BECKMAN COULTER

P/ACE Setter

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The worldwide newsletter for capillary electrophoresis

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PACE Setter

The worldwide newsletter for capillary electrophoresis

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CE-SSCP Screening for BRCA1 and BRCA2—Allelic Mutations Associated with Breast Cancer

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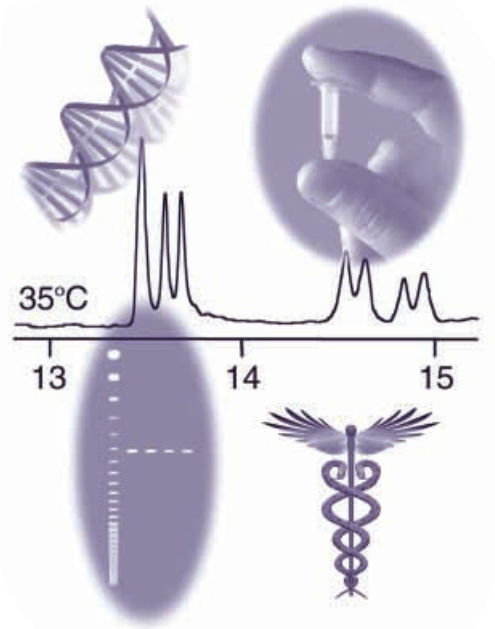
Cancer Susceptibility Genes

Extensive studies on hereditary factors of breast and ovarian cancers have resulted in the identification of two breast cancer susceptibility genes, BRCA1 and BRCA2[13,22]. In each gene, a small number of specific mutations have been found at high frequency in the Ashkenazi Jewish population. The mutations, 185delAG and 5382insC in BRCA1, and 6174delT in BRCA2, have been identified as having a combined frequency of 2.0% to 2.5%[4,8]. Based on studies of breast cancer families, it was determined that mutations in either gene conferred up to an 85% lifetime risk of breast cancer, and that mutations in BRCA1 itself correlated with a 63% lifetime risk of ovarian cancer[20]. Studies of less selected cancer cases within this population suggest that the estimated lifetime breast cancer risk to the carriers of any of the above three common mutations is 56% and an ovarian cancer risk of 16.5%[18]. Therefore, accurate, cost-effective detection of these three mutations has important implications.

SSCP Analysis

There are two kinds of mutation detection methods: specific and scanning. The former is used to identify specific, well-characterized sequence variations, while the latter is used to



detect uncharacterized sequence mutations. Single-strand conformation polymorphism (SSCP) is an example of mutation scanning technology. Mutation detection via conventional SSCP requires PCR* amplification of the DNA fragment of interest, denaturation of the double-stranded product, followed by non-denaturing slab gel electrophoresis. The need for higher-efficiency detection, greater automation, and safety have led to studies using capillary electrophoresis-based SSCP analysis[2,7,9,10,11,15,21].

In this report, we apply CE-based SSCP analysis as a screen for the three common breast cancer mutations, 185delAG, 5382insC, and 6174delT. We present a fast and simple separation/detection method and describe the steps we have taken to optimize it.

Materials and Methods

Reagents

GeneAmp thin-walled PCR tubes, 10x PCR buffer, 25 mM $MgCl_2$, 100 mM dNTPs stock solutions and Taq DNA polymerase (5 unit/mL) were from Perkin-Elmer (Norwalk, CT). Boric acid, EDTA, Tris(hydroxymethyl)-aminomethane, polyethylene glycol (MW 8,000), mixed-bed resin, pBR322 Hae III digest (609 µg/mL) and 50 bp ladders were from Sigma Chemical (St. Louis, MO). Hydroxyethylcellulose (HEC, MW 250,000) was from Aldrich Chemical Co. (Milwaukee, WI). PicoGreen and YO-PRO-1 were from Molecular Probes (Eugene, OR). Fluorocarbon polymer (FC) coated capillaries were from J & W Scientific, Inc. (Folsom, CA). Ultra-pure formamide and 2 mL disposable polystyrene cuvettes were from Fisher Scientific, Inc. (Pittsburgh, PA). Microcon YM-100 filters were from Millipore Corp. (Bedford, MA). Polyvinylpyrrolidone (PVP, MW 360,000) was from Acros Organics (NJ).

Deionized formamide is made as follows: 0.1 g mixed-bed resin is added into 1 mL formamide, the mixture is shaken for 0.5 hour, then centrifuged. The clear upper solution is collected and stored at -20°C.

Genomic DNA Isolation

Blood was taken by venapuncture to a glass tube containing EDTA. DNA was purified directly from the whole blood or cell lines by the solid-phase extraction (SPE) method described by Tian, *et al*[19]. Blood was diluted 50–60 fold with a guanine hydrochloride (GuHCl)-based buffer (6M GuHCl and 1% Triton-100 as the final concentration) and loaded on a silica SPE cartridge (Supelco). Eighty percent isopropanol (20 bed volumes) was used to wash the cartridge. DNA was eluted from the SPE cartridge, using 10 mM TE at pH 8.4 (3–18 bed volumes), and used directly for PCR amplification.

Genomic DNA for positive controls was isolated from lymphoblastoid cell lines obtained from individuals heterozygous for the 185delAG, 5382insC, and 6174delT mutations (Coriell Cell Repositories, Camden, NJ). All were used in an anonymous fashion. The concentrations of purified human genomic DNA were measured by a PicoGreen dsDNA quantitation assay[17]. The presence of BRCA1 or BRCA2 mutations was confirmed by fluorescent dideoxy sequencing.

