Current Practices and Developing Methods in Forensic Serology

1

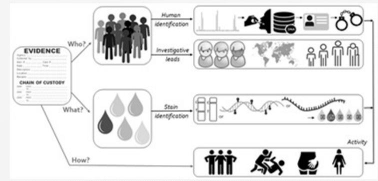
Green Mountain DNA Conference
July 2024

Sarah Williams
Virginia Commonwealth University

Body Fluid Detection and Identification

2

- First step in the forensic biology workflow
  - Screening tool for presence of biological fluids
    - Locate best stain area to swab/cut for DNA analysis
  - Investigative leads
  - Story corroborations

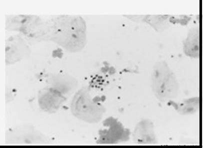


Sijen, Titia, and SallyAnn Harbison. 2021. "On the Identification of Body Fluids and Tissues: A Crucial Link in the Investigation and Solution of Crime" *Genes* 12, no. 11: 1728. https://doi.org/10.3390/genes12111728

Where does BFID stand in the US today?

3

- Full Serology services
- Efficient approaches:
- No Serology
  - DNA based quants



Where does BFID stand in the US today?

4

	Blood		Semen			Saliva		Urine	Feces
	Presumptive	Immuno assay	Presumptive	Microscopic ID	P30	Presumptive	Immuno assay	Pres.	Pres.
VA DFS	X		X	X	X*				
NY OCME	X				X*				
TX DPS	X*		X*	X*	X*				
AK DPS	X	X*	X		X				
Houston FSC	X	X*	X	X*	X*				
MI SP	X	X*	X	X	X*		X		
ID SP	X	X*	X	X	X*	X		X	X
Suffolk Co	X	X*	X	X	X*	X		X	X

Where does BFID stand in the US today?

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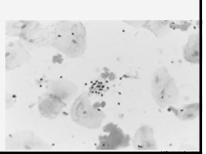
- Full Serology services
- Efficient approaches:
  - VA DFS
    - Blood: Luminol screening, PTMB presumptive test
    - Semen: ALS, AP presumptive test, microscopic ID, p30 under specific circumstances
      - Abbreviated screening possible
    - Vag. Secretions, Saliva, Perspiration/Touch, Urine, Feces: no testing
  - NY OCME
    - ALS + Phenolphthalein
    - All sexual assault samples: Zygem Y-screening prior to traditional extraction
      - Sperm ID on F2 only under special circumstances
    - P30 and a-amylase LF tests for certain circumstances
- No Serology
  - DNA based quants

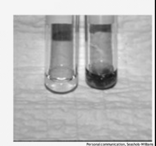


Where does BFID stand in the US today?

6

Hybrid Approaches for Semen testing:

- AP Presumptive tests with no microscopy
  - Direct to DNA
- AP Presumptive tests with P30
  - Reduce hours spent screening slides



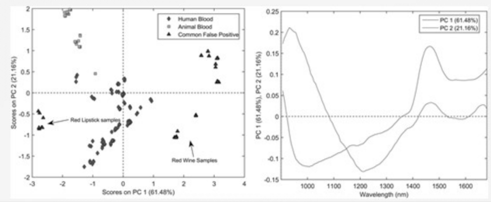
Where does BFID stand in the US today?
7

Sperm and Nonsperm fraction quantification data used to infer presence of sperm

- NOT male and female fractions
- Sperm and Nonsperm fractions?
- Better called Fractions 1 and 2?

Where does BFID stand in the US today? Rapid on-site approaches: Spectroscopy
8

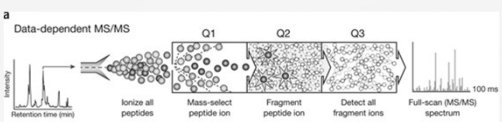
Near Infrared and hyperspectral detection of latent blood using handheld device



José et al, *Microchemical Journal*, 133 (2017) 561-566 https://doi.org/10.1016/j.microc.2017.04.038.

