Video Article

Preparation and Using Phantom Lesions to Practice Fine Needle Aspiration Biopsies

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Abstract

Currently, health workers including residents and fellows do not have a suitable phantom model to practice the fine- needle aspiration biopsy (FNAB) procedure. In the past, we standardized a model consisting of latex glove containing fresh cattle liver for practicing FNAB. However, this model is difficult to organize and prepare on short notice, with the procurement of fresh cattle liver being the most challenging aspect. Handling of liver with contamination-related problems is also a significant draw back. In addition, the glove material leaks after a few needle passes, with resulting mess.

We have established a novel simple method of embedding a small piece of sausage or banana in a commercially available silicone rubber caulk. This model allows the retention of vacuum seal and aspiration of material from the embedded specimen, resembling an actual FNAB procedure on clinical mass lesions.

The aspirated material in the needle hub can be processed similar to the specimens procured during an actual FNAB procedure, facilitating additional proficiency in smear preparation and staining.

Video Link

The video component of this article can be found at https://www.jove.com/video/1404/

Protocol

1. Preparation of *phantom lesion* (prepare phantom about 38 to 48 hours prior to actual practicing session of FNA proficiency (Figure 1) or utilize ready to use 'AV Phantom' from FNA Source, USA. The phantom lesion (stored under refrigeration without freezing) may be kept prepared even 3 to 4 days prior to practice session.
2. Practicing FNA proficiency: Although we have demonstrated the procedure using Shidham's THFV (Tissue Harvester with Functional Valve) (FNA Source, USA), any other FNA system may be used for practicing (Figure 2).
3. Spreading and processing the aspirate on the glass slides (Figure 3).

Material required

1. Clear silicone rubber caulk- available at many construction/home improvement stores. Many brands are available including GETM, DAP, Red Devil, White Lightning, and local chain brands. We used Clear Silicone Rubber, White Lightning Products, Cleveland, Ohio, USA, http://www.wlcaulk.com/products/all_purpose/silicone_rubber/wl09010.html)
2. Caulking gun
3. A piece of plastic wrap sheet (we used Saran Cling Plus Wrap, SC Johnson & Son Inc, Racine, WI, USA http://www.saranbrands.com/plastic-wrap/index.asp
4. Latex gloves
5. Wide diameter banana or sausage.
6. Firm and flat base plate (approximately 6 inches X 6 inches) made of thick card board, acrylic plate, or other.
7. FNA performing equipments
   - 25 gauge hypodermic needles
   - Shidham's THFV (or other components for performing FNA)
   - glass slides
   - staining kits

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Step-by-step details

A. Preparation of phantom lesion (prepare phantom 1 to 2 days prior to practicing FNA proficiency (Figure 2) or utilize ready to use 'AV Phantom' from FNA Source, USA.

1. Cut a 6 X 6 inch size of supporting board.
2. Cut a 2.5 inch long segment of *'phantom lesion core'* (banana or sausage).
3. Expel 0.5 inch thick layer of caulk on the base plate.
4. Press the *'phantom lesion core'* lightly in to this layer of caulk.
5. Add more caulk over the *'phantom lesion core'* so that it is completely embedded in the mound of caulk, without trapping air bubbles.
6. Adjust and shape the caulk over *'phantom lesion core'* with gloved hand.
7. Take a 6 X 6 inch size piece of plastic wrap and cover the caulk mound and adjust the surface of the mound into a smooth convex configuration with a clean, gloved hand.
8. Let the caulk mound cure for 6-8 hours longer than the curing time mentioned under the product instructions, (usually for 24 hours), in warm place.
9. After curing, the caulk should be firm, and the plastic wrap can be peeled off easily.
10. The FNA phantom lesion is ready to be used for practicing the FNA procedure after about 8 hours when the entire mound of caulk has completely cured to the center containing the *'phantom lesion core'* (banana or sausage).

