

## The use of replicates in the WHO laboratory manual for examination and processing of human semen

The WHO manual (fifth edition 2010) recommends to make replicate measurements in the routine semen analyses as a general method for internal quality assurance.

In the following extracts from this manual will be quoted which are related to the use of replicates for motility, concentration and morphology measurements.

### Motility

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- Evaluate **at least 200 spermatozoa** in a total of at least five fields in each replicate, in order to achieve an acceptably low sampling error (see Box 2.5).
- **Calculate the average percentage and difference** between the two percentages for the most frequent motility grade (PR, NP or IM) in the replicate wet preparations.
- **Determine the acceptability of the difference from Table 2.1** or Fig. A7.2, Appendix 7. (Each shows the maximum difference between two percentages that is expected to occur in 95% of samples because of sampling error alone.)
- **If the difference between the percentages is acceptable, report the average percentage for each motility grade (PR, NP and IM). If the difference is too high, take two new aliquots from the semen sample, make two new preparations and repeat the assessment** (see Box 2.6).
- **Report the average percentage** for each motility grade to the nearest whole number.

When the difference between percentages is greater than acceptable, discard the first two values and reassess. **(Do not count a third sample and take the mean of the three values, or take the mean of the two closest values.)**

**Table 2.1** Acceptable differences between two percentages for a given average, determined from replicate counts of 200 spermatozoa (total 400 counted)

Average (%)	Acceptable Difference*	Average (%)	Acceptable Difference*
0	1	66–76	9
1	2	77–83	8
2	3	84–88	7
3–4	4	89–92	6
5–7	5	93–95	5
8–11	6	96–97	4
12–16	7	98	3
17–23	8	99	2
24–34	9	100	1
35–65	10		

\*Based on the rounded 95% confidence interval.

## Concentration

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- Determine the acceptability of the difference from Table 2.4 or Fig. A7.1, Appendix 7. (Each shows the maximum difference between the counts that is expected to occur in 95% of samples because of sampling error alone.)
- If the difference is acceptable, calculate the concentration (see Section 2.8.4). If the difference is too high, prepare two new dilutions as described in Section 2.8.2 and repeat replicate counts (see Box 2.10).

**Note 2:** Assessing the same chamber twice or assessing both chambers filled from a single dilution is not true replication, as this will not allow detection of errors of sampling, mixing and dilution.

When the difference between the counts is greater than acceptable, discard the first two values, and prepare and assess two fresh dilutions of semen. (Do not count a third sample and take the mean of the three values, or take the mean of the two closest values.)

Table 2.4 Acceptable differences between two replicate counts for a given sum

Sum	Acceptable Difference*	Sum	Acceptable Difference*
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.

## Morphology

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- Evaluate **at least 200 spermatozoa in each replicate**, in order to achieve an acceptably low sampling error (see Box 2.5).
- Tally the number of normal and abnormal spermatozoa with the aid of a laboratory counter.
- **Repeat the assessment of at least 200 spermatozoa**, preferably on the replicate slide, but alternatively on the same slide.
- **Determine the acceptability of the difference from Table 2.1 or Fig. A7.2, Appendix 7.** (Each shows the maximum difference between two percentages that is expected to occur in 95% of samples because of sampling error alone.)
- If the difference between the percentages is acceptable, report the average percentage normal morphology. If the difference is too high, **repeat** the assessment **on the same slides** (see Box 2.6).

**Table 2.1** Acceptable differences between two percentages for a given average, determined from replicate counts of 200 spermatozoa (total 400 counted)

Average (%)	Acceptable Difference*	Average (%)	Acceptable Difference*
0	1	66–76	9
1	2	77–83	8
2	3	84–88	7
3–4	4	89–92	6
5–7	5	93–95	5
8–11	6	96–97	4
12–16	7	98	3
17–23	8	99	2
24–34	9	100	1
35–65	10		

\*Based on the rounded 95% confidence interval.