm, white blood cells, or other debris. In our comparison of the Irvine and PureSperm methods, we determined that neither method was superior at time 0; however, the Sperm Wash Medium produced significantly lower sperm counts than the PureSperm wash method 2 hours after collection. This suggests that PureSperm may be better at maintaining and supporting specimen viability for subsequent analysis. This difference could prove critical for successful IUI results, especially in suboptimal cases (eg, hyperviscosity) where specimen manipulations are more challenging.

Although this study investigated 2 aspects of SA that could optimize specimen quality and decrease variability, additional studies will be required to assess other factors that may contribute to variability in SA results. Because the SA procedure may never be completely standardized, the prediction and treatment of infertility will continue to present challenges due to the complex nature of the sample. However, the development of procedures and use of products that provide optimal results may reduce the necessity of recollections, increase laboratory efficiency, and improve the overall experience of patients being evaluated for infertility. LM

Disclosure
The views expressed in this manuscript are those of the authors and do not reflect the official policy or position of the Department of Army, Department of Defense, or the US Government.

References

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