B. Practicing FNA proficiency on AV Phantom lesion.

Any approach in obtaining high quality, adequate FNA aspirates with good quality direct cytology smears should address following critical points-

1. The needle gauge- as implied in the title of the procedure, the finer needles (higher gauge number) yield better aspirates with minimal blood dilution. For critical balance of benefits and limitations, 22 to 25 gauge needles are preferred for optimal mechanical stability of the needle without affecting the FNA aspirate quality.
2. Controlling appropriate sequences in vacuum application and release during the FNA procedure- This is critical in order to advance the sampled cells with each stroke of the needle, towards the needle hub. However, the aspirated material should not be allowed to reach into the area of the needle aspiration assembly with syringe. If this is not prevented, the material may be *difficult to retrieve for making smears* (this material may, however, be used for cell block preparation or for concentration methods such as Cytospin, filter methods, or recent liquid based cytology preparations).
3. Developing appropriate skill in *processing of the aspirated specimen,* including *direct smear preparation* and *on-site adequacy evaluation with appropriate triaging* for ancillary tests such as cell-block preparation for elective immunocytochemistry, elective flow cytometry for immunophenotyping of lymphoproliferative lesions, submitting sterile passes for microbiology studies, and material for molecular tests (cytogenetics, etc.).

Ba. Performing FNA procedure (shown with Shidham's THFV [1] (FNA Source, USA); however, any other FNA system may be used for practicing) (Figure 3,4,5).

1. Prepare the FNA assembly with the valve closed.
2. Locate the lesion and insert the needle tip in the lesion.
3. Open the valve to connect the vacuum, through the needle to its tip, to aspirate representative cellular components of the lesion with each *to and fro* stroke into the lesion.
4. Maintain the vacuum and sample different areas of the lesion by inserting the needle into the lesion in different directions.
5. Close the valve to disconnect the vacuum in the needle lumen.
6. Equalize the pressure with ambient conditions (E.g. by disconnecting the Syringe-valve from the needle for a brief moment & reconnect. If the special device with inbuilt steps, such as 'THFV' is used, this and other steps from Ba1 through Ba6 are concerted automatically by following different steps in the valve system) [1].
7. Remove the needle from the lesion with the valve closed, in order to prevent loss of the specimen by capillary action into the lesion unless there is slightly negative pressure at the hub end.
8. Dislodge the aspirated content onto the glass slide to prepare direct smear (see below) (figure 6).

Bb. Processing of the aspirated specimen with appropriate triage
Spreading the aspirate on the glass slides and preparing good quality direct smears (Figure 6) followed by proper staining and triage (Figure 7,8).

1. A drop of aspirate dislodged on the glass slide is spread by a variety of approaches as shown in (Figure 6) [2]. The usual approach is 'a'. However, if the specimen is blood diluted with some microfragments, the excess blood is drained away by tilting the slide, and the microfragments then are spread between this slide and second glass slide similar to 'a'. If the aspirate is predominantly a suspension without microfragments, such as from lymphoproliferative lesions, it is spread similar to 'b' or 'c'.
2. The smears may be processed and fixed in variety of ways for different staining methods during on-site adequacy evaluation. However, the best approach is to air-dry all the smears. The air-dried smears can be stained with Romanowski stains (such as Difff-Quik stain, Wright stain) after methanol fixation or with Pap stain after saline-rehydration and post-fixation (in 95% ethanol or preferably in 95% ethanol with 5% acetic acid) [3]. Elimination of interference due to the obscuring blood is a significant benefit [3].
3. Depending on the initial cytomorphologic findings during on-site adequacy evaluation, additional triage (such as elective cell block, electron microscopy, microbiology cultures, flow cytometry, molecular tests-cytogenetics etc.) is recommended as indicated.

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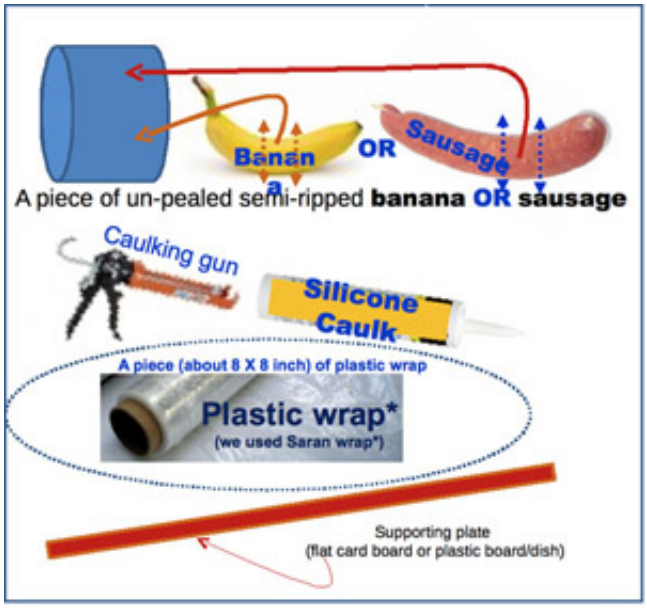
Representative Results:



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Please click here to see a larger version of figure 1.

