

GeneXpert® Quality Controls

for All Cepheid Assays

The GeneXpert system is a closed platform using a single use, fully integrated, unitized test cartridge. Within each cartridge (and therefore for every patient sample tested) are controls that check the system, the test reagents, the patient sample, lysis, amplification, and the integrity of the cartridge itself.

The internal quality controls included in each Cepheid cartridge check every step of the assay for every patient specimen, not just once a day. Daily use of external controls provides no additional information about a specific patient run than the individual internal quality controls within each Cepheid cartridge. This applies to each individual module of the GeneXpert system.

The internal quality controls used for each patient sample are (see table format below):

- 1. Sample Processing Control (SPC):** This control co-extracts and co-amplifies with the patient sample. It controls for the sample processing steps including lysis and extraction, and the integrity of the nucleic acid and reagent bead rehydration. For genotyping assays, the sample functions as its own internal control since both normal and mutant gene sequences are detected, and each individual tested is expected to have one or the other of these sequence variants.
 - If the SPC fails in a negative test, an **INVALID** test result will be reported.
 - If no signal appears in a genotyping test, an **INVALID** test result will be reported.
- 2. Amplification Control (AC):** This control detects enzyme integrity and possible PCR inhibition. For genotyping assays, the sample functions as its own internal control since both normal and mutant gene sequences are detected, and each individual tested is expected to have one or the other of these sequence variants. For assays requiring a minimum amount of cells within the specimen, known genetic targets in human cells are co-extracted and co-amplified ensuring adequate sample, collection and preparation.
 - If the amplification control fails in a negative test, an **INVALID** test result will be reported.
 - If no signal appears in a genotyping test, an **INVALID** test result will be reported.
- 3. Reagent Controls (Probe Check):** This control checks the integrity of the probes and proper reconstitution of reagents within each cartridge.
 - Depending on the test, there are 2 to 6 probes with specific fluorescent criteria measured. If the probe check control fails, an **ERROR** test result will be reported.

4. Instrument System Control (Check Status): This control checks the optics, temperature of the module and mechanical integrity of each cartridge.

- Thermal and mechanical checks are run. If the system controls fail, an **ERROR** test result will be reported.

5. Reliable results:

- Multiplex genetic and infectious disease assays employ relational algorithms for each analyte amplified. **ERROR** or **INVALID** is reported if the criteria within the algorithms are not met.

When one of the controls described above does not meet specification, an error code, no result or invalid test result is produced specific to the patient sample and the test run. The result of an external control run in a separate cartridge does not predict the outcome of the test sample.

External controls can provide useful information regarding shipment, lot, storage conditions, operator competency and peer comparison. For this reason, after verification studies of the performance of the test in each laboratory, external controls are still recommended for lot changes, new shipments, after a major service event, or a minimum of every 30 days. Cepheid recognizes that these are our recommendations based on sound scientific evidence, validation studies and clinical trials.

The ultimate decision and responsibility for the type and frequency of controls remains with the laboratory director. Laboratories should follow their applicable federal and local regulations.

Table of Controls by Process within each Xpert Cartridge*

Procedural step or condition controlled		SPC	AC	Probe Check	System	Final Results
Pre Test and during test Self-checks	Sonication Control				X	Failure results in an ERROR
	Heating, cooling and optics for PCR				X	Failure results in an ERROR
Sample Processing Control	Bead rehydration	X	X	X		Failure results in an ERROR or INVALID
	Cell Lysis	X				Failure results in an INVALID
	DNA extraction	X				Failure results in an INVALID
	Presence of Inhibitors	X	X			Failure results in an INVALID
	Adequate Specimen		X			Failure results in an INVALID
Probe Check Test	PCR Tube Fill			X		Failure results in an ERROR
	Fluorogenic detection			X		Failure results in an ERROR
	Bead rehydration			X		Failure results in an ERROR
PCR	Thermal cycling				X	Failure results in an ERROR
	Primer or target annealing	X	X			Failure results in an INVALID
Presence of PCR inhibitors	Amplification	X	X			Failure results in an INVALID

* specific control utilized is dependant upon target analyzed.