

Review

Sample Adequacy Control for Negative Molecular Results: An Integrated Specimen-Process Framework to Strengthen Result Interpretation

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Abstract

Sample adequacy control (SAC) is a validated specimen-specific marker or metric, threshold, and reporting rule used to decide whether a negative molecular result is supported by the submitted specimen. SAC is not simply a cell-count or biomass check. Depending on specimen type and target, an unsupported negative result may reflect insufficient or nonrepresentative material, adequacy-marker degradation, inefficient transfer or extraction, matrix-associated inhibition, or mismatch between marker and anatomical compartment. SAC is therefore best understood as an integrated specimen-process adequacy control for negative-result interpretation. Its result should be reported as valid, borderline, or invalid for the intended negative interpretation, not as detected/not detected like the disease-specific biomarker. This perspective synthesizes quantitative polymerase chain reaction (qPCR) control guidance, preanalytical-quality literature, respiratory and enteric sampling studies, human papillomavirus (HPV) evidence, cartridge-assay precedents, and decentralized workflows. Direct prospective evidence that SAC improves patient or epidemiologic outcomes remains limited; available evidence is outcome-adjacent or operational, including HPV cellularity-control associations, Chlamydia trachomatis/Neisseria gonorrhoeae (CT/NG) and Ebola cartridge precedents, respiratory workflows showing lower pathogen positivity at higher SAC quantification cycle (Cq) strata, and reporting rules that change validity or recollection decisions. We propose a risk-based framework for selecting, validating, reporting, and monitoring SAC, with particular value in direct-amplification, point-of-care, and self-collection workflows where preanalytical failures affect negative-result interpretation.

Keywords: sample adequacy control; specimen adequacy; specimen integrity; integrated specimen-process adequacy control; disease-specific biomarker negative result; molecular diagnostics; qPCR; preanalytical quality; process monitoring; point-of-care testing; self-collection; direct polymerase chain reaction; direct amplification; laboratory-developed test (LDT); human papillomavirus (HPV) screening; nucleic-acid lateral-flow; ambient-temperature hybridization; fixed-stringency diagnostics



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1. Introduction

Nucleic-acid amplification tests (NAATs), including PCR, reverse-transcription PCR (RT-PCR), and quantitative PCR (qPCR), are central to clinical microbiology, infectious-

disease screening, antimicrobial-resistance surveillance, and public-health response. Their design and reporting are supported by qPCR and molecular-control guidance [1–5], and in clinical or public-health use they can guide isolation, cohorting, hospital placement, treatment, outbreak control, and occupational or school clearance decisions [6–10]. In practice, a negative NAAT is not a direct statement that the disease-specific biomarker is absent from the patient; it is a conditional statement that the biomarker was not detected in the material that was collected, transported, processed, and amplified.

Quality control in molecular diagnostics must therefore span more than the amplification reaction. Positive and no-template controls address assay response and contamination; internal amplification and process controls address inhibition, reagent performance, extraction, or recovery; and external quality and risk-management systems monitor laboratory performance [1–5,11–14]. These safeguards are necessary but can still pass when the submitted patient material is nonrepresentative, nearly acellular, degraded, diluted, poorly transferred, poorly released in direct amplification, inhibited, or taken from the wrong anatomical compartment for the intended negative interpretation.

Specimen-adequacy logic also has practical precedent. The Xpert Chlamydia trachomatis/Neisseria gonorrhoeae (CT/NG) assay incorporated an explicit sample adequacy control that detects a single-copy human gene to verify human-cell or human-DNA input in relevant specimens [15]. During COVID-19, the Centers for Disease Control and Prevention (CDC) 2019-nCoV Real-Time RT-PCR Diagnostic Panel made a related respiratory implementation highly visible by including a human RNase P target in clinical specimens and requiring RNase P amplification for a valid negative interpretation [6]. These examples do not establish a universal marker, but they show the central principle: a negative molecular result sometimes requires a specimen-specific signal linked to an assay-specific threshold and reporting rule.

SAC addresses this specimen-process blind spot. In this manuscript, SAC means a validated specimen-specific adequacy/integrity marker or metric, a threshold or classification rule, and a reporting consequence. The purpose is not to prove that the patient is uninfected or that the disease-specific biomarker is truly absent. The purpose is to decide whether the specimen and process provide enough support for a negative result for the disease-specific biomarker being assayed. The question is therefore not only whether relevant biological material was present, but whether the submitted specimen and workflow had the capacity to support the intended negative interpretation, including adequacy-marker quality, transfer or release, extraction or direct-amplification compatibility, and inhibition.

This perspective makes a measured claim. Direct outcome studies showing that SAC reduces confirmed false negatives or improves patient outcomes remain limited. Nevertheless, specimen-adequacy evidence can make negative reports more transparent, trigger recollection or qualification in selected workflows, and provide a quantitative process-monitoring signal. This is especially relevant when extraction-free, decentralized, or home workflows leave few downstream checkpoints after collection. The goal is to define SAC precisely, prevent overgeneralization of any one marker, and provide a practical framework that laboratories, manufacturers, and researchers can test.

