Mind Your X's and Y's: Screening with QIAGEN Casework GO!

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Disclaimer

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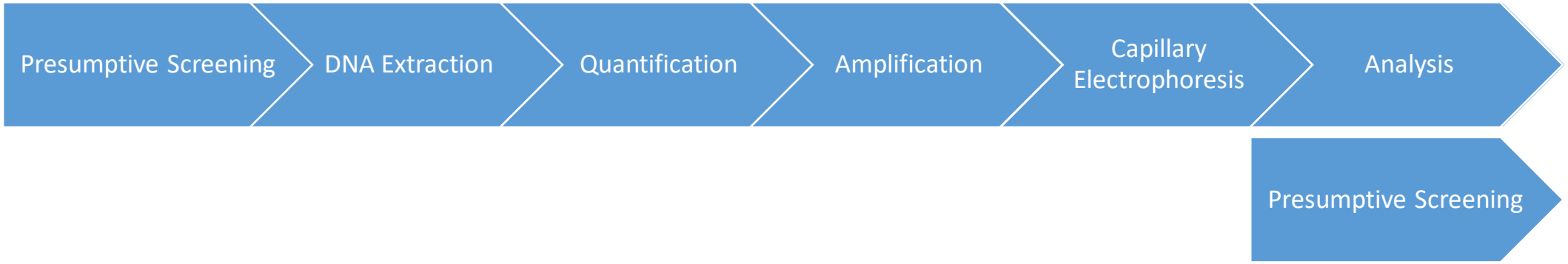
Standard Sexual Assault Evidence Processing

Y-Screening


Presumptive Screening
DNA Extraction
Quantification
Amplification
Capillary Electrophoresis
Analysis
Presumptive Screening

QIAGEN Investigator Casework GO!

- Kit designed for direct amplification workflow
- Lysate can be directly quantified or amplified for STR analysis

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

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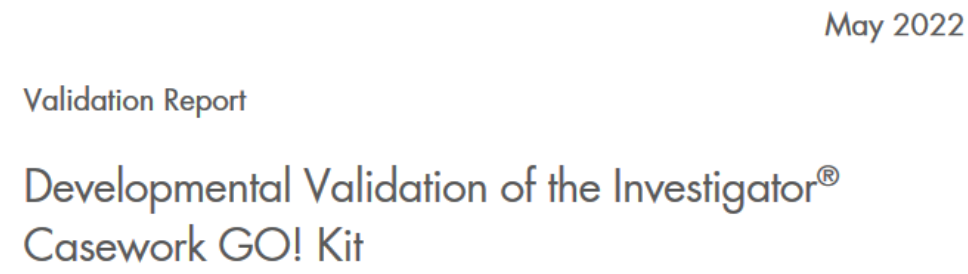
(2019):

