Levenshtein Distance as a Measure of Accuracy and Precision in Forensic PCR-MPS Methods

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ABSTRACT

Accuracy and precision determinations are standard components of method validations where they help to describe the performance of the method. Despite their importance, a standard approach to calculating these parameters is not available for forensic PCR-MPS methods that detect sequence-based alleles. In this paper, we describe a method based on the Levenshtein distance metric which aptly summarizes method accuracy in terms of the closeness of read sequences to reference sequences, and method precision in terms of the agreement among read sequences. Inaccuracy or imprecision in forensic methods can lead to wrong allele calls. By expressing method performance in terms of a distance metric, this method places PCR-MPS on equal footing with distance-based measures in PCR-CE methods where inaccuracy or imprecision can result in wrong microvariant allele calls. Summary statistics based on the Levenshtein distance can be used to compare performance of different kits, markers, sequencers, or methods.

KEYWORDS

Accuracy, Precision, Massively Parallel Sequencing

1. Introduction

Performance evaluations of quantitative analytical methods used in forensics often includes consideration of accuracy and precision [1]. These measures are particularly useful when describing the suitability of a method in an intended application or when comparing the performance of alternative methods [2]. Method accuracy and precision are core components of forensic method validations under SWGDAM and ENFSI guidelines [3-5]. In this context, accuracy has been defined as the degree of conformity of measurements to the true value; and precision has been defined as the degree of mutual agreement among a series of individual measurements [3].

1.1. Accuracy and Precision in PCR-CE Methods

The goal of PCR-CE methods is to determine the true length of PCR-targeted markers in the genome. In these methods, the measurement of interest is electrophoretic retention time from which the length of

PCR amplicons is derived. Electrophoretic retention times are routinely transformed into fractional base pair units. Even though "base pair" is a discontinuous measure, the fractional units are essentially continuous. The transformation from seconds to fractional base pairs does not alter the underlying distribution, meaning that means and standard deviations can be calculated in units of fractional base pair under an assumption that Normal distributions fit the data [6, 7]. To avoid length-miscalls, PCR-CE methods must be accurate to retention times that are within ± 0.5 base pairs of the true amplicon length [5, 8]. It is not possible to measure accuracy at the individual PCR amplicon level. Rather, the entity measured is the mode of retention times of an aggregate of same-length amplicons that migrate together under electrophoresis [9]. Precision is commonly described in terms of a standard deviation of measurement error of the aggregate [10, 11]. That is, the standard deviation of the electrophoretic peak retention time.

In addition to error present in electrophoretic retention times, PCR-CE methods can introduce error in PCR amplicons due to PCR misincorporation and PCR stutter. PCR misincorporation possibly contributes to variation in electrophoretic retention times. Stutter error holds a special status in forensic STR analysis. Stutter peaks are expected in electropherograms, and presence (or absence) of stutter contributes to visual assessments of the sensitivity of the method. Stutter artifacts are not considered in measures of accuracy or precision in PCR-CE methods.

1.2. Accuracy and Precision in PCR-MPS Methods

In contrast to PCR-CE methods, the goal of PCR-MPS methods is to determine the nucleotide sequence of PCR-targeted markers in the genome. Experimental measurement of a marker sequence results in a sequencer read (hereafter simply called a read) expressed as a string of letters following the IUPAC coding convention [12] for the four nucleotides in the DNA backbone (A, C, G, T)[1]. Like PCR-CE, the genomic segments targeted by the assay are defined by the PCR primers, which for most STR markers, includes flanking sequence that may contain additional polymorphisms. Targeted segments can be bioinformatically trimmed to shorter extents, and typically at least the PCR primer sequences are trimmed away. The read sequence that remains becomes the sequence-based allele under consideration. Like PCR-CE, accuracy and precision should be sensitive to single nucleotide differences[2] because authentic allele sequences can differ by a single nucleotide due to point polymorphisms [13, 14]. PCR-MPS methods generate a read sequence for each individual PCR amplicon, and a base call for each nucleotide position. Thus, accuracy and precision can be determined at several levels: nucleotide, read, and sequence-based allele.

Consensus has not yet been reached for reporting accuracy and precision for forensic PCR-MPS methods. Accuracy of PCR-MPS methods has been reported either as a call rate [15] or as a concordance to CE allele numbers. Precision is often not reported at all [16-21] or has been reported as a modified call rate [15].

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[1] The IUPAC ambiguity code N can appear in sequencer output but reads containing N characters are typically not considered in forensic analysis and will be disregarded here.

[2] Our use of the term nucleotide is functionally equivalent to the term base pair commonly used in forensics. The term nucleotide is preferred because the object of most bioinformatic analysis is the sequencer read, which represents just one DNA strand.

1.3. Distance Measures

Accuracy and precision are often expressed in distance units. The measurement result of a PCR-MPS method is a read string which may be close to or distant from the expected sequence. The distance between two DNA sequences is called an edit distance. The concept of edit distance is tightly associated with sequence alignment, which can be thought of as a transcript of the edits (nucleotide substitutions, insertions, and deletions) required to convert one sequence string into another [22] (p 125). An optimal alignment may not correspond to the minimum possible number of edits due to weightings that may be assigned to transitions versus transversions and on opening and extension of gaps in the aligned sequences. The minimum number of equally-weighted edit operations is called the Levenshtein distance [22, 23].

