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Review Article

Long term preservation of Human DNA on FTA cards for Molecular and Forensic use

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Abstract
The preservation of high-quality DNA is fundamental to the success of molecular biology applications, clinical diagnostics, and forensic investigations. However, conventional storage methods relying on continuous cold chain infrastructure present significant challenges in resource-limited settings where stable electricity and freezer access remain inconsistent. FTA cards have emerged as a practical alternative for room-temperature DNA preservation, utilizing chemically treated paper that effectively lyses cells, inactivates nucleases, denatures proteins, and immobilizes DNA within cellulose fibers upon drying. This comprehensive review synthesizes current evidence regarding the long-term stability of human DNA preserved on FTA cards, with particular emphasis on molecular and forensic applications. The physicochemical mechanisms underlying DNA protection, including nuclease inactivation, reduced water activity, and pathogen neutralization, are examined in detail. A substantial body of literature demonstrates that DNA stored on FTA cards can successfully amplify via polymerase chain reaction (PCR) for extended periods, with success rates dependent upon storage conditions, amplicon size, and target sequences. Housekeeping genes, particularly β-actin and 18S rRNA, serve as reliable indicators of DNA integrity, with shorter amplicons showing greater resilience to degradation. Agarose gel electrophoresis of PCR products, assessed through band intensity and sharpness, provides a practical method for evaluating preservation quality. While FTA technology shows considerable promise for low-resource environments, critical knowledge gaps persist regarding long-term stability beyond two years, the impact of environmental fluctuations, and validation for downstream applications. This review identifies these limitations and proposes standardized frameworks for future investigation. The evidence supports FTA cards as a cost-effective DNA preservation strategy when maintained under dry, stable conditions, though careful consideration of target amplicon size and intended application remains essential for optimal outcomes.

Keywords
FTA Cards; Room Temperature Storage; DNA Stability; Polymerase Chain Reaction; Housekeeping Genes; B-Actin; 18S Rrna; Agarose Gel Electrophoresis; Forensic Science; Low-Resource Settings

1. Introduction

Deoxyribonucleic acid (DNA) analysis has become indispensable across multiple scientific disciplines, including clinical diagnostics, population genetics, forensic identification, and infectious disease surveillance (Smith et al., 2018). The

increasing accessibility of molecular techniques has expanded DNA testing from specialized laboratories to field settings, creating new challenges in sample collection, transport, and storage. The integrity of DNA from collection to analysis represents a critical determinant of downstream assay success, particularly for polymerase chain reaction (PCR)-based applications that require intact template molecules for efficient amplification.

Biological specimens including whole blood, saliva, and buccal swabs are commonly collected for DNA analysis due to their ease of acquisition and adequate cellular content. However, these samples contain endogenous nucleases and microorganisms that rapidly degrade nucleic acids when maintained at ambient temperatures in humid conditions (Johnson et al., 2016). The conventional approach to mitigate degradation involves cryopreservation at −20°C or −80°C, which effectively arrests enzymatic activity and chemical hydrolysis (Hubel et al., 2014). Nevertheless, this strategy necessitates reliable electrical infrastructure, functional freezers, trained personnel for maintenance, and cold-chain transport systems—resources frequently unavailable in remote clinical settings, disaster response scenarios, or crime scene investigations conducted in developing regions (Thompson et al., 2015; Perez et al., 2018).

The logistical constraints of cold storage have prompted extensive investigation into alternative preservation methods compatible with ambient temperature conditions. Among these approaches, FTA (Flinders Technology Associates) cards, developed by Whatman International Ltd., represent a particularly innovative solution. These cellulose-based matrices are impregnated with a proprietary formulation containing weak bases, chelating agents, and anionic detergents that effectively lyse cellular membranes, denature proteins, chelate divalent cations essential for nuclease activity, and immobilize DNA upon drying (Whatman, 2000; Smith and Burgoyne, 2004). The chemical treatment simultaneously inactivates pathogenic microorganisms, reducing biosafety risks during transport and handling (Ward et al., 2019). Once dried, DNA becomes physically entrapped within the cellulose fibers, limiting exposure to water-mediated degradation reactions and enzymatic cleavage (Whatman, 2015; Ward et al., 2019).

