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When and "Y" to Screen with QIAGEN Investigator Casework GO!

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Disclaimer

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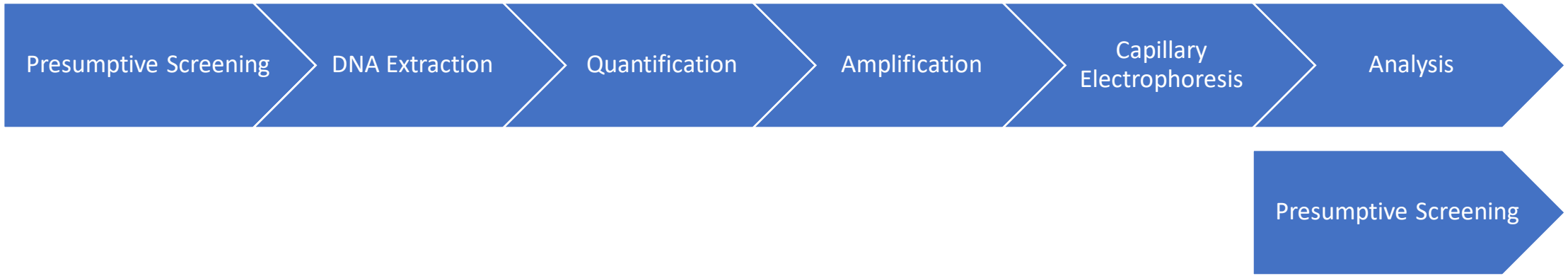
Standard Sexual Assault Evidence Processing

Y-Screening


Presumptive Screening
DNA Extraction
Quantification
Amplification
Capillary Electrophoresis
Analysis
Presumptive Screening

QIAGEN Investigator Casework GO!

- **<u>Lysis</u>** kit designed for direct amplification workflow along with Investigator® 24plex GO! kit
- Compatible with quantification or amplification for STR analysis

Direct PCR

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/fsigss

How the Investigator Casework GO! Kit provides sensitive, fast and robust direct amplification of low copy number samples

Kathrin Wolf, Sarah Pakulla-Dickel, Antonio Girela del Río, Anke Prochnow*, Keith Elliott, Mario Scherer

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

(2019):

- Blood and semen dilutions
- Direct PCR (Casework GO!) comparable to traditional method (EZ1) for blood dilutions
- 90-100% alleles recovered in semen dilutions up to 1:1000

