

# Accelerated Tissue Processing With Minimal Formalin Fixation Time for 9-Gauge Vacuum-Assisted Breast Biopsy Specimens

## A Proof of Principle

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**Key Words:** Breast cancer; Diagnosis; Fixation; Accelerated processing; Core needle biopsy

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## ABSTRACT

**Objectives:** Vacuum-assisted biopsy (VAB) of the breast seems unsuitable for rapid processing due to large size. We tested microwave-based acceleration.

**Methods:** As a proof-of-principle study, 9-gauge VAB specimens were taken from eight mastectomy specimens. Forty-two biopsy specimens were processed. Quality of H&E was evaluated in 84 slides, and estrogen receptor (ER), progesterone receptor (PR), E-cadherin, and human epidermal growth factor receptor 2 (HER2) stains were evaluated in six slides. Preoperative biopsy specimens were used as a control.

**Results:** Diagnostic quality of H&E slides was good in 87%, reasonable in 12%, and low in 1%. Quality of E-cadherin was good in 75% and reasonable in 25%. Quality of ER was good in 83% and reasonable in 17%. PR and both HER2 immunohistochemistry and fluorescence in situ hybridization were good in all slides. Quality of experimental slides was similar to control slides.

**Conclusions:** Nine-gauge VAB specimens can be processed within 4 hours. Slides are suitable for all routine pathologic stains. This enables a same-day diagnosis.

With increasing emphasis on expediting the diagnostic process of breast cancer, physicians aim to improve diagnostic tracks in the breast clinic. There have been big advancements for patients with suspicious breast lesions, using techniques such as fine-needle aspiration cytology, core wash cytology, and accelerated core needle biopsy (CNB) in enabling a pathologic diagnosis within 1 day in most cases.<sup>1-3</sup> Patients with microcalcifications on screening mammograms, however, did not yet benefit from these developments. The number of patients referred with microcalcifications varies, depending on the screening program. In our clinic, 40% of biopsy specimens are stereotactic.<sup>1</sup> A recent large French single-institution series reviewing all new referrals showed that 69% of referrals were for masses and 31% were for microcalcifications or other nonmass lesions in 10,000 patients.<sup>4</sup> The number of referred patients with microcalcifications has risen since the introduction of digital mammography.<sup>5</sup> Most screened patients with microcalcifications (65%) turn out to have benign breast disease.<sup>6</sup> Nonetheless, the period between screening and final diagnosis is not any less stressful for these patients than those receiving a malignant diagnosis. Women with benign microcalcifications and those requiring magnetic resonance imaging (MRI)-guided biopsy (MRGB) would therefore definitely benefit from an expedient, one-stop-shop diagnosis.

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American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines dictate all histologic breast pathology specimens (both CNB and excision specimens) should receive at least 6 hours of formalin fixation.<sup>7,8</sup> This makes it virtually impossible to run a true one-stop-shop breast clinic using CNB. For the thinner, mostly 14-gauge (~1.6-mm diameter) handheld ultrasound-guided CNBs that are used for most mass lesions (eg, patients with a palpable breast lump), there is increasing literature available supporting the use of accelerated processing, with less than 6 hours of formalin fixation.<sup>1,9,10</sup>

In patients with microcalcifications, two problems arise that make these lesions less amenable to fast-track diagnostics. Microcalcifications almost always require stereotactic biopsy, which is more time- and resource-consuming than handheld ultrasound-guided biopsy. Moreover, vacuum-assisted biopsy (VAB) needles (11 to 7 gauge, approximate diameter 2.3-3.7 mm) are typically used to retrieve larger biopsy specimens.<sup>5</sup> These biopsy specimens are thought not to be suitable for accelerated processing. If patients with microcalcifications are selected at referral, and their first clinic visit is planned with a corresponding stereotactic biopsy slot, it is possible (although logistically demanding) to immediately perform a stereotactic biopsy. We hypothesized that the second problem, more time-consuming fixation of the biopsy specimen, can be overcome using adjusted parameters for the accelerated processing technique previously described.<sup>1</sup> The current study was designed to test whether this is technically feasible.