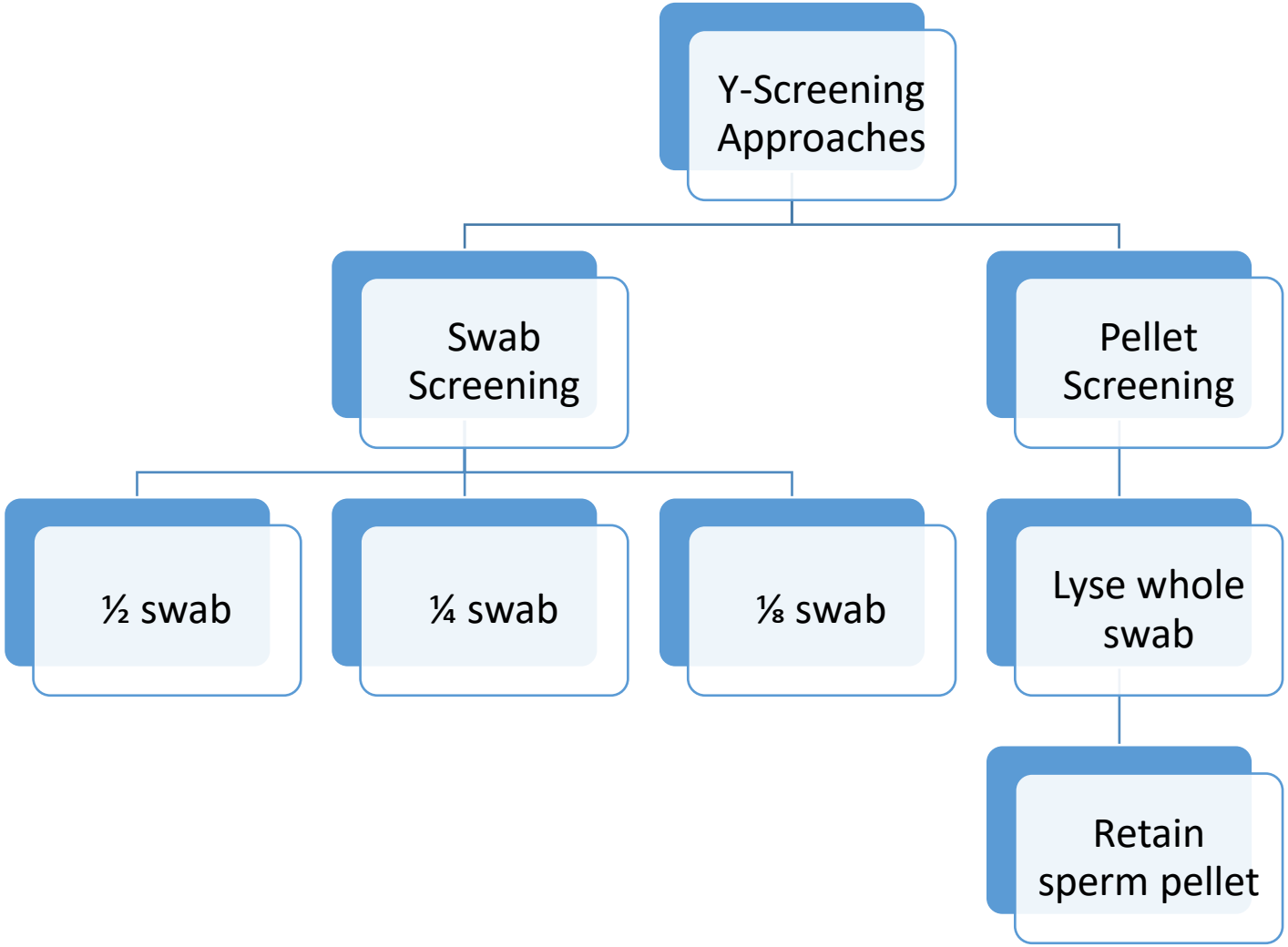
- Blood and semen dilutions
- Direct PCR (Casework GO!) comparable to traditional method (EZ1) for blood dilutions
- 90-100% alleles recovered in semen dilutions up to 1:1000



(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!