Polymerase Chain Reaction

Primers used to flank the three mutations were designed based on the BRCA1 and BRCA2 mRNA sequences in GenBank, and the genomic sequences on the website of the Breast Cancer Information Core **(http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/)**. The primers were evaluated by the http://www.williamstone.com/primers/calculator/ program and are listed in Table 1. Primers tagged with 6-FAM were used to obtain the SSCP profiles. The sizes of the DNA fragments amplified were 256 bp, 296 bp, and 201 bp for 185delAG allele, 5382insC, and 6174delT allele, respectively. PCR amplifications of BRCA1 and BRCA2 alleles were carried out in a Progene thermocycler (Techne, Princeton, NJ) with the following reagents in 50 mL reaction mixtures: 60-80 ng of genomic DNA, 0.2 mM of the proper primers, 1 mM dNTPs, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$ and 5.0 units of AmpliTaq polymerase. Each PCR reaction mixture was heated for 5 minutes at 95°C, followed by 35 cycles of 1 minute at 94°C, 0.5 minute at the annealing temperature (Tm) listed in Table 1, and 0.5 minute at 72°C. A final 10-minute extension at 72°C was used following the final temperature cycle.

Table 1. Primers used for SSCP Analysis of 3 common mutations in BRCA1 and BRCA2

Position	Primers	Tm (°C)	Size (bp)
185delAG (Exon 2, BRCA1)	Forward: 5'-GAAGTTGTCATTTTATAAACCTTT-3'	55	256
	Reverse: 5'-TGTCTTTTCTTCCCTAGTATGT-3'		
	Forward1: 5'-ATATGACGTGTCTGCTCCAC-3'	58	257
	Reverse1: 5'-AGTCTTACAAAATGAAGCGG-3'		
5382insC (Exon 20, BRCA1)	Forward1: 5'-ATATGACGTGTCTGCTCCAC-3'	58	296
	Reverse2: 5'-CCTGTGTGAAAGTATCTAGCAC-3'		
	Forward1: 5'-ATATGACGTGTCTGCTCCAC-3'	58	399
	Reverse3: 5'-GGGAATCCAAATTACACAGC-3'		
6174delT (Exon 11, BRCA2)	Forward: 5'-CACCTTGTGATGTTAGTTTGGA-3'	60	201
	Reverse: 5'-TGGAAAAGACTTGCTTGGTACT-3'		

CE Instrumentation and Conditions

The PCR products were analyzed on a P/ACE™ 5510 System from Beckman Coulter (Fullerton, CA). Laser-induced fluorescence (LIF) detection was used with the excitation at 488 nm (Argon ion laser) and emissions collected at 520 nm. Capillary electrophoresis conditions were as follows: the FC-coated capillary was 50 µm (I.D.) by 27 cm (effective length 20 cm); the running buffer was 1.5% (w/v) HEC in 1x TBE buffer (89 mM Tris, 89 mM borate, 2 mM EDTA, pH 8.6) containing 1 mM YO-PRO-1 as the fluorescent intercalator. Samples were electrokinetically injected for 10 seconds at 10 kV. The separation was carried out at 148 V/cm using reversed polarity (inlet as cathode and outlet as anode), and the capillary was maintained at 20°C. The capillary was flushed at the start of each day with water for 5 minutes, followed by fresh buffer for 15 minutes and rinsed with the buffer for 4 minutes between runs.

CE-Based SSCP Analysis

One microliter of PCR product (without purification) was combined with 10 µL deionized formamide and 0.5 µL 0.3 M sodium hydroxide. The mixture was heated for 5 minutes at 95°C and snap-cooled on ice for 2 minutes before injection, unless specified.

The denatured PCR products were introduced into the capillary by electrokinetic injection for 20 s at 10 kV. The sieving matrix was 1.5% or 2% HEC plus 10% glycerol in 1x TBE buffer (no intercalator). The separation voltage was in the range of 370-481 V/cm.

Results

Standard SSCP analysis involves the denaturation of the PCR product followed by rapid chilling on ice prior to analysis. Under these conditions, a difference in electrophoretic mobility between the wild type and mutant alleles is indicative of the presence of a mutation.

185delAG Mutation

Figures 1(a) and (b) illustrate examples of SSCP profiles derived from both the wild type and mutant 185delAG alleles. As shown, the profiles for both the forward and reverse strands of wild type are qualitatively similar [Figures 1A(a) and (b)], as are those for the mutant 185delAG allele [Figures 1B(a) and (b)]. Most importantly, there are consistent qualitative differences between the wild type and the mutated strands in the profiles. These differences manifest themselves in the forms of three peaks in the SSCP profile of the wild type, where there are five peaks in the SSCP profile of the mutant allele—allowing the wild type and mutant alleles to be easily discriminated via visual inspection.

6174delT Mutation

As shown in Figure 1(c) and (d), the mutated strands have three peaks at about 4.5 minutes while the wild type strands only have a single peak. The wild type and the 6174delT alleles can be easily distinguished based on its peak number at about 4.5 minutes.

Assay Optimization

DNA Fragment Size

In order to evaluate the effect of DNA fragment size on this analysis, three separate pairs of primers were designed and synthesized for amplification. These consisted of 257 bp, 296 bp, and 399 bp fragments which included the 5382insC allele in exon 20 of BRCA1. In each amplification reaction, one 6-FAM-tagged primer (forward or reverse) and its corresponding untagged primer (listed in Table 1) were used to amplify the DNA fragment for SSCP analysis. Figure 2 shows the SSCP profiles of the three sizes of DNA fragments, derived from the wild type and 5382insC alleles. A single peak migrating at about 5.5–5.7 minutes is associated with the wild type DNA [Fig. 2A(a), (b)], while the amplified DNA from a 5382insC heterozygote [Fig. 2B(a), (b)] is observed as three peaks. Using the additional fragment sizes of 296 and 399 bp, the SSCP profiles of the wild type and 5382insC alleles could be distinguished in a similar manner (*i.e.*, a single peak in the SSCP profiles of the wild type allele [Fig. 2A (c), (d)] corresponding to two or three peaks in the profiles of the mutant 5382insC allele [Fig. 2B (c), (d)]). Despite relatively poor resolution, two peaks in the SSCP profile of the mutant allele were observed (at approximately 6.5 minutes) with the 400-bp fragment. The difference is less obvious between wild type and mutant alleles with 400 bp fragment compared to the 258 and 297 bp alleles. No obvious improvement was achieved using 258 bp instead of 297 bp.

