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Quantitative PCR analysis of the decay and recovery of biothreat agents during sample storage.

William E. Lee, Thompson Tang and Douglas E. Bader
Defence R&D Canada – Suffield

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Abstract

Real-time quantitative polymerase chain reaction (PCR) is widely employed for detection and identification of genetic markers in DNA-containing samples. This report describes methods to use quantitative PCR in conjunction with reaction rate kinetics and thermodynamics to determine recovery of sample material from a commercially available storage medium, FTA® cards. The report shows practitioners of PCR a method to assess analyte recovery, plus the kinetic and thermodynamic parameters associated with sample decay during storage.

Résumé

L'amplification en chaîne par polymérase (PCR) quantitative en temps réel sert couramment à la détection et à l'identification de marqueurs génétiques dans des échantillons contenant de l'ADN. Le présent rapport traite des méthodes d'utilisation de la PCR quantitative en combinaison avec la thermodynamique et la cinétique des réactions en vue d'évaluer la récupération d'échantillons depuis un milieu de stockage commercial, soit les cartes FTA^{MD}. Il vise à enseigner aux spécialistes de la PCR une méthode d'analyse de la récupération d'analytes, en plus des paramètres cinétiques et thermodynamiques connexes à la détérioration des échantillons durant le stockage.

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Executive summary

Quantitative PCR analysis of the decay and recovery of biothreat agents during sample storage.

William E. Lee, Thompson Tang and Douglas E. Bader;
DRDC Suffield TM 2009-279; Defence R&D Canada – Suffield; December 2009.

Introduction: Polymerase chain reaction (PCR) is a widely used gene amplification technique for detection and identification of genetic markers found within biothreat agents. Sample storage can play a role in the detection and identification processes when a sample is collected and not analysed immediately but rather stored for a period that may extend from of hours to years. The problem of sample storage of biothreat agents was the subject of a trinational research project comprising Canada (DRDC Suffield), Sweden (Swedish Defence Research Agency, FOI) and the Netherlands (TNO Defence, Security and Safety). In that body of work a solid state matrix, commercially available FTA® cards, was evaluated for storage of DNA from *Bacillus anthracis* prior to PCR amplification. The work in this report provides a theoretical model based on quantitative PCR, reaction rate kinetics and thermodynamics to quantify the storage process.

Results: The report contains an overview of real-time quantitative PCR and thermodynamics in order to foster a better understanding of real-time PCR data. The derivations describe a method for obtaining relevant parameters for recovery of DNA from FTA® cards and the decay of the DNA during storage. Such knowledge is useful for assessing the stability of stored analytical materials. In this work the stability of the DNA template (i.e., analyte material) for PCR was studied, however the theoretical models can be applied to reagents used in PCR or in other bio-analytical techniques. The methods are also useful for designing accelerated storage experiments.

Significance: Since sample storage is a component of the detection and identification process, easy to use mathematical models are useful for developing protocols and standard operating procedures for stability (shelf-life) of samples and reagents for fieldable analysis systems for biothreat agents.

Sommaire

Analyse PCR quantitative de la détérioration et de la récupération d'agents de bioterrorisme durant le stockage

William E. Lee, Thompson Tang et Douglas E. Bader
RDDC Suffield TM 2009-279; R & D pour la défense – Suffield; novembre 2009

Introduction : L'amplification en chaîne par polymérase (PCR) est une technique d'amplification du gène couramment utilisée pour détecter et identifier des marqueurs génétiques dans des agents de bioterrorisme. Le stockage peut influer sur les processus de détection et d'identification d'un échantillon lorsque ce dernier n'est pas immédiatement analysé, mais plutôt stocké pendant une période pouvant aller de quelques heures jusqu'à des années. Le stockage d'échantillons d'agents de bioterrorisme a été le sujet d'un projet de recherche trinational mené par le Canada (RDDC Suffield), la Suède (Swedish Defence Research Agency - FOI) et les Pays-Bas (TNO Defence, Security and Safety). Dans le cadre du projet, nous avons évalué une matrice à semi-conducteurs (soit des cartes FTA^{MD} commerciales) en tant que méthode de stockage d'ADN provenant de *Bacillus anthracis* avant la PCR. Les travaux décrits dans le présent rapport offrent un modèle théorique fondé sur la PCR quantitative, ainsi que sur la thermodynamique et la cinétique des réactions pour quantifier le processus de stockage.

Résultats : Le rapport comporte un aperçu de la PCR quantitative en temps réel et de la thermodynamique en vue de favoriser une meilleure compréhension des données PCR en temps réel. Les dérivations traitent d'une méthode d'obtention de paramètres pertinents pour la récupération d'ADN depuis des cartes FTA^{MD}, ainsi que la détérioration de l'ADN durant le stockage. Ces connaissances sont utiles pour évaluer la stabilité de matières analytiques stockées. Bien que dans le cadre des travaux nous ayons étudié la stabilité d'une matrice d'ADN (matière analytique) aux fins de la PCR, les modèles théoriques peuvent être appliqués à des réactifs utilisés dans d'autres techniques bioanalytiques. Les méthodes sont également utiles pour la conception d'expérimentations de stockage accéléré.

Portée : Le stockage d'échantillons fait partie du processus de détection et d'identification. C'est pourquoi les modèles mathématiques simples sont utiles sur le plan de l'élaboration de protocoles et de procédures d'utilisation normalisées (SOP) favorisant la stabilité (durée de conservation) d'échantillons et de réactifs pour les systèmes d'analyse utilisables sur le terrain qui visent les agents de bioterrorisme.

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Introduction

Incidents that constitute a health or security threat such as bioterrorism, biological warfare or natural outbreaks of disease require the collection of samples containing (or suspected to contain) pathogenic agents. Such samples would be stored for subsequent analysis and likely archived for use at later times as well. Storage could extend from hours to years. Polymerase chain reaction (PCR) is a widely used gene amplification technique for detection and identification of genetic markers found within biothreat agents. Although the current PCR instrumentation permits real-time on-site amplification and identification, additional biothreat analysis can occur "behind the scenes" in laboratories. Thus sample collection and storage are important issues that affect the physical condition of analytes and the overall results of identification analysis.