Where does BFID stand in the US today? Proteomics
9

- MS for Proteomics: Produces spectra of the masses of the molecules comprising sample
  - By ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios



Nature Methods 10, 28–34 (2013)

Where does BFID stand in the US today? Proteomics
10

838 K. M. Legg et al. *Electrophoresis* 2017, *38*, 833–845

Table 2. Results of targeted ion Q-TOF multiplex assays of 50 samples each of saliva, seminal fluid, vaginal/menstrual fluid, and peripheral blood and 25 samples each of female urine and male urine

	Semen Biomarkers						Urine Biomarkers		Saliva Biomarkers						Vaginal and Menstrual Fluid Biomarkers						Peripheral Blood Biomarkers		
	Semenogelin-1	Semenogelin-2	Epididymal protein E1	PSA	PAP	Glycodelin	Uromodulin	Osteopontin	Submaxillary gland	Cystatin_D	Cystatin_SA	Statherin	Histatin 1	mucin 5b	Cornulin	Neutrophil gelatinase-associated lipocalin	Ly6/PLAUR domain-containing protein 3	IgGFc-binding protein	Matrix mmp-9	Suprabasin	Hemoglobin subunit beta	Complement C3	Hemopexin
Semen	100%	100%	100%	100%	100%	82%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saliva	-	-	-	-	-	-	-	-	100%	76%	94%	90%	30%	96%	-	-	-	-	-	-	4%	-	-
Urine (male)	24%	20%	40%	60%	80%	-	100%	100%	-	-	-	-	-	4%	-	-	-	-	-	-	-	-	-
Urine (female)	-	-	30%	-	-	-	100%	100%	-	-	-	-	-	4%	4%	-	4%	-	-	-	12%	-	6%
Vaginal Fluid	-	-	2%	-	-	-	-	-	-	-	-	-	-	38%	100%	100%	100%	68%	20%	22%	6%	-	4%
Menstrual Fluid	-	-	-	-	-	16%	-	-	-	-	-	-	-	20%	20%	6%	4%	-	-	-	100%	54%	76%
Peripheral Blood	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%	100%	96%

Targeted biomarkers are listed across the top of the table and the body fluids tested are listed along the left side of the table. Values indicate percent of samples where target protein marker was confidently identified based on the detection of at least one target peptide using a validated database search.

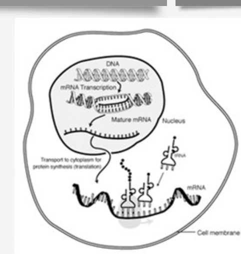
What is the rest of the world doing?
11

- Traditional Serological methods
- Validated molecular approaches

What is the rest of the world doing?
12

mRNA Analysis:

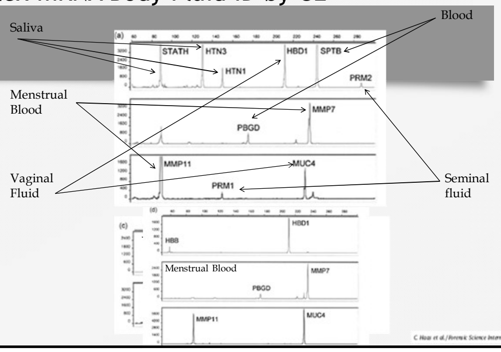
- The intermediate between DNA and protein
- Exists as a "usable mobile copy" of DNA to make proteins
- DNA transcribed only for proteins made in/used by the cell and/or tissue
  - Spatial & temporal differences derive from combinatorial interactions b/t transcription factors, chromatin state, and enhancer/silencer regions in DNA



http://www.daviddarling.info/encyclopedia/M/mRNA.html

Multiplex mRNA Body Fluid ID by CE

13



C Haas et al. / Forensic Science International: Genetics 3 (2009) 80–88

What is th… Consideri…

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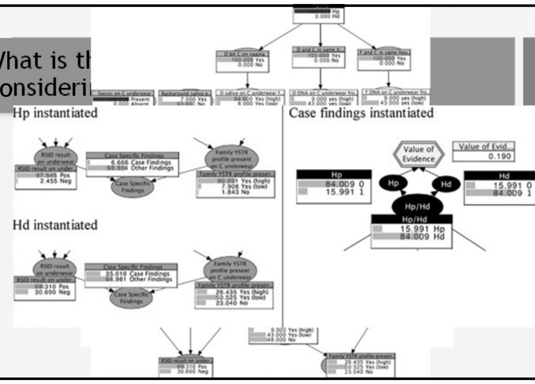


Duncan Taylor, Alex Biedermann, Tacha Hicks, Christophe Champod. A template for constructing Bayesian networks in forensic biology cases when considering activity level propositions. Forensic Science International: Genetics, 33, 2018,136-146. https://doi.org/10.1016/j.fsigen.2017.12.006.

Where is the research going?
mRNA analysis via high-throughput sequencing

15

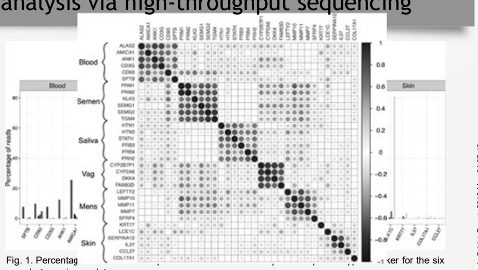


Fig. 1. Percentag… …ker for the six sample types in our data.