Denaturing Conditions

As the denaturing step is critical in SSCP analysis[5,6], we studied the effects of varying the heating and chilling time during the denaturing step. Figure 3A shows the effect of varying denaturing conditions of the reverse strand derived from the 6174delT allele. While the three peaks at ~15 minutes remain qualitatively unaffected, the different denaturing conditions have a marked impact on the relative intensities of the last four peaks.

Sample Stability

Since CE allows for automated analysis, it is important to understand the rate at which denatured samples re-anneal and how quickly the sample must be analyzed after loading on the sample tray. Experiments were carried out to observe the stability of the denatured PCR products derived from the 185delAG, 5382insC, and 6174delT alleles. The SSCP profile of the denatured reverse strand amplified from the 185delAG allele was monitored over a period of 12 hours at room temperature. Over this time course the SSCP profile remained almost unchanged (data not shown), whereas the SSCP profile of the forward strand derived from the 5382insC allele (297bp) only remains stable up to 2 hours at ambient temperature (data not shown). The stability of the SSCP profile derived from the reverse strand of the 6174delT allele was monitored over a period of 3 hours and was found to be stable for up to 1.5 hours (Figure 3B).

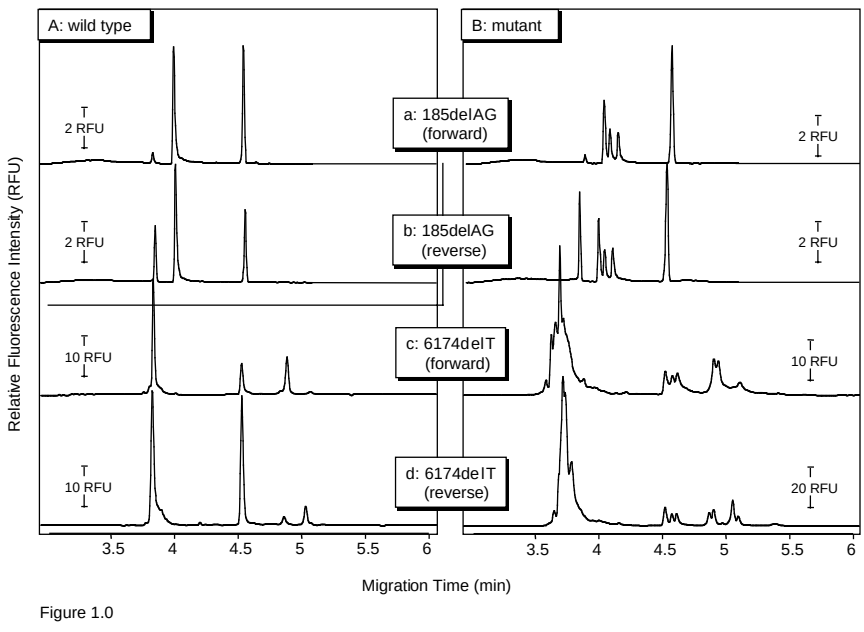


Figure 1.0

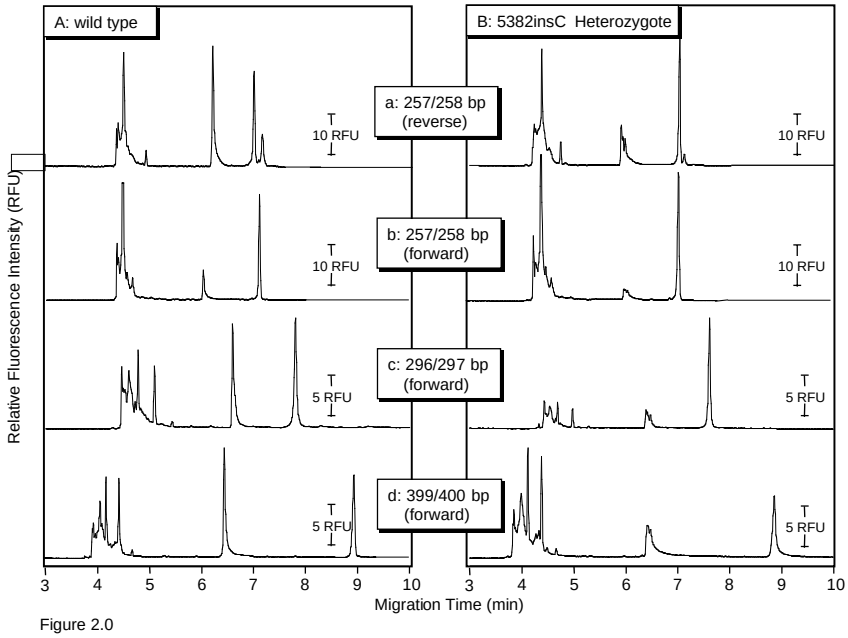


Figure 2.0

Run Temperature

Several electrophoresis temperatures were used. The peak number and the relative intensities in the SSCP profiles derived from 185delAG allele are similar in the range of 20-50°C; those derived from 5382insC allele are similar in the range of 20-40°C with a slight change at 50°C (data not shown). The 6174delT allele, the most sensitive to the electrophoresis temperature, resolved best between 30-35°C (Figure 3C). A temperature of 30°C was found to be suitable for the CE-SSCP analysis of all three mutations studied.