FTA® cards are commercially available sample storage media made from a chemically treated fibre matrix. They are routinely used in research, clinical and forensic laboratories to collect, store and transport biological samples of blood, saliva, tissue, cells or microorganisms [1, 2]. Samples can be applied as water-based liquid or smear, and according to manufacturer's literature, after the moisture dissipates, nucleic acids are immobilized and protected from nuclease, oxidation or UV damage. The convenience and ease of use provided by FTA® cards in handling samples destined for genetic analysis led our laboratory and partners in Sweden (Swedish Defence Research Agency, FOI) and the Netherlands (TNO Defence, Security and Safety) to evaluate FTA® cards for laboratory and/or field-based PCR [3, 4]. A recent report from our laboratory [1] described the thermal stability of biothreat samples in solution and on solid-phase FTA® matrix. In that work, FTA® cards were evaluated for convenience and reliability as a medium to maintain the integrity of genetic material for subsequent PCR amplification. The samples were stored for up to 4 weeks at temperatures from -20 to 50 ^{o}C. The present work builds upon this study using the principles of quantitative PCR [6–8] and reaction kinetics [9] to extract recovery and decay rates of the samples during storage. The data used herein was obtained from PCR amplification of genomic DNA from *Bacillus anthracis,* stored in liquid medium and on solid-phase FTA® cards [5]. The analysis presented is applicable to all storage methods and gene targets. The analytical approach is useful for validating standard methods for sample collection and storage.

Real-time quantitative PCR is widely employed in research in conjunction with commercial PCR thermocycler instruments that monitor PCR product by fluorescence intensity [10–12]. The instrument uses computer software to display the progress of the PCR reaction in amplification plots of fluorescence intensity versus cycle number (see Figure 1, top panel). This figure is an example of PCR data and shows the important features of the plot needed for quantitative PCR analysis [13]. The baseline is defined as the intensity of the PCR reaction prior to formation and accumulation of measureable amounts of amplicon. By default, the software of the PCR instrument determines the baseline from the fluorescence intensity during cycles 3 to 15, although it can be set manually if needed [14,15]. In the early cycles of the PCR reaction, the fluorescence intensity is not significantly different than the baseline. The threshold is an arbitrary intensity selected by the software, based on the variability of the baseline. It is usually set as the mean baseline plus 10 times the standard deviation of the mean. Ct is the interpolated (fractional cycle number) at which the fluorescence intensity surpasses the threshold. Ct is the principal determinant in quantitative PCR.

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Experimental Section

Materials and methods

Detailed experimental methods are provided elsewhere [5]. Purified DNA from *Bacillus anthracis* was spotted onto Whatman FTA® cards, air-dried, placed inside a multi-barrier pouch along with a desiccant package and stored at specific temperatures from -20 to +50 ^{o}C for 29 days. Simultaneously, purified DNA in liquid medium was subjected to similar storage conditions.

For PCR analysis of the samples on FTA® cards, 1.2 mm (diameter) discs were punched from the spotting zone. Experimental controls provided direct comparison by weight of DNA on the card and in the solution sample. The amount of DNA per run was 4 ng for the liquid sample and 2 ng for the disc punched from the FTA® card.

PCR analysis

The PCR assay evaluated in this study was directed against the lethal toxin (*lef*) gene on the pXO1 plasmid of *B. anthracis* [5]. Prior to PCR analysis, samples were retrieved from their storage locations, placed in the biosafety cabinet, and equilibrated to room temperature. Three PCR runs were performed for each storage time point. For PCR analysis of liquid sample, 1 µL of sample was added to a Smart Cycler® tube containing 25 µL of 1x PCR mastermix. For FTA® cards, a 1.2 mm disc was punched out, placed into a 1.5 mL Simport tube and then processed according to the procedure described previously [5].

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Results and Discussion

The PCR process is described by the following equation

$$Nc = (E+1)^c N_0 \quad (1)$$

where c is the number of thermocycles, E is the amplification efficiency, Nc is the number of amplicon molecules and N_0 is the initial number of target analyte molecules. The efficiency of PCR is high, approximately 0.9, so equation 2 can be approximated as

$$Nc = 2^c N_0 \quad (2)$$

Thus the number of DNA amplicons effectively doubles with each cycle.

The progress of PCR amplification can be monitored by fluorescent dyes or fluorogenic probe molecules whereby the increase in Nc in the reaction mixture is proportional to the increase in probe fluorescent intensity. In our laboratory we employ two amplicon detection chemistries: the intercalating dye, SYBR Green I, and fluorogenic hydrolysis probes (Taqman) [16]. SYBR Green dye binds preferentially to double-stranded (ds) DNA and fluoresces upon excitation by a light source when bound to dsDNA. The greater the number of ds amplicons, the greater the fluorescence signal relative to the background. Taqman probe detection chemistry utilizes a gene probe that is complementary to the amplicon sequence. The probe contains a fluorescent label and a fluorescent quencher. The probe in the amplicon/probe ds hybrid molecule is enzymatically cleaved, which separates the fluorescent label from the quencher, allowing the label to fluoresce upon excitation with a light source. In each case the effect is the same on fluorescence intensity (I).

$$I \propto Nc \quad (3)$$

In this work, data was obtained using Taqman chemistry, but the analysis is applicable to Taqman and SYBR Green or any other amplification method adhering to equations 1-3.

In quantitative PCR, individual amplification reactions are compared at a point of equal fluorescence intensity and thus equal numbers of DNA amplicons [8]. This point on the curve is the threshold intensity. The number of thermocycles that are required to achieve this intensity is Ct (see Figure 1, upper panel). Therefore in Figure 1, for all amplification curves at threshold the number of amplicons, Nc, is equal even though the specific number of amplicons may be undetermined. Plots such as those shown in Figure 1 are well known and form the basis of quantitative PCR [7].

