

# VALIDATION OF THE JANUS AUTOMATED LIQUID HANDLING SYSTEM

A Major Qualifying Project Report

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## **ABSTRACT**

Validation of forensic laboratory procedures and instruments is crucial for quality assurance and control. JANUS™, a novel automated workstation, was validated for accurate liquid handling at specified volume ranges and to show that PCR amplifications set up by JANUS™ provide results comparable to amplifications set up manually. PCR amplification of normalized DNA samples was performed by JANUS™ and compared to manual PCR. The results indicated that JANUS™ accurately dispensed specified volumes and is competent for PCR.

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## **BACKGROUND**

Violent crimes such as rape and murder are an unfortunate part of our society. Thankfully though, in many cases the perpetrators of these crimes do not go unpunished for long due to the advent of DNA fingerprinting. DNA fingerprinting or profiling is a technique used to identify individuals based on their unique genetic makeup. Most often criminals leave their DNA behind at crime scenes (i.e. blood, saliva, or semen) and this evidence is collected by crime scene personnel where they are taken to crime labs for testing. DNA is extracted from the samples and “typed” for specific lengths of repeat DNA sequences. Finally the generated profile is compared to a reference profile where a match signifies that the reference sample donor was at the crime scene.

### **DNA Fingerprinting History**

DNA fingerprinting was invented in 1985 by British geneticist Alec Jeffreys. While doing research on human genetics, Dr. Jeffreys discovered that certain regions of DNA contained DNA sequences that were repeated next to each other. These “stutter” regions repeated multiple specific patterns that were found at the same location on chromosomes for each individual. What differed between individuals were the lengths of repeated sections present in the sample, ranging from six to five hundred base pairs (Butler 2005). The repeat regions became known as “minisatellites” or variable number tandem repeats (VNTRs) that range from 10-100 bases in length (Butler 2005). Dr. Jeffreys’ technique of identifying them was called restriction fragment length polymorphism (RFLP). His technique cuts the VNTRs at specific regions using enzymes called restriction endonucleases to produce DNA fragments of varying lengths. These

fragments are then separated using gel electrophoresis and compared side by side to different samples for a potential match (Butler 2005).

### **STR PCR Genotyping**

Recently, new PCR STR methods of DNA typing have been developed that are faster, more accurate, and overcome some of the downfalls of the RFLP technique. RFLP requires large amounts of DNA (50-500 nanograms, or ng) and cannot be used to analyze samples degraded by environmental factors such as dirt or mold ("DNA Forensics" 2008). This is problematic because more than 2 out of 3 forensic cases are rape cases that involve mixture DNA samples that must be resolved and DNAs are often degraded (Butler 2009). The new method most commonly used in DNA analysis in forensic laboratories, the short tandem repeat (STR) technique, is better at resolving these issues. STR technology is a forensic analysis tool that evaluates loci that are found on nuclear DNA ("DNA Forensics" 2008). Loci are the chromosomal positions or locations of a gene or a DNA marker in a non-coding region. The repeat regions used in STR are "microsatellites" that are 2-6 basepairs in length. Tetranucleotide repeats (4 bp) are most often used as STR markers because they are more easily and accurately amplified by polymerase chain reaction (PCR). For example, the 16 bp sequence of "catacatacatacata" would represent 4 head-tail copies of the tetramer "cata". Due to the smaller marker size, discrete alleles from STR systems may be obtained by PCR, and DNA fragments differing by a single basepair in size may be differentiated ("STR DNA Internet Database"). Also, smaller quantities of DNA (0.1-1 ng), including degraded DNA, may be typed using STRs making the quantity and integrity of the DNA sample less of an

issue with PCR-based typing methods than with the traditional RFLP methods (Butler 2005).

There is a general procedure for the STR typing method. As with the RFLP technique, DNA must first be extracted from its source sample (collected evidence) and reference samples. As mentioned before, at a crime scene it can be in the form of blood, saliva or even hair. Some specific sources include semen from rape cases, and buccal cells from the inside of the cheek for paternity testing (Hallick 2000). Afterwards, unlike RFLP, the DNA is amplified using PCR. PCR is a molecular 'xeroxing' process in which a specific region of DNA is replicated to yield many copies of a particular sequence. Invented by Kary Mullis, who earned a Nobel Prize for its development, it copies DNA by cooling and heating samples in a precise thermal cycling pattern over approximately 30 cycles (Butler 2005).

Many commercial kits are available with the pre-mixed components of the PCR. There are two main vendors for STR kits used by the forensic DNA community; Promega Corporation from Madison, Wisconsin, and Applied Biosystems in Foster City, California (Butler 2005). Both companies have marketed multiplex PCR reactions that permit co-amplification of all 13 core loci STRs in a single reaction, along with the amelogenin sex-typing marker, and two additional STR loci. The AmpF/STR<sup>®</sup> IDENTIFILER<sup>™</sup> typing kit by Applied Biosystems is such a kit. The kit has an allelic ladder containing an amazing 205 alleles for accurate genotyping, and an internal size standard. Finally, this kit involves the use of a new 5-dye detection system (previously 4 dyes) where four of the dyes (6FAM, VIC, NED, and PET) are used to label the PCR products (previously 5FAM, JOE, and NED) and the fifth dye, LIZ (ROX in 4-dye

system) is used to label the internal size standard (Butler 2005). The use of these kits has greatly simplified PCR use in forensic laboratories.

The crucial components of any PCR reaction are the two primers. These primers must flank the target region to be amplified, and if not properly constructed can result in faulty PCR products. Their flanking regions of the primers remain constant although the number of repeats within the flanked region is not ("STR DNA Internet Database"). The primers used for STR analysis have fluorescent molecules covalently linked to them. Moreover, to extend the number of different loci that can be analyzed in a single PCR reaction, multiple sets of primers with different "color" fluorescent labels are used (Hallick 2000). This is necessary for DNA detection and comparison after PCR.

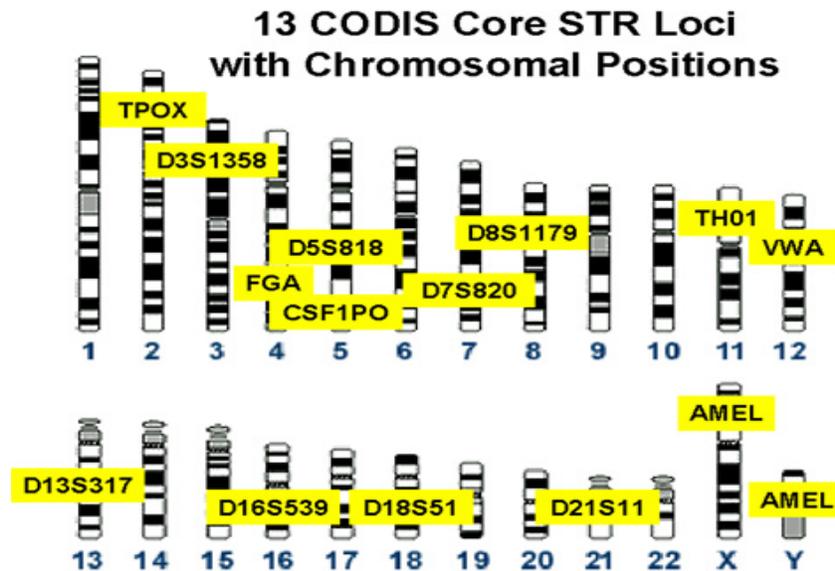
In STR analysis the PCR primers must amplify certain loci for human identification. The main criteria for the loci are that they are:

- Highly discriminating, with observed heterozygosity > 70%
- Provide robust amplification and reproducibility
- In separate chromosomal locations, so that closely linked loci are not chosen
- Distinguishable alleles with low stutter characteristics and low mutation rates. (Butler 2005)

### **CODIS 13 Core Loci**

In 1997, the Federal Bureau of Investigation (FBI) announced the selection of 13 STR loci to constitute the core of the United States nation-wide DNA database named the COMbined DNA Index System or CODIS (Hallick 2000). The 13 CODIS core loci are

CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11 (*see Figure 1*) (Butler 2005) and all 13 satisfy the main criteria for forensic testing. These chosen loci are tetranucleotides, most are located on separate chromosomes and have several alleles each. For ethical purposes they are in non-coding regions of the DNA that have no genetic value, and are variable among individuals in the population. When all 13 CODIS core loci are tested, the average random match probability is rarer than one in a million among unrelated individuals (Butler 2005). This is because the probability of two individuals having the same alleles for each locus decreases as the number of loci increases (*see Table 1*). If any two samples have matching genotypes at all 13 CODIS loci, it is a virtual certainty that the two DNA samples came from the same individual (or an identical twin).



**Figure 1: The FBI Chosen Thirteen STR Loci.**

This figure shows the 13 core loci chosen by the FBI for the CODIS DNA database. With the exception of two, all the loci are on separate chromosomes so that closely linked loci were not chosen. Also, this increases their discriminatory capability between individuals when doing profile comparison. (Source: <http://www.cstl.nist.gov/div831/strbase/fbicore.htm>)

**Table 1 : Example DNA Profile.**

Locus	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818
Genotype	15, 18	16, 16	19, 24	12, 13	29, 31	12, 13	11, 13
Frequency	8.2%	4.4%	1.7%	9.9%	2.3%	4.3%	13%

Locus	D13S317	D7S820	D16S539	THO1	TPOX	CSF1PO	AMEL
Genotype	11, 11	10, 10	11, 11	9, 9.3	8, 8	11, 11	X Y
Frequency	1.2%	6.3%	9.5%	9.6%	3.52%	7.2%	(Male)

This table shows a sample DNA profile for a male individual using the 13 core loci. The genotype shows the alleles present at each locus for that individual and the frequency of those specific alleles at that locus within the population is also shown. By combining the frequency information for all 13 CODIS loci, one can calculate that the frequency of this profile would be 1 in 7.7 quadrillion.

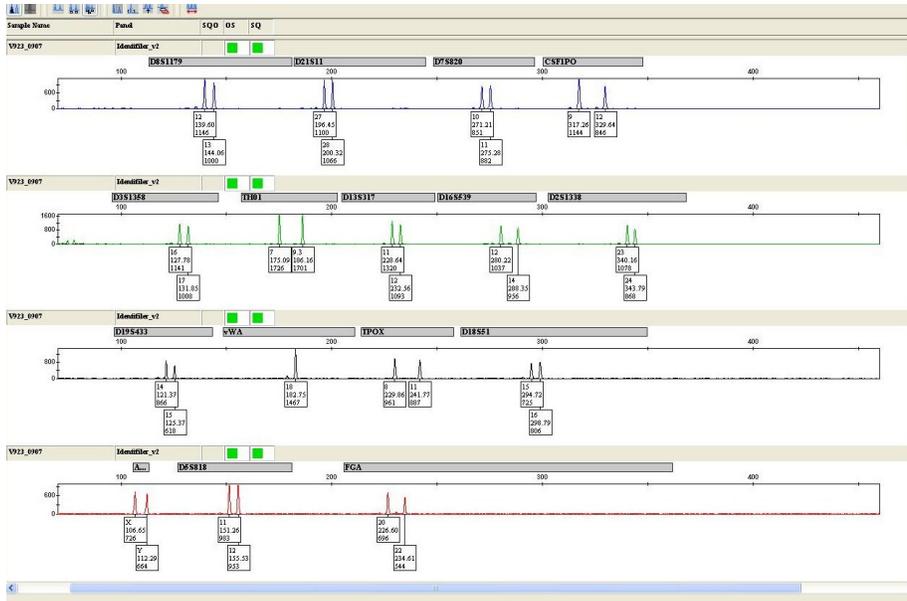
(Source: [http://www.biology.arizona.edu/human\\_bio/activities/blackett2/str\\_codis.html](http://www.biology.arizona.edu/human_bio/activities/blackett2/str_codis.html))

## Capillary Electrophoresis

A PCR reaction only amplifies the DNA fragments, but a step is needed to resolve the fragments from one another. Electrophoresis is a process that separates charged molecules by creating an electric field in the buffer. DNA is a negatively charged molecule and in gel electrophoresis it will move towards the positively charged electrode or anode of the gel with the smallest fragments moving faster than the larger fragments (Butler 2005). Gel separation has been used for decades, however there is a relatively new technique called capillary electrophoresis (CE) that has some advantages over the traditional gel method. CE separates DNA fragments through a capillary tube made of silica that has an electric field. The CE instrument itself contains a narrow capillary, two buffer vials, two high-voltage powered electrodes, a laser excitation source, and a fluorescence detector (Zacharis 2009). The capillary is filled with polymer solution, and one DNA PCR sample is loaded and the fragments move to the anode. The CE columns can be reused and, depending on the system, can be reused serially for 100 to 150 sample runs (Applied Biosystems 3130 and 3130xl”). Detection occurs when the sample passes

the laser detector near the end of the capillary, with the smallest fragments reaching the anode first. These DNA fragments will be illuminated as they pass the laser because of the attached PCR fluorescence emission primer dyes, and the instrument automatically measures the time span from injection it took them to reach the laser (Butler 2005).

The ABI 3130xl and GeneMapper ID software by Applied Biosystems is a CE instrument containing software that generates STR profiles. Its advantages include fully automated injection, separation, and detection of DNA samples not available with gel electrophoresis. Only a small amount of sample is needed and the samples do not cross-contaminate each other because each sample is contained in its own capillary. Also, separation takes place in minutes rather than hours as with gels, and the data is available electronically following the completion of a run (Butler 2005). In the STR technique, the profiles generated from the CE appear as peaks and not as bands from the traditional gel method (*see Figure 2*). The 13 loci and their respective alleles are shown with the size and peak heights labeled.



**Figure 2: Sample STR Genetic Profile.**

This is a sample STR profile generated by the ABI 3130xl and GeneMapper ID software by Applied Biosystems. All 13 core loci are labeled with boxes, and the peaks indicate which alleles are present at each particular locus and its size and peak height. (Source: Dindinger, Massachusetts State Police Forensic and Technology Center).

Currently, the use of DNA fingerprinting has grown immensely. Hundreds of thousands of DNA tests are conducted in the United States by public and private forensic laboratories (Butler 2005). These analyses are not limited to crime scene investigations but are also used in determining paternity, and identifying remains of missing persons or mass disaster victims (i.e. World Trade Center 2001 tragedy). Furthermore, more DNA fingerprinting techniques such as mitochondrial DNA analysis and Y-chromosome analysis are in use for cases with highly degraded DNA or male DNA mixtures. Finally, DNA databases have also been created that store DNA profiles of individuals. Although there are ethical controversies linked to databases, it shows the growing knowledge and popularity of DNA profiling in society.

## **DNA Sample Backlog Problem**

DNA fingerprinting is used in almost every criminal case, so long as there is DNA evidence to process. Given the high number of crimes, for example, an estimated 1,408,337 violent crimes occurred nationwide in the United States in 2007 (“Violent Crime” 2008), this has led to massive amounts of DNA evidence to be processed by forensic labs that have limited analytical capacities (i.e. limited resources, staff, and funding). Therefore one of the biggest problems facing the criminal justice system today is the substantial backlog of unanalyzed DNA samples and biological evidence from crime scenes. Data collected from a large sampling of local law enforcement agencies for the *National Forensic DNA Study Report* from 2003 made the following estimates:

- The number of rape and homicide cases with possible biological evidence which local law enforcement agencies have not submitted to a laboratory for analysis is over 221,000.
  - Homicide cases - 52,000 (approximate)
  - Rape cases - 169,000 (approximate)
- The number of property crime cases with possible biological evidence which local law enforcement agencies have not submitted to a laboratory for analysis is over 264,000.
- The number of unanalyzed DNA cases reported by State and local crime laboratories is more than 57,000.
  - State laboratories - 34,700 cases (approximate)
  - Local laboratories - 22,600 cases (approximate)
- Total crime cases with possible biological evidence either still in the possession of local law enforcement, or backlogged at forensic laboratories is over one half million (542,700). (“Backlog of DNA Samples” 2009)

The sample backlog is not static, but rather is subject to change depending on the available resources, number of crimes committed, and law statutes. This is evident because the backlog expanded by about 80,000 samples in 2006 when a law took effect requiring that all federal convicts, rather than just violent felons, submit DNA samples (Willing 2007). Potential legislation, such as California's Proposition 69, will only

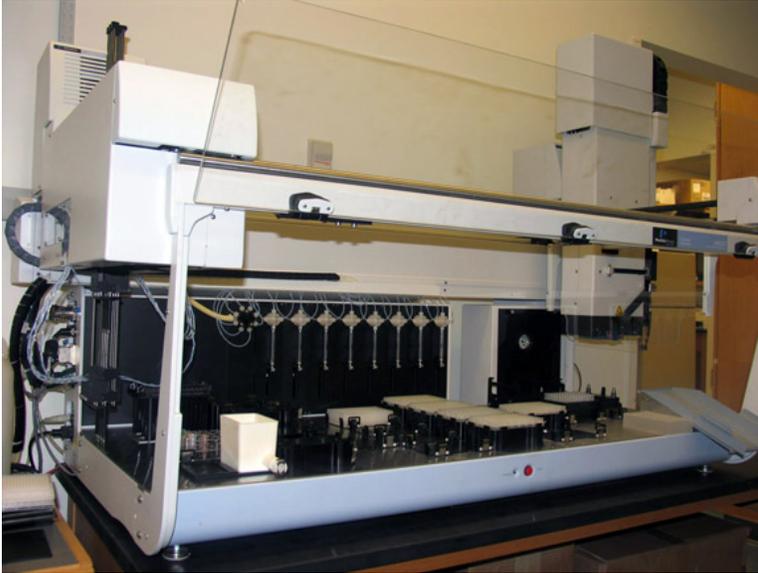
magnify the problem. This legislation calls for DNA profiling and entrée into CODIS DNA from *arrestees* not yet convicted of a crime (Pinchin 2007). Due to the backlog's unfixed nature, it is difficult to measure the precise number of unanalyzed DNA samples for a state or for the nation as a whole. The only definite thing with this backlog crisis is that if it continues to grow, more crimes that could have been prevented may occur simply because samples were not processed through CODIS leading to a match that could have taken a criminal off the streets.

