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Reliability of the Acid Phosphatase Test for the Identification of Seminal Fluid

From the day in 1677 when a medical student named Johann Ham first discovered spermatozoa swimming about in a microscopic field to the present, it has been traditional to identify seminal fluid by finding spermatozoa in the specimen. It has been pointed out [*1-4*], however, that conditions exist in which the ejaculate bears no spermatozoa. Because of this situation, forensic scientists have been searching for a foolproof method of identifying seminal fluid in the absence of spermatozoa.

A successful method depends upon the presence of a singular substance, different in some way from any other material. Unfortunately, seminal fluid is a veritable repository of chemicals, both organic and inorganic, running the gamut from amino acids, cholesterol, fructose, lactic acid, and acid phosphatase to calcium, magnesium, potassium, and sodium.

In 1896, Florence [*5*] devised the first microchemical test for seminal stains. The reaction is based upon the presence of free choline, a degradation product of phosphorylcholine and glycerylphosphorylcholine. These two products of the seminal vesicles liberate choline through catalytic conversion of phosphatase; after 6 h outside the body, high levels (2120 mg/100 ml) are produced. The test was enthusiastically received in medicolegal circles and within a few decades was recognized as the classical test for the identification of seminal fluid. Nevertheless, some individuals detected the test's fallibility. Kind [*6*] has shown that occasionally the test remained negative in the presence of definite seminal stains; conversely, it has occasionally turned positive in the absence of semen. Pollack [*1*] believed results with the Florence test could be ambiguous, thereby making the test unreliable and hence, useless. Gradwohl [*7*] phrased it thus: "Weisman states that a positive reaction is not significant since other body fluids give such results with this test. Our own experience confirms this statement. A negative test, however, informs the investigator that the suspected substance is not semen and this is the only value of the Florence test." Nevertheless, the Florence test continued to be used. However, because of the persistent increase in sex crimes [*8*] through the ensuing years, a search for a better and more affirmative means of identifying seminal fluid has pushed forward.

As early as 1923, Robison [*9*] demonstrated the presence of an enzyme capable of hydrolyzing calcium hexosemonophosphate in bone. The name given to this enzyme was phosphate esterase or phosphatase. A study was initiated to learn where else in nature this newly discovered enzyme could be located. In short order, phosphate esterase was found in kidney, intestine, liver, striated muscle, and lung in addition to the teeth of young animals, milk, and the floral parts of plants. In 1935, Kutscher and Wolbergs [*10*] made a major breakthrough in the identification of seminal fluid by discovering that normal human prostatic tissue is exceptionally rich in phosphatase, having optimal activity at

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a pH of 4.9. A year later, Gutman et al [*11*] cautiously associated increased serum phosphatase activity with metastasizing carcinoma of the prostate gland; in 1938, both Gutmans [*12*] found in 11 of 15 patients with metastasizing prostatic carcinoma "significantly increased 'acid' phosphatase activity of the serum." Gomori [*13*] added a new and brilliant chapter to the story in 1941 by introducing a histochemical method for demonstrating acid phosphatase in tissue. In 1943, Pollack [*1*] declared unequivocally that "the chemical composition of human semen gives little hope that a suitable test will be detected." Nevertheless, many researchers in the field were spurred on by Gomori's work.

Two years later, Lundquist [*2*], in a brief but historical paper, reviewed some of the older methods including the classical Florence test and listed their shortcomings. Noting "the colossal amounts of a phosphatase with a pH optimum in the acid region," he proposed that these "extraordinary amounts" be used as the basis for the identification of seminal fluid "independent of the presence of sperm cells." Others [*14–16*] were quick to pick up the suggestion and by the mid-century, answers to the problem had been worked out.