Equation 2 can be rewritten as

$$N_0 / N_{Ct} = 2^{-Ct} \quad (4)$$

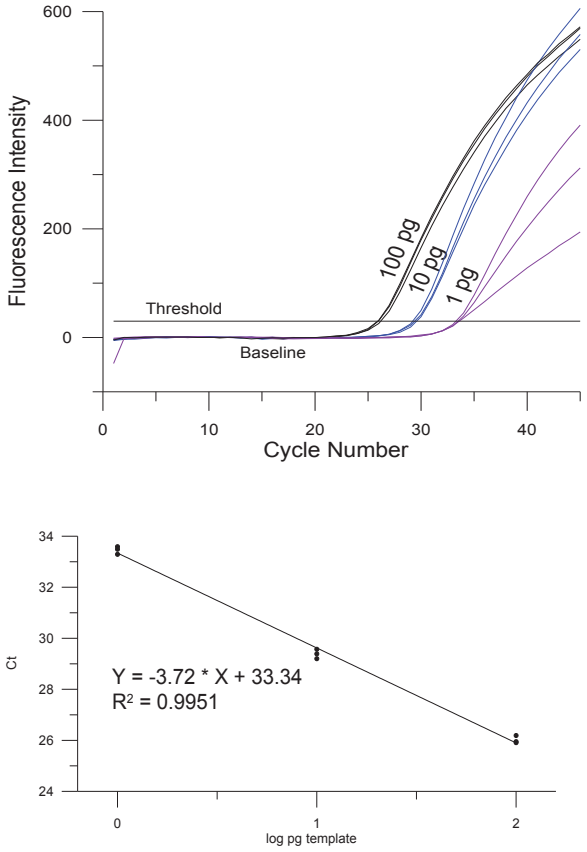

Figure 1 : Example of real-time PCR: target is genomic DNA from Y. pestis strain CO92 amplified and detected using ypo2130 primer/probe set (D.E. Bader and G.R. Fisher unpublished data).

The top panel of Figure 1 shows overall fluorescence signal (arbitrary units) during the amplification process of serial diluted DNA target. The threshold is set (usually by the instrument software) at a signal level above baseline. Ct is the fractional cycle number where the fluorescence intensity surpasses the baseline. The bottom panel shows the linear relationship between Ct (threshold number) and logarithm of starting number of templates (copy number). The Ct values from the top panel are plotted against the log of amount of nucleic acid template used.

Clearly, a plot of Ct versus log N_0 (as in Figure 1, lower panel) will be linear [7], and the greater the initial number of genetic target molecules the fewer amplification cycles required to generate the threshold level of intensity and hence the threshold level of amplified products. Actual quantitation of amplicon number, if required, can be made using a standard curve.

Storage and degradation of biothreat samples

The amounts of DNA used in PCR in the liquid samples and on the 1.2-mm disks punched from the FTA® cards were 4 and 2 ng, respectively.

The results of the PCR amplifications are shown in Figures 2 to 5, wherein the threshold cycle, Ct, is plotted against days of storage for FTA® samples and for liquid samples at the indicated storage temperature. The general features of the plots show that greater number of threshold cycles (Ct) were required for the FTA® samples. The Ct values increased slightly after storage at -20 to +7 ^{o}C, but there was a marked increase of Ct values after storage at 50^{o}C.

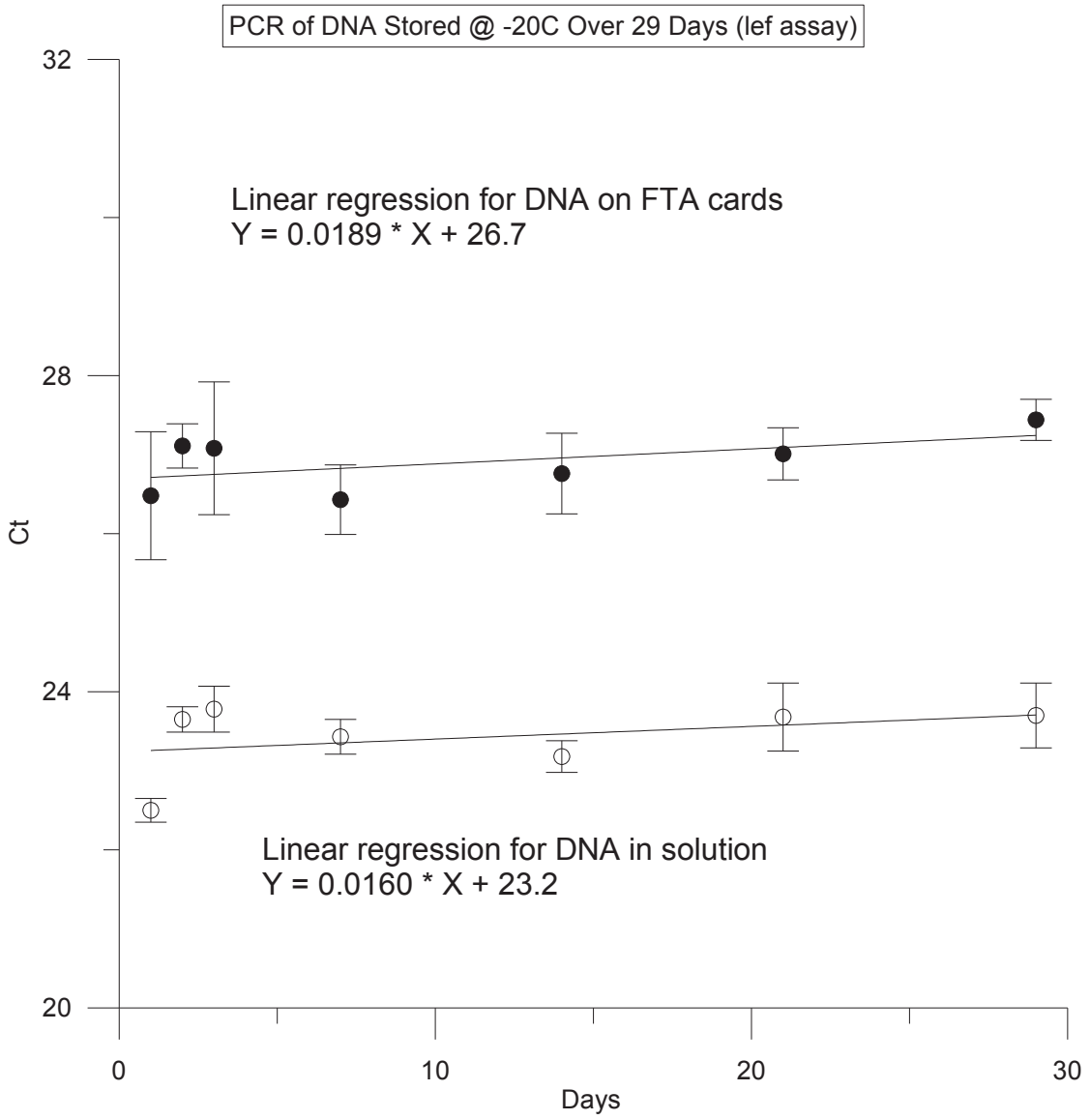

Figure 2 : Plot of threshold cycle number (Ct) versus storage time for purified DNA stored at -20 ºC over 29 days. The target of the PCR amplification was the lef gene on the pXO1 plasmid of B. anthracis.