Discussion

Parameters for CE-Based SSCP Analysis

The underlying principle of SSCP analysis is that single-stranded DNA, under non-denaturing conditions, will adopt a conformation dependent on its sequence composition. This conformation generally will be different in physical configuration (secondary structure) even if only a single base change has occurred. As a result of different secondary structural conformations, fragments will have different electrophoretic mobilities. In this study, the effect of DNA fragment size, polymer network composition, buffer additives, and running temperature were all evaluated. Consistent with slab gel-based SSCP analysis, optimization of these parameters was found to be critical to effective mutation detection.[16] We have shown that there is an obvious correlation between the DNA fragment size and the ability to discern the presence of a mutation. In our study, a size range of 200 to 300 bp is optimal for CE-based SSCP analysis and is consistent with the 150-200 bp range suggested for slab gel-based SSCP analysis[14].

The type of sieving matrix used is also very important[14]. A number of sieving matrices were evaluated including polyvinylpyrrolidone (PVP), linear (uncrosslinked) polyacrylamide, and HEC (data not shown). Under our conditions and with these particular BRCA1 and BRCA2 mutations, HEC (1.5%-2%) was found to provide the best electrophoretic discrimination between wild type and mutant DNA.

While running temperature has less effect on the 185delAG and 5382insC allele, it is as important in CE-based SSCP analysis as in slab-gel-based SSCP analysis[14]. In our study, a single running temperature (30°C) was found to be effective to screen for all three common mutations in BRCA1 and BRCA2.

Summary

Laser-induced fluorescence detection, in combination with a cellulose-based polymer network (*i.e.*, HEC) and a commercially-available fluorocarbon

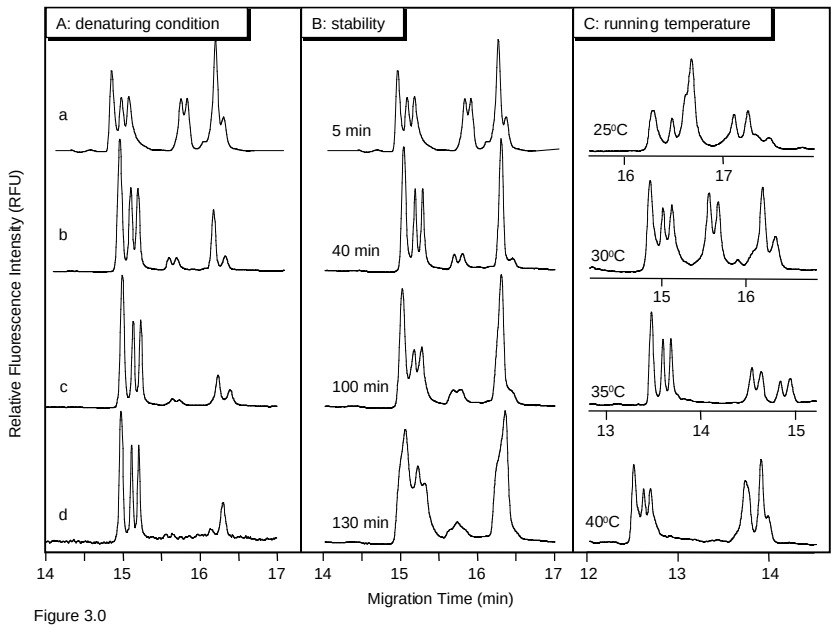


Figure 3.0

polymer-coated (FC) capillary, provides an ideal analytical system for CE-based SSCP analysis. In this assay, the total time (including DNA purification and DNA amplification by multiplex PCR) for screening is about 3.5 hours, which is much more rapid than the slab-gel heteroduplex analysis protocol (about 7 hours[12]). CE-based SSCP assays have a very bright future for screening mutations in select genes in research or clinical diagnostic laboratories.

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We thank Dr. Saijun Fan (Long Island Jewish Medical Center) and Dr. Jeffery Kant (Department of Pathology, University of Pittsburgh Medical Center) for providing samples. We thank Therese Liebert, Jennifer L. Profozich, and Bob F. Friday for oligonucleotide synthesis; and acknowledge the National Cancer Institute for funding (AR-07591) and Beckman Coulter, Inc., for the equipment, along with Dr. David Mao, J & W Scientific, Inc., for providing FC-coated capillaries for the study.

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Editor's Note:
Part II of the article on Binding Isotherms by David D. Y. Chen is not included in this newsletter but will appear in a future issue of *P/ACE Setter*.

Organizing the Data on Your Lab Computer

I frequently receive questions from our customers asking me how I organize the data on my lab computer. In response, here are some of my views on data organization and batch table optimization.

