Xpert® Assays

GeneXpert® Quality Control Features
for All Cepheid Xpert® Assays***

Cepheid's GeneXpert System is a closed 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 test is performed, the Instrument Status, Sample Processing, Integrity of PCR Reagents, and PCR Efficiency are evaluated. 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 onboard 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)/Cepheid Internal Control (CIC):** The SPC and CIC are exogenous (non-sample, non-analyte) nucleic acid pre-loaded in the cartridge that co-extracts and co-amplifies along with the sample nucleic acids. The SPC and/or CIC 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.
   - If the SPC or CIC Ct is not within the valid range and the endpoint is below the minimum setting 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
4. **Internal Quantitative Standard High and Low (IQS-H and IQS-L):** IQS-H and IQS-L are two Armored RNA® constructs in the form of a dry bead that goes through the whole assay process. They are used for quantification by using lot specific parameters for the calculation of

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assay specific RNA concentration in the sample. Additionally IQS-H and IQS-L detect specimen associated inhibition of the RT-PCR reaction.

5. **Sample Volume Adequacy (SVA):** Ensures the sample was correctly added to the cartridge. The SVA verifies that the correct in-volume of sample has been added in the sample chamber. The SVA verifies the sample addition.
   - If there is no sample or not enough sample added, an ERROR will be displayed.

6. **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.
   - If the SAC is not within the valid range and the endpoint is below the minimum setting in an analyte-negative test, an **INVALID** result is reported

7. **Other Control Features (all assays):** Cepheid's Xpert assay software employs proprietary 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.

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

Test Step	Aspect Verified	Check Status	Probe Check	SPC*/ CIC*	SAC*	SVA*	IQS*	Test Result (If control fails)
Instrument Status	Optics, mechanics, temperature, cartridge integrity	X						ERROR
	Sonication control*	X						ERROR
Sample Processing, Integrity of PCR Reagents, PCR Efficiency	Sufficient amount of human cells in sample*				X			INVALID**
	Sample Addition*			X	X	X	X	INVALID**
	Nucleic acid extraction			X	X		X	INVALID**
	Appropriate number of reagent beads		X				X	ERROR
	Probe integrity		X					ERROR
	Complete filling of PCR reaction tube		X				X	ERROR
	Favorable PCR reaction conditions	X		X	X		X	ERROR
	Absence of excess PCR inhibitors			X	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, the SPC and SAC analyte result will be NA.*
**** "All Cepheid Xpert® Assays" includes, but is not limited to CE-IVD, US-IVD, IUO, and RUO.*

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