The hypothesis is that expedited tissue processing of large core biopsy specimens does not compromise subsequent histologic diagnosis. We tested routine H&E staining (results immediately available) and suitability of processed tissue for subsequent immunohistochemistry (IHC) testing of estrogen receptor (ER), progesterone receptor (PR), and E-cadherin status, as well as human epidermal growth factor receptor 2 (HER2) status assessment with both IHC and fluorescence in situ hybridization (FISH) (results available the next day).

## Materials and Methods

### Source of Material

In our university hospital, the use of anonymous leftover material acquired as a by-product of routine care processes for scientific purposes is governed by a strict guideline. The local ethical review board judged our study protocol to be in full compliance with this guideline;

therefore, a full review by the board was deemed not necessary.

### Stage 1

For the first stage of our study, we used archival material from surgical mastectomy specimens after overnight fixation in formalin. The tissue was cut into fragments resembling size and shape of 9-gauge (3-mm) VAB specimens. Tissue was then processed using a number of different program settings for the rapid microwave histoprocessor (Pathos; Milestone Srl) to test the feasibility of each individual processing step. Different durations of formalin fixation, durations of dehydration steps, and the use of two different dehydrating agents (JFC solution [Milestone Srl] and isopropyl alcohol) were tested. All fixation and dehydration steps were accelerated by microwave processing. Minimal fixation time in all programs was 20 minutes of (microwave-assisted) formalin fixation, with a maximum of 30 minutes. All specimens were then paraffin embedded, cut, and stained according to standard clinical practice. Technical specifications of IHC and FISH staining procedures are provided as supplemental digital content (all supplemental materials can be found at *American Journal of Clinical Pathology* online). Afterward, specimens were evaluated for ease of cutting, preservation of tissue architecture, and quality of H&E staining. An experienced breast pathologist (P.B.) and senior technician (L.J.M.B.) selected the shortest possible best-performing program for stage 2.

### Stage 2

For the second stage, we used eight surgical mastectomy specimens: six with known malignant disease and two prophylactically removed. A breast radiologist (R.M.M. or M.v.d.L.) took vacuum-assisted 9-gauge (2.9-mm) biopsy specimens from each mastectomy specimen under ultrasound guidance. Three to seven VAB rounds were performed in every specimen: in most cases, normal breast tissue in the four quadrants and centrally behind the nipple and, if present (six cases), one of the malignancy. Cores were prefixed in formalin, and the time of biopsy and the time of automatic processing were recorded. The resulting material was included in the regular assessment of the mastectomy specimens.

Material from each biopsy location was divided equally into pairs, cassettes were filled with three to five biopsy cores each, and each pair was processed in duplicate using the program selected in stage 1 by two different (but very similar) microwave-assisted histoprocessors, "Pathos" and "Logos," both from the same supplier (Milestone Srl). The main difference in processing between

**Table 1**  
Quality of Material by Study Stage

Characteristic	Stage 2	Benchmark
Patients, No.	8	6
Biopsy specimens, No.	42	7
Paraffin blocks, No.	84	9
H&E-stained slides, No. <sup>a</sup>	84	44
Low quality, %	-1	-50
Reasonable quality, %	-12	-50
Good quality, %	-87	
ER-stained slides, No. <sup>a</sup>	6	3
Low quality, %	-83	-33
Reasonable quality, %	-17	-33
Good quality, %		-33
PR-stained slides, No. <sup>a</sup>	6	3
Low quality, %	-100	-17
Reasonable quality, %		-33
Good quality, %		-50
E-cadherin-stained slides, No. <sup>a</sup>	6	3
Low quality, %	-25	-17
Reasonable quality, %	-75	-17
Good quality, %		-66
HER2 IHC, No. <sup>a</sup>	6	
Good quality, %	-100	
HER2 FISH, No. <sup>a</sup>	6	
Good quality, %	-100	

ER, estrogen receptor; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; PR, progesterone receptor.