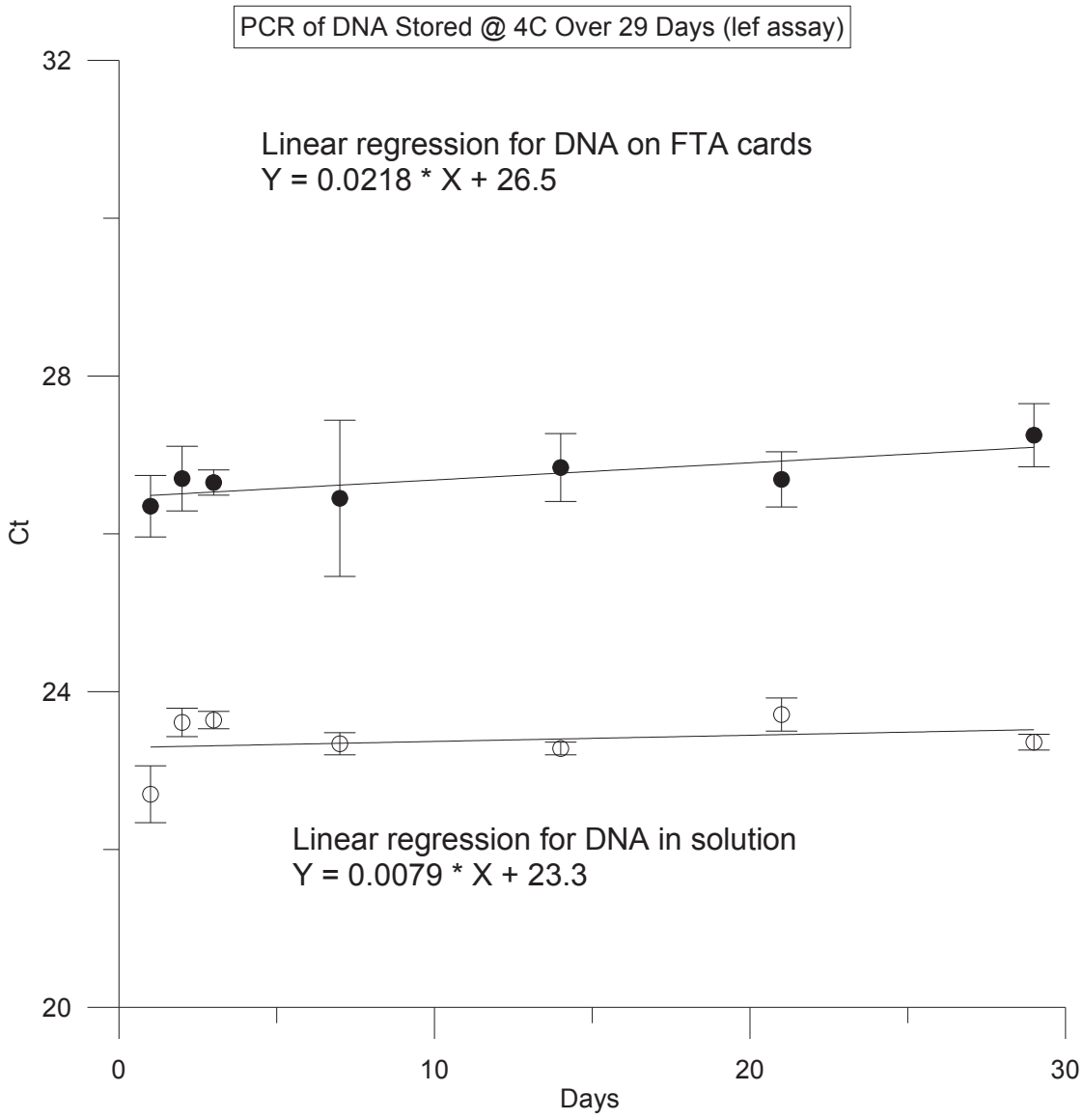

Figure 3 : Plot of threshold cycle number (Ct) versus storage time for purified DNA stored at 4 ºC over 29 days. The target of the PCR amplification was the lef gene on the pXO1 plasmid of B. anthracis .