2. Methodological Approach and Scope

This article is a structured narrative review and perspective, not a systematic review or meta-analysis. It was designed to clarify terminology, compare SAC with conventional molecular controls, summarize relevant evidence across specimen types, and propose a risk-based framework for implementation and future research.

Searches for the revision used PubMed/MEDLINE, Embase, Web of Science or Scopus, Google Scholar for citation chasing, and official or manufacturer sources when regulatory or

instructions-for-use details were needed. The search window covered January 2004 through May 2026, with targeted follow-up checks through 16 June 2026 for regulatory updates, direct-amplification workflows, nucleic-acid lateral-flow formats, and fixed-stringency readouts. Search terms included combinations of sample adequacy control, specimen adequacy, internal control, RNase P, RPP30, beta-globin, CCR5, 16S rRNA gene, qPCR, PCR inhibition, extraction control, false negative, HPV, SARS-CoV-2, point-of-care testing, self-collection, direct PCR, direct amplification, Simplexa, in vitro diagnostic (IVD), laboratory-developed test (LDT), risk-based quality control, nucleic-acid lateral-flow assay, room-temperature hybridization, and probe design.

Because this article is not a systematic review, the search was not designed to generate Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-style retrieved/included record counts; instead, retained sources were categorized by the level of inference they could support.

Eligible sources were retained when they addressed conventional qPCR or molecular controls, specimen adequacy or sample quality, sampling- or processing-related false-negative mechanisms, self-collection or decentralized testing, HPV or other IVD examples in which human-cell controls affect result validity, direct-amplification workflows, or risk-based laboratory-quality management. Because this was a framework-building narrative review rather than an intervention meta-analysis, source appraisal was qualitative and question-specific. Formal risk-of-bias tools and pooled estimates were not applied because the retained material included heterogeneous guidance documents, instructions for use, regulatory materials, validation studies, observational sampling studies, and implementation examples rather than a common intervention-outcome design. Each source was used for the level of inference it could support: analytical-control guidance, preanalytical risk, operational precedent, distributional evidence, clinical linkage, or limitation. Several operational or validation studies cited here come from the authors' group; these are used only for the workflow-specific claims they directly support and are balanced against independent IVD precedents, HPV evidence, respiratory studies, and laboratory-quality guidance.

3. Terminology and Control Hierarchy

A marker becomes SAC only when it is linked to a validated interpretation. This manuscript uses sample adequacy control for a system with three elements: a specimen-specific adequacy or integrity marker/metric; a threshold or classification rule validated for the specimen type, disease-specific biomarker, and platform; and a reporting, recollection, or quality-monitoring consequence.

The phrase integrated specimen-process adequacy control is the conceptual advance of this manuscript, whereas SAC remains the operational label used in reports, validation files, and workflow rules. The phrase clarifies that a validated SAC may integrate evidence about collection, transfer, extraction or direct release, inhibition-sensitive amplification, and marker integrity. It should not be read as a claim that one marker identifies a single mechanism of failure or replaces contamination and analytical controls; these limits are returned to in Section 9. The useful claim is narrower: SAC can tell the laboratory whether the specimen-process evidence supporting a negative result is valid, borderline, or invalid under the validated workflow.

An adequacy/integrity marker is the measurable signal itself, such as a human-cell target, bacterial biomass marker, site-specific host or microbial biomarker, total nucleic-acid signal, physical acceptability criterion, inhibition-sensitive comparator, or matrix-specific marker. A conventional internal amplification or extraction control can be essential, but it is not automatically SAC because it may pass even when the specimen lacks relevant patient, microbial, or site-specific material [4,16].

SAC does not replace contamination or analytical controls. No-template or negative controls remain necessary for contamination monitoring, and positive or analytical controls remain necessary for assay-performance verification. The specific value of SAC is different: when validated, one specimen-specific adequacy/integrity signal can link the negative result to otherwise hidden questions about relevant material, marker integrity, transfer, release or extraction, inhibition-sensitive amplification, and processing compatibility. This makes SAC particularly useful in direct-amplification, decentralized, home, or limited-observation workflows, where there may be no extraction yield, visible pellet, or separate recovery signal to inspect. SAC remains limited to the failure modes validated for that marker and assay.

Table 1 summarizes the functional distinction between SAC and conventional molecular controls.

Table 1. Conceptual distinction between SAC and conventional molecular controls.