<sup>a</sup>No slides were scored as poor quality.

these machines is that Pathos uses vacuum to speed up paraffin infiltration (at 65°C), while Logos uses a higher paraffin temperature (80°C) to speed up this process. Fixation and dehydration processes are identical. From every paraffin block, one H&E-stained slide was made.

The VAB specimens were processed and evaluated for integrity of tissue architecture and quality with H&E staining independently by a pathology resident (A.H.) and an experienced breast pathologist (P.B.). Both evaluations were valued equally and included separately in the final analysis. Of three cases with invasive tumor, ER, PR, E-cadherin IHC, and HER2 status (by both IHC and FISH) were assessed. Technical specifications of IHC and FISH staining procedures are provided as an online supplement (online resource 1). IHC was also independently evaluated by A.H. and P.B., and FISH was evaluated (as in routine clinical practice in our laboratory) by two trained laboratory technicians. This means that for the final program, 84 slides were evaluated for tissue architecture and quality with H&E staining and six slides for ER, PR, E-cadherin, and HER2 IHC. There were six slides of three biopsy specimens available for HER2 FISH assessment (Table 1). Quality of the H&E- and IHC-stained slides was scored as “good,” “reasonable,” “low,” or “poor,” parallel to daily clinical practice for diagnostic purposes, which inherently means it is subjective

to some degree. A good score indicated optimal quality, and a poor score indicated the slide was of insufficient quality to use for diagnostics. Reasonable and low scoring were in between these two extremes, containing varying suboptimal features, not influencing diagnostic use.

### Benchmark

As a benchmark, the quality of 44 H&E slides from seven corresponding preoperative biopsy specimens (nine paraffin blocks) was scored per case (total of six cases; two preventive mastectomy specimens were not biopsied preoperatively). Three IHC-stained E-cadherin, ER, and PR slides were similarly independently scored in the corresponding “regular,” diagnostic CNB specimens obtained prior to surgery by the same two observers (A.H. and P.B.). Processing of these biopsy specimens was according to our laboratory’s standard clinical practice: five of these biopsy specimens (14 gauge; five paraffin blocks) were processed by our previously described accelerated processing program for thinner breast biopsy specimens.<sup>1</sup> Two biopsy specimens (9-gauge VAB; four paraffin blocks) were processed by conventional, overnight formalin fixation and standard processing methods.

A schematic visualization of the methods is depicted in Figure 1, and a short description per study stage is included in Table 2.