In 1945, Rasmussen [*17*] speculated that "in those cases where no spermatozoa can be demonstrated in the stain, a determination of sF [acid phosphatase] may be of value for deciding the origin of the stain." In 1949, Kaye [*3*] recommended "a microchemical test for the identification of semen and seminal stains by an acid phosphatase reaction that appears to be both specific and sensitive" which "may answer the need for a reliable test." He added that "none of the tests so far proposed can positively indicate or exclude the presence of human semen." He analyzed 37 substances including vaginal fluids, urine, feces, blood, saliva, and beet juice; in these substances, he found less than 5 King-Armstrong (K-A) units of acid phosphatase per millilitre of concentrated pure substance. On the other hand, fresh seminal fluid contained from 2000 to 2800 K-A units/ml. Arbitrarily established by comparison and observation, an acid phosphatase activity of 25 K-A units/ml of extract from an area of approximately 1 cm^2 should be considered positive for seminal stains, according to Kaye. One of his conclusions was that the reaction "is very sensitive and appears to be specific for seminal fluid of man or monkey only."

In the same year, Seligman and Manheimer [*18*] produced a test based on the acid phosphatase activity of seminal fluid. They obtained a highly colored product at the optimum pH of 4.9 by employing stabilized diazonium salts which couple freely with the liberated radical of a suitable substrate.

A year later, Walker [*19*] acknowledged the need for a test to identify seminal stains independent of the presence or absence of spermatozoa and introduced the procedure based on the histochemical technic of Seligman and Manheimer. He wrote, "With the discovery of the intense acid phosphatase activity of prostatic secretion, new definitive test methods for semen have become available." This procedure, which came to be known as the Walker test, "is considered today to be the most conclusive test for the presence of semen; in the absence of spermatozoa, this test has sometimes been regarded as being as confirmatory as if spermatozoa had actually been found" [*20*].

Riisfeldt [*15*] demonstrated increased phosphatase activity in vaginal fluid 6 and 12 h after coitus, but not after 24 h. Fischer [*21*] prophesized, "There is, however, little reason to doubt that the test will be used frequently, and no difficulty is anticipated in having such evidence accepted by the Court, provided it is introduced by a qualified expert." In my opinion, the last phrase is the most important thought of the whole paragraph; I will elaborate on this later.

In 1955, focusing on the presence of large quantities of acid phosphatase in seminal fluid, Berg [*22*] described a procedure in which he used a substrate and a suitable diazonium salt to produce an intense violet color. In discussing the Walker test, he "admitted it to be suitable for medicolegal use. However, it did not give such clear results as I have just described."

The various tests developed since 1950 have usually centered about the acid phosphatase

contents of seminal fluid. In general, the techniques use a substrate, usually a phenolic ester, which is hydrolyzed in the presence of acid phosphatase. The reaction is occult. Therefore, a diazonium salt which reacts quickly in a medium adjusted to the right degree of acidity is added. "The coupling reaction," Fieser [*23*] has written, "is spectacular because of the rapid formation of brightly colored products from colorless components."

Investigators began searching for an ideal substrate and a perfect coupling agent. Babson et al [*24*] evaluated six different substrates and concluded that for prostatic acid phosphatase, α-naphthyl phosphate was twice as specific as β-glycerophosphate. In addition, it was 40 to 100 times as specific as the other substrates investigated. Nine years later, Babson [*25*] still held the same opinion. He quoted Bruhn and Keller [*26*] as saying, "Because of its greater specificity alpha naphthyl phosphate is the substrate of choice for the estimation of prostatic acid phosphatase." Babson and his group employed diazodi-orthanisidine reagent or Brentamine fast Blue B salt as the chromogen.

