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Issue	Possible Cause	Solution
Outliers/poor duplicates	Issues with **washing steps**. It is vital that all the **washing** steps in the ELISA protocol is performed thoroughly. Any unbound material left in the wells increases level of background noise and can cause poor duplicates and/or false absorbance values.	Aspirate each well and wash with wash buffer, repeating the process for a minimum of 3 washes. Wash by forcefully filling each well with wash buffer (300 µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash buffer by aspirating and by inverting the plate and blotting it against clean paper toweling. If using an automatic plate washer, check that all the ports are clean and free of obstructions. Adding a 60 second soak step at each wash can improve the results.
Outliers/poor duplicates	Excessive t**ime** was taken to add samples controls or reagents to the assay plate	Be sure to have all materials set up and ready to use quickly.
Outliers/poor duplicates	Dilution error /incorrect pipetting	Careful and precise **pipetting** at every step in the assay. Reverse pipetting is recommended to increase reliability. Verify pipette calibration and check that tips are on tight. If using a multichannel pipette; Be sure all channels of the pipette draw and dispense equal volumes. Addition of sample and standard dilutions to the plate in **triplicates**, instead of duplicates, can increase reliability.

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Issue	Possible Cause	Solution
Low absorbance values	**Incubation time** were too short.	Follow protocol for incubation times. Time each plate separately to ensure accurate incubation periods
Low absorbance values	Substrate solution were too cold.	The TMB solution should be brought to **room temperature** before the development reaction.
Low absorbance values	Incorrect/expired **wash solution**	Make sure that the correct wash solution was used, and that the wash solution has not expired. Make sure that the components used has not expired.
Low absorbance values	Absorbance read at incorrect **wavelength**	Make sure that the absorbance was read at the correct wavelength and that the plate reader is operating correct.
No colour development		Read the complete assay procedure again and repeat the assay
Low absorbance values	Problem with water quality	Check water cleaning systems

Issue	Possible Cause	Solution
Low absorbance of Vtg standard curve	Lyophilized Vtg escaped during **vacuum release** or not completely dissolved	The vacuum in the vial with lyophilized Vtg standard must be released carefully. Ensure that all material in the vial is completely dissolved. NB! Reconstituted Vtg can not be frozen and re-used quantitatively.

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Issue	Possible Cause	Solution
High background (NSB) values	Issues with **washing** steps	Make sure that all the **washing** steps were performed thoroughly and verify the performance of the washer system. Make sure that the correct **wash solution** was used, and that the wash solution has not expired
High background (NSB) values	**Contamination** of Substrate	The substrate solution should be colourless and clear prior to adding. Make sure the substate is not contaminated or expired.
High background (NSB) values	Issue with reader	Make sure that the absorbance was read at the correct wavelength and that the **plate reader** is operating correct