If you have issues viewing or accessing this file contact us at NCJRS.gov.

U.S. Department of Justice
National Institute of Justice

This document has been reproduced exactly as received from the person or organization originating it. Points of view or opinions stated in this document are those of the authors and do not necessarily represent the official position or policies of the National Institute of Justice.

Permission to reproduce this copyrighted material has been granted by

Public Domain

US Department of Justice

to the National Criminal Justice Reference Service (NCJRS).

Further reproduction outside of the NCJRS system requires permission of the copyright owner

The laboratory's role in investigating rape

Biochemical and genetic testing can be pivotal in the investigation and prosecution of rape.

GEORGE F. SENSABAUGH, DCrim, JAN BASHINSKI, MCrim, and EDWARD T. BLAKE, DCrim

The incidence of sexual assault has increased dramatically over the last decade. At the same time, laboratory techniques for analyzing and interpreting evidence have become increasingly sophisticated. As a result, the laboratory now plays a significant role in investigating rape and identifying or eliminating suspects. This article discusses some of the methods we use to do so, including enzyme marker determination and genetic typing.

Evidence in sexual assault cases

Investigations usually try to determine three main points: 1) sexual contact; 2) the victim's lack of consent; and 3) the assailant's identity. Laboratory analysis usually focuses on the first and third, although it may help in the second as well.

Rape is a violent physical crime during which many kinds of physical evidence may be transferred to the victim or suspect or left at the scene. Physical evidence from the victim is collected during the physical examination; it includes blood, saliva, swabs of vaginal and cervical secretions, and pubic hair combings. Table 1 lists evidence and reference samples required by the state of California in its guideline protocol for examining rape victims.

Clothing, bedding, and other items may be gathered by the police and examined for semen, saliva, feces, or blood stains. These are often more valuable for testing than the biologic samples collected during the victim's physical examination. For example, semen stains on the victim's panties may contain more semen or be less contaminated by vaginal fluids than vaginal swabs are. Analyzing foreign matter—hairs, fibers, soil, or plant material—may also be helpful in reconstructing the assault and in identifying the assailant.

Scheme for analyzing semen as evidence

In most cases the laboratory focuses on analyzing stains and vaginal swabs. Detecting semen provides evidence of sexual contact, and genetic markers give information about the assailant.

Because the swabs and stains usually contain a limited amount of material, it is important to use an analytic approach that extracts maximum information from the material in hand. Figure 1 outlines a basic scheme used in many crime laboratories. The first series of tests, which use only half the swab or a portion of the stain, indicates the presence and quantity of semen. If there is enough semen, we then use the other half for further genetic testing. Since our protocol requires collecting two swabs from the victim, one is available for duplicate tests.

Key to this approach is using quantitative information to evaluate and interpret test results. The pivotal point is estimation of the amount of semen in the swab or stain material, based on a quantitative acid phosphatase (ACP) assay. This result should be compatible with other measures of semen content such as sperm density. If a marked discrepancy exists, we use another quantitative procedure—the semen p30 test—as a means of confirming the content.

The estimated minimum semen content also helps in two other ways: to interpret the results of qualitative assays for ABO blood group substances and to assess whether the swab has enough semen for electrophoretic enzyme marker typing. Qualitative observations enter into the picture as well. If we find a high microbial or white cell count in the swab, for example, we have to interpret other results with caution.

Analytic techniques

Seminal fluid is usually detected by finding sperm in the supporting evidence. When the victim is being examined, the physician can obtain a vaginal sample for a wet slide preparation and immediately examine it by microscope for sperm motility. These smears should be dried and included in the evidence kit sent to the laboratory. There, the slides are stained for microscopic examination. Staining may demonstrate sperm and sperm parts that cannot be seen in fresh preparations. The nuclear-fast, red-picroindigocarmine stain is superior for sperm,[1] dyeing the heads red and the tails green. The Papanicolaou stain is also useful.

