

Lysate Calculation Worksheet (M-AMS)

First, determine the number of wells to be tested:

Two wells will be needed for the Negative Control.

Two wells will be needed for the lysate control reagent.

Two wells will be needed for each patient sample to be tested (pre, post, current, etc.)

1. Calculation for testing determining total wells to be tested for either Class I or Class II:

$$\frac{4}{\text{(Control Wells)}} + \frac{\quad}{\text{(Patient Wells)}} + \frac{2}{\text{(Pipetting Loss)}} = \frac{\quad}{\text{(Total Wells)}} \times \frac{15 \mu\text{L}}{\text{(Vol/Well)}} = \frac{\quad \mu\text{L (a)}}{\text{(Vol Diluted Lysate Needed)}}$$

Next, determine the quantity of undiluted lysate needed.

2. Calculation for determining the amount of undiluted lysate needed for Class I strips:

Divide the total volume of diluted lysate by 8 to determine the amount of undiluted lysate required for the Class I strips.

$$\frac{\quad \mu\text{L (a)}}{\text{(Vol Diluted Lysate Needed)}} \div \frac{8}{\text{(Class I Dilution)}} = \frac{\quad \mu\text{L (b)}}{\text{(Vol Undiluted Lysate Needed for Class I Strips)}}$$

3. Calculation for determining the amount of undiluted lysate needed for Class II strips:

Divide the total volume of diluted lysate by 4 to determine the amount of undiluted lysate required for Class II strips.

$$\frac{\quad \mu\text{L (a)}}{\text{(Vol Diluted Lysate Needed)}} \div \frac{4}{\text{(Class II Dilution)}} = \frac{\quad \mu\text{L (c)}}{\text{(Vol Undiluted Lysate Needed for Class II Strips)}}$$

4. Calculation for determining the total amount of lysate for both Class I and Class II strips:

Add the volume of undiluted lysate required for Class I and Class II strips to determine the total volume of undiluted lysate required.

$$\frac{\quad \mu\text{L (b)}}{\text{(Class I)}} + \frac{\quad \mu\text{L (c)}}{\text{(Class II)}} = \frac{\quad \mu\text{L (d)}}{\text{(Total Vol Undiluted Lysate Required)}}$$

5. From the total volume of undiluted lysate required, determine the volume of packed cells to prepare:

$$\frac{\quad \mu\text{L (d)}}{\text{(Vol Undiluted Lysate Needed)}} \div \frac{10}{\text{(Lysis Dilution)}} = \frac{\quad \mu\text{L (e)}}{\text{(}\mu\text{L Packed Lymphocytes)}}$$

Note: 30 μL of packed cells or approximately 90 x 10⁶ cells will supply enough lysate to test 20 patient samples in duplicate for both Class I and Class II reactivity.

10 μL of packed cells or approximately 30 x 10⁶ cells will supply enough lysate to test 20 patient samples in duplicate against Class I strips only.

20 μL of packed cells or approximately 60 x 10⁶ cells will supply enough lysate to test 20 patient samples in duplicate against Class II strips only.

Record of dilutions:

Volume of packed cells used **(e)** _____

Volume of diluted Lymphocyte Lysis Buffer added _____ (100 μL / 10 μL packed cell volume)

Class I Lysate Dilution Record:

Total Volume Diluted Class I Lysate **(a)** _____

Volume undiluted lysate used **(b)** _____

Volume LCD used **(a-b)** _____

Class II Lysate Dilution Record:

Total Volume Diluted Class II Lysate **(a)** _____

Volume undiluted lysate used **(c)** _____

Volume LCD used **(a-c)** _____