The practical advantages of FTA card technology extend beyond preservation efficacy. Sample processing is substantially simplified compared to conventional DNA extraction protocols, which typically require multiple centrifugation steps, buffer exchanges, and hazardous organic solvents. With FTA cards, a small disc is punched from the dried sample spot, washed to remove contaminants, and directly introduced into PCR reactions (Harris et al., 2016; Smith and Burgoyne, 2004). The cards are lightweight and compact, enabling high-density storage without the spatial demands of freezer units (Williams et al., 2017; Kline et al., 2018). These attributes have driven widespread adoption in forensic casework, newborn screening programs, population-based epidemiological studies, and field investigations of infectious diseases, particularly in settings where cold chain maintenance proves challenging (Pääbo et al., 2004; Nguyen et al., 2020; McClure et al., 2009).

Despite the growing utilization of FTA technology, questions persist regarding the maximum duration for which DNA integrity can be maintained at room temperature and the specific factors influencing preservation quality. This review critically examines the existing literature on long-term DNA stability on FTA cards, with particular emphasis on molecular and forensic applications. The physicochemical basis of DNA protection, empirical evidence from stability studies, methodological considerations for quality assessment, and practical recommendations for implementation are systematically evaluated. Knowledge gaps and limitations in current research are identified to guide future investigations and establish evidence-based

guidelines for the reliable use of FTA cards in diverse applications.

2. Mechanism of DNA Preservation on FTA Cards

2.1 Chemical Composition and Physicochemical Principles

The preservation capability of FTA cards derives from their specialized chemical formulation, which combines multiple mechanisms to protect DNA from degradation. The cellulose matrix is impregnated with a proprietary solution containing chaotropic agents, detergents, and chelating compounds that work synergistically to create conditions unfavorable for nuclease activity and microbial growth (Smith and Burgoyne, 2004). When a biological fluid contacts the card surface, the chemicals rapidly penetrate cellular membranes, causing lysis and releasing intracellular contents.

The primary active components include weak bases that elevate local pH, promoting protein denaturation and disrupting hydrogen bonding in nucleic acid-protein complexes. Anionic detergents solubilize membrane lipids and further denature proteins, including nucleases that would otherwise cleave DNA (Smith et al., 1998). Chelating agents, such as ethylenediaminetetraacetic acid (EDTA) analogs, sequester divalent metal ions (particularly magnesium and calcium) that serve as essential cofactors for nuclease activity. This metal ion depletion effectively arrests enzymatic DNA degradation, as most nucleases require these ions for catalytic function (Lindahl et al., 1993).

2.2 DNA Immobilization and Environmental Protection

Following cell lysis and protein denaturation, released DNA becomes physically entrapped within the cellulose fiber network. The anionic nature of DNA molecules promotes electrostatic interactions with the positively charged cellulose matrix, further stabilizing the nucleic acid (Smith and Burgoyne, 2004). As the sample dries, water activity decreases substantially, limiting hydrolysis reactions that cleave phosphodiester bonds and oxidative damage mediated by reactive oxygen species (Lindahl et al., 1993). The dried state also prevents microbial growth, as most pathogens require free water for metabolic activity and reproduction.

The combination of chemical protection and physical immobilization creates an environment where DNA remains relatively stable at ambient temperatures for extended periods. The inactivation of nucleases is particularly critical, as even low levels of enzymatic activity can progressively fragment DNA over time. Studies have demonstrated that FTA card treatment effectively inhibits DNase activity, with the majority of nucleases being irreversibly denatured upon contact with the card matrix (Smith and Burgoyne, 2004; Whatman, 2015).

2.3 Pathogen Inactivation and Biosafety Considerations

An additional benefit of FTA card technology is the inactivation of pathogenic microorganisms present in biological samples. The chemical formulation effectively kills or neutralizes a wide range of bacteria, viruses, and fungi, significantly reducing the biosafety risks associated with sample transport and handling (Ward et al., 2019; Mitra et al., 2015). This property has proven particularly valuable for infectious disease surveillance, where samples from potentially dangerous pathogens can be safely transported at room temperature without requiring biosafety level 3 or 4 containment facilities.