May 2022

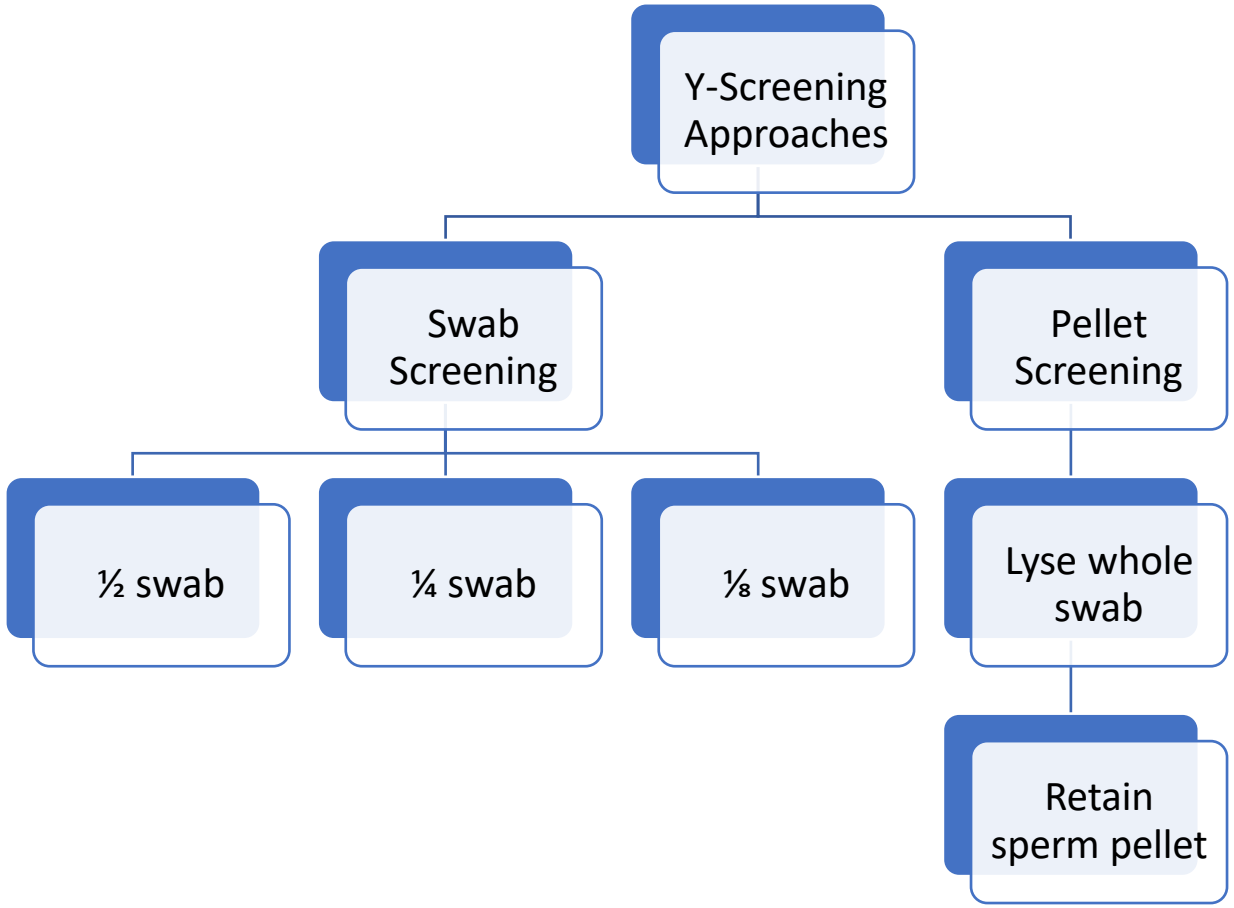
Validation Report

Developmental Validation of the Investigator® Casework GO! Kit

(2022):

- Marginal DNA loss in direct PCR approach using semen dilutions
- In mock sexual assault samples, Y-STRs were recovered up to 100% after lysis with Casework GO! and differential washes
- Could not successfully perform autosomal STR analysis because of high epithelial background

Investigator Casework GO!