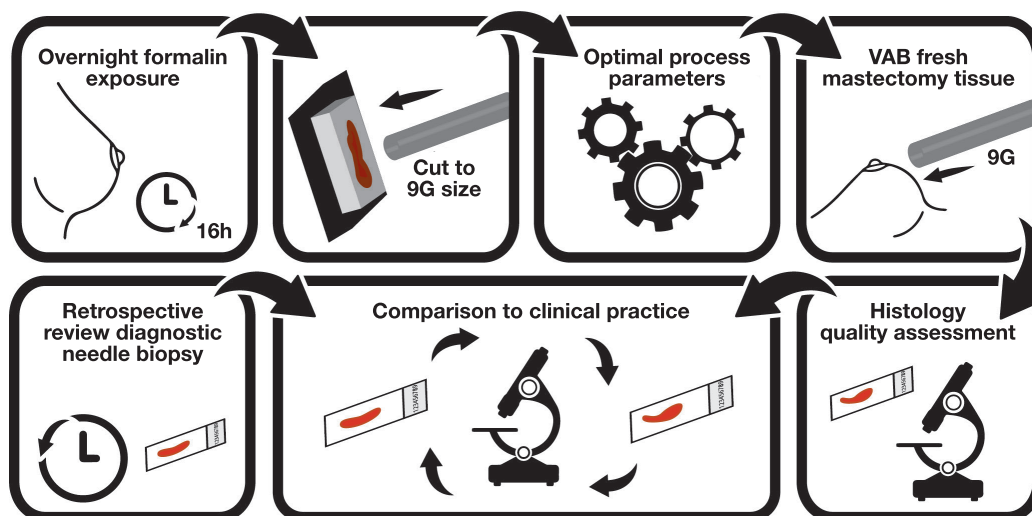
## Results

### Stage 1

Stage 1 resulted in the optimal program parameters for the shortest program that still produced stable, good-quality paraffin blocks that were easily sectioned to good-quality slides. The program consists of a 20-minute formalin fixation step, followed by rinsing with ethanol 60%, then 20 minutes of ethanol 100%, followed by 40 minutes of isopropyl alcohol, and finishing with 55 minutes of paraffin infiltration (either vacuum assisted at 65°C in Pathos or nonvacuum assisted at 80°C in Logos).

These settings were adopted as our standard program for VAB processing in stage 2, and all slides further described in the Results section were processed using this program.

Alternative programs that were tested but yielded lesser quality results consisted of combinations of 25 and 30 minutes of formalin fixation and 20 or 55 minutes of either ethanol 100% or “JFC” solution. Variation in dehydration steps was more important for results than variation in formalin fixation duration.



**Figure 1** Schematic visualization of the study. VAB, vacuum-assisted biopsy.

**Table 2**

**Description of Study Stages**

Characteristic	Stage 1	Stage 2	Benchmark
Material	Archive material Cut to 9-gauge size and shape	Eight fresh mastectomy specimens Three to seven VAB rounds each Nine-gauge VAB (taken ex vivo)	Diagnostic biopsy specimens (retrospectively reviewed)
Preprocessing formalin exposure	Overnight formalin fixation prior to sampling	Fifteen to 70 minutes of prefixation after biopsy, before processing	Variable
Processing	Different durations of formalin fixation, dehydration, and dehydrating agents	Optimal parameters from stage 1: -20 minutes formalin fixation -Rinsing in ethanol 60% -20 minutes ethanol 100% -40 minutes isopropyl alcohol -55 minutes paraffin infiltration	Either: -Conventional overnight processing -“Conventional” accelerated processing
Result	Optimal processing parameters	Forty-two paraffin blocks, 84 H&E slides, six slides of E-cadherin/ER/PR/HER2 IHC/HER2 FISH each	Nine paraffin blocks, 44 H&E slides, three slides of E-cadherin/ER/PR each
Shows	9-gauge size tissue fragments can be processed using an accelerated processing program	Quality of slides of 9-gauge VAB specimens subjected to accelerated processing is good	Quality of slides of 9-gauge aCNB VAB specimen is similar to regular slides

aCNB, accelerated core needle biopsy; ER, estrogen receptor; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; PR, progesterone receptor; VAB, vacuum-assisted biopsy.