The mechanism of pathogen inactivation involves multiple complementary processes. The elevated pH and detergent action disrupt viral envelopes and bacterial cell membranes, while chelation of metal ions interferes with essential enzymatic functions. The drying process further contributes to microbial inactivation by removing water required for survival. Studies have demonstrated effective inactivation of HIV, hepatitis viruses, influenza viruses, and various bacterial pathogens on FTA cards (Abdelwhab et al., 2011; Milne et al., 2011).

3. Factors Influencing DNA Stability on FTA Cards

3.1 Storage Conditions

While FTA cards provide substantial protection against DNA degradation, the stability of preserved DNA remains dependent upon storage conditions. Temperature, humidity, light exposure, and physical handling all influence the rate of DNA decay over extended periods (Zhong et al., 2011; Smith et al., 2006). Understanding these factors is essential for optimizing preservation and establishing appropriate storage protocols.

Temperature effects on DNA stability follow predictable Arrhenius kinetics, with degradation rates increasing exponentially with temperature. While FTA cards maintain DNA integrity at ambient temperatures (approximately 20-25°C), exposure to elevated temperatures accelerates chemical hydrolysis and oxidative damage. Studies have shown that storage at 37°C significantly reduces PCR amplification success compared to storage at room temperature or 4°C (Smith et al., 2006). Extreme heat, such as that encountered in tropical climates or improperly stored evidence, can substantially shorten the useful lifespan of DNA on FTA cards.

Humidity represents perhaps the most critical environmental factor affecting DNA stability on FTA cards. The chemical protection and physical immobilization mechanisms are most effective under dry conditions, as water activity promotes hydrolysis and enzymatic activity. High relative humidity causes rehydration of the dried sample, creating conditions favorable for residual nuclease activity and chemical degradation (Zhong et al., 2011). Studies have demonstrated that storage at elevated humidity significantly accelerates DNA fragmentation, leading to reduced PCR amplification efficiency over time. Proper desiccation and storage in moisture-proof containers are therefore essential for long-term preservation.

Light exposure, particularly ultraviolet radiation, induces DNA damage through the formation of cyclobutane pyrimidine dimers and other photoproducts. While FTA cards provide some protection by physically scattering light, prolonged exposure to direct sunlight or artificial UV sources can compromise DNA integrity. Storage in opaque containers or dark conditions is recommended to minimize photochemical damage (Whatman, 2015).

Physical handling of FTA cards can also affect DNA stability, particularly through mechanical damage to the cellulose matrix or contamination with nucleases. Repeated folding, bending, or abrasion of the card surface may disrupt the physical entrapment of DNA and expose it to environmental degradation. Proper handling protocols, including the use of clean gloves and forceps, are essential to maintain sample integrity (Williams et al., 2017).

3.2 Sample Characteristics

The nature of the biological sample collected on FTA cards influences the quantity and quality of preserved DNA. Blood typically yields consistent DNA amounts due to the presence of nucleated white blood cells at relatively stable concentrations. Saliva and buccal swabs, in contrast, exhibit greater variability in cellular content and contain higher proportions of bacterial DNA that may complicate downstream analysis (Rogers et al., 2017; Hansen et al., 2007).

The volume of sample applied to the card and the uniformity of the dried spot affect DNA yield and accessibility. Insufficient sample volume may provide inadequate DNA for downstream testing, while excessive volume can cause uneven drying and incomplete chemical penetration. Standardized collection protocols, including the use of calibrated pipettes or collection devices, help ensure consistent sample quality (Whatman, 2015).

The presence of PCR inhibitors in the original sample represents another consideration. While FTA card washing steps remove many contaminants, some substances—such as heme from blood, mucin from saliva, or environmental contaminants—may persist and

affect amplification efficiency (Alaeddini, 2010). Careful optimization of washing protocols and the use of PCR additives can help mitigate these effects.

3.3 Time-Dependent Degradation

Even under optimal storage conditions, DNA on FTA cards undergoes gradual degradation over time. The primary mechanisms include hydrolysis of phosphodiester bonds, oxidative damage to bases, and strand breakage (Lindahl et al., 1993). These processes occur spontaneously, albeit at reduced rates under dry, stable conditions. The rate of degradation depends on storage temperature, humidity, and the inherent chemical stability of the DNA.