The FBI has taken the initiative in resolving the backlog issue by creating the Forensic DNA Backlog Reduction Program. The aim of this program is to assist eligible States and units of local government to reduce forensic DNA case turnaround time, increase the throughput of public DNA laboratories, and reduce DNA forensic casework backlogs ("Forensic DNA Backlog Reduction Program" 2009). It does this by providing much needed funding to chosen eligible states so that their labs can acquire necessary resources.

### **The Need for Automation**

Although the process of DNA fingerprinting can be quite successful, it is tedious and can lead to inconsistency depending on the ability and training of the analyst performing the steps in the process. One way to address these issues and the backlog crisis is through automation and the use of expert software systems. Automation is commonly viewed by the forensics community as a way of increasing the efficiency and throughput of DNA extraction, as well as other processes in the laboratory workflow (such as qPCR setup, normalization, STR setup, etc.) (Cowan 2008).

The robotic system allows the analyst to load more samples, assay more plates, and walk away from that process for a longer period of time, reducing the time spent performing bench work, and increasing the time spent performing tasks that are more difficult or controversial to automate. Overall, using robotic instruments to remove mundane duties for staff have proved hugely successful, reducing the margin for error and contamination (Pinchin 2007). While humans can get careless while repeatedly transferring liquid, especially into the tiny wells of something like a 96-well plate, a robot does not. Such a robot is the JANUS™ Automated Liquid Handling System by PerkinElmer Inc. (*see Figure 3*). PerkinElmer Inc. unveiled the JANUS™ Automated Liquid Handling System at LabAutomation 2006 in Palm Springs, CA (“PerkinElmer Unveils” 2006). This is a liquid handling robot capable of automated liquid transfers in an effort to reduce or eliminate human interaction in sample and reagent transfer. Its design allows for up to three arms for dispensing and moving labware. It also includes 4- and 8-tip pipeting capability plus 96- or 384-tip MDT dispense head options in a single platform for precision pipeting and performance (“PerkinElmer Unveils JANUS” 2006). Overall it is hoped that JANUS™ and robots like it will make forensic laboratories more efficient.



**Figure 3: Photo of the JANUS™ Automated Liquid Handling System.**

This robot completely automates liquid pipeting tasks allowing analysts to move on to more pressing tasks. (Source: [http://www.roswellpark.org/Research/Shared\\_Resources/small-molecule-screening-core/smsc-infrastructure](http://www.roswellpark.org/Research/Shared_Resources/small-molecule-screening-core/smsc-infrastructure))

### **Robot Validation**

DNA evidence is crucial to linking suspects to a crime. It can lead to the loss of someone's liberty and even their life if they are found guilty and sentenced to death. Therefore the method in which DNA evidence is processed must be reliable and done with great care to ensure that the right person is charged of the crime in question. Also, the evidence has to hold up in court. For the past decade or so automation has been investigated as a means to increase lab efficiency, however DNA processing cannot simply be entrusted into the 'arms' of a robot without proof that it works. In other words, the robot, like all laboratory instrument and procedures must be validated.

Validation is the process of demonstrating that a laboratory procedure is robust, reliable, and reproducible in the hands of the personnel performing the test in that laboratory (Butler 2005). A robust method is one in which successful results are obtained a high percentage of the time, and few, if any, samples need to be repeated. A reliable

method refers to one in which the obtained results are accurate and correctly reflect the sample being tested. A reproducible method means that the same or very similar results are obtained each time a sample is tested. All three criteria are important for techniques performed in forensic laboratories. Validation involves performing laboratory tests to verify that a particular instrument, software program, or measurement technique is working properly. These validation experiments typically examine precision, accuracy, and sensitivity, which all play a factor on reliability, reproducibility, and robustness (Butler 2007). The DNA Advisory Board (DAB) standards govern DNA analysis for casework and database applications, and its section 8 speaks specifically of validation broadly, simply saying that it must be done, be documented, and pass a qualifying test ("Standards for Forensic DNA Testing Labs" 2009). Validation is necessary for laboratory accreditation, which means that the lab has successfully completed an inspection or audit by an accrediting body such as the Scientific Working Group on DNA Analysis Methods or SWGDAM established in 1988 (Butler 2005). Organizations such as this recommend and oversee quality assurance in lab products and services while providing quality control measures that are done daily to fulfill quality requirements. This ensures that the accredited lab maintains good laboratory practices.

Even with organizations like SWGDAM and DAB, there are no set standards for how to perform validations in all forensic DNA laboratories, only guidelines and requirements they must meet. Nevertheless, bringing a procedure (assay, instrument, or software) "on-line" in a forensic lab typically includes the following steps: (a) installation of the instrumentation or software and purchase of assay reagents, (b) learning about the technique and how to perform it properly, (c) validation of the analytical procedure to

define its range and reliability, (d) creation of the standard operating procedures with interpretation guidelines based on the validation studies, (e) training of other personnel on the technique, and (f) each trained analyst passing a qualification test for initial use in forensic casework. After a procedure has been successfully implemented into use with forensic casework, proficiency tests are performed on a regular basis to demonstrate successful application of the technique over time by qualified analysts (Butler 2007).

Validations can be performed on robotic instruments like the JANUS™ Automated Liquid Handling System. First, these instruments undergo validation by the company that designed them, in this case by PerkinElmer Inc. that did their own validation to credit its use. The JANUS™ Automated Liquid Handling System has also been validated by law enforcement personnel and is currently in use in four states' labs, including the New York Regional Crime Laboratory (WCFL), Pennsylvania State Police Crime Lab (PACL), Indian River Crime Lab in Florida, and the Montgomery County Crime Laboratory in Maryland. The Centre of Forensic Sciences in Toronto, Canada is also using it (Dindinger). The robot has been validated for DNA analysis processes such as qPCR, normalization and profiler STR setup. The Massachusetts State Police Forensic and Technology Center hopes to soon use the JANUS™ Automated Liquid Handling System for DNA fingerprinting. It is currently undergoing validation for PCR amplification setup, and is the subject of this project.

## **PROJECT OBJECTIVE**

The purpose of this project was to validate the JANUS™ Automated Liquid Handling System for use in the Massachusetts State Police Forensic and Technology Center. First the JANUS™ system was validated for its liquid handling ability at specified volumes and volume ranges to see how accurate and precise the instrument is. Then the JANUS™ system was validated for its ability to set up PCR amplifications in a 96-well plate accurately and without sample contamination across wells. The goal is for JANUS™ to be “on-line” in our lab to increase laboratory efficiency and throughput.

# METHODS

## Liquid Handling Validation

The following experiments were done to ensure that JANUS™ accurately handles liquid samples at specified volumes or volume ranges consistently. Each experiment was done twice.

### *25 $\mu$ L Tip Test*

1. Labeled 30 microcentrifuge tubes with the numbers 1-30.
2. Measured and recorded the weight of each tube using the analytical balance.
3. Placed the tubes into the Janus microcentrifuge tube racks and placed on the racks on the deck of the robot.
4. Set up Janus to dispense distilled water into the tubes as follows using 25  $\mu$ L conductive tips:
  - a. Tubes 1-10: 5  $\mu$ L dH<sub>2</sub>O
  - b. Tubes 11-20: 10  $\mu$ L dH<sub>2</sub>O
  - c. Tubes 21-30: 15  $\mu$ L dH<sub>2</sub>O
5. Measured and recorded the new weight of each tube. Then calculated the difference.

### *175 $\mu$ L Tip Test*

1. Labeled 40 microcentrifuge tubes with the numbers 1-40.
2. Measured and recorded the weight of each tube using the analytical balance.
3. Placed the tubes into the Janus microcentrifuge tube racks and placed on the racks on the deck of the robot.
4. Set up Janus to dispense distilled water into the tubes as follows using 175  $\mu$ L conductive tips:
  - a. Tubes 1-10: 25  $\mu$ L dH<sub>2</sub>O
  - b. Tubes 11-20: 50  $\mu$ L dH<sub>2</sub>O
  - c. Tubes 21-30: 100  $\mu$ L dH<sub>2</sub>O
  - d. Tubes 31-40: 150  $\mu$ L dH<sub>2</sub>O
5. Measured and recorded the new weight of each tube. Then calculated the difference.

### *1000 $\mu$ L Tip Test*

1. Labeled 30 microcentrifuge tubes with the numbers 1-30.
2. Measured and recorded the weight of each tube using the analytical balance.

3. Placed the tubes into the Janus microcentrifuge tube racks and placed on the racks on the deck of the robot.
4. Set up Janus to dispense distilled water into the tubes as follows using 1mL conductive tips:
  - e. Tubes 1-10: 250  $\mu\text{L}$  dH<sub>2</sub>O
  - f. Tubes 11-20: 500  $\mu\text{L}$  dH<sub>2</sub>O
  - g. Tubes 21-30: 750  $\mu\text{L}$  dH<sub>2</sub>O
5. Measured and recorded the new weight of each tube. Then calculated the difference.

In addition, the experiments were repeated twice manually. A 2-20  $\mu\text{L}$  pipette was used for the 25  $\mu\text{L}$  tip test, a 20-200  $\mu\text{L}$  pipette was used for the 175  $\mu\text{L}$  tip test, and a 100-1000  $\mu\text{L}$  pipette was used for the 1000  $\mu\text{L}$  tip test. The tubes were weighed before and after the same specified volumes were added, and the differences were recorded for comparison with JANUS™.

### **Amplification Setup Validation**

The following experiments were done to show that PCR amplifications setup by JANUS™ provide results comparable to amplifications set up manually, and that the robot does not introduce or cause any contamination between wells of the 96 well plate.

#### *JANUS™ Amplification Setup*

1. Formatted a 96 well amplification plate with 44 normalized DNA extracts separated by negative controls such that no two extracts are adjacent to each other. The layout resembled a checkerboard (*see Figure 4*). Eighty-eight wells (11 rows) of the plate were utilized.
2. Prepared enough Identifiler™ master mix for 92 samples.
3. Ran the normalized extract amplification setup program on JANUS™ (*see Figure 5*). The total reaction volume in each well was 25  $\mu\text{l}$ .
4. Covered the plate with a plate seal.
5. Pulse spun the plate in the swinging bucket centrifuge in the Post-PCR room.

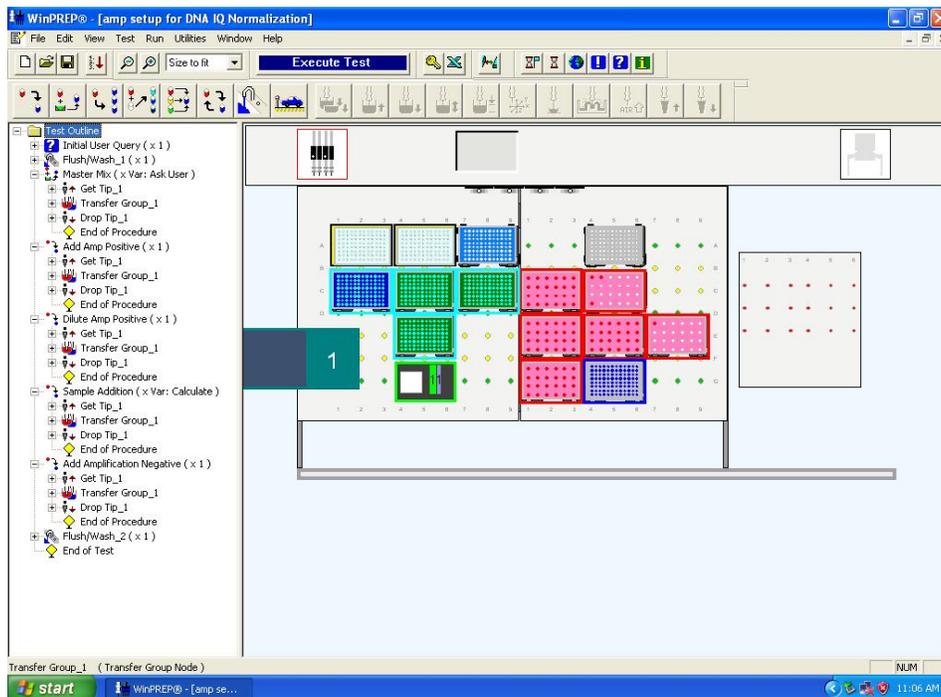
6. Placed the plate on the thermalcycler and started the Identifiler program.
7. Analyzed the amplified samples and controls using the 3130xl and GeneMapper ID software.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Amp + control	Blank	V876 BR	Blank	V401 EXP6	Blank	V919 SR	Blank	V880 KSS-KW	Blank	V284 EXP6	
B	Blank	V991 KSS-KW	Blank	V268 0907	Blank	V906 0208A	Blank	V904 EXP5	Blank	V1020 EXP4	Blank	
C	V923 0907	Blank	V1044 KS	Blank	V901 SR	Blank	V921 AT	Blank	V1031 CQ	Blank	V882 KSS-KW	
D	Blank	V926 0208C	Blank	V874 EXP8	Blank	V907 0208B	Blank	V1029 BF	Blank	V1041 CD	Blank	
E	V924 0208A	Blank	V878 BR	Blank	V903 AT	Blank	V879 KSS-KW	Blank	V911 EXP8	Blank	V1043 AT	
F	Blank	V873 EXP8	Blank	V997 KSS-KW	Blank	V902 EXP8	Blank	V912 AT	Blank	V1042 BX	Blank	
G	V925 0208B	Blank	V1045 Ext Neg	Blank	V905 0907	Blank	V910 SR	Blank	V1040 EXP9	Blank	V913 EXP5	
H	Blank	V992 KSS-KW	Blank	V283 EXP6	Blank	V908 0208C	Blank	V920 EXP8	Blank	V881 KSS-KW	Blank	

**Figure 4: PCR Design for a 96 Well Amplification Plate.**

Above is a representation of the 96 well PCR amplification plate. The grey shaded areas are samples, separated by amp negative blanks (white). The samples shown in red were also amplified manually for comparison.

Twelve of the normalized extracts (shown as red in the figure) were manually amplified using the same lot of amplification reagents and a total reaction volume of 25  $\mu$ l. The amplification protocol used was from the AmpF/STR<sup>®</sup> IDENTIFILER<sup>™</sup> STR TYPING KIT (see Appendix D). All amplified samples and controls were analyzed using the 3130xl and GeneMapper ID software (**Figure-5**), and profiles were generated. Peak heights of the twelve manual amplifications were compared to peak heights of the same twelve amplifications setup by JANUS<sup>™</sup> to ensure concordance.



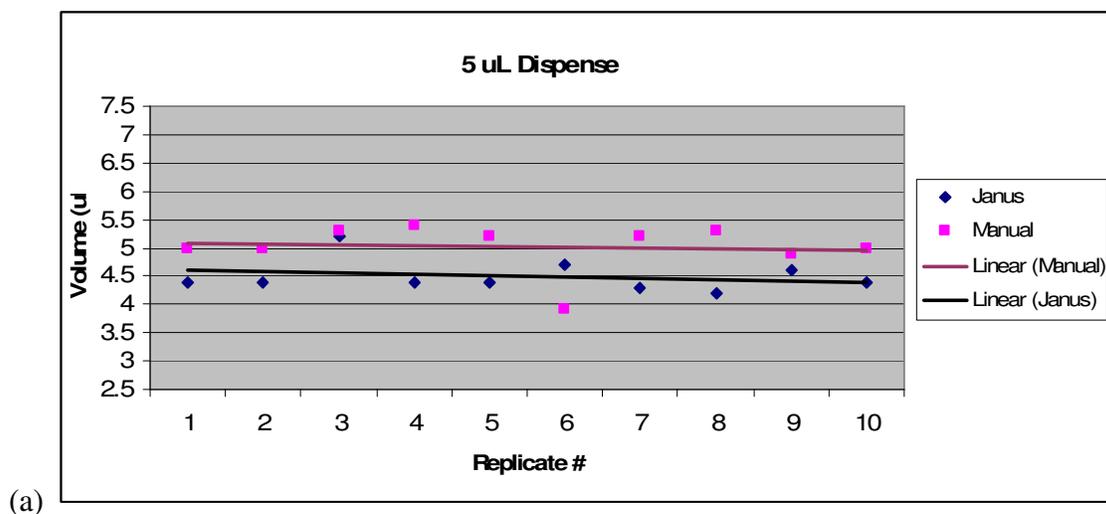
**Figure 5: JANUST™ Amplification Setup Program.**

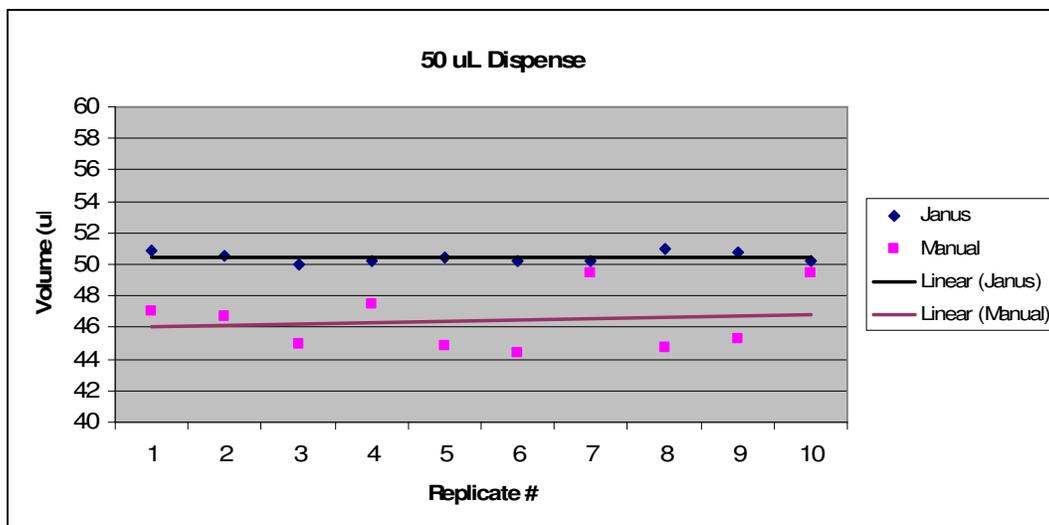
This figure shows the protocol and the desk layout for the JANUST™ normalized extract amplification setup. On the left is a test outline showing the procedure Janus follows when dispensing the reagents and samples into the 96 well plate. To the right is the desk layout showing the placement of the reagents (master mix, amp positive, amp negative, and all samples) and the 25  $\mu$ l and 175  $\mu$ l conductive disposable tips.

