Getting to “DNA-free”: The Quest for Forensic-Grade Certification




Promega Corporation

November 14, 2013

Phantom of Heilbronn

GERMANY

Contaminated Cotton Swabs Send Police on Search for Phantom Killer

DNA traces of an unknown woman have been found at crime scenes spanning 16 years. Police admitted on Thursday that they were chasing a phantom killer when swab sticks used for testing were found to be contaminated.

Phantom's DNA surfaced in a high profile Heilbronn murder case

One of Germany's most wanted criminals may not even exist. Investigators who found DNA traces of a mystery woman suspected of committing at least three murders and numerous break-ins over the past 16 years, admitted on Thursday, March 26, they might have been chasing a phantom.

The first DNA trace from the female suspect turned up at the scene of a murder in May 1993. Later her DNA fingerprint matched the 2001 killing of a 61-year-old man and the cold-

http://dw.de/p/HKMq
26.03.2009

Potential Sources of Human DNA Contamination

Crime scene

- Environmental events
- Law enforcement personnel
- Sample collection devices

Crime lab

- Lab staff
- Other samples in lab
- Lab consumables

Poll Question 1

ENFSI, SWGDAM, & BSAG Recommendations

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Letter to the Editor

Manufacturer contamination of disposable plastic-ware and other reagents—An agreed position statement by ENFSI, SWGDAM and BSAG

We propose that a new product grade be introduced for forensic applications that should include:

ENFSI, SWGDAM, & BSAG Recommendations

Follow Good Manufacturing Processes	• Minimize interaction of staff with products • When contact is necessary, ensure products are protected
Perform Post-production Treatment	• Ethylene oxide gas treatment • UV cross-linking
Perform Continual QC Checks	• Use a sensitive method of detection • Test an adequate number of samples
Maintain Elimination Database	• Check in the event of suspected contamination

Certification – What Does it Mean?

Test methods

- … using a sensitive STR profiling assay
- … using a sensitive real-time PCR assay
- … analyzed on a 2% agarose gel stained with EtBr

Pass criteria

- Test sensitivity: ≤ 32 pg
- 2 pg of genomic DNA, equivalent to less than one human cell
- Limit of quantitation: ≤ 0.2 pg/µl

UK Guidelines (June 2012)

PAS 377:2012

Specification for consumables used in the collection, preservation and processing of material for forensic analysis

Requirements for product, manufacturing and forensic kit assembly

A consumable used in DNA casework sample analysis shall have no detectable human DNA under the following conditions by either using:

a) enhanced PCR and analysis conditions, such as increased cycle number and/or increased capillary injection [7] for the STR profiling kit used

For each batch of consumables, samples shall be tested from which no individual sample shall have either more than 1 allelic peak of greater than 50 relative fluorescent units (rfu) or the threshold value for calling a heterozygote allele peak by the analytical method used as reproduced by replicate analysis.

International Effort to Standardize

ISO TC PC 272/SC N

Date: 2013-06-3

ISO/CD 18385

Minimizing the risk of human DNA contamination in products used to collect and analyze biological material for forensic purposes.

The objective of this Standard is to provide requirements for the production of products used in forensic analysis in order to minimize contamination with human DNA during the production process.