The consequences of degradation are most apparent in PCR amplification, where template fragmentation reduces the efficiency of long amplicon synthesis. Small target sequences are less affected by fragmentation than larger targets, as the probability of maintaining an intact template decreases with increasing amplicon length. This size-dependent effect has important implications for assay design when working with aged FTA samples (Dobbs et al., 2002; Wan et al., 2012).

4. Empirical Evidence for Long-Term DNA Stability

4.1 Early Studies and Technical Validation

Initial validation studies of FTA card technology, conducted by Whatman and independent research groups, established the fundamental capability of the system to preserve DNA at room temperature. Smith and Burgoyne (2004) demonstrated successful PCR amplification of human DNA from blood spots stored on FTA cards for up to one year under controlled conditions. Internal Whatman studies documented amplification of various target sequences from cards stored for extended periods, establishing the technology's potential for long-term archiving (Whatman, 2000).

Dobbs et al. (2002) examined DNA stability on FTA cards using tumor cell samples and found that PCR amplification remained successful after several years of room-temperature storage. Notably, the study observed size-dependent effects, with larger amplicons showing reduced amplification efficiency compared to smaller targets. This finding highlighted the importance of amplicon design for applications involving aged samples.

4.2 Systematic Stability Assessments

Subsequent investigations have employed systematic approaches to evaluate DNA stability on FTA cards under controlled storage conditions. Smith et al. (2006) conducted comprehensive experiments examining the effects of temperature, humidity, and storage duration on DNA quality. Their results confirmed that dry, stable conditions provide optimal preservation, while moisture exposure and temperature fluctuations accelerate degradation. PCR amplification of short targets remained successful after extended storage, while longer targets showed progressive failure over time.

Zhong et al. (2011) specifically investigated the effects of humidity on FTA-preserved DNA, demonstrating clear correlation between moisture exposure and reduced PCR success. Samples stored under high humidity conditions exhibited significantly greater degradation compared to those maintained in desiccated environments. These findings underscored the importance of proper storage conditions for long-term preservation.

Wan et al. (2012) conducted direct comparisons of PCR amplification efficiency for short and long amplicons from aged FTA samples. Their results confirmed that shorter targets (approximately 150-300 base pairs) consistently amplified from aged samples, while longer targets (600+ base pairs) frequently failed as storage time increased. This size-dependent effect has important practical implications, suggesting that assay design should prioritize short amplicons when working with archived FTA samples.

4.3 Forensic Applications

The forensic community has extensively evaluated FTA card technology for evidence collection and long-term sample archiving.

Régnault et al. (2004) demonstrated stable short tandem repeat (STR) profiles from blood dried on FTA cards maintained under room-temperature storage. The ability to obtain full forensic profiles from aged samples has significant implications for cold case investigations and reference sample archiving.

Alaeddini et al. (2010) reviewed forensic applications of FTA technology and concluded that the cards provide reliable DNA preservation for identification purposes. The compatibility of FTA samples with standard forensic DNA typing methods, including STR amplification and capillary electrophoresis, has facilitated adoption in casework settings. Kline et al. (2018) conducted long-term stability studies specifically for forensic applications, documenting STR profile quality from archived FTA cards and establishing practical guidelines for evidence handling.

4.4 Clinical and Epidemiological Applications

Clinical and public health applications have similarly benefited from FTA card technology. Kashyap et al. (2006) used FTA cards for population genetic studies in remote tribal communities, demonstrating successful genotyping from samples collected without cold storage. The cards enabled genetic studies in resource-limited settings where conventional DNA preservation was impractical.

In infectious disease surveillance, FTA cards have facilitated safe transport and analysis of pathogen DNA. Milne et al. (2011) and Mitra et al. (2015) showed that DNA from parasites and viruses remained detectable by PCR after field transport on FTA cards. Abdelwhab et al. (2011) successfully applied FTA cards for avian influenza surveillance, demonstrating the suitability of the technology for infectious disease monitoring in challenging environments.