Levenshtein distance has been shown to satisfy [24] the axioms [25] (p.147) required of distance metrics (see Appendix A for details). Distance metrics imply the existence of a corresponding metric space. The concept of a metric space where the elements of the space are DNA (or protein) sequence strings has a well-established foundation. Maynard Smith [26] first described sequence spaces in the context of natural selection, while Eigen [27] provided a formal topological/geometrical interpretation of sequence spaces. In short, Levenshtein distance is a distance metric that can be used in mathematical and statistical calculations. By employing the Levenshtein distance metric, accuracy and precision can be expressed in accord with SWGDAM guidance. That is, accuracy in amplicon sequencing can be expressed as the degree of conformity of read sequences to the true DNA sequence, and precision can be expressed as the degree of mutual agreement among a series of individual read sequence determinations.

Here, we define concepts for accuracy and precision in sequence-based PCR-MPS methods that are 1) compliant with SWGDAM and ENFSI guidelines, 2) analogous to the concepts of sizing accuracy and precision currently used for PCR-CE methods [28], and 3) can be expressed in units that can be used in statistical calculations.

2. Material and Methods

2.1. Samples and Sequencing

One ng aliquots of four DNA standards were sequenced in triplicate: 2800M (Promega, Madison, WI), and NIST standard reference material 2391d components A, B, and E (NIST, Gaithersburg, MD). Sequencing was performed at Intermountain Forensics using the ForenSeq™ kit and MiSeq™ sequencer (Verogen, San Diego, CA). Raw FASTQ data files were analyzed using MixtureAce™ (NicheVision Forensics, Akron, OH) with an analytical threshold of zero. Read sequences were bioinformatically trimmed to match the Verogen recommended analyzable regions [29] and exported to comma separated value (CSV) files along with their read count intensities (RCI). Stutter artifacts identified by MixtureAce were deleted from the CSV files. A custom Python script was used to modify the CSV files for statistical analysis such that each data row corresponded to an individual sequencer read (RCI = 1). Sixteen alleles were selected for analysis based on being homozygous in one of the DNA standards (Table 1). Selecting homozygous alleles allowed unambiguous association of erroneous sequences to parent alleles.

Table 1. Alleles selected for analysis. Allele sequences have not been published for NIST standard reference materials. Therefore, allele IDs [30] were determined by MixtureAce and cross-referenced to STRSEQ accessions through the MixtureAce internal allele frequency tables.

Standard[1]	Locus	Allele ID[1]	STRSEQ[2] Accession No.
2800M	CSF1PO	12 PD	MH085189.1
	D5S818	12 RR	MH167007.1
	D22S1045	16 AW	MH167279.1
	TPOX	11 UN	MF044255.1
NIST SRM 2391d Component A	D2S441	11 RC	MH167320.1
	D3S1358	17 ZU	MH166980.1
	D4S2408	9 ZI	MH039589.1
NIST SRM 2391d Component B	D7S820	10 TO	MH167034.1
	D13S317	11 AQ	MH167219.1
	TH01	7 CS	MH085116.1
NIST SRM 2391d Component E	D6S1043	11 BZ	MH166925.1
	D9S1122	11 KZ	MH085132.1
	D10S1248	14 TJ	MH167062.1
	D19S433	14 WU	MH174820.1
	D20S482	15 QX	MH085169.1
	PentaD	14 WB	MH232690.1

[1] Allele identifiers consist of the CE-equivalent allele number and the first two digits of the SID label [30].
[2] https://www.ncbi.nlm.nih.gov/bioproject/380127

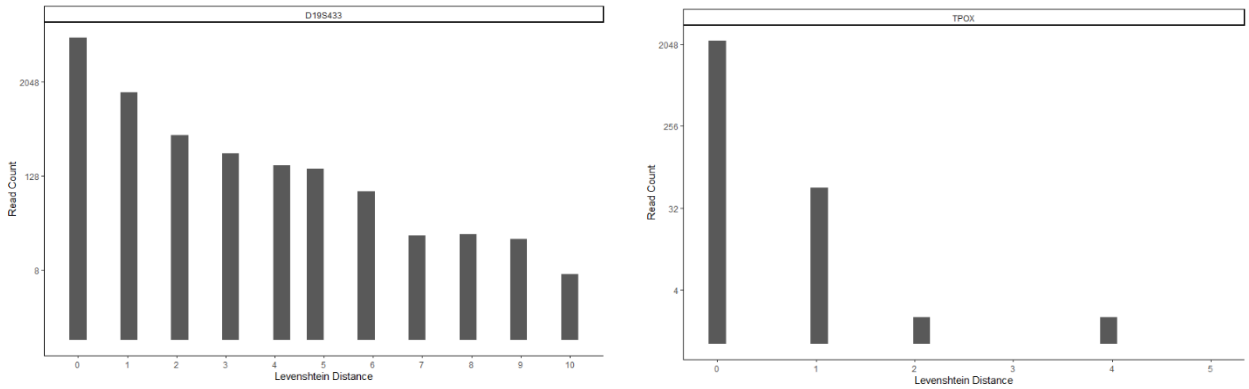
Reads exhibiting Levenshtein distances > 10 were omitted from the data set. Reads with extreme Levenshtein distances may arise from trace level contamination, or PCR mis-priming of non-human sequence, and therefore are not representative data. Of 125,994 reads in the dataset, only 107 exhibited Levenshtein distances of > 10 from the expected sequences. The maximum Levenshtein distance was 78. The truncated data set included 125,887 reads. Levenshtein distances were calculated using a custom Python script employing the Jellyfish module (https://github.com/jamesturk/jellyfish). All other data analysis was performed using custom R scripts.