## Stage 2

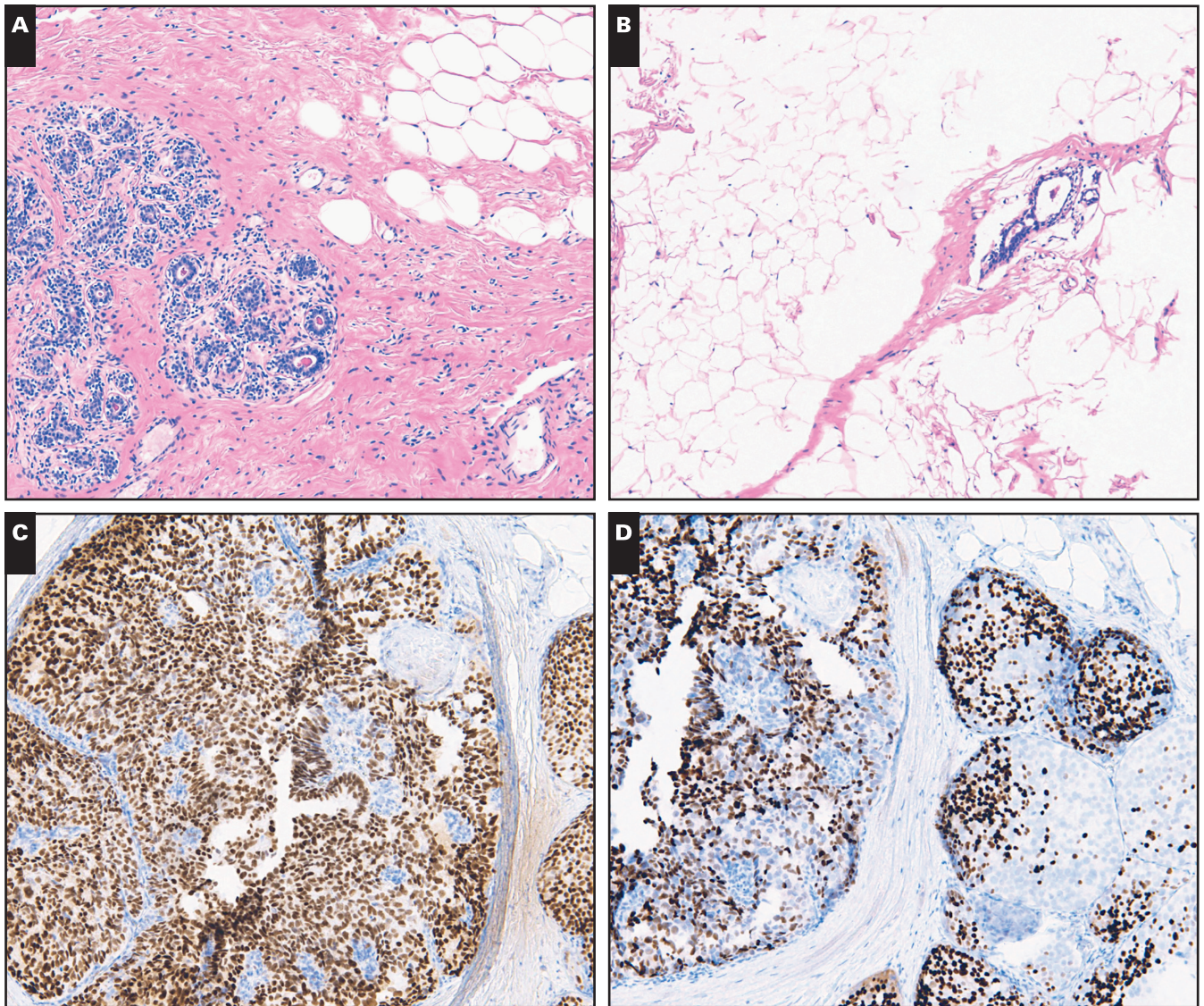
In stage 2, prefixation time varied from 15 to 70 minutes. Duration of prefixation had no measurable effect on slide quality. The tissue architecture and quality of H&E-stained slides of 42 biopsy specimens were evaluated. It was scored as good in 87%, reasonable in 12%, and low in 1%. Most frequently, a lower score than good was due to a focal or partial collapse of fatty tissue processed by Pathos: a characteristic that does not affect diagnostic assessment but can affect preservation and DNA quality. None of the slides were scored as poor. Quality of the IHC-stained slides for E-cadherin (six slides) was scored

as good in 75% and reasonable in 25%. ER IHC (six slides) was in 83% good and 17% reasonable. PR IHC (six slides) was scored good in all slides (100%). Both HER2 IHC (six slides) and HER2 FISH (six slides) were scored as good in 100% (Table 2). Examples of slides are shown in Image 1.

