Frequently Asked Questions:

• I don't have heparinized tubes. Can I use another tube?

Heparinized (green top) tubes are STRONGLY recommended for sample collection. If heparinized tubes are not available, sodium heparin can be added to <u>sterile</u>, <u>no</u> <u>additive</u> tubes. 'Red top' tubes commonly have a silicone coating, and sometimes have a silica clot activator coating. There are also 'no additive' red top tubes. 'No additive' red tops are ideal, but silicone coating can be used if 'no additive' tubes are not available. Silica clot activator red top tubes are not acceptable for sample submission (these tubes may appear to have a hazy/cloudy surface).

For a 3-5 mL blood sample, we recommend adding 140 units of sodium heparin. For a 1000 U/mL sodium heparin, this would be 0.14 mL sodium heparin added to the tube prior to blood addition. Tubes should be mixed by inversion immediately.

Note that our assay has not been validated for this approach, and this should only be done in an emergency when green top tubes cannot be obtained.

• My patient is a cat. Can you perform your assay on samples from cats?

Unfortunately, currently, the answer is no. We have not yet developed or validated the assay in cat

• Why did the laboratory report that my sample had 'insufficient RNA for analysis'?

Uncommonly, we are unable to extract insufficient RNA from samples for qRT-PCR analysis. Since we extract RNA from activated T-cells, the most likely reason for an 'insufficient RNA for analysis' is that the patient has very low lymphocyte numbers. While we don't encounter this issue very often, when it occurs it is most likely to occur in patients with marked lymphopenia, as can be seen with severe illness or exposure to glucocortioids. Insufficient RNA results could also potentially occur with suboptimal sample transportation and handling conditions such as a greater than two days transport time, or samples that become too warm. 'Insufficient RNA' could also result from laboratory error, but we have controls and measures to minimize this potential pitfall. Unfortunately, if we cannot extract sufficient RNA, we cannot perform our qRT-PCR analysis.