3. Results

3.1. Distribution of Levenshtein Distances

Histograms of Levenshtein distances of the individual reads generated by a PCR-MPS method exhibit a consistent shape independent of locus. Most reads match the expected sequence and therefore, exhibit a zero Levenshtein distance from the expected sequence. The most abundant error type consists of reads exhibiting a single point error and a corresponding Levenshtein distance from expected equal to one and frequencies tend to decline exponentially with increasing Levenshtein distance (Figure 1). Assuming point errors in reads arise from random sequencing error, the read count distribution should be Poisson. However, it is known that sequencing error rates increase over the length of DNA templates, and that other error processes including PCR misincorporation contribute to the variance of the distribution; thus, leading to overdispersion. Tests for overdispersion indicates the Poisson does not fit the data. This is consistent with variances exceeding means at all loci tested (Table 2). In addition,

140 the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) values consistently show
141 that the negative binomial fit is better than the Poisson fit.



142

143 **Figure 1. Levenshtein distance profiles for reads from selected loci after filtering stutter. Read count intensities**
144 **are expressed on a log scale to aid visualization. A) Sequencer reads from D19S433 exhibit significant numbers**
145 **of erroneous reads as measured by Levenshtein distance. B) Sequencer reads from TPOX exhibit a higher degree**
146 **of accuracy, with the highest error category corresponding to reads with a Levenshtein distance of one from the**
147 **authentic allelic sequence.**
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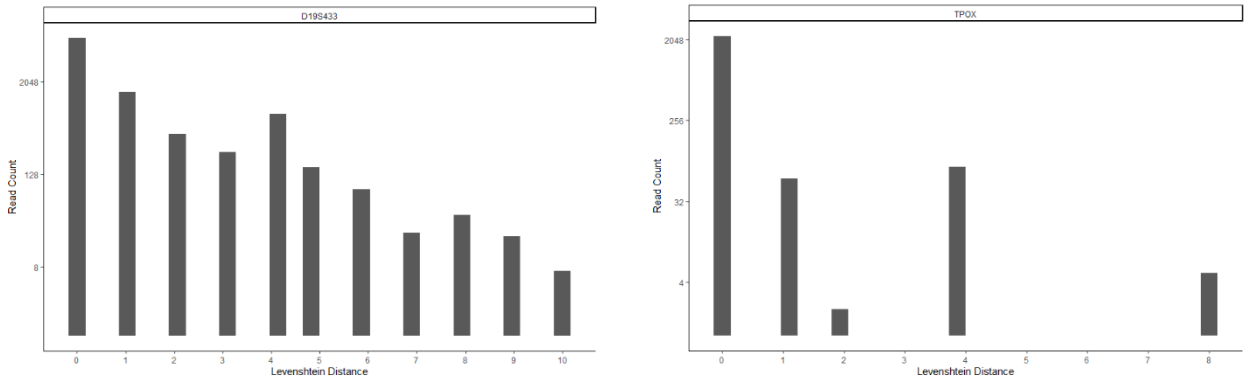

150

151 **Figure 2. Levenshtein distance profiles for reads from selected loci with stutter included. Read count intensities**
152 **are expressed on a log scale to aid visualization. Inclusion of stutter increases the read count intensity of reads**
153 **exhibiting Levenshtein distances of 4 and 8.**
154

155

156 **Table 2. Parameter values and fit statistics for the Poisson and negative binomial distributions to Levenshtein**
157 **distance data from 16 loci that were homozygous in the data set.**

Locus	Reads (N)	Mean	Variance	Standard Deviation	Poisson		Negative Binomial	
					AIC	BIC	AIC	BIC
TPOX	2,296	0.02918	0.04052	0.2013	655	661	606	617
D2S441	5,809	0.06335	0.1110	0.3332	2918	2925	2699	2713
D3S1358	10,822	0.1351	0.3811	0.6173	10088	10095	8092	8106
D4S2408	4,881	0.03176	0.04878	0.2209	1430	1436	1334	1347
CSF1PO	1,170	0.06838	0.1782	0.4221	653	658	531	541
D5S818	1,062	0.06309	0.1213	0.3483	603	608	493	503
D6S1043	15,335	0.1111	0.3090	0.5559	12355	12363	9985	10001
D7S820	3,747	0.1703	0.4354	0.6598	4074	4080	3347	3359
D9S1122	8,670	0.03956	0.09382	0.3063	3134	3141	2605	2619
D10S1248	6,407	0.1313	0.3294	0.5739	5741	5748	4824	4837
TH01	24,670	0.07937	0.1824	0.4271	15112	15120	12967	12983
D13S317	4,185	0.1101	0.2065	0.4544	3185	3192	2906	2918
D19S433	10,271	0.5516	1.643	1.282	24843	24850	19489	19504
D20S482	18,769	0.09414	0.2498	0.4998	13340	13348	10760	10776
PentaD	2,292	0.2133	0.3834	0.6192	2731	2737	2567	2578
D22S1045	5,501	0.1736	0.4270	0.6534	655	661	606	618
TOTAL	**125,887**							
Average		**0.1291**	**0.3213**	**0.5668**				

