

## Method development and validation

Category	Feasibility	Method Development	Method Qualification	Method Validation
Validation or sample analysis study plan		Optional	Required	Required
Assay design	3 designs per target	3 designs per target	3 designs per target	3 designs per target
Assay efficiency and amplicon confirmation	✓	✓	✓	✓
Establish calibration curve parameters	✓	✓	✓	✓
Linear range of assay		✓	✓	✓
Accuracy and precision in surrogate matrix buffer		2 runs, 5 levels, 3 sets		
Extraction efficiency		3 kits, 1 level of spike, QC of nucleic acids Quality and integrity, 1 repeat	1 kit, 3 levels of spike, QC of nucleic acids Quality and integrity, 2 repeats	1 kit, 3 levels of spike, QC of nucleic acids Quality and integrity, 2 repeats
Minimum Required Dilution MRD		✓	✓	✓
Accuracy and precision in authentic matrix		2 runs, 5 levels, 3 sets	3 runs, 5 levels, 3 repeats	6 runs, 5 levels, 3 repeats
Selectivity and specificity in authentic matrix		Optional	✓	✓
Dilution linearity		Optional	✓	✓
Stability		Optional	Optional	✓
Data QC		20%	50% or 100%	100%
QA involvement			Optional	✓
Report		✓	✓	✓
Acceptance criteria		Depending on Context of Use	Depending on Context of Use	Depending on Context of Use
QC levels		LLOQ, LQC, MQC, HQC, ULOQ	LLOQ, LQC, MQC, HQC, ULOQ	LLOQ, LQC, MQC, HQC, ULOQ

