OFFICE OF CHIEF MEDICAL EXAMINER
THE CITY OF NEW YORK

Presentation Prepared for the LT-DNA Panel

Theresa Caragine Ph.D.
Deputy Director
October 15, 2009

The allotted time for each question was brief; thus, this presentation does not represent the practices and protocols of the NYC OCME in their entirety.

How do you define or use the term “LCN”?

Stochastic or Random Effects HT-DNA and LT-DNA samples

- Heterozygous Peak Imbalance
  - Extreme imbalance results in dropout of one or both alleles at a locus.
- Stutter
- Detection of an allele(s) from a very minor contributor that was originally in sample or was deposited after the commission of the crime
  - These alleles are termed “drop-ins”.

LT-DNA: Exaggerated Stochastic Effects- Threshold 100 pg

- Peak Imbalance greater (28 cycle data)
  - 500 pg = 86 RFUs +/- 6 RFUs
  - 100 pg = 77 RFUs +/- 8 RFUs
  - 50 pg = 50 RFUs +/- 11 RFUs

Dropout may occur below 100 pg

- Stutter more frequent and may be taller.
- Greater propensity to detect minor components or drop-ins

Continuum

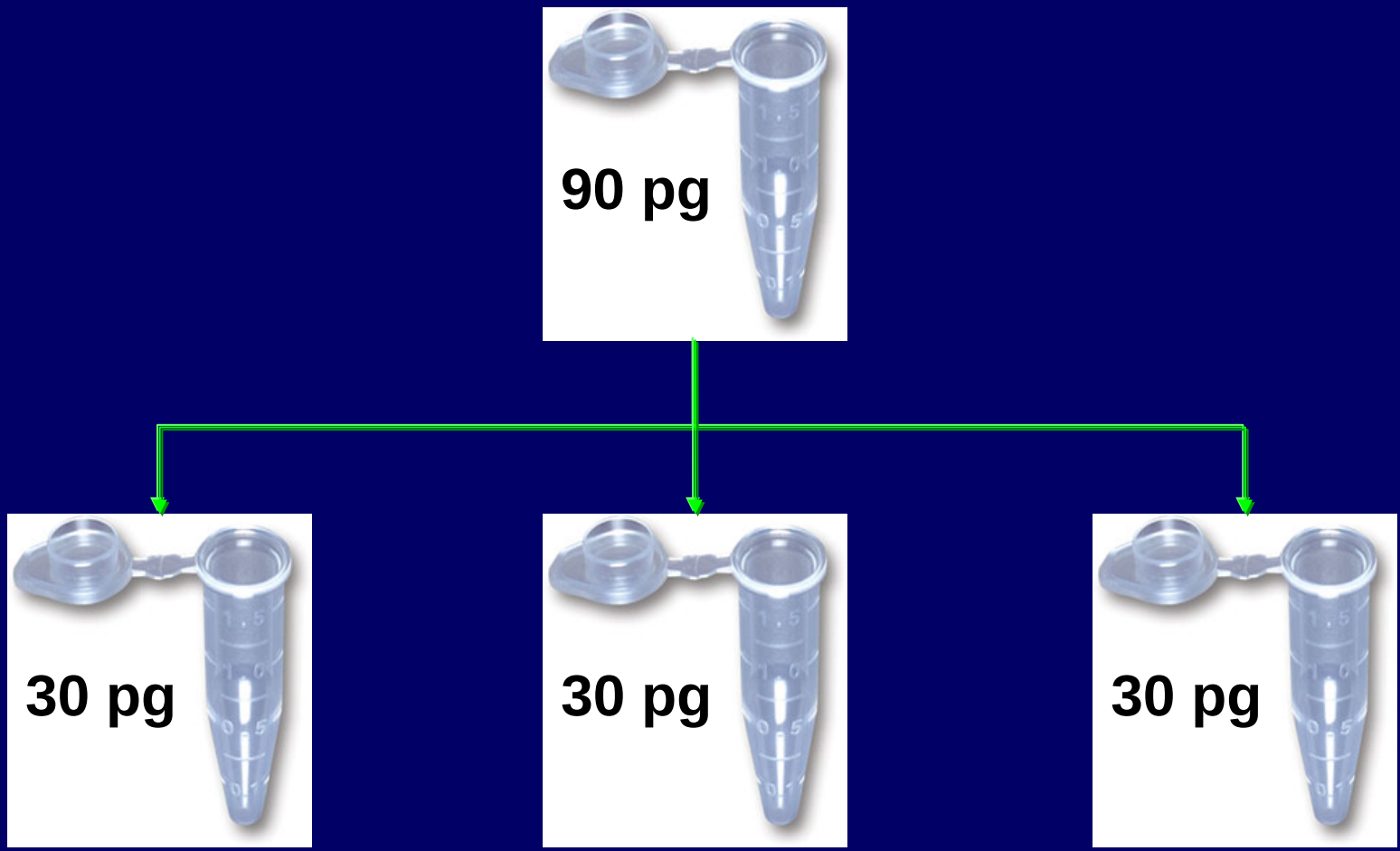
- All LT-DNA samples amplified in triplicate
- HT-DNA samples, if unique in a case or a mixture, amplified twice.

  Concordance Policy

LT-DNA Protocols to Accommodate Stochastic Effects

Issue	Resolution
Peak Imbalance	•3 replicates and interpretation protocols to assign alleles for •Single source samples •Mixed samples
Stutter	More stringent guidelines for assigning alleles in stutter position
Drop-ins	•Consensus approach •Enhanced Quality Control practices •Such as irradiation of labware and water

Amplify Three Replicates



1st Step: COMPOSITE or CONSENSUS PROFILES

Alleles must be labeled in 2 out of the 3 amplifications to be confirmed.