NOTES FROM THE LAB

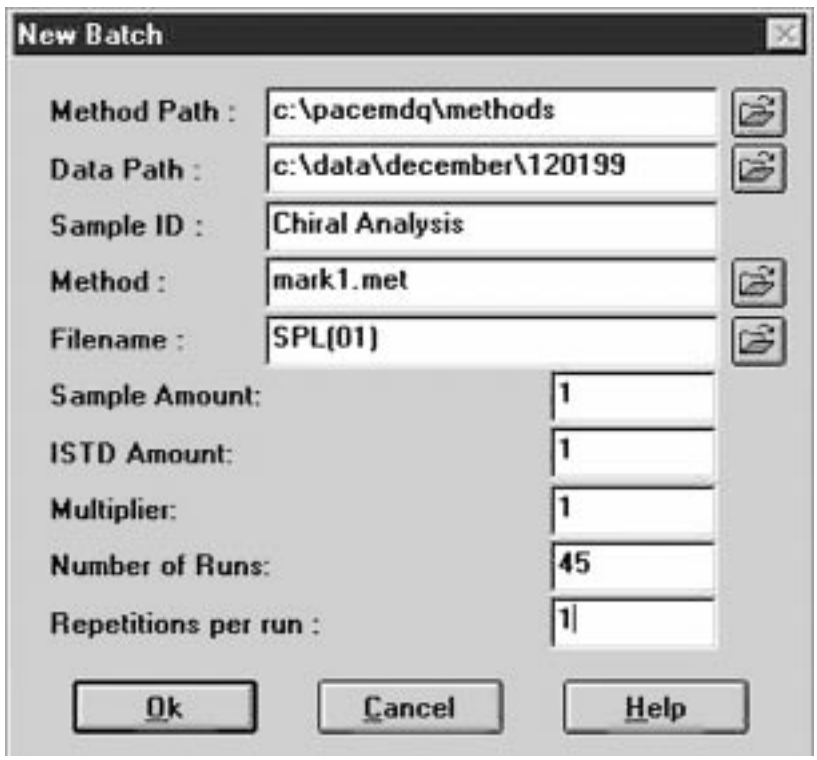
MARK T. FLOCCO

In my lab, I organize all data either on the secondary "D" drive or in a separate folder labeled "Data"—I prefer to keep this folder outside the MDQ or P/ACE™ Station directories to maintain the integrity of the program organization. Using this main folder, I then structure my data in subfolders both by month and by day. Once a week or once a month (depending on my workload), I back up this data to an external drive to free up needed space on the hard drive. To achieve this, I first create a new Data folder from Windows Explorer, pulling down the menu under File and selecting "New Folder" and then labeling it "Data." While still in Windows Explorer, I double-click the Data folder and repeat the task, creating 12 subfolders, one for each month.

Every day I set up a new batch table by selecting New Batch in the File drop down menu of the CE software. Where it specifies data path, I select my new folder entitled Data, then the new subfolder (by month). I usually create an additional subfolder by day by simply typing in the current date in the directory path (*i.e.*, D:\data\december\120199). This new folder—by day—will then be created before the batch table starts. Actually, the software will indicate that this subdirectory does not exist: "Do you wish to create it?" In the dialogue box, click "Yes." You should now have the data in one folder divided by both month and day. It makes it very easy to find a backup copy or transfer your data.

With regard to filenames, I like to keep them short and concise. I use acronyms such as "SPL" (short for "sample") with a corresponding starting run number in brackets, (*e.g.*, "01"). This allows the software to generate automatically a large number of runs with minimal input from the operator. In the example shown in Figure 1, I have created a sample batch of 45 different samples by simply selecting SPL(01) and telling the software I wish to run 45 samples. In this way 45 lines will be created with filenames labeled 01–45. So now all of your data will be stored in a directory sorted by month, day, and number designation. In the "Sample ID" dialogue box, I usually include a word or two that briefly identifies the project: in this case, I chose "Chiral Analysis." If more detail is required, I use the description box in the batch table.

While in the batch table: if you specified auto-increment in the injection line in your "Time Program," the injection will increment from the starting point specified. Example: If you specified "SI: A1," the program will increment one space per injection in a serpentine fashion from that point. If you checked "allow override," you can change any sample introduction position in the batch table. I prefer to operate this way—keeping my methods independent from my samples. It is also very important to get into the habit of entering information into the description box (concentration of



sample, lot number, sample matrix, or anything pertinent to your analysis). This can be copied and pasted from other programs and can be included in the custom report. It is not necessary to enter physical parameters from the method because the sample is always saved with the method. Even if you have one method and change it on a run-to-run basis, the method that was used to run your sample will be saved with the data.

Helpful Tip: Did you know that you do not need to completely rewrite names, methods, or descriptions if you edit a line in the batch table? If you want to change the sample name for a specific sample, simply click on the name and press the ESC key. The name will be highlighted in blue. Click the right mouse button and a flashing cursor will appear. You may now edit a section of the sample name without having to retype the whole thing. This can be done in any field of the batch table you wish to edit.

In the next article, we will talk about setting up calibration or suitability runs and more detailed options in the batch table.

Please send us suggestions that you have used to organize your data. We welcome your comments and tips, as will your peers who read this newsletter.

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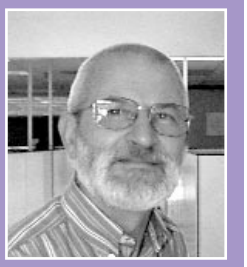
Simple Tips to Improve the Robustness of CE Methods

The purpose of this column to provide you with "tips" that we use in our lab to improve the robustness of capillary electrophoresis methods. In this issue we will focus on procedures you can use to improve peak area reproducibility—which is especially important if your sample is contained in an organic phase and introduced into a capillary containing aqueous buffers.

PRACTICAL POINTERS



ROLAND CHEVIGNE

TIP 1: POST – INJECTION WATER PLUG

The introduction of a water plug after your sample is injected may substantially improve the robustness of your method. In our hands, we have seen examples where C.V.s of 15% were reduced to 2% when employing this method. This procedure consists of performing a one-second post-injection of water after injecting the sample. It is important to note that the post-injection of a water plug will work much better than the post injection of a buffer plug. When using either a P/ACE™ 2000 or 5000, we recommend a one-second (0.5 psi) post-injection. If using the P/ACE MDQ, we recommend a post-injection using 0.1 psi for 10 sec.

The introduction of this water plug prevents:

- Loss of sample during the movement from sample to buffer.
- Contamination of inlet buffer by the backward movement of the sample off the capillary.
- Loss of sample when applying voltage (because the sample is usually less conductive than buffer, local heating will result in the expansion of the sample zone, resulting in a backward movement off the capillary).

> Significant improvements to peak area reproducibility can be achieved with only small adjustments to the operating method.

