

# A new approach to postvasectomy semen analyses eliminates the need to evaluate a fresh specimen

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## Abstract

**Background:** According to current guidelines, confirmation that vasectomy results in sterility depends on microscopic examination of postvasectomy semen for the presence of spermatozoa. Guidelines established in 2012 require examination of a fresh specimen within 2 h of collection, which necessitates the patient making an appointment with either the surgeon's office or a licensed clinical laboratory. Twenty-five to 42% of patients fail to comply with postvasectomy semen analysis (PVSA).

**Objectives:** To determine if an at-home semen collection kit can substitute for the evaluation of a fresh specimen and improve patient compliance with postvasectomy spermatozoa assessment.

**Materials and methods:** The kit contains a patented aldehyde-fixative that maintains spermatozoa and semen cells in suspension for quantitation. Patients order a PVSA kit to be delivered to their home and can collect a semen specimen and return it to the laboratory through regular US mail.

**Results:** From January 2011 through December 2018, 6096 men undergoing vasectomy by 184 urologists in 33 states in the US ordered PVSA kits, of which 5408 (89%) returned at least one for analysis. Of those, 398 men (7.4%) returned the first kit with greater than 10,000 spermatozoa/ml within a year of vasectomy, of which only 4.4% contained greater than 100,000 spermatozoa/ml 12 weeks postsurgery. This suggests that fewer than 5% of postvasectomy patients might need follow-up fresh semen analyses, greatly easing the logistical burden of PVSA. Ninety percent of surgeons returning a patient satisfaction questionnaire said their patients "never" complained about using PVSA kits.

**Discussion and conclusion:** These data support the adoption of a new standard for PVSA that does not involve an initial evaluation of a fresh semen specimen.

## KEYWORDS

confirmation, fixed specimen, fresh specimen, guidelines, hemacytometer, postvasectomy, PVSA, semen analyses, spermatozoa count, vasectomy

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## 1 | INTRODUCTION

Vasectomy is the simplest, most cost effective approach to permanent fertility control, but according to the National Center for Health Statistics,<sup>1</sup> vasectomy is used for contraception by only 5.6% of women, in contrast to 18.1% who use tubal ligation for contraception. This preference for tubal ligation is despite evidence that vasectomy may provide more reliable contraception with a reported one pregnancy per 1000 vasectomies compared to one pregnancy per 200 tubal ligations.<sup>2,3</sup> Following a comprehensive review of vasectomy literature from 1949 to 2011, an American Urological Association panel concluded in 2012 that "Vasectomy should be considered for permanent contraception much more frequently than is the current practice in the US and many other nations".<sup>4</sup> The highest rate of vasectomy, only 1.3%, was reported among men aged 35–44 years.<sup>5</sup>

There are several surgical techniques for vasectomy,<sup>4</sup> but whatever the approach, it is necessary to verify the absence of ejaculated spermatozoa to guarantee sterility because of spermatozoa storage on the abdominal side of the ligation site and the potential for recanalization of the cut ends of the vas deferens.<sup>4,6</sup>

Postvasectomy semen analyses are routinely ordered by surgeons performing the vasectomy, but patient compliance is low, ranging from 58% to 75%.<sup>7,8</sup> The 2002 British Andrology Society guidelines recommended three postvasectomy semen analyses: two within several weeks of the procedure and a third at 1 year to rule out re-canalization. Vasectomy was considered a success if no motile spermatozoa were observed in the centrifuged pellets of fresh specimens submitted for evaluation within 2 h of collection.

In 2012, the American Urological Association published revised guidelines that vasectomy be considered a failure if any motile spermatozoa are seen on a PVSA scan at 6 months after vasectomy, and that successful vasectomy specimens contain fewer than 100,000 spermatozoa/ml after 6 months.<sup>4</sup> These same guidelines outlined that one postvasectomy semen analysis (PVSA) might be sufficient if there were fewer than or equal to 100,000 spermatozoa/ml, and none were motile, in a noncentrifuged specimen evaluated within 2 h of collection. The same year, the European Association of Urology published an extensive literature review<sup>9</sup> that concluded sterility was evidenced by fewer than 100,000 spermatozoa/ml of a semen specimen produced 3 months after vasectomy. In 2016, the British Andrology Society and the Canadian Urological Association published similar guidelines,<sup>10,11</sup> although the Canadian guidelines suggest examination of two semen specimens after surgery, both centrifuged and noncentrifuged, to assess the number of motile spermatozoa.