Swab and stain samples are also examined for sperm. After the samples are extracted, the cellular debris is collected by centrifugation, then smeared on a slide, dried, and stained. Sperm density should then be assessed semiquantitatively, from 0 for no visible sperm, to 4+ for many in each field.[2]

Other cellular material, such as vaginal epithelium, white blood cells, and microorganisms should also be noted because they may indicate the degree of sample contamination. If no sperm are present, the laboratory may test for other seminal constituents such as

TABLE 1
Evidence to be collected from rape victims

Physical evidence

- Clothing (optional, depends on circumstances of the case)
- Foreign material: soil, plant matter, extravaginal blood and semen stains, and saliva traces
- Pubic hair combings
- Specimen for motile sperm
- Vaginal swabs and smears
- Swabs and smears from other areas, such as the rectum and mouth, if indicated

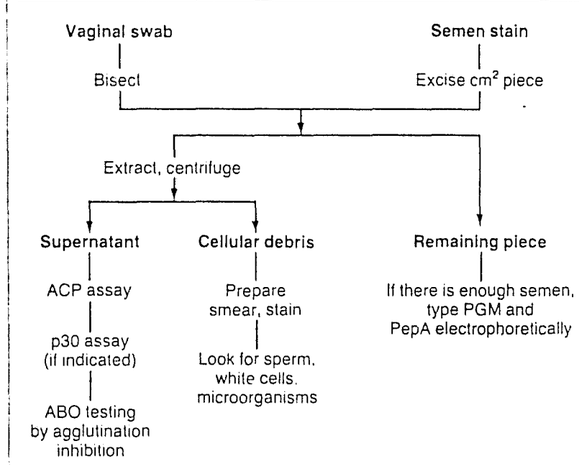
Reference standards

- Pubic hair
- Head hair
- Blood
- Saliva

Medical specimens

- Blood sample for VDRL
- Sample for GC culture (*Neisseria gonorrhoeae*)
- Pap smear (optional)
- Blood sample for alcohol level (optional)

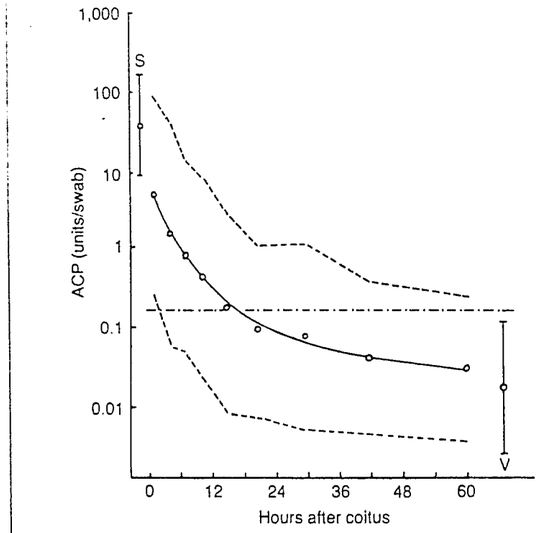
FIGURE 1
Scheme for analyzing semen evidence



Dr Sensabaugh is associate professor, forensic science group, school of public health, University of California, Berkeley. Ms Bashinski is director, criminalistics laboratory of the Oakland Police Department, and Dr. Blake is at Forensic Science Associates, Emeryville, Calif.

FIGURE 2

Postcoital decline of ACP activity in the vagina



ACP units are expressed as micromoles of *p*-nitrophenylphosphate hydrolyzed per minute at 25 C in pH 5.5 acetate buffer (0.1M). The solid line indicates a decline in mean enzyme level after coitus. The other two lines encompass the two standard deviation range of values; 95% fall between them. The S bar shows the distribution of undiluted semen on a swab; the V bar noncoital vaginal ACP distribution (mean = 2SD). The horizontal line represents the 99% threshold for noncoital vaginal ACP levels (0.165 units/swab). ACP levels in semen, vaginal fluids, and postcoital vaginal fluids are log normally distributed. This represents the analysis of more than 400 postcoital data points.

