## **TECHNICAL NOTE**

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# A Method for the Determination of Syringe Needle Punctures in Rubber Stoppers Using Stereoscopic Light Microscopy

**ABSTRACT:** The ability to accurately determine the number of syringe needle penetration holes through the rubber stoppers in pharmaceutical vials and rubber septa in intravenous (IV) line and bag ports has been a critical factor in a number of forensic cases involving the thefts of controlled substances or suspected homicide by lethal injection. In the early 1990s, the microscopy and microanalysis group of the U.S. Food and Drug Administration's Forensic Chemistry Center (FCC) developed and implemented a method (unpublished) to locate needle punctures in rubber pharmaceutical vial stoppers. In 1996, as part of a multiple homicide investigation, the Indiana State Police Laboratory (ISPL) contacted the FCC for information on a method to identify and count syringe needle punctures through rubber stoppers in pharmaceutical vials. In a joint project and investigation using the FCC's needle hole location method an applying a method of puncture site mapping developed by the ISPL, a systematic method was developed to locate, identify, count, and map syringe punctures in rubber stoppers or IV bag ports using microscopic analysis. The method requires documentation of punctures on both sides of the rubber stoppers and microscopic analysis of each suspect puncture site. The final result of an analysis using the method is a detailed diagram of puncture holes on both sides of a questioned stopper and a record of the minimum number of puncture holes through a stopper.

**KEYWORDS:** forensic science, rubber stopper, septa, syringe, needle, intravenous bags, pharmaceutical bottle, puncture, hole, stereoscopic light microscopy, potassium chloride

Historically, there have been two primary reasons for the forensic analysis of rubber pharmaceutical container stoppers and septa in intravenous (IV) line and bag ports. The first reason is the theft of controlled substance liquid pharmaceuticals such as morphine sulfate and Ketamine<sup>®</sup> from sealed vials in hospitals and medical offices. The theft of these liquid pharmaceuticals, whether for illegal sale or personal use, can pose a serious health risk for the intended recipient/patient if undetected. Individuals stealing the drugs will usually remove a significant volume of the drug product from a commercially sealed container and replace the volume with water or saline to hide the theft. The plastic or metal flip-off tamper-evident cap that covers the rubber stopper access is often reattached using glue or the vial is placed in the same location as previously opened vials in use. Some cases have involved inserting the syringe directly through the flip-off cap and never removing the cap itself. The legitimate recipient/patient ultimately receives a di-

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luted dosage, or a volume of water or saline instead of the intended dosage of drug. The sterility of the injected liquid is a health concern as well as the complications and side effects, which may result from not receiving the prescribed medication.

The second principal reason for the analysis of puncture holes in rubber stoppers and septa is related to the investigation of hospital and nursing home homicide cases usually cited as "mercy killings." In recent years, there have been a number of multiple homicide cases involving the alleged killing of hospital and nursing home patients by so called "angels of death" (1,2,4). The perpetrators in many of these hospital deaths have used a variety of methods to kill patients, who are primarily (but not limited to) elderly and seriously or terminally ill. The most common method is lethal injections with epinephrine, insulin, potassium chloride, morphine, Pavulon<sup>®</sup>, succinylcholine chloride, arsenic, or cyanide. The route of lethal injection was often reported to be directly into the patient or through the patient's intravenous (IV) tube or bag via a needle port. Historically, 15 "angels of death" admitted to causing or were convicted of causing 156 known deaths. However, there is evidence and/or reasons to suspect that the same individuals may be responsible for many more deaths, with possible associated deaths totaling more than 1099 (1-4). Other associated internet-based references include the following sites.

http://www.channel2000.com/news/stories/news-980327-235547.html

http://www.angelfire.com/oh/yodaspage/angelo.html

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http://www.terryhayden.treeonline.co.uk/murder/serialkillers/b evallit.html

http://www.law.cornell.edu/ny/ctap/I96\_0061.htm http://www.massline.com/indepth/nurse/nurse1.html http://www.quasar.ualberta.ca.ddc/ICAD/digests/murders.html http://www.dispatch.co.za/1998/07/27/foreign/WORLD1.HTM http://www.augustachronicle.com/stories/072898/tec\_124-6523.shtml

http://www.cnn.com/2001/WORLD/europe/UK/06/19/ship-man/index.html

http://www.cnn.com/2001/LAW/01/09/angel.death.crim/in-dex.html

http://www.cnn.com/2000/LAW/07/18/angel.death.crim/in-dex.html

http://allafrica.com/stories/20009170090.html

http://www.geocities.com/Area51/Comet/4190/swango.htm http://www.cincypost.com/news/1997/harvey053197.html http://www.histroychannel.com/crime/1029.html

Following medical safety and standard operations procedures (SOPs) now used by most hospitals and nursing homes, individual pharmaceutical bottles of all drugs are closely tracked by recording the storage site, date opened, administered volume(s), and date(s), and the final disposition/destruction of remaining contents and bottle. Unless heavily used, the rubber stoppers, primarily in pharmaceutical containers but also in some styles of IV needle access ports, will usually have a needle hole or track for each time a needle completely penetrated the stopper/access port to withdraw or inject. The determination of the number of needle holes and the remaining volume and integrity of the liquid pharmaceutical remaining in the container, in conjunction with evaluation of the hospital pharmacy/patient records, can be most valuable in determining if the remaining liquid volume is consistent with the recorded medical usage.

