

$$s = \sqrt{\frac{\sum_{i=1}^n (X_i - \bar{X})^2}{n-1}}$$

Where:

$X_i$  = a result in the method reporting units obtained from analysis of a sample aliquot,  $i=1$  to 3

$\bar{X}$  = mean of the three results, and

$n$  = number of sample aliquots (3 in this case)

4.4.3.3 Calculate a preliminary MDL as follows:

Preliminary MDL = 6.96s

Where:

6.96 = Student's  $t$ -value appropriate for a 99% confidence level and two degrees of freedom

$s$  = standard deviation of the results of analyses of the three replicates from section 4.4.3.2

4.4.3.4 If the preliminary MDL is in the range of 0.2–1.0 times the concentration in the spiked sample (section 4.3), analyze a minimum of four additional aliquots and proceed using the procedure in section 4.5. Use all seven measurements for calculation of the MDL. Otherwise, produce a new bulk

sample per section 4.3, with the analyte at the concentration of the preliminary MDL and either repeat section 4.4.3, or proceed to section 4.5 for determination of the MDL.

#### 4.5 MDL determination

4.5.1 Process at least seven aliquots of the test sample prepared in section 4.4 or section 4.4.3 through the entire analytical method.

4.5.2 Make all computations as specified in the method, with final results in the method-specified reporting units.

4.5.3 To obtain a valid MDL, all of the analytical results must be positive numbers. If any of the results are negative or zero, increase the test concentration (per section 4.3) and repeat the MDL procedure.

4.5.4 If more than seven aliquots are prepared and analyzed, the results from all the aliquots must be used to calculate the MDL, except as described in section 4.9.

4.6 Calculate the standard deviation,  $s$ , as follows:

$$s = \sqrt{\frac{\sum_{i=1}^n (X_i - \bar{X})^2}{n-1}}$$

Where:

$X_i$  = a result, in the method reporting units, obtained from analysis of a sample aliquot,  $i=1$  to  $n$

$\bar{X}$  = mean of the results, and

$n$  = number of sample aliquots

**Note to Section 4.6:** When using a program such as a spreadsheet to calculate the standard deviation ( $s$ ), make certain that the sample standard deviation, which uses  $(n-1)$  in the denominator, is calculated, rather than the population standard deviation ( $\sigma$ ), which uses  $n$  in the denominator.

#### 4.7 Calculate the MDL

The MDL is calculated as:

$$\text{MDL} = s \times t_{(n-1, 1-\alpha=0.99)}$$

Where:

$s$  = standard deviation of the results calculated in section 4.6

$t_{(n-1, 1-\alpha=0.99)}$  = Students'  $t$ -value appropriate for a 99% confidence level and  $(n-1)$  degrees of freedom, from the table below.

TABLE OF STUDENT'S  $t$ -VALUES AT THE 99% CONFIDENCE LEVEL

Number of replicates for		Degrees of freedom (df)	$t_{(n-1, 1-\alpha=0.99)}$
Singles MDL (df = $n-1$ )	Iterative MDL (df = $n-2$ )		
7	N/A	6	3.143
8	N/A	7	2.998
9	N/A	8	2.896
10	N/A	9	2.821
11	N/A	10	2.764
12	N/A	11	2.718
13	14	12	2.681
14	15	13	2.650
15	16	14	2.624
16	17	15	2.602
17	18	16	2.583
18	19	17	2.567
19	20	18	2.552

**Note to Section 4.7:** Degrees of freedom =  $(n-1)$  if a single MDL study is performed. If an iterative MDL study is performed, degrees of freedom =  $(n_h + n_l - 2)$ , as described in section 4.8; N/A indicates that the number of degrees of freedom in this row does not apply to an iterative MDL study.

4.8 Iterate and verify the reasonableness of the MDL

When developing an MDL for a new or revised method, or when developing a matrix-specific MDL, the MDL procedure must be iterated and the reasonableness of the MDL determined using an F-test, as described in sections 4.8.1 through 4.8.4. When verifying a method-, matrix-, program-, or study-specific MDL, the MDL is determined as described in section 4.8.5 and iteration may not be necessary.

#### 4.8.1 Iteration

When developing an MDL for a new or revised method, the spiking, analysis, and

calculation steps (sections 4.3 to 4.6) must be repeated using a spike at no more than five times the MDL determined initially or in the most recent iteration, to confirm the reasonableness of the MDL.

4.8.2 Once the iteration is complete (*i.e.*, two successive MDL estimates have been produced), calculate the F-ratio (F) as:

$$F = \frac{\left( \frac{s_h^2}{n_h - 1} \right)}{\left( \frac{s_l^2}{n_l - 1} \right)}$$

Where:

$s_h^2$  = variance estimate from the higher spike concentration

$s_l^2$  = variance estimate from the lower spike concentration

$n_h$  = number of observations at the higher concentration

$n_l$  = number of observations at the lower concentration

4.8.3 For seven replicates at each concentration, the 90th percentile of the distribution of the F-statistic is 3.055.

4.8.3.1 If seven replicates were analyzed at each spike concentration and  $F > 3.055$ , the two variances are different and the MDL determined at the higher spike concentration is not a reasonable estimate. In this case, return to section 4.3 and produce another sample at a test concentration below the higher of the two previous iterations, analyze a minimum of seven aliquots, calculate the MDL, and repeat the F-test in section 4.8.2.

4.8.3.2 If  $F \leq 3.055$  for seven replicates at each concentration, the two variances are not different. Proceed to section 4.8.4.

**Note to Section 4.8.3.2:** If more than seven replicates are used, the appropriate F-statistic is determined from the table below.