Control Type	Primary Question Answered	Why It Is Not Sufficient as SAC
Positive control	Can the assay detect the target under expected analytical conditions?	Does not evaluate the patient specimen or site-specific collection adequacy.
No-template or negative control	Is reagent or setup contamination detected?	Does not evaluate specimen adequacy or biological representativeness.
Internal amplification control	Is amplification inhibited in the reaction?	May amplify normally when the collected specimen is nonrepresentative or lacks disease-specific biomarker-relevant material.
Extraction/process control	Did extraction and amplification work technically?	May monitor recovery or inhibition but may not measure site-specific material, degradation, or anatomical targeting.
Adequacy/integrity marker	Is a potentially relevant sample signal present?	Becomes SAC only after validation of the marker, threshold, specimen type, disease-specific biomarker context, and reporting consequence.
Sample adequacy control	Is the negative disease-specific biomarker result supported by validated specimen-specific adequacy/integrity evidence?	Requires specimen-specific validation; reduces uncertainty about specimen/process adequacy but does not prove true absence of the disease-specific biomarker or replace no-template, positive, or other analytical controls.

The most important reporting distinction is therefore simple: SAC should be valid, borderline, or invalid for the intended negative interpretation. The phrase negative SAC should be avoided. A disease-specific biomarker result of not detected accompanied by invalid SAC is best reported as uninterpretable, inadequate, indeterminate, or qualified according to a validated policy, not as a simple negative.

4. Candidate Markers and Validation Boundaries

No marker is universal. Human-cell markers may be appropriate in host-cell-rich specimens, bacterial biomass markers may be more appropriate in rectal swabs, and stool or enteric assays may require matrix-specific interpretation, inhibition assessment, or quantitative normalization. The marker must match the biology of the specimen and the question attached to the negative result.

Multiplex panels add another validation boundary. A single SAC marker may be adequate for high-shedding or high-burden targets but marginal for low-burden, compartment-

specific, or degradation-sensitive targets within the same panel. Thresholds and reporting consequences may therefore need to be target-class-specific rather than panel-wide. The same issue applies to quantitative assays: an SAC cutoff calibrated for a qualitative detected/not-detected rule may need a different threshold or report comment when the disease-specific biomarker is reported quantitatively, because inadequacy can bias magnitude as well as detection.

Endogenous-gene normalization is not automatically SAC. In gene-expression qPCR, endogenous reference genes are used to reduce variation in input amount, extraction, and reverse transcription; qPCR and digital PCR (dPCR) reporting standards also emphasize transparent control selection, validation, and performance characterization [17–19]. The ongoing International Organization for Standardization (ISO) 20395 revision is not cited here as current ISO guidance. Instead, this manuscript cites a personal communication from an ISO/TC 276/SC 1/WG3 participant stating that SAC-related work is expected to be referenced in a future ISO 20395 revision [20]. In diagnostics, the same signal may function as a cellularity marker, a denominator, an internal reference, or a reportable SAC. It becomes SAC only when validated against a specimen-specific threshold and linked to a reporting consequence.

Crawford et al. [21] provide an important validation-boundary example because they show the danger of marker generalization. In their nasopharyngeal-swab study, RPP30 and beta-globin were valid measurable endogenous signals, and average Cq values differed by collection category, but the Cq distributions overlapped enough that these markers did not reliably classify the swab collection method. This does not invalidate SAC; it narrows the claim. SAC should not be equated with any convenient endogenous gene, and it should not be described as measuring sampling technique, sampling mode, or anatomical optimality unless that endpoint has been validated. The interpretation is more complex than the simplified conclusion that SAC does not depend on sampling mode. Their data are consistent with substantial individual biological variability in adequacy-marker signals, and that variability contributes to the frequency distribution expected for a defined specimen–biomarker–platform workflow. For this reason, distribution information is useful: absence or invalidity of SAC, especially together with absence of the disease-specific biomarker, means that confidence in a negative result is not absolute.

Candidate marker and control classes relevant to SAC design are summarized in Supplementary Table S1.

Box 1 lists the risk-assessment questions that should be considered before SAC implementation and that are used again in Section 7.

Box 1. Risk-assessment checklist before implementing SAC.

1. What clinical or public-health action follows a negative result?	2. How variable is collection quality in the local workflow?
3. Can inadequacy be detected by another control, physical criterion, or process check?	4. Is recollection feasible and acceptable to patients and clinicians?
5. Is the marker validated for this specimen type, disease-specific biomarker, and platform?	6. What residual risk remains if SAC is not feasible?

5. Evidence by Application and Specimen Type

The evidence base is heterogeneous, so the most useful main-text structure is not a study-by-study catalog. The recurring pattern is that SAC is most valuable when specimen quality is variable, negative results drive action, and a validated adequacy/integrity signal can change reporting, recollection, or process monitoring. Box 2 summarizes the application-

specific message; individual studies should be interpreted only at the level of inference their design supports.

Box 2. Condensed evidence map by application area.