Since all current guidelines require an assessment of spermatozoa motility, the potential burden of delivering a fresh specimen within 2 h of collection to either the surgeon's office or a laboratory is thought to be a persistent barrier to patient compliance.<sup>4,12</sup>

spermatozoa concentration reports are complicated by the use of multiple methods to count spermatozoa. Many clinical laboratories use a standard hemacytometer with nine one millimeter (mm) square grids

each with a volume of 0.1  $\mu$ l. The limit to detection of spermatozoa in the specimen if all nine square grids are counted is one spermatozoa/0.9  $\mu$ l, equaling 1110 spermatozoa/ml of semen. An alternate counting chamber developed for andrology laboratories is the Makler Chamber, which has a 1-mm square grid holding 0.01  $\mu$ l; a single spermatozoa in a Makler Chamber is 100,000 spermatozoa/ml of semen.

Another traditional spermatozoa counting method is spermatozoa per "high powered field" (HPF). This involves putting a drop of semen under a standard cover slip and examining a few fields at 40X with a 10X eye piece = 400X. The estimated volume of each field is four nanoliters (nl). Therefore, one spermatozoa/HPF equals 250,000 spermatozoa/ml of semen, necessitating the examination of multiple HPFs.

Therefore, counting spermatozoa in all nine squares of a standard hemacytometer grid is at least an order of magnitude more sensitive than other counting methods.

PVSA kit is a home collection method, originally developed to research the burden of HIV in semen in the early 1990s.<sup>13</sup> It has been adapted for postvasectomy semen analyses. The method employs an aldehyde-based fixative specifically formulated to maintain all cells in suspension for analysis, which renders the spermatozoa nonmotile, so vasectomy success needs to be based on spermatozoa count alone, not spermatozoa motility. The concern about being unable to assess motility in a fixed specimen has recently been addressed by an assessment of spermatozoa motility in postvasectomy specimens with low spermatozoa count.<sup>14</sup> Of 6491 vasectomies, 2100 (32%) had spermatozoa seen in the range of 100–99,999/ml in a postsurgery specimen, of which only 29 (1.4%) had a few motile spermatozoa, with 20 of those (2.2%) in the 907(14%) specimens with spermatozoa seen in the range of 100–9999.<sup>14</sup> Therefore, 99.7% of the postvasectomy specimens with spermatozoa counts less than 9999/ml had no motile spermatozoa.

To compare PVSA kit results to the guidelines requiring examination of fresh specimens, we have retrospectively analyzed 8 years of PVSA kit test results. Our results support a modification to the current guidelines wherein fewer than 10,000 spermatozoa/ml in fixed specimens are evidence of sterility, thus negating the need for evaluation of a fresh specimen.

## 2 | METHODS

Semen contains a high concentration of protein, which causes it to congeal in conventional 10% formaldehyde fixatives. For this reason, an aldehyde-based semen fixative (Patent # 5,618k,664, 1997) was developed specifically to maintain all semen cells in suspension for analysis of HIV-infected leukocytes in semen.<sup>13</sup> Neither freeze-thaw, nor heating to 65°C, altered the analysis of spermatozoa and leukocytes in fixed specimens, which were found to be stable for at least 5 years.

