Objective: The aim of this in vitro study was to evaluate the effects of clear and opaque fissure sealants on readings of laser fluorescence (LF) and light-emitting diode (LED) based caries detection devices. Background data: When planning patient care, the practitioner needs to consider any changes in the status of the sealed surface for the long-term success of the sealant. As visual inspection is difficult to perform on sealed surfaces, adjunct diagnostic methods must be used to improve follow-up assessments and increase the accuracy of caries diagnosis. Methods: Forty-six freshly extracted permanent human molars were selected and divided into two groups. Each group was treated with a different sealant (clear and opaque). The teeth were measured twice by two blinded observers using an LF-based and an LED-based device before and after sealing. The data were analyzed using Wilcoxon’s matched-pairs signed-rank test and a paired t-test. Cohen’s j and the intraclass correlation coefficient were used to examine intra- and inter-examiner repeatability. Results: The values of the LED device were significantly higher after the application of the opaque sealant, but there was no statistically significant difference after the application of the clear sealant (p = 0.15). The LF-based device readings were also significantly lower after both the clear and the opaque sealant applications (p < 0.001). Conclusions: The readings from the LF-based device were affected by both sealants. The readings from the LED-based device were affected by the opaque sealant but not by the clear sealant.