Application Area	Evidence Signal	Implication for SAC
Rectal/enteric	Specimen biology matters: fecal or microbial biomass markers may be more informative than human housekeeping targets; stool workflows add matrix, inhibitor, and extraction effects [5,11,12,22,23]. Evidence level: analytical/operational.	Do not generalize one marker across matrices. A low or absent human-gene signal should not invalidate a detected enteric target, but may qualify a negative result or trigger repeat/recollection or inhibition assessment.
Respiratory/pandemic response	Sampling variability, timing, transport, inactivation, and marker choice can affect negative-result confidence. Higher RNase P SAC Cq strata were associated with lower influenza/respiratory syncytial virus (RSV) positivity, while respiratory SAC failure/no-Cq fractions varied markedly across workflows [6–10,21,24–37]. Evidence level: operational/outcome-adjacent.	Use respiratory SAC as a validated negative-result support and process-monitoring signal, not as proof of correct sampling mode or absence of infection.
HPV/cartridge precedents	HPV workflows and automated cartridge assays show that cellularity, sample-processing, or adequacy controls can be incorporated into routine validity logic [15,38–43]. Evidence level: IVD precedent/outcome-adjacent.	Adequacy thresholds can be operationalized and linked to clinical endpoints or high-consequence workflows, but endpoint benefit still requires direct evaluation.
Direct amplification/point-of-care (POC)/home/ambient lateral-flow assay (LFA)	Extraction-free or fixed-stringency readouts reduce observation of collection, transfer, release, inhibition, and specimen integrity. Probe/capture design can improve specificity but does not assess specimen adequacy [44–47]. Evidence level: implementation/workflow precedent.	SAC is a complementary specimen-process fail-safe for negative interpretation, especially when sampling or processing occurs outside laboratory supervision.

Respiratory testing illustrates the calibration problem. Human-cell markers such as RNase P, RPP30, beta-globin, or beta-actin can reveal nearly acellular specimens or monitor distribution shifts, but the marker endpoint must be validated. The Crawford et al. nasopharyngeal-swab study is therefore best read as a boundary condition: endogenous-marker Cq distributions may overlap enough that they cannot classify collection method, even though late or absent adequacy signals can still weaken confidence in a negative result [21].

HPV screening remains the strongest outcome-adjacent example because cellularity or internal-control signals can affect the validity of HPV-negative reports [40,43], large clinical datasets have evaluated internal-control cycle-threshold (Ct) values against cervical intraepithelial neoplasia grade 2 or worse (CIN2+) endpoints [38], and CCR5 has been evaluated as an HPV adequacy marker [39]. These associations demonstrate that adequacy thresholds can be operationally integrated and evaluated against clinical endpoints, but they do not establish that SAC implementation reduces CIN2+ incidence or improves screening-program effectiveness.

Ambient nucleic-acid lateral-flow formats after PCR, recombinase polymerase amplification (RPA), loop-mediated isothermal amplification (LAMP), or other direct-amplification chemistries make this distinction especially important. Once the disposable device, buffer,

temperature, and capillary-flow conditions are fixed, there is limited opportunity to rescue hybridization stringency at the point of interpretation [46,47]. Probe and capture-design choices remain important for analytical specificity under fixed stringency, but they do not evaluate collection, transfer, release, inhibition, or specimen integrity. At the workflow level, an in-process dense-city pandemic framework likewise argues that diagnostic systems should be evaluated by sample-to-action time, isolation feasibility, decentralized logistics, and equity rather than analytical performance alone [48]. SAC therefore complements these analytical strategies as a specimen-process fail-safe for negative-result interpretation. For endpoint lateral-flow readouts, the absence of real-time amplification curves can further limit reaction-level interpretation; SAC may therefore need to be implemented as a validated specimen-process or pre-readout adequacy signal rather than as a conventional Cq-based control.

6. Reporting Logic and Minimum Requirements

SAC primarily adjudicates negative results. A detected disease-specific biomarker target generally remains reportable if analytical controls pass and contamination risk is addressed; invalid SAC may be documented but usually does not negate a valid target detection. When the disease-specific biomarker target is not detected, SAC status determines whether the negative result is interpretable, qualified, or invalid under the laboratory policy.

Table 2 translates this reporting logic into result categories and suggested reporting consequences.

Table 2. Reporting logic for disease-specific biomarker detection and SAC validity.

Disease-Specific Biomarker Result	Technical Controls	SAC Result	Suggested Category	Reporting Implication
Detected	Pass or acceptable	Valid, borderline, or invalid	Positive; target detected	Report detected. SAC status may be documented, but it usually does not override valid target detection.
Not detected	Pass	Valid	Interpretable negative	Report not detected with standard limitations; the SAC was valid for this specimen-target context.
Not detected	Pass	Borderline	Qualified negative or indeterminate	Use predefined policy: repeat extraction/test, comment, or recollect if clinically indicated.
Not detected	Pass	Invalid/below threshold	Uninterpretable or inadequate negative	Do not report as fully reassuring negative. Recommend recollection or repeat testing, or include an explicit limitation.
Not detected or inconclusive	Fail	Any	Invalid assay or process failure	Repeat test or request a new specimen according to the assay-specific failure mode.