2nd Step: Interpretation Protocols

- Alleles assigned according to rules, which include parameters for peak ratios in mixtures and single sour samples etc
- If allele cannot be clearly assigned, loci deemed inconclusive.

Interpretation Protocols Verified

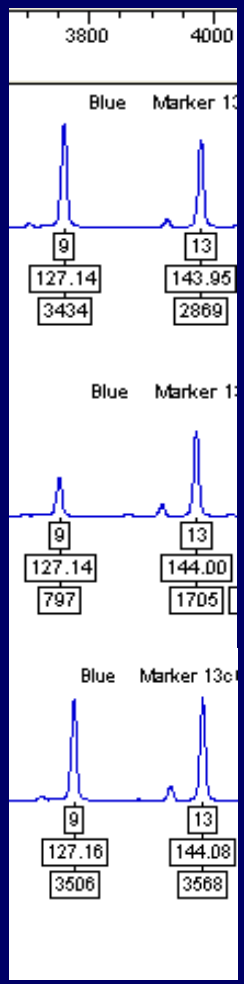
- During the validation, DNA donors to the samples tested were known.
- Data was interpreted according to our guidelines for allelic assignments.
- In no case was an incorrect allele assigned to a profile.

Accommodating Heterozygous Imbalance to Assign Allelic Pairs in Non-Mixtures.

83%

46%

98%



- Assign the two highest peaks in two injections (must distinguish highest peaks or no assignment).
- However, if one of the two peaks is consistently below a heterozygote balance of 0.3 the possibility of this locus being homozygous must be considered.
- Protocol for alleles in stutter position

Consequence of Allelic DROPOUTS

(ultimate imbalance of allelic pairs)

False Homozygote: One Allele of a heterozygous pair not detected

Guidelines to determine the presence of a true Homozygote

Determining Homozygotes

- Normally an RFU threshold can help distinguish heterozygote from homozygote types.
- Based on our validation, due to the sensitivity of the equipment and assays, tall peak heights result for even 12.5 pg and 6.25 pg of DNA**.** However, dropout can still be observed.
- Therefore, peak height thresholds cannot be implemented to establish homozygote assignments.

Stringent Protocols to Assign Homozygotes

- Dropout rate for largest loci of each color and for TH01 and D16 was 2.2 times higher than for other loci; thus these loci are always considered to be potential false homozygotes.
- All loci in samples with less than 20 pg in each replicate considered potential false homozygotes.
  - A true heterozygous allele in a 6.25 or 12.5 pg amps were noted to be less than 30% of the main allele in 3 replicates in some samples.

Additional Requirements to Assign Homozygote Alleles

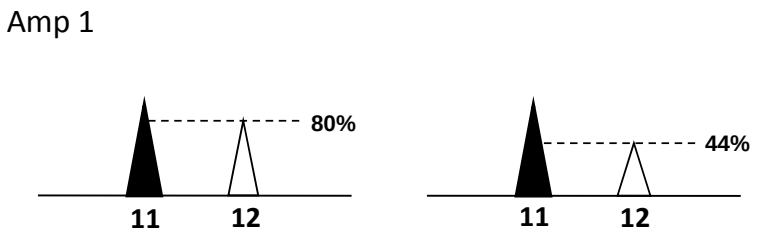
- Assign A “Z” to denote the presence of another allele also if:
  - A different allele >30% of main allele is present in one of the three amplifications.
  - The homozygote allele is present in only 2 of 3 amplifications.

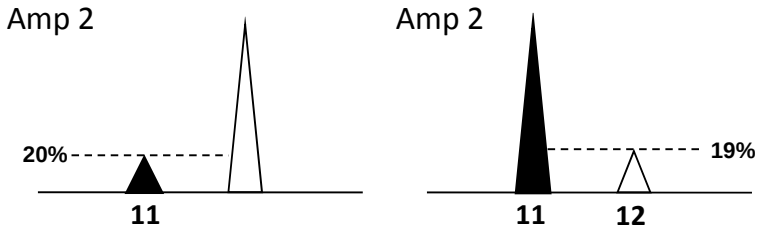
Stutter Rates and the Effect of Filters

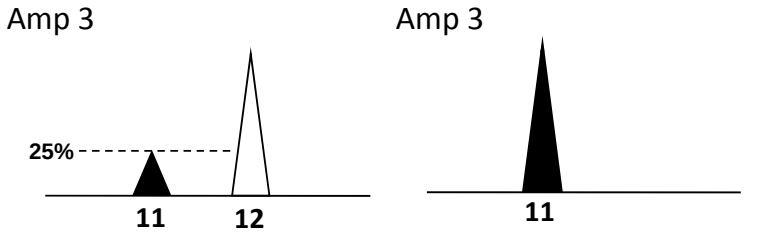
	Observed stutter	% Potential stutter peaks that did not occur or were removed by filters
28 cycles 500 pg	28.2%	100%
31 cycles 100 pg	58.5%	96.9%
31 cycles < 50 pg	51.2%	94.6%

Not occurring in repeatedly in 3 amps.

LT-DNA Interpretation Protocols to Assign Alleles in Minus or Plus 4 Stutter Positions







D13	
Assigned	11, 12
Correct	11, 12

D13	
Assigned	11, Z
Correct	11, 11

Stutter in Mixtures
HT-DNA and LT-DNA

- Potential stutter alleles in mixtures may be minor components.
- For non-deconvoluted mixtures for comparison only
  - alleles consistent with a comparison sample are not observed in the stutter position throughout all or most loci.