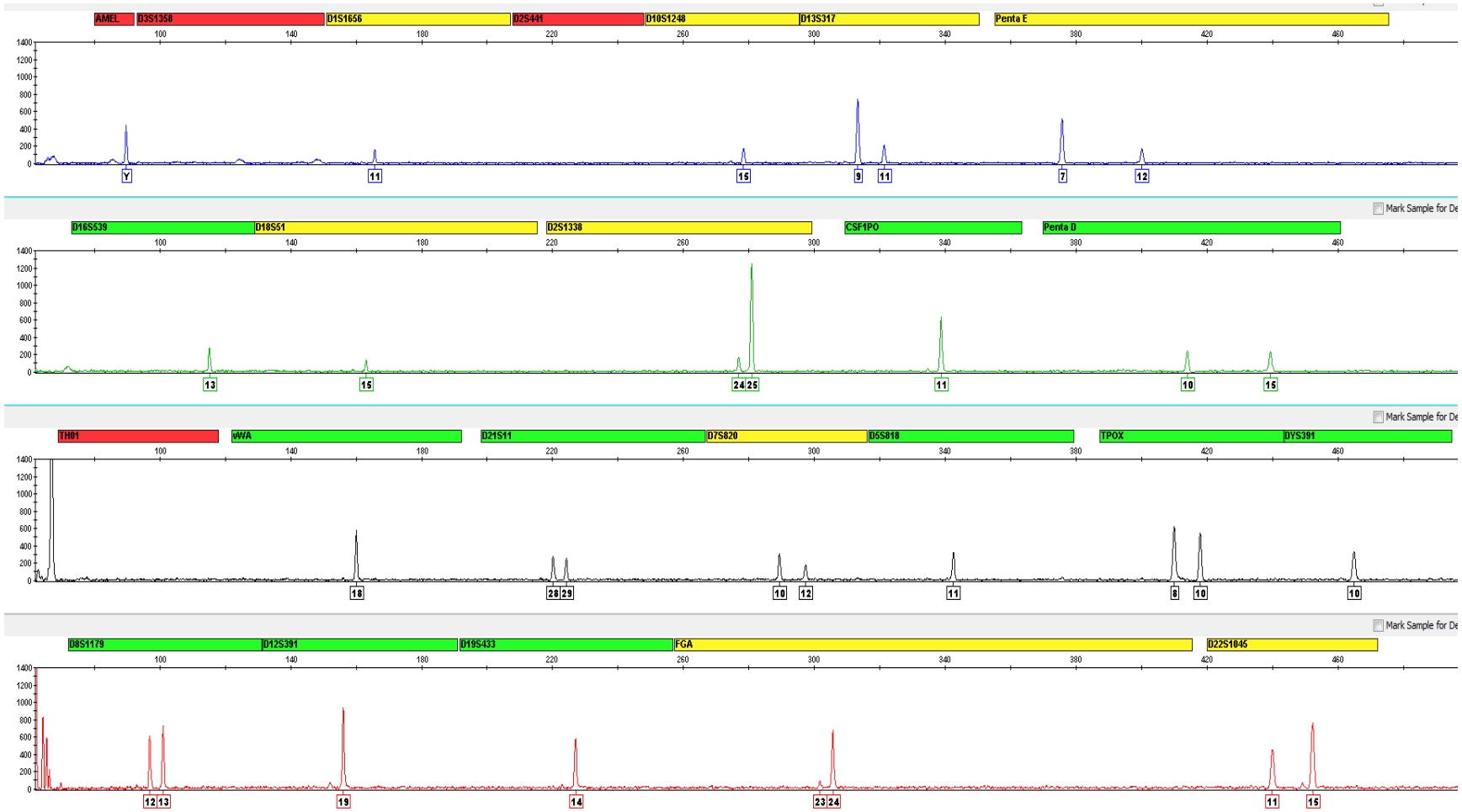
ISO 18385 Draft: Some Definition

3.1.3
Contamination
The introduction of detectable DNA during the manufacturing or assembly processes that would compromise the forensic DNA analysis.

3.1.4
Contamination detection limit
Value at or above which human DNA is deemed to be 'detected' and below which the compound is deemed to be 'not detected'. The value should be set so that human DNA fragments are of a sufficient size and/or quantity that they do not interfere with current forensic DNA analysis methods.

3.1.7
Forensic DNA Grade
Products that have been produced in accordance with this Standard and from which human DNA is minimized and present at a level or concentration lower than the limit of detection using current methods in forensic laboratories.

Partial Profile from ~ 6.7pg DNA (24sec, 90RFU)



Y-axis: 1,400RFU

Poll Question 2

What is Forensic Grade?

- What limit of detection is acceptable to crime labs?
- What detection method is acceptable to crime labs?
- What level of “undetectable” is achievable by manufacturers?
- What level of “undetectable” is practical?