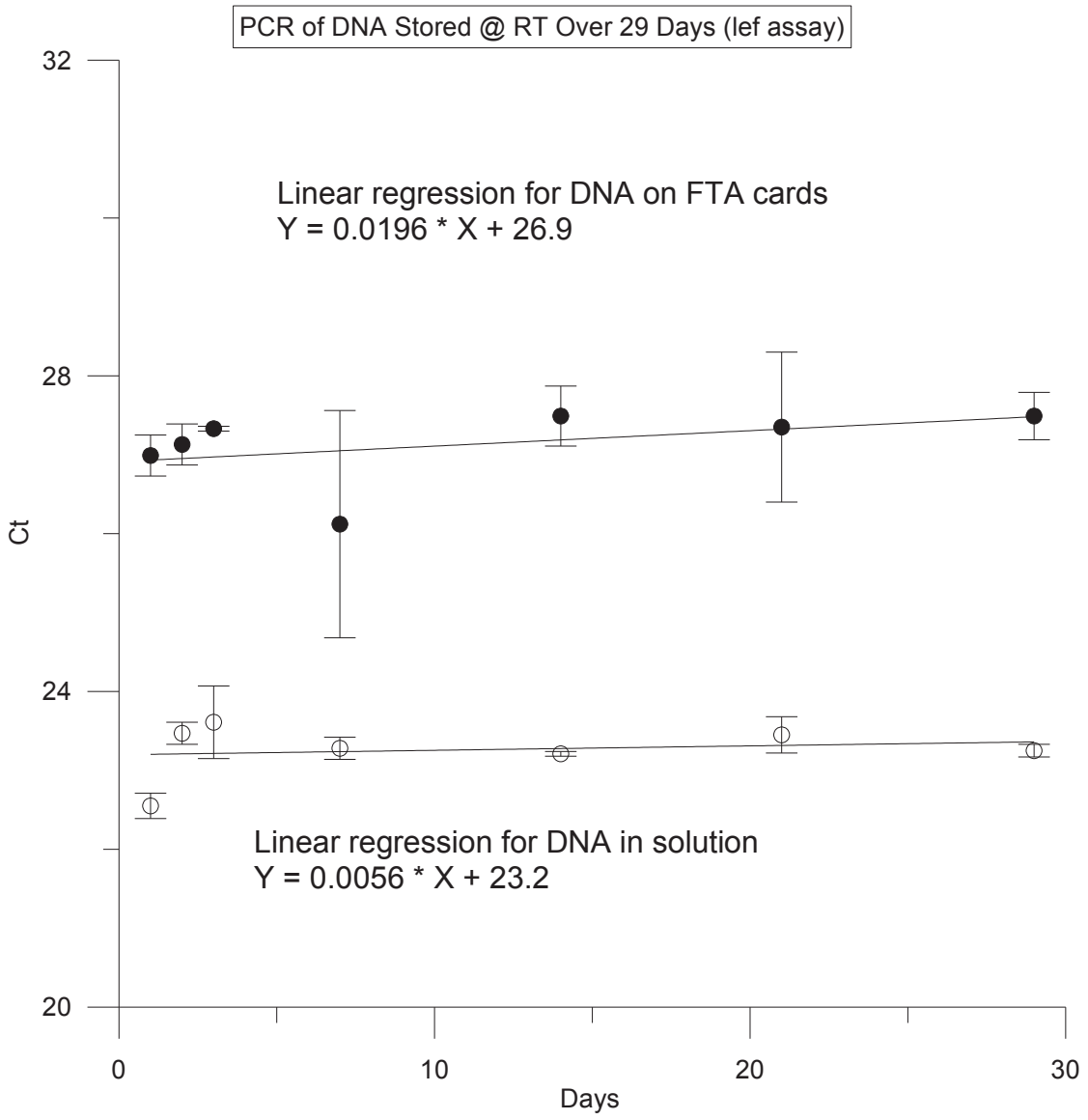

Figure 4 : Plot of threshold cycle number (Ct) versus storage time for purified DNA stored at room temperature (ca. 25 ºC) over 29 days. The target of the PCR amplification was the lef gene on the pXO1 plasmid of B. anthracis.

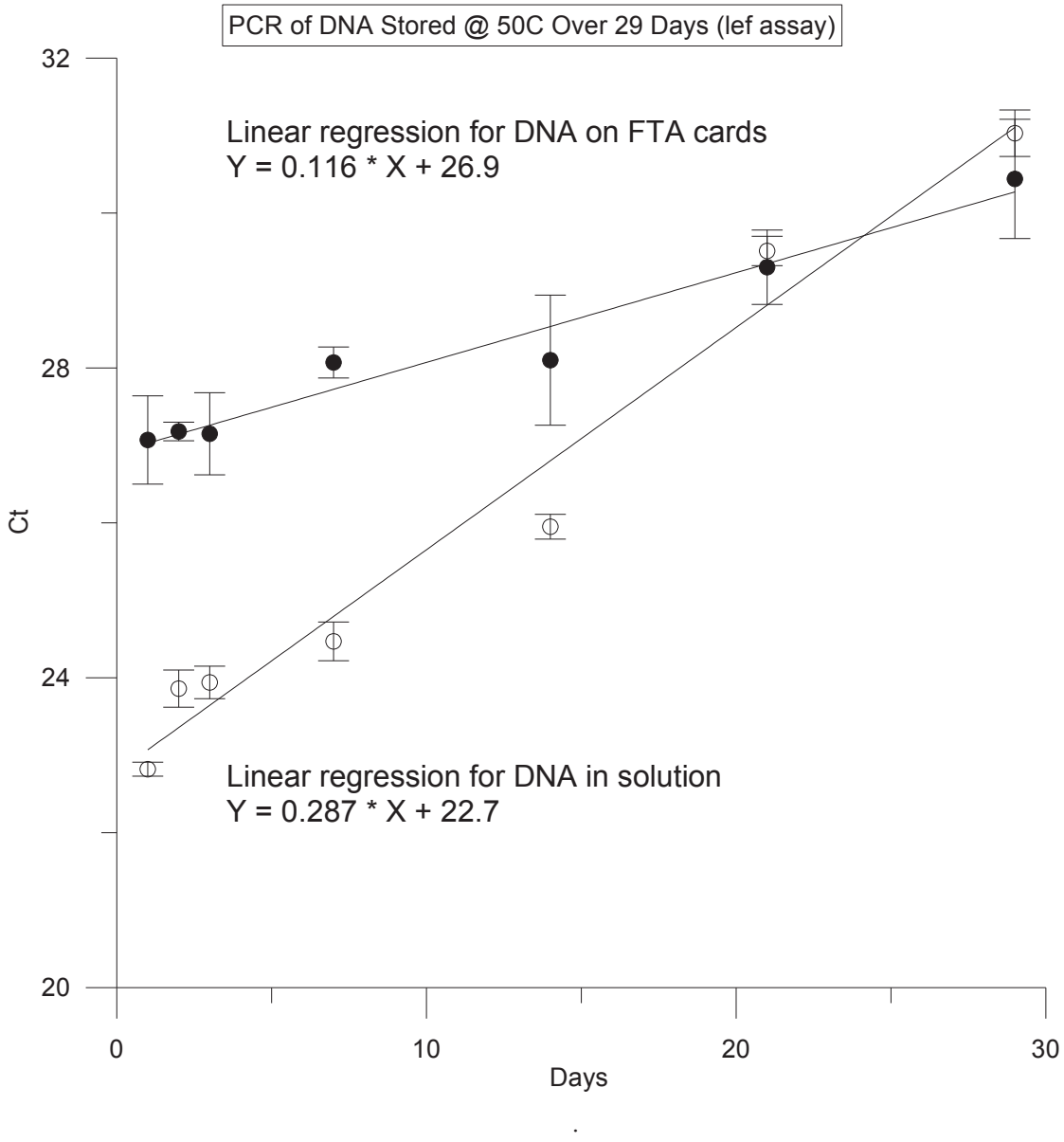

Figure 5 : Plot of threshold cycle number (Ct) versus storage time for purified DNA stored at 50 ºC over 29 days. The target of the PCR amplification was the lef gene on the pXO1 plasmid of B. anthracis

Recovery of DNA from solid state matrix

For the recovery of DNA template from the FTA® cards consider Figure 3 (4 °C). On day 1, Ct was 22.7 and 26.3 for DNA in solution and for DNA on the FTA® card, respectively. We know that the final amounts of amplicon DNA were equal at threshold and that prior to storage the initial amounts of DNA were in known ratio of 2 (liq/FTA®).