Composite Profile is not the Assigned Profile. Evaluate replicates in their entirety and follow interpretation protocols!

	Alleles labeled
Replicate “A”	12, 13
Replicate “B”	12, 13
Replicate “C”	14, 15
Composite Profile	12, 13
Assigned Profile	INC

What do you see as the biggest scientific issue/challenge/limitation with "LCN" testing in forensic cases and how do you think it should be addressed by the scientific and legal communities?

Application of Statistics

Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Forensic Science International 160 (2006) 90–101
Forensic Science International
www.elsevier.com/locate/forsciint

DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures

P. Gill [a,*], C.H. Brenner [b], J.S. Buckleton [c], A. Carracedo [d], M. Krawczak [e], W.R. Mayr [f], N. Morling [g], M. Prinz [h], P.M. Schneider [i], B.S. Weir [j]

[a] *Forensic Science Service, Trident Court, 2960 Solihull Parkway, Birmingham, UK*
[b] *Forensic Science Group, School of Public Health, University of California, Berkeley, CA 510-339-1911, USA*
[c] *ESR, Private Bag 92021, Auckland, New Zealand*
[d] *Institute of Legal Medicine, Faculty of Medicine, University of Santiago de Compostela, 15705 Santiago de Compostela, Spain*
[e] *Institute of Medical Informatics and Statistics, Kiel, Germany*
[f] *Division of Blood Group Serology, Medical University of Vienna, Austria*
[g] *Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark*
[h] *Office of the Chief Medical Examiner, Department of Forensic Biology, 520 First Avenue, New York, NY 10016, USA*
[i] *Institute of Legal Medicine University Clinic of Cologne, Melatengürtel, 60-62 D 50823 Köln, Germany*
[j] *University of Washington, Department of Biostatistics, Box 357232, Seattle, WA 98195, USA*

Received 4 April 2006; accepted 10 April 2006
Available online 5 June 2006

Likelihood Ratio

- NYC OCME developing a statistical software tool using the likelihood ratio method and submitting for review to the NY State DNA Subcommittee and the Forensic Science Commission.
- This statistical tool will employ empirically determined dropout and drop-in rates.
  - Single source and mixtures amplified with 28 and 31 cycles
  - A range of template DNA amounts
  - Degradation factor depending upon the sample.

Random Match Probabilities

- Used only with…
  - Single source profiles
  - Resolved profiles from a mixture
- Calculation performed independently of a comparison with a known sample.
- Recommendation of the National Research Council I (1992) and II (1996) - The Evaluation of Forensic DNA Evidence1996

LT-DNA Samples

- From validation, there was no peak height threshold that could accurately serve as a "stochastic threshold."
- Due to sensitivity of techniques, tall peak heights (~2000 RFUs) can result for even 6.25 pg DNA. However, dropout may still be observed.
- Instead interpretation protocols define allelic assignments.

Hardy Weinberg:
p2 + 2pq + q2 = 1

p= allele 1

q= allele 2

Allele Assignments	HW
8, 8	p^2
8, 10	2pq
8, Z	2p (q=1)

- If only one allele is determined at a locus, the other allele is termed “Z”.
- Statistical calculation uses frequency of the one allele only.
- Frequency or number will be more common, and favor the defendant.

RMNE (CPE/CPI) method justified under the following (for mixtures suitable for comparison):

DNA Commission of the International Society of Forensic Genetics: Recommendations on the Interpretations of Mixtures. 2006.

- All relevant alleles are “unmistakable”.

OR

- All relevant alleles are “unmistakable” or “masked by stutter”.
- All of the suspect’s alleles are present.

NYC OCME does not use RMNE if any alleles of the known are not labeled.

Omitting Loci

- Pg 2[1]: “if a locus is eliminated from analysis because it is a poor result showing no alleles at all, then of course there is no prejudice ignoring it.”
  - NYC OCME does not use loci that are deemed inconclusive in the composite profile.
    - No repeating allele
  - Alleles from all other loci used in calculation.

[1]DNA Commission of the International Society of Forensic Genetics: Recommendations on the Interpretations of Mixtures. 2006.

Calculation of CPE/CPI

- Alleles in the mixture are labeled prior to making comparisons.
- Know which locus is inconclusive in the composite profile and will not be used.
- Need suspect's profile to subsequently make the comparison.

RMNE
Conservative Approach

- M Krawczack: “the RMNE method does not make efficient use of the available information.”
- Usually results in an underestimate of the strength of the evidence.

[1]DNA Commission of the International Society of Forensic Genetics: Recommendations on the Interpretations of Mixtures. 2006.

Other Outcomes for Mixtures

- If ~1 or 2 alleles of known sample not apparent (LT-DNA) or below threshold (HT-DNA): known cannot be excluded as a contributor.
  - No statistics are calculated at this time.
  - Qualitative statement still has value, must explain statement.
- In some cases, no conclusions can be drawn regarding the comparison.
- Or known sample can be excluded as a contributor to the DNA detected.

Scientific Community: Cooperation to develop and train practitioners in the best methods.

Legal Community: Statistics go to weight. Make a qualitative statement quantitative. A qualitative statement still has value.

What advice do you have to offer to forensic scientists working with attorneys on cases that may be considered “LCN”. What materials should be routinely provided in discovery when DNA testing is challenged?