TIP 2: INCREASE RAMP TIME OF APPLIED VOLTAGE

Once the sample and water plug are injected, the voltage is applied to the capillary. We recommend increasing the length of the voltage ramp to about 1 minute. However if a given buffer generates amperage above 80–100 µA, a longer ramp time should be employed. This ramping action acts to mnimize local heating of the injected sample zone. The voltage ramp default on a P/ACE system is 0.17 minutes.

TIP 3: DEDICATE YOUR RINSE VIAL

Always use a separate vial to rinse or fill the capillary. A common practice that has led to much error has been the use of the inlet separation vial to fill the capillary between runs. The problem with this is that the buffer pH and concentration will be changing with time (the longer the analysis time, the more change occurs), resulting in a slightly different buffer for each run. If you fill the capillary with buffer from a dedicated vial that never sees an "applied voltage," you will ensure that the buffer remains constant from run to run.

TIP 4: INTRODUCE SAMPLE WHILE THE CAPILLARY OUTLET IS IN BUFFER

Ensure that the capillary outlet is placed into buffer during the introduction of sample. Sample injection toward an empty outlet vial may result in a buffer droplet being expelled from the capillary. This will create a suction effect, increasing the amount of sample injected. If this droplet does not leave the capillary but re-enters when the capillary is introduced into the outlet buffer, some injected sample may be reversed and expelled from the capillary.

In summary, significant improvements to peak area reproducibility can be achieved with only small adjustments to the operating method. In the next issue, we will discuss mechanisms by which we can improve the inter-capillary migration time reproducibility.

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The Cat Case: P/ACE™ MDQ Resolves Feline Mystery

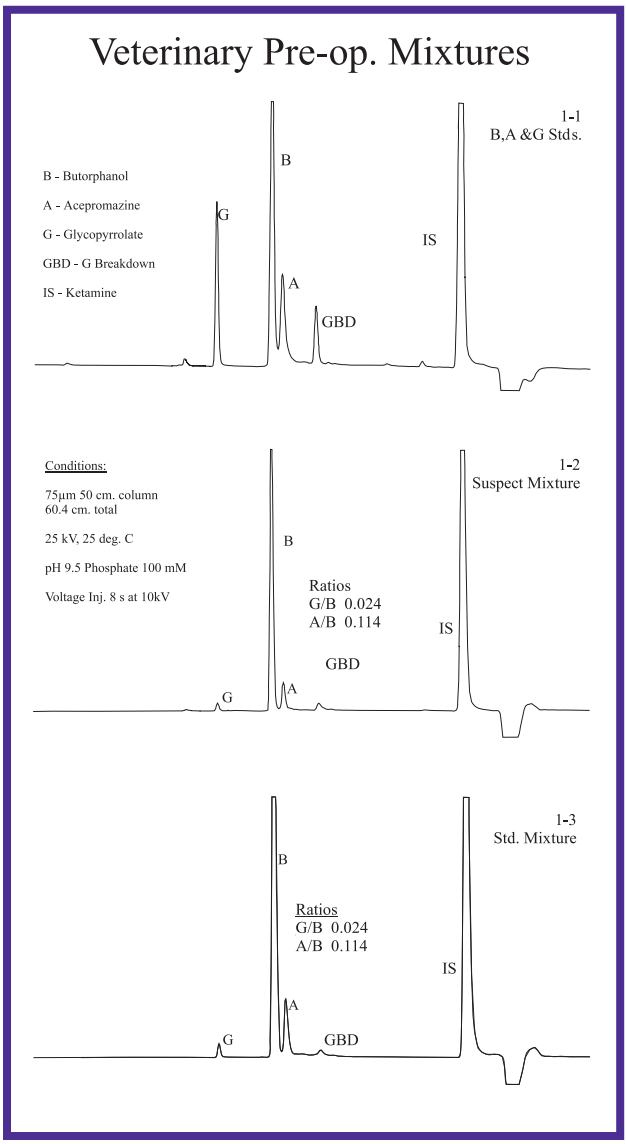
John C. Hudson, Royal Canadian Mounted Police Forensic Laboratory, Regina, SK, Canada

Case History

A clear liquid injectable solution was submitted in a suspected case of pharmaceutical product tampering. A veterinarian was losing feline patients during open-heart surgery at a higher rate than expected and suspected that something may be askew with the pre-operative anaesthetic mixture. The question was whether someone had tampered with the concentrations of the components or whether a mistake in preparation had been made.

The Analytical Challenge

The preoperative mixture was a cocktail of three drugs: acepromazine (A), butorphanol (B), and glycopyrrolate (G), an aqueous mixture presenting a unique analytical challenge to any toxicologist. At the center of this challenge is glycopyrrolate, an anticholinergic agent. This compound is a quaternary ammonium ion which is very water soluble and will not extract from aqueous solutions by conventional liquid–liquid methods. Unfortunately, this compound cannot be analyzed easily by traditional chromatographic means, yet appears to be a perfect candidate for analysis by capillary electrophoresis. After all, glycopyrrolate is just a big cation.



Direct Analysis by CZE

The P/ACE MDQ is designed for rapid methods development: Using a buffer array with vial incrementing capability, a sample was analyzed using a series of buffers at varying pH values. This all happens rapidly and automatically. This capability was just "what the doctor ordered" for the pre-operative mixture. The sample was simply diluted and analyzed using four different run buffers in series (pH 2.38, 6.00, 8.20, to 9.50). In just less than two hours, a suitable separation (See Figure 1-1) was obtained and applied to the case and standard samples. The resulting electropherograms are shown as Figures 1-2 and 1-3.

Results and Conclusion

Ratios (G/B and A/B) were calculated and compared for both the suspect mixture and the standard mixture. Analysis that might have taken days or that even could not have been done at all was accomplished effectively by CZE in a short period of time.

The results provided proof that the injections had NOT been tampered with. The end result was that a most appreciative vet changed anaesthetic to a "safer" preoperative cocktail. Cat Case closed.

