BACKGROUND

The measurement of AMH has now become widespread practice within the field of fertility treatment and research, so require detailed examination and comparison under different preanalytical conditions. Testing of clinical samples at central labs introduces potential for increased pre-analytic variability, with phlebotomy, sample preparation, transportation, and storage all being critically important. In this study, various pre-analytical conditions that could affect AMH results were assessed.

MATERIALS AND METHODS

Following the International Society for Biological and Environmental Repositories, five volunteers with AMH levels between 3.92 - 6.39 ng/mL were recruited. Peripheral blood was drawn using VACUETTE® tubes (Greiner Bio-One, Austria).

Four types of samples were created for each participant:

- Serum (Type n° 1).
- Plasm (Type n° 2).
- Serum separated from freeze-thaw whole blood gel tubes (Type n° 3).
- Serum separated after centrifugation from closed gel tubes using Vacuette closed tube system (Type n° 4).

Aliquots from each group were subjected to varying numbers of freeze-thaw cycles (1 to 5 freeze-thaw cycles at -80°C) and at 12 different temperature conditions: at room temperature (RT) and at 4°C for 1, 2, 4, 24, 72 and 168 hours, respectively (Table 1). As reference condition, the blood collected and fresh samples prepared according to Access AMH manufacturer instructions.

Samples were then stored at -80°C until analysis. Separate aliquots were stored -20°C for 30 days. AMH was measured on UniCel DxI 800 (Beckman Coulter, Inc.).

RESULTS

The mean coefficients of variation (CV%) for groups Type n°1,2,3 and 4 for 1-5 freeze-thaw cycles at -80°C were 2.55%, 2.65%, 3.73% and 3.01% respectively. The highest variation of mean AMH concentration and CV% was shown for Type n°3 (Fig 1; Fig 4).

Among mean coefficients of variation (%) for groups n°1,2,3 and 4 for different temperature conditions (six stored at RT and six at 4°C for 1, 2, 4, 24, 72 and 168 hours), the highest variation of mean AMH concentration and CV% were achieved in group Type n°3 in comparison to groups Type n°1,2,4: 5.5% vs. 3.02%, 2.22% and 2.8%, respectively (Fig. 2 and 3; Fig 5).

CONCLUSION

Changes in AMH results at different freeze-thaw cycles and temperature conditions were non-significant. The highest CV% was observed the freeze-thaw whole blood gel tube group. These results demonstrate the stability of the AMH molecule under different pre-analytical conditions when measured with the Access AMH assay.

Table 1 List of different types of samples analyzed in different storage conditions

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>Type of the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh sample</td>
<td>Serum (Type n°1)</td>
</tr>
<tr>
<td>RT (22°C) for 1 hr</td>
<td>Plasma (Type n°2)</td>
</tr>
<tr>
<td>RT (22°C) for 2 hrs</td>
<td>Serum separated from freeze-thaw whole blood gel tubes (Type n°3)</td>
</tr>
<tr>
<td>RT (22°C) for 4 hrs</td>
<td>Serum separated after centrifugation from closed gel tubes using Vacuette closed tube system (Type n°4)</td>
</tr>
<tr>
<td>RT (22°C) for 24 hrs</td>
<td></td>
</tr>
<tr>
<td>RT (22°C) for 72 hrs</td>
<td></td>
</tr>
<tr>
<td>Fridge (4-8°C) for 1 hr</td>
<td></td>
</tr>
<tr>
<td>Fridge (4-8°C) for 2 hrs</td>
<td></td>
</tr>
<tr>
<td>Fridge (4-8°C) for 4 hrs</td>
<td></td>
</tr>
<tr>
<td>Fridge (4-8°C) for 24 hrs</td>
<td></td>
</tr>
<tr>
<td>Fridge (4°C) for 72 hrs</td>
<td></td>
</tr>
<tr>
<td>Fridge (4-8°C) for 168 hrs</td>
<td></td>
</tr>
<tr>
<td>Freezer (-20°C) for 30 days</td>
<td></td>
</tr>
<tr>
<td>Frozen to -80°C 1 cycle (frozen to -80°C thaw 1x and frozen to -80°C)</td>
<td></td>
</tr>
<tr>
<td>Frozen to -80°C 2 cycles (frozen to -80°C thaw 2x and frozen to -80°C)</td>
<td></td>
</tr>
<tr>
<td>Frozen to -80°C 3 cycles (frozen to -80°C thaw 3x and frozen to -80°C)</td>
<td></td>
</tr>
<tr>
<td>Frozen to -80°C 4 cycles (frozen to -80°C thaw 4x and frozen to -80°C)</td>
<td></td>
</tr>
<tr>
<td>Frozen to -80°C 5 cycles (frozen to -80°C thaw 5x and frozen to -80°C)</td>
<td></td>
</tr>
</tbody>
</table>

© 2019 Beckman Coulter. All rights reserved.
Beckman Coulter, the stylized logo, and the Beckman Coulter product and service marks mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries.

References

1) R. Fleming et al. HUMAN FERTILITY, 2017; 2) D.L. Garcia Biopreservation and Biobanking, Vol. 12, No. 6, 2014