Example report comments may include: “Target not detected; SAC valid. Result is interpretable within standard assay and clinical limitations.” For an inadequate negative: “Target not detected; SAC invalid or below validated threshold. The negative result is not fully interpretable because specimen-process adequacy criteria were not met. Repeat testing or recollection is recommended if clinically indicated.”

To avoid relabeling ordinary endogenous signals as SAC, the designation should be reserved for controls that meet minimum validation and reporting requirements.

Minimum validation and reporting requirements before a marker or metric is called SAC are summarized in Supplementary Table S2.

At minimum, the laboratory should define what the SAC marker measures, what failure modes it can and cannot detect, how valid/borderline/invalid thresholds were derived, how invalid SAC changes a negative report, and whether the added control affects disease-specific biomarker sensitivity or workflow burden.

7. Implementation and Distribution Monitoring

Implementation should begin with the risk-assessment checklist in Box 1 rather than with a universal mandate. A laboratory should define the action attached to a negative result, local collection variability, alternative adequacy checks, recollection feasibility, marker validation status, and residual risk if SAC cannot be implemented. The broader workflow is summarized in Supplementary Table S3 and Figure 1.

Distribution monitoring is useful after validation because SAC is quantitative. In qPCR-based SAC, higher Cq usually means lower recovered marker input or poorer marker recovery; with approximately 100% amplification efficiency, a ΔCq of d cycles corresponds roughly to a 2^d -fold difference in starting marker quantity. The exact relationship depends on assay efficiency and should not be treated as an absolute biological ratio. Shifts in the center, dispersion, high-Cq tail, or no-Cq fraction can flag changes in collection, transport/storage, extraction or release, inhibition, reagent lots, device function, or self-collection quality. Between-person biology can also broaden the distribution, including pediatric versus adult sampling, immunocompromised hosts, inflammation, mucus, microbiota, and nucleic-acid stability. SAC distributions are therefore process-performance signals rather than standalone failure-mode diagnoses.

An SAC Cq distribution should not be assumed to be normal. Cq is approximately $\log_2$ -scaled, so multiplicative variation may make the central region of a stable workflow look roughly symmetric, but real distributions can be skewed, heavy-tailed, censored at the assay cutoff, or accompanied by a separate no-Cq category. Distribution figures should therefore show observed local data, including undetected SAC results, and should use any smooth curve only as a workflow reference, not as a normality claim.

Respiratory data do not support a universal 5% SAC-failure expectation. Reported examples range from very rare RNase P nonamplification in a large self-collected anterior-nares program (13/115,435; 0.011%) to approximately 2.4% late or absent RNase P in a CDC-protocol COVID-19 series and marker-dependent nondetection around 0–5% for RPP30 or 5–10% for beta-globin in a controlled nasopharyngeal-swab study [21,34,35]. Other respiratory-panel data similarly reported hRNase-P failure in 94/4000 specimens (2.35%) [36]. Thus, approximately 5% may be a conservative planning or monitoring trigger in selected respiratory workflows, not a transferable cutoff. The clinically relevant denominator is the fraction of negative-result interpretations that also have late, invalid, or absent SAC, not the overall disease-biomarker no-Cq fraction.

An initial SAC cutoff can be defined as a nonparametric upper-tail threshold from the local acceptable-specimen distribution:

$$T_\alpha = Q(1 - \alpha; C | A)$$

where C denotes SAC Cq values among specimens meeting predefined acceptability criteria A , and α is the tolerated upper-tail fraction of otherwise acceptable specimens that will be flagged. Equivalently, $Q(1 - \alpha; C | A)$ denotes the $(1 - \alpha)$ quantile of the acceptable-specimen SAC Cq distribution. In plain terms, choosing $\alpha = 0.05$ sets T_α near the 95th percentile and flags the highest-Cq 5% of otherwise acceptable specimens as borderline or invalid, depending on clinical consequence and operational tolerance. Because higher

Cq indicates lower recovered adequacy-marker input, the low-adequacy region lies in the upper tail. SAC results with no measurable Cq should be handled as censored observations or as a separate categorical invalid class, not imputed as numeric Cq values unless that convention has been validated. This percentile threshold is a process adequacy flag, not a patient-level probability of a false-negative disease-specific result.

