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To whom it may concern,

Emicizumab Application Details

The emicizumab assay is run using the same methodology as a standard one-stage clot-based factor assay:

1. One volume of patient sample is diluted **1:80** (50 μ L) with the system assay buffer,
2. One volume of factor VIII deficient plasma (50 μ L) is added to the cuvette, and
3. One volume of APTT (50 μ L) is added to the same cuvette.
4. The mixture is then incubated for 3-4 minutes at 37C.
5. The reaction is initiated with one volume of 20mM Calcium Chloride (50 μ L).
6. Clot time is then converted to μ g/mL from a corresponding calibration curve.

An example calibration includes points at 10, 25, 50, 75, and 100 μ g/mL emicizumab. The log of clot time is plotted against the log of concentration and a linear regression is performed across all calibration points. Sample clot times can then be converted to μ g/mL emicizumab from clot time using the regression from the calibration curve.