# RESULTS

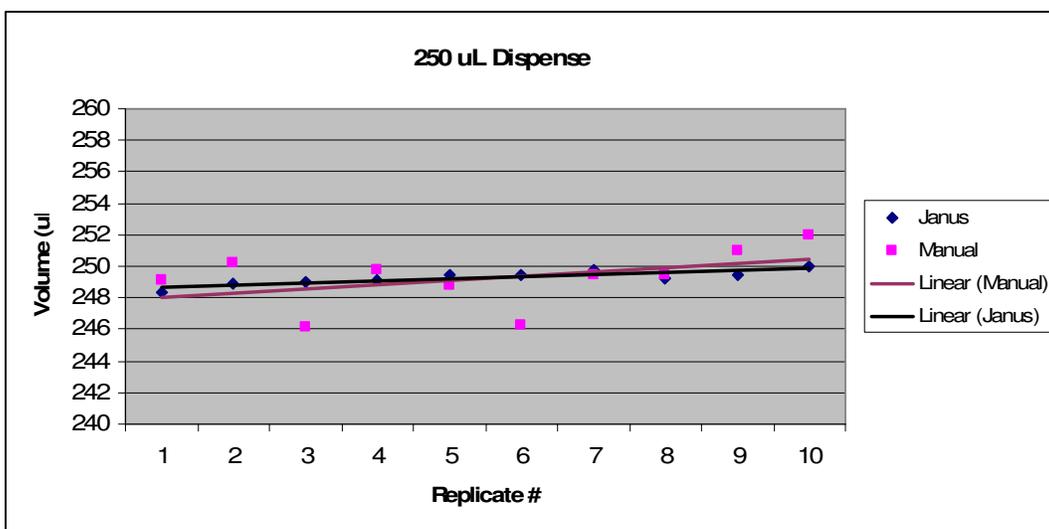
## Liquid Handling Validation

Distilled water was added to pre-weighed microcentrifuge tubes in specified volumes by JANUS™, and also manually. The difference in tube weight with and without water was recorded. Calculations were done based on the assumption that 1 g dH<sub>2</sub>O is equal to 1 ml dH<sub>2</sub>O to convert the weights into microliters dispensed. **Figure 6** shows a comparison between manual and JANUS™ liquid handling for three of the specified volumes tested. At low volumes such as 5 uL (6a) it can be seen that JANUS™ dispenses at about 4.5 uL of dH<sub>2</sub>O rather than the full requested 5 uL. The manual dispenses though are closer to the specified volume. However, at 50 uL (6B), JANUS™ was much closer to the specified volume than when manually dispensed. Both manual and JANUS™ dispenses were similar at the higher volume of 250 uL (6C). The exact volumes dispensed in each tube for all specified volumes are shown in Appendix A.





(b)



(c)

**Figure 6: Liquid Handling Comparison.**

At low volumes such as 5 uL (a) the manual dispense (pink) was more accurate than JANUS™ (blue). At higher volumes however 50 uL (b) or 250 uL (c), the JANUS™ system was just as competent as manual distribution.

**Table 2** compares the amount of liquid dispensed by JANUS™ versus manually when the specified volumes were 5, 10, and 15 µl. JANUS™ was able to dispense the specified volumes with less than 5 % error, as was the case manually, but not the 5 µl dispense, in which there was an 11.1% error.

**Table 2: Comparison of JANUS™ Robot and Manual Liquid Dispense (5 µl – 15 µl)**

Dispense Amount	5 µl			10 µl			15 µl		
	Mean (µl)	STDEV	% Error	Mean (µl)	STDEV	% Error	Mean (µl)	STDEV	% Error
Janus	4.445	0.317	11.1 %	9.55	1.003	4.2%	14.66	0.843	2.27%
Manual	5.025	0.315	0.2%	9.965	0.35	1.63%	14.95	0.285	1.34%

The average amount of liquid dispensed manually and by JANUS™ was calculated. Manual liquid dispense of the 5 µl volume was more accurate than JANUS™. However, JANUS™ liquid handling was comparable to manual handling at the other volumes.

**Table 3** compares the amount of liquid dispensed by JANUS™ versus manually when the specified volumes were 25, 50, 100 and 150 µl. In all the volumes, JANUS™ was able to dispense the requested amount with less than 5% error. This was not so manually, indicating that at these higher volumes JANUS™ was more accurate.

**Table 3: Comparison of JANUS™ Robot and Manual Liquid Dispense (25 µl – 150 µl)**

Dispense Amount	25 µl			50 µl			100 µl			150 µl		
	Mean (µl)	STDEV	% Error									
Janus	25.54	0.601	2.16%	50.22	0.974	0.47%	101.34	0.424	1.34%	151.93	2.195	1.29%
Manual	22.77	1.215	8.58%	47.48	1.813	7.27%	97.89	1.441	3.75%	147.42	1.336	2.58%

JANUS™ is more accurate dispensing these volumes than manual dispensing. Overall there was less variation in the amounts dispensed into each tube by JANUS™ and those amounts were closest to the requested volumes than the manual dispenses were.

**Table 4** compares the amount of liquid dispensed by JANUS™ versus manually when the specified volumes were 250, 500, and 750 µl. Again, JANUS™ accurately dispensed the specified volumes with less than 5% error. This was also true for the manual dispenses.

**Table 4: Comparison of JANUS™ Robot and Manual Liquid Dispense  
(250 µl – 750 µl)**

Dispense Amount	250 µl			500 µl			750 µl		
	Mean (µl)	STDEV	% Error	Mean (µl)	STDEV	% Error	Mean (µl)	STDEV	% Error
Janus	248.86	0.627	0.46%	503.43	0.613	0.69%	761.62	1.137	1.55%
Manual	249.37	1.145	0.26%	497.79	0.769	0.45%	745.73	0.97	0.57%

The volumes dispensed by JANUS™ are similar to those dispensed manually. For the 150 µl volume, however, JANUS™ consistently dispensed about 10 µl more water than requested. With such high volume though, that amount is not too significant and the percent error of JANUS™ still remains low.

### Amplification Setup

Normalized DNA samples were set up for PCR amplification manually and by the JANUS™ robot. The same kit and reagents were used to set up both amplifications. The only difference was that different thermocyclers were used to run the two PCRs.

Following amplification, the samples were profiled using the 3130xl and GeneMapper ID software. Profiles were generated for all 44 samples from the JANUS™ amplification setup. For the purpose of this project, only the profiles from the twelve samples that were also amplified manually were analyzed and compared. **Table 5** shows the genetic profiles of the twelve samples that were amplified both by JANUS™ and manually. Many of the samples were repeats having the same genetic profile so that all twelve samples had one of the profiles shown below.

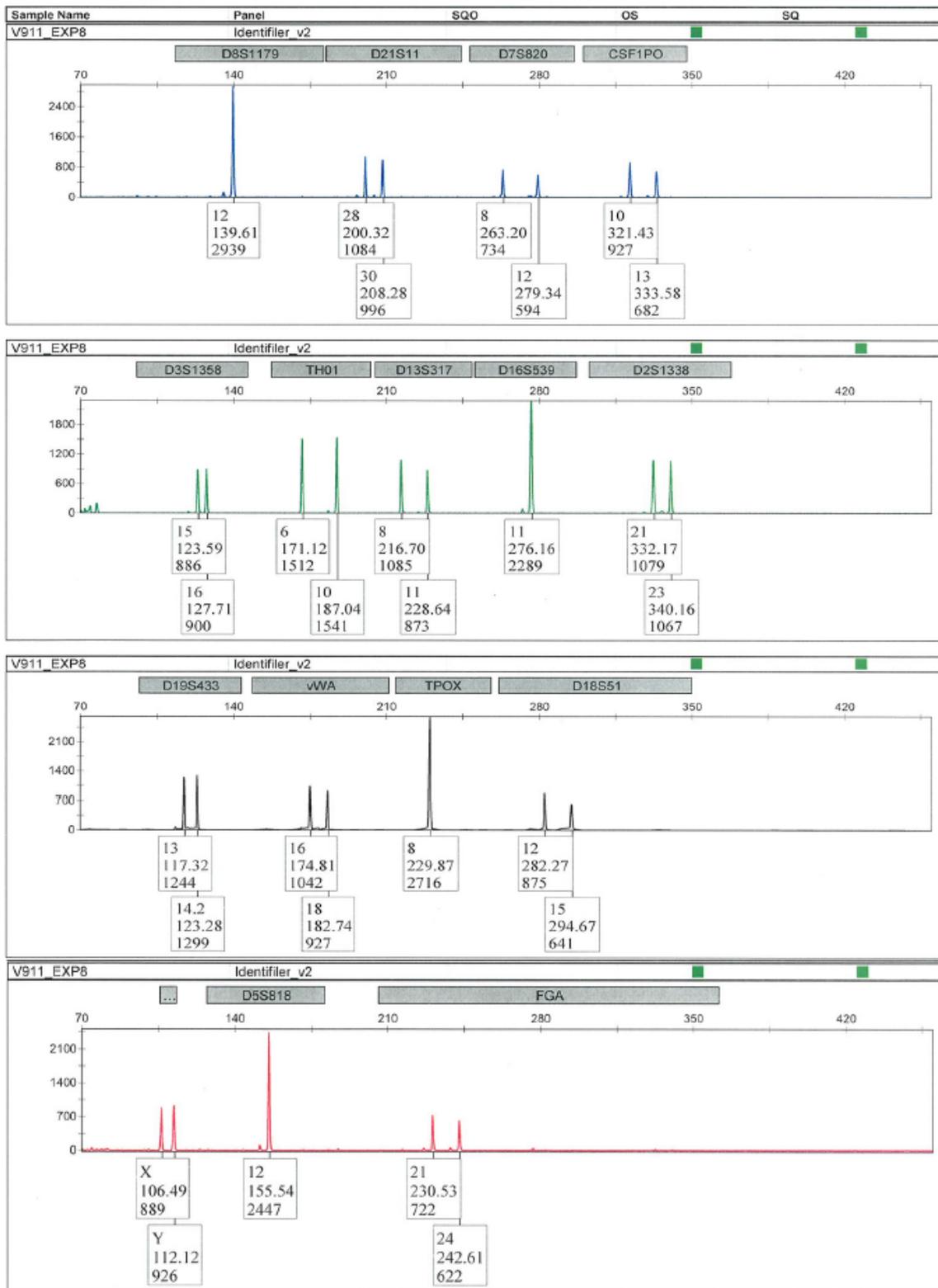
**Table 5: Genetic Profiles of Normalized DNA Extracts.**

Profile	Sample	Blue Loci				Green Loci				
		D8	D21	D7	CSF	D3	TH01	D13	D16	D2
AT	V912	10,13	28,29	11,11	12,12	14,17	7,7	8,10	12,13	25,NR
KW	V879, V880, V881	14, 14	27, 30	8, 11	10, 12	15, 16	9.3,9.3	11,?	11, 12	17, 20
SR	V876, V878, V910	11, 14	28, 31.2	11, 12	10, 14	15, 17	7, 9.3	11, 12	13, 13	17, 19
Exp5,6,8	V873,V874, V911,V913, V284	12,12	28,30	8,12	10,13	15,16	6,10	8,11	11,11	21,23

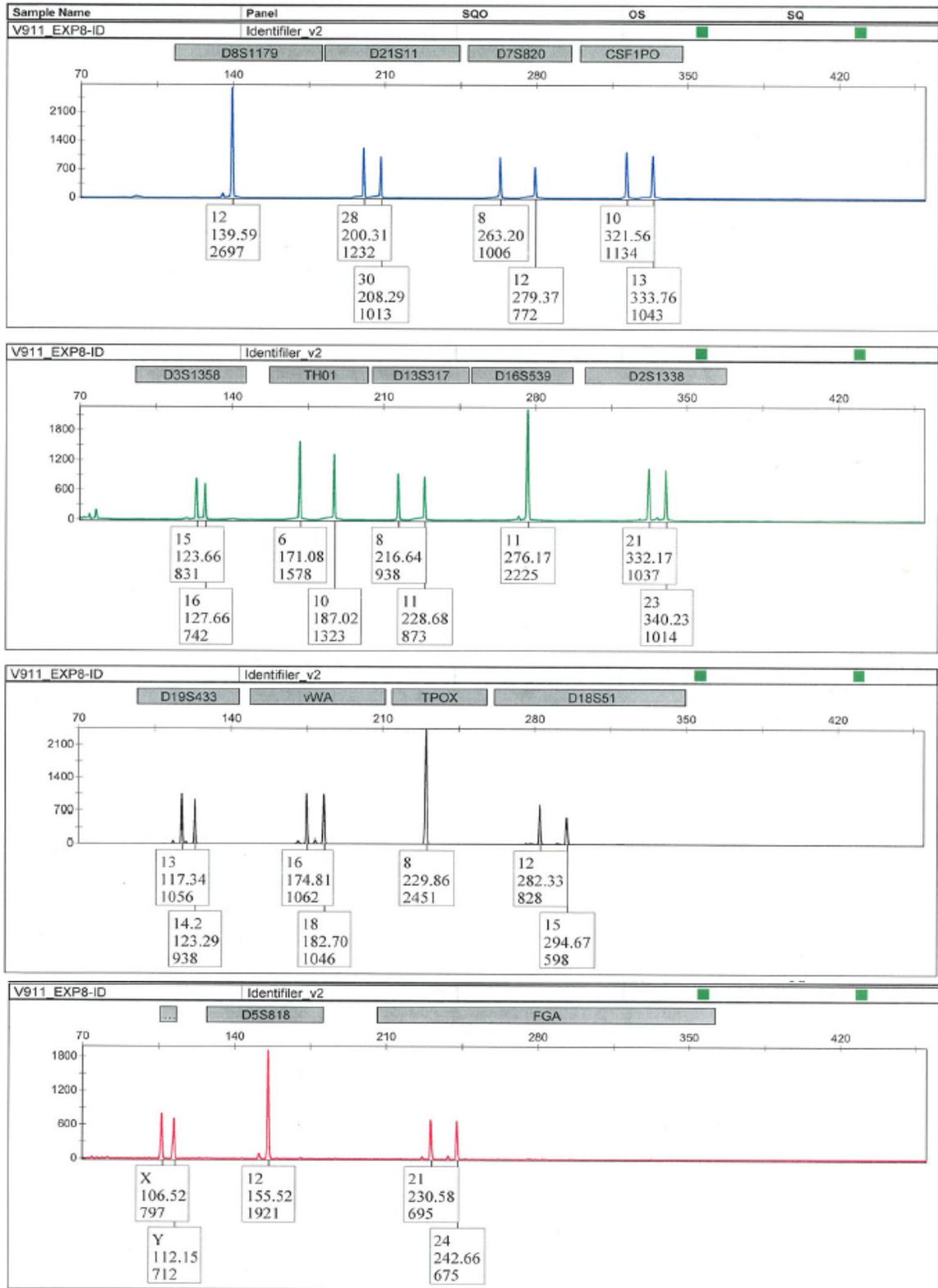
Profile	Sample	Yellow Loci				Red Loci			# homozyg alleles	TOTAL # of alleles
		D19	vWA	TPOX	D18	amel	D5	FGA		
AT	V912	16,17.2	15,18	8,8	14,16	x, x	11,12	21,24	5	27
KW	V879, V880, V881	13, 16	16, 17	8, 11	15, 17	x, x	12, 13	20, 23	4	28
SR	V876, V878, V910	12, 13	14, 17	8, 11	16, 17	x, x	11, 12	23, 24	1	31
Exp5,6,8	V873,V874, V911,V913, V284	13,14.2	16,18	8,8	12,15	x, y	12,12	21,24	4	28

Shown above are the alleles (number combinations within the parentheses) present at each of the 13 core loci (blue, green, yellow), two additional loci, and amelogenin (sex-type) (red) for the twelve normalized DNA extracts used for amplification.

STR genetic profiles were produced electronically after each CE run. The electropherogram charts **Figure-7** show the peak heights and size (in basepairs) for each allele (in boxes) for sample V911 using JANUS™ PCR (Figure 7a) or manual PCR (Figure 7b). Results show the exact same male genetic profile was generated for sample V911 for both types of PCR amplification. This was also the case for 11 of the samples tested. For unknown reasons no profile was generated for sample V284, and it is assumed that JANUS™ might not have aspirated that sample. Besides that, since the profiles were identical to each other and matched its known profile, it is deduced that JANUS™ does not cause contamination between wells during PCR amplification set up.



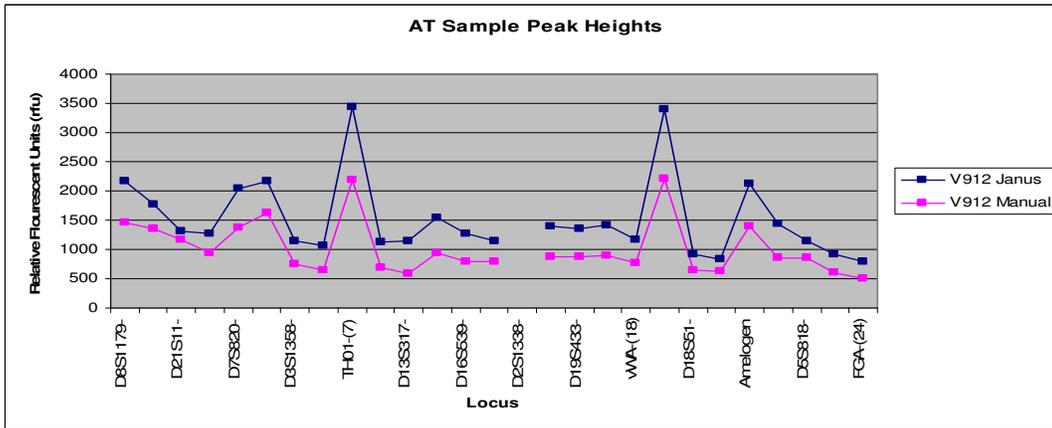
**Figure 7(a): CE Electropherogram for DNA Extract V911, PCR Set Up By JANUS™**  
 For all 13 core loci the peak heights and size (in basepairs) for each allele (top number) are shown in boxes.



