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| **New Vision Medical Laboratories****Joint Township District Memorial Hospital (St. Marys, Ohio)** **Technical Procedure** |  | **Procedure Number: nvml.jtdmh.URN-304**POLICY INITIATED: 06/24/2013 |

**SUBJECT: Semen Count Analysis**

**1.0 Scope:**

Semen count analysis is part of the testing performed during a complete semen analysis. A semen count analysis determines the number of sperm in a semen sample per unit volume. This policy applies to all testing personnel.

**2.0 Reagents/Supplies:**

hemocytometer

diH20

**3.0 Specimen Requirements:**

It is usually recommended that semen sample be collected following a 3 to 7-day period of continence. It is essential that the specimen not be subjected to temperature extremes and must be received in the lab within one hour following collection. Collection is in a polypropylene container.

**4.0 Procedure:**

4.1 Determining what dilution to use:

The dilution of semen required to allow sperm number to be measured accurately is assessed from an undiluted semen preparation. Use a wet preparation (fill a Kova chamber).

4.1.1 Examine the wet preparation to estimate the number of spermatozoa per HPF (×200 or ×400).

4.1.2 One HPF is equivalent to approximately 16 nl (at ×200) or 4 nl (at ×400).

4.1.3 If spermatozoa are observed, count them, determine the necessary dilution from Table 2.1, and proceed as in Section 4.2.1.

4.1.4 If no spermatozoa are observed, examine the replicate wet preparation. If no spermatozoa are found in the second preparation, report as “None Seen”.

4.1.5 Table 2.1 of semen dilutions required, how to make them, which chambers to use:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Spermatozoa per ×400 field | Spermatozoa per ×200 field | Dilution required | Semen (l) | Fixative (l) | Chamber |
| >101 | >404 | 1:20 (1+19) | 50 | 950 | Improved Neubauer |
| 16-100 | 64-100 | 1:5 (1+4) | 50 | 200 | Improved Neubauer |
| 2-15 | 8-60 | 1:2 (1+1) | 50 | 50 | Improved Neubauer |
| <2 | <8 | 1:2 (1+1) | 50 | 50 | Improved Neubauer |

4.2 Sperm Count (SCONT):

4.2.1 Thoroughly mix liquefied semen sample (if sample does not liquify, see procedural notes below).

4.2.2 With extreme attention to accuracy, typically prepare two 1:20 dilutions using cold H20 (different dilutions may be required depending

4.2.3 Mix thoroughly.

4.2.4 Charge hemocytometer chambers. Use one dilution per side of hemacytometer.

4.2.5 Using one side of hemacytometer, count sperm in 2 sq. mm. (2 of 9 large squares). Note result

4.2.6 Repeat procedure using the other side of the chamber. Note result.

4.2.7 Total the two counts and note result. In a separate calculation determine the difference between the counts. Using the total refer to table 2.2 and find the result within the ranges listed. Once the range has been found, look at the adjacent column and compare the difference determined in the second calculation to the number in the column for “acceptable difference between counts”. If the difference calculated is less than the acceptable number from the table, the counts match sufficiently. If not then the hemocytometer must be cleaned, recharged and counts repeated until the difference determined each time from the appropriate sum cell is below the “acceptable difference”. If there is any doubt that the dilutions prepared initially are proper and accurate, prepare new dilutions.

Table 2.2 Acceptable differences between two replicate counts for a given sum

|  |  |  |  |
| --- | --- | --- | --- |
| Sum of 2 counts | Acceptable difference between counts | Sum of 2 counts | Acceptable difference between counts |
| 144–156 | 24 | 329–346 | 36 |
| 157–169 | 25 | 347–366 | 37 |
| 170–182 | 26 | 367–385 | 38 |
| 183–196 | 27 | 386–406 | 39 |
| 197–211 | 28 | 407–426 | 40 |
| 212–226 | 29 | 427–448 | 41 |
| 227–242 | 30 | 449–470 | 42 |
| 243–258 | 31 | 471–492 | 43 |
| 259–274 | 32 | 493–515 | 44 |
| 275–292 | 33 | 516–538 | 45 |
| 293–309 | 34 | 539–562 | 46 |
| 310–328 | 35 | 563–587 | 47 |

\*Based on the rounded 95% confidence interval

4.2.8 Once an acceptable number is determined, divide it by two and multiply the result by 100,000. This is the result reported in millions/ml..

**5.0 Expected Values**

5.1 Sperm Count: >20 million/ml

**6.0 Procedural Notes:**

6.1 Result via manual result entry using template SEMEN

6.2 Adequate mixing of semen specimen is of great importance. The specimen must be thoroughly mixed before and after diluting.

6.3 Failure to liquify poses a problem due to the high viscosity of the semen specimen. In these instances, a mucolytic agent, such as Acytelcystene should be used (Acetylcystene is available from Pharmacy in a 20% solution. Dilute with equal volume of Di H2O. Stable 96 hours after opening). The semen is diluted 1:1 prior to dilution for sperm count. The result must then be multiplied times 2 to compensate for dilution.

6.4 Do not confuse immature forms with WBC.

6.5 Use caution when entering the result value for count. A numeric result (expressed in MILLIONS/ML) is to be entered in this field. Refer to calculation below:

Calculation: 20 X 10 X1000 = 100,000

2

20 = dilution

10 = depth to obtain answer in mm3. (0.1 mm depth is counted)

1000 = to convert mm3. to ml.

2 = 2sq. mm. counted

6.6 **NOTE:** If a dilution other than 1:20 is utilized, substitute the dilution factor in the first position of the numerator of the calculation above. The result will change the number the count result is multiplied by:

1:5 dilution

Calculation: 5 X 10 X1000 = 25,000

2

5 = dilution

10 = depth to obtain answer in mm3. (0.1 mm depth is counted)

1000 = to convert mm3. to ml.

2 = 2sq. mm. counted

In this case the count result would be multiplied by 25, 000 and results reported as the result millions/ml.

1:50 dilution

Calculation: 50 X 10 X1000 = 250,000

2

50 = dilution

10 = depth to obtain answer in mm3. (0.1 mm depth is counted)

1000 = to convert mm3. to ml.

2 = 2sq. mm. counted

In this case the count result would be multiplied by 250, 000 and results reported as the result millions/ml.

**7.0 References:**

Urinalysis and Body Fluids, 5th Ed., Strasinger and Schaub Di Lorenzo, pp 202-203.

Clinical Diagnosis and Management, 18th Ed., Henry, pp 497-500.

WHO laboratory manual for the Examination and processing of human semen, 5th Ed.

Manual on Basic Semen Analysis, NAFA based on the WHO recommendations.

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**Date: 2/24/2025**