Xpert®
Assays

GeneXpert® Quality Control Features
for All Cepheid Xpert® Assays

Cepheid's GeneXpert System is a closed *in vitro* diagnostic assay platform that employs single-use, self-contained Xpert cartridges. Each Xpert cartridge contains all necessary reagents for the detection of target nucleic acids using the polymerase chain reaction (PCR). As each individual assay is performed, external system and internal cartridge quality control factors check the instrument system, cartridge reagents, on-board sample processing, and presence of favorable reaction conditions for PCR performance. Certain Xpert cartridges include a sample adequacy control to verify that the sample contains sufficient patient cells to generate reliable results. These internal quality control features verify multiple aspects of assay performance for every sample tested.

1. **Instrument System Control (Check Status):** Once an Xpert cartridge has been loaded into a GeneXpert module and before the sample processing steps begin, the software checks the optics, the readiness of the module's mechanical components, the ambient temperature of the module to assure proper performance of PCR, and the physical integrity of the cartridge.
   - If Check Status fails for any reason, the assay is terminated and an **ERROR** is reported
2. **Reagent Control (Probe Check):** Probe Check is performed on every cartridge after on-board sample preparation, reagent mixing, probe integrity, and reaction tube filling, but before initiation of PCR. During Probe Check, fluorescence readings are measured in the reaction tube for each probe and compared to default settings established by Cepheid.
   - If the readings match the default settings, the Probe Check control passes
   - If the readings do not match the default settings, the assay is terminated and an **ERROR** is reported
3. **Sample Processing Control (SPC):** The SPC is an exogenous (non-sample, non-analyte) nucleic acid pre-loaded in the cartridge that co-extracts and co-amplifies along with the sample nucleic acids. For DNA assays, the SPC is an encapsulated DNA. For RNA assays, the SPC is an encapsulated RNA. The SPC verifies the (i) effectiveness of on-board sample processing, (ii) integrity of extracted nucleic acids, (iii) favorable reaction conditions for PCR performance, and (iv) absence of excess PCR inhibitors.
   - The SPC can be positive or negative in an analyte-positive test
   - If the SPC is negative in an analyte-negative test, an **INVALID** result is reported

   Genotyping assays do not include an SPC because the presence of at least one target allele indicates that sample processing and PCR reaction conditions were favorable.
   - If no target allele is detected in a genotyping assay, an **INVALID** result is reported

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4. **Sample Adequacy Control (SAC):** The SAC is employed in assays that require that the sample contains sufficient patient cells for reliable assay performance. The SAC reagents amplify and detect an endogenous single-copy human gene in the patient sample. The SAC co-extracts and co-amplifies with the other nucleic acids in the sample. It controls for the (i) sufficiency of sample collection, (ii) effectiveness of on-board sample processing, (iii) integrity of the extracted nucleic acid, (iv) favorable reaction conditions for PCR performance, and (v) absence of excess PCR inhibitors.
   - The SAC can be positive or negative in an analyte-positive test
   - If the SAC is negative in an analyte-negative test, an **INVALID** result is reported
5. **Other Control Features (all assays):** Cepheid's Xpert assay software employs relational algorithms for each amplified analyte. These can include minimum and maximum PCR cycle cutoffs, baseline correction, and additional processes to reduce the effects of unusual curves or target relationships in multiple-target assays.
   - If the criteria of these algorithms are not met, an **ERROR** or **INVALID** result is reported

Summary. Each Xpert cartridge produces a valid (positive or negative) test result, an **ERROR** result, or an **INVALID** result, depending on performance characteristics of the above internal controls that are run on every cartridge. Additionally, external controls can be run to assess performance of assays from new shipments, new lots, in different inventory conditions, to monitor operator competence, for peer comparisons, after major service events, in the event of a suspected or actual contamination event, and/or periodically (e.g., at least monthly).

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

Internal Quality Control Features of Cepheid Xpert Assays

Assay Step	Aspect Verified	Check Status	Probe Check	SPC	SAC	Test Result (If control fails)
Instrument Check Status	Optics, mechanics, temperature, cartridge integrity	X				ERROR
	Sonication control*	X				ERROR
Sample Processing and Preparation of PCR Reaction Mixture	Sufficient amount of human cells in sample*				X	INVALID**
	Nucleic acid extraction			X	X	INVALID**
	Appropriate number of reagent beads		X			ERROR
	Probe integrity		X			ERROR
	Complete filling of PCR reaction tube		X			ERROR
	Favorable PCR reaction conditions	X		X	X	ERROR
	Absence of excess PCR inhibitors			X	X	INVALID**

** Applies only to certain assays. Please refer to package inserts for details of specific assays.*
*** Applies only to analyte-negative results. For analyte-positive results, SPC and SAC can be positive or negative.*

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