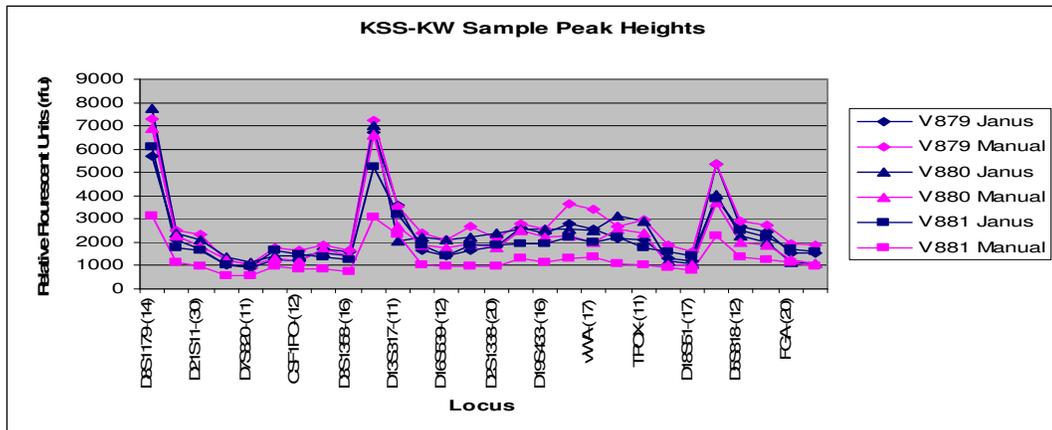
**Figure 7(b): Electropherogram for DNA Extract V911 PCR Set Up Manually.**  
 For all 13 core loci the peak heights and size (in basepairs) for each allele (top number) are shown in boxes.

To compare each peak height by locus of the samples amplified manually to those amplified by JANUS™, the samples were first separated by genetic profile. **Figure 8** shows the comparison between JANUS™ amplification (blue) and manual amplification (pink) for the twelve samples organized by the four DNA profiles (a) AT, (b) KW, (c) SR, and (d) EXP5,6,8 from Table 5 (most samples had the EXP5,6,8 profile while only sample V912 had the AT profile). The peak heights at each locus are shown. Overall, the peak heights of the samples amplified by JANUS™ are very similar to those of the peaks amplified manually. Their heights vary slightly but are close enough to each other to say that the two methods of amplification set up are almost equivalent.

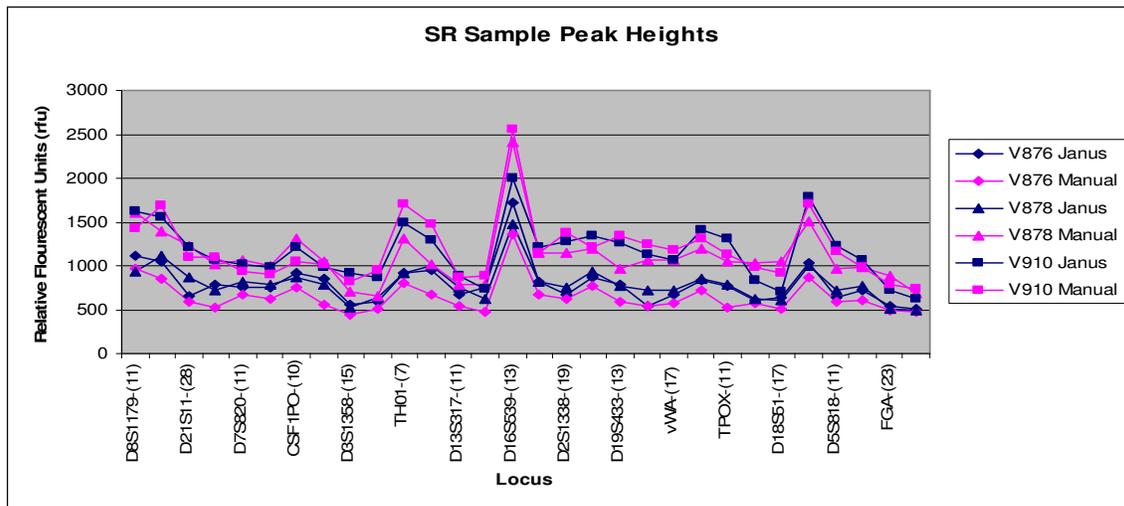
(a)



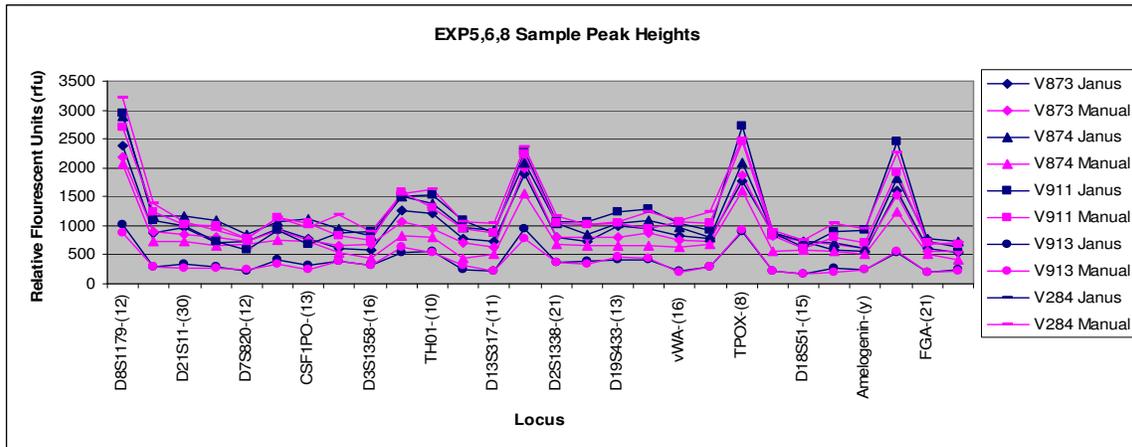
(b)



(c)



(d)

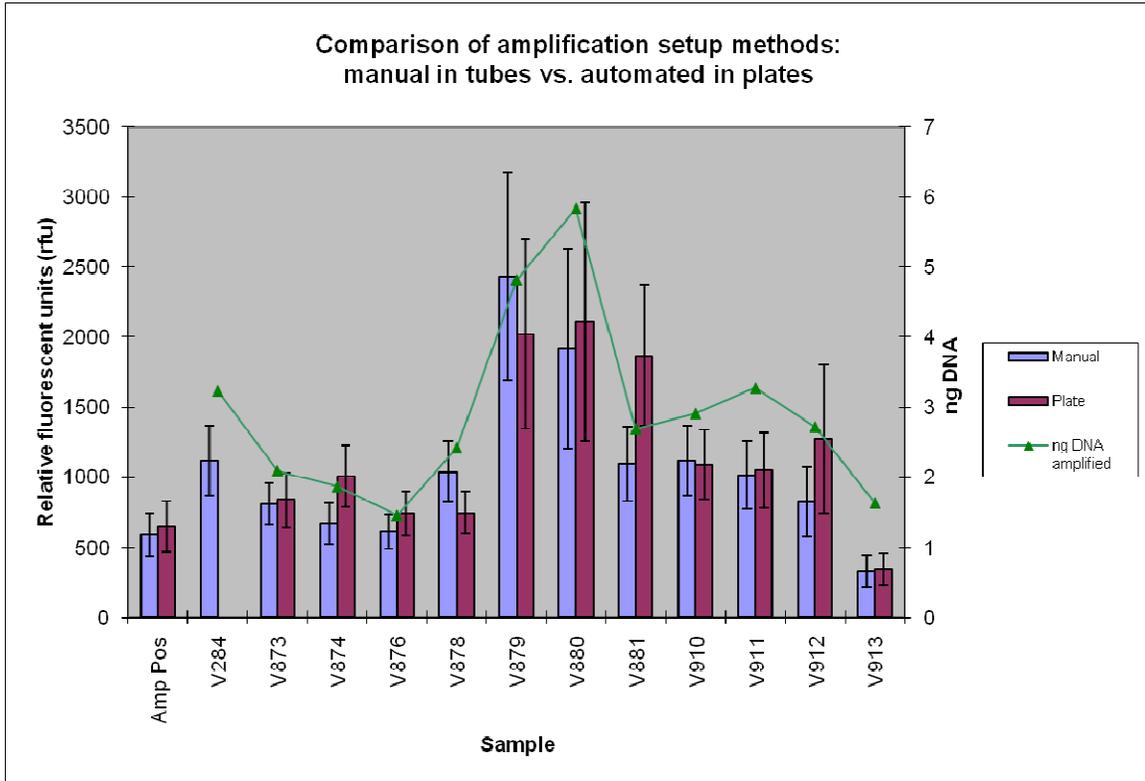


**Figure 8: Allele Peak Height Comparison by Genetic Profile.**

Samples were separated by CE profile, and the peak heights quantitated (a) AT, (b) KW, (c) BR, and (d) EXP5,6,8. Peak heights of all the twelve samples for all loci were very similar in size when amplified by Janus (blue) or manually (pink), resulting in the nearly direct overlay of Janus peaks on the manual peaks.

JANUS™ and manual amplifications were also compared by averaging the peak heights of all the loci of each sample. **Figure 9** shows a graph of the average peak heights for each sample amplified by JANUS™ and manually alongside each other. Also shown is the amount of DNA that was amplified for each sample in nanograms. The average peak heights of the samples are once again very close (about 500 or less rfu) in height. Also the peak heights in both amplification setup methods corresponded with the amount

of DNA that was amplified; the more DNA amplified, the higher the peak height. Once more, JANUS™ is found to be nearly equivalent to manual in PCR amplification setup by generating nearly identical genetic profiles for the samples tested.



**Figure 9: Comparison of Amplification Setup Methods by Sample.**

Each histogram denotes the average peak height for all 13 core loci from a particular DNA sample amplified by Janus (purple) or manual (blue). The two techniques produce average band heights almost analogous in size, varying in most cases by less than 500 relative fluorescence units (rfu). X-axis labels denote the various DNA samples analyzed. For unknown reasons, no profile was generated for sample V284 when amplified by Janus.

## DISCUSSION

The goal of this project was to validate the JANUS™ Automated Liquid Handling System for accurate liquid handling and PCR amplification setup for potential future applications to DNA fingerprinting analyses. The automatic JANUS™ system was found to accurately dispense liquid at specified volumes as well as, and sometimes better than, manual liquid handling (especially at medium to high volumes). The percent error was consistently low for liquid dispensed by JANUS™ compared to that dispensed manually (*see Figure 6 and Tables 3 & 4*). An exception was at low volumes such as 5 uL, where JANUS™ tends to dispense slightly less than the requested amount of liquid, and the percent error is much higher (*see Table 2*). This is not surprising because humans have one advantage that the robot does not. At low volumes sometimes a drop of liquid might remain in the pipette tip that the robot does not realize but an analyst would. The analyst responds by possibly shaking the pipette to ensure that that last drop makes it in, giving manual dispenses the edge at this specified volume. Overall it can be seen that JANUS™ is in fact accurate and precise in liquid handling. We were able to obtain about the same correct liquid volumes when the test was repeated. This is important for validation because a part of validation is to ensure that the procedure (in this case instrument) being validated is robust, reliable, and repeatable, all of which JANUS™ reflects in liquid handling.

In addition, the PCR results indicated that the DNA PCR amplifications set up by the JANUS™ Automated Liquid Handling System provide results comparable to amplifications set up manually. The samples amplified by JANUS™ had the exact same known profile as their corresponding sample amplified manually (*see Figure 7*). This

means that there were no extra or unknown peaks, and that JANUS™ does not introduce or cause any contamination between the wells of a 96-well plate. Furthermore, the peak heights of the alleles for the samples were very close in height between JANUS™ and manual amplification (*see Figures 8 & 9*). In summary, the PCR amplification procedure in the JANUS™ system can be run and the instrument will dispense the necessary reagents into their appropriate locations in the 96-well plate. This way, that somewhat mundane task can be accomplished by the robot while the analyst moves on to more pressing matters. All the analyst must do is carry the plate to the thermalcycler to actually run the PCR.

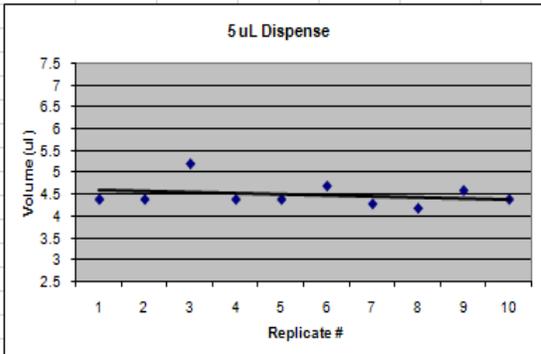
Although the JANUS™ Automated Liquid Handling System has been shown to be accurate in liquid dispensing and PCR set up, it is not quite ready for use yet in the Massachusetts State Police Forensic and Technology Center. There are several general “steps” to the validation process and this project has only accomplished up to defining the range and reliability of two JANUS™ procedures. In the future JANUS™ will probably have to be validated for CE set up because that is another procedure the laboratory wants to automate. Afterwards the later steps of validation such as creating standards for JANUS™ use and training other personnel will have to be done to fully implement JANUS™.

# APPENDIX I: LIQUID HANDLING

This appendix contains all results from the liquid handling experiments. First the JANUS™ results are shown followed by the manual results for each specified volume.

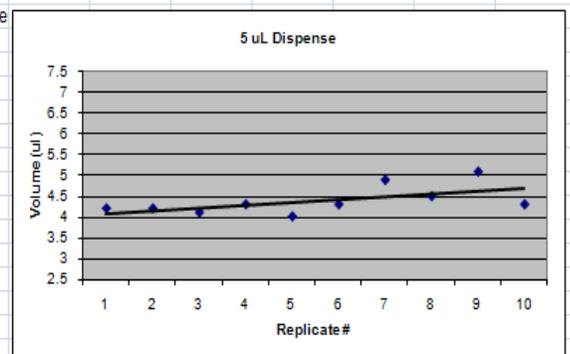
**Janus 25 uL Tip Test 1**

Tube #	Difference
1	4.4
2	4.4
3	5.2
4	4.4
5	4.4
6	4.7
7	4.3
8	4.2
9	4.6
10	4.4
Average	4.5
STDEV	0.28284
% cv	6.29%
% error	10.00%

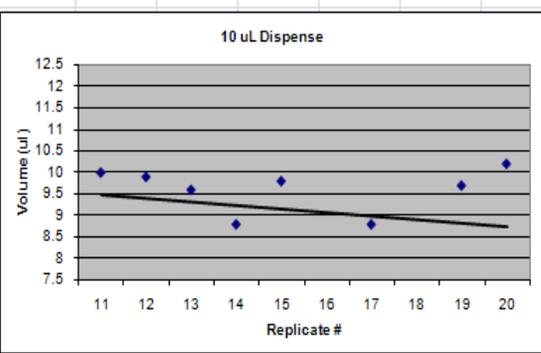


**Janus 25 uL Tip Test 2**

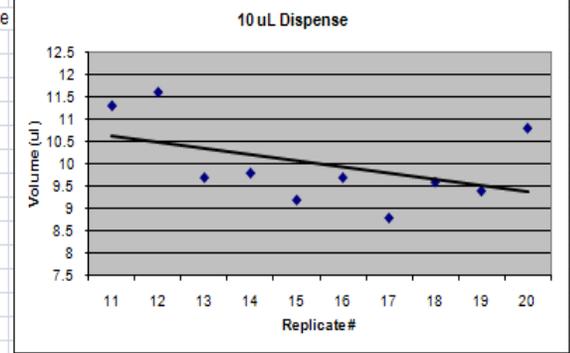
Tube #	Difference
1	4.2
2	4.2
3	4.1
4	4.3
5	4
6	4.3
7	4.9
8	4.5
9	5.1
10	4.3
Average	4.39
STDEV	0.35103
% cv	8.00%
% error	12.20%



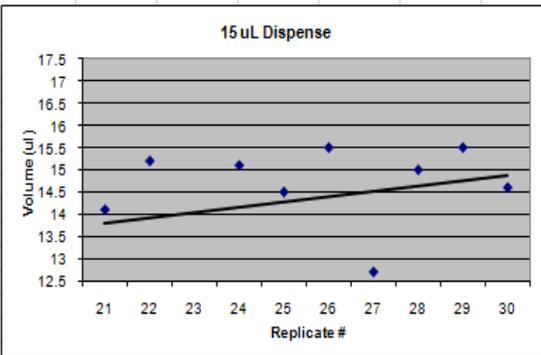
Tube #	Difference
11	10
12	9.9
13	9.6
14	8.8
15	9.8
16	6.9
17	8.8
18	7.4
19	9.7
20	10.2
Average	9.11
STDEV	1.08023
% cv	11.90%
% error	8.90%