PVSA kits include tamper-evident bottles of eight ml of the fixative, spiked with glutaraldehyde-fixed red blood cells to serve as an internal control for deleterious conditions during transport (Figure 1). The kit also includes a condom and instructions in a US Postal



**FIGURE 1** Picture of postvasectomy semen analysis (PVSA) kit

Service approved seamless plastic transport box with absorbent liners, originally designed to mail 15-ml urine specimens for testing. The instructions direct the patient to collect a semen specimen in the condom by either masturbation or intercourse, wait 30 min, then add the entire volume of the specimen to the bottle of fixative. The fixed specimen of approximately 8.5–13 ml is returned to the seamless plastic transport box with absorbent liners placed inside the pre-addressed cardboard mailer for return to a clinical laboratory for hemacytometer analysis.

Spermatozoa counts reported include the assumption that there is a 1:5 dilution of the semen specimen (average volume of 2.0 ml) when added to the 8 ml of fixative. The range of error in this assumption relates to the actual volume of semen, for example, 0.5 ml of semen would have a 1:13 dilution factor, whereas 5 ml of semen would have a 1:2.5 dilution factor. The total volume in the PVSA bottle is measured when returned to the laboratory, and the internal control red blood cells are counted to estimate fixative loss or specimen degradation during handling and transport. If the total volume is less than 8 ml, and/or the red blood cell count is outside the accepted range of 0.2–2 million/ml of fixative, the specimen is considered not acceptable for counting, and a repeat specimen is requested.

Manual counts of all nine squares of the Neubauer hemacytometer represent cells/0.9  $\mu$ l of fixed specimen; applying a multiplication factor of five to adjust for the 1:5 average specimen dilution in the fixative yields an estimate of spermatozoa concentration in the original semen specimen. The fixed specimens are archived for 2 years.

Specimens that contain a high background of fixed protein debris are stained with the fluorescent dye, Hoechst, to aid in identifying

spermatozoa heads, and scanned in an EVOS 7000 inverted fluorescent microscope for spermatozoa head identification (Figure 2).

The surgeon determines the length of time between the vasectomy and the PVSA, and whether or not reports are mailed/faxed directly to the patient as well as to the surgeon's office.

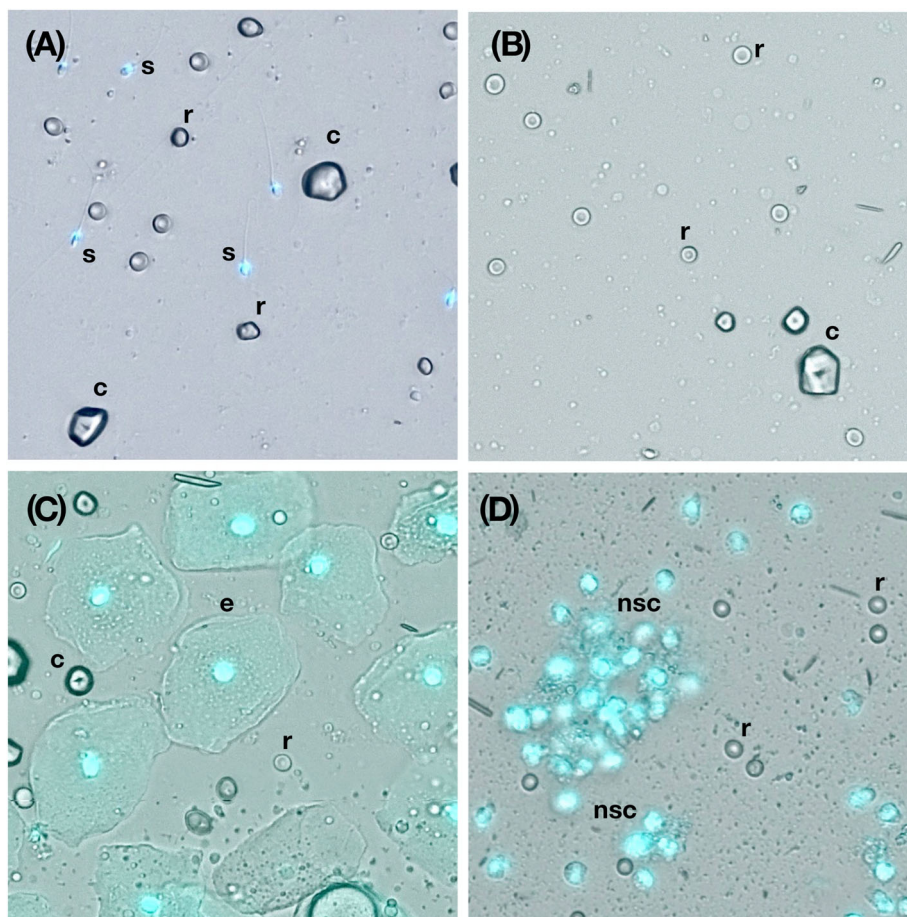
The first few thousand PVSA kits included a blinded questionnaire about the ease of use of the kit and the demographics of the patient (Figure 3), at the suggestion of a Food and Drug Administration officer who indicated a need to demonstrate that men of all ethnic backgrounds from all over the country could understand and use the kit. The cost of each kit is approximately \$50 including mailing and reporting, which includes Current Procedural Terminology code information for insurance claims.