ACP,[3,4] choline,[5] spermine,[6] and semen protein p30.[7,8]

ACP assay is the best-established technique for detecting semen. This enzyme, secreted by the prostate, is found in much higher concentrations in seminal fluid than elsewhere in the body. Although improved methods for ACP determination have been developed, it is still difficult to distinguish prostatic acid phosphatase from the ACP found in vaginal secretions. The ACPs in semen, vaginal fluids, and in some tissues comprise the family of related enzymes that are essentially identical in substrate and inhibitor specificities as well as in immunologic cross-reactivity.

The only significant difference between vaginal and seminal fluid ACP is in electrophoretic motility.[9] There is evidence that this is caused by differences in bound carbohydrate rather than in altered protein sequence,[9,10] so electrophoretic analysis doesn't necessarily yield useful results. We thus rely primarily on quantitative enzyme differences.

Noncoital and postcoital ACP activity levels in vaginal fluids have been statistically characterized, which has made it possible to define thresholds that distinguish endogenous from elevated levels (Figure 2).[11] Using an assay with a *p*-nitrophenylphosphate substrate (pH 5.5 at 25 C), the mean level of activity in semen-free vaginal swabs is about 0.025 units per swab. Activity levels in other fluids are typically lower. Statistical analysis of vaginal swab levels indicates a 99% threshold value of about 0.165 units per swab, and a 99.9% value of about 0.355 units per swab. Case samples with enzyme activity higher than these thresholds are considered semen-positive. Postcoital swab values begin to fall below these values as early as 3 to 6 hours after intercourse; and by 12 to 15 hours, fully 50% of the swab values are below the threshold. Thus a positive ACP test is meaningful, but a negative test is not.

p30 testing is relatively new.[7,8] A major seminal plasma glycoprotein in the prostate—weighing about 30,000 daltons—it is identical to the prostate-specific antigen currently being studied as a prostatic cancer marker.[12] p30 has not been found in any female secretion or tissue and is possibly a male-specific protein.[13] It is detected by double diffusion immunoassay or crossover immunoelectrophoresis.[14] It can be assayed quantitatively by rocket electrophoresis[14] or by enzyme-linked immunosorbent assay.[13]

It is important to remember that the absence of detectable semen cannot be interpreted as evidence of no assault. Semen may not be found if more than 24 hours elapse between the assault and collecting the evidence or if the assailant did not ejaculate. The latter is fairly common among sex offenders.[15] Our experience[2] indicates that semen may not be found in some 25% to 30% of otherwise well-documented sexual assaults. This underscores the need for the physician to observe other evidence, such as torn clothing, bruises, and bite marks, while examining the victim.

Assessing postcoital interval

Precisely determining when intercourse occurred is precluded by the widely varying rates of semen clearance from the vagina; the best that can be done in most cases is estimate a broad postcoital interval range.

Finding motile sperm indicates recent intercourse, but the absence of motility means nothing. Sperm can normally cease moving in the vagina within 30 minutes; and in one controlled study, only 50% of the vaginal samples collected within three hours of intercourse showed motile sperm.[16] Estimating sperm density on smears is also of limited value. Very high densities are more compatible with short than long intervals, but moderate densities are found in most cases, and they can be interpreted either way. Sperm can survive in the vagina for about three days and even longer in the cervix. Because of this, it is important to find out about consensual intercourse up to 72 hours before the assault.

ACP levels are only marginally more informative in estimating postcoital interval. Figure 2 shows that high ACP levels (>10 units) on a vaginal swab are unlikely to last more than 10 hours. Levels from 1 to 3 units per swab are more typical, and are compatible with a broad span of postcoital intervals.

Genetic marker typing

Genetic typing to determine the assailant's blood group and type can be done if there is enough semen in the evidence samples. Although such typing cannot uniquely specify an individual, it significantly restricts the suspect population; its value is thus exclusionary. The more definitive the genetic profile, the greater the chance of excluding false suspects, and the smaller the population of possible suspects.

Semen contains three genetic markers at high enough levels to permit routine typing for evidence.[17,18] They are the ABO blood group antigens, and the enzyme markers peptidase A (PepA) and phosphoglucomutase (PGM). The three also appear at lower levels in vaginal secretions; as a result, testing samples that contain both seminal and vaginal fluid includes the exercise of deciding which genetic marker types can originate from the victim and which cannot. Those that are not the victims's are presumed to be the assailant's. These three markers are also expressed in blood. The genetic types of victims and suspects can be established by typing reference blood samples.