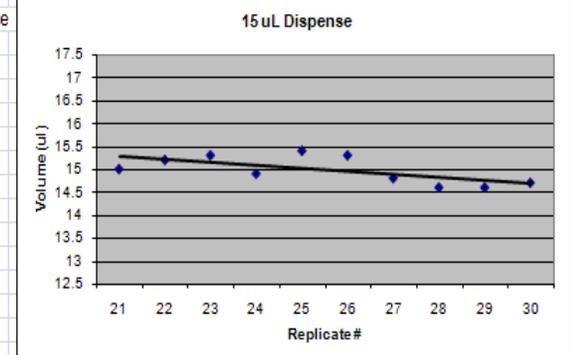
Tube #	Difference
11	11.3
12	11.6
13	9.7
14	9.8
15	9.2
16	9.7
17	8.8
18	9.6
19	9.4
20	10.8
Average	9.99
STDEV	0.9255
% cv	9.27%
% error	0.10%



Tube #	Difference
21	14.1
22	15.2
23	11.2
24	15.1
25	14.5
26	15.5
27	12.7
28	15
29	15.5
30	14.6
Average	14.34
STDEV	1.38018
% cv	9.62%
% error	4.40%

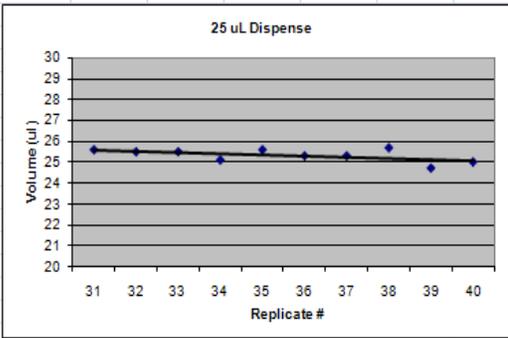


Tube #	Difference
21	15
22	15.2
23	15.3
24	14.9
25	15.4
26	15.3
27	14.8
28	14.6
29	14.6
30	14.7
Average	14.98
STDEV	0.30478
% cv	2.04%
% error	0.13%



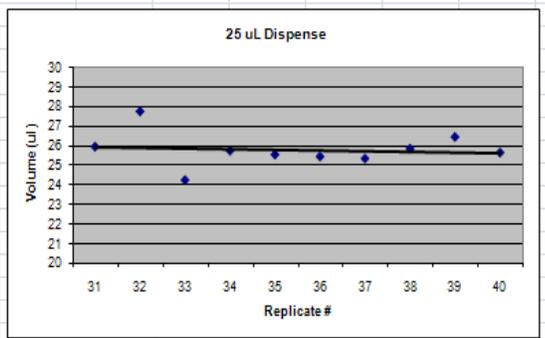
**Janus 175 uL Tip Test 1**

Tube #	Difference
31	25.6
32	25.5
33	25.5
34	25.1
35	25.6
36	25.3
37	25.3
38	25.7
39	24.7
40	25
Average	25.33
STDEV	0.3164
% cv	1.25%
% error	1.32%

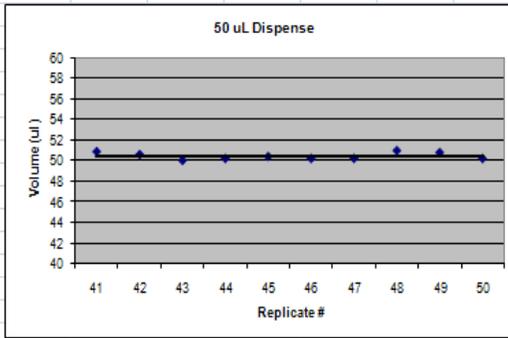


**Janus 175 uL Tip Test 2**

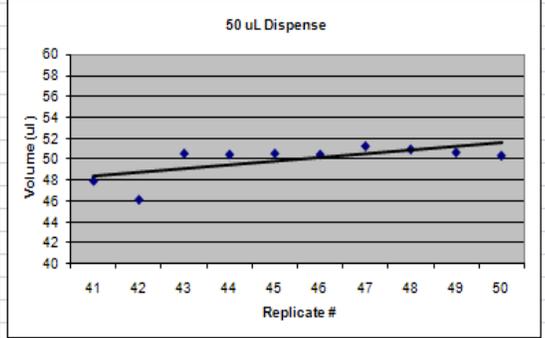
Tube #	Difference
31	25.9
32	27.7
33	24.2
34	25.7
35	25.5
36	25.4
37	25.3
38	25.8
39	26.4
40	25.6
Average	25.75
STDEV	0.886
% cv	3.44%
% error	3.00%



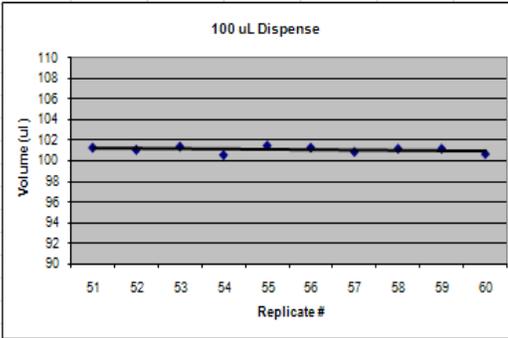
Tube #	Difference
41	50.9
42	50.6
43	50
44	50.2
45	50.4
46	50.2
47	50.2
48	51
49	50.8
50	50.2
Average	50.45
STDEV	0.3504
% cv	0.69%
% error	0.90%



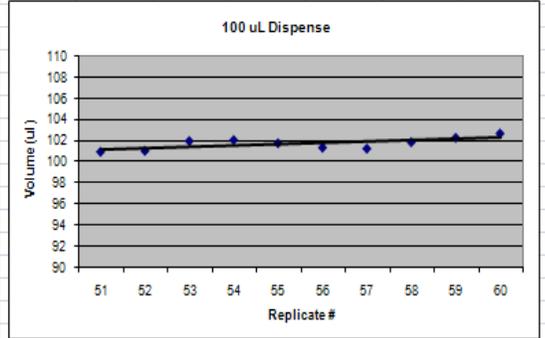
Tube #	Difference
41	48
42	46.2
43	50.6
44	50.5
45	50.6
46	50.5
47	51.3
48	51
49	50.7
50	50.4
Average	49.98
STDEV	1.59847
% cv	3.20%
% error	0.04%



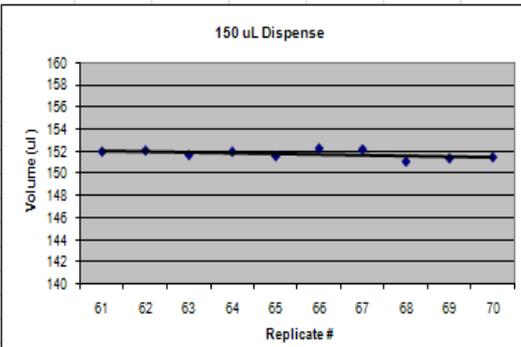
Tube #	Difference
51	101.2
52	101
53	101.3
54	100.5
55	101.4
56	101.2
57	100.8
58	101.1
59	101.1
60	100.6
Average	101.02
STDEV	0.2974
% cv	0.29%
% error	1.02%



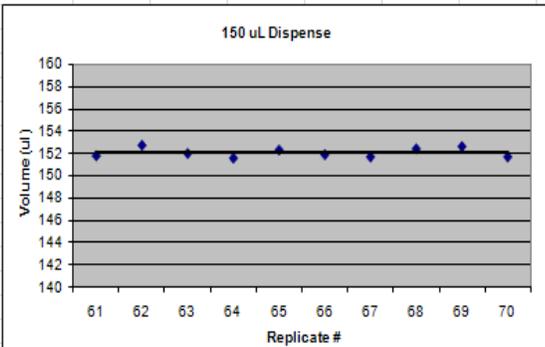
Tube #	Difference
51	100.9
52	101
53	101.9
54	102
55	101.7
56	101.3
57	101.2
58	101.8
59	102.2
60	102.6
Average	101.66
STDEV	0.55015
% cv	0.54%
% error	1.66%



Tube #	Difference
61	152
62	152.1
63	151.7
64	152
65	151.6
66	152.3
67	152.2
68	151.1
69	151.4
70	151.5
Average	151.79
STDEV	0.39001
% cv	0.26%
% error	1.19%

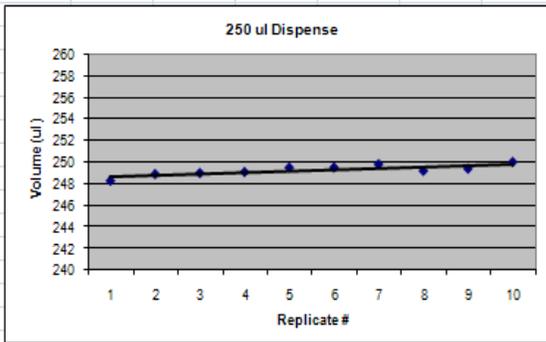


Tube #	Difference
61	151.8
62	152.7
63	152
64	151.6
65	152.3
66	151.9
67	151.7
68	152.4
69	152.6
70	151.7
Average	152.07
STDEV	0.40014
% cv	0.26%
% error	1.38%



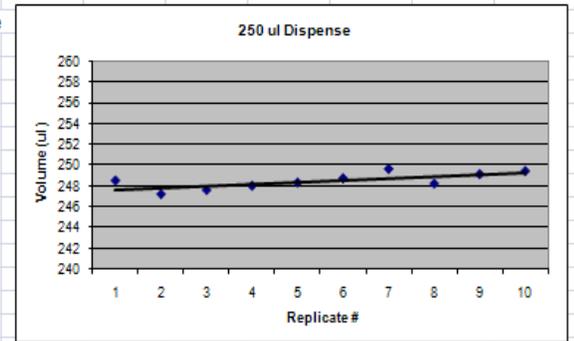
### Janus 1000 ul Tip Test 1

Tube #	Difference
1	248.3
2	248.9
3	249
4	249.1
5	249.5
6	249.5
7	249.8
8	249.2
9	249.4
10	250
Average	249.27
STDEV	0.48546
% cv	0.20%
% error	0.29%

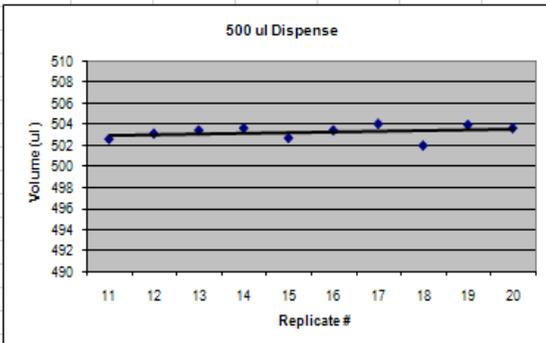


### Janus 1000 ul Tip Test 2

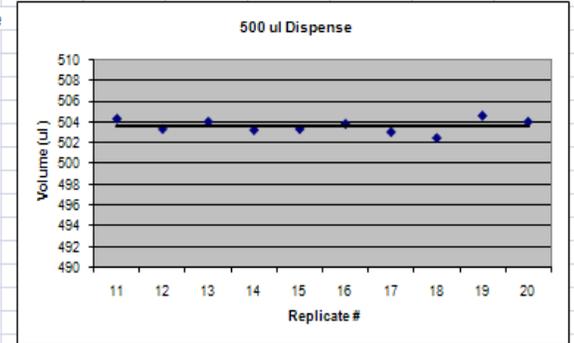
Tube #	Difference
1	248.5
2	247.2
3	247.6
4	248
5	248.3
6	248.7
7	249.6
8	248.2
9	249.1
10	249.4
Average	248.46
STDEV	0.76623
% cv	0.31%
% error	0.62%



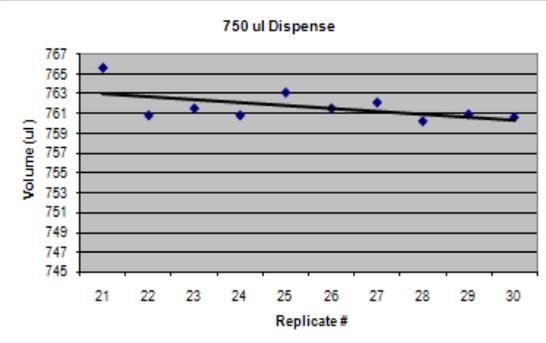
Tube #	Difference
11	502.6
12	503.1
13	503.4
14	503.6
15	502.7
16	503.4
17	504
18	502
19	503.9
20	503.6
Average	503.23
STDEV	0.63078
% cv	0.13%
% error	0.65%



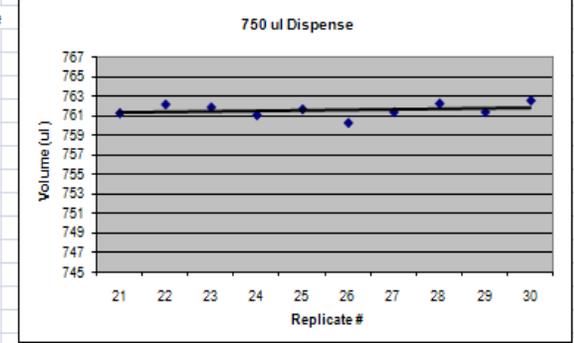
Tube #	Difference
11	504.3
12	503.3
13	504
14	503.2
15	503.3
16	503.8
17	503
18	502.4
19	504.6
20	504
Average	503.59
STDEV	0.66575
% cv	0.12%
% error	0.72%



Tube #	Difference
21	765.6
22	760.8
23	761.5
24	760.8
25	763.1
26	761.5
27	762.1
28	760.2
29	760.9
30	760.6
Average	761.71
STDEV	1.60309
% cv	0.21%
% error	1.56%

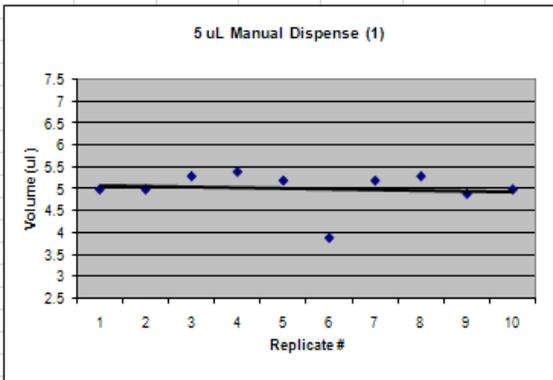


Tube #	Difference
21	761.2
22	762.1
23	761.8
24	761
25	761.6
26	760.2
27	761.3
28	762.2
29	761.3
30	762.5
Average	761.52
STDEV	0.67132
% cv	0.09%
% error	1.54%



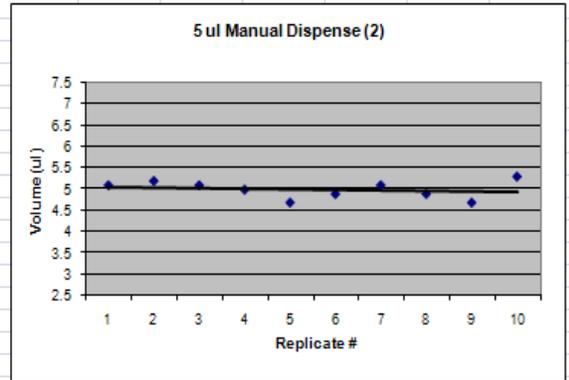
**Manual 25 uL Tip Test 1**

Tube #	Difference
1	5
2	5
3	5.3
4	5.4
5	5.2
6	3.9
7	5.2
8	5.3
9	4.9
10	5
Average	5.02
STDEV	0.43
% CV	8.49%
% error	0.40%

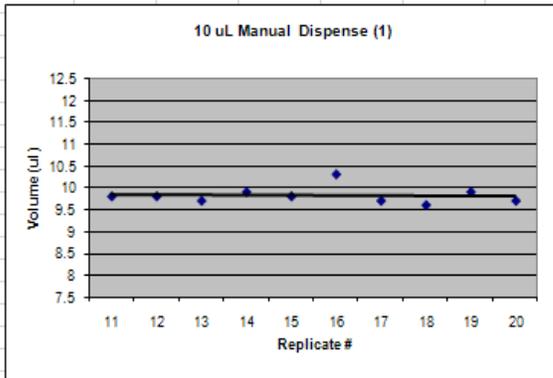


**Manual 25 uL Tip Test 2**

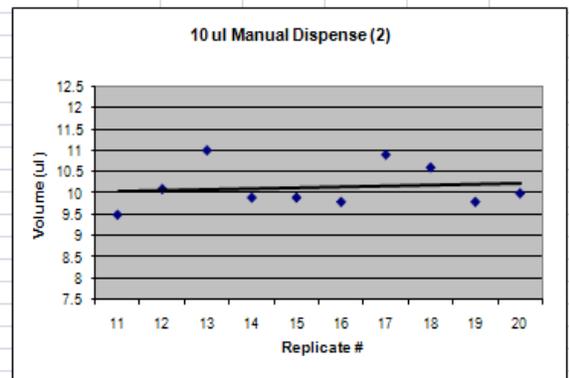
Tube #	Difference
1	5.1
2	5.2
3	5.1
4	5
5	4.7
6	4.9
7	5.1
8	4.9
9	4.7
10	5.3
Average	5
STDEV	0.20
% CV	4.00%
% error	0.00%



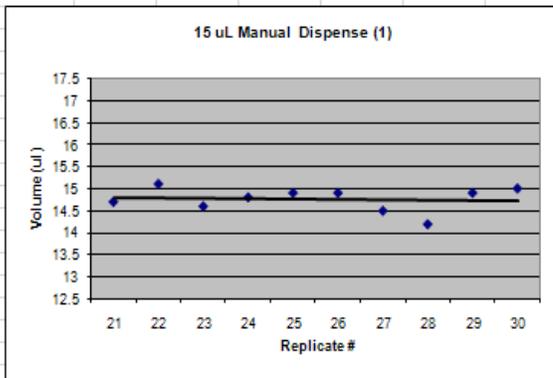
Tube #	Difference
11	9.8
12	9.8
13	9.7
14	9.9
15	9.8
16	10.3
17	9.7
18	9.6
19	9.9
20	9.7
Average	9.82
STDEV	0.19
% CV	1.97%
% error	1.80%