## Benchmark

Quality of our benchmark assessment of the preceding diagnostic core needle biopsy specimens was scored for H&E as good in 50% and reasonable in 50%. E-cadherin was scored as good in 66%, reasonable in





**Image 1** Examples of slides from stage 2 (×100). **A**, Example of an H&E-stained slide, rated as “good” quality. **B**, Example of an H&E-stained slide, rated as “low” quality. **C**, Example of an immunohistochemistry (IHC)–stained slide for estrogen receptor, rated as good quality. **D**, Example of an IHC-stained slide for progesterone receptor, rated as good quality.

17%, and low in 17%. Quality of ER staining was scored as 33% good, 33% reasonable, and 33% low. PR quality was scored as good in 50%, reasonable in 33%, and low in 17%. In both experimental and control cases, no slides were scored as poor (Table 2).

### Differences Between Pathos and Logos

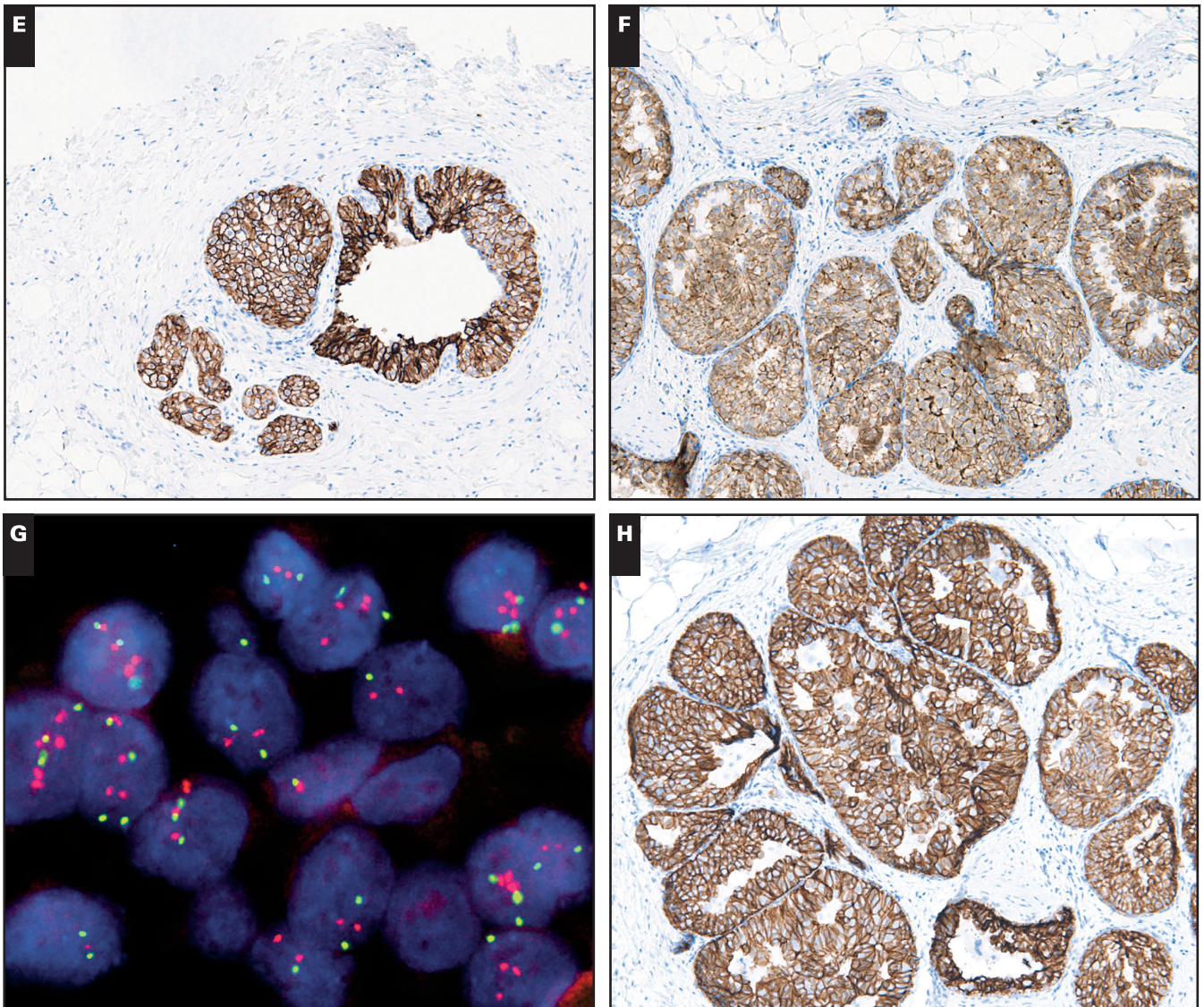
There were minor differences between the tissues processed by Pathos and Logos. The paraffin blocks from tissues processed by Pathos were less well dehydrated than the tissues processed by Logos. The collapse of the fat tissue in the H&E-stained slides was seen more often in the tissues processed by Pathos than by Logos. Overall