A high percentage (80% to 85%) of the population secretes blood group antigens into other body fluids; these people are known as secretors. Secretor status is established by testing saliva for soluble antigens or by typing RBC's for Lewis antigens.[19] Soluble ABO antigen testing is done on cell-free extracts using a semiquantitative agglutination inhibition assay; the results are read microscopically. Cell-free extracts are used to preclude interference from cell-bound antigens such as those on vaginal epithelial cells or microorganisms that may be in the sample.

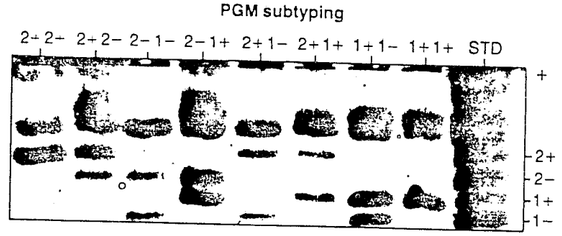
Interpreting ABO typing results depends on antigens found, their titers, and estimated semen content of the extract. If antigens foreign to the victim are found and if their titer is compatible with the estimated semen content, they are presumed to be of seminal origin.

More commonly, no foreign antigens are found, making interpretation more difficult. If antigens matching the victim's are found at titers greatly exceeding normal vaginal fluid levels and if the semen content is high, then it may be inferred that the semen donor is the same ABO type as the victim. If we find no antigens and if the semen content is high, then a nonsecretor semen source is possible. Finally, if the content is low, we can draw no conclusion about the semen type, and make no genetic exclusion. Interpreting these results obviously requires experience in assessing normal ABO substance levels in vaginal fluids and semen.

The enzyme markers PGM and PepA occur in semen regardless of ABO type and secretor status; both are typed by electrophoresis, and conventional typing shows that both have three phenotypes, 1, 2-1, and 2. Extended electrophoretic analysis further subdivides PGM into 10 phenotypes, several of which are shown in Figure 3. PGM is genetically variable in all populations; but PepA electrophoretic variants are common only in blacks, so its typing is usually done only when the assailant may have been black.

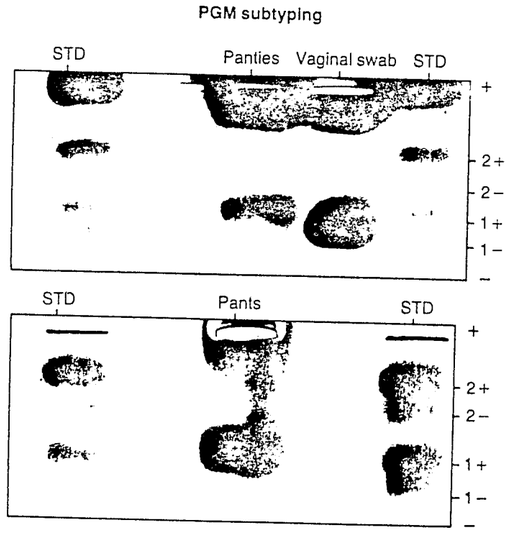
PGM and PepA activities decrease quite rapidly in the vagina after coitus; meaningful PGM results are rarely obtained from vaginal swabs collected more than six hours after intercourse, and PepA

FIGURE 3
Electrophoretic separation of PGM subtypes



This shows eight of the 10 common PGM subtypes with the four type-indicating bands designated from cathode to anode: 1−, 1+, 2−, and 2+. There's a 75% chance that two individuals selected at random will differ in PGM subtype.