### 3 | RESULTS

#### 3.1 | Demographics

From June 2009 through June, 2014, 2530 kits were returned with completed multiple-choice questionnaires. The questionnaire tallies (Table 1) supported the ability of men of all ages and ethnicities to use PVSA, and revealed a majority of vasectomies were undertaken by white (90%) college educated (82%) men, 31–50 (90%) years of age.

Thirty urologists returned a questionnaire asking about patient and physician satisfaction with PVSA, 27 (90%) of whom circled “never” for patient complaints, and 28 (93%) circled “always” for satisfaction with reports received.



**FIGURE 2** Fixed Semen Photomicrograph. Merged bright field and fluorescent images of four fixed postvasectomy semen specimens stained with Hoechst 33342 which fluoresces specifically when bound to DNA. In all frames, s = spermatozoa, r = internal control fixed red blood cell, c = semen crystal, e = epithelial cell, nsc = nonspermatozoa cell. Frame A is an example of a specimen containing spermatozoa, Frames B–D are examples of specimen containing no spermatozoa greater than 10,000/ml. Frame C illustrates abundant epithelial cells in a fixed specimen, and frame D illustrates abundant “nonspermatozoa cells,” morphology consistent with leukocytes, in a fixed specimen.

### 3.2 | Patient compliance

From January, 2011, through December, 2018, 6096 men undergoing vasectomy by 184 urologists in 33 states in the US ordered PVSA kits for semen analyses. Five thousand four hundred and eight (89%) returned at least one PVSA kit, and 1956 (30%) returned at least two kits.

### 3.3 | Vasectomy results

Of the 5408 first kits returned, only 398 (7.4%) overall had greater than 10,000 spermatozoa/ml of semen, and only 213 (3.9%) contained greater than 100,000 spermatozoa/ml, broken down by interval postvasectomy in Table 2.

As indicated in Table 2, although residual spermatozoa are detectable in the semen of some men up to a year following vasectomy, sterility is achieved within a few weeks of vasectomy for most patients, with 95% of men ejaculating fewer than 100,000 spermatozoa/ml

12–16 weeks following vasectomy. Of the 168 men submitting semen specimens with greater than 100,000 spermatozoa/ml within 20 weeks of their vasectomy, 139 (83%) submitted a second PVSA kit an average of 11 weeks later, of which 55 (40%) still contained greater than 100,000 spermatozoa/ml. Thirty-one (56%) of those 55 returned a third PVSA specimen of which 15 (48%) still contained greater than 100,000 spermatozoa/ml. Therefore, of the 4260 men contributing first PVSA specimens within 20 weeks of their vasectomy, only 16 (0.38%) of them still ejaculated greater than 100,000 spermatozoa an average of 10 weeks later. It is this small subset of patients that might need to submit fresh specimens for further analyses.

It is interesting that 23 (3.1%) of the specimens from the 751 patients that delayed past 20 weeks to submit their first PVSA specimen contained greater than 100,000 spermatozoa/ml. Of those only 14 men submitted a second kit an average of 13 weeks later, of which six still contained greater than 100,000 spermatozoa/ml.