Factors Influencing STR Analysis Sensitivity

- STR Kit
- PCR Cycle Number
- CE Instrument
- CE Run Parameters
- Data Analysis

Sensitivity: CE Instrument

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Instrument Sensitivity

Without stochastic influence of amplification

500pg DNA amplified with PowerPlex® Fusion System

→

Serially dilute amplification product

↓

Condition	3500 CE
Default	15sec, 1.2kV, 175RFU
Enhanced	24sec, 1.2kV, 90RFU

↓

- Total the number of alleles called in all 3 replicates
- Divide by 135 (total possible alleles)

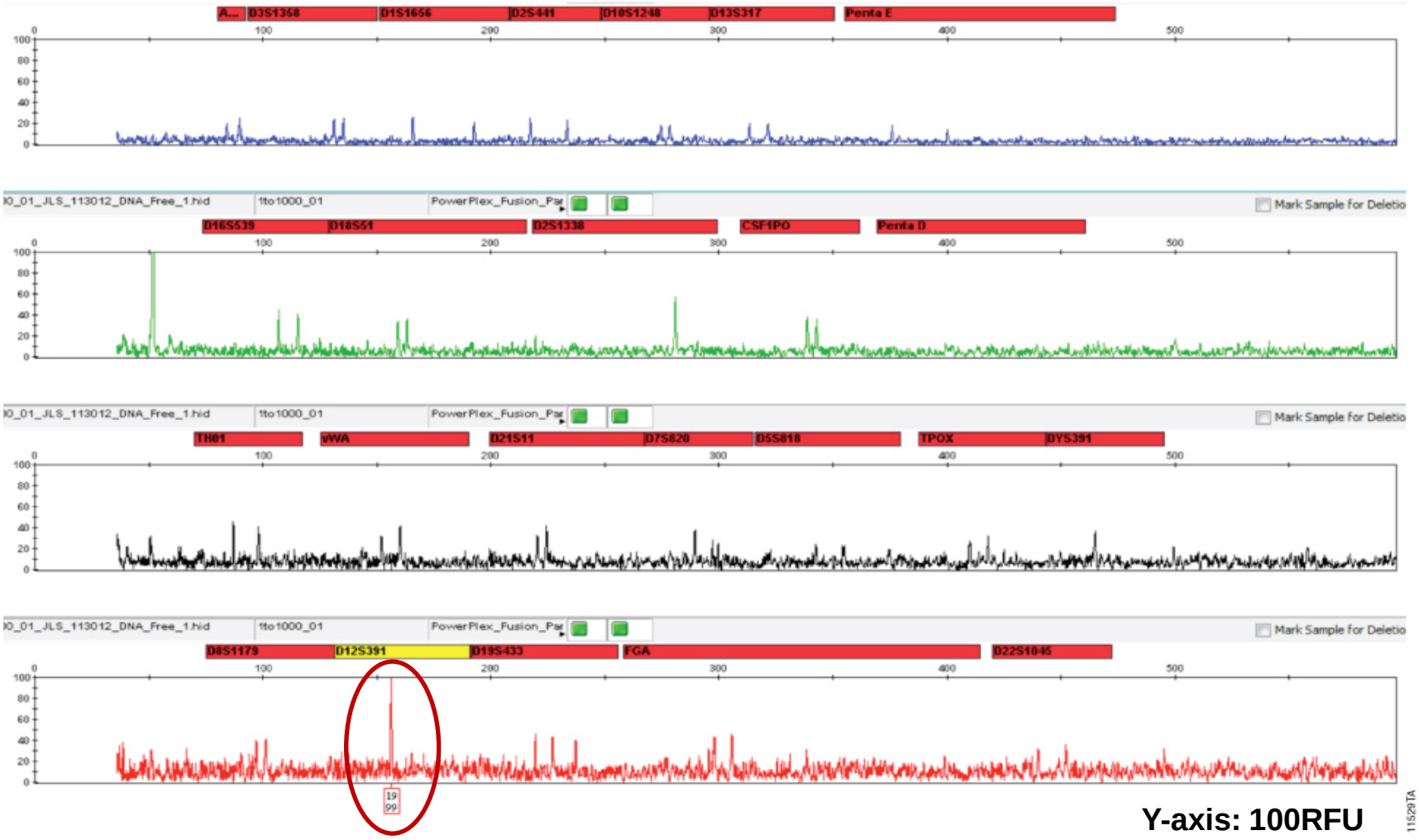
Instrument Sensitivity

Without stochastic influence of amplification

Dilution	Calculated DNA Amount (pg)	% Alleles Called on 3500 CE			
		15sec 175RFU	15sec 90RFU	24sec 175RFU	24sec 90RFU
1:10	50	100	100	100	100
1:50	10	73	97	95	100
1:100	5	11	83	61	96
1:200	2.5	2.9	20	5.1	67