FIGURE 4
Genetic typing from vaginal stains



Genetic typing of stains from vaginal drainage is often more informative than typing vaginal swabs. In this case, the victim was a PGM subtype 1−. The vaginal swab shows a prominent 1− band with weak 1+ staining. The stains from the victim's panties and pants show prominent 1+ banding. The stains contained more semen than the swab. By deduction, the semen donor is a PGM subtype 1+.

does not appear to survive longer than three hours.[20,21] Even if the samples are collected within the intervals, they may not contain enough semen for enzyme typing; assessing the sample's semen content can determine whether there is enough for typing. Both markers are relatively labile in improperly preserved specimens containing mixtures of semen and vaginal fluids. These considerations again point out how critical it is to collect and preserve evidence as soon as possible after the assault.

Since seminal markers remain in the vagina for only a short time, the most useful evidence for genetic typing is often stained material from the victim's body, clothing, or bedding. Because semen drains readily from the vagina, drainage stains on panties have a higher semen content than do vaginal swabs. Figure 4 illustrates this point. The victim's pants had the highest semen concentration and showed a foreign PGM subtype. The panties had less semen and demonstrated the foreign type as well as traces of her own. The vaginal swab contained little semen, and the PGM type was predominantly hers. The ABO antigen titers were also higher on the pants and panty samples than on the swab.

The value of genetic profiling in investigating sexual assault is considerable. Each of the four ABO groups is subdivided by the three independently inherited PGM types to yield 12 ABO-PGM combinations; PGM subtyping extends this to 40 combinations. Combined ABO typing and PGM subtyping thus allow the genetic differentiation of a random pair of individuals about 90% of the time.

The following case nicely illustrates the way genetic typing can be used in a rape investigation. Semen evidence was analyzed from victims of a series rapist, and a genetic profile was developed. The assailant's combination of types occurred in only about 2% of the general population. Several prowlers who were picked up in the neighborhood where the assaults had occurred did not fit the genetic profile and so were rejected as suspects. Finally, someone was arrested who did fit the profile, and further investigation turned up the evidence that implicated him. The genetic evidence was thus pivotal in clearing several innocent persons, as well as pointing to the correct suspect.

Coordinating medical and emergency room personnel

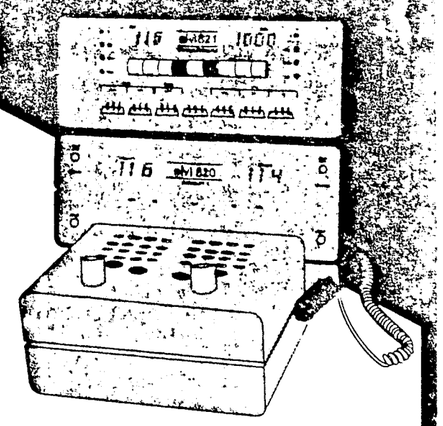
The laboratory has a primary responsibility to work with the appropriate medical and emergency room (ER) personnel to develop protocols regarding collection of evidence. These should describe what evidence is to be collected, how it is to be collected, and how it should be preserved for transport to the laboratory for analysis. The laboratory should also be involved in training medical and ER personnel to treat rape victims and collect evidence.

Prepackaged kits are often used to facilitate collection. This is excellent if they are sealed to keep them from being disturbed until they are used.

All specimens should be dated, timed, and initialed by the doctor, as well as labeled with the patient's name. Ideally, specimens are sent to as few people as possible, preferably carried by hand and with extensive use of receipts to maintain a chain of evidence. Custody must be accounted for from the time evidence is collected to the time of trial, or it is useless in court. The critical nature of this chain of custody cannot be overemphasized.

Efforts should be made to preserve the evidence collected. Degradation can be halted by drying or freezing the specimen. Stability studies in our laboratories indicate that the specimen's wetness is a critical factor in marker deterioration. Figure 5 illustrates the loss of PGM activity under various conditions of swab storage; the rapid loss of enzyme activity in swabs held at room temperature is evident. The markers are stable when dry, and frozen dried swabs retain their marker activity for many months. Because drying swabs and stains does not interfere with semen detection or ABO typing, we

elvi 820



THE PERFORMANCE YOU NEED AND THE FLEXIBILITY YOU WANT WITH THE

elvi 820 DIGICLOT II

PERFORMANCE:
Dynamic ranging clot detection gives high sensitivity to clot formation and precise results with any reagent system and all samples. Ideal for special coagulation work. Dual channel for faster testing