A whole new field of investigation centered about the identification of seminal fluid through the use of a substrate, generally α-naphthyl phosphate, and a color developer, usually Brentamine fast Blue B. Shupe [*27*] attempted to popularize the Babson and co-workers [*24*] assay method by using two tablets (Tablet 1 was the "buffer substrate" and Tablet 2 the "color developer") and a small, 25-mm (1-in.) long test tube called a "Teswell." This method is time-consuming when compared to the test described below and takes at least 42 min to complete. Reinstein et al [*28*] (disclosing that Fishman and Lerner [*29*] noted prostatic acid phosphatase is inhibited by tartrate but that blood acid phosphatase is not) devised a method that they believed was specific for acid phosphatase of prostatic origin. They concluded, "The method is extremely sensitive. As little as 0.001 ml of seminal fluid was detected on a fabric on which it had been dried." Kind [*6*] introduced a stable form of acid phosphatase reagent containing in a spray bottle α-naphthyl phosphoric acid and diazodiorthanisidine as the major components. In his experience, the solution was stable for several months at 4°C. Raju and Iyengar [*30*] tested 100 stains of various animal and plant origins ranging from asparagus, garlic, emblic myrobalan, date, and fig to secretions of crow, duck, and hen. They dissolved α-naphthyl phosphoric acid and the diazonium salt in acetate buffer of pH 5. Like Kind, they pressed dampened filter paper firmly on the suspected stain, leaving it in position for 5 min. They then removed the filter paper and sprayed it with their prepared reagent; they looked for an intense purplish color to appear on the filter paper. This process reminds one of lifting fingerprints from smooth surfaces.

In a short communication, Babson and Phillips [*31*] wrote of using the substrate they had previously recommended [*24*], but as a coupling agent, substituting 5-nitro-*o*-anisidine (fast Red B salt). They concluded the sensitivity was almost twice as great as in their original method and the end color was more stable.

Schiff [*32*] modified the test in the following manner: he impregnated vaginal swabs with an acidified, saturated solution of the relatively stable sodium α-naphthyl phosphate and, after allowing them to dry, placed them in sterile test tubes and stored them in a refrigerator at 4.4°C. In use, the swab was rubbed lightly against any suspected material, either in the vagina, rectum, vulva, or moistened stains, and then treated only with a coupling agent. The purpose was to eliminate two steps from the usual method; the chemical reaction, however, was the same as with the other methods. After six years, the treated swabs are still serviceable.

Sivaram [*33*] modified the test in yet another fashion. Assuming prostatic acid phosphatase is "almost completely inhibited by 0.04*M* L-(+)-tartrate," he claimed he was able to differentiate human semen from other acid phosphatase-bearing substances. Two years later Willott [*34*] produced evidence that L-tartrate-inhibitable acid phosphatase is found in vaginal secretions as well as in seminal fluid. Gomez et al [*35*] agreed with Willott's findings when they concluded, "Inasmuch as endogenous vaginal ACP (acid

phosphatase] is inhibited by tartrate to the same extent as seminal ACP, this inhibitor is useless in resolving the two types of activity."

Material and Method

The materials and method were the same as previously used [*32*]:

(1) a buffer solution, 0.07*M*, prepared by dissolving 14.7 g citric acid monohydrate in 700 ml of distilled water and adjusting the pH to 5.6 with 2*N* sodium hydroxide;

(2) a substrate, sodium α-naphthyl phosphate (Borden Chemical Co., Phila., Pa.), 20 mg in 2 cm^3 distilled water (the substrate should be freshly prepared);

(3) a chromagen, Brentamine fast Blue B (Borden Chemical Co.), 20 mg in 2 cm^3 distilled water (this should be freshly prepared);

(4) disks made from Whatman No. 1 filter paper upon which seminal fluid has been smeared; after drying, the disks are punched out with a standard paper punch (the author has found these disks to be serviceable at least six years after having been made); and

(5) control disks made in the same fashion as above except that the paper had not been smeared with seminal fluid.

Vaginal aspirates were collected from 100 alleged rape victims. An acid phosphatase (AP) test was first performed on each aspirate. Then a careful search was made of at least two slide preparations for spermatozoa.

