IN THE LOOP

False Positives and Negatives in PCR: How to Prevent Them



Polymerase chain reaction (PCR) technology is characterized by high specificity and sensitivity hence, considered as the gold standard for confirmatory diagnostic testing of many diseases. However, being highly specific means it may not detect other variations of the target sequence causing false negatives. Also, contaminating nucleic acids may still be amplified generating false positives.

FALSE POSITIVES

CAUSES:

- Introduction of random contaminant to the samples
- Reagents are not specific such that it can bind to non-specific targets
- Operator errors during PCR workflow

PREVENTION:

- Clean work area properly using 70% ethanol or 10% bleach.
- **Disinfect through UV irradiation.** This method keeps the works surface clean by inactivating nucleic acid contaminants.
- Use appropriate reagents for the accurate detection of the disease.
- Ensure that operators are well-trained or supervised before performing PCR.

FALSE NEGATIVES

CAUSES:

- Presence of inhibitors
- Suboptimal reagents (e.g., primers, probes)
- Poor sample and/or reagent storage
- Improper sample collection and/or wrong timing
- Uncalibrated pipettes and equipment
- Operator errors during the workflow

PREVENTION:

- Use improved polymerase to reduce the effect of inhibitors.
- Perform in an aseptic and suitable environment to prevent the addition of inhibitors.
- Use appropriate reagents for the accurate detection of the disease.
- Ensure that the pipettes and equipment are calibrated and maintained.
- Ensure that operators are well-trained or supervised before performing PCR.



Actual Status With Disease No Disease



Figure 1. Illustration of false positives and negatives. Actual disease states may be confirmed through sequencing or viral/ bacterial culture.



ENSURE RELIABLE RESULTS WITH ESCO PCR CABINETS!

To attain reliable results, possible inhibitors and contaminants affecting the PCR process must be removed. Esco PCR cabinets provide HEPA-filtered airflow to prevent the introduction of inhibitors and contaminants to the samples and reagents. These cabinets are also equipped with UV lighting for decontamination of random nucleic acids.

Reference: [1] Clinical Lab Manager. May 2020. Preventing False Positives and False Negatives in PCR.https://www.clinicallabmanager.com/ trends/infectious-diseases/preventing-false-positives-and-false-negatives-in-pcr-22271