Communication

- Pre-trials in advance to explain testing results
- Periodic training sessions with attorneys
- Specialized training with person(s) in each of the attorney's offices that can serve as a liaison for the other attorneys in their office.

Analysts from the NYC OCME meet with defense attorneys when requested.

Limitations of Testing

- Forensic analysts report the DNA results.
- Cannot determine
  - when the sample was deposited.
  - how long the sample was there.
  - how the sample was deposited.
- Can relay experience from research studies
  - Highly unlikely that one could attribute a DNA profile from a touched item (skin cells) in a case to secondary or tertiary transfer. This depends upon the circumstances of the case.

Conclusions that can be Drawn

- DNA profile (from a single source sample or mixture deconvolution)
  - DNA alleles that are consistent with a known profile may be consistent with those of the profile generated.
- Mixture may only be suitable for comparison
  - At most, a known sample could be a possible contributor to a mixture.
- There is insufficient evidence to support that the known sample contributed to a mixture (excluded).
- No conclusions can be drawn regarding the sample and/or comparison.

Exclusion

If a contributor is excluded as a contributor to a mixed sample, an insufficient number of alleles consistent with his/her DNA profile were identified.

Relevance depends upon the circumstances of the case.

1st Case Processed: EXONERATION!

- Semen stain tested on a slip taken from a victim that was sexually assaulted and murdered in 1968.
- DNA profile determined with LT-DNA testing.
- The DNA profile of the individual recently arrested for this crime was not the same as the semen donor to the slip.
- DNA profile generated with LT-DNA testing resulted in a database hit to an individual who had been one of the original suspects in 1968.

NYC OCME: Independent Agency

- Several requests processed for post-conviction testing.
- If DNA found to be consistent with a person of interest (not the convicted offender) this may be exculpatory.

NYC OCME analysts called to testify by the defense.

Effort Must be Made to Share all Relevant Information.

- NYC OCME
  - Defense experts may observe testing.
  - Supply protocols and copy of case file(s) upon request.
  - Defense may review records in house that are too cumbersome to copy.

Defining what constitutes discovery material is a prosecutorial issue.

Elevated Standard of Interpretation of Criminal Procedure Law § 240.20 (1)(c) and Federal Rule of Criminal Procedure 16(a)(1)(F) met with Copy of Case file

- Case file:
  - Summary report
  - Printouts of the electronic data
  - Printouts of results of all testing
  - Notations from the analysts (in accordance with People v. DaGata, 86 NY2d 40 (1995)

The electronic data files not discoverable under the statutes cited.

Raw data does not exist in a readily accessible form that can be disclosed to the defense.

- Raw Data for up to 96 samples from many cases saved together.
  - Sample files
  - Project file: processed raw data
  - Genotyper file: analyzed data for each “run”
    - Contains the DNA profiles for each sample
    - Generates the print outs contained in the case files
- in Liquid Sugars and W.R. Grace courts did not require the gov’t to create or compile complex files that did not exist at the time the discovery demand was made.

Raw electronic data does not have to be disclosed since it could result in the manipulation of data.

People v. Marcoux, No. 2009-1176-FH (Mich. Cir. Ct., Macomb County, June 24, 2009

Final Response and Future Aims of LT-DNA Testing

Future Aims of LT-DNA Testing

- Continue to progress while providing service to current victims and suspects of crimes.
  - It is a disservice to these victims and suspects not to utilize the available technology.
  - Provide qualitative findings and a quantitative weight to those findings when possible.

Evaluation of Current Practitioners and Evaluation of New Techniques

- Must be data driven.
- Allow that there can be different approaches which achieve the same end.
- Receptive to evaluate new methodologies or approaches to old methods that may be better suited to resolve issues.

Define our Role and Appreciate the Limitations of that Role

- Forensic Scientists are tasked with identifying the potential sources of recovered DNA.
- What that finding means depends upon the context of the case and is determined by the finders of fact, the jury.

LT-DNA Mixture Deconvolution

Sample Interpretation Protocols Determined from Validation

- Consensus or composite profile (alleles that repeat twice)
- Decide whether to apply mixture or single source protocols.
- Apply interpretation protocols determined from the validation.
- Confirm with the pooled sample and the overall sample results.
- Supervisory review

Indications of a Mixture

- At least two contributors
  - three or more repeating peaks
  - or inconsistencies among the replicates at two or more loci.
- At least three contributors if five or more alleles in the composite profile in at least two loci.

Mixture or Non-Mixture?