Figure 1. Material required for preparing AV Phantom for practicing FNA proficiency (*We use *Saran plastic wrap* which did not stick to the caulk at the end, while removing after complete curing of the caulk.)

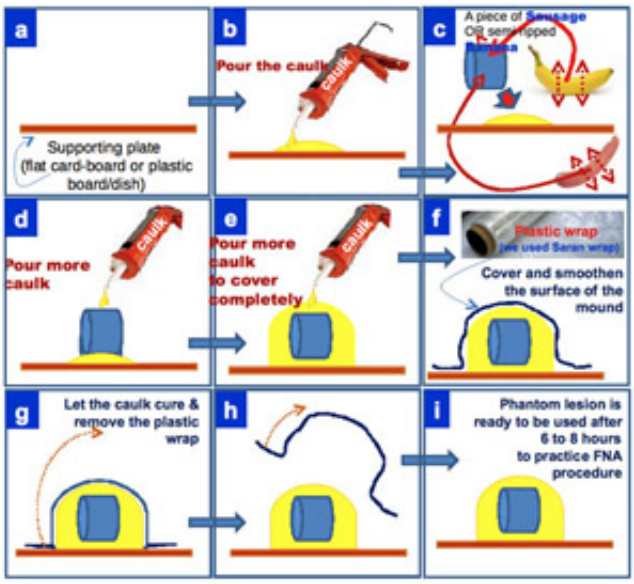

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Figure 2. Preparing AV Phantom lesion

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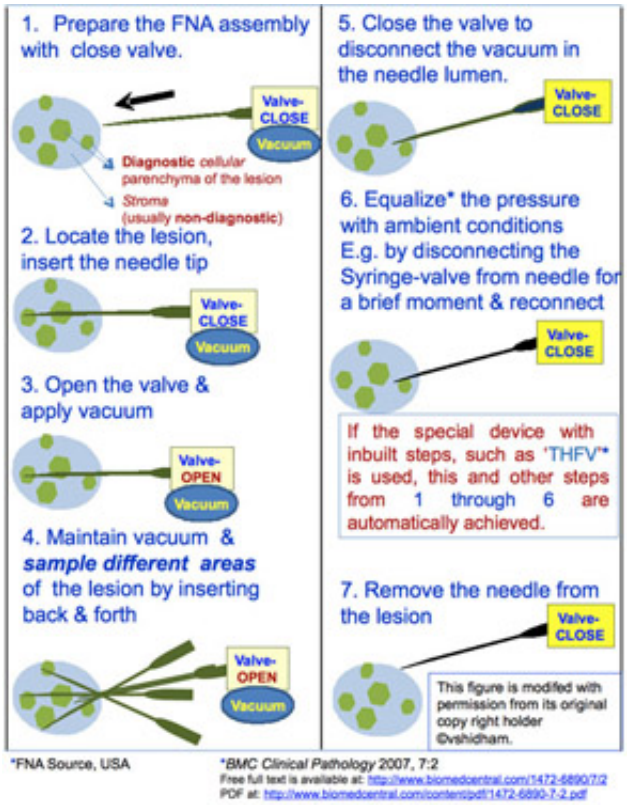

Please click here to see a larger version of figure 3.
Figure 3. Critical steps in FNA procedure.