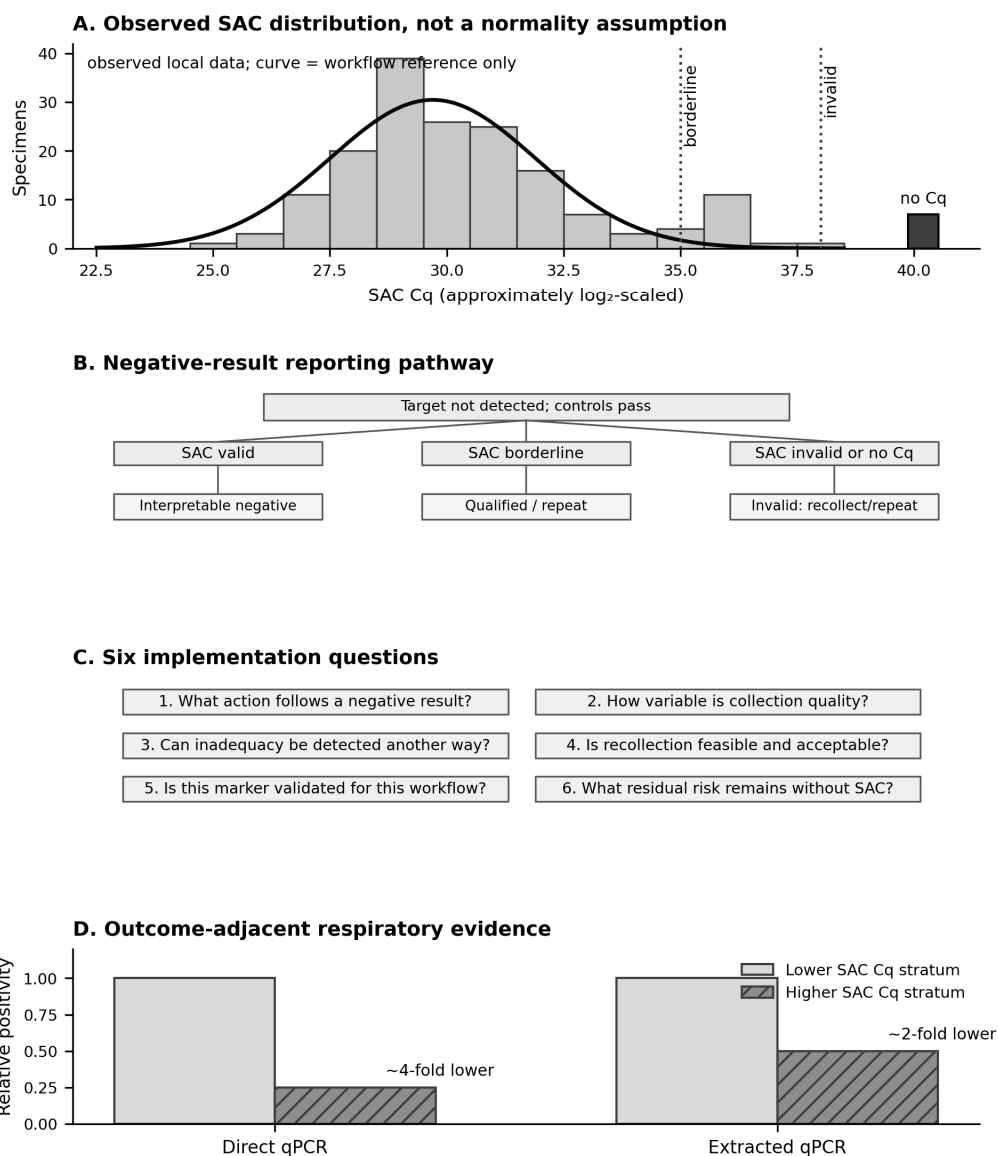


Figure 1. Risk-based implementation and distribution monitoring of sample adequacy control (SAC). (A) Observed local SAC Cq values are plotted with a separate no-Cq category; the smooth line is a workflow reference, not a normality assumption. Because Cq is approximately $\log_2$ -scaled, central values may appear symmetric, but real distributions can be skewed or censored and reflect collection, biology, transport/storage, processing, inhibition, and marker integrity. (B) Negative-result reporting pathway. (C) Six risk-assessment questions. (D) Empirical respiratory-screening evidence showing that higher SAC Cq strata can have lower pathogen positivity; (D) summarizes respiratory-screening data reported by Oughton et al. [33]. Note that thresholds and no-Cq fractions are workflow-specific.

Thresholds should remain workflow-specific. SAC signal distributions depend on sampling method, anatomical site, device, transport medium, sampling force, marker biology, primer/probe design, PCR cycling, direct amplification versus nucleic-acid isolation, inhibition tolerance, and reporting consequence. Once these elements are fixed, the laboratory can record the local observed distribution and define an expected range,

borderline zone, invalid zone, and no-Cq rule. A nasopharyngeal swab with very late SAC Cq, for example approximately Cq 39, or no SAC Cq at all, together with a non-detected disease-specific biomarker would be a local repeat, qualification, or recollection trigger, not a universal cutoff.