Thus the difference in Ct values reflects the overall recovery of target from the FTA® card matrix. Using equation 2 we write

for solution $Nc_{sol} = N_{0sol} \cdot (2)^{Ctsol}$

and

for FTA® $Nc_{FTA} = N_{0FTA} \cdot (2)^{CtFTA}$

Since by definition $Nc_{liq} = Nc_{FTA}$

$$N_{0sol} \cdot (2)^{Ctsol} = N_{0FTA} \cdot (2)^{CtFTA}$$

The ratio of starting template molecules of FTA® disc to liquid sample is

$$N_{0FTA} / N_{0sol} = (2)^{Ctsol} / (2)^{CtFTA} = 2^{\Delta Ct}$$

$$= 2^{(Ctsol - CtFTA)} = 2^{(22.7-26.3)} = 2^{(-3.6)} = 0.082$$

Since the amount of DNA spotted on the FTA® card was half that of the liquid DNA sample (4 vs 2 ng), the actual recovery is twice the above, 0.164 or 16.4%.

The same calculation of recovery can be made directly from the linear regression analysis by the difference in y-intercept. For example using the 4 °C storage data (Figure 3)

$$\Delta Ct = 23.29 - 26.46 = -3.17$$

The ratio of starting template molecules on the FTA® disc to liquid sample is

$= 2^{-3.17} = 0.11$. The actual recovery (as above) is double this value: 0.22 or 22%.

Calculations of recovery for all temperatures are summarized in Table 1.

Table 1 : Recovery of purified B. anthracis DNA template from FTA® cards and decay of template during storage

			% Recovery of template from FTA®						First order decay		
Temperature			From day1 data			From intercept			k (day^{-1})		
°C	K		ΔCt	%recv		ΔCt	%recv		FTA®	solution	
-20	253		3.97	12.8		3.5	17.6		0.013	0.011	
4	277		3.60	16.4		3.17	22		0.015	0.0054	
25	298		4.93	9.2		3.7	15.4		0.014	0.0039	
50	323		4.25	6.6		4.1	11.4		0.080	0.20	

Decay of DNA template during storage

Typical decay processes exhibiting first order or pseudo-first order kinetics include radioactive decay, radiative (fluorescence) decay and uni-molecular chemical reactions. Such processes are described by

$$A(t) = A_0 \exp e^{-kt} \qquad (5)$$

where A(t) is concentration at time t, A_0 is initial concentration, k is the first order rate constant and t is time [9]. Thus, a plot of log $(A(t)/A_0)$ vs t should be linear having a slope -(k /2.303).

Inspection of Figure 5 (50 °C) shows a positive slope for the plots of Ct vs t, indicating that N_0 (the initial amount of template in each PCR run) decreased over the 29 day period. We compare day 1 and day 29 for DNA in solution using equation 4.

Day 1: $N_{0day1} \times 2^{Ctday1} = Nct$

Day 29: $N_{0day29} \times 2^{Ctday29} = Nct$

$$N_{0day29} / N_{0day1} = 2^{\Delta Ct} = 2^{(22.8 - 31.0)} \qquad (7)$$

$$= 2^{-8.2} = 0.0034$$

Using equation 7 we can calculate the N_0 values (normalized to day 1) for the solution sample stored at 50 °C. The N_0 values for each day are given in Table 2 and plotted as a decay curve in Figure 6. Linear regression of the plot gives a slope of 0.92×10^{-2}. Returning to equation 6

slope = -k/2.303

k = $0.92 \times 10^{-2} \times 2.303$ = 0.20/day

Comparison of the Figure 6 decay plot with the corresponding plot in Figure 5 (open circles) shows similarity, although the y-axis is inverted

From equation 6 we write

$$\ln(A(t)/Ao) = -k \cdot t \qquad (6)$$

or in terms of starting template (starting copy number)

$$\ln(N_{0day\text{-}x} / N_{0day1}) = -k \cdot t$$

$$\log_2 (N_{0day\text{-}x} / N_{0day1}) = -k \cdot t/\ln 2$$

Substituting for ln2 we write

$$\text{Log}_2 (N_{0day\text{-}x} / N_{0day1}) = -k \cdot t/\ln 2 = -k \cdot t/(0.693)$$

The slope of the plot in Figure 5 for the solution sample at 50 ^{o}C is -0.287.

Thus, slope = -k / (0.693) = -0.287

k = 0.287 x (0.693) = 0.20/day

Figure 6 and the subsequent determination of k were based on the normalized N_0 values in Table 2, which were extracted from the original data in Figure 5. Upon closer consideration, with the help of the kinetic analysis, it becomes apparent that the Ct plot in Figure 5 and the decay curve in Figure 6 are essentially the same. Figure 5 has a base-2-logarithm, Figure 6 base-10. Thus the fact that the decay rate constant calculated from each is the same is to be expected. However without this analysis, and for many researchers who practice quantitative PCR, these relationships are not obvious.

Table 1 summarizes the decay rates for the liquid samples and the FTA®-spotted samples stored at temperatures -20 to +50 ^{o}C determined from the plots in Figures 2-5. Overall the plots can be expressed as first order kinetics. For storage temperatures -20 ^{o}C to room temperature (approximately 25 ^{o}C) samples are relatively stable over the storage period. There was a small amount of decay of analyte template. For the FTA® cards the recovery of template was 10-20% but additional losses due to storage were minimal. At 50 ^{o}C storage, the recovery from the FTA® card was within the same range, 10%, but the decay of template was significant. Decay of the solution sample was significantly greater than FTA®, so after about 25 days more template was available from FTA® even though the recovery was only about 10%.