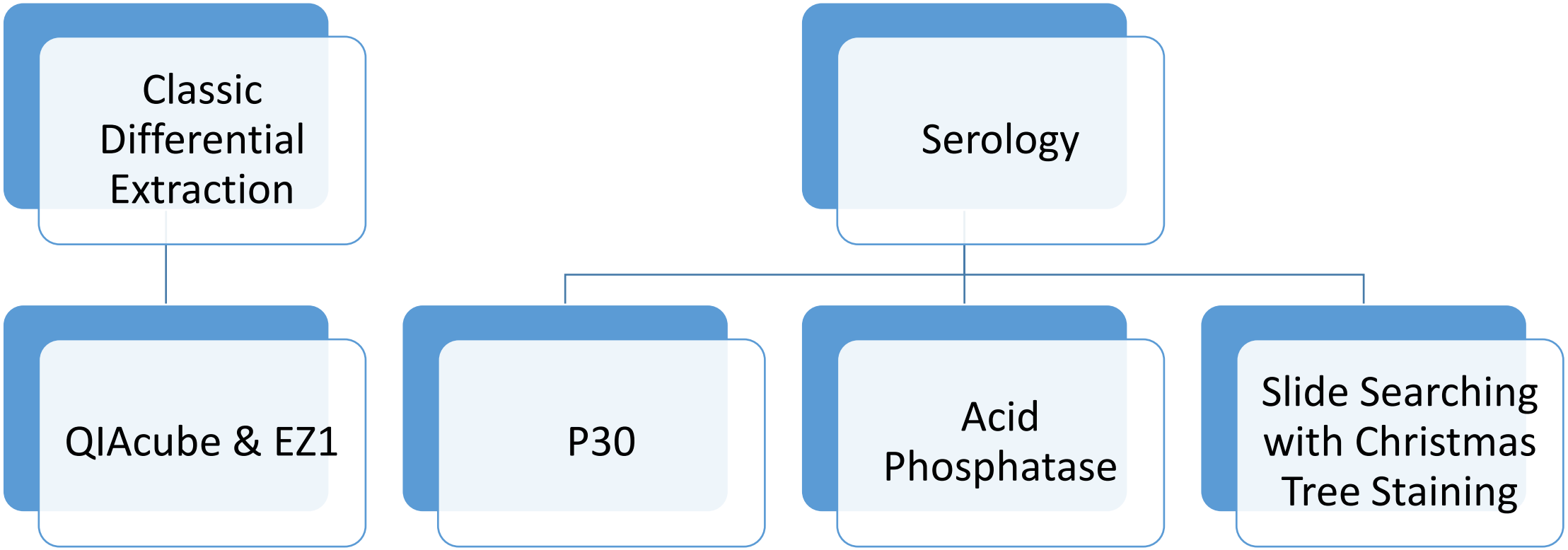
Swab Screening

- 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

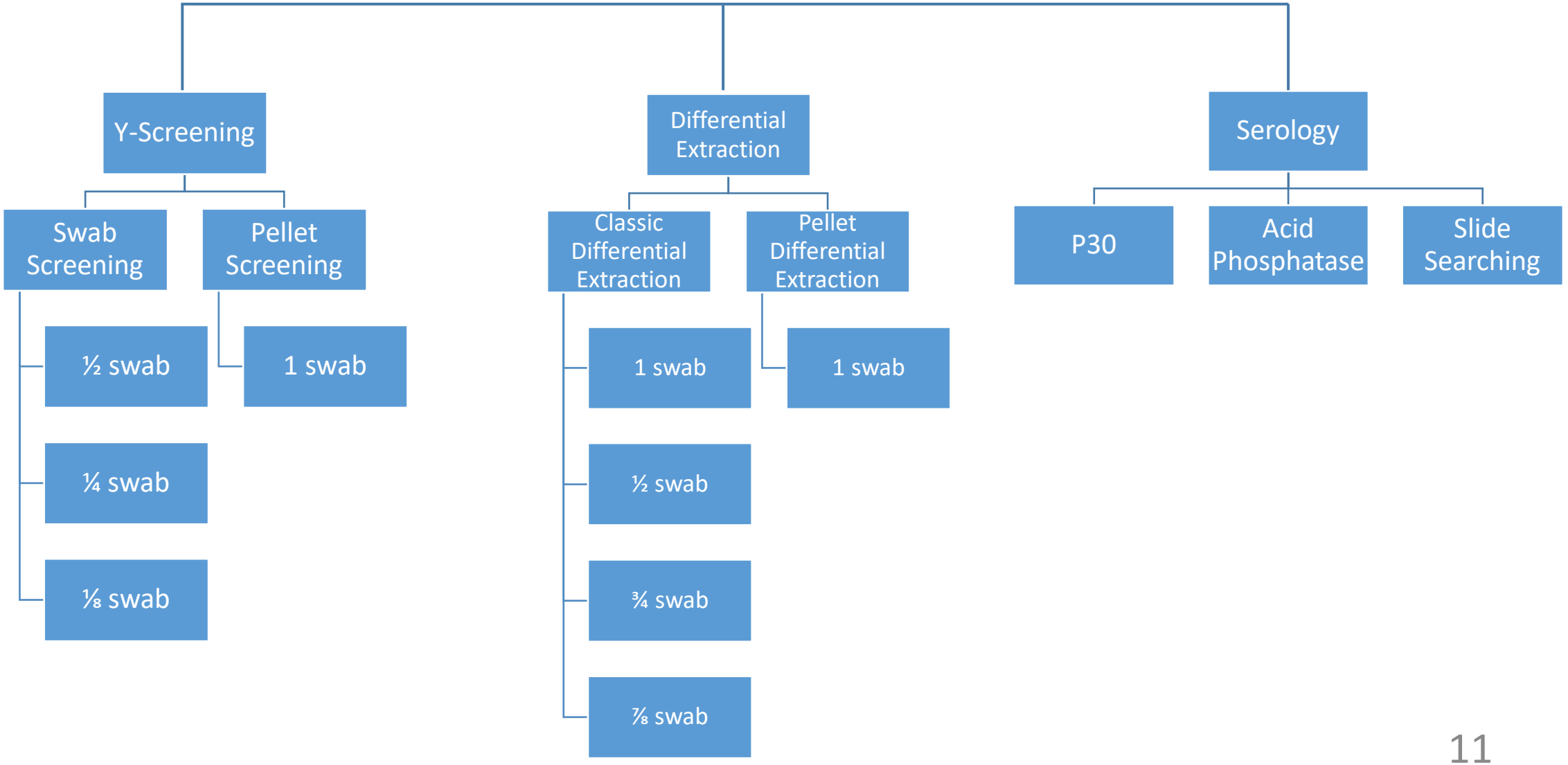
Pellet Screening

- 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

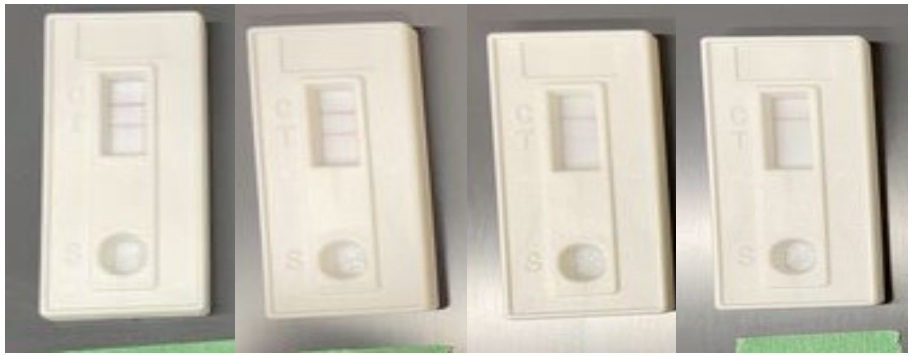

Y-Screening
Swab Screening
Pellet Screening
½ swab
¼ swab
⅛ swab
1 swab
Differential Extraction