The method of testing for AP is simple: three glass slides are placed side by side on a sheet of white paper and labeled known (K), unknown (U), and control (C). A disk having seminal fluid on it is positioned on the K slide while untreated disks are positioned on the U and C slides. Onto the U disk is dropped a minute quantity of the vaginal aspirate. To each disk is added a drop of the buffer solution, then a drop of substrate solution, and, finally, a drop of the chromagen. The K disk develops a rich purple color, the U disk may or may not depending upon whether AP is present in the aspirate, and the C disk remains a pale yellow.

Results

Table 1 reflects that the series consists of 61 white and 39 black females. The victims range in age from 8 to 85 years old, with a mean of 25. Fifty-five percent are in the 16- to 26-year-old age group. The time interval between the assault and the examination was anywhere from 1 h to 4 days. It was a tribute to the cooperative efforts of the police and the rape treatment center (RTC) that 45% of the victims were examined within 3 h of the assault, and an additional 25% within 6 h. Eight percent reported to the RTC more than 24 h after the assault. Both AP and spermatozoa have been recorded in each case. A definite correlation has been noted between the presence of AP and spermatozoa or the absence of both in the same cases. In almost half the cases (49%) no spermatozoa or AP was discovered; in Table 1 explanations are given: one assailant used a condom, one did not have time to complete the sexual act, and several victims denied coitus had been performed. It is interesting to note regarding the assailant who used a condom that this situation was encountered only one other time in more than 1500 alleged sexual assaults. This is far less than the 4% reported by Amir [*36*] in 173 rapes.

Given the time factor and the various explanations in Table 1, there appears to be an absolute parallel between the presence and absence of AP and the presence and absence of spermatozoa.

TABLE 1—*Summary of cases.*

Number	Case	Race	Age	Time After Intercourse, h	AP	Spermatozoa	Comments
1	325	w	34	2½	0	0	forced fellatio; mouth swabs negative
2	327	b	22	2½	strong	numerous nonmotile	...
3	333	w	27	3	strong	numerous nonmotile	...
4	334	w	14	2	strong	numerous motile	...
5	335	b	15	5½	0	0	questionable as to whether assault actually occurred
6	338	w	20	2¼	moderate	nonmotile	all 3 attackers had been smoking "pot"
7	339	w	19	5½	0	0	victim and assailants had been smoking "pot"
8	341	b	43	2	strong	motile	...
9	342	b	25	3	strong	motile	victim was a drug addict
10	345	b	65	1½	strong	nonmotile	victim was intoxicated
11	347	w	35	4	0	0	victim was "bombed out"
12	348	w	22	3½	weak	motile	assailant may have used condom
13	349	w	45	3	strong	nonmotile	penetration uncertain
14	358	b	25	3½	strong	nonmotile	...
15	360	b	18	3	strong	nonmotile	...
16	361	w	55	3	0	0	although there was penetration, assailant did not have time to ejaculate
17	368	b	20	3	0	0	no penetration occurred
18	369	b	19	24	0	0	...
19	374	w	37	1	strong	motile	victim assaulted by 3 males
20	376	w	26	3	strong	nonmotile	not certain whether ejaculation occurred
21	378	b	13	14	moderate	nonmotile	...
22	379	w	36	7	0	0	...
23	380	b	21	2¾	moderate	nonmotile	...
24	381	w	26	10	0	0	...
25	386	w	15	3	strong	nonmotile	...
26	387	w	26	4	0	0	sexual assault not completed
27	390	b	21	48	0	0	victim had bathed and douched
28	392	w	22	6	moderate	nonmotile	victim was assaulted by two men
29	393	w	20	5	strong	nonmotile	...
30	394	b	12	over 24	0	0	...