Fig. 2. Correlation plot for the 33 markers based on CLR transformed read counts from the 183 samples. The darker and bigger the circles, the more correlated the markers are. Blue denotes a positive correlation, red denotes a negative correlation.

Forensic Sci Int Genet. 2018 May;34:37-48.

Where is the research going?
mRNA HTS to connect tissue to donor

16

- cSNPs – coding region SNPs
- Provide connection between mRNA and DNA of donor

Mixture	Gene	cSNP	Donor 1	Obs	Donor 2
VS-BD-1	ANK1	rs504574	CG	CC	CC
Donor 1 – VS		rs7816734	GG	AG	AG
Donor 2 - BD	CD3G	rs3753059	TT	TT	TT
	SPTB	rs1741488_rs1741487	CA/TG	TG/TG	TG/TG
		rs229592	AA	AG	AG
		rs229586	CT	CC	CC
	MUC22	rs3869098_rs4248153	GG/GA	GG/GA	GG/AA
		rs1419664_rs3094672	CA/CA	CA/CA	CA/CC
		rs12110470_rs12110785	TC/TC	TC/TC	GT/GT
		rs10947121	CC	*(CC)*	TT
VS-SE-3	KLKL3	rs11573_rs1135766	TA/CG	TA/CG	TA/CG
Donor 1 – VS	PRM1	rs737008	GT	TT	TT
Donor 2 - SE	SEMG2	rs2233896	CC	CC	CC
	TGM4	rs1995640	CT	CT	CT
		rs1995641	AG	AG	AG
	MUC22	rs3869098_rs4248153	AA/GG	AA/GG	AA/AA
		rs1419664_rs3094672	TA/CA	TA/CA	CA/CA
		rs12110470_rs12110785	GT/GT	GT/GT	GT/GT
		rs10947121	TC	TC	TT
VS-SA-2	HTN3	rs1849937_rs1136515	CT/CT	CT/CT	CT/CT
Donor 1 – VS		rs75067954	CC	CC	CC
Donor 2 - SA	MUC7	rs2306948	CC	CC	CC
	PRB4	rs1052808	CG	GG	GG
	PRH2	rs10772391	CT	CT	CT
	CYP2A6	rs8192721	CT	CT	CT
	MUC22	rs3869098_rs4248153	AA/AA	AA/AA	AG/GG
		rs1419664_rs3094672	CT/TA	CT/TA	CA/TA
		rs12110470_rs12110785	GT/GT	GT/GT	TC/TT
		rs10947121	TT	TT	CT

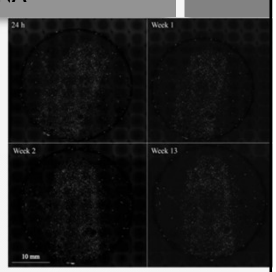
Color-shaded cells indicate a match of the observed genotype to the reference sample (red, blood (BD); yellow, semen (SE); blue, saliva (SA); green, vaginal secretions (VS)). Gray cells indicate non-concordance demonstrating the association of the body fluid with the other donor. Below thresholds are shown in italicized parentheses; markers in which no counts were observed are not shown. For BFID, see Fig. 3. Each mixture contains unique donors (no shared donors among the mixture sets)

Hanson et al. IJLM 2023

Where is the research going?
Fluorescence to locate touch DNA

17

- Diamond Nucleic Acid Dye
  - External groove binding dye
- Demonstrated on objects, fingerprint lift tape, saliva, hair, lip prints



Hughes et al. FSI 2022

Where is the research going?
Cell capture methods

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- If we can separate cells *prior* to extraction, we can minimize mixture deconvolution issues
  - Reduce analysis time
  - Reduced court challenges of mixture interpretations
- DEP Array
- Micromanipulation
- Optical Trapping
- Flow Cytometry

...ing?