observed quality seems similar for both processors. Limited sample size and small differences in results mean no reliable formal comparison between Pathos and Logos can be made in this study.

### Discussion

Our study shows that it is technically feasible to use accelerated microwave-based processing for 9-gauge VAB biopsy specimens in approximately 2 hours, 20 minutes. Taking into account 15 minutes of prefixation/transport time from the radiology department and 1 hour, 25 minutes for routine paraffin embedding, cutting, and staining





**Image 1** (cont) **E**, Example of an IHC-stained slide for human epidermal growth factor receptor 2 (HER2), rated as good quality. **F**, Example of an IHC-stained slide for HER2, rated as low quality. **G**, Example of a fluorescence in situ hybridization–stained slide for HER2; green spots are CEP17 (chromosome 17 centromere) as a reference, and red spots are HER2. **H**, Example of an IHC-stained slide for E-cadherin, rated as good quality.

in the pathology laboratory, the slides can be ready for evaluation by the pathologist within 4 hours after the biopsy specimen has been taken. This means that it is technically feasible to include patients requiring a large core breast biopsy in a true one-stop-shop and 1-day diagnosis breast program. Quality of the resulting slides was similar to the quality of our (small) benchmarking set of regularly processed CNB specimens.

A (slightly quicker) variant of this technique for the thinner 14-gauge, 16-gauge, and 18-gauge (1.6-, 1.2-, and 0.8-mm, respectively) CNB specimens has been used routinely in our breast clinic for many years. The results of this program have been published previously, including

the reliability for basic histologic assessment and subsequent IHC for hormone receptor assessment, HER2 FISH, and E-cadherin staining.<sup>1,9</sup> All these tests proved to yield reliable results in the thinner specimens. Reliability of ER and PR status in specimens subjected to accelerated processing was also confirmed by a study by another pathology department,<sup>10</sup> and reliability of Ki-67 marker and HER2 IHC was the subject of joint publications.<sup>11,12</sup>

So far, patients with microcalcifications or lesions only visible on MRI had to forego the accelerated processing technique. These patients often require a stereotactic biopsy or MRGB during a second appointment and then subsequently a third appointment when definitive

results are available.<sup>5</sup> This category of patients actually has a somewhat lower probability of malignancy than patients with mass lesions requiring biopsy (26% vs 46% in a recent single-institution series of 10,000 patients and 36% vs 40% in our own institution).<sup>1,4</sup> However, the psychological stress is no less intense and is prolonged due to the multiple appointments required. This eventually benign category of patients will likely gain the most from implementing the accelerated processing method, especially since no additional stains will be required. Patients who do receive a malignant result, however, will still have to wait an additional day for the results of additional stains (eg, hormone receptor and HER2 status) before a complete treatment plan can be formulated and communicated. By the time this treatment plan is available, most patients will have overcome the initial shock of a cancer diagnosis and are more likely to adequately process and consider treatment options.