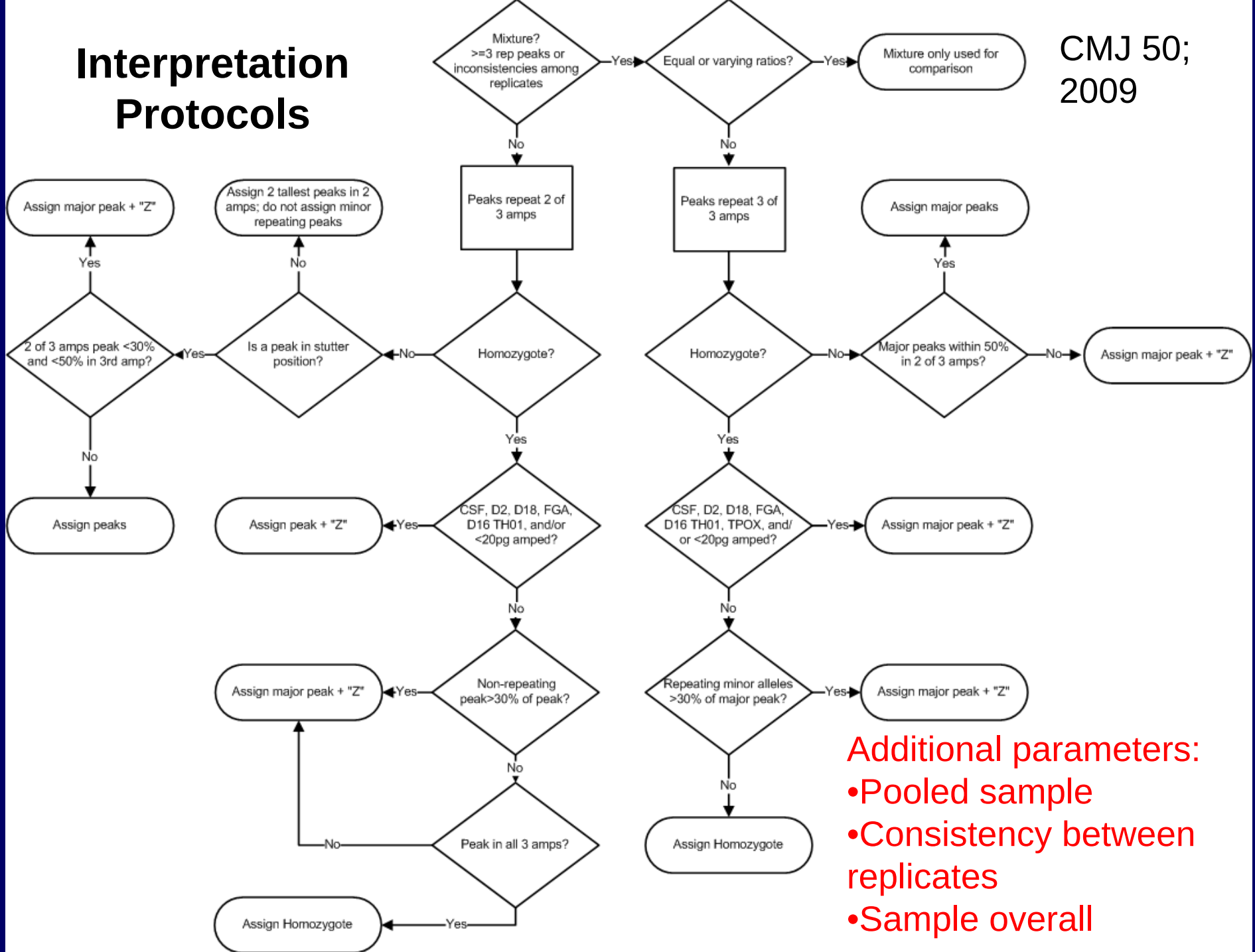
- During the validation, the DNA profiles of samples with 1 or 2 repeating peaks and no other indications of a mixture were correctly determined with the first set of guidelines for single source samples.
- At a locus, if two repeating peaks are clearly major peaks, any additional repeating peaks at a locus are not assigned to the profile.
- The minor peaks may represent very small amounts of a minor component evident at only one locus for example.

Mixture Categories

- Deducible mixtures (may be deconvoluted)
  - Sometimes the Major contributor's DNA profile can be determined.
  - NO conclusions drawn regarding the DNA profile of the minor component (LT-DNA) at this time.
- Non-deducible mixtures for comparison only
  - 1:1 and usually 1:2 mixtures cannot be deduced independently.
  - In some instances, may be able to subtract out the victim's profile.

Interpretation Protocols

CMJ 50; 2009



Additional parameters:

- Pooled sample
- Consistency between replicates
- Sample overall

Sample "Pooling" Helps to Determine Mixture Ratios

- Replicates are run on CE independently and as a "pooled" (combined) sample
  - Sample 1a injected
  - Sample 1b injected
  - Sample 1c injected
  - Sample 1a+1b+1c combined and injected
- Therefore, each sample yields 4 lanes to analyze
- Heterozygote pairs are more balanced.