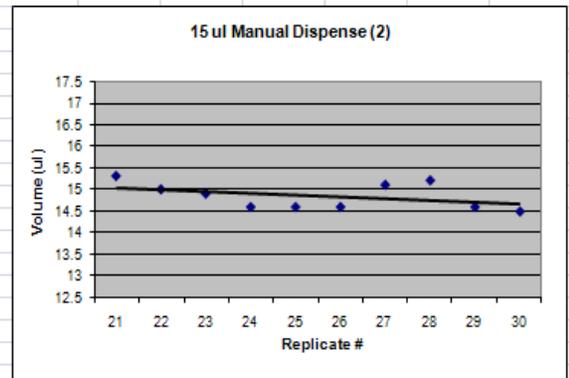
Tube #	Difference
11	9.5
12	10.1
13	11
14	9.9
15	9.9
16	9.8
17	10.9
18	10.6
19	9.8
20	10
Average	10.15
STDEV	0.51
% CV	4.99%
% error	1.50%



Tube #	Difference
21	14.7
22	15.1
23	14.6
24	14.8
25	14.9
26	14.9
27	14.5
28	14.2
29	14.9
30	15
Average	14.76
STDEV	0.27
% CV	1.81%
% error	1.60%

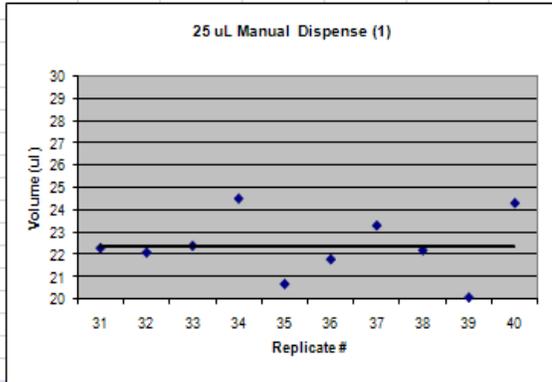


Tube #	Difference
21	15.3
22	15
23	14.9
24	14.6
25	14.6
26	14.6
27	15.1
28	15.2
29	14.6
30	14.5
Average	14.84
STDEV	0.30
% CV	1.99%
% error	1.07%

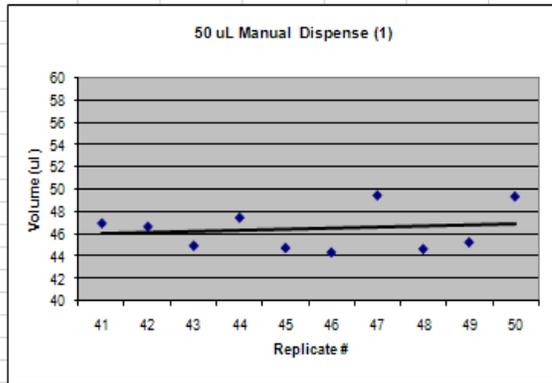


**Manual 175 uL Tip Test 1**

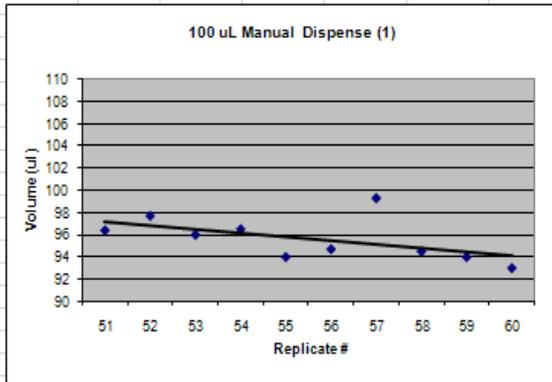
Tube #	Difference
31	22.3
32	22.1
33	22.4
34	24.5
35	20.7
36	21.8
37	23.3
38	22.2
39	20.1
40	24.3
Average	22.37
STDEV	1.39
% CV	0.62%
% error	10.52%



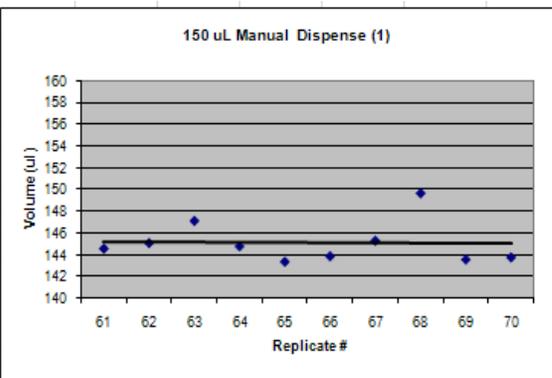
Tube #	Difference
41	47
42	46.7
43	45
44	47.5
45	44.8
46	44.4
47	49.5
48	44.7
49	45.3
50	49.4
Average	46.43
STDEV	1.91
% CV	4.12%
% error	7.14%



Tube #	Difference
51	96.4
52	97.7
53	96
54	96.5
55	94
56	94.7
57	99.3
58	94.5
59	94
60	93
Average	95.61
STDEV	1.93
% CV	2.02%
% error	4.39%

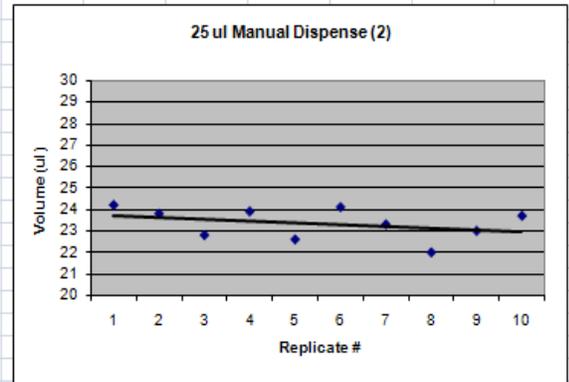


Tube #	Difference
61	144.6
62	145.1
63	147.1
64	144.8
65	143.4
66	143.9
67	145.3
68	149.6
69	143.6
70	143.8
Average	145.12
STDEV	1.91
% CV	1.32%
% error	3.25%

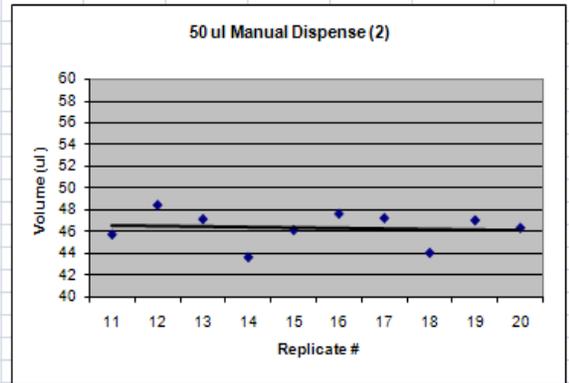


**Manual 175 uL Tip Test 2**

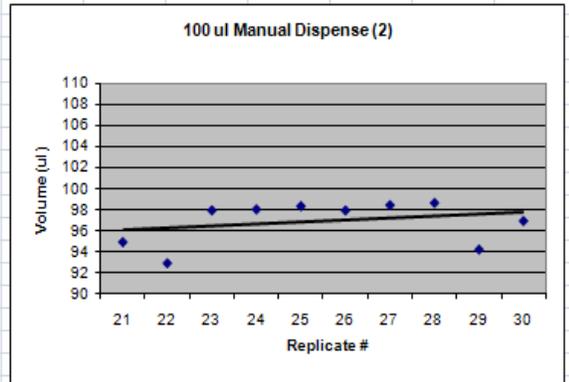
Tube #	Difference
1	24.2
2	23.8
3	22.8
4	23.9
5	22.6
6	24.1
7	23.3
8	22
9	23
10	23.7
Average	23.34
STDEV	0.72
% CV	3.10%
% error	6.64%



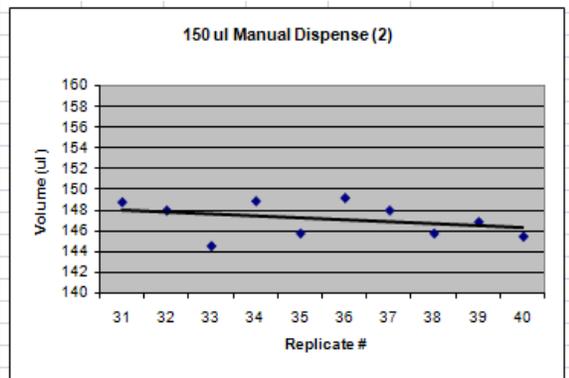
Tube #	Difference
11	45.7
12	48.4
13	47.1
14	43.6
15	46.1
16	47.6
17	47.2
18	44
19	47
20	46.3
Average	46.3
STDEV	1.53
% CV	3.30%
% error	7.40%



Tube #	Difference
21	95
22	93
23	98
24	98.1
25	98.4
26	98
27	98.5
28	98.7
29	94.3
30	97
Average	96.9
STDEV	2.04
% CV	2.93%
% error	3.10%

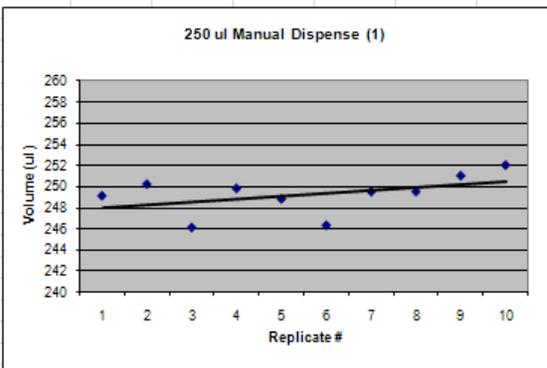


Tube #	Difference
31	148.8
32	148
33	144.6
34	148.9
35	145.8
36	149.2
37	148
38	145.8
39	146.9
40	145.5
Average	147.15
STDEV	1.65
% CV	1.12%
% error	1.90%

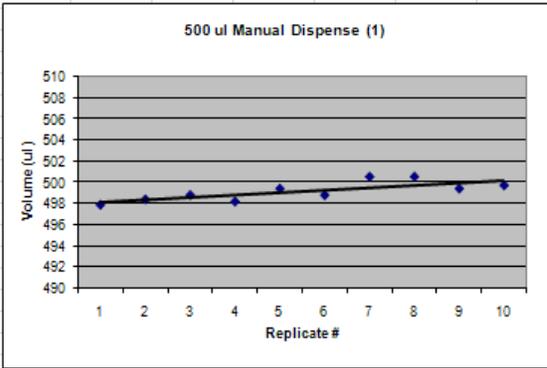


**Manual 1000 uL Tip Test 1**

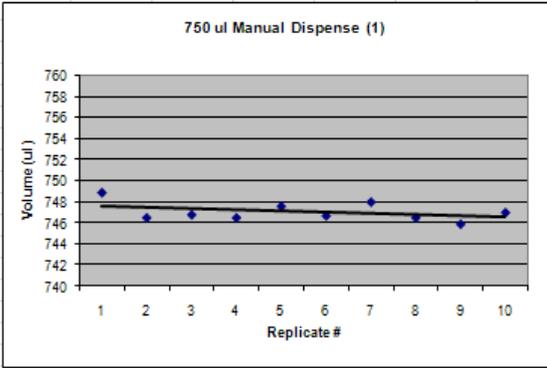
Tube #	Difference
1	249.1
2	250.2
3	246.1
4	249.8
5	248.8
6	246.3
7	249.5
8	249.5
9	251
10	252
Average	249.23
STDEV	1.85
% CV	0.74%
% error	0.31%



Tube #	Difference
11	497.9
12	498.4
13	498.8
14	498.2
15	499.4
16	498.8
17	500.5
18	500.5
19	499.4
20	499.7
Average	499.16
STDEV	0.90
% CV	0.18%
% error	0.17%

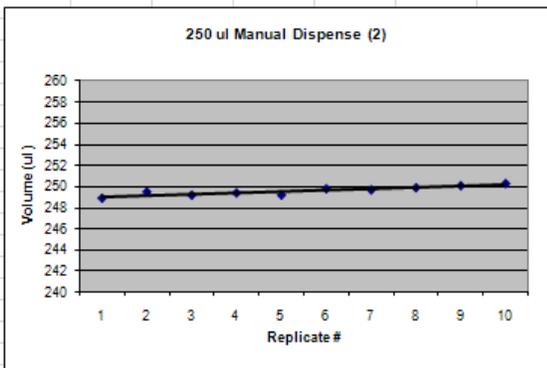


Tube #	Difference
21	748.9
22	746.5
23	746.8
24	746.5
25	747.6
26	746.7
27	748
28	746.5
29	745.9
30	747
Average	747.04
STDEV	0.88
% CV	0.12%
% error	0.39%

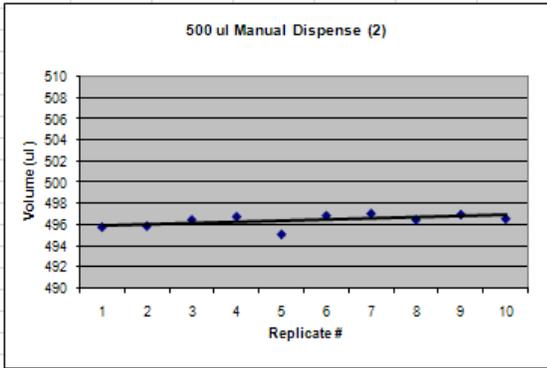


**Manual 1000 uL Tip Test 2**

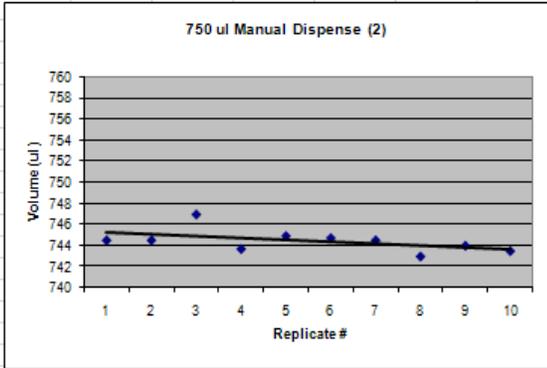
Tube #	Difference
1	248.8
2	249.4
3	249.1
4	249.3
5	249.1
6	249.7
7	249.6
8	249.8
9	250
10	250.2
Average	249.5
STDEV	0.44
% CV	0.00176
% error	0.20%



Tube #	Difference
11	495.8
12	495.9
13	496.5
14	496.8
15	495.1
16	496.9
17	497.1
18	496.5
19	497
20	496.6
Average	496.42
STDEV	0.63
% CV	0.13%
% error	0.72%



Tube #	Difference
21	744.5
22	744.5
23	746.9
24	743.7
25	744.9
26	744.7
27	744.5
28	743
29	744
30	743.5
Average	744.42
STDEV	1.06
% CV	0.14%
% error	0.74%



## APPENDIX II: METHODS

This appendix contains the procedure followed to set up the PCR amplification manually and also the procedure followed to set up the CE after amplification.

DNA-15 (Version 4.1)

### AMPLIFICATION - AmpF/STR<sup>®</sup> IDENTIFILER<sup>™</sup> STR TYPING KIT

#### **PURPOSE**

The purpose of this procedure is to outline the DNA amplification procedure that utilizes the Polymerase Chain Reaction. All reaction components (except template DNA) are provided within the commercial kit (AmpF/STR<sup>®</sup> Identifiler<sup>™</sup>).

#### **MATERIALS AND SUPPLIES**

##### Equipment

Thermal Cycler(s) (GeneAmp Model 9700 or higher)  
Vortex  
Microcentrifuges  
Pipettors  
PCR Prep Station and/or laminar flow biological hood

##### Supplies

Barrier pipet tips and/or Combi-tips  
MicroAmp PCR tubes and racks  
Autoclaved 1.5mL microcentrifuge tubes and racks  
AmpF/STR<sup>®</sup> Identifiler<sup>™</sup> containing the following:  
(1) \*Reaction Mix  
(2) \*Primer set  
(3) AmpliTaq Gold DNA Polymerase  
(4) Amplification Positive Control DNA 9947A

\* These 2 components may be combined for use as a MSPCL Master Mix. (See page 3, step 2)

TE<sup>-</sup> Buffer (10mM Tris-HCL, 0.1mM EDTA, pH 8.0)  
Kimwipes/ Tube de-capping tool

##### Standards and Controls

Amplification Positive Control(s)  
Amplification Negative Control(s)  
Extraction Positive, if necessary  
Extraction Negative, if necessary

##### Safety Considerations

Good laboratory practice dictates the use of appropriate precautions to preclude inhalation, ingestion or skin contact with chemicals, whether in the preparation or use of reagents and test media. Ventilation, protective gloves and hand washing are definitely required. Storage of prepared chemicals and test media should be such that no outside contamination or intracontamination is possible. Storage containers should be kept sealed until the contents are needed. Please refer to the "[Crime Laboratory Safety Manual](#)" and to the appropriate Materials Safety Data sheet that corresponds to the materials being used.

NOTE: Body fluid and tissue samples must be handled using universal precautions and appropriate personal protective equipment must be donned to limit the exposure to potential bloodborne pathogens. Analysts must follow the Exposure Control Plan described within the "[Crime Laboratory Safety Manual](#)".