It is important to realize that there is some evidence that the period between the start of investigations and the final diagnosis is the most stressful of the entire disease process.<sup>13</sup> The duration of this period is dictated by two factors: quick access to stereotactic biopsy and MRGB, as well as the pathologic processing required for the thicker biopsy specimens. This study shows that processing of large core biopsy specimens does not preclude a same-day diagnosis. While logistically demanding, upon referral of patients with microcalcifications, it is possible to schedule stereotactic biopsy at the time of their first hospital visit.

Since VAB specimens are deposited in formalin-filled transportation containers in the radiology department, total formalin exposure is somewhat more than just the 20 minutes of microwave-assisted fixation. In practice, this “prefixation” can be anywhere between 15 and 45 minutes, which means a total formalin exposure in a clinical 1-day diagnosis program would be between 35 and 65 minutes. Our study mimics this, as it was performed in a clinically active pathology laboratory. As a result, there is also some variation in “prefixation” duration for our experimental samples: between 15 and 70 minutes of prefixation formalin exposure. Slides with optimal and suboptimal results were spread out evenly along this interval. All cases were well under the 6 hours minimum that is considered required by the ASCO/CAP guidelines for breast biopsy specimens.<sup>8,14</sup> There is already sufficient evidence available that smaller 16- to 18-gauge biopsy specimens do not necessarily require the full 6 hours of formalin fixation.<sup>1,9,10,15</sup> The current study suggests the same holds true for the thicker 9-gauge biopsy specimens. Currently, the ASCO/CAP guidelines do not differentiate minimal formalin fixation time between CNBs and surgical resections. The next update of the ASCO/CAP guidelines should reduce

minimal formalin fixation required for CNBs when microwave-assisted processing is used.

While the accelerated processing procedure uses specialized equipment, it does not use breast-dedicated equipment. In our clinic, the same histoprocessors are used for accelerated processing of skin, colonic, lymph node, and soft tissue biopsy specimens. Furthermore, it is used for all regular (nonaccelerated) histology workflow. Naturally, facilitating a same-day diagnosis clinic does require additional runs of the histoprocessors with subsequently smaller batches, while overnight processing can be run with larger batches.

This study did not attempt to investigate the processing of biopsy specimens thicker than 9 gauge. While it is theoretically plausible that it would be possible to adapt the accelerated processing method for larger tissue specimens, this would most likely increase the total processing program time, which would eventually eliminate the clinical advantage of this method over traditional, overnight processing.

This study has some inherent drawbacks: it is a single-center proof-of-principle study, and for the large core biopsy specimens, only small numbers were available. Because of the exploratory nature of the study, no attempt was made to achieve a sample size sufficient to statistically prove noninferiority. While this would likely require a very large sample size, we have no arguments to believe increasing the number of cases is unlikely to change the study’s conclusion. For the same reason, no formal comparison between the two histoprocessors (Pathos and Logos) was included. The study builds on extensive clinical experience and multiple publications of applying the same technique on thinner breast biopsy specimens.<sup>1,9</sup> Results for thinner breast biopsy specimens have been reproduced in an independent pathology department.<sup>10-12</sup> The material used for stage 1 and stage 2 of this study was different: material for stage 1 had already been prefixed by longer formalin exposure. This means that data from stage 1 are not by definition completely applicable to stage 2. However, the results of stage 2 speak for themselves. While our quality grading system is inherently subjective, this does mean it answers the essential question: is the quality of these slides good enough for clinical practice? The current study shows that with minor modifications, the accelerated processing technique can be applied to biopsy specimens of a much wider breast patient group.

## Conclusion

Microwave-based accelerated processing with minor modifications can be applied to large core (9-gauge) VAB



specimens from breast lesions. Applying this technique, VAB specimens can be reliably processed within 4 hours from biopsy. The slides are suitable for all routine pathologic stains and tests. This enables a same-day diagnosis for patients requiring VAB.

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