1:300	1.67	0	2.9	2.2	18
1:400	1.25	0	2.2	0.7	8.9
1:500	1.0	0	0.7	0	2.2
1:1000	0.5	0	0	0	2.2
1:5000	0.1	0	0	0	0

Default condition: no alleles called at/below ~1.67pg
Enhanced condition: no alleles called at/below ~0.1pg

One Allele Called with 0.5pg (24sec, 90RFU)



Poll Question 3

No CE Instrument Baseline Noise

No amplification mix:
1X PowerPlex® Fusion Primer Pair Mix
1X PowerPlex® Fusion Master Mix

1µL no amp mix
10µL Hi-Di™ formamide
1µL ILS 500

Condition	3500 CE
Default	15sec, 1.2kV, 175RFU
Enhanced	24sec, 1.2kV, 90RFU

No allelic peaks observed in 94 no-amplification injections

Sensitivity:
Low Template Condition

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Sensitivity with Consensus Allele Calling

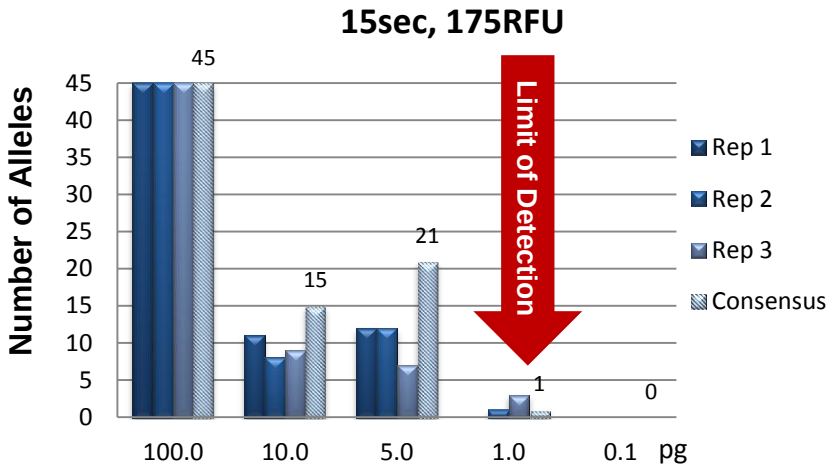
Serially dilute DNA

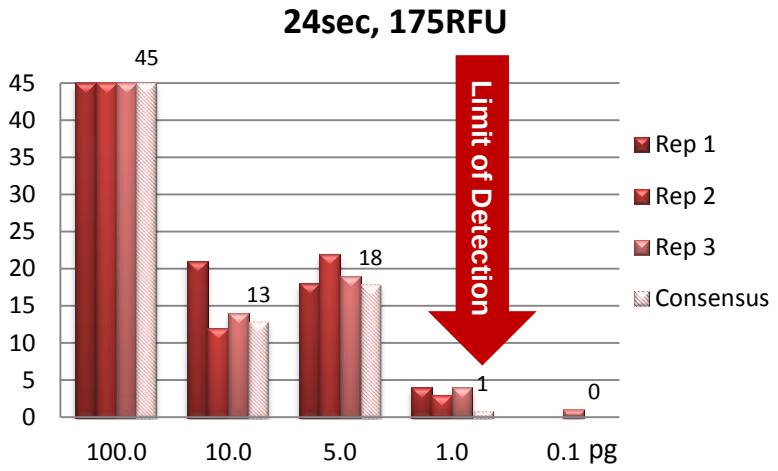
Amplify with PowerPlex® Fusion System; 3 replicates