Table 2 : Determination of starting copy number of purified B. anthracis DNA template (normalized to day 1) versus storage time for solution sample at 50 °C

day	Ctday-n – Ctday-1	N_{0day-n} / N_{0day-1}	log (N_{0day-n} / N_{0day-1})	
1	0	1	0	
2	-1.0	0.50	-0.30	
3	-1.1	0.47	-0.33	
7	-1.7	0.31	-0.51	
14	-3.0	0.0125	-0.90	
21	-6.7	9.62×10^{-3}	-2.02	
28	-8.2	3.40×10^{-3}	-2.47	

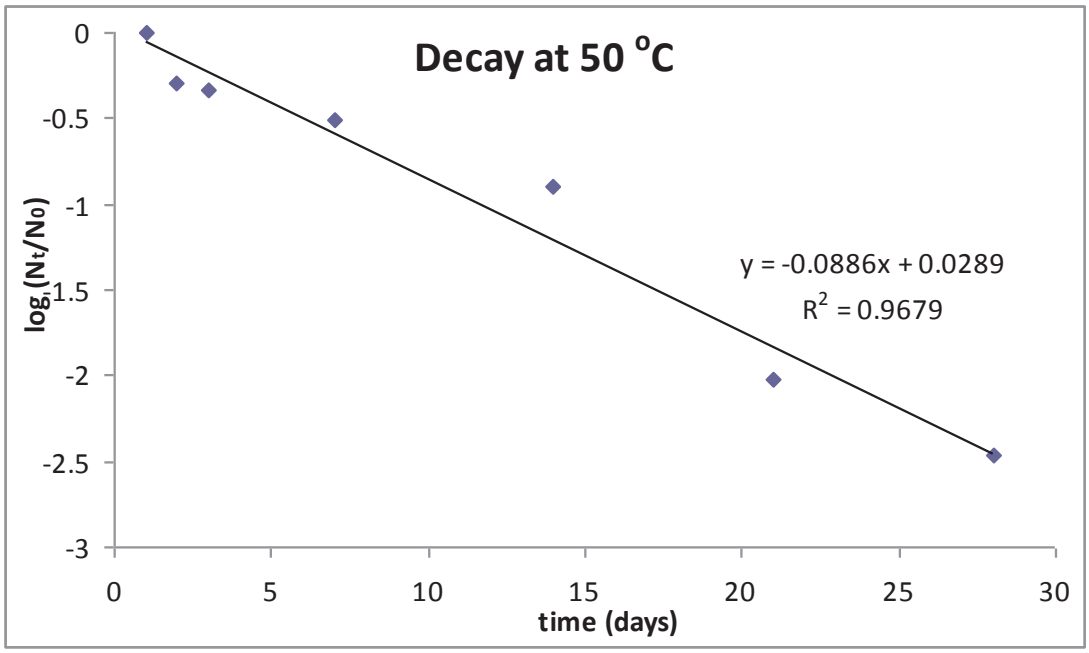

Figure 6 : Decay plot of solution sample of purified B. anthracis DNA stored at 50 oC: log (N0day-n / N0day-1) vs time (days). The y-axis gives the ratio of the number of starting templates on day n vs number on day 1. The x-axis is the storage time. Data is from Table 2.

Thermal stability and activation energy

In the above sections we were able to use the threshold cycle number in PCR, Ct, to determine decay rate constants of analyte template during storage. The model employed first order kinetics

$$D \xrightarrow{k} P$$

where D is the intact analyte template, P represents products, in this case degraded DNA target that no longer functions as template in the PCR process, k is the rate constant (same k as above, equation 5). The Arrhenius equation provides a simple formula to express the dependence of the rate constant of a chemical reaction on the temperature [9].

$$k = Ae^{-(\Delta E/RT)} \qquad (8)$$

where A is a pre-exponential frequency factor having dimension of reciprocal time (such as sec^{-1} or day^{-1}, ΔE is the activation energy, R is the gas constant and T is absolute temperature (Kelvin).

From equation 8 we can write

$$\log k = \text{Log } A - \Delta E/(2.303R \cdot T) \qquad (9)$$

A plot of log k vs 1/T is predicted to be linear (i.e., y = mx + b), slope = $-\Delta E/(2.303R)$, y-intercept = log A. The Ct plots in Figure 2-5 provide sufficient information to solve the Arrhenius equation for thermodynamic parameters associated with the stored samples.

Figure 7 shows plots of log k (from Table 1) vs 1/T for DNA stored in liquid and on FTA® cards. Activation energies and the frequency factors derived from equation 9 are shown in Table 3. The plots tell us that the activation energy in solution was higher than on FTA® card, 58.1 vs 18.5 kJ mol^{-1}, and that the frequency factor for DNA in liquid was also higher 8.1×10^8 vs 1.4×10^2 day^{-1}.

Table 3 : Storage stability parameters for purified B. anthracis from Arrhenius plots DNA

Sample Storage	Activation Energy	Pre-exponential frequency factor
	ΔE (kJ mol^{-1})	A (day^{-1})
FTA®	18.5	1.4×10^2
solution	58.1	8.1×10^8