158

159 ### 3.2. Accounting for Levenshtein distances of Four After Filtering Stutter

160 Modeling was performed on data after stutter artifacts were removed. However, despite their removal,
161 stutter artifacts can still contribute to overdispersion in a cryptic way. Frequencies of reads with
162 Levenshtein distances in multiples of tandem repeat motifs are slightly elevated relative to the overall
163 pattern. For example, counts of reads with Levenshtein distances equal to four and eight are slightly
164 elevated for loci with tetramer repeats (Figure 1). This phenomenon is a consequence of defining
165 stutter as a pure motif shift and combined with how Levenshtein distances are calculated. In PCR-MPS,
166 stutter artifacts formed during the PCR step can be subject to sequencing error during the sequencing
167 step. If a sequencing error occurs inside the tandem repeat region of a stutter artifact, then a doubly
168 mutated read results where the total number of nucleotide changes can be said to be five (for a
169 tetramer repeat locus). However, the minimum number of steps to convert the stutter sequence to the
170 correct parent allele sequence is only four. In the case of N+1 stutter only the repeat motif containing
171 the sequencing error must be deleted for an edit distance equal to four. In the case of N-1 stutter, the
172 structure of certain repeat motifs the presence of a single nucleotide substitution inside the repeat
173 structure can allow the conversion of one sequence to another to be solved using only four edits despite
174 the doubly mutated sequence. Loci that exhibit high stutter can also show elevated RCIs for sequences
175 with Levenshtein distances from the authentic allele of five and nine. These sequences can be explained
176 as sequencing error in stutter artifacts that occurs outside the STR repeat motif. Mechanisms other
177 than those described here may also be involved. Illustrations of selected mechanisms are presented in
178 Appendix B.

179 ### 3.3. Levenshtein Distance as an Estimator of Error Rates

180 Locus and profile error rates can be estimated from mean Levenshtein distances. The mean Levenshtein
181 distances for the sixteen loci studied here ranged from 0.58 errors per 1,000 nucleotides (D9S1122) to

3.73 errors per 1000 nucleotides (D19S433, Table 3). Error rates for most loci were less than 1 per 1,000 nucleotides, which is consistent with published error rates for Illumina sequencing [31].

Table 3. Inference of error rate using Levenshtein distance statistics.

Locus	Sample	Allele	Allele Length	Mean Levenshtein Distance	Inferred Error Rate	
					Per nt	Per 1000 nt
TPOX	2800M	11 UN	49	0.02918	5.96×10^{-4}	0.596
D2S441	2391d_A	11 RC	96	0.06335	6.60×10^{-4}	0.660
D3S1358	2391d_A	17 ZU	134	0.1351	1.01×10^{-3}	1.01
D4S2408	2391d_A	9 ZI	50	0.03176	6.35×10^{-4}	0.635
CSF1PO	2800M	12 PD	68	0.06838	1.01×10^{-3}	1.01
D5S818	2800M	12 RR	68	0.06309	9.28×10^{-4}	0.928
D6D1043	2391d_E	11 BZ	129	0.1111	8.61×10^{-4}	0.861
D7S820	2391d_B	10 TO	95	0.1703	1.79×10^{-3}	1.79
D9S1122	2391d_E	11 KZ	68	0.03956	5.82×10^{-4}	0.582
D10S1248	2391d_E	14 TJ	97	0.1313	1.35×10^{-3}	1.35
TH01	2391d_B	7 CS	72	0.07937	1.10×10^{-3}	1.10
D13S317	2391d_B	11 AQ	122	0.1101	9.02×10^{-4}	0.902
D19S433	2391d_E	14 WU	148	0.5516	3.73×10^{-3}	3.73
D20S482	2391d_E	15 QX	102	0.09414	9.23×10^{-4}	0.923
PentaD	2391d_E	14 WB	228	0.2133	9.36×10^{-4}	0.936
D22S1045	2800M	16 AW	174	0.1736	9.98×10^{-4}	0.998
Overall Average				**0.1219**	**1.13×10^{-3}**	**1.13**

3.4. Accuracy and Precision

Method accuracy can be expressed as the mean Levenshtein distance of reads relative to zero. The mean Levenshtein distance is a metric expression of the degree of conformity of measurements to the true value. In the data presented here, the overall method accuracy is 0.122 distance units. The variance of Levenshtein distance values is a metric expression of the degree of mutual agreement among a series of individual measurements. In the data presented here, the overall method precision is the overall standard deviation of 0.3213.

