

The Y's and How's of Screening with QIAGEN Casework GO!

Julia Wang, MS*; Grace Rutledge, MS; Rachel Houston, PhD; Sheree Hughes, PhD

Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340



ABSTRACT

Serological testing and differential extraction have been the standard approach to processing sexual assault evidence. More recently, laboratories have begun to apply the use of Y-screening methods to detect semen and spermatozoa. Additionally, direct-to-DNA workflows that omit conventional quantification and purification steps have been developed to reduce sample consumption, DNA loss, and processing time.

In this study, various Y-screening and sample processing methods with the QIAGEN Investigator Casework GO! kit were compared to classic differential extraction and serological tests. From simulated sexual assault evidence and genuine post-coital samples, Y-screening methods using Casework GO! were equivalent or more sensitive at detecting semen.

INTRODUCTION

Prevalence of sexual assault and rape in the U.S. is high, with over 400,000 victimizations of rape and sexual assault occurring in 2019¹. Forensic DNA laboratories frequently process sexual assault kit evidence. Conventional methods of screening evidence include detection of acid phosphatase reaction and testing for the presence of antigen p30. Once semen is detected, DNA is extracted using preferential lysis extraction.

This conventional workflow is vulnerable to the loss of sample and final DNA product². This can be prevented by implementation of direct DNA workflows, which bypass DNA purification. Additionally, Y-screening can be performed with currently offered quantification kits that include human male-specific targets. As recommended by NIJ and SWGDAM, this Y-screening approach can also delay or eliminate upfront sample consumption for presumptive screening^{3,4}.

ACKNOWLEDGEMENTS

Samples were collected from donors using SHSU IRB protocol 2020-166. This work was supported by a collaboration with QIAGEN. The opinions, findings, and conclusions expressed in this presentation are those of the authors and do not necessarily reflect those of QIAGEN.

RESULTS AND DISCUSSION

Mock Sexual Assault Evidence Swabs

Spiked Semen (ng)	[Male Target] (ng/μL) & Male:Human Ratio						Serology		
	Classic Differential	Y Pellet Differential	½ Swab Screen	¼ Swab Screen	⅓ Swab Screen	Y Pellet Screen	P30	AP	Staining
500	5.80 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	2.81 1:1	0.24 1:2	0.04 1:1E2	0.07 1:36	0.09 1:38	3.42 1:51	3	3	++
20	0.43 1:4	0.12 1:24	0.02 1:2E2	0.03 1:1E2	0.01 1:2E2	0.74 1:3E2	2	3	++
4	0.04 1:17	0.02 1:21	0.003 1:2E3	0.01 1:2E2	0.01 1:4E2	0.08 1:7E2	1	2	+
0.8	0.02 1:81	0.001 1:2E2	0.0003 1:2E5	0.002 1:4E3	0.002 1:2E3	0.04 1:4E3	0	2	0
0.16	0.002 1:3E2	0.001 1:2E2	0.0001 1:4E5	0.0007 1:3E3	0.007 1:2E3	0.009 1:2E5	0	2	0
0.032	0.0003 1:2E3	0.0004 1:4E2	0.00 N/A	0.0008 1:2E3	0.001 1:5E3	0.0006 1:3E5	0	1	0
0.0064	0.00 N/A	0.0003 1:1E3	0.0001 1:3E5	0.0009 1:4E3	0.0005 1:2E3	0.00007 1:2E6	0	1	0

Table 1. Screening with Casework GO! is comparable to traditional serological screening methods. Sensitivity of screening the Y pellet with Casework GO! is comparable to all three of the traditional serological methods in detection of sperm.

Post-Coital Swab 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

Table 2. Y pellet differential extraction methods are as sensitive as classic differential extraction. Likelihood ratios were conditioned on female donors. STRmix™ analysis on post-coital swabs reveal that Y pellet differentially extracted profiles can be as informative as classical differentially extracted profiles up to 48 hours post-coitus.

- Although acid phosphatase screening was the most sensitive of the screening methods, Y pellet screening with Casework GO! most closely matched the results after a full differential extraction (**Table 1**).
- Likelihood ratios from Y pellet screening and differential extraction workflow performed on post-coital samples were as powerful as 10²⁵ up to 48 hours post-coitus. (**Table 2**).