Classic Differential Extraction
Pellet Differential Extraction
1 swab
½ swab
¾ swab
⅞ swab
1 swab
Serology
P30
Acid Phosphatase
Slide Searching

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

Results: P30

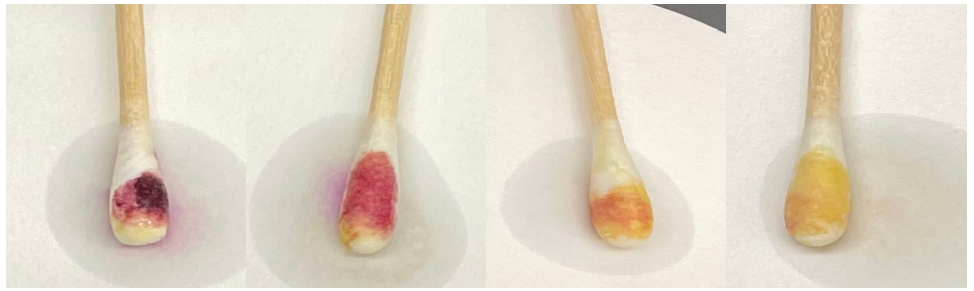
Semen DNA	P30 Result
500 ng	3
100 ng	3
20 ng	2
4 ng	1
0.8 ng	0
0.16 ng	0
0.032 ng	0
0.0064 ng	0