TABLE 1—(*Continued*)

Number	Case	Race	Age	Time After Intercourse, h	AP	Spermatozoa	Comments
31	395	b	20	2½	0	0	assailant (about 15 or 16 years old) had smell of transmission fluid on his breath; may not have ejaculated
32	396	w	27	2½	moderate	nonmotile	...
33	397	b	17	2	0	0	...
34	398	b	20	5	strong	nonmotile	assailant had intercourse with victim twice
35	399	b	14	13	0	0	no penetration
36	400	w	28	3	strong	nonmotile	...
37	401	b	25	3	strong	nonmotile	...
38	402	b	24	1	0	0	assailant may not have ejaculated
40	403	b	58	8	0	0	no ejaculation
41	404	w	55	3½	moderate	nonmotile	...
42	405	b	18	3	moderate	0	three men allegedly had intercourse with victim who had had sexual intercourse with boyfriend 12 h previously
43	406	w	16	4	strong	motile	two men assaulted victim
44	407	w	21	4	0	0	manual manipulation only
45	409	w	18	4	strong	0	two men involved; vaginal aspirate diluted
46	413	b	23	3	strong	nonmotile	three men involved
47	414	w	16	6	strong	nonmotile	...
48	415	w	24	14	0	0	...
49	418	w	77	3	0	0	...
50	419	w	18	3	strong	nonmotile	...
51	422	w	15	3	moderate	both motile and nonmotile	victim was not certain whether assailant had ejaculated
52	423	w	13	5	0	0	false allegation; no intercourse occurred
53	424	b	12	2	0	0	condom was used
54	425	w	22	4	strong	motile	...
55	427	w	25	5	0	0	victim had heavy menses
56	428	w	23	3	strong	nonmotile	...
57	429	w	11	18	0	0	...

58	430	w	18	10	0	0	victim unaware if assailant ejaculated
59	431	w	20	13	moderate	nonmotile	...
60	432	b	11	19	0	0	false allegation
61	433	b	16	96	0	0	...
62	444	w	16	29	0	0	assailant supposed to have had vasectomy
63	445	b	8	16	0	0	Victims 63 and 64 were sisters
64	446	b	8	16	0	0	...
65	448	w	16	4	0	0	doubt as to validity of story
66	450	w	21	4	moderate	nonmotile	3 men had sexual intercourse
67	451	w	19	4	0	0	doubt as to validity of story
68	452	w	30	5	strong	some motile, some nonmotile	...
69	453	w	30	1	0	0	no penetration; no ejaculation
70	455	w	18	4	strong	motile	...
71	457	w	27	2	strong	nonmotile	victim unaware if assailant ejaculated
72	459	w	23	2½	strong	nonmotile	...
73	460	w	85	8	0	0	assailant could not get erection; did not penetrate
74	461	b	31	8	strong	nonmotile	...
75	463	w	18	1½	strong	nonmotile	victim claimed to have been assaulted by two men, but did not believe ejaculation occurred; she may have had intercourse with a man who was not an offender
76	464	b	16	28	0	0	...
77	466	w	44	2½	0	0	no penetration
78	467	b	17	12	strong	0	...
79	468	w	29	27	0	0	victim showered before reporting to police
80	474	b	24	2	strong	half motile, half nonmotile	...
81	478	b	18	3	strong	nonmotile	...
82	480	w	17	10	moderate	0	victim did not know whether sexual assault had occurred because she had been knocked out
83	481	w	75	2	strong	motile and nonmotile	...
84	482	w	35	6	strong	0	...
85	485	w	38	1½	moderate	nonmotile	victim assaulted sexually by 3 males

TABLE 1—(*Continued*)