3.5. Comparison to Other Measures of Accuracy and Precision

Currently published methods for expressing accuracy and precision in PCR-MPS methods involve either allele call rates [15] or standard deviations of allele read count intensities [32]. These measures are indicators of PCR efficiency and variability but are less informative for whole method accuracy and precision. In many cases where inhibition or low template is not involved, percent call rate can be an insensitive measure (e.g. see Table 4, Figure 3). Levenshtein distance is more closely related to total method error and is sensitive to differences in method error rates. In this study, we demonstrated the utility of Levenshtein distance-based accuracy and precision by discriminating performances at different loci within a single forensic kit. However, this same method can be employed in the comparison of other factors to address differences between kits, sequencers, technicians, or laboratories.

205

206 **Table 4. Comparison of Levenshtein distance measures with call rate and standard deviations**
207 **of read count intensities.**

Locus	Call Rate (%)	Read Count Intensity			Levenshtein Distance	
		Mean	Standard Deviation	Standard Error	Mean	Standard Deviation
TPOX	100	722	149	60.8	0.02918	0.04052
D2S441	100	1,884	421	172.0	0.06335	0.1110
D3S1358	100	3,040	817	333.4	0.1351	0.3811
D4S2408	100	1,561	507	206.8	0.03176	0.04878
CSF1PO	100	352	104	42.3	0.06838	0.1782
D5S818	100	426	146	59.5	0.06309	0.1213
D6D1043	100	4,745	621	358.7	0.1111	0.3090
D7S820	100	1,204	182	74.5	0.1703	0.4354
D9S1122	100	2,809	92	53.1	0.03956	0.09382
D10S1248	100	1,948	142	82	0.1313	0.3294
TH01	100	7,571	1,378	562.4	0.07937	0.1824
D13S317	100	1,482	559	228.3	0.1101	0.2065
D19S433	100	2,537	892	515.0	0.5516	1.643
D20S482	100	5,876	687	396.8	0.09414	0.2498
PentaD	100	639	315	181.8	0.2133	0.3834
D22S1045	100	1,942	420	171.5	0.1736	0.4270
Average	**100**	**2,421**	**464**	**218.7**	**0.1291**	**0.3213**

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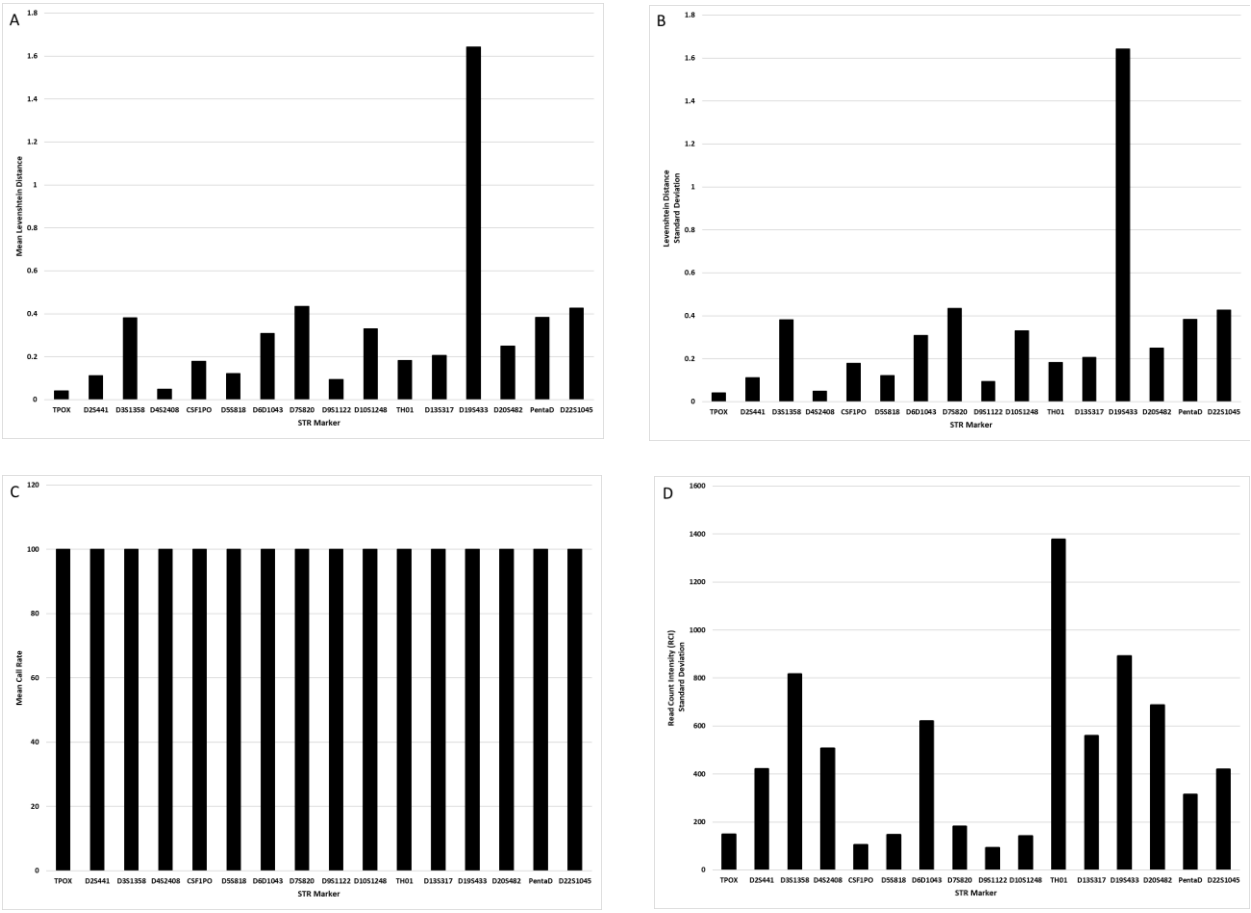