An exploratory baseline of 50–100 acceptable specimens can reveal gross shifts, but stable upper-tail cutoffs usually require more data; when local volume permits, approximately 200–500 acceptable specimens provide a practical basis for 95th–97.5th percentile thresholds, while 1–2% tails should be treated as provisional until supported by larger local datasets or external/manufacturer evidence. This 200–500 range is a practical recommendation for more stable upper-tail cutoffs, not a prerequisite for implementation. High-consequence or low-volume assays may legitimately start with smaller datasets, conservative manufacturer guidance, external evidence, historical data, or Bayesian updating, provided the threshold is labeled provisional and reviewed as data accumulate. High-consequence settings may justify smaller α , whereas lower-risk screening may use a larger α , such as 0.05, to reduce unnecessary recollection.

When compromised-specimen distributions or adjudicated outcomes are available, additional models can be used. A Bayesian classifier can estimate the posterior probability of specimen/process inadequacy from the observed SAC Cq, and outcome-linked models can estimate false-negative risk conditional on disease-specific biomarker nondetection, specimen type, timing, and clinical covariates. These extensions should remain secondary to local validation because they require representative compromised specimens, stable case mix, and calibrated outcome adjudication. Until such linkage exists, SAC should be reported categorically as valid, borderline, or invalid for negative-result interpretation.

For LDTs, thresholds are especially assay-dependent: a cutoff derived for a nucleic-acid-isolation workflow should not be transferred unchanged to direct amplification because lysis or release efficiency, extraction loss, dilution, inhibition tolerance, input volume, amplification chemistry, and reporting consequences differ [22,23,49].

8. Regulatory and Oversight Considerations

A risk-based regulatory posture is preferable to a universal requirement [13,14,50–52]. Laboratories and manufacturers should document how specimen adequacy is addressed when negative results drive high-consequence decisions. That documentation can include the SAC marker and threshold, the reason no SAC is feasible, the residual risk for negative results, and how that uncertainty is communicated. The same elements can be mapped onto existing risk-management and quality-system files, including Clinical and Laboratory Standards Institute (CLSI) EP23 and ISO 15189, by specifying the hazard, control measure, acceptance criterion, corrective action, and residual risk [13,14]. Published qPCR/dPCR method-evaluation standards such as ISO 20395 [19] and ongoing ISO/TC 276/SC 1/WG3 revision discussions [20] provide an additional standards context, but the latter is treated as personal communication rather than formal published guidance.

The distinction between laboratory-developed tests and manufacturer IVD platforms is secondary to the interpretive question: what evidence supports the negative result? A manufacturer may incorporate SAC into chemistry, cartridges, software, or instructions for use. A clinical laboratory may add a local adequacy marker, report comment, or aggregate monitoring program. A regulator or accreditor may ask how the negative-result risk is mitigated, not mandate a single design for all assays. If accreditation bodies, payers, or health systems request objective specimen-quality metrics, this framework can justify either SAC implementation or a documented rationale for why SAC is not feasible and what residual risk remains.

Independent precedents support this measured position. HPV screening workflows show that cellularity/internal-control failure can alter validity of otherwise negative results [38–40,43]. Xpert CT/NG and Xpert Ebola show that sample adequacy or process controls can be incorporated into automated high-consequence molecular platforms [15,41,42]. These examples support feasibility and risk-management logic, not universal outcome claims.

9. Limitations and Unresolved Questions

The evidence base remains incomplete. Current evidence supports a rationale for SAC and provides examples of marker design, IVD integration, and operational reporting logic. It does not yet provide strong prospective studies showing that SAC implementation reduces transmission, improves patient outcomes, or changes epidemiologic decisions. The manuscript therefore uses calibrated language such as may support, can identify, and risk-based framework.

The proportion of false-negative results attributable specifically to sample inadequacy is not generalizable. It depends on the disease-specific biomarker, anatomical site, sampling time, organism or biomarker burden, collection device, transport medium, extraction method, assay chemistry, inhibition, and reference standard. Pandemic false-negative reports illustrate this complexity [8–10,21,24–32].

An invalid or absent SAC, especially when the disease-specific biomarker is also not detected, does not prove a false negative. It means that confidence in the negative result is incomplete. A defensible attributable-fraction estimate requires a defined specimen–biomarker–platform combination, parallel SAC measurement, repeat collection or alternative-specimen adjudication, and linkage to clinical or infection-control outcomes.

The integrated-control language also has limits. An SAC marker can support whole-process interpretation only for the failure modes it has been shown to detect. A human gene or tissue-specific RNA biomarker may detect adequate host-tissue recovery or sampling of the intended anatomical compartment, but it cannot guarantee capture of the disease-specific biomarker. A co-amplified marker may help detect inhibition but not wrong anatomical site. An endogenous marker may reflect extraction recovery but not transport degradation unless integrity has been evaluated. In direct amplification, an absent SAC may indicate poor collection, poor transfer, failed nucleic-acid release, inhibition, degradation, or reaction failure, but validation is required to decide which explanation is most plausible. A low microbial biomass signal can also reflect patient biology as well as collection failure. These limits should be stated in validation files and, when relevant, in reports.