The 397 men submitting first kits more than 1 year following vasectomy, 58 of whom submitted kits 5 years postvasectomy, represent

**Questionnaire for Food and Drug Administration**

Today's Date: \_\_\_\_\_

I live in: \_\_\_\_\_  
(name of state, if in the U.S.; name of country, if outside the U.S.)

My highest level of education is: (circle one)

high school      college      post-graduate

My ethnic background is: (circle one)

caucasian    asian    black    hispanic    other

My age range is: (circle one)

21-30    31-40    41-50    51-60    over 60

Please circle your answer to the following questions about using PVSA. Rate 1-5, with 1 indicating 'strongly agree' to 5 indicating 'strongly disagree'.

The directions were easy to understand.

1 2 3 4 5  
Agree Disagree

My sample was easy to collect.

1 2 3 4 5  
Agree Disagree

A condom is the best way to collect my sample.

1 2 3 4 5  
Agree Disagree

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P.O. Box 1028 | Bedford, MA 01730 | 617.623.7447  
[www.pvsa.org](http://www.pvsa.org)

Thank you. Please return with your kit.

**FIGURE 3** Questionnaire

**TABLE 1** Demographics of postvasectomy semen analysis (PVSA) users and patient satisfaction assessment

Highest level of education	High school 18%	College 56%	Postgraduate 26%		
Ethnicity	Caucasian 90%	Asian 1%	Black 2%	Hispanic 6%	Other 1%
Age range	21-30 6%	31-40 52%	41-50 38%	51-60 3%	Over 61 0
	Agree (1)	(2)	(3)	(4)	Disagree (5)
PVSA directions were easy to understand	78%	15%	2%	4%	1%
Sample was easy to collect	70%	19%	7%	3%	1%
A condom is the best way to collect a sample	63%	17%	12%	4%	4%

**TABLE 2** Postvasectomy semen analysis (PVSA) spermatozoa counts at intervals postvasectomy

Weeks post vasectomy	First kits returned	<sup>a</sup> Specimens with >15,000 sp/ml (%)	<sup>b</sup> Specimens with >100,000 spermatozoa/ml (%)
1-4	172	23 (13%)	10 (5.8%)
4-8	967	117 (12%)	60 (6.2%)
8-12	1300	98 (7.5%)	57 (4.4%)
12-16	1280	66 (5%)	32 (2.5%)
16-20	541	26 (5%)	9 (1.7%)
20-52	751	29 (4%)	23 (3.1%)
>52	397	39 (10%)	22 (5.5%)

Note: The date of vasectomy was listed by the patient; no data about number of ejaculations prior to specimen submission were obtained.

<sup>a</sup>The threshold of spermatozoa detection by PVSA.

<sup>b</sup>Current guideline for possible fertility.

a separate subset of men who may have been double-checking their sterility for reasons not disclosed to the PVSA lab.

One pregnancy was reported following an initial spermatozoa count of 155,600/ml, 6 weeks after vasectomy. The patient had not returned a second kit for a repeat analysis.

The 304 semen specimens containing greater than 10,000 spermatozoa/ml at 16 weeks postvasectomy were distributed among 56 surgeons, indicating that surgeon and surgical approach were less important than patient variability.

A few patients exhibited unusual spermatozoa counts: one 35-year-old sent in a total of five kits: the first kit at 8 weeks postvasectomy was fewer than 10,000 spermatozoa/ml; a second kit submitted 11 weeks postvasectomy contained 39,000 spermatozoa/ml; a third kit submitted 20 weeks later contained 800,000 spermatozoa/ml. After a 5-month interval, two repeat specimens 2 months apart were fewer than 10,000 spermatozoa/ml. Another 38 years old also submitted five kits: the first had fewer than 10,000 spermatozoa/ml, 8 weeks after vasectomy; a second had 33,000 spermatozoa/ml at 10 weeks; a third had 25,000 spermatozoa/ml at 15 weeks after vasectomy; a fourth at 26 weeks had fewer than 10,000 spermatozoa/ml, and a fifth at 34 weeks also had fewer than 10,000 spermatozoa/ml. Another patient submitted four kits: first specimen at 6 weeks postsurgery had 117,000 spermatozoa/ml; the second specimen at 7 weeks had fewer than 10,000 spermatozoa/ml; the third specimen at 9 weeks had 133,000 spermatozoa/ml; a fourth specimen at 14 weeks had 19,400 spermatozoa/ml. Another patient submitting three kits had 1.1 million spm/ml 9 weeks postvasectomy, fewer than 10,000 spermatozoa/ml at 12 weeks postvasectomy and 22,000 spermatozoa/ml at 16 weeks postvasectomy. Occasional reappearance of spermatozoa postvasectomy has been reported.<sup>10</sup>