## SAMPLE PREPARATION

Step	Action												
1	Fill out the appropriate worksheet (Identifiler™ Amplification Worksheet, Appendix A). Circle the sample type(s) (exemplars, questioned stains or semen). Note: Questioned and semen samples may be amplified together. Document the results obtained from the quantitation step in the column labeled "DNA conc. (ng/μl)" and note the volume. Document the volume of DNA extract and the volume of TE <sup>-d</sup> buffer required. Record lot numbers and expiration dates of reagents used.												
2	Utilize the Amplification Sample Preparation Worksheet, Appendix B, as necessary to make extract dilutions prior to step 3. Make copy/copies of the worksheet for case file folder(s). The original worksheet is stored in the Amplification Sample Preparation binder.												
3	For each sample to be amplified label the side of a new, 1.5-mL autoclaved microcentrifuge tube with the unique identifier number.												
4	To each labeled tube, add the appropriate amount of TE <sup>-d</sup> buffer and DNA extract as documented on the Amplification Worksheet(s). <b>NOTE:</b> Samples may be diluted for amplification and placed in a rack and stored refrigerated at 2-8 °C, for amplification at a later time/date.												
5	Add the following amount of TE <sup>-d</sup> buffer and sample extract to new labeled tubes: <table border="1" style="margin-left: 20px;"> <thead> <tr> <th>Sample name</th> <th>TE<sup>-d</sup></th> <th>Sample</th> </tr> </thead> <tbody> <tr> <td>*Amplification positive control(s)</td> <td>---</td> <td>10μl</td> </tr> <tr> <td>Amplification negative control(s)</td> <td>10μl</td> <td>---</td> </tr> <tr> <td>Extraction negative(s)</td> <td>QS to 10uL</td> <td>&lt;10μl</td> </tr> </tbody> </table>	Sample name	TE <sup>-d</sup>	Sample	*Amplification positive control(s)	---	10μl	Amplification negative control(s)	10μl	---	Extraction negative(s)	QS to 10uL	<10μl
Sample name	TE <sup>-d</sup>	Sample											
*Amplification positive control(s)	---	10μl											
Amplification negative control(s)	10μl	---											
Extraction negative(s)	QS to 10uL	<10μl											

### PCR GUIDELINES:

1. If the Quantifiler result is  $\geq 100$  ng /  $\mu$ l, dilute the sample with TE<sup>-d</sup> buffer and re-quantitate (e.g., 1:10 dilution of DNA extract to TE<sup>-d</sup> buffer). The procedure may be repeated if the diluted sample is still  $\geq 100$  ng /  $\mu$ l. Use the dilution result for amplification set-up.
2. It is critical to determine whether or not to proceed with amplification based on the quantitation results or save the sample for future technologies. In order to do this, you must consider the sensitivity of the quantitation kit, (human 6pg/ul). If the Quantifiler result is none detected and the extract was brought up to a volume of 50 $\mu$ l, it is appropriate to concentrate this extract to 12-13 $\mu$ l and requant. Based on the sample type an analyst may choose not to bring the volume up 50 $\mu$ l. If for example, in the 2ul that was quanted 6pg/ $\mu$ l was detected in the remaining extract (10 $\mu$ l) you could estimate that you would have 60pg, which based on the sensitivity of the 3130xl would not be sufficient to obtain a DNA profile and therefore the sample could be retained for future technologies. Consult with DNA supervisor(s) and/or Technical Leader if necessary.
3. The volume of Amplification Positive Control used may be adjusted based on the in-house quality control of the commercial kits (e.g., a 1:1 ratio may be considered optimum for the desired relative fluorescent units (rfus) on the Genetic Analyzer). If applicable, this will be noted on the Amplification Positive Controls' storage container(s) and this will be reflected on the Amplification Worksheet(s).
4. The optimal amount of target DNA for the Identifiler™ amplification system is 2 ng.
5. The extraction negative(s) should be run at the highest stringency of the related samples in the batch (i.e. if sample extracts in a questioned sample batch are run at 1, 3 and 5 uL, run the extraction negative at 5 ul and if sample extracts in a semen sample batch are run at  $\leq 1$  uL for epithelial fractions and at 5 uL for sperm fractions, run the extraction negatives at 1 uL and 5 uL, respectively). A negative control (e.g., amplification negative or extraction negative) should be amplified at the end of the batch.
6. Individual batches of PCR samples may be set-up, stored and then amplified together in the same thermocycler (e.g., several smaller sample sets of 24 may be combined to reach the full capacity of a 96 well thermocycler block). Prepared samples contained in MicroAmp PCR tubes and racks may be stored

refrigerated prior to amplification (note: it is desirable to wrap the amp rack in aluminum foil to protect the samples from the light). If applicable, ensure that multiple batches do not have the same label on the caps of the PCR tubes, use consecutive numbering and document on the corresponding amplification worksheet(s).

Note: Individual batches may also be distinguished with symbols (e.g., \*).

**Alternate PCR Sample Preparation Strategies**

• **INHIBITOR(S)**

If inhibitors are suspected prior to amplification or on observation of the results obtained from RT-PCR and / or on the Genetic Analyzer, an analyst may amplify a dilution of the extract (e.g., 1:10 of DNA extract to TE<sup>-4</sup> Buffer). The dilution utilized will be documented on the amplification worksheet(s).

• **CONCENTRATION**

Concentrate the sample volume required (and related extraction negative control(s)) and if necessary, bring the volume to 10µl with TE<sup>-4</sup> buffer for Identifiler™. To concentrate DNA extracts refer to the DNA protocol "Extract Concentration Protocol" DNA-12.

**NOTES:**

- *If inhibitors are suspected, it may be prudent to bring up the volume to greater than the required volume after concentration e.g., 13-15µl in order to allow for the amplification of diluted extracts e.g. 1:10, 1: 20, 1:50. etc.*
- *STR results must be interpreted with caution when amplifying small quantities of DNA.*

• **MINOR CONTRIBUTOR(S)**

If the minor contributor(s) to a biological stain is determined to be the DNA profile of interest (e.g., a low sperm count for a suspect on a vaginal swab) prior to amplification or after obtaining results on the Genetic Analyzer, an analyst may opt to amplify both a 2ng and a higher target amount of DNA (e.g., 4 ng). This will be documented on the amplification worksheet.

**NOTE:** *Amplifying a significant amount of DNA may result in saturation of the Genetic Analyzer's detector at one or more loci. STR results must be interpreted with caution when using peak heights that are saturated on the Genetic Analyzer.*

**AMPLIFICATION SET-UP**

The following steps should be conducted within a PCR Prep-Station hood and / or within designated PCR prep room. An amplification positive control and an amplification negative control should be included with each batch of samples being amplified to demonstrate procedural integrity. Extraction controls should be included in the amplification set-up, if applicable.

Step	Action
1	Obtain samples to be amplified from the appropriate rack in the biological refrigerator. Determine the number of samples to be amplified (including controls) and complete the amplification worksheet. When completing the worksheet, use the standard format for sample identification. List the unique identifier number and the MSP Case Number/Item Number/Stain Number sequence (e.g. R000 03-0000 1-1.1). If desired, the analyst may opt to add a brief sample description.
2	Obtain appropriate stock MSPCL Master Mix from the reagent refrigerator. The stock Master Mix consists of the reaction mix and the primers, dispensed into single use aliquots.  Routinely the following aliquots are prepared: <ul style="list-style-type: none"> <li>• 320µl Master Mix - enough for 20 reactions (labeled EB)</li> <li>• 224µl Master Mix - enough for 14 reactions (labeled S)</li> </ul>

	<ul style="list-style-type: none"> <li>• 128µl Master Mix - enough for 8 reactions (labeled M)</li> </ul> <p>Vortex briefly and spin briefly in a microcentrifuge. From the reagent freezer, obtain a microcentrifuge tube containing an aliquot of Taq Gold.</p> <p>Routinely the following aliquots are prepared:</p> <ul style="list-style-type: none"> <li>• 10µl Taq Gold - enough for 20 reactions (labeled EB)</li> <li>• 7µl Taq Gold - enough for 14 reactions (labeled S)</li> <li>• 4µl Taq Gold - enough for 8 reactions (labeled M)</li> </ul> <p>Vortex briefly and spin briefly in a microcentrifuge.</p>
3	Add the entire volume of Master Mix to the microcentrifuge tube containing the appropriate aliquot of Taq Gold. Vortex briefly to mix and centrifuge briefly.
4	<p>Alternately, the volume of required reagents may be calculated and aliquoted into a autoclaved microcentrifuge tube as follows:</p> <ul style="list-style-type: none"> <li>• Number of samples x 10.5µl of AmpF/STR<sup>®</sup> PCR Reaction Mix</li> <li>• Number of samples x 5.5µl of AmpF/STR<sup>®</sup> Primer Set</li> <li>• Number of samples x 0.5µl of AmpliTaq Gold DNA</li> </ul> <p>The above formulation provides a slight overfill to allow for volume which may be lost in pipetting. Document the lot #s of the PCR reaction mix and primer set on the worksheet.</p>
5	Place appropriate number of MicroAmp PCR tubes into a rack designated for PCR sample transfer. Label the caps of the PCR Reaction tubes by placing an "I" and labeling each tube numerically, corresponding to the amplification worksheet.
6	Pipet 15µl Identifiler <sup>™</sup> Master Mix (containing the Taq Gold) into each PCR reaction tube and close. Pipet carefully at a slight angle to avoid splashing of the solution. Use either a barrier tip or a combi-tip on the pipettor.
7	Ensure that the Master Mix is at the bottom of each PCR reaction tube by gently tapping the tubes down onto a clean work surface or by centrifuging briefly.
8	Label microcentrifuge tubes (containing prepared sample/extract dilutions), corresponding to the amplification worksheet and PCR reaction tubes i.e., 11, 12...
9	Reference the PCR Logbook and obtain the next consecutive PCR Run Number. Complete an entry in the logbook and record the PCR Run Number on the amplification worksheet(s). Appendix C consists of a PCR run log sheet.
10	Have another individual check the tube labels against the worksheet and initial the worksheet as Tube Set-up Witness.
11	<p>Using a new barrier pipet-tip for each sample, add 10µl of sample DNA or control to its correspondingly labeled PCR tube, mixing thoroughly with the pipet tip. The final volume in each MicroAmp PCR tube is 25µl.</p> <p>Note: Prior to the adding the samples to the PCR tube and Master Mix the samples should have been prepared (i.e., diluted or concentrated as required). See Sample preparation section, page 2.</p> <p>Note: Open caps using the microcentrifuge tube de-capping tool or a new Kimwipe. Avoid touching the inside surface of the tube caps.</p>
12	Transport samples and the amplification worksheet to a DNA post laboratory.

### *Post Amplification Room*

Step	Action
1	Briefly spin the tubes in a microcentrifuge and transfer the tubes in a rack to the designated thermal cycler and close the heat cover. Record the thermal cycler used on the amplification worksheet(s). Make copy/copies of worksheet(s) for case file folder(s). Original worksheet is stored in the appropriate binder.
2	Turn on the thermal cycler.
3	Select the appropriate program ensuring that the following parameters are shown:  <b>Initial Incubation Step:                    95°C for 11 minutes</b>



**CAPILLARY ELECTROPHORESIS OF AMPLIFIED DNA FRAGMENTS ON  
THE ABI 3130xl GENETIC ANALYZER**

**Purpose** The purpose of this procedure is to electrophorese DNA samples amplified with AmpF $\ell$ STR<sup>®</sup> Identifiler<sup>™</sup> or Yfiler<sup>™</sup> PCR Amplification Kits using an ABI 3130xl Genetic Analyzer capillary electrophoresis instrument.

**Materials**

Equipment

ABI 3130xl Genetic Analyzer  
Thermal Cycler or 95°C heat block  
Bucket centrifuge with 96 well plate capacity  
Microcentrifuge  
Pipettes  
LIMS  
Printer  
Scanner

Supplies

96 well reaction plate  
3130xl plate bases  
3130xl plate covers  
3130xl 96 well septa  
Reservoir septa  
1.5 ml Microcentrifuge tube(s)  
Ice or cooling block (if available)  
16 Capillary array (36 cm)  
Pipette tips  
Kimwipes

Solutions and Reagents

Performance Optimized Polymer (POP4, 3130xl specific)  
Genetic Analyzer Buffer, 1X working solution (see Solution Log)  
Deionized water  
Hi-Di Formamide

Standards and Controls

Allelic Ladder (Identifiler<sup>™</sup> or Yfiler<sup>™</sup>)  
GeneScan-500 LIZ size standard  
Hi-Di Formamide/LIZ 500 (dform-LIZ) blank  
Extraction positive, if applicable  
Extraction negative, if applicable  
Amplification positive, if applicable  
Amplification negative, if applicable

**Safety  
Considerations**

Good laboratory practice dictates the use of appropriate precautions to preclude inhalation, ingestion or skin contact with chemicals, whether in the preparation or use of reagents and test media. Ventilation, protective gloves and hand washing are definitely required. Storage of prepared chemicals and test media should be such that no outside contamination or intracontamination is possible. Storage containers should be kept sealed until the contents are needed. Please refer to the “Crime Laboratory Safety Manual” and to the appropriate Materials Safety Data sheet that corresponds to the materials being used.

**General**

In the **Data Collection Software** toolbar, there are **Wizards** to aid in instrument maintenance and procedures. The **Wizards** list step by step how to perform that particular function on the 3130xl Genetic Analyzer.

Running samples on the 3130xl Genetic Analyzer consists of setting up the instrument, the software and the samples to be run.

On the instrument level, preparing for a run consists of:

- checking the capillary array count and changing if necessary
- changing expired POP4, cleaning the pump block and the pump block water trap.
- changing the buffer and water in the reservoirs and replacing the septa

On the software level, preparing for a run consists of:

- restarting the computer at least once a week or before each run
- ensuring the Data Collection Software is launched
- filling out the plate record for the run

**Launching Instrument and Software**

**DO NOT OPEN THE DOORS WHILE THE INSTRUMENT IS PROCESSING PLATES OR WHILE THE AUTOSAMPLER IS MOVING - THE PROGRAM WILL FREEZE!!**

1	Turn the computer on first, then the instrument.
2	Wait for green light on the instrument before launching <b>3130xl Data Collection Software</b> (version 3.0 or higher) by double-clicking on the desktop icon or, through the start menu: Applied Biosystems / Data Collection / run 3130xl Data Collection.
3	The <b>Service Console</b> window will open and proceed through its diagnostics, this may take several minutes. This window will display green squares before launching the Data Collection Software. <b>NOTE:</b> If this window does not display all green squares, the Data Collection Software will not launch, please see ABI

	troubleshooting guide.
4	In the <b>Foundation Data Collection</b> window, click on “+” to expand the folders in the Navigation Pane (left pane) of the window. All application programs are located here.
5	To shut down or restart the computer, close the <b>Data Collection Software</b> first, then power off the instrument, then choose either restart or shut down from the computer’s Start menu.

### **Remove Plate(s) of Previously Run Samples**

1	Press the "Tray" button on the left-hand side of the instrument. Wait for the autosampler to stop before opening the doors.
2	Gently rock the plate assembly to remove from the autosampler.
3	Refer to the CE run number on the plate. Determine the age of the samples by checking the run date in the run log and/or consulting with the analyst who set up the run.
4	Discard expired samples (greater than <b>4</b> days old).

### **Checking the Capillary Array Count**

#### *BEFORE STARTING, CHECK THE CAPILLARY ARRAY COUNT!*

The number of injections on the capillary array cannot exceed the determined maximum of 150. Change the capillary array before or when this limit is reached. The capillary array may also need to be changed if the electrode end of the capillary has remained outside of the buffer for an extended period of time.

1	Check the capillary array count by checking in the run log and/or in the <b>Data Collection Software &gt; Instrument Status</b> window. The capillary injection count will be displayed at the top right of the pane as “Array Usage”.
2	If the number of injections to be run will exceed the maximum number of injections allowed, 150, the capillary array must be changed. In the <b>3130xl Run Log</b> (Appendix B), indicate the number of injections the capillary serviced prior to being changed. Proceed to <b>Changing the Capillary Array</b> .
3	If the capillary array does not need to be changed, proceed to <b>Replacing the Performance Optimized Polymer (POP4)</b> .

## Changing the Capillary Array

1	In the <b>Data Collection Software &gt; Wizards &gt; Install Array Wizard</b> . Follow the <b>Wizard</b> directions for changing the capillary array. Record the serial number of the capillary array in the <b>3130xl Maintenance Log</b> (Appendix A). Note: The capillary length is 36cm.
2	When the <b>Install Array Wizard</b> is complete, proceed to <b>Spatial Calibration</b> and then to <b>Spectral Calibration</b> .

## Spatial Calibration

A spatial calibration provides information about the position of the capillaries to the CCD camera to obtain maximum fluorescence. A spatial calibration must be performed every time the detection-end assembly of the capillary array is moved. This includes when the array is replaced or installed.

1	In the Navigation Pane, select <b>Spatial Run Scheduler</b> .
2	Select <b>Protocol: 3130SpatialFill_1</b> or select <b>3130SpatialNoFill_1</b> if the capillaries contain fresh polymer, and select <b>Start</b> , this will take several minutes.
3	Guidelines for passing a Spatial Run: <ul style="list-style-type: none"><li>• Similar peak heights.</li><li>• An orange cross-hatch at the top of each peak.</li><li>• Spacing difference between adjacent positions between 13 to 16 pixels: check the Left spacing and Right spacing columns.</li></ul>
4	If the calibration passed: select <b>Accept</b> .  If the calibration failed: select <b>Reject</b> and repeat the calibration from Step 2. Also, refer to the <b>ABI 3130 xl Maintenance, Troubleshooting and Reference Guide</b> . <b>Note:</b> If the spatial calibration fails again, open the laser detection window to ensure the detection window is seated properly and re-run the spatial calibration. It may also be necessary to clean the detection window with methanol on a kimwipe.

## Spectral Calibration

The purpose of a spectral calibration is to generate matrices that are used by the data analysis software to correct for spectral overlap. This spectral is applied by the software to remove the overlap in order to generate multicomponent data.

It is recommended to run a Spectral Calibration whenever a capillary array has been replaced or installed. **Note:** A spectral should be run if pull-up is seen consistently in the data. A spectral calibration must be performed before using samples labeled with a dye set not previously used on the instrument.