In one particular "angel of death" case, bottles of potassium chloride were found in the suspect's residence and vehicle (2). These bottles were collected as evidence. A method for counting and documenting puncture holes in rubber stoppers was developed as a result of that case and used to present the forensic evidence in the subsequent multiple-count, homicide trial (2,4).

In other hospital cases processed by the FCC, injection containers of controlled substances such as morphine sulfate had been suspected of theft and tampering with possible removal of product and substitution with a visually similar liquid. In some cases, tamper evident "flip caps" had been reattached with glue to hide the suspected theft and tampering. Still other theft and removal of controlled substances was made through the rubber stoppers of prefilled injectable syringes.

Several of the cases noted above have presented a need for the determination of syringe needle puncture holes through rubber stoppers, but only one has been determined to have had syringe needle puncture hole analyses entered as case evidence. The elastic properties of the rubber in stoppers create a somewhat unique problem in that the material nearly closes or reseals itself following penetration by a syringe needle. The site of a single needle penetration therefore becomes nearly if not completely invisible to examination even by light microscopy. Multiple penetration sites also remain nearly invisible as long as they are not positioned at the same location or do not cut out pieces of the stopper.

In order to locate, count, and accurately record each needle hole through a suspect rubber stopper, a systematic method was jointly developed by the U.S. Food and Drug Administration's Forensic Chemistry Center (FCC) and the Indiana State Police Laboratory (ISPL). The resultant method was found to be effective in determining the minimum number of needle penetration holes through a rubber stopper with accurate hole location and stopper orientation mapping.

## **Materials and Methods**

## Materials

A high-quality stereoscopic light microscope (SLM) is mandatory for this method. The SLM should have a long and variable focal length to accommodate stopper mounts in small bench vises as well as permit the analyst/examiner manipulation during analysis. The objective lens should be 0.5 to 1.0X with a variable zoom magnification from 2 to  $\sim$ 50X. Analysis was routinely performed at a magnification of 10X or less. A trinocular SLM head to permit the attachment of a camera to the SLM is necessary for photodocumentation. Measuring devices such as micro-rulers (gradations in 0.1 mm units) and/or calipers or micrometers are required to calibrate the SLM and perform any size measurements. External illumination, preferably by variable intensity halogen lamp and flexible arm fiber optics, is necessary to provide multiple direction illumination and selective, low-angle surface shadowing.

A color video camera coupled to a computer-operated image (frame) capture device is used for photomicrography. The captured image should be stored in a unique case file using the computer-operated, image capture, and storage software. Hardcopy images for both case analysis and court visual displays can be printed using a color video printer.

A small table vise or clamping device is necessary for holding stoppers during SLM examination. Also required are flat and rounded tip stainless steel probes with no sharp points to manipulate the rubber stopper, and forceps to remove any extraneous surface material. The authors have found that using a small (<1.0 mm diameter) ball-tip burnishing tool (available at most art and craft supply stores) or stainless steel probes, manufactured for snake gender determination [Fuhrman Diversified, Inc., Seabrook, TX (281)474-1388] work well for stopper surface manipulation. It may be necessary for the analysts to prepare their own blunted probes by grinding the tip of a stainless steel surgical probe to produce a rounded "ball-shaped" tip measuring 1 to 2 mm in diameter. The analyst/examiner may find other hand instruments (i.e., scalpel, probes, clamps, etc.) useful depending upon the sample condition and analysis requirements. A rubber stopper reference collection produced by making puncture holes in stoppers with a wide variety of puncturing objects (including analysis probes) is helpful for evaluation and comparison of suspect hole(s). At the very least, analyst/examiners should thoroughly familiarize themselves with the characteristic hole shape made by the insertion and removal of syringe needles in rubber stoppers and be able to discern differences in holes made by other objects.

## Evidence Packaging

Rubber stoppers and stopper components (overcaps, tamper-evident seals, etc.) should be wrapped in a soft, protective material (cotton or cotton gauze, Parafilm®, lab tissues, etc.) and placed in labeled protective cardboard or plastic containers. If a suspect direct-delivery-type vial or intravenous (IV) bag is submitted with the rubber stopper/septum still attached, it should be considered to be a biohazard and labeled and handled accordingly.