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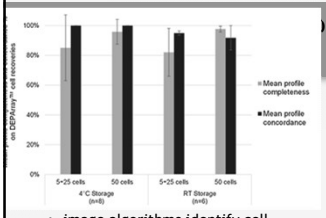

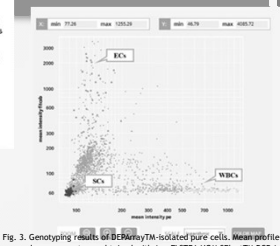

- image algorithms identify cell type
- Electric gates combine similar cells for fractionation and lysis

Fig. 3. Genotyping results of DEPArray™-isolated pure cells. Mean profile completeness and concordance percentages obtained with AmpFLSTR® NGM SElect™ PCR Amplification Kit on homogeneous cell pools recovered from simulated forensic mixtures adsorbed on swabs. RT = room temperature. Error bars show the standard deviation

https://www.fsigenetics.com/article/S1872-4973(17)30107-2/pdf

Menarini Biosystems

Where is the research going? Cell capture methods

20

- Micromanipulation
- Use of microneedles or pipets to pluck individual cells from a matrix or slide
  - With or without adhesive
  - With or without robotic arm manipulation

Typically performed using a standard stereoscope

Cell staining optional, some methods demonstrate lifting from substrates

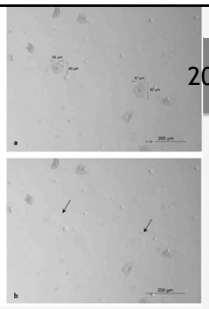

Fig. 1. Confirmation of Cell Recovery from Trypan Blue-stained buccal epithelial cells on a Gel-Film affixed microscope slide. Recovery of two cells using a tungsten needle and 3M adhesive

Huffman et al., Sci Justice 2021

Where is the research going? Cell capture methods

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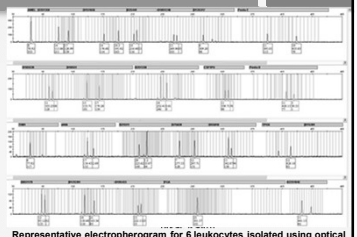
Optical Trapping

- Laser beam that uses an objective lens on an inverted microscope to create an optical trap
- Optical trap can hold a particle in its center (focus spot)
- Allows the trapped particle to be gently maneuvered
- Research in biomedical applications



Representative electropherogram for 6 leukocytes isolated using optical trapping. This data demonstrates the ability to obtain a full high quality, single-source STR profile from as few as 6 cells.
Valle et al. JFS 2023

Where is the research going? Cell capture methods

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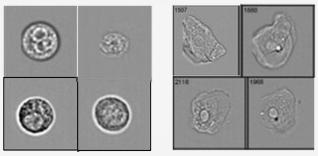
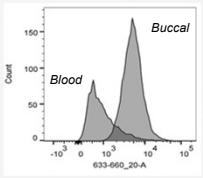
Flow Cytometry

- Can we use autofluorescence signatures to separate cell populations?



Blood Buccal

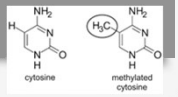


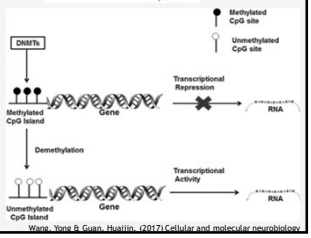
Where is the research going? Methylation signatures

23

Methylation signatures for body fluid ID

- Methylation of DNA causes suppression of transcription.
- Active genes are not methylated – tissue specificity





Wang, Yong & Guan, Huailin. (2017) Cellular and molecular neurobiology.

Where is the research going? Methylation signatures

24

Analysis Methods:

- Bisulfite Treatment: (deamination of cytosine into uracil, (read as thymine in sequencing analysis). Methylated residues are resistant to this conversion and read as cytosine. (sensitive to degraded samples)
- Enzymatic digestion: methylation dependent endonuclease digestion (followed by qPCR or sequencing) - denatured DNA will not work for this assay

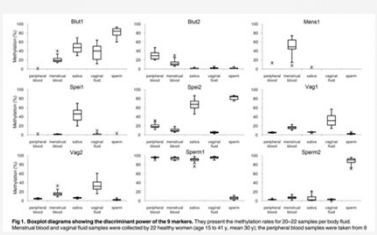


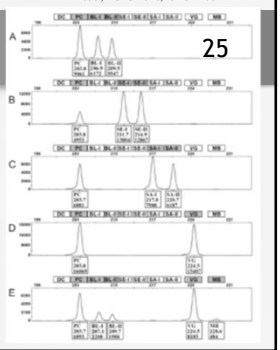
Fig 1. Boxplot diagrams showing the discriminant power of the 9 markers. They present the methylation rates for 20–22 samples per body fluid. Menstrual blood and vaginal fluid samples were collected by 20 healthy women (age 19 to 41 y, mean 30 y); the peripheral blood samples were taken from 9

PLOS ONE | DOI:10.1371/journal.pone.0143973 February 1, 2016

Where is the research going? Methylation signatures

25

Lin et al, FSI:G 2016,25:157-165



Analysis method: Methylation SNaPshot

A methylation-sensitive single nucleotide primer extension (Ms-SNuPE)