Figure 3. Alternative measures of accuracy and precision in PCR-MPS methods. Each plot represents data pooled across triplicate analyses of DNA standards. Only homozygous STR markers were analyzed to allow unambiguous assignment of erroneous reads to parent alleles. Stutter was removed prior to analysis, and flanking regions recommended for analysis by Verogen were included with the STR loci. Levenshtein distances were calculated for each read relative to the known allele sequences. A) The mean Levenshtein distance is a measure of method accuracy where larger values indicate lower method accuracy. Perfect accuracy would result in a mean Levenshtein distance of zero. B) The standard deviation of Levenshtein distance is a measure of method precision where larger values correspond to lower precision. C) Call rate has been employed as a measure of method accuracy [15], but at high template concentrations are high, and in the absence of degradation, call rate can be an insensitive measure. Call rate is a better measure of PCR efficiency than PCR-MPS whole method accuracy. D) The standard deviation of repeated measurements has been employed as a measure of method precision. When applied to read count intensity measurements, the standard deviation of RCI is a better measure of PCR precision than PCR-MPS whole method precision.

4. Conclusions

Levenshtein distance is a metric that can be used to calculate accuracy and precision of forensic PCR-MPS methods in compliance with SWGDAM and ENSFI guidelines. The Levenshtein distance has an intuitive interpretation as the edit distance between experimentally determined reads and authentic reference sequences.

Acknowledgements

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Appendix A

Definitions for mean and variance imply the need for a metric that can be used to quantitatively calculate accuracy and precision. A measure that satisfies the axioms of a metric is essential for calculating accuracy and precision in a way analogous to the approach used for PCR-CE methods, including calculation of standard deviations. Metrics are distance functions that must satisfy four axioms [25] p.147. Let $d(x, y)$ be a function measuring the distance between two elements ***x*** and ***y*** in a set of elements S.

1. The distance between two elements in the metric space is non-negative: $d(x, y) \geq 0$
2. The distance between two entities is zero if and only if the two entities are the same: $d(x, x) = 0$
3. The order of comparison does not matter: $d(x, y) = d(y, x)$
4. The distance between two elements is less than or equal to the sum of the distances obtained when an intermediate element is inserted. In other words, the triangle inequality is satisfied: $d(x, z) \leq d(x, y) + d(y, z)$

These properties hold for common metrics such as meters or inches in metric spaces of ordinary experience. These properties also hold when the metric $d(x, y)$ is Levenshtein distance and the elements in the metric space are DNA sequence strings [24].

Appendix B

Explanation of Elevated Frequencies of Levenshtein Distances of Four and Five at Loci with Tetrameric Tandem Repeat Motifs

```
D19S433_14_WU      1 AATAAAAATCTTCTCTCTTTCTTCCTCTCTCC----TTCCTTCCTTCCTT     46
                     ||||||||||||||||||||||||||||||||    |||||||||||||||
D19S433_15_AH      1 AATAAAAATCTTCTCTCTTTCTTCCTCTCTCCTTCTTTCCTTCCTTCCTT     50

D19S433_14_WU     47 CCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTACCTTCTTTCCTTCA     96
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
D19S433_15_AH     51 CCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTACCTTCTTTCCTTCA    100

D19S433_14_WU     97 ACAGAATCTTATTCTGTTGCCCAGGCTGGAGTGCAGTGGTACAATTATAG    146
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
D19S433_15_AH    101 ACAGAATCTTATTCTGTTGCCCAGGCTGGAGTGCAGTGGTACAATTATAG    150

D19S433_14_WU    147 CT    148
                     ||
D19S433_15_AH    151 CT    152
```

Figure B-1. Alignment of the D19S433 14 WU and 15 AH sequences. The 15 AH sequence is an N+1 stutter artifact of the 14 WU allele sequence with the addition of a sequencing error inside the repeat motif of the stutter artifact. Alignment can be solved using only four edit operations. The bracketed STR sequences are $[CCTT]_{12}[CCTA]_1[CCTT]_1[CTTT]_1[CCTT]_1$ and $[CCTT]_1[CTTT]_1[CCTT]_{11}[CCTA]_1[CCTT]_1[CTTT]_1[CCTT]_1$ respectively. Alignment was performed using EMBOS Needle with default parameter values.

```
D19S433_14_WU      1 AATAAAAATCTTCTCTCTTTCTTCCTCTCTCCTTCCTTCCTTCCTTCCTT     50
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
D19S433_13_TA      1 AATAAAAATCTTCTCTCTTTCTTCCTCTCTCCTTCCTTCCTTCCTTCCTT     50

D19S433_14_WU     51 CCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTACCTTCTTTCCTTCAACAG    100
                     |||||||||||||||||||| |  || |||||||||||||||||||||||
D19S433_13_TA     51 CCTTCCTTCCTTCCTTCCTT-C--CC-TCCTACCTTCTTTCCTTCAACAG     96

D19S433_14_WU    101 AATCTTATTCTGTTGCCCAGGCTGGAGTGCAGTGGTACAATTATAGCT    148
                     ||||||||||||||||||||||||||||||||||||||||||||||||
D19S433_13_TA     97 AATCTTATTCTGTTGCCCAGGCTGGAGTGCAGTGGTACAATTATAGCT    144
```