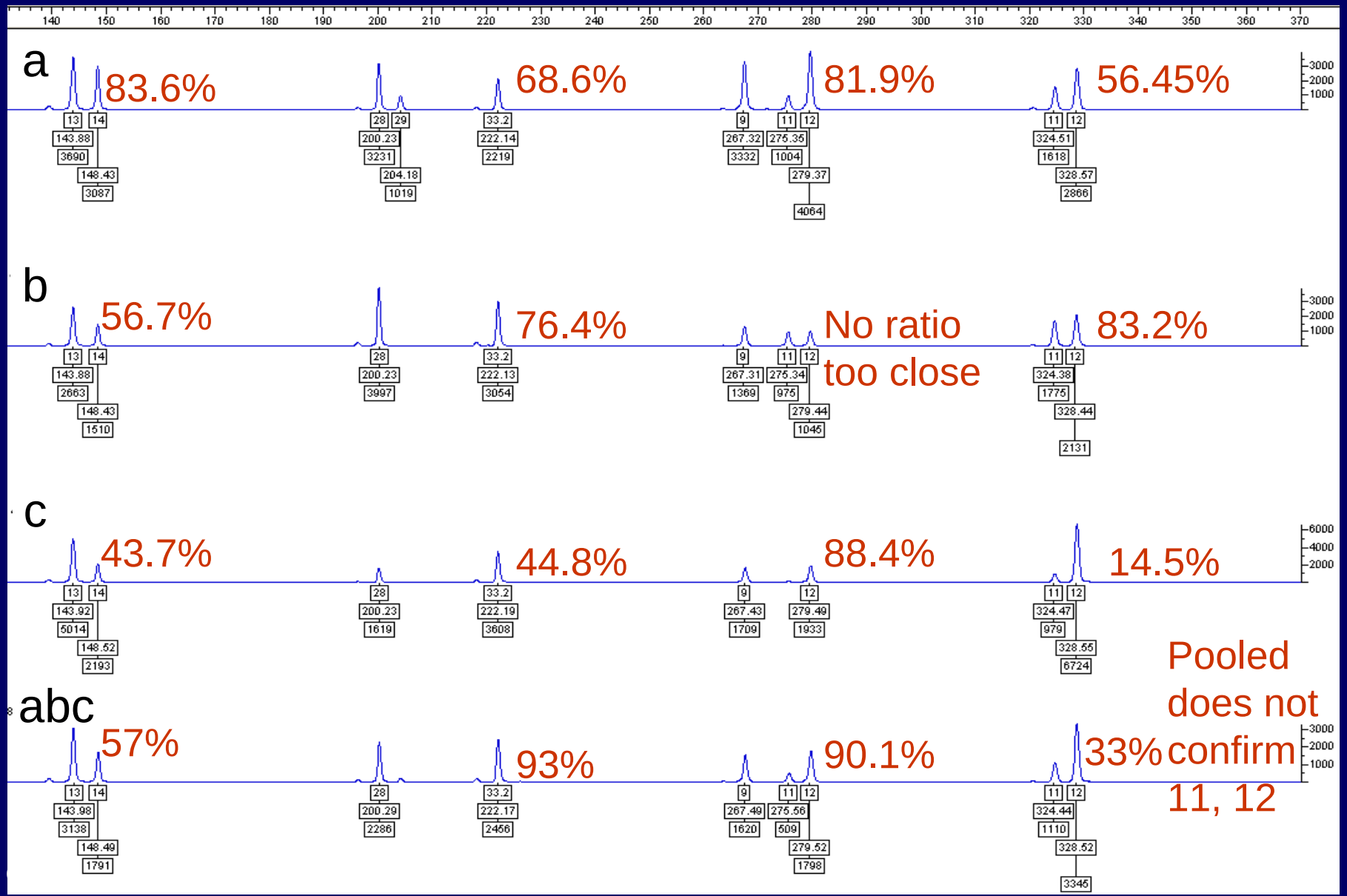
Assignment of Major Component

- Allele must appear in all three amplifications.
- Allele must be clearly the major component in two of three amplifications.

Peak Ratio	Allele Assigned
>0.5	Heterozygote
0.3-0.5	Z: another allele may be present
< 0.3	Homozygote

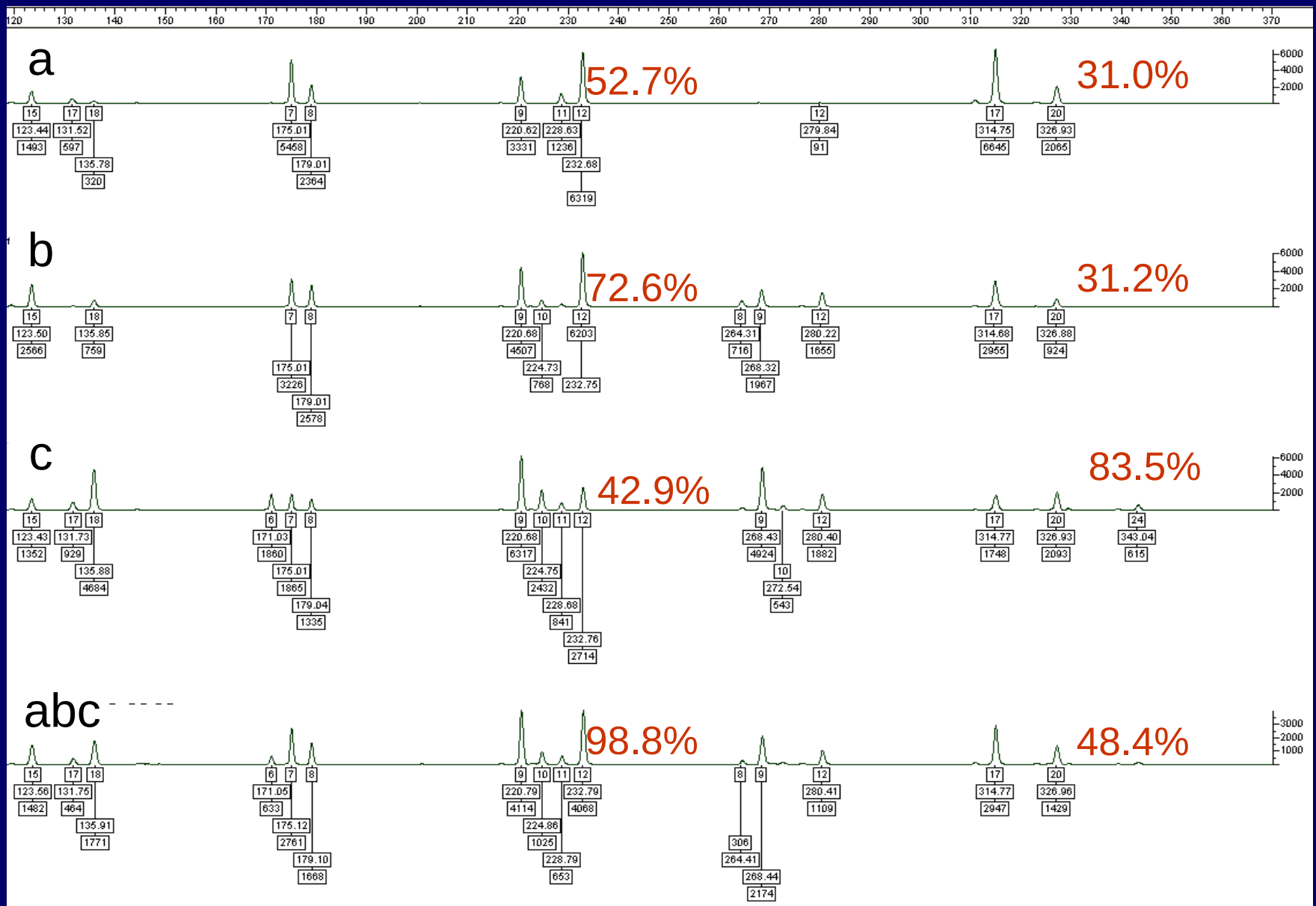
Assigned	13, 14	29, 33.2	9, 12	12, Z
Known	13, 14	29, 33.2	9, 12	12, 12

