

REAL-TIME PCR TROUBLESHOOTING

QUICK REFERENCE | Symptoms, first checks, and prevention

SYMPTOM	POSSIBLE CAUSES	CHECK FIRST
No amplification - positive control fails	Reagent/setup issue; cycling program; wrong channel	Check mix, expiry, cycling, detection settings
No amplification - samples only	Absent target; inhibition; extraction or sample issue	Review internal control and extraction QC
Late or drifting Cq/Ct	Low input; inhibition; reagent or instrument drift	Check controls, input, lots; evaluate dilution series
Amplification in NTC	Carryover/contamination; primer-dimer in dye assays	Fresh aliquots; separation; melt curve if applicable
Replicates disagree	Pipetting, bubbles, evaporation; stochastic low copy	Inspect sealing and mixing; review input level
Efficiency or standard curve off	Dilution errors, inhibition, assay conditions	Remake standards; inspect slope and residuals
Abnormal curves	Baseline/threshold error; optical or plate artifacts	Inspect raw fluorescence; settings; bubbles
Internal control fails	Inhibition; extraction/setup failure; multiplex competition	Check control addition and assay instructions

PREVENTION CHECKLIST

- ☐ Separate pre- and post-PCR areas; use dedicated supplies
- ☐ Include controls based on assay requirements
- ☐ Check extraction performance and sample quality
- ☐ Use filter tips and aliquot reagents appropriately
- ☐ Track control Cq/Ct values, lots, and instrument status
- ☐ Inspect seals, pipettes, calibration, and analysis settings

Need workflow or panel support? jantdx.com/contact/

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Research-use educational reference. Follow validated assay instructions and your laboratory QC procedures. RUO products are not for diagnostic use.