Figure B-2. Alignment of the D19S433 14 WU and 13 TA sequences. The 13 TA sequence is an N-1 stutter artifact of the 14 WU allele sequence with the addition of a sequencing error inside the repeat motif of the stutter artifact. Alignment can be solved using only four edit operations. The bracketed STR sequences are $[CCTT]_{12}[CCTA]_1[CCTT]_1[CTTT]_1[CCTT]_1$ and $[CCTT]_{10}CCCT[CCTA]_1[CCTT]_1[CTTT]_1[CCTT]_1$ respectively. Alignment was performed using EMBOS Needle, with gap open = 5.0 and gap extend = 0.0005.

```
D19S433_14_WU      1 AATAAAAATCTTCTCTCTTTCTTCCTCTCTCCTTCCTTCCTTCCTTCCTT     50
                     ||||||||||||||||||||||||||||||    |||||||||||||||||
D19S433_13_PG      1 AATAAAAATCTTCTCTCTTTCTTCCTCTC----TCCTTCCTTCCTTCCTT     46

D19S433_14_WU     51 CCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTACCTTCTTTCCTTCAACAG    100
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
D19S433_13_PG     47 CCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTACCTTCTTTCCTTCAACAG     96

D19S433_14_WU    101 AATCTTATTCTGTTGCCCAGGCTGGAGTGCAGTGGTACAATTATAGCT    148
                     ||||||||||||||||||||||||||||||.||||||||||||||||||||
D19S433_13_PG     97 AATCTTATTCTGTTGCCCAGGCTGGAGTACAGTGGTACAATTATAGCT    144
```

Figure B-3. Alignment of the D19S433 14 WU and 13 PG sequences. The 13 PG sequence is an N-1 stutter artifact of the 14 WU allele sequence with the addition of a sequencing error outside the repeat motif of the stutter artifact. Alignment can be solved using five edit operations. The bracketed STR sequences are $[CCTT]_{12}[CCTA]_1[CCTT]_1[CTTT]_1[CCTT]_1$ and $[CCTT]_{11}[CCTA]_1[CCTT]_1[CTTT]_1[CCTT]_1$ respectively. Alignment was performed using EMBOS Needle, with default parameter values.

References

[1] S. Bell, Measurement uncertainty in forensic science : a practical guide, CRC Press, Taylor & Francis Group, Boca Raton, 2017.
[2] T. Vosk, A.F. Emery, Forensic metrology : scientific measurement and inference for lawyers, judges and criminalists, CRC Press,Taylor & Francis Group, Boca Raton, 2015.
[3] SWGDAM, Scientific Working Group on DNA Analysis Methods Validation Guidelines for DNA Analysis Methods, 2016.
[4] SWGDAM, Addendum to "SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories" to Address Next Generation Sequencing, 2019.
[5] ENFSI, Recommended Minimum Criteria for the Validation of Various Aspects of the DNA Profiling Process, 2010.
[6] D.Y. Wang, C.W. Chang, R.E. Lagace, L.M. Calandro, L.K. Hennessy, Developmental validation of the AmpFlSTR(R) Identifiler(R) Plus PCR Amplification Kit: an established multiplex assay with improved performance, J Forensic Sci 57(2) (2012) 453-65.
[7] R.L. Green, R.E. Lagace, N.J. Oldroyd, L.K. Hennessy, J.J. Mulero, Developmental validation of the AmpFlSTR(R) NGM SElect PCR Amplification Kit: A next-generation STR multiplex with the SE33 locus, Forensic Sci Int Genet 7(1) (2013) 41-51.
[8] T.R. Moretti, A.L. Baumstark, D.A. Defenbaugh, K.M. Keys, A.L. Brown, B. Budowle, Validation of STR typing by capillary electrophoresis, J Forensic Sci 46(3) (2001) 661-76.
[9] R.M. Goor, L. Forman Neall, D. Hoffman, S.T. Sherry, A mathematical approach to the analysis of multiplex DNA profiles, Bull Math Biol 73(8) (2011) 1909-31.
[10] M.G. Ensenberger, K.A. Lenz, L.K. Matthies, G.M. Hadinoto, J.E. Schienman, A.J. Przech, M.W. Morganti, D.T. Renstrom, V.M. Baker, K.M. Gawrys, M. Hoogendoorn, C.R. Steffen, P. Martin, A. Alonso, H.R. Olson, C.J. Sprecher, D.R. Storts, Developmental validation of the PowerPlex((R)) Fusion 6C System, Forensic Sci Int Genet 21 (2016) 134-44.
[11] L.K. Hennessy, N. Mehendale, K. Chear, S. Jovanovich, S. Williams, C. Park, S. Gangano, Developmental validation of the GlobalFiler((R)) express kit, a 24-marker STR assay, on the RapidHIT((R)) System, Forensic Sci Int Genet 13 (2014) 247-58.
[12] I.-I.C.o.B. Nomenclature, Abbreviations and symbols for nucleic acids, polynucleotides, and their constituents, Biochemistry 9(20) (1970) 4022-4027.
[13] K.B. Gettings, L.A. Borsuk, D. Ballard, M. Bodner, B. Budowle, L. Devesse, J. King, W. Parson, C. Phillips, P.M. Vallone, STRSeq: A catalog of sequence diversity at human identification Short Tandem Repeat loci, Forensic Sci Int Genet 31 (2017) 111-117.
[14] K.B. Gettings, L.A. Borsuk, C.R. Steffen, K.M. Kiesler, P.M. Vallone, Sequence-based U.S. population data for 27 autosomal STR loci, Forensic Sci Int Genet 37 (2018) 106-115.
[15] A.C. Jager, M.L. Alvarez, C.P. Davis, E. Guzman, Y. Han, L. Way, P. Walichiewicz, D. Silva, N. Pham, G. Caves, J. Bruand, F. Schlesinger, S.J.K. Pond, J. Varlaro, K.M. Stephens, C.L. Holt, Developmental validation of the MiSeq FGx Forensic Genomics System for Targeted Next Generation Sequencing in Forensic DNA Casework and Database Laboratories, Forensic Sci Int Genet 28 (2017) 52-70.
[16] R.S. Just, L.I. Moreno, J.B. Smerick, J.A. Irwin, Performance and concordance of the ForenSeq system for autosomal and Y chromosome short tandem repeat sequencing of reference-type specimens, Forensic Sci Int Genet 28 (2017) 1-9.
[17] J.D. Churchill, S.E. Schmedes, J.L. King, B. Budowle, Evaluation of the Illumina((R)) Beta Version ForenSeq DNA Signature Prep Kit for use in genetic profiling, Forensic Sci Int Genet 20 (2016) 20-29.

