Insufficient Color Development

Possible causes	Recommended actions
Laboratory temperature was too low.	Maintain the room temperature within 18–25°C. Avoid running assays under air conditioning vents or near cold windows.
Wash solution was prepared incorrectly or the wrong wash solution was used.	Be sure to use the wash solution recommended for the kit and that it is prepared correctly. Label each unique wash solution to avoid using the wrong one.
Washer system had microbial contamination or contained an alternate wash formulation.	Clean out microbial contamination by flushing the system with a dilute solution of bleach (10% by volume) followed by a large amount of distilled or deionized water, then prime the system with the appropriate wash solution. Be sure each unique wash solution is properly labeled. Prime the system thoroughly when switching between solutions.
Too many wash cycles were used.	Stay within the recommended range for the number of wash cycles. Try to use the lowest number of washes recommended for the assay.
Incubation periods were too short.	Follow protocol for incubation times. Time each plate separately to ensure accurate incubation periods.
Reagents and plates were too cold.	Make sure plates and reagents are at room temperature by taking them out of the refrigerator, and the test components out of the box, at least 2–3 hours before starting the assay.
Reagents were expired or intermixed from a different lot number.	Verify the expiration dates and lot numbers on the reagents.
Wrong conjugate was used, conjugate was prepared incorrectly or has deteriorated.	Be sure that the conjugate used is the one that came with the test. All conjugates are test and lot-specific. If preparation of a working conjugate is needed, be sure that the concentrate and diluent are mixed in appropriate volumes. Do not prepare the working solution too far in advance, and do not save any unused portion for future use. If no conjugate preparation is necessary, be sure to pour out only the amount required for immediate use, and do not return any unused portion to the stock bottle.
Assay plate was read at wrong wavelength, or reader was malfunctioning.	Verify the correct wavelength for the assay and read the plate again. Verify reader calibration and lamp alignment.
Positive control was diluted (indirect format only).	Do not dilute controls unless specified in the package insert.
Excessive test stress occurred.	Check records to see how many times the test has cycled from the refrigerator. Check to see if the test was left out on a loading dock or other area for too long or at extreme temperatures.
Assay plates were compromised or previously used.	Be sure to refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them to equilibrate to room temperature while in the packaging. If partial plates are used, be sure to label used wells to prevent reuse; cover them with sealing tape and use the remaining wells as soon as possible. Do not store partially used plates with other plates. Include a desiccant in the storage bag.

IDEXX Laboratories, Inc.
One IDEXX Drive, Westbrook, Maine 04092 USA, idexx.com
1-207-556-0300 1-800-548-6733 f. 1-207-556-4346