## Analytical Policy

- 1. Rubber stopper examination will ordinarily be conducted only when appropriate comparison standards are submitted or obtained. Rubber stoppers (and septa) may be obtained by acquiring control samples of the product, bottle, or IV bag in question. Often, contacting the manufacturer or pharmacy directly can quickly provide a source of the appropriate comparison sample(s).
- 2. All items submitted for examination will be analyzed using a variety of visual, microscopic, instrumental, and lighting meth-

ods and techniques depending upon the type of evidence and analysis requested.

- 3. Other laboratory techniques may be necessary following initial stopper examination including the determination of external stopper surface/cap residues [by FTIR spectrometry, polarized light microscopy (PLM), or scanning electron microscopy/energy dispersive X-ray analysis (SEM/EDX), etc.] or bottle/bag contents analysis (GC-MS, Headspace GC, etc.).
- 4. Care must be exercised not to damage the stopper during removal from the container. A decrimping tool (commercially available) can make stopper removal easy and safe. It is important to note that if analysis is to include fingerprint examination, content analysis, sterility checks, etc., the stopper must be protected from any subsequent damage. If liquid product is to be withdrawn for any subsequent analyses, the stopper should be removed as described in "Step 4" of the stopper examination methodology below. No additional holes or cuts should be made through the stopper. Clearly, analyses such as checking for sterility must be performed immediately upon removal of the stopper, but other analyses may be ordered in a number of sequences. The actual order of the forensic examinations must be determined by the analyst based upon the analyses requested and condition of the sample (undamaged/intact, damaged/broken bottle, leaking liquid, obvious evidence of product tampering, etc.).

## Initial Sample Treatment

Upon receipt of evidence for rubber stopper examination, all items and containers are to be identified with the appropriate case/sample numbers and initials consistent with the examining laboratory's evidence policy. The analyst/examiner must further determine which examinations are to be performed and in what order. Prior to removal of a sample stopper from a pharmaceutical bottle/vial or IV bag, etc., the container description should be carefully noted and recorded, including size dimensions, color (of each component), and, if available, the label type/style, production name, and manufacturer, lot number, batch number, expiration date, etc. If applicable, dates, specific patient name, or hospital/pharmacy codes or other annotation should be fully recorded. Photodocumentation of all bottle/vial or bag surfaces and labels by standard laboratory methods or SLM photomicrograph preparation should also be performed.

The rubber stopper should be further examined (not removed from bottle/vial or bag) for class characteristics including but not limited to the color, size, markings, molding marks, and style. The analyst/examiner should make every effort to determine the stopper manufacturer and, if possible, any lot, batch, or other identification number by comparison to a laboratory reference set (established and maintained by the investigating laboratory) or by follow-up communication to the manufacturer.

#### Stopper Examination Methodology

- Figure 1 is an illustration of a cross-sectional view of a typical stopper with specific nomenclature and regions identified. Following photodocumentation of the sample container and labels as received, the overcap and all visible stopper area including the target area or ring are examined by SLM. Any evidence of tampering is noted and photodocumented, including examination for any trace evidence such as glue residue under the safety cap, broken safety cap attachments, missing safety cap, damaged overcap, etc. All analysis observations are to be recorded on the analyst's stopper worksheet (Fig. 2).
- The target area of the rubber stopper is closely examined for defects, obvious penetration/puncture holes, missing rubber

## **Rubber Stopper Cross Section**



## **Corkage Side (Bottom)**

FIG. 1—Cross-section illustration of rubber stopper and nomenclature.

## Laboratory/Agency Name

## STOPPER WORKSHEET



FIG. 2-Sample of stopper worksheet.

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material, or marks of any type. Any of the above irregularities are photodocumented. Surface residues, particles, or fibers are to be carefully collected, appropriately labeled, and retained without damaging the stopper surface.