[18] J.D. Churchill, J. Chang, J. Ge, N. Rajagopalan, S.C. Wootton, C.W. Chang, R. Lagace, W. Liao, J.L. King, B. Budowle, Blind study evaluation illustrates utility of the Ion PGM system for use in human identity DNA typing, Croat Med J 56(3) (2015) 218-29.
[19] S. Kocher, P. Muller, B. Berger, M. Bodner, W. Parson, L. Roewer, S. Willuweit, D.N. Consortium, Inter-laboratory validation study of the ForenSeq DNA Signature Prep Kit, Forensic Sci Int Genet 36 (2018) 77-85.
[20] V. Sharma, H.Y. Chow, D. Siegel, E. Wurmbach, Qualitative and quantitative assessment of Illumina's forensic STR and SNP kits on MiSeq FGx, PLoS One 12(11) (2017) e0187932.
[21] A.L. Silvia, N. Shugarts, J. Smith, A preliminary assessment of the ForenSeq FGx System: next generation sequencing of an STR and SNP multiplex, Int J Legal Med 131(1) (2017) 73-86.
[22] D. Gusfield, Algorithms for Strings, Trees, and Sequences, Cambridge University Press, Cambridge, 1997.
[23] V. Levenshtein, Binary codes capable of correcting deletions, insertions, and reversals, Soviet Physics Doklady 10(8) (1965) 707-710.
[24] G. Navarro, A guided tour to approximate string matching, ACM Comput. Surv. 33(1) (2001) 31–88.
[25] S. Axler, Measure, Integration & Real Analysis, Springer, Cham, Switzerland, 2020.
[26] J. Maynard Smith, Natural selection and the concept of a protein space, Nature 225(February 7) (1970) 563-564.
[27] M. Eigen, R. Winkler-Oswatitsch, A. Dress, Statistical geometry in sequence space: a method of quantitative comparative sequence analysis, Proc Natl Acad Sci U S A 85(16) (1988) 5913-7.
[28] A.R. Isenberg, R.O. Allen, K.M. Keys, J.B. Smerick, B. Budowle, B.R. McCord, Analysis of two multiplexed short tandem repeat systems using capillary electrophoresis with multiwavelength fluorescence detection, Electrophoresis 19(1) (1998) 94-100.
[29] K.B. Gettings, D. Ballard, M. Bodner, L.A. Borsuk, J.L. King, W. Parson, C. Phillips, Report from the STRAND Working Group on the 2019 STR sequence nomenclature meeting, Forensic Sci Int Genet 43 (2019) 102165.
[30] B. Young, T. Faris, L. Armogida, A nomenclature for sequence-based forensic DNA analysis, Forensic Sci Int Genet 42 (2019) 14-20.
[31] M. Schirmer, U.Z. Ijaz, R. D'Amore, N. Hall, W.T. Sloan, C. Quince, Insight into biases and sequencing errors for amplicon sequencing with the Illumina MiSeq platform, Nucleic Acids Res 43(6) (2015) e37.
[32] R. Tao, W. Qi, C. Chen, J. Zhang, Z. Yang, W. Song, S. Zhang, C. Li, Pilot study for forensic evaluations of the Precision ID GlobalFiler NGS STR Panel v2 with the Ion S5 system, Forensic Sci Int Genet 43 (2019) 102147.