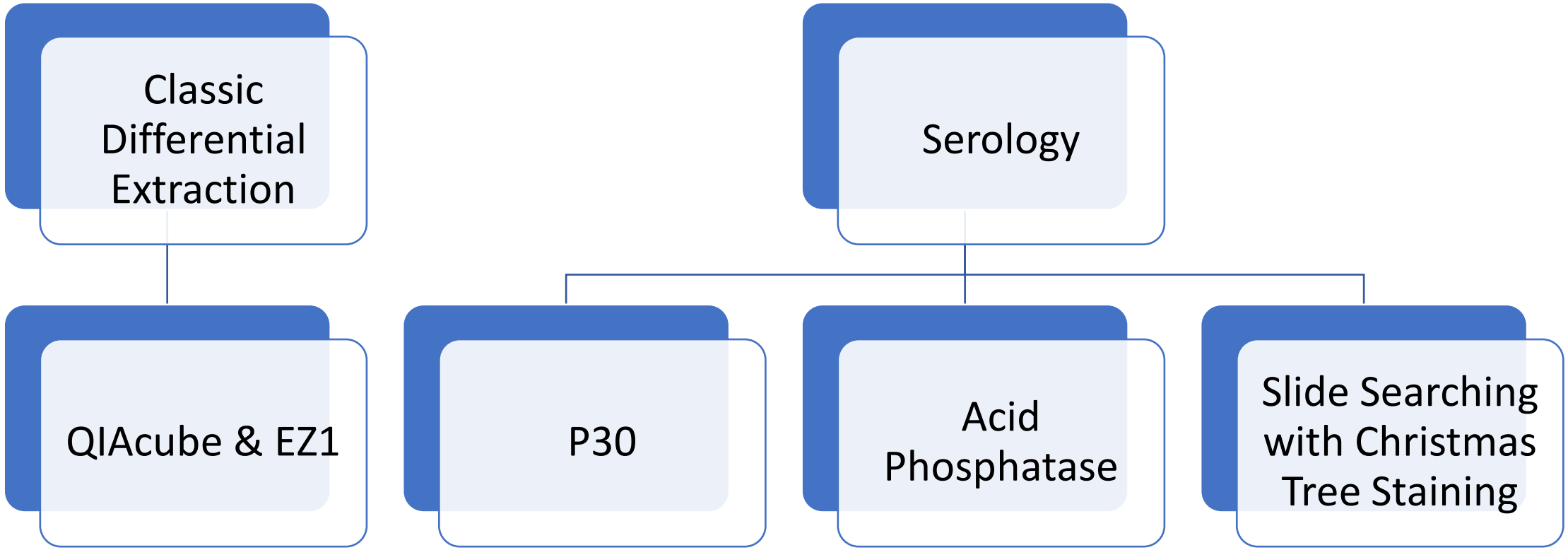
Swab Screening

- Add 187 µL Casework GO! buffer, 7 µL ProK, 6 µL DTT (10 mM) to sample
- Vortex
- Incubate 60°C for 25 min at 900 rpm
- Incubate 80°C for 5 min
- Transfer lysate
- Quantification