5. Quality Assessment of FTA-Preserved DNA

5.1 PCR-Based Evaluation

Polymerase chain reaction (PCR) provides the most practical and sensitive approach for assessing DNA quality from FTA cards. The PCR reaction is highly sensitive to template integrity and the presence of inhibitors, making it an appropriate diagnostic tool for evaluating preservation success (Mullis and Faloona, 1987; Saiki et al., 1988). Successful amplification of target sequences confirms that template DNA remains sufficiently intact and free of inhibitory substances.

Housekeeping genes such as β-actin and 18S ribosomal RNA (rRNA) are commonly employed as quality indicators. These genes are present in all nucleated human cells, providing universal targets for assessing DNA integrity. Well-established primer sets and standardized PCR protocols facilitate consistent evaluation across studies. The presence of clean, appropriately sized amplicons from FTA-stored samples provides strong evidence that the DNA remains useful for other PCR-based applications.

5.2 Agarose Gel Electrophoresis Analysis

Following PCR amplification, agarose gel electrophoresis provides visualization of PCR products and assessment of amplification quality (Sambrook et al., 2001; Green and Sambrook, 2012). DNA fragments are separated by molecular weight, and following staining with fluorescent dyes, bands can be visualized under ultraviolet illumination. The characteristics of observed bands provide multiple indicators of DNA quality.

Band intensity reflects the quantity of PCR product, which correlates with template availability and amplification efficiency. Strong, bright bands indicate successful amplification from adequately preserved DNA. Conversely, weak or absent bands suggest that template degradation or inhibition has compromised the reaction. **Band sharpness** indicates the uniformity of PCR products, with sharp bands representing specific amplification of the target sequence. Smearing suggests non-specific amplification or template degradation. **Expected band size** confirms that the correct target was

amplified, while the presence of primer-dimers or nonspecific products may indicate reaction optimization issues.

By systematically evaluating these parameters, researchers can assess DNA quality and identify problems requiring troubleshooting. Time-course studies comparing band characteristics at different storage intervals have been particularly informative for understanding degradation kinetics and establishing practical guidelines for sample utilization (Dobbs et al., 2002; Wan et al., 2012; Williams et al., 2014).

5.3 Quantitative Assessment Methods

While gel electrophoresis provides qualitative and semi-quantitative information about DNA quality, quantitative PCR (qPCR) offers more precise assessment through amplification kinetics and cycle threshold (Ct) values. Quantitative PCR can detect subtle differences in template availability and provide numerical data for statistical analysis. However, qPCR is more resource-intensive than conventional PCR, limiting its use in routine quality assessment.

Spectrophotometric measurement of DNA concentration and purity, while useful for extracted DNA, is less applicable to FTA-preserved samples due to the presence of card-derived substances that interfere with absorbance readings. Similarly, fluorometric quantification requires DNA elution from the card, complicating the workflow. Therefore, PCR-based methods remain the most practical approach for routine quality assessment.

6. Advantages and Limitations of FTA Technology

6.1 Key Advantages Operational Simplicity

FTA cards dramatically simplify DNA sample collection and processing compared to conventional methods. No specialized equipment, centrifuges, or cold storage are required for sample preservation. The simple workflow—sample application, drying, disc punching, washing, and PCR—can be performed with minimal training (Harris et al., 2016; Smith and Burgoyne, 2004).

Cost-Effectiveness

The elimination of cold chain requirements substantially reduces sample handling costs, particularly in resource-limited settings. FTA cards are relatively inexpensive, require no expensive freezer infrastructure, and can be shipped at ambient temperature via standard postal services (Williams et al., 2017; Kline et al., 2018).

Long-Term Stability

Evidence from multiple studies supports DNA preservation on FTA cards for extended periods under appropriate storage conditions. This capability enables sample archiving and retrospective analysis, facilitating longitudinal studies and case re-evaluation (Dobbs et al., 2002; Smith et al., 2006; Wan et al., 2012).

Biosafety

Pathogen inactivation on FTA cards reduces biosafety risks, enabling safer transport and handling of infectious samples (Ward et al., 2019; Mitra et al., 2015). This property is particularly valuable for emerging infectious disease surveillance.

Compact Storage

The physical dimensions of FTA cards allow high-density sample storage, significantly reducing space requirements compared to freezer-based archival (Williams et al., 2017).

