Henderson-Hasselbalch Calculations
for 100 ml of 500 mM Tris-Cl, pH 9

- Mathematical Proof -

$HA \Leftrightarrow H^+ + A^-$	HA = Undissociated Acid	A^- = Conjugate Base of HA	H^+ = Hydrogen Ion
$K_a = [H^+][A^-]/[HA]$	*Therefore:* $-\log[H^+] = -\log[K_a] + \log[A^-] - \log[HA]$		
$pK_a = -\log[K_a]$	$pH = -\log[H^+]$	$\log[A^-] - \log[HA] = \log[A^-/HA]$	
Henderson-Hasselbalch Equation: $\mathbf{pH = pK_a + \log[A^-/HA]}$			
Tris-base : MW = 121.14 ; pK_a = 8.12 at 25°C			

Since the desired pH is 9, then $9 = 8.12 + \log[A^-/HA]$

Therefore, $\log[A-/HA] = 0.880 \therefore [A^-/HA] = 10^{(0.880)} = 7.586$

The concentration of conjugate base $[A^-]$ is equal to the concentration of base (in this case 500 mM) minus the concentration of H^+ (which is what we are solving for). Similarly, the concentration of the undissociated acid is equal to the concentration of H^+. Thus, [A-/HA] = ([Base] - [Acid]) / [Acid] = 7.586

Multiply both sides of the equation by [Acid] to get : [Base] - [Acid] = (7.586) * [Acid]

Add [Acid] to both sides of the equation to get : [Base] = ((7.586) * [Acid])+ [Acid]

Divide both sides of the equation by [Acid] to get : [Base]/[Acid] = (7.586) + 1

Invert the equation and get : [Acid] = [Base] / (7.586 + 1) ∴ [Acid] = [500 mM] / (7.586 + 1)

[Acid] = 58.24 mM

If 12.1 M Hydrochloric Acid is used then [12.1] / [58.24] = 0.208 (dilution factor)

Divide the desired volume (100 ml) by the dilution factor to get the required volume of HCl to add:

100 ml / 0.208 = **481 uL of 12.1 M HCl needed**

To determine the amount of Tris-base needed, multiply the desired volume (100 ml), by the desired concentration (500 mM), by the molecular weight (121.14) :

(100 ml) x (0.5 M) x (121.14) = **6.057 g**