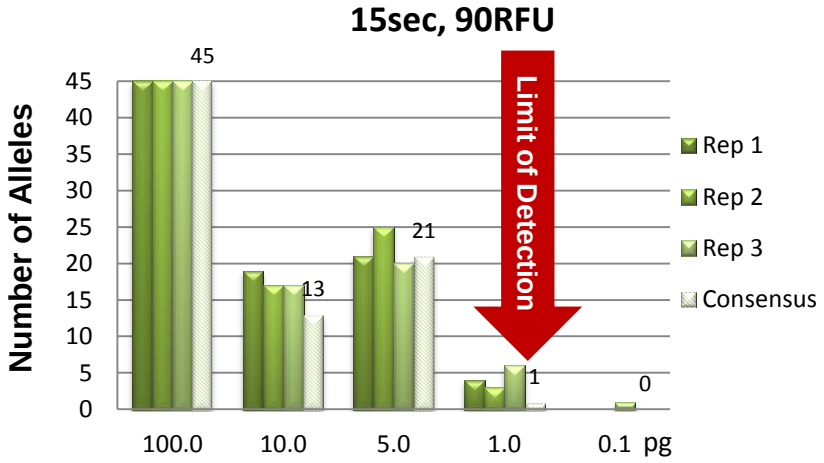
Condition	3500 CE
Default	15sec, 1.2kV, 175RFU
Enhanced	24sec, 1.2kV, 90RFU

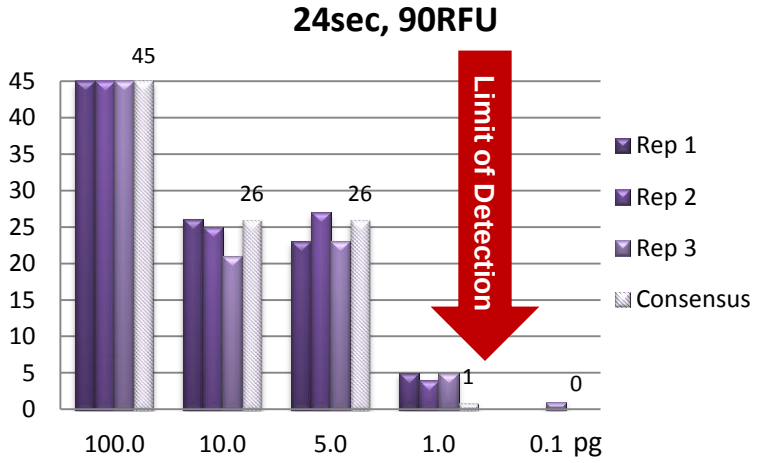
Count number of alleles called in at least 2 of 3 replicates

STR Analysis is Sensitive Down to 1 pg









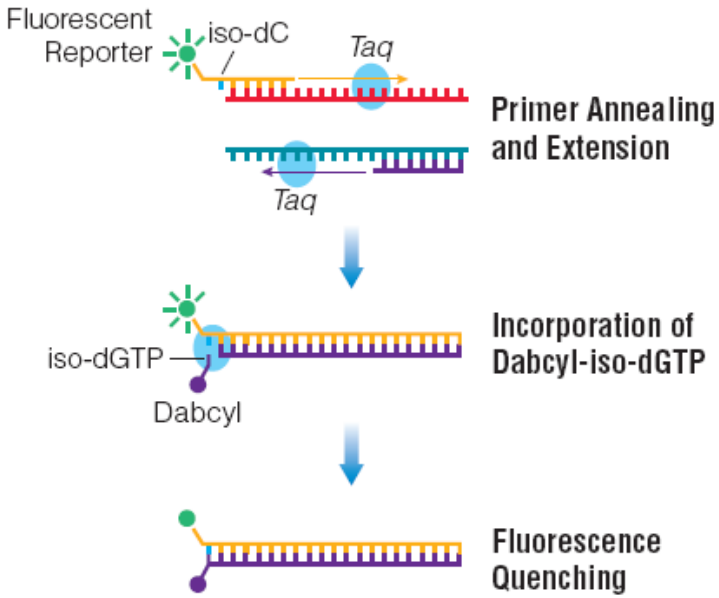
qPCR: Suitable Option for Human DNA Detection?