Number	Case	Race	Age	Time After Intercourse, h	AP	Spermatozoa	Comments
86	487	w	58	26	0	0	victim had been intoxicated; allegation open to suspicion
87	488	b	15	3	0	0	allegation open to suspicion
88	489	w	15	15	moderate	nonmotile	allegation open to suspicion; voluntary sexual intercourse may have occurred later
89	490	b	24	32	0	0	victim had bathed and douched
90	492	b	19	5	0	0	intact hymen; victim did not believe penetration or ejaculation occurred
91	493	w	22	2	0	0	victim believes assailant did not penetrate, but ejaculated outside body
92	494	b	15	15	0	0	mother gave victim bath and vinegar douche
93	496	w	17	2	0	0	victim did not believe ejaculation occurred
94	497	w	19	3	0	0	victim did not believe ejaculation occurred; Victims 93 and 94 were part of the same assault with assailant alternating between the two
95	498	w	15	4½	0	0	there was penetration and injury, but victim did not believe known offender had ejaculated nor even penetrated very far since she was a virgin
96	499	w	16	4	weak	rare sperm	victim did not know whether there was penetration or ejaculation
97	500	w	25	18	0	0	...
98	501	b	20	2	strong	nonmotile	victim assaulted by two men
99	502	b	14	5½	strong	nonmotile	victim assaulted by two men
100	504	w	64	12	0	0	victim slightly intoxicated

Discussion

When the AP test first became known and used, it was greeted with unreserved acclaim by many medicolegal experts. Lundquist [*37*] declared, "We do not hesitate to consider the positive acid phosphatase test as proof of the presence of semen as valid as the demonstration of spermatozoa." Jones [*38*] stated, "Many workers are now convinced that the fairly rapid appearance of an intense colour on applying the test is almost certainly diagnostic of the presence of human semen or, more accurately, human prostatic secretion. The word 'almost' is used in order to include the possibility, however remote, of monkey semen." Levonen [*39*] affirmed, "It is generally considered to have almost the same specificity as the microscopic recognition of spermatozoa." Schumann et al [*40*] concluded that vaginal fluid AP "is a reliable and sensitive method for the identification of semen."

On the other hand, some workers disagreed. Gradwohl [*7*] stated, "With these points in mind, it will be found that this test is very useful as an indicative or screening test and cannot, under any circumstances, be used unconfirmed as a conclusive test for semen." Polson [*41*] agreed, "It cannot, however, displace the microscopical demonstration of sperms as proof of seminal fluid." Sivaram [*33*] wrote that although the presence of acid phosphatase activity was strongly suggestive of the presence of semen, it was nevertheless not specific because of the widespread occurrence of the enzyme in nature.

Evrard [*42*] pointed out that "few medical examiners are willing to testify on the basis of elevated vaginal acid phosphatase without the presence of sperm."

Kind [*6*] stated bluntly that "in the present state of knowledge acid phosphatase activity per se is not proof of semen." His laboratory, however, has used the method as a screening method.

Henningsen[2] has written as recently as 1976 that the AP test "is very sensitive and is *not* conclusive evidence of semen, but if negative it is generally accepted as disproof or anyway as sufficient evidence against semen to omit further examination." Yet, he concluded, "Summarizing, we generally use the acid phosphatase test as a screen test but in certain cases and subject to special criteria, it is used as a reliable indication of the presence of seminal fluid."

Pinto [*43*] stated quite definitely, "We do not, in any circumstances, advocate the substitution of this test for the microscopic identification of spermatozoa." Then, he became slightly ambiguous by commenting on what is done in a legal case where the test is positive and yet "an exhaustive examination" reveals no spermatozoa: "The testimony in Court should be that a positive chemical reaction for semen was obtained which was not confirmed by the microscopic identification of spermatozoa. It would then be left to the Court to assess the value of this evidence in the light of the experience of the expert with the phosphatase test." This is hardly a fair proposition to place upon the shoulders of a judge or a jury, laymen, the onus of making a decision which, here, is the function and responsibility of the expert. The court has the right to expect relatively direct and authoritative answers regarding opinion as well as fact from one who is qualified as an expert.

After 14 year's experience with the AP test, the author has found it to have great merit as a test for the identification of seminal fluid in the absence of spermatozoa. Relying on the test, the author has had no hesitancy in testifying in court regarding the presence or absence of seminal fluid. Because he has used the test qualitatively rather than quantitatively, he has established no arbitrary, numerical cutoff as to when the test is to be declared positive and when negative.

[2]K. Henningsen, personal communication, 15 Sept. 1976.