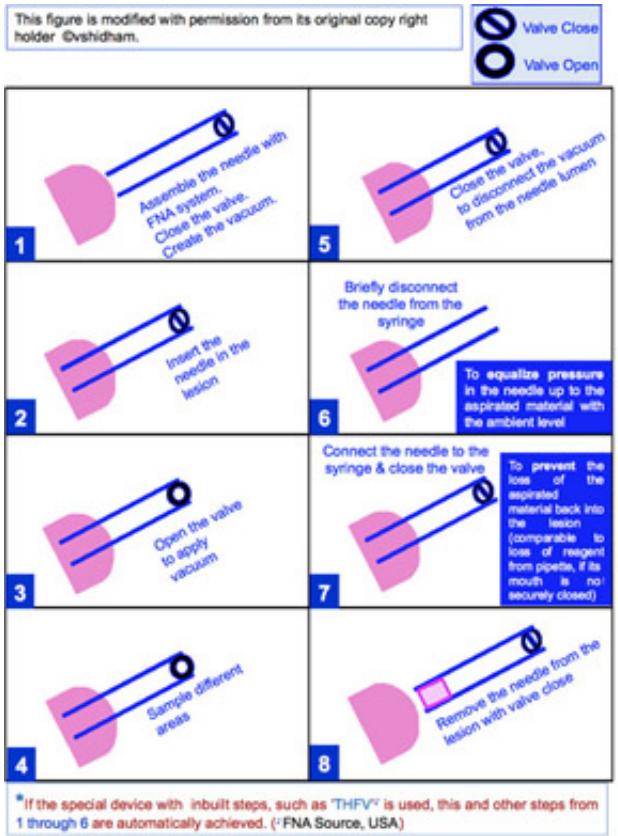

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Figure 4. EUS-FNA- Summary of Critical steps* (see Fig 3)

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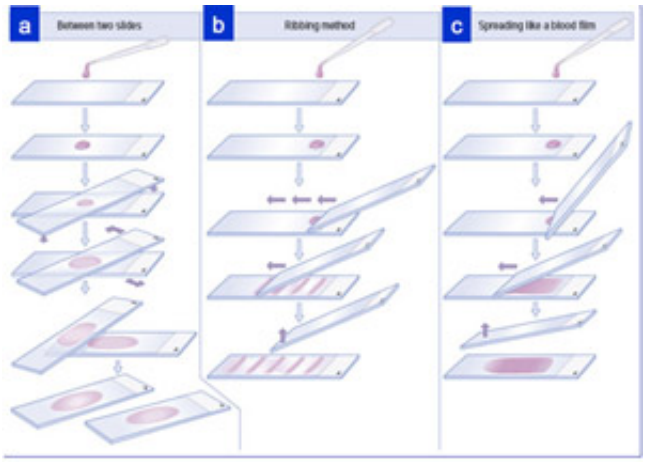
From: Vinod B. Shidham VB, Dua K, Evans D. EUS-FNA procedure for Pancreatic lesions- A brief review [Endoscopic Ultrasound-Guided Fine Needle Aspiration (EUS-FNA)]. CytoJournal (In press).

Please click here to see a larger version of figure 5.

Figure 5. Pitfalls related to the critical steps in FNA procedure.



Modified from: Shidham VB and Atkinson BF. Cytopathologic Diagnosis of Serous Fluids, Elsevier, 2007 (2).

Please click here to see a larger version of figure 6.

Figure 6. Preparation of direct smears of FNA specimen between two slides.

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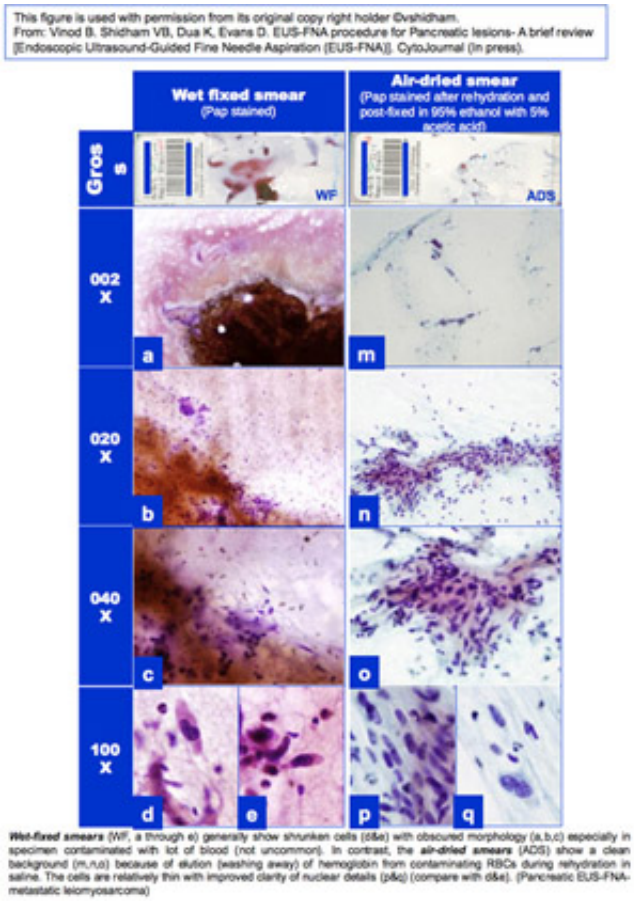
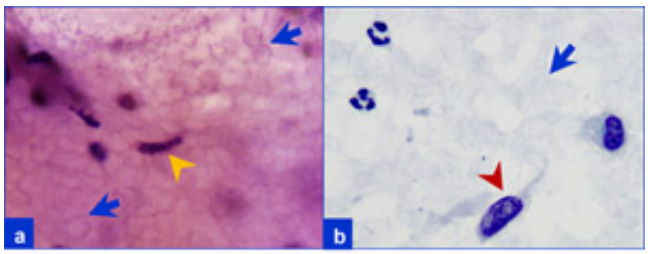