Practical advantages for non-STR kits:

- Straightforward data interpretation
- Can test more samples
  - Faster
  - More economical
- More sensitive with mtDNA primers
  - mtDNA present at higher copies (~ 500 copies per cell)

qPCR Analysis

Serially dilute DNA → Plexor® qPCR System; 45 cycles; 3 replicates → Determine Cq value



qPCR is Sensitive Down to 0.25pg

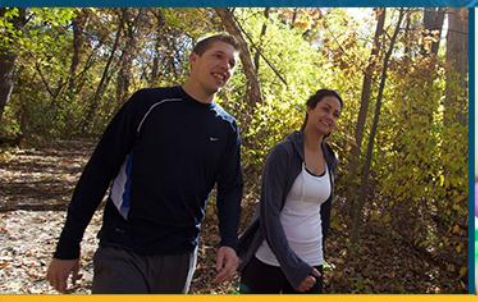
C_q Values Using 45 Cycles.			
	C_q Value		
DNA Amount	**Replicate 1**	**Replicate 2**	**Replicate 3**
250pg	25.4	25.3	25.5
25pg	29.0	28.9	28.6
2.5pg	33.7	33.5	33.2
1.25pg	33.7	33.5	34.2
0.25pg	37.1	37.4	37.1
0.025pg	39.1	ND	ND
0.0025pg	ND	ND	ND
0.00025pg	ND	ND	ND
ND = Not detected.			