Attorneys, judges, and other legal authorities recognize well that the polygraph is only as unquestioned as the skill of a particular operator. This same belief holds true for the AP test. It is only as dependable as the physician, chemist, or pathologist who performs it. Certain guidelines must be set down and adhered to. First, the reagents must be freshly prepared. Table 2 shows that solutions left standing at room temperature for 44 h will still be usable. However, a rare lawyer has questioned the author as to when the solutions were prepared, hinting at the fact, with an eye on the jury, that if 2 or 3 h had passed, the test would be void. The diazo-coupling agent that originally was clear and lightly yellow tinted begins to precipitate after 12 h. This may leave doubt in an inexperienced examiner's mind as to its acceptability. Second, the examiner must follow the same protocol in every case. If he places three drops of buffer solution on each disk, then he must do the same with each case. If two drops of Brentamine fast Blue B are used in one case, then two drops must be used in every case. Third, and most important, the examiner must not deviate from his method of reading the test. An intense purple color must appear immediately or up to 5 s after the application of the chromagen.Then the test is positive.

The RTC with which the author was formerly associated listed four classifications under AP: negative, weak, medium, and strong. Under these circumstances, it is obvious that test results were meaningful only if all six examiners on the panel agreed on strict criteria of uniformity in their reports and only if each stage was defined. This was not done; what one physician would interpret as medium, another might read as strong.

It must be emphasized that the author is not comparing a qualitative with a quantitative method, but, rather, is underscoring his belief that the AP test employed in many centers is a reliable indicator of the presence of seminal fluid if one follows the three guidelines detailed herein.

The test described is very simple and can be performed in several minutes. By comparison, the least involved test making the most unsophisticated attempt at quantification consumes a minimum of 15 min and involves incubation of the unknown material. Hence, one must have access to an incubator. After incubation, one makes comparisons with three different colors: normal (about 0.6 Bodansky units), questionable (about 1.0 Bodansky unit), and markedly elevated (over 2.5 Bodansky units).

A much more sophisticated and accurate method of determining AP quantitatively employs a spectrophotometer, which is not customarily found in an RTC. Only a trained and knowledgeable person can use this laboratory apparatus. In addition, under ideal conditions, the test consumes approximately 40 min.

Some believe the quantitative method is more accurate than the qualitative one and assists in pinpointing the time of the assault, but the vagaries of the enzyme in vivo must be reckoned with. For example, one must question why, in one woman, with either method, the enzyme has a high value 10 h after coitus, yet in another woman 10 h after coitus

TABLE 2—*Stability of reagents.*

Time, h	Blank Disk	Seminal Fluid Disk
0	0	++++
1	0	++++
6	0	++++
12	0	++++
16	0	++++
24	0	++++
30	0	++++
36	0	++++
44	0	++++
50	+	++++

no enzyme is found. If a victim had to hike 10 km (six miles) to the nearest police station, very little AP would be found in the vaginal barrel because of her vigorous exercise and, possibly, the loss of much of the seminal fluid. It is also possible that the vaginal environment might be so hostile that—as sometimes in the case of spermatozoa—the enzyme might be destroyed within a few hours. There would be little difference as to whether a qualitative or a quantitative method were employed. If a true quantitative test, with a spectrophotometer and established standards, were to be performed in the RTC and if, for example, the test should yield a value of 2000 K-A units, then one would certainly have to agree that sexual intercourse had occurred recently. Then, in this context, the word "recently" would have to be defined. To one examiner "recently" might be within 5 h; to another, the word might extend to twice that time. With the entire concept being so nebulous, one must concede that a qualitative AP test resulting in an intense purple color would serve the same purpose. To fix the approximate time of the assault, the author draws upon three sources: (1) official police reports, (2) the victim's statement, and (3) the qualitative AP test.

Summary

Data from 100 cases of alleged rape victims demonstrate why the author believes the AP test to be a reliable tool, when employed by an expert for the identification of seminal fluid.

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