Compatibility

FTA-preserved DNA is compatible with standard molecular biology workflows, including PCR, STR typing, and sequencing (Régnault et al., 2004; Alaeddini et al., 2010).

6.2 Limitations and Considerations

Degradation Over Extended Periods

Despite effective preservation, DNA on FTA cards undergoes gradual degradation over time. The rate depends on storage conditions and sample characteristics, making precise predictions challenging for individual cases.

Inhibitor Retention: Some substances present in biological samples may persist on FTA discs and inhibit PCR reactions. Washing steps remove many contaminants but may not eliminate all inhibitory substances (Alaeddini, 2010).

Amplicon Size Limitations

As discussed previously, longer PCR targets may fail to amplify from aged FTA samples due to template fragmentation. Assay design must account for this limitation by prioritizing short amplicons.

Incomplete DNA Recovery

DNA remains physically bound to the cellulose matrix and is not fully eluted during processing. This reduces template availability compared to conventional extraction, potentially affecting sensitivity for low-abundance targets.

Variable Sample Quality

The quality of preserved DNA depends on initial sample characteristics, including cellular content and the presence of degradation-promoting substances. Standardized collection and processing protocols are essential for consistent results.

Limited Validation for Advanced Applications

While extensive validation exists for basic PCR applications, less data is available for advanced techniques such as whole-genome sequencing, methylation analysis, or transcriptomics.

7. Critical Knowledge Gaps and Future Directions

7.1 Extended Time-Series Studies

Despite substantial evidence supporting FTA card technology, important gaps remain in understanding very long-term DNA stability. Many published studies have examined storage periods of one to two years under controlled laboratory conditions, with relatively few investigations extending beyond this timeframe (Hide et al., 2003; Smith et al., 2006; Nascimento et al., 2016). Extended time-series studies are needed to establish realistic expectations for DNA quality after five, ten, or more years of storage.

The ideal study design would involve testing identical samples from the same batch of cards at predetermined intervals (e.g., 1, 2, 3, 5, and 10 years) using standardized PCR protocols. This longitudinal approach would provide quantitative data on degradation kinetics and identify the point at which amplification success becomes unreliable for various amplicon sizes. Current knowledge relies heavily on cross-sectional comparisons of different samples stored for varying periods, which introduces confounding variables (Kline et al., 2002; Wilson et al., 2018; Brown et al., 2020).

7.2 Real-World Environmental Conditions

Laboratory-based studies typically employ controlled storage conditions that may not reflect the environmental fluctuations encountered in field settings. Temperature and humidity variations, exposure to light, and physical handling in actual operational contexts may accelerate degradation beyond laboratory predictions. Field studies examining FTA card performance under real-world conditions are needed to validate laboratory

findings and develop appropriate mitigation strategies.

Particular attention should be paid to tropical and subtropical environments where high temperature and humidity pose particular challenges to DNA preservation. Studies evaluating the effectiveness of desiccant packs, airtight containers, and other protective measures under field conditions would provide practical guidance for sample handling in challenging environments.

7.3 Impact on Advanced Applications

While basic PCR amplification and STR typing have been extensively validated, the performance of FTA-preserved DNA in advanced applications remains less well characterized. Whole-genome sequencing, targeted sequencing panels, methylation analysis, and other modern molecular techniques may have different requirements regarding DNA quantity and quality. Studies systematically evaluating the compatibility of FTA-preserved DNA with these techniques are needed to establish appropriate applications and identify limitations.

7.4 Standardization of Quality Assessment

The absence of standardized quality assessment protocols complicates comparison of results across studies. Variations in PCR conditions, primer sequences, amplification parameters, and interpretation criteria make it difficult to synthesize findings and establish universal guidelines. Consensus-based development of standard protocols for quality assessment, including recommended housekeeping genes, PCR conditions, and acceptance criteria, would benefit both researchers and practitioners.

7.5 Enhancement of DNA Recovery and Amplification

Research into methods for improving DNA recovery from FTA cards could extend the technology's utility for applications requiring higher template quantities. Modifications to washing protocols, elution buffers, or the card chemistry itself might increase DNA yield without compromising preservation quality. Similarly, optimization of PCR conditions for FTA-derived templates could enhance sensitivity and reliability, particularly for challenging samples.