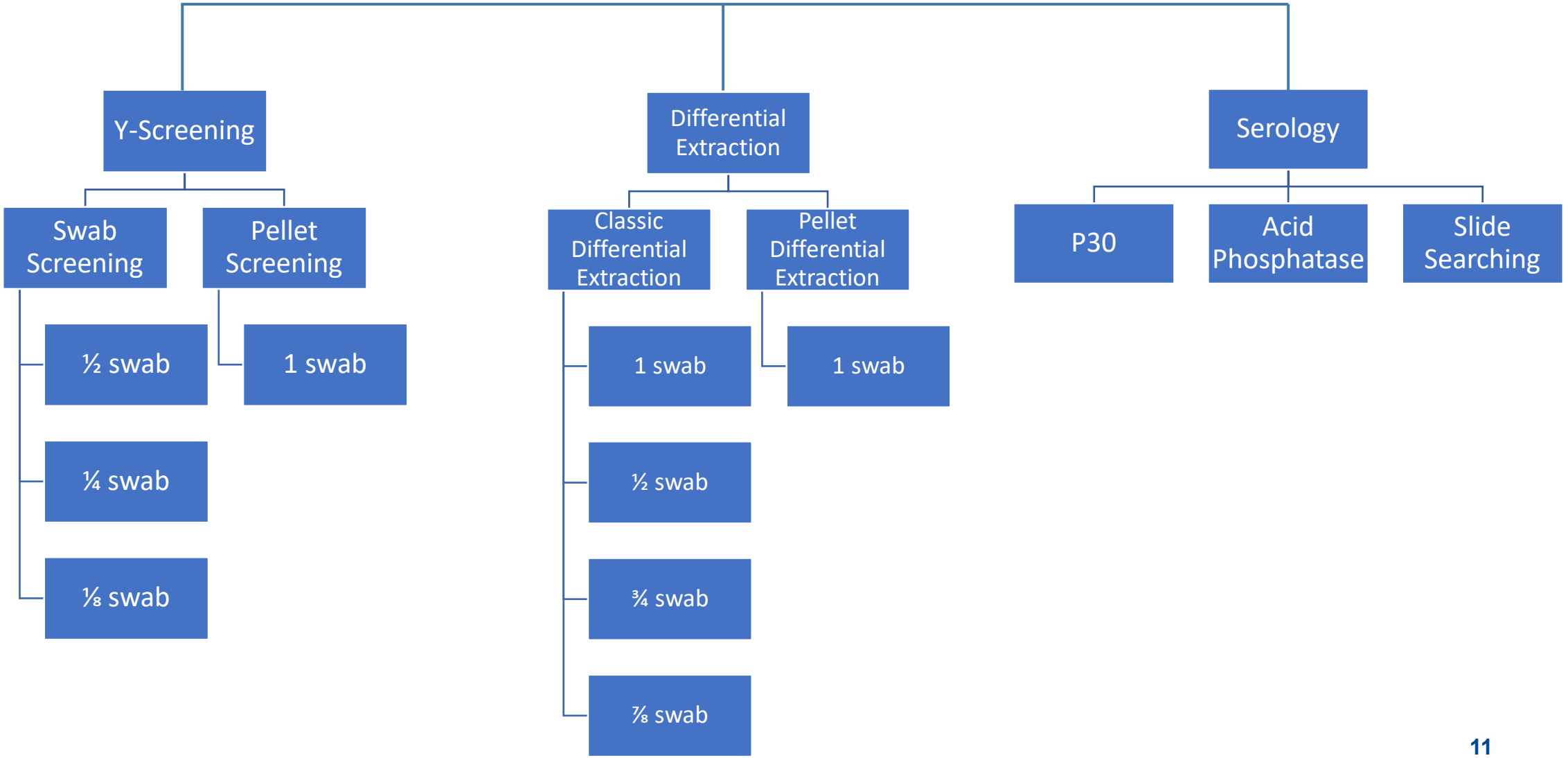
Pellet Screening

- Add 290 µL of Casework GO! buffer and 10 µL ProK to whole swab
- Incubate 60°C for 25 min at 900 rpm
- Place swab into spin basket
- Centrifuge for 5 min at 4500 rpm
- Remove 250 µL (epithelial fraction) from top
- Vortex pellet and remaining 50 µL of sample (sperm fraction)
- Remove 10 µL of pellet fraction for screening
- Add 1 µL ProK and 1 µL DTT (10 mM) to screening sample
- Quantification

Methods of Comparison



Methods

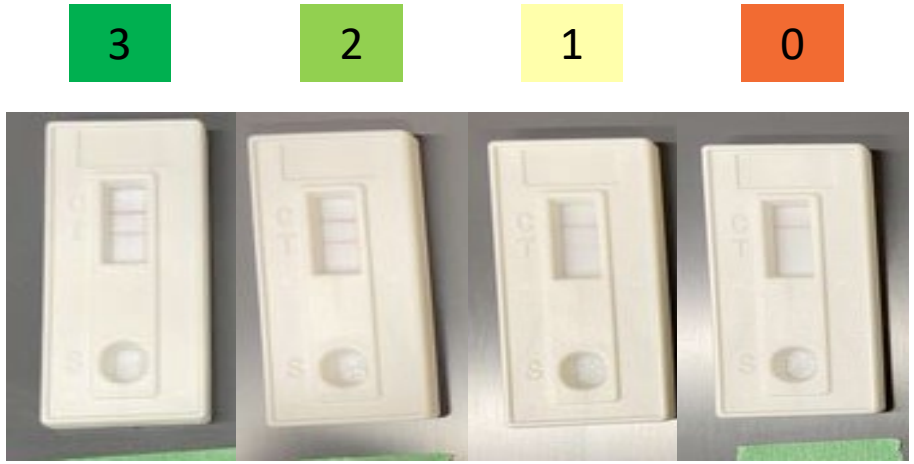

Y-Screening
Swab Screening
Pellet Screening
½ swab
¼ swab
⅛ swab
1 swab
Differential Extraction
Classic Differential Extraction
Pellet Differential Extraction
1 swab
½ swab
¾ swab
⅞ swab
1 swab
Serology
P30
Acid Phosphatase
Slide Searching