Please click here to see a larger version of figure 7.

Figure 7. Wet fixed versus Air-dried smears.



As compared to ***wet-fixed Pap stained smears*** (a), the ***air-dried smears stained with Pap stain* (after saline rehydration and post-fixation in 95% ethanol with 5% acetic acid)** (b) show a clean background because of elution (washing away) of hemoglobin from contaminating RBCs (blue arrows) during rehydration in saline. The cells are relatively thin with improved clarity of nuclear details (brown arrowhead in 'b') (compare with 'a'- Yellow arrowhead) and improved staining quality. (see also Figure L) (Pancreatic EUS-FNA- metastatic leiomyosarcoma, 100Xoil)

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Please click here to see a larger version of figure 8.

Figure 8. Wet fixed versus Air-dried smears.

Discussion

Fine-needle aspiration biopsies (FNAB) are widely performed as an economical, safe, and rapid minimally invasive technique for tissue diagnosis of various tumors and lesions. In this procedure a 18 to 27 gauge needle is inserted into the lesion, moved back and forth multiple times in different areas of the lesion under controlled vacuum (suction) of variable intensity ranging from minimal in the form of only capillary action to about 10 ml vacuum in syringe to retrieve diagnostic tissue material. A finer gauge needle with higher number is preferred to retrieve good cytology material to prepare direct smears. Wider gauge needle of 18G retrieves more sample suitable for cell block, flow cytometry, electron

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microscopy, cytogenetics, microbiology cultures etc. Although the procedure is simple, controlling various steps with proper *application* and *release* of vacuum at appropriate steps in concerted manner is extremely crucial for optimum results.

Due to non-availability of specially designed high-yield needles, conventional hypodermic needles or their modifications are used to perform this procedure. However, the hypodermic needles have many limitations leading to a low yield of specimen if used in conventional manner. The requirement of special syringe grips usually adds to the complexity of the procedure. Thus, due to the lack of commercially available suitable FNAB needle device, this highly versatile procedure suffers a frequent problem of inadequate specimen retrieval. Recently, a device increasing the yield with reproducibility has been reported [1].

In addition, currently there is a lack of suitable model for practicing proficiency in performing FNAB by health personnel in training including residents and fellows. We had standardized a model with a piece of fresh cattle liver stuffed in latex glove for practicing FNAB proficiency. However, this model is difficult to organize at a short notice. Procurement of fresh cattle liver is usually the limiting predicament. In addition, handling of liver may introduce contamination-related factors as a significant draw back. The tendency for the latex glove to leak after a few needle pricks leads to potential mess during the practice session.

We report and describe a novel but simple method of embedding a small piece of banana (or sausage) in a commercially available silicone rubber caulk and other easily available material. The FNAB phantom retains vacuum seal thus permitting aspiration of material from the embedded specimen, equivalent to the actual FNAB procedure on a lesion in clinical setting. In this video, we demonstrate the procedure of making FNAB phantom lesion and practicing FNAB procedure. It also demonstrates in brief the processing of retrieved specimen including smear preparation and staining [2,3].

Because the caulk takes time to cure, the phantom lesion should be prepared in advance about 24 to 48 hours prior to the practicing session. Comparable ready to use phantom lesion may be obtained from a commercial source (FNA Source, USA).

The phantom lesion for practicing FNAB proficiency may be used for training the residents and fellows prior to the beginning of FNAB performance on patients in the clinical setting.

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