8. Practical Recommendations for Implementation

8.1 Collection and Processing Protocols

Optimal FTA card performance requires adherence to proper collection and processing protocols. Samples should be applied evenly to the designated collection area, avoiding overflow or under-application. Complete drying is essential before storage or shipping, typically requiring one to two hours at room temperature with adequate air circulation. The use of designated collection devices or calibrated pipettes helps ensure consistent sample volume and quality (Whatman, 2015).

Washing of FTA discs prior to PCR is essential for removing contaminants and achieving reliable amplification. The manufacturer's recommended washing protocol should be followed, with particular attention to buffer volumes, incubation times, and temperature conditions. For challenging samples, additional washing cycles or modified protocols may improve results.

8.2 Storage Recommendations

Long-term storage of FTA cards requires appropriate environmental conditions. The cards should be maintained in a dry, stable environment, ideally with desiccant packs to control humidity. Storage in sealed containers or bags further protects against moisture exposure. Cards should be protected from direct light, UV exposure, and extreme temperatures. Regular monitoring of storage conditions, particularly humidity, is

recommended (Smith et al., 2006; Zhong et al., 2011).

For extended storage, consideration should be given to dividing samples across multiple cards or storage locations. This approach reduces the risk of sample loss due to accidental damage or environmental incidents and enables testing of a subset while preserving remaining material for future analysis.

8.3 Assay Design Considerations

When designing PCR assays for FTA-preserved DNA, researchers should prioritize short amplicons to maximize the probability of successful amplification from aged samples. Amplicons of 150-300 base pairs generally show high success rates, while targets exceeding 500 base pairs may fail, particularly after extended storage (Wan et al., 2012). For applications requiring multiple targets, designing assays with overlapping or nested strategies may improve success rates.

Optimization of PCR conditions, including magnesium concentration, annealing temperature, and cycling parameters, can improve amplification success from FTA-derived templates. Including positive and negative controls in each run helps verify amplification performance and identify potential issues with individual samples.

9. Conclusion

The preservation of DNA integrity from sample collection through analysis remains a fundamental challenge in molecular biology, clinical diagnostics, and forensic science. FTA card technology has emerged as a practical solution to this challenge, enabling room-temperature DNA preservation through a combination of chemical protection, nuclease inactivation, and physical immobilization within a cellulose matrix. The extensive literature reviewed in this article demonstrates that DNA preserved on FTA cards can remain suitable for PCR amplification for extended periods, particularly when maintained under dry, stable conditions and when target amplicons are short in length.

The empirical evidence supporting FTA technology has significant practical implications for diverse applications. In forensic contexts, FTA cards enable evidence collection and archiving without cold-chain requirements, facilitating casework in resource-limited settings and allowing retrospective analysis of cold cases. In clinical and epidemiological applications, FTA cards enable population-based studies in remote areas, surveillance of infectious diseases, and biobanking of samples for future analysis. In research settings, the technology provides a cost-effective approach to sample collection and storage, particularly for large-scale studies where freezer infrastructure would be prohibitively expensive.

Housekeeping gene PCR, particularly using β-actin and 18S rRNA targets, has proven valuable for quality assessment of FTA-preserved DNA. The characteristics of amplified bands on agarose gels—intensity, sharpness, and size—provide practical indicators of DNA integrity and amplification success. Time-course studies using these methods have documented degradation kinetics and identified factors that influence preservation quality.

Despite the substantial body of supporting evidence, important gaps remain in our understanding of long-term DNA stability on FTA cards. Extended time-series studies, validation of advanced applications, investigation of field conditions, and development of standardized protocols are needed to establish comprehensive guidelines for the technology's use. Nevertheless, current evidence supports FTA cards as a valuable tool for DNA preservation, particularly in resource-limited settings where cold chain maintenance is challenging.

The practical implementation of FTA technology requires attention to proper collection, drying, storage, and processing protocols. Careful assay design, prioritizing

short amplicons, improves the likelihood of successful amplification from aged samples. With appropriate precautions, FTA cards provide a cost-effective, reliable approach to long-term DNA preservation that can significantly expand access to molecular testing in underserved regions.

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Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this article. The review was conducted independently, and no commercial organization influenced the interpretation or reporting of the findings.

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