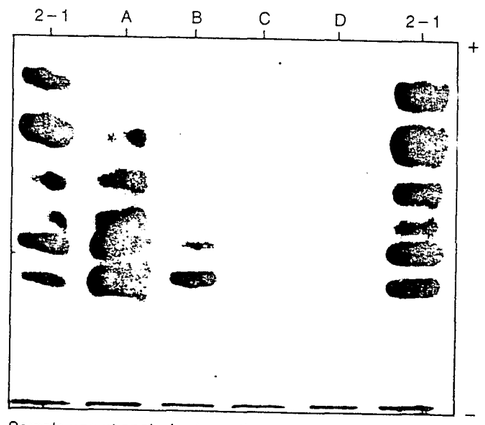
FLEXIBILITY:
The turbine testing system provides increased testing capabilities. The Digiclot II has the ability to perform PT's, APTT's, Thrombin times, factor assays, and fibrinogen.

Logos elvi

700 Sunset Road Henderson, NV 89015
800-821-2495

For more information, circle 147 on the R.S.C.

FIGURE 5
How storage affects PGM activity



Sample age at analysis: 4 months

A: Dried in a cool air flow and frozen
B: Frozen while wet
C: Refrigerated wet for three days and then frozen
D: Left wet at room temperature for three days and then frozen

PGM survival on vaginal swabs depends on preservation conditions. Replicate postcoital swabs were collected and treated as shown in the key. The swab samples were frozen for four months before electrophoresis. The samples flanking the swab samples are PGM type 2-1 standards. This electrophoretic analysis distinguishes the three conventional types, but not the subtypes.

recommend it for preserving swabs and stains.

Freezing may be convenient; but if samples have to be transported any distance, they may thaw. Drying takes longer but yields a more resilient product. To encourage swab drying in our ER, we have provided boxes that contain a small stand to hold the swabs upright in front of a small fan. It takes about an hour for them to dry that way. In California, failure to preserve rape evidence adequately can result in its being excluded from the trial.[22]

Reports and court testimony

The laboratory communicates its results to the police and attorneys through reports and to the courts by testimony. Since we are addressing a lay audience, the report must be as complete and informative as possible. Inadequate statements tend to leave interpretation to the audience, and could thus be easily misunderstood.

A good report interprets what the findings mean in the context of the case. For example, in reporting the absence of detectable semen on a vaginal swab, it would be appropriate to add that this is compatible with 1) lack of sexual contact; 2) sexual contact without ejaculation into the vagina; or 3) sexual contact with loss of semen between the time of contact and collection of evidence. The third possibility can be further evaluated in terms of the alleged postcoital interval and knowledge of the rates at which seminal components are lost from the vagina.

Similarly, if the types on a swab are compatible only with a suspect, the report should establish that the types are attributable only to semen and not to the victim's own secretions. The report should also note the frequency with which that type occurs in the general population. A simple statement of compatibility without qualification could possibly lead to a mistaken inference of identity.

Should the laboratory analyst be called to testify, he or she should know what the evidence says and what it does not. Incomplete answers can give a misleading impression of the evidence, so complete responses with appropriate qualifications are necessary.

The laboratory should not only establish a good working relationship with police investigative units but also make regular presentations to legal associations, to provide information about the strengths and limitations of sexual assault evidence.

Summary

Crime laboratories have made significant advances in their ability to analyze and interpret evidence in rape cases. Particularly noteworthy is the contribution of genetic typing to identify assailants. To make the most of this potential, however, laboratory personnel must get involved in various facets of managing sexual assault cases that relate to the collection and use of its evidence. This includes the development of specimen collection protocols, the training of police and hospital personnel, and even public education. Taking this broad view, the laboratory can make significant