Process:

- Sodium bisulfate conversion of C to U (read as T), methylated C remains C.
- Primer adjacent to methylation site and appropriate ddNTP incorporated
- Fragment analysis on CE

Where is the research going? Methylation signatures

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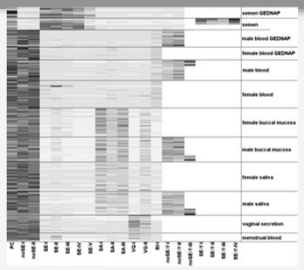

Yellow to red color scale presents high to low digestion score values. Number of samples: 10 semen, 49 buccal mucosa, 49 saliva, 46 blood, 7 menstrual blood, and 17 vaginal secretion samples from own sample collection, as well as 38 blood and 10 semen GEDNAP samples. White gaps represent no data, because no Y-chromosomal marker can be detected for female samples

Int J Legal Med. 2024; 138(2): 375–393.

Where is the research going? Microbial signatures

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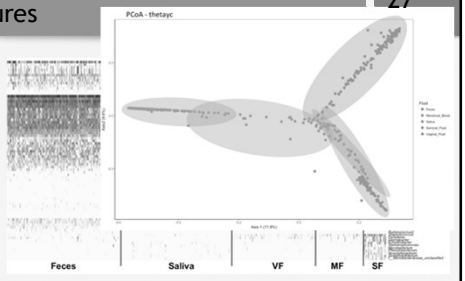
- 16S ribosomal RNA gene
- Single conserved primer locations, sequence variation between families/genus/species
- qPCR or HTS applications



FSIG 2023, Wohlfahrt et al

Where is the research going? Microbial signatures

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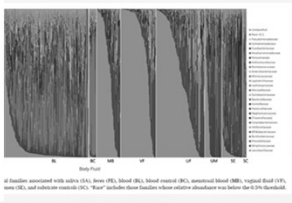
Table 4
Confusion matrix based on the XG Boost model showing the actual and predicted body fluid in blind validation samples (%) at a 6000 read cutoff for blood (BL), feces (FE), female intimate (FI), saliva (SA), semen (SE), and male urine (UM). Female intimate samples include menstrual blood, vaginal fluid, and female urine.

Actual		BL	FE	FI	SA	SE	UM
Predicted	BL	100	0	0	0	8	0
XG Boost	FE	0	95	0	0	0	0
	FI	0	5	100	0	42	63
	SA	0	0	0	100	0	0
	SE	0	0	0	0	17	0
	UM	0	0	0	0	33	38



Wohlfahrt et al FSIG 2023

Where is the research going? miRNA panels

29

- Small, non-coding RNAs (18-25 nucleotides) that suppress protein expression by binding to messenger RNA in the cytosol
- Assist in regulatory processes and therefore can be:
  - Consistently expressed in all tissues
  - Tissue-specific
- Encapsulation in proteins and lipid vesicles provide protection and stability
- Detectable in DNA extracts

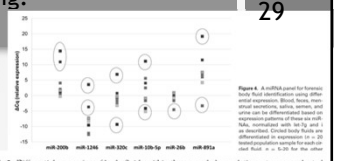


Table 2. Differential expression of body fluids within the expanded population set were evaluated with the trained QDA model. ΔCq values from the samples were imported into the model. Overall classification percentages are displayed above. (Mens. = menstrual secretions, Vag. = vaginal secretions).

Body Fluid	n	Correct Body Fluid Classification	Classification as Another Body Fluid	Classification as Other
Blood	49	97.96%	2.04%	0.00%
Mens.	50	72.00%	28.00%	0.00%
Feces	50	98.00%	2.00%	0.00%
Urine	46	84.80%	15.20%	0.00%
Saliva	50	84.00%	16.00%	0.00%
Semen	50	90.00%	10.00%	0.00%
Vag.	50	72.00%	28.00%	0.00%

Seashols-Williams et al. Electrophoresis 2016
Rhoden and Lewis et al. Genes 2023

Where is the research going? Combinatorial approaches

30

Build on the strengths of each biomarker – none are perfect!

- mRNA panels + lactobacillus primer sets for increased confidence in vaginal secretions (in practice)
- Combined microbial and miRNA panel (qPCR-based)
- Combined methylation and microbial DNA panel (qPCR-based)

Conclusions

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BFID continues to be an important part of the analysis workflow for many forensic cases

New Markers/Systems should be:

- Informative with confidence intervals
- Robust
- Sensitive
- Able to discriminate mixtures
- Streamlined implementation –OR- NO extra effort – put the markers into a larger HTS panel