Samples

- Semen spiked onto vaginal swabs in triplicate at:
  - 500 ng
  - 100 ng
  - 20 ng
  - 4 ng
  - 0.8 ng
  - 0.16 ng
  - 0.032 ng
  - 0.0064 ng

P30

Semen DNA	P30 Result
500 ng	3
100 ng	3
20 ng	2
4 ng	1
0.8 ng	0
0.16 ng	0
0.032 ng	0
0.0064 ng	0



Score	Description
3	Immediate strong reaction
2	Moderate reaction
1	Weak reaction
0	No reaction; negative reaction

Acid Phosphatase

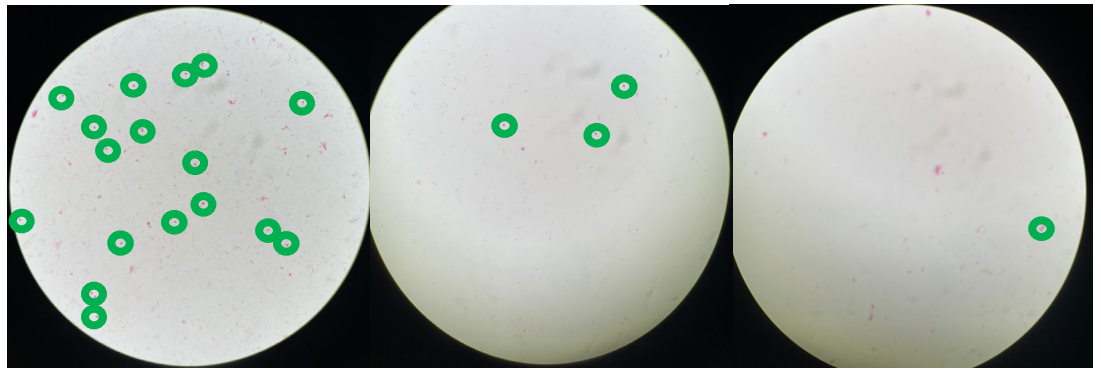
Male DNA	AP Result
500 ng	+4
100 ng	+3
20 ng	+3
4 ng	+2
0.8 ng	+2
0.16 ng	+2
0.032 ng	+1
0.0064 ng	+1

Score	Description
+4	Intense purple color develops and bleeds into test paper
+3	Intense purple color develops and develops quickly
+2	2: Slight purple color develops on and may develop slowly
+1	1: Slow pink color develops on stain material

Slide Searching

Male DNA	Slide Searching Result
500 ng	+++
100 ng	++
20 ng	++
4 ng	+
0.8 ng	0
0.16 ng	0
0.032 ng	0
0.0064 ng	0

++++ ++ +



Score	Description
++++	Many spermatozoa in every field
+++	Many or some spermatozoa in most fields
++	Some spermatozoa in some fields, easy to find
+	Hard to find
0	No spermatozoa observed