### 3.4 | Hoechst staining

Some semen specimens exhibit more random-sized clumps of fixed protein and cellular structures than other specimens. We have taken advantage of the DNA-specific dye, Hoechst 33258, that fluoresces when exposed to ultraviolet light if bound to DNA, to more readily visualize spermatozoa heads in specimens with a high background of semen crystals and subcellular structures such as prostasomes, as shown in Figure 2. A comparison of counts with Hoechst-stain detection of spermatozoa heads agreed with nonstained specimens with low background clumps, and easier visualization of spermatozoa heads in specimens with high background, so the counts by both methods have been included in this study.

## 4 | DISCUSSION

The finding that only 5% of semen specimens contained greater than 100,000 spermatozoa/ml 12 weeks following vasectomy by 184 different urologists strongly supports the efficacy of this cost-effective

contraceptive approach. spermatozoa counts this low fall within the 0.3% of postvasectomy specimens that might have some motile spermatozoa.<sup>14</sup> Repeat PVSA specimens a few weeks later usually resulted in fewer than 10,000 spermatozoa/ml, thus only a small percentage of vasectomized men need to submit fresh specimens for motility assessment, determined by the surgeon.

The compliance rate for patients ordering PVSA test kits is 89% for at least one kit. This could be due to the commitment to following through if a test kit is ordered, and the ease of collecting the postvasectomy semen sample at home with no 2 h time constraint for delivery.

For physician's offices wishing to perform the spermatozoa counts themselves, the kits can be returned directly to that office. They can also be counted by any clinical laboratory licensed to perform semen analyses.

An additional advantage to examining a preserved semen specimen is the ability to detect elevated concentrations of other cells, such as inflammatory cells. PVSA scientists are in the process of analyzing PVSA specimens for incidence and concentrations of inflammatory cells by immunostaining specimens for leukocyte-specific and prostate-specific antigens.<sup>15,16</sup> It will be possible in the future to notify surgeons of leukocyte and prostate cell concentrations that may be clinically significant, for example, greater than two million/ml of semen.

The addition of fluorescent staining for DNA improves the ease of obtaining spermatozoa counts in semen specimens with a high background of crystals and subcellular entities, such as prostasomes<sup>16</sup> and fixed proteins.

Although this study provides data that nearly 90% of men ordering a PVSA kit return it for spermatozoa count, a limitation to understanding an effect on overall compliance with postvasectomy testing is lack of information on the number of men who never order any semen test after vasectomy.

## 5 | CONCLUSION

Sterility confirmation is necessary after vasectomy. On the order of 95% of patients ejaculate too few spermatozoa 12 weeks after surgery to warrant the need to evaluate fresh semen for motile spermatozoa. In addition to the cytologic advantages of a suitably fixed specimen that can be archived for several months and evaluated for specific cell types, the improved convenience for both the patient and the evaluating laboratory or doctor's office provides strong support for this new paradigm in postvasectomy semen analyses.

### AUTHOR CONTRIBUTIONS

In addition to being the corresponding author, Ryan Kiessler's contribution to this manuscript was research design oversight, data analysis, and manuscript preparation. Alex Hauser gathered data and organized and prepared data for publication. Ann Kiessler and Robert Eyre were responsible for research design oversight, data organization and analysis, and manuscript preparation.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. cd\_value\_code=text

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