1	To perform a spectral calibration follow the directions outlined in <b>QCDNA-23A Creating Spectrals for the 3130xl Genetic Analyzer.</b>
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**Replacing the Performance Optimized Polymer (POP4)**

1	<p>The POP4 should be changed or replenished for the following reasons:</p> <ul style="list-style-type: none"> <li>• <i>The POP4 is expired.</i> Once installed on the instrument, 3130xl POP4 has an expiration date of <b>7</b> days. Check the <b>3130xl Maintenance Log</b> (Appendix A) to determine if the POP4 is expired.</li> <li>• <i>The amount of POP4 left in the bottle is not sufficient for the run.</i></li> </ul> <p>Note: Remove the POP4 from the refrigerator and allow it to warm to room temperature before using.</p>
2	<ul style="list-style-type: none"> <li>• <u><i>If POP4 is expired:</i></u></li> </ul> <p>Clean the pump block using <b>Wizard &gt; Water Wash Wizard</b>, then follow the remaining directions in Water Wash Wizard for replenishing the POP4. Record the polymer change in the <b>3130xl Maintenance Log</b>. Changing the water in the pump block water trap is performed after the <b>Water Wash Wizard</b>. See direction in Step 3.</p> <ul style="list-style-type: none"> <li>• <u><i>If the amount of POP4 left in the bottle is not sufficient to complete the run, there are 2 options:</i></u> <ul style="list-style-type: none"> <li>○ <u>Set up the instrument following the “If POP4 is expired” directions.</u></li> <li>○ <u>Replenish the POP4 by adding more to the supply bottle without doing the Water Wash Wizard.</u></li> </ul> </li> </ul> <p>This can only be done if the lot number for the replenishing POP4 is the same as the POP4 currently installed on the instrument. Apply the expiration date of the POP4 currently installed on the instrument to the new POP4 added for replenishment.</p> <p>Use the <b>Wizard &gt; Replenish Polymer Wizard</b> to install more POP4</p>

	<p><b>Note: DO NOT REMOVE the polymer bottle cap from the pump block tubing as stated in the directions for replenishing polymer.</b></p> <p>-If the POP4 is from a different lot, follow the directions above for “<i>If POP4 is expired.</i>”</p>
3	Maintenance of the pump block water trap is performed when the <b>Water Wash Wizard</b> is completed. Fill the supplied 20mL syringe with deionized water. Remove any bubbles from the syringe.
4	Attach the syringe to the forward-facing ferrule by holding the ferrule with one hand and threading the syringe into the ferrule with the other hand.
5	Open the ferrule approximately one-half turn counter-clockwise.
6	Open the waste ferrule on the left side of the pump block approximately one-half turn counter-clockwise.
7	Hold an empty tube or beaker under the waste ferrule. Flush the trap by pushing <b>SLOWLY</b> and steadily on the syringe plunger. It should take approximately 30 seconds to flush 5 mL of deionized water through the trap.
8	Close the forward-facing syringe ferrule first, then the waste ferrule. Do not over tighten the ferrules.
9	Remove the syringe from the ferrule by holding the ferrule with one hand while turning the syringe counter-clockwise with the other hand.

### Changing the Buffer and Water Reservoirs

**Note: Buffer and water reservoirs should be changed every 24 hours or before each run.**

1	Press the "Tray" button on the left-hand side of the instrument. Wait for the autosampler to stop before opening the doors.
2	Fill the anode buffer chamber with 1X Genetic Analyzer Buffer up to the fill line and fit securely to its position on the lower polymer block.
3	Fill the cathode buffer reservoir with 1X Genetic Analyzer Buffer and fill the other 3 reservoirs with deionized water to their fill lines. <b>Important:</b> Ensure that the septa groove is completely dry, as the septa will not stay properly seated and the capillary array may become damaged. Add new septa to each reservoir. Make sure each one is securely seated in the reservoir. Ensure that all reservoirs are dry before placing on the instrument.
4	Close the instrument doors, the autosampler will home itself.

### Filling out the Plate Record

Before starting a run you must fill out a **3130xl Plate Map** in the LIMS DNA Matrix and export it to the appropriate run folder (e.g. \3130 Import Export File\CE004). The Plate Record associates sample information (name and type of analysis) with the plate position. The information recorded in the Plate Record becomes the sample identification.

1	<p>Using the LIMS DNA Matrix, create and complete a <b>3130xl Plate Map</b>. A plate run number will automatically be assigned. A date and time will appear on the worksheet corresponding to the date and time created. Update the date and time if the procedure is conducted on a different date and time from when the worksheet was created. Use the following plate set-up rules as a guide to complete the plate map:</p> <ul style="list-style-type: none"> <li>• Questioned samples (semen and blood) must be run separately from exemplar samples. (i.e., they cannot be on the same plate). Questioned and exemplar plates may be run consecutively on the same instrument.</li> <li>• CO samples must be run independently from all other sample types.</li> <li>• Multiple amplifications of the same type, either questioned or exemplar, can be run on one plate. (i.e., amplifications from several analysts may be run together on the same plate).</li> <li>• Samples amplified with Identifiler and Yfiler may be run on the same plate. It is recommended that samples amplified with Identifiler and Yfiler not be combined into the same capillary array injection.</li> <li>• It is recommended that each injection contain an Allelic Ladder. (i.e., each Identifiler injection contains an Identifiler ladder and each Yfiler injection contains a Yfiler ladder). If only one injection is being run on a plate it is highly recommended to include two Allelic Ladders in that injection.</li> <li>• A dform-LIZ blank is run once per plate. However, if different injection times are run on the same plate (i.e. a 10 second injection and a 20 second injection) a dform-LIZ can be run for each injection time OR with the most stringent injection time (i.e. run the dform-LIZ blank with the 20 second injections).</li> </ul>
2	In the Navigation Pane, select <b>Plate Manager</b> . Select <b>Import</b> .
3	Locate and highlight the file in the instrument folder that was previously exported from the LIMS DNA Matrix Select <b>Open</b> .
4	A progress window will appear and indicate if the plate imported successfully. Select <b>OK</b> .

	Note: The illegal characters ( / \ : * " < > ? ' space) will automatically be taken out of the sample identifier information by the LIMS DNA Matrix. If the plate does not import successfully, please see a supervisor.												
5	Select <b>Find All</b> to refresh the plate ID list. Proceed to the next section if the Plate Map has imported successfully.  Note: With the permission of a supervisor, you may manually create a Plate Map. Follow steps 6 through 11.												
6	In the Navigation Pane, select <b>Plate Manager</b> . Select <b>New</b> .												
7	In the <b>New Plate Dialog</b> box, name the Plate Record using the <b>Plate ID #</b> assigned by the LIMS DNA Matrix.  Under <b>Application</b> select <b>GeneMapper-Generic</b> . Plate type is <b>96-Well</b> . Enter your initials in <b>Owner Name</b> and <b>Operator Name</b> fields. Select OK.												
8	In the <b>Plate Editor</b> box, fill in the following fields: <ul style="list-style-type: none"> <li>• <b>Sample Name:</b> fill out with the complete sample identification information including the case number, sample description and injection time (if not the default). Other identifying information such as the analyst's initials or PCR run number may be added to aid in sample sorting. Spaces between sample information are not allowed by the software, use “-“or “_”. (e.g. 01-0000-1-1-01.1 or 01-0000_1-1-01.1)</li> <li>• <b>Comment:</b> number the samples consecutively on the plate 001, 002, 003, etc. This provides a means of sorting the Plate Record during subsequent data analysis.</li> <li>• <b>Results Group 1</b> column: select 3130xl-(<i>instrument #</i>)-Generic_Results_Group</li> <li>• <b>Instrument Protocol 1</b> column: select the appropriate instrument protocol for the samples:</li> </ul> <table border="1" data-bbox="558 1415 1380 1677"> <thead> <tr> <th><b>Amp. Kit</b></th> <th><b>Instrument Protocol</b></th> <th><b>Parameters</b></th> </tr> </thead> <tbody> <tr> <td>Identifiler and Yfiler</td> <td>G5_POP4_ Instrument_Protocol</td> <td>10 seconds, 3kV injection (<i>default</i>)</td> </tr> <tr> <td>Identifiler and Yfiler</td> <td>G5_POP4_IP_5-sec-inj</td> <td>5 seconds, 3kV injection</td> </tr> <tr> <td>Identifiler and Yfiler</td> <td>G5_POP4_IP_20-sec-inj</td> <td>20 seconds, 3kV injection</td> </tr> </tbody> </table> <p><b>NOTE:</b> Negative controls need to be run at the same injection time as the samples (e.g., samples at 20 seconds and negative controls at 20 seconds). An Allelic Ladder should be included in each injection regardless of injection time.</p>	<b>Amp. Kit</b>	<b>Instrument Protocol</b>	<b>Parameters</b>	Identifiler and Yfiler	G5_POP4_ Instrument_Protocol	10 seconds, 3kV injection ( <i>default</i> )	Identifiler and Yfiler	G5_POP4_IP_5-sec-inj	5 seconds, 3kV injection	Identifiler and Yfiler	G5_POP4_IP_20-sec-inj	20 seconds, 3kV injection
<b>Amp. Kit</b>	<b>Instrument Protocol</b>	<b>Parameters</b>											
Identifiler and Yfiler	G5_POP4_ Instrument_Protocol	10 seconds, 3kV injection ( <i>default</i> )											
Identifiler and Yfiler	G5_POP4_IP_5-sec-inj	5 seconds, 3kV injection											
Identifiler and Yfiler	G5_POP4_IP_20-sec-inj	20 seconds, 3kV injection											
9	The <b>Fill-down</b> (Ctrl + D) function can be used to fill out the												

	Results Group and Instrument Protocol columns.
10	When all fields are filled out select OK.
11	Proceed to <b>Preparing and Running DNA Samples</b> .

## Preparing and Running DNA Samples

### **Important Notes:**

- Use one Allelic Ladder per capillary array injection. Do not place the Allelic Ladder in the same capillary position in every array injection.
- LIZ 500 is the size standard used for 5-dye chemistry (Identifiler™ and Yfiler™)
- If only running a partial tray of injections, ensure that all the empty wells for the array injection contain 10 µL dform.
- Note: Samples from plate can be re-injected for up to four days from date of preparation.

1	Check for bubbles in the pump block, lower polymer block, tubing and channels. Remove all bubbles using <b>Wizard &gt; Bubble Remove Wizard</b> . If there are no bubbles present proceed to Step 2.
2	Each array injection consists of 16 wells for injection. All 16 wells for an injection must be filled with either sample (e.g., amplicon or ladder) or with d-formamide. <b>Important: There cannot be any empty wells in an array injection.</b>
3	Prepare enough dform-LIZ solution mix for n+4 samples to be run; include samples, Allelic Ladders and a dform-LIZ blank in your calculations. Check your calculations on the <b>3130xl Plate Map</b> . Note: For each well in a set of 16 (one array injection) that does not have a sample, add 10 µL of dform to the well. This well will be designated as “BLANK” on the Plate Map and the Plate Record.  <b><u>LIZ 500 for Identifiler and Yfiler samples:</u></b>  (# of samples + 4) x 8.9 µl d-formamide (# of samples + 4) x 0.1 µl LIZ size standard  Add dform and LIZ size standard to a 1.5 mL microcentrifuge tube, mix by briefly vortexing and pulse spin the tube in a microcentrifuge.
4	Label the Genetic Analyzer sample plate with the date, run number and your initials on the short side of the plate to the left of the notch.
5	Cover the plate with a piece of aluminum foil, pressing down to reveal the outline of the wells. This functions as a place marker

	for aliquotting dform-LIZ or dform blank to the appropriate wells.
6	Pierce the foil over each well with the pipet tip to aliquot 9 $\mu$ L dform-LIZ into each well. For wells designated as BLANK, aliquot 10 $\mu$ L dform only.
7	Remove the aluminum foil and replace with a fresh piece, pressing down to reveal the outline of the wells. This will aid in placing the samples into the correct wells.
8	If needed, briefly spin amplicons if condensation is noted.
9	Have another individual witness plate set-up and electronically initial the <b>3130xl Plate Map</b> in the LIMS DNA Matrix.
10	Pierce the foil over each well with the pipet tip to add 1 $\mu$ L of PCR product or Allelic Ladder to each well according to the <b>3130xl Plate Map</b> . When adding the sample, make sure to pipet the sample into the dform-LIZ solution. For the dform-LIZ blank, add an extra 1 $\mu$ L of the mix to that well.
11	Carefully remove the aluminum foil and discard. Cover the plate with plate septa. Pulse spin the plate in the bucket centrifuge.
12	Heat denature the plate in a heat block or a thermal cycler at 95°C for 3 minutes. Immediately chill the plate on ice or a cooling block, if available, or refrigerate for at least 3 minutes.
13	Dry the plate with kimwipes, checking between the wells for any residual moisture. Place the plate in the black plate base and cover with the white plate cover ensuring that the cover clicks into place on both sides.
14	Present the autosampler by pressing the “Tray” button on the left-hand side of the instrument. Wait for the autosampler to stop before opening the doors.
15	Place the plate assembly so that position A1 sits at the right rear corner of the autosampler platform and the notch in the black base plate is in the back. The plate assembly will only fit in this position.
16	Close the instrument doors, the autosampler will home itself.
17	In the navigation pane, click on <b>Run Scheduler</b> . When the plate assembly is in place, the autosampler deck image will be yellow indicating an unlinked plate.
18	The <b>Run Scheduler</b> view links the plate record with the plate on the autosampler. Select <b>Find All</b> to find your plate record. Click on the plate record, and then select the autosampler deck position of your plate (i.e., A or B) by clicking on the corresponding yellow plate. When the Plate Record is linked to the chosen plate on the autosampler, the yellow plate will change to green. Wait for Ready status in the Data Collection software.
19	Click on green “go” arrow on toolbar to start the run. A message window will appear confirming “You are about to start processing plates,” select OK.

## Instrument Maintenance

### Weekly Maintenance

- Restart the computer and the instrument weekly. Follow the directions in the **Launching Instrument and Software** section of this protocol.
- Check the storage conditions of the used arrays (if applicable). Replenish anode and cathode vials with deionized water.

### Computer Maintenance

- Performed as needed. Can be incorporated into a quality control schedule to be performed every 6 or 12 months.
- Computer maintenance must be performed when the instrument gives an error message and prevents samples being run. Runs cannot be started until the database is cleaned up.

1	When the drive and/or database is full, the Data Collection Software will prevent any runs from starting and give an error message such as:  “The limit for a results group is 7000 samples. Please use a different results group or remove samples from the results group.”  or  “The results group has exceeded the maximum number of samples allowed for this results group.”
2	Data is backed up onto a CD using Sonic Record Now Plus (usually pre-installed onto the 3130xl Dell computer with Windows XP Professional software) or similar CD burning software. These instructions are more specific to the Sonic Record Now Plus software and the Data Collection software (version 3.0 or higher), but the same general procedure is used to create a data CD.
3	Open the program by going to <b>Start menu &gt; All Programs &gt; Sonic &gt; Record Now Plus</b> . In the Sonic window choose Data Disc. Select Add Files and Folders.
4	In the “Select files and folders to add” window, go to:  <b>E: Applied Biosystems / UDC / Data Collection / data / ga3130xl / instrument number or name</b>  Select all files in this window and select Add.
5	Then create a temporary folder for the next step of writing the plate records to the CD. The Data Collection Software does not allow these to be directly exported to a CD. (A temporary folder can be created on the server in a place such as the 3130 Extracted

	Runs folder).
6	In the Data Collection Software, in the Navigation Pane select Plate Manager.
7	In the Plate Manager window select a plate record (the Data Collection Software does not allow selecting more than one plate record at a time).
8	Select Export (export to the created temporary folder).
9	Repeat for all plate records displayed, including spectral runs.
10	After all plate records have been copied/exported to the temporary folder, select Add Files and Folders in the CD writing software and add this folder to the CD.
11	Select Burn to write this data to the CD. If deemed necessary, a second data CD can be created before closing the CD writing program. Label the CD <i>Instrument number or name Backup</i> , date and initial.
12	After the disc has been successfully written, go back to:  <b>E: Applied Biosystems / UDC / Data Collection / data / ga3130xl / instrument number or name</b>  and delete all files in this folder – do <b>NOT</b> delete the folder.
13	Delete all files in Plate Manager by selecting the plate record and then select Delete (the Data Collection Software does not allow selecting more than one plate record at a time). Select Yes when prompted to agree to delete the record.
14	Repeat for all plate records displayed.
15	Delete the temporary folder created for the plate records.
16	Empty the recycle bin.
17	Then, defragment <b>ONLY</b> the C and E drives.
18	Right click on <b>My Computer</b> and select <b>Manage</b> . In the navigation pane select <b>Computer Management (Local) &gt; Storage &gt; Disk Defragmenter</b> .
19	Choose the C drive and select <b>Defragment</b> . This may take several minutes.
20	When the <b>Defragmentation Complete</b> window is displayed, click <b>Close</b> .
21	Repeat for the E drive.
22	Close the <b>Computer Management</b> window, and then restart the computer.
23	To restart the computer, close the Data Collection Software first, then power off the instrument, then choose either restart or shut down from the computer's Start menu.

## **Long-Term Shutdown**

If the instrument will be idle for more than 7 days, it is recommended to perform a long-term shutdown.

1	In the <b>Data Collection Software</b> select > <b>Wizards &gt; Instrument Shutdown Wizard</b> . Follow the wizard directions for shutting down the instrument. Note: Make sure all parts are completely dry before long-term storage. Record the shut down date in the 3130xl Maintenance Log.
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### **Appendices:**

3130xl Maintenance Log (Appendix A)  
3130xl Run Log (Appendix B)  
3130xl Run Control Sheet (Appendix C)

### **Revision History:**

May 23, 2007      Version 2.0 –  
Incorporated Yfiler Amplification kit to protocol.  
Removed references to Profiler Plus and COfiler Amplification kits, and ROX 500 size standard.  
Moved Spectral Calibration to beginning of protocol, following Spatial Calibration.  
Clarify POP4 expiration date when POP4 is replenished.  
Moved Maintenance of Pump Block Water Trap into the Replacing the Performance Optimized Polymer (POP4) section.  
Language clarifications throughout.protocol

September 1, 2008      Protocol updated to incorporate the LIMS DNA Matrix system.  
Addition of Computer Maintenance section to Instrument Maintenance section  
Added note about expiration of plate for re-injection section

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