Male Target Quantification (ng/µL) & Male:Human Ratio

	Casework GO! Screening				Differential Extraction					Serology		
	½ Swab Screen	¼ Swab Screen	⅛ Swab Screen	Y Pellet Screen	Whole Swab DE	½ DE	¾ DE	⅞ DE	Y Pellet DE	P30	AP	Slide Searching
500 ng	0.28 1:30	0.53 1:9	0.32 1:9	14.89 1:23	5.80 1:1	2.42 1:1	1.50 1:1	0.82 1:1	3.12 1:1	3	4	+++
100 ng	0.04 1:1E2	0.07 1:36	0.09 1:38	3.42 1:51	2.81 1:1	0.33 1:1	0.22 1:3	0.22 1:2	0.24 1:2	3	3	++
20 ng	0.02 1:2E2	0.03 1:1E2	0.01 1:2E2	0.74 1:3E2	0.43 1:4	0.03 1:6	0.06 1:8	0.11 1:2	0.12 1:24	2	3	++
4 ng	0.003 1:2E3	0.01 1:2E2	0.01 1:4E2	0.08 1:7E2	0.04 1:17	0.01 1:21	0.004 1:27	0.02 1:32	0.02 1:21	1	2	+
0.8 ng	0.0003 1:2E4	0.002 1:4E3	0.002 1:2E3	0.04 1:4E3	0.02 1:81	0.001 1:53	0.003 1:41	0.007 1:2E2	0.001 1:2E2	0	2	0
0.16 ng	0.0001 1:4E4	0.0007 1:3E3	0.007 1:2E3	0.009 1:2E4	0.00 1:3E2	0.0004 1:23	0.0007 1:2E2	0.0005 1:5E2	0.001 1:2E2	0	2	0
0.032 ng	0.00 N/A	0.0008 1:2E3	0.001 1:5E3	0.0006 1:3E4	0.00 1:2E3	0.0002 1:2E2	0.0003 1:3E2	0.0006 1:2E2	0.0004 1:4E2	0	1	0
0.0064 ng	0.0001 1:3E4	0.0009 1:4E3	0.0005 1:2E3	0.00007 1:2E5	0.00 N/A	0.00 N/A	0.00007 1:4E2	0.0001 1:2E2	0.0003 N/A	0	1	0

Results: STR Mixture Analysis

Range of log(*Likelihood Ratios*) from Spiked Swabs					
	500 ng	**100 ng**	**20 ng**	**4 ng**	**0.8 ng**
Classic Differential	19-24	24	21-37	5-11	0-1
Y Pellet Differential	24	24	2-14	4-9	0-3

Post-Coital Swab Testing

Post-coital swabs collected at:

- 6 hr
- 12 hr
- 24 hr
- 48 hr

Screening

- Serology
- Swab Screening with Casework GO!
- Pellet Screening with Casework GO!

Extraction

- Classic Differential Extraction
- Y Pellet Differential Extraction

Mixture Analysis with STRmix™ 2.9.1

Male Target Quantification (ng/µL) & Male:Human Ratio

	Casework GO! Screening			Differential Extraction		Serology	
	¼ Swab Screen	⅛ Swab Screen	Y Pellet Screen	Whole Swab DE	Y Pellet DE	AP	Slide Searching
6 hr	0.14 1:82	0.22 1:1E2	0.81 1:1E3	5.16 1:1	3.32 1:1	3	+++
12 hr	0.03 1:1E3	0.07 1:2E2	0.21 1:4E2	0.86 1:1	0.42 1:2	3	+
24 hr	0.01 1:1E3	0.02 1:9E2	0.05 1:3E4	0.14 1:3	4.48 1:24	3	+
48 hr	0.02 1:3E3	0.02 1:5E3	0.16 1:5E4	0.80 1:5	0.33 1:21	2	0

Results: Post-Coital STR Mixture Analysis

Range of log(*Likelihood Ratios*) from Post-Coital Swabs				
	6 hr	**12 hr**	**24 hr**	**48 hr**
Classic Differential	25	25	12-25	8-25
Y Pellet Differential	25	25	24-25	17-25

Conclusions

- Y Pellet screening with QIAGEN Casework GO! was the most successful method.
  - Screening most closely matched results observed after full differential extraction.
- Higher Human:Male ratios in screened samples were indicative of epithelial crossover from lack of differential washes.
  - Can still successfully screen for presence of male DNA.
- Results with genuine post-coital showed successful Y Pellet differential workflow.
  - LRs within the same orders of magnitude between Y Pellet and Classic differential extraction.

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Questions?

Julia Wang, MS

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