50 pg



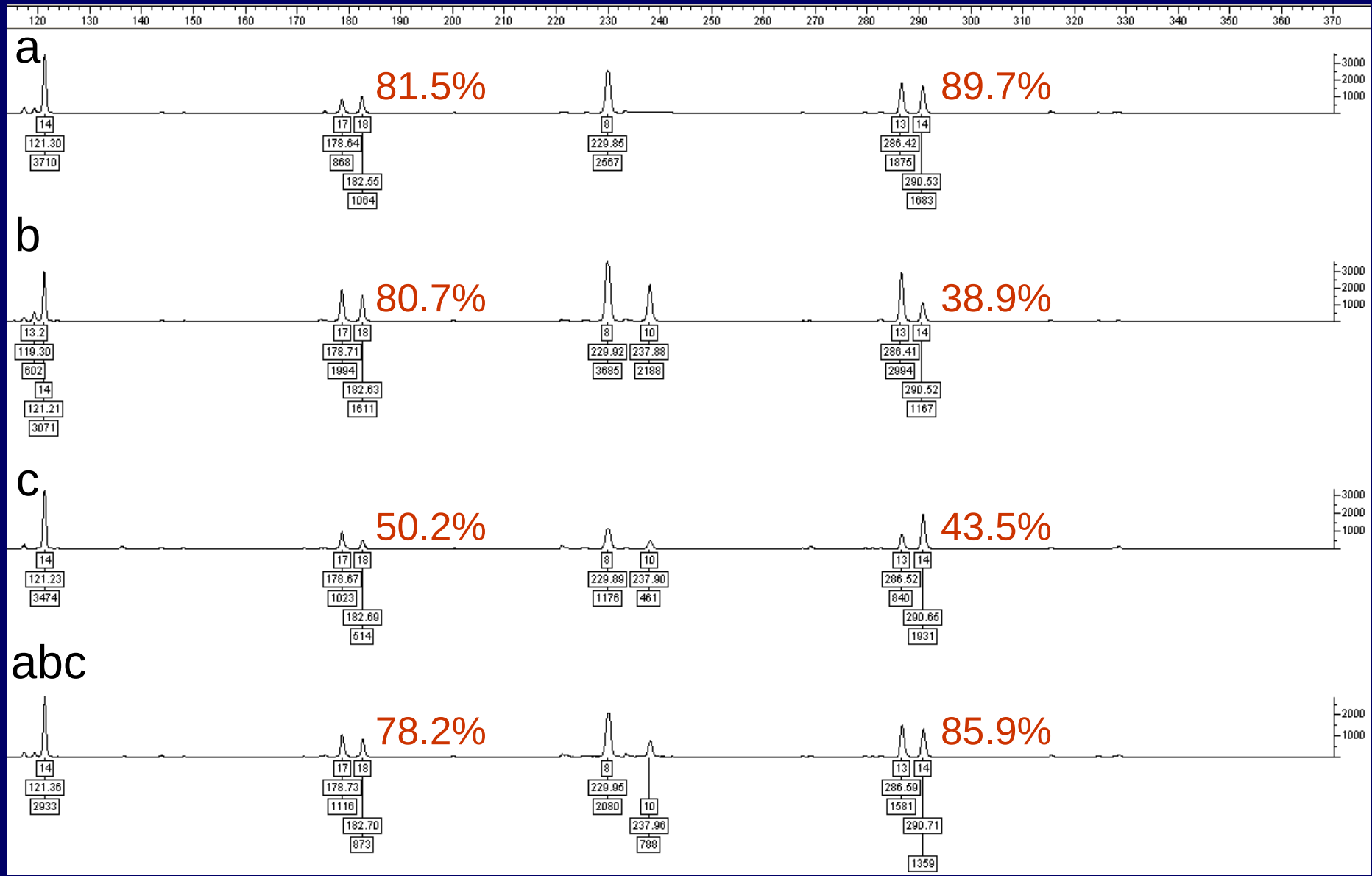
Assigned	15, Z	7, Z	9, 12	12, Z	17, Z
Known	15, 15	7, 8	9, 12	9, 12	17, 20