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

Results: Acid Phosphatase

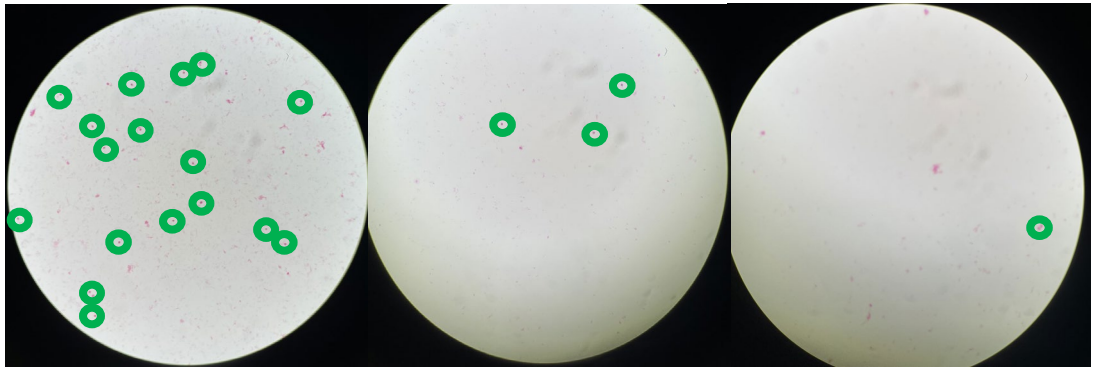
Male DNA	AP Result
500 ng	+4
100 ng	+3
20 ng	+3
4 ng	+2
0.8 ng	+2
0.16 ng	+2
0.032 ng	+1
0.0064 ng	+1



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

Results: Slide Searching

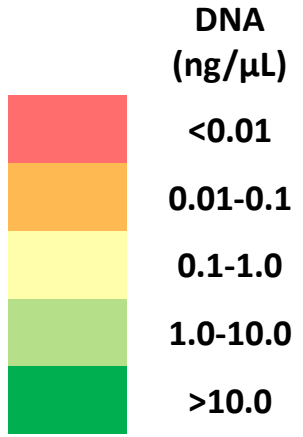
Male DNA	Slide Searching Result
500 ng	+++
100 ng	++
20 ng	++
4 ng	+
0.8 ng	0
0.16 ng	0
0.032 ng	0
0.0064 ng	0



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

Results: Human Target Quantification

Human Target Concentration (ng/µL) & Male:Human Ratio									
	Whole Swab DE	½ DE	¾ DE	⅞ DE	Y Pellet DE	½ Swab Screen	¼ Swab Screen	⅛ Swab Screen	Y Pellet Screen
500 ng	5.29 1:1	2.59 1:1	1.7 1:1	0.90 1:1.1	3.32 1:1	81.8 1:30	51.5 1:9	16.7 1:9	325.2 1:23
100 ng	3.68 1:1	0.46 1:1	0.52 1:3	0.39 1:2.0	0.42 1:2	52.7 1:134	25.1 1:36	22.5 1:38	166.8 1:51
20 ng	1.53 1:4	0.18 1:6	0.35 1:8	0.24 1:2.2	4.48 1:24	43.2 1:195	30.1 1:108	17.8 1:153	236.2 1:307
4 ng	0.54 1:17	0.19 1:21	0.11 1:27	0.36 1:31.5	0.33 1:21	52.7 1:1805	29.0 1:236	45.5 1:370	60.8 1:695
0.8 ng	1.06 1:81	0.07 1:53	0.15 1:41	0.56 1:161.0	0.19 1:164	43.0 1:18430	44.5 1:4276	19.3 1:1596	159.4 1:4057
0.16 ng	0.98 1:344	0.18 1:23	0.12 1:172	0.18 1:490.7	0.17 1:161	67.1 1:36279	21.5 1:3402	24.3 1:2427	166.1 1:24187
0.032 ng	0.96 1:1753	0.06 1:160	0.26 1:319	0.17 1:226.4	0.31 1:370	42.1 N/A	22.7 1:1979	35.2 1:4815	81.2 1:32693
0.0064 ng	0.79 N/A	0.17 N/A	0.15 1:350	0.09 1:248.1	0.59 N/A	64.5 1:28933	29.4 1:3851	21.2 1:2152	141.8 1:202479