Limit of Detection

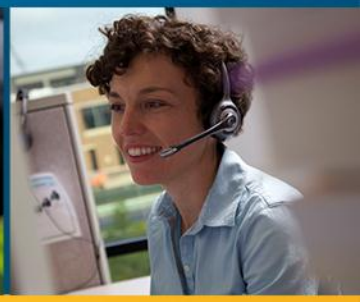
11527LA

Poll Question 4

Towards ISO 18385







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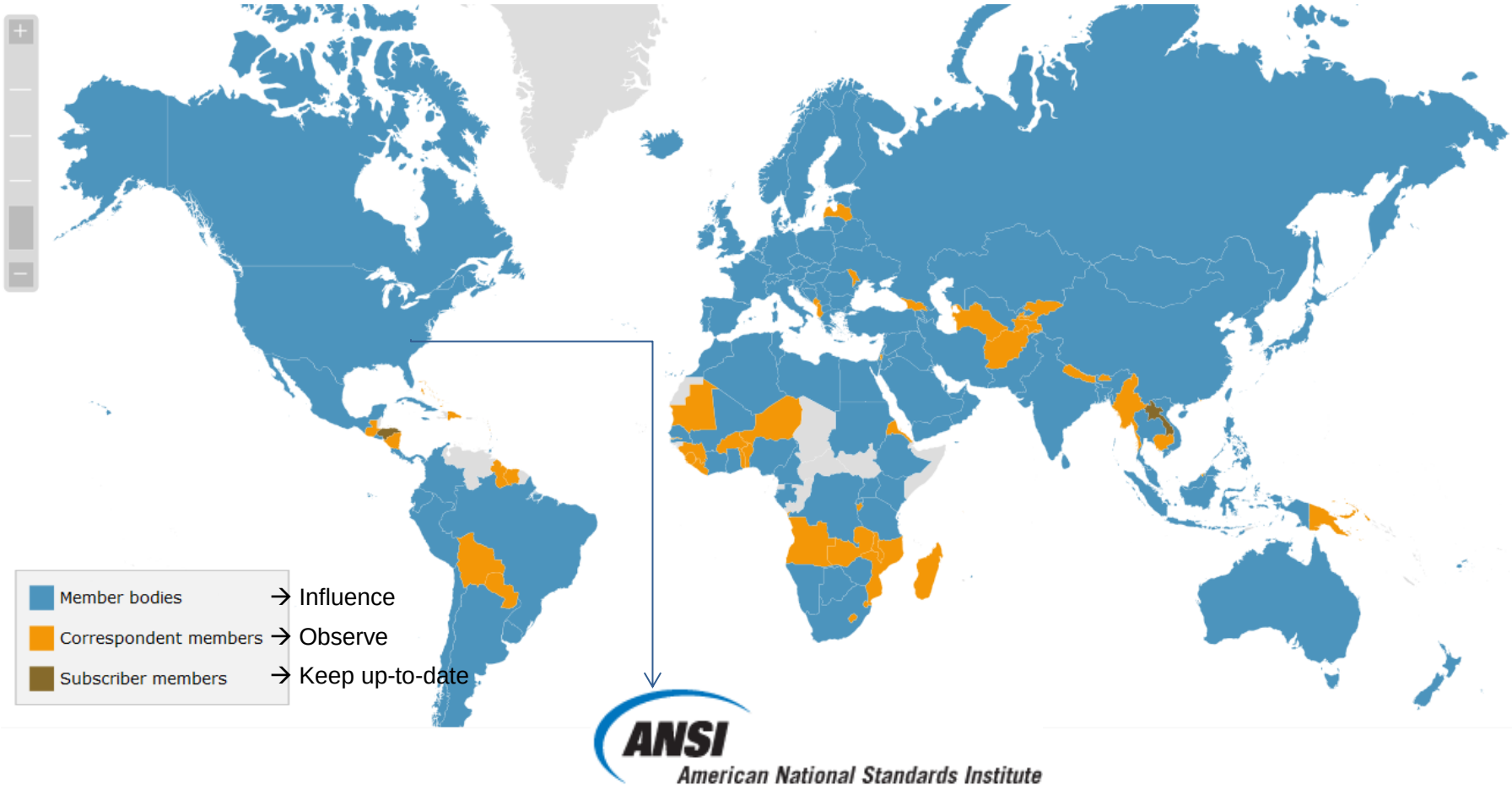
ISO Develops International Standards



Relevant ISO Standards

Forensic Labs:

ISO/IEC 17025:2005 specifies the general requirements for the competence to carry out tests and/or calibrations, including sampling. It covers testing and calibration performed using standard methods, non-standard methods, and laboratory-developed methods.

Forensic Manufacturers:

ISO 9001:2008 specifies requirements for a quality management system where an organization needs to demonstrate its ability to consistently provide product that meets customer and applicable statutory and regulatory requirements, and aims to enhance customer satisfaction through the effective application of the system, including processes for continual improvement of the system and the assurance of conformity to customer and applicable statutory and regulatory requirements.

Developing a New ISO Standard

ISO/PC 272: Forensic Sciences

ISO 18385

12 Participating Countries	13 Observing Countries
• Australia	• Argentina
• Belgium	• Austria
• France	• Bulgaria
• Germany	• Denmark
• Japan	• Ecuador
• Korea	• Finland
• Netherlands	• Iran
• Singapore	• New Zealand
• Sweden	• Poland
• Switzerland	• Romania
• Thailand	• Slovakia
• United Kingdom	• Spain
	• United States

US Planning to Become a Participating Country

To provide input on achievable standard

Requirements for Switching

- ANSI (American National Standards Institute) needs to create TAG (Technical Advisory Group)
- TAG requires:
  - Administration by non-biased entity – ASCLD
  - Monetary support of administrative cost

Summary

- CE instruments are very sensitive
  - No alleles called from ~0.67 pg under default conditions
  - No alleles called from ~0.1 pg under enhanced conditions
- STR kits are very sensitive
  - Full profile from 50 pg DNA
  - Partial profile from one cell
  - One allele called from 1pg under low template conditions
- qPCR analyses can detect 0.25pg DNA

Thank You!

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