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Aligning Methods Between Different CE Models or Brands

Capillary Dimensions

Capillaries purchased from different manufacturers often utilize unique dimensions. It is important to ensure you that you match the internal diameter (I.D.), external diameter, and even the dimensions of the external coatings when aligning methods between different lots or brands of capillaries. Slight differences in capillary I.D. will affect both sample loading and electroosmotic flow, while the thickness of the external coating can impact capillary resistance. Also make sure to match the capillary distance from the point of sample introduction to detection. This is referred to as the capillary effective length. Although most instruments will allow flexibility with this dimension, do not assume the distances—it is important to confirm with a measuring device.

Applied Voltage

As all CE manufacturers use a unique fixed distance from the point of detection to the capillary outlet, it is important to ensure that you match the field strength in the method rather than the applied voltage. "Field strength" is defined as the applied voltage/total capillary length and is commonly expressed as Volts cm^{-1}.

Capillary Temperature

Alignment of capillary temperature is an important element for the successful transfer of methods between instrument models or brands. A difference in absolute temperature will impact:

- buffer pH, which affects analyte mobility
- buffer viscosity, which affects both electroosmotic flow and sample introduction volume
- kinetics of surface interaction or solute partitioning (depending upon mode), affecting analyte retention

Not surprisingly, capillary temperature is the parameter which is most often misinterpreted—and most often responsible for troublesome methods transfer. The reason for this inconsistency is understandable: internal capillary temperature is very hard to measure. All CE manufacturers thermostat the environment in which the capillary is bathed—not the temperature of the buffer in the capillary. In our experience, it is not uncommon to see temperature differences of greater than 10°C between different instrument brands. For this reason, it is important to express the capillary temperature in a method as a function of both the programmed temperature and the actual current generated. If you have fixed the capillary dimensions, buffer concentration, and applied field strength, and the actual observed temperature was the same, then the resulting current should also be the same. However, most of the time you will find that this is not the case, as most commercial instruments differ in the way they thermostat the capillary. Therefore, when transferring methods between different instrument models or brands, use the programmed temperature only as a starting point. Once all parameters are set, compare the current between the two systems in question. If they are not equivalent, change the temperature until the current generated from the method on the new instrument matches that of the original. Do this only after you are sure that you have fixed or normalized all other parameters. Because different CE brands have different capacities in their ability to regulate the temperature, it is wise to then monitor the stability of the current. If the current is unstable, you may need to lower the applied field strength. This is where a well-engineered capillary thermoregulatory system really pays off.

Detection Wavelength

UV/Vis Detection

When transferring methods between different instruments, be sure to match both the detection wavelength and bandwidth. If using a diode-array detector, be sure that the detection system is calibrated appropriately. This is done most often with holmium oxide, D2, or Hg calibration. It is good practice to routinely calibrate your detection system and to test it against the spectrum of a known standard you use. You may wish to make this an automated as part of your system suitability prior to the analysis of unknowns.

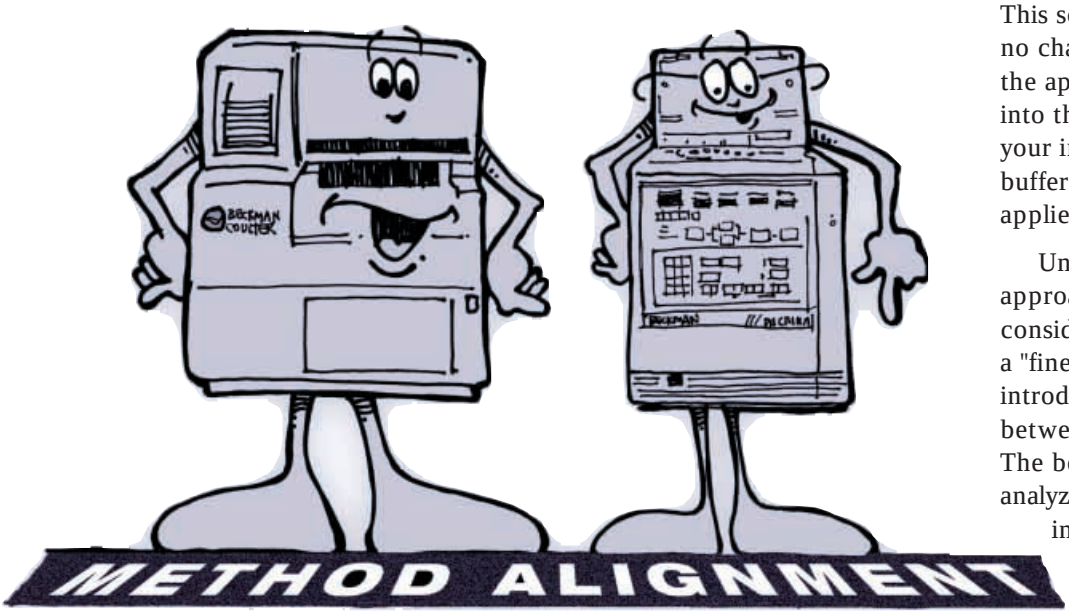
Laser-Induced Fluorescence Detection

Since fluorescence is a relative measurement, it is important that you normalize signal between instruments by calibrating it to a standard of known concentration. This calibraion procedure should be considered routine and repeated every time the capillary is replaced or cartridge exchanged. Ensure that the emission wavelength and bandwidth are the same—and that the same laser wavelength and energy are used. You also will want to ensure that you have a notch filter in place to block laser light from returning to the detector and—if using UV lasers—be sure that all fiber and optical path components are UV-compatible.