SAC also creates operational trade-offs. Recollection can delay care, increase cost, and frustrate users. In low-prevalence screening, over-qualification of negative results may increase workload and anxiety. In high-risk settings, however, failing to qualify inadequate negatives may cause greater harm. Clinician interpretation is also a risk: an invalid SAC flag may be mistaken for poor laboratory performance rather than a protective warning against over-interpreting an unsupported negative result. Report comments, interpretive footnotes, and laboratory-clinician feedback loops should therefore be part of implementation. Pilot implementation with structured feedback from ordering clinicians, such as brief surveys or laboratory liaison meetings, can help refine report comments and reduce misinterpretation. SAC policies should be piloted, audited, and adjusted to local consequence and feasibility.

In pandemic response or local infectious outbreaks, the operational cost of SAC, repeat testing, or recollection may be small compared with the downstream cost of an unsupported negative result that contributes to delayed isolation, inappropriate cohorting, preventable exposure, outbreak investigation, workforce disruption, or loss of confidence in screening

programs [48]. This supports risk-based SAC use in high-consequence workflows while still requiring local validation and audit rather than universal implementation.

The main unresolved questions are how SAC signal relates to adjudicated false-negative risk; which thresholds or gray zones are optimal; whether distributions are transferable across sites; which failure modes each marker detects; how clinicians interpret qualified negatives; and whether SAC improves workflow, outcomes, or cost-effectiveness.

10. Conclusions

A negative molecular result is not only a statement about assay performance; it is also a statement about the specimen. Conventional controls are necessary, but they do not necessarily show that the submitted material was biologically adequate and process-compatible for the intended negative interpretation.

SAC is best defined as a validated specimen-specific marker or metric, threshold, and reporting consequence that supports disease-specific biomarker negative-result confidence. It is not simply sufficient material, and it is not any endogenous gene. It becomes clinically meaningful only when the marker, threshold, and action are validated for the specimen, platform, disease-specific biomarker context, and decision attached to the negative result.

The practical recommendation is measured: laboratories and manufacturers should assess specimen-adequacy risk during assay design, validation, and reporting-policy development; implement SAC when a validated marker exists and the negative-result consequence justifies it; use aggregate monitoring when individual thresholds are immature; and communicate residual uncertainty when SAC is not feasible.

This distinction is especially relevant for direct-amplification, POC, home-collection, home-testing, and ambient lateral-flow workflows. In those settings, SAC may be the most compact integrated specimen-process control for negative-result interpretation, while remaining an interpretive fail-safe rather than a mechanistic fault detector. No-template, contamination, and analytical-performance controls still remain necessary.

For high-consequence molecular diagnostics, SAC is appropriately treated as an integrated specimen-process adequacy control for negative-result interpretation. It does not catch every error and does not guarantee true absence of the disease-specific biomarker. Its value is narrower and important: it identifies when the specimen-specific evidence supporting a negative result is valid, borderline, or invalid, and it creates a framework for safer reporting, recollection, quality monitoring, and future outcome studies.

11. Future Directions

Future studies should distinguish four endpoints: analytical performance of the SAC marker, operational performance of the reporting or recollection rule, clinician or public-health decision impact, and patient or epidemiologic outcomes. A pragmatic near-term design would be a multicenter before/after or stepped-wedge evaluation in selected high-consequence workflows, measuring recollection rates, repeat-test burden, qualified-negative reporting, clinician acceptance, turnaround time, and downstream adjudicated outcomes. Without this distinction, SAC evidence will remain difficult to compare across assays and specimen types. Priority research and reporting items are summarized in Supplementary Table S4.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/labmed3030016/s1>, Table S1: Candidate markers and control classes relevant to SAC design; Table S2: Minimum validation and reporting requirements before a marker or metric is called SAC; Table S3: Risk-assessment framework for SAC implementation; Table S4: Priority research and reporting items for future SAC studies.

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Abbreviations

SAC	sample adequacy control
NAAT	nucleic-acid amplification test
qPCR	quantitative polymerase chain reaction
PCR	polymerase chain reaction
RT-PCR	reverse-transcription polymerase chain reaction
dPCR	digital polymerase chain reaction
CT/NG	Chlamydia trachomatis/Neisseria gonorrhoeae
Cq/Ct	quantification cycle/cycle threshold
POC	point-of-care
IVD	in vitro diagnostic device
LDT	laboratory-developed test
LFA	lateral-flow assay
NALFA	nucleic-acid lateral-flow assay
RPA	recombinase polymerase amplification
LAMP	loop-mediated isothermal amplification
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
ISO	International Organization for Standardization
CIN2+	cervical intraepithelial neoplasia grade 2 or worse
RSV	respiratory syncytial virus
MIQE	Minimum Information for Publication of Quantitative Real-Time PCR Experiments

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