contributions to investigating and prosecuting sexual assault. ■

1. Oppitz E. Eine neue Farbemethode zum Nachweis der Spermien bei Sittlichkeitsdelikten. *Arch kriminol* 1969;144:145-148
2. Willott GM, Allard JE. Spermatazoa—their persistence after sexual intercourse. *Forensic Sci Int* 1982;19:135-194
3. Kind SS. The acid phosphatase test. *Methods Forensic Sci* 1964;4:267-288
4. Sensabaugh GF. The acid phosphatase test. In *Proceedings. Symposium on the Analysis of Sexual Assault Evidence*. Washington DC: US Government Printing Office (in press)
5. Suzuki O, Matsumoto T, Oya M, et al. A new enzymatic method for the demonstration of choline in human semen stains. *J Forensic Sci* 1981;26:420-415
6. Suzuki O, Oya M, Katsumata Y, et al. A new enzymatic method for the demonstration of spermine in human seminal stains. *J Forensic Sci* 1980;25:99-102
7. Sensabaugh GF. Isolation and characterization of a semen specific protein from human seminal plasma: a potential new marker for semen identification. *J Forensic Sci* 1978;23:105-115
8. Baechtel FS. Immunological methods for seminal fluid identification—prostatic antigen. In *Proceedings. Symposium on the analysis of sexual assault evidence*. Washington DC: US Government Printing Office (in press)
9. Sensabaugh GF. Isozymes in forensic science. In Rattazzi MC, Scandalios JG, Whitt GS (eds). *Isozymes: Current topics in biological and medical research*. New York, Alan R. Liss, Inc., 1982, vol 6, pp 247-282
10. Toates P. The forensic identification of semen by isoelectric focusing of seminal acid phosphatase. *Forensic Sci Int* 1979;14:191-214
11. Sensabaugh GF. The quantitative acid phosphatase test. *J Forensic Sci* 1979;24:346-365
12. Wang MC, Valenzuela LA, Murphey GP, et al. Purification of a human prostate specific antigen. *Invest Urol* 1979;17:159-163
13. Graves HC, Sensabaugh GF, Blake ET. Postcoital detection of a male-specific semen protein by ELISA. Application to rape investigation. *N Engl J Med* 1985;312:338-343
14. Rawlinson LE, Wraxall BG. Semen quantitation utilizing p30 antigen. *J Forensic Sci Soc* 1983;23:175
15. Groth AN, Burgess AW. Sexual dysfunction during rape. *N Engl J Med* 1977;297:764-766
16. Soules MR, Pollard AA, Brown KM, et al. The forensic evaluation of evidence in alleged rape. *Am J Obstet Gynecol* 1976;130:142-147
17. Blake ET, Sensabaugh GF. Genetic markers in semen: A review. *J Forensic Sci* 1976;21:784-796
18. Blake ET, Sensabaugh GF. Genetic markers in Semen II: Quantitation of polymorphic proteins. *J Forensic Sci* 1978;23:707-719
19. Race RR, Sanger R. *Blood groups in man*, ed 5. Philadelphia: FA Davis Co, 1968
20. Price CJ, Davies A, Wraxall BG, et al. The typing of phosphoglucomutase in vaginal material and semen. *J Forensic Sci Soc* 1976;16:29-42
21. Parkin B. The evidential value of peptidase A as a semen typing system. *J Forensic Sci* 1981;26:398-404
22. *People vs Nation* (1980) 26 Cal 3rd 169

Is your service on-line and on-time?

Ours is.

Coulter customer service. It's real service, not lip service. When a Coulter* system is installed in your lab, you become part of our nationwide service program. Should a problem arise, there's a problem-solver a toll-free call away. Usually with a solution that get's your instrument up to speed within hours.

We do it with people. We do it with computer-assisted diagnostics. We do it anyway that's needed to keep your instrument performing.

And we do it with the CARDS* remote diagnostic system—our instrument-to-computer direct telephone link. It brings hands-off, on-line trouble shooting to your laboratory.

Automated S-PLUS and S series systems. Compatible diagnostics. Tailored training. On-line, on-time service. Flexible financing. All from Coulter, the leader in hematology for over 25 years. Call toll free 800 526 6632 in Florida 800 432 6516 Ex 2720.

Coulter Electronics Inc.
P.O. Box 2145 Hialeah, FL 33012-0145

Coulter... Quality Counts!

For more information, circle 128 on the R.S.C.

END