Results: Male Target Quantification

	Male Target Concentration (ng/µL) & Male:Human Ratio									Serology		
	Whole Swab DE	½ DE	¾ DE	⅞ DE	Y Pellet DE	½ Swab Screen	¼ Swab Screen	⅛ Swab Screen	Y Pellet Screen	P30	AP	Slide Searching
500 ng	5.80 1:1	2.42 1:1	1.50 1:1	0.82 1:1.1	3.12 1:1	0.28 1:30	0.53 1:9	0.32 1:9	14.89 1:23	3	4	+++
100 ng	2.81 1:1	0.33 1:1	0.22 1:3	0.22 1:2.0	0.24 1:2	0.04 1:134	0.07 1:36	0.09 1:38	3.42 1:51	3	3	++
20 ng	0.43 1:4	0.03 1:6	0.06 1:8	0.11 1:2.2	0.12 1:24	0.02 1:195	0.03 1:108	0.01 1:153	0.74 1:307	2	3	++
4 ng	0.04 1:17	0.01 1:21	0.004 1:27	0.02 1:31.5	0.02 1:21	0.003 1:1805	0.01 1:236	0.01 1:370	0.08 1:695	1	2	+
0.8 ng	0.02 1:81	0.001 1:53	0.003 1:41	0.007 1:161.0	0.001 1:164	0.0003 1:18430	0.002 1:4276	0.002 1:1596	0.04 1:4057	0	2	0
0.16 ng	0.00 1:344	0.0004 1:23	0.0007 1:172	0.0005 1:490.7	0.001 1:161	0.0001 1:36279	0.0007 1:3402	0.007 1:2427	0.009 1:24187	0	2	0
0.032 ng	0.00 1:1753	0.0002 1:160	0.0003 1:319	0.0006 1:226.4	0.0004 1:370	0.00 N/A	0.0008 1:1979	0.001 1:4815	0.0006 1:32693	0	1	0
0.0064 ng	0.00 N/A	0.00 N/A	0.00007 1:350	0.0001 1:248.1	0.0003 N/A	0.0001 1:28933	0.0009 1:3851	0.0005 1:2152	0.00007 1:202479	0	1	0

STR Recovery

	Y-Screening with Casework GO!				Serology			Male Profile STR Recovery	
	½ Swab Screen	¼ Swab Screen	⅛ Swab Screen	Y Pellet Screen	P30	AP	Slide Searching	Autosomal Profile (%)	Y-STR Profile (%)
500 ng	0.28 1:30	0.53 1:9	0.32 1:9	14.89 1:23	3	4	+++	100%	
100 ng	0.04 1:134	0.07 1:36	0.09 1:38	3.42 1:51	3	3	++	100%	
20 ng	0.02 1:195	0.03 1:108	0.01 1:153	0.74 1:307	2	3	++	85%	
4 ng	0.003 1:1805	0.01 1:236	0.01 1:370	0.08 1:695	1	2	+	44%	
0.8 ng	0.0003 1:18430	0.002 1:4276	0.002 1:1596	0.04 1:4057	0	2	0	39%	90%
0.16 ng	0.0001 1:36279	0.0007 1:3402	0.007 1:2427	0.009 1:24187	0	2	0		52%
0.032 ng	0.00 N/A	0.0008 1:1979	0.001 1:4815	0.0006 1:32693	0	1	0		19%
0.0064 ng	0.0001 1:28933	0.0009 1:3851	0.0005 1:2152	0.00007 1:202479	0	1	0		1%

Conclusions

- AP testing was the most sensitive of the traditional serology methods
- Y-pellet screening with QIAGEN Casework GO! was the most successful method.
  - Screening most closely matched results observed after a full DE
  - Best predicted autosomal STR profile recovery.
- Y-STR profiles were recovered from samples that were screened negative or at very low concentrations
- Higher Human:Male ratios in screened samples were indicative of epithelial crossover or lack of differential washes.
  - Can still screen for presence of male DNA
- Project currently being continued with genuine post-coital vaginal swabs collected at various time points

Acknowledgements

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Questions

Julia Wang, MS

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