50 pg



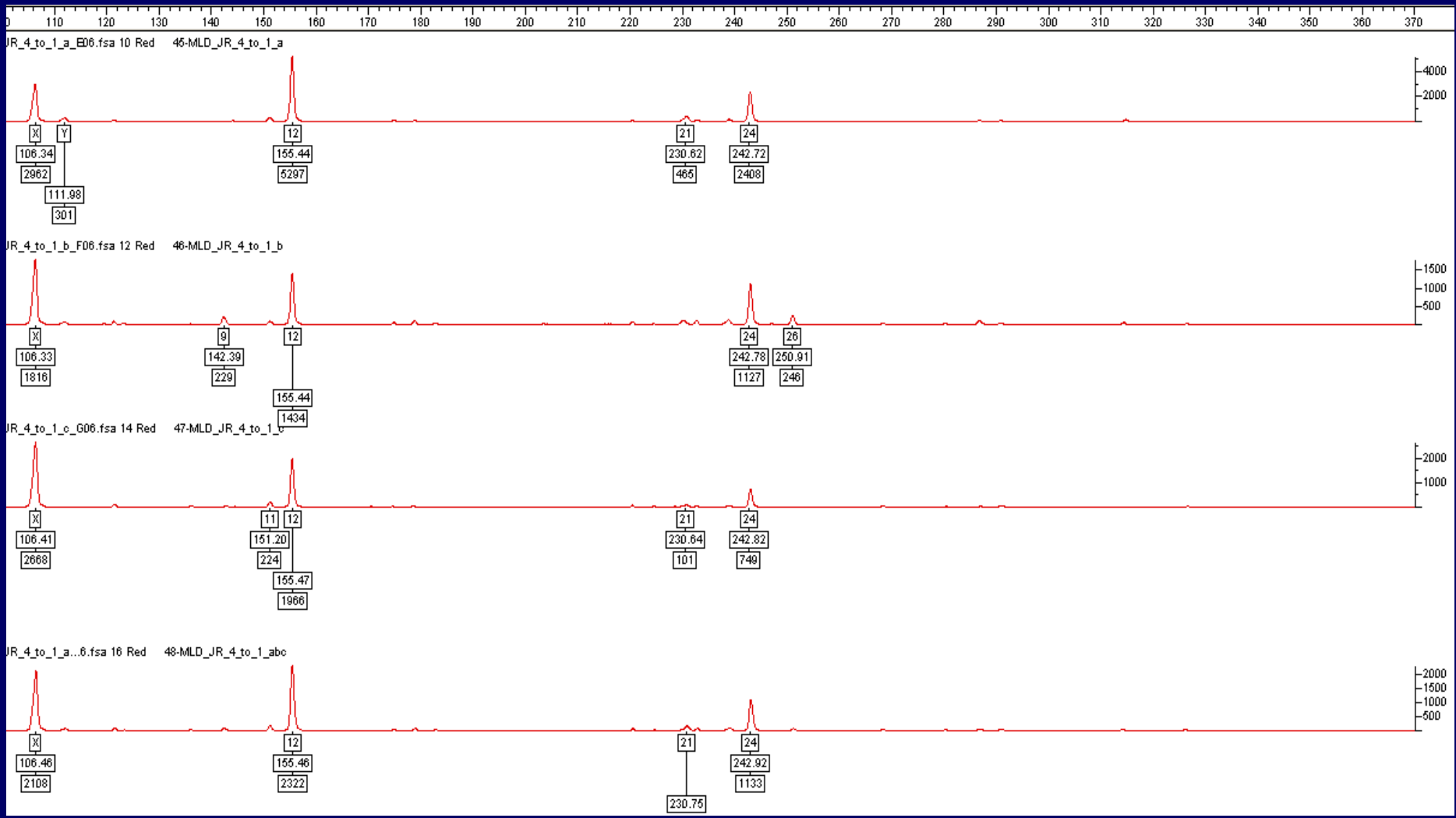
Assigned	14, 14	17, 18	8, Z	13, Z
Known	14, 14	17, 18	8, 8	13,14

50 pg



Assigned	X, X	12, 12	24, Z
Known	X, X	12, 12	21, 24

50 pg



Advantage of Replicate Amplifications for Samples Approaching 100 pg of DNA.

- If a sample is a mixture, although it has 75 to 100 pg of DNA, the major component actually contains less DNA, and therefore would benefit from replicate amplifications.

- Many touched objects are low level mixtures with mixtures only apparent at small loci.

A 100 pg Sample may have LT-DNA Components if a Mixture

Triplicates: More Alleles Assigned for Small Amounts of DNA (single source samples)

	Duplicates					
AMP	7, 8	7, 8	7 , 8	7	7	8
AMP	7, 8	7	8	8		
Consensus	7, 8	7, Z	8, Z	INC	INC	INC

	Triplicates					
AMP	7, 8	7, 8	7, 8	7	7	7
AMP	7, 8	7, 8	7	8	8	8
AMP	7, 8	7	8	7	8	
Consensus	7, 8	7, 8	7, 8	7, Z	8, Z	INC

Testing is Reliable

- We based our protocols on the methodology already implemented in Great Britain.
- Since our instruments and testing kits differed, we performed additional work in our laboratory and adjusted the protocols in order to ensure reliable results.

Reliability of Results

- When our data does not meet the requirements for alleles to be assigned, the sample or a locus is deemed inconclusive.
- When alleles are determined according to our interpretation protocols, we are 100% confident of the results as demonstrated in our testing of known samples through the validation.

LT-DNA Results are Reliable when…

- A laboratory bases their protocols on previously established and validated procedures and

- demonstrates through their own validation procedures that their methods, which might include any adjustments, are robust and reproducible.


THE CITY OF NEW YORK
OFFICE OF CHIEF MEDICAL EXAMINER