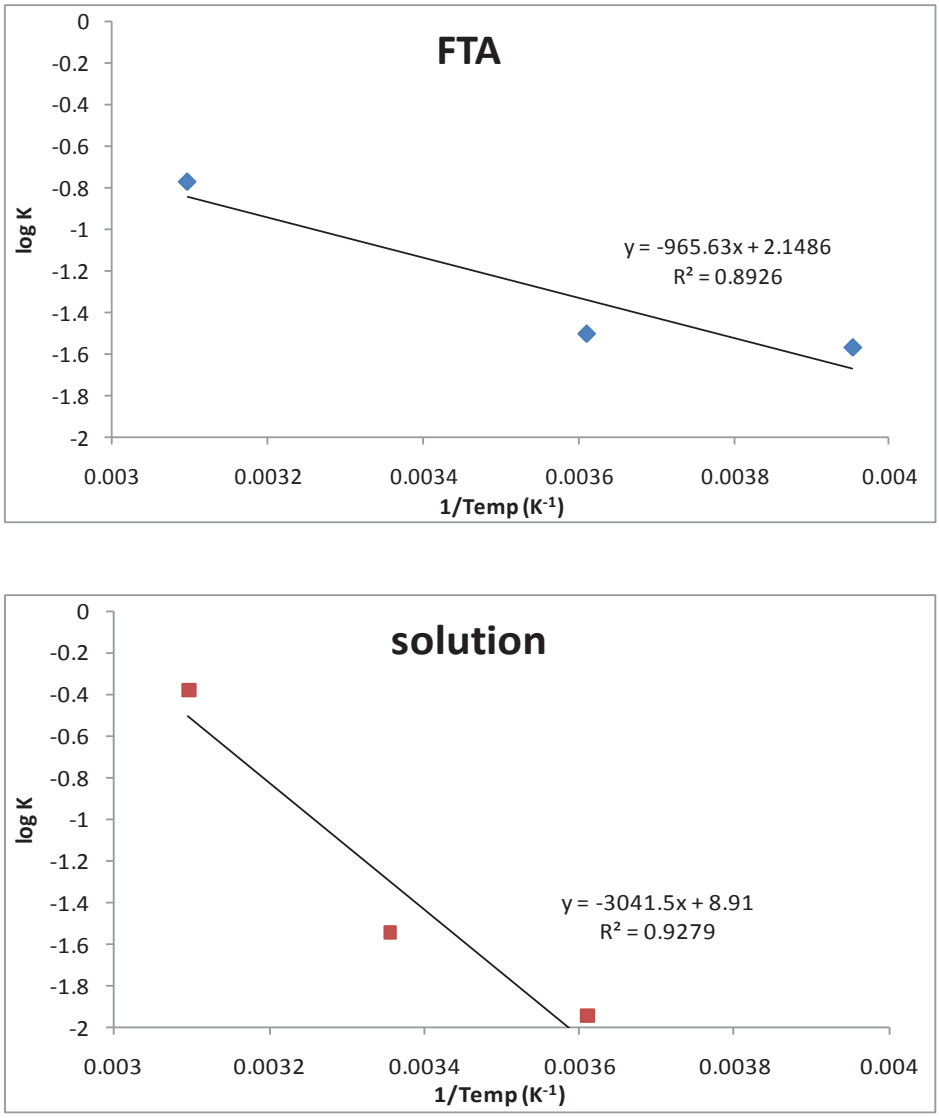

Figure 7: Arrhenius plots of rate constants vs 1/T. The rate constants for decay of purified B. anthracis DNA were derived from the data in Figures 2 to 5. Top panel, samples stored on FTA® cards; bottom panel, samples stored in solution.

Knowledge of the parameters in equation 9 allows calculation of the rate constant over a wide range of temperatures. This is useful in predicting the storage stability. Many substances such as analytes and reagent formulations for bioassays are stable over the medium time run, e.g., one year, especially when refrigerated. Predicting the stability for long term unrefrigerated storage is important in developing assay protocols. It is also highly inefficient to run test and evaluation experiments for up to five years. Arrhenius theory assists in the storage stability problem by allowing accelerated storage experiments whereby storage at elevated temperature increases the decay rates (i.e., the breakdown) of analytes or reagents undergoing storage. Arrhenius theory provides the method to correlated accelerated high temperature storage data to typical temperature that would be experienced in normal use.

In this work the efficiency of the PCR process was approximated as 1. Such approximation simplified the derivations that followed equation 1. However if desired, the efficiency of the specific PCR conditions used can be determined from a standard curve such as shown in Figure 1 (lower panel) [16, 17]. Derivations that follow for recovery and decay rates can then be made for efficiency, E, less than 1.0.

Conclusions

The purpose of this work was to illustrate that quantitative PCR, such as that given in this report and elsewhere, can yield a breadth of physical parameters when subjected to physical chemical analysis. The previous report [5] examined the use of and advantages of solid matrix FTA® cards for storing biothreat agents and genomic DNA for later PCR analysis. This report describes a method to extract relevant physical parameters associated with the storage process. Such method allows comparisons of starting template, recovery, and storage stability to be made independently of instrument, storage devices, operator and other variables.

Although this work contains numerous derivations and kinetic plots which are given for didactic purposes, the basic analytical methods are simple and can readily be implemented from the initial PCR data obtained directly from the PCR instrument, which in this case was the Cepheid Smart Cycler. The same methods can be used with any PCR instrument that provides Ct data. In Figure 2-5, recovery of sample from a solid-phase card (or from any other storage device) compared to the control sample of DNA template is obtained from ΔCt at t = 0 or day 1. The decay rate is obtained from the slope of Ct vs time, where decay rate, k = slope/0.693. If PCR samples were stored over a range of temperatures then a plot of log k vs (1/T) gives activation energy (ΔE) and Arrhenius constant (A).

Sample collection and storage are important issues for detection and identification of biothreat agents. Together, quantitative PCR, reaction kinetics and Arrhenius theory provide a powerful analytical method for extracting physical parameters of sample recovery and sample decay. Furthermore these concepts provide a frame of reference for designing experiments to characterize the processes and reactions that occur during sample storage. Since sample storage is an essential component of detection and identification, the more that is known about the physical processes that occur during sample storage the better will be the performance of such analytical systems.

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Real-time quantitative polymerase chain reaction (PCR) is widely employed in research and biotechnology for detection and identification of genetic markers in DNA-containing samples. This report describes methods to use quantitative PCR in conjunction with reaction rate kinetics and thermodynamics to determine recovery of sample material from a commercially available storage medium, FTA® cards. The report shows practitioners of PCR a method to assess analyte recovery, plus the kinetic and thermodynamic parameters associated with sample decay during storage.

L'amplification en chaîne par polymérase (PCR) quantitative en temps réel sert couramment à la détection et à l'identification de marqueurs génétiques dans des échantillons contenant de l'ADN. Le présent rapport traite des méthodes d'utilisation de la PCR quantitative en combinaison avec la thermodynamique et la cinétique des réactions en vue d'évaluer la récupération d'échantillons depuis un milieu de stockage commercial, soit les cartes FTA^{MD}. Il vise à enseigner aux spécialistes de la PCR une méthode d'analyse de la récupération d'analytes, en plus des paramètres cinétiques et thermodynamiques connexes à la détérioration des échantillons durant le stockage.

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Real-time polymerase chain reaction kinetics

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