Data Acquisition/Filtering

The way in which data is acquired will often vary between different manufacturers. Data systems capture information through a sampling process, which can be modulated to favor increased resolution or sensitivity—one usually at the cost of anoth-

er. For this reason, it is important to understand the factors involved with your particular data system when making comparisons or method transfers between different software programs. How is my data being sampled? What is the filtering mechanism—is it fixed or variable? What variables do I have control over? Do not simply match data rates and assume that you have normalized your comparison; some data systems link their data rate with electronic filters while others make them independent. If you are not careful, you may end up with a smooth baseline at the cost of peak resolution, resulting in impurities being "lost" into the baseline. The key is to understand well the parameters of your data system. It is helpful to use peak resolution, peak efficiency, and peak asymmetry as a means to compare the effect of data sampling settings between systems.



SAMPLE INTRODUCTION

Although the term sample "injection" is often used, a more accurate terminology for the process in CE is sample "introduction." There are primarily two methods by which samples are introduced: either through pressure displacement (either positive pressure or vacuum) or through electromigration. In the former case, a volume of sample is introduced into the capillary while, in the latter case, a quantity of mass is migrated into the capillary. We have positioned this topic as the last of our "six points to consider" to reinforce the need to align all other parameters before addressing sample introduction. The reason is straightforward: sample introduction is impacted by the capillary I.D., total capillary length, sample viscosity, and the buffer temperature (viscosity). Once you have normalized these variables, you have a better starting point for aligning sample introduction between instrument types.

POSITIVE PRESSURE DISPLACEMENT

With this mode, one typically generates a low-pressure head on a vial containing sample (or vacuum onto an outlet vial), creating a pressure differential that forces a plug of sample into the capillary. Assuming the five points listed above are normalized, your next step should be to align the pressure/time variable that is used to generate the pressure differential across the capillary. Some instruments fix the pressure and allow you to vary a time parameter, while other instruments allow you to vary both pressure and time. In either case, you should consider this step as a "course adjustment" toward defining equivalent sample loads between instrument types. We recommend that you download CE Expert software from the Beckman Coulter website (www.beckmancoulter.com) to help facilitate this task. This software, which is available at no charge, will allow you to calculate the approximate volumes introduced into the capillary as a function of your input for a given temperature, buffer viscosity, capillary dimensions, applied pressure and time.

Unfortunately, this theoretical approach does not take all into consideration and you will require a "fine-adjustment" if you wish to introduce equivalent sample loads between different instruments. The best way to facilitate this is to analyze the data from the different instruments on the same data system. By simply up/down regulating the delivered pressure or increasing/decreasing the time variable, you should be able to match the peak area generated from the two different systems, allowing you to successfully align the volume introduced with each system.

ELECTROMIGRATION

In the case of sample introduction via electromigration, act first to align the field strength for the course adjustment and then fine-tune as above by changing the voltage and/or sample introduction time until the peak areas between systems match.

So there you have it: six points that—when considered—will ensure the successful transfer and/or comparison of methods between different instrument types.

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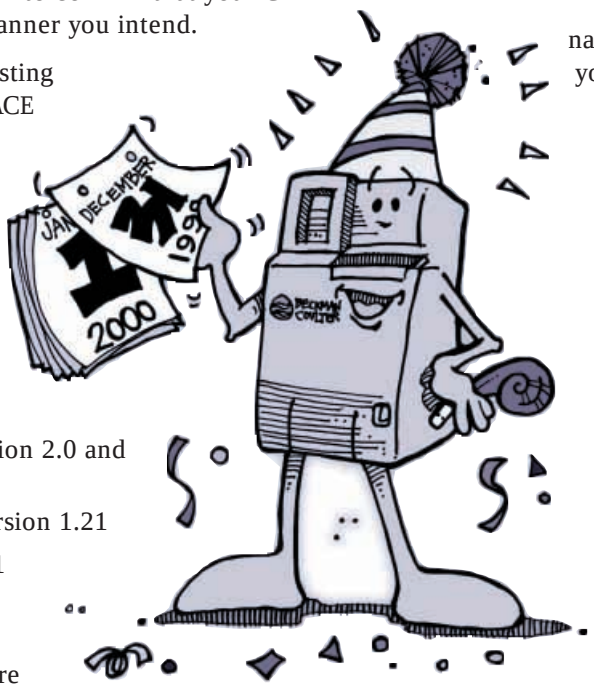
Year 2000—Are You Ready?

I am sure that by now you are probably weary about yet another reminder of Y2K. However, as the new year is now upon us, you may wish to confirm that your CE continues to operate in the manner you intend.

We have done extensive testing on both P/ACE™ MDQ and P/ACE Station software using a verification process designed around the rules of the British Standards Institute (BSI) definition (DISC PD2000-1) of Year 2000 conformity. This testing has verified 100% conformity with the following versions:



- P/ACE MDQ Software Version 2.0 and greater
- P/ACE Station Software Version 1.21
- Gold™ Software Version 8.1 and greater

For systems using P/ACE Station or P/ACE MDQ Software prior to the versions listed, the automatic sample-naming feature will not function after the year 2000. This is the feature that automatically creates a filename when the filename field is left blank in either the batch table or in the start single run dialog. This does not affect operation when the filename is specified in the filename field.

Even though this automatic sample-naming feature is rarely used, we do offer you an upgrade path.

With P/ACE Station software, a copy of version 1.2 can be downloaded at no charge from the Beckman Coulter Internet site. If you do not have Internet access, diskettes may be purchased by ordering P/N 713065.

With P/ACE MDQ, both the software diskettes and required firmware will be made available to you at no charge. However, service installation of this upgrade will be required. A labor charge may apply depending upon your current agreements. Please contact your local Beckman Coulter Service group to inquire about this upgrade.

Once again, these upgrades are required only if you need to use the automatic file-naming feature. For more information about Beckman Coulter's Year 2000 testing procedures, visit the company's website at: www.beckmancoulter.com

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