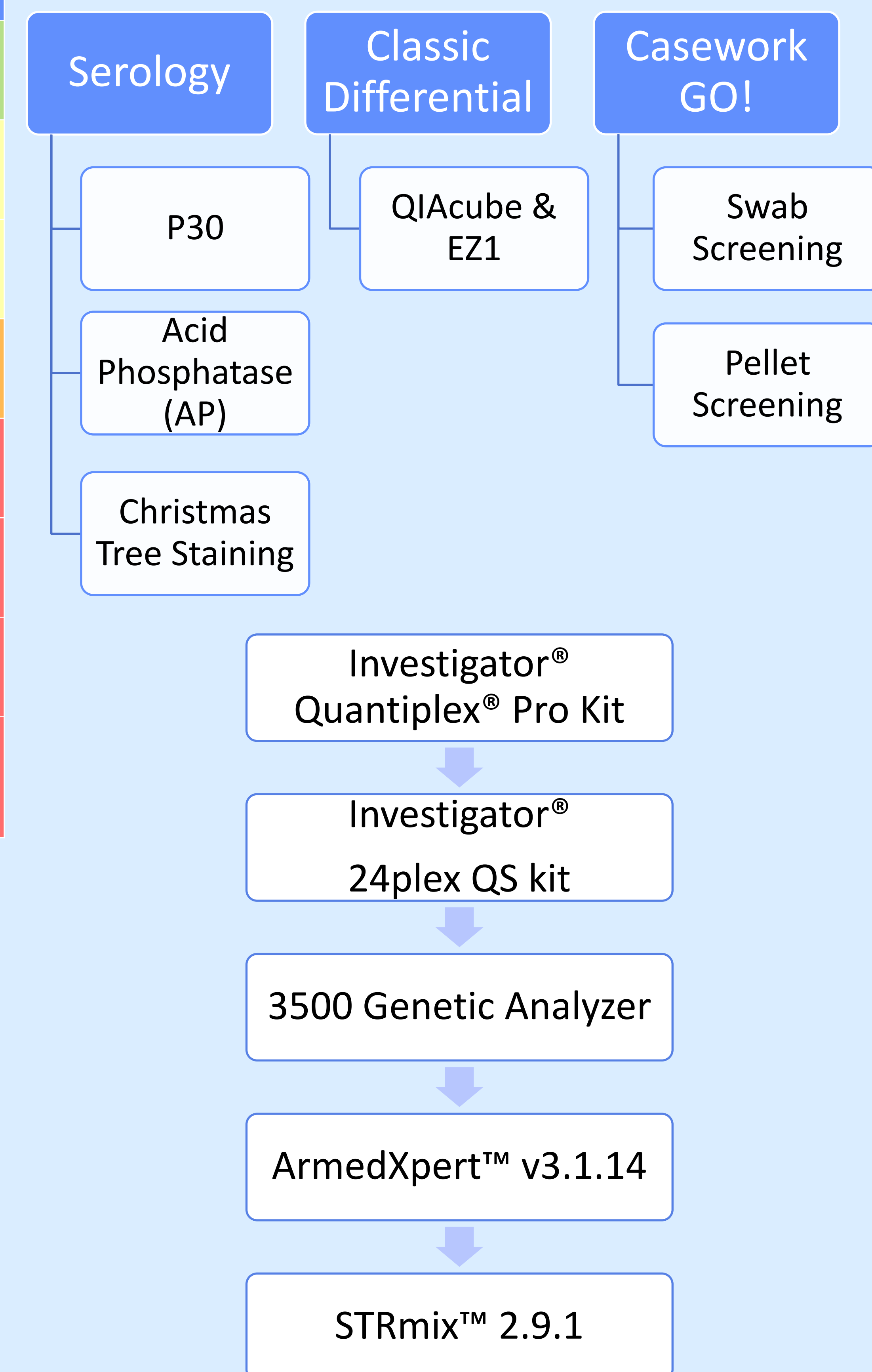
REFERENCES

1. Morgan RE, Truman JL. Criminal Victimization, 2019. Bureau of Justice Statistics, 2020.
2. Vuichard S, Borer U, Bottinelli M, Cossu C, Malik N, Meier V, et al. Differential DNA extraction of challenging simulated sexual-assault samples: a Swiss collaborative study. *Investig Genet* 2011;2:11. <https://doi.org/10.1186/2041-2223-2-11>.
3. National Institute of Justice. National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach. 2017.
4. Scientific Working Group on DNA Analysis Methods. Report on Y-Screening of Sexual Assault Evidence Kits (SAEKs). 2020.

MATERIALS AND METHODS

Samples

- Mock sexual assault evidence swabs: vaginal swabs spiked with various amounts of semen
- Post-coital swabs: 6, 12, 24, and 48 hours post-coital



CONCLUSIONS

- In mock sexual assault swabs, Y pellet screening was the most successful as it most closely matched results after a full differential extraction.
- Post-coital swabs extracted using the Y pellet screening and differential extraction workflow yielded LR's within the same orders of magnitude as classic differential extraction.