- 3. If the bottle/vial or bag still contains visible liquid product, a clean and suitable container is prepared and labeled to correspond to the sample.
- 4. The overcap is removed and placed in an appropriately labeled container. This is done by the use of a decrimping tool or by cutting the overcap with wire cutters. If wire cutters are to be used, the overcap is initially cut on the bottom or lower side with pre-cleaned, particle-free wire cutters and then spread with pliers. Care must be taken not to damage the stopper.
- 5. The stopper is removed and examined on both the head (top) and the corkage side (bottom) by SLM. Care must be taken to note and record any observed pressure change (positive = pushes out of container, or negative = pulls into container) in the product container as the stopper is removed. Any immediate marks or damage are also noted. If applicable, the liquid is poured from the suspect bottle/vial or bag into the previously prepared and labeled container and appropriately retained (refrigerated, protected from light, etc.).
- 6. Using a permanent ink marker (Sharpie<sup>®</sup>, etc.), an index mark is made on the top and side of the head surface of the stopper wrapping around and onto the corresponding site on the bottom or corkage side. The index mark is placed in a region away from any previously noted marks or damage sites. This index mark will be used to orient the stopper and reference all observations including hole location and orientation.
- 7. Using the stopper worksheet (Fig. 2), the top of the stopper is first examined using the SLM and variable intensity and fiber optic illumination with the position of the index mark on the stopper noted. All visible holes or cuts are recorded on the worksheet, keeping their orientation to the index mark. The location, shape, and orientation are recorded on the stopper worksheet.
- 8. Next, the bottom or corkage side of the stopper is examined and all visible holes, cuts, etc. are recorded on the worksheet as in Step 7 above, keeping their orientation to the index mark. All surfaces including the outer and inner corkage walls must be carefully examined.
- 9. The stopper is gently secured in a small bench vise with protective rubber/plastic jaws and positioned to view the top surface. Noting the position of the index mark and placing the mark in the 12 o'clock position for viewing, the stopper is slightly compressed in the vise. Using a rounded or blunted probe, the surface of the stopper is slowly rubbed from the left side to the right side in a 12 o'clock to 6 o'clock plane. The surface is then gently rubbed from top to bottom in a 9 o'clock to 3 o'clock plane. Any puncture holes are noted, counted, and their position and entrance hole shape recorded on the stopper worksheet on the "TOP" view template. Cuts, incomplete holes, missing surface rubber, double holes, etc. are all recorded.
- 10. The stopper is then rotated 90° in the vise and Step 9 repeated, always referencing any punctures found to the index mark. This helps to confirm previously located holes as well as to locate other punctures. Again, any puncture holes are noted, counted, and the entrance hole position and shape recorded on the stopper worksheet on the "TOP" view template. The stopper is again re-analyzed in both the 180 and 270° position for a total of four positions of analysis (90, 180, 270, and 0°/360°).
- 11. Next, the stopper is gently secured in small bench vise jaws with the corkage (bottom) side up for examination. Noting the

position of the index mark, the stopper is slightly compressed in the vise. Using a rounded or blunted probe, the flat surface (corresponds to the target area) of the stopper is gently rubbed in a 12 o'clock to 6 o'clock plane. The circular bottle flange of the stopper is then carefully rubbed and examined on the inner and outer surfaces for cuts and holes. The flat surfaces (both below the target area and around the outer rim) are then gently rubbed in a 9 o'clock to 3 o'clock plane. The circular bottle corkage of the stopper is again carefully rubbed and examined on the inner and outer surfaces. Any puncture holes are noted, counted, and their position recorded on the stopper worksheet on the "BOTTOM" view template.

- 12. Again, the stopper is rotated 90° in the vise and Step 11 repeated. Any puncture holes are noted, counted, and their position recorded on the stopper worksheet on the "BOTTOM" view template. The stopper is again re-analyzed in both the 180 and 270° position for a total four positions of analysis (90, 180, 270, and 0°/360°).
- 13. All puncture holes are counted on the top surface first. The analysis of the bottom of the stopper also serves as an attempt to locate all corresponding or exit holes. It is important to note that preliminary studies at both laboratories have shown that the number of top and bottom (entrance and exit) punctures may not always be the same. Syringe needle punctures on the top may share the same entrance hole with nearly indistinguishable separation between the two needle tracks observed. Further probe manipulation at a higher magnification may show the bifurcation of the two needle tracks 1 to 2 mm below the surface (Fig. 3). This often helps to account for differences in the number of tracks noted on the head versus the corkage side of the stopper. The same phenomenon can sometimes be observed on the bottom of the same location.
- 14. The location and number (and sometimes the size) of all observed needle punctures holes are recorded on the stopper worksheet (Fig. 2). Conclusions or interpretation of the analysis of a rubber stopper or rubber stopper component including characterization, defects, or punctures are based on the significance of similarities or dissimilarities in the characteristics observed.
- 15. The above procedures are basically the same for needle puncture hole determination in rubber septa such as those used with intravenous bags and lines. The primary difference is the removal of the septum from the port. The position of the septum in the port is noted prior to removal. A sharp razor blade is used to cut the sides of the port on two sites directly opposite each other. The port is then pried open like a clamshell and the septum removed.

## Reporting Results

Rubber Stopper Case Reports:

- 1. Rubber Stopper or Rubber Stopper Component Characterization
  - a. Examination of the stopper in sample/item \_\_\_\_\_ was found to be typical of a (physical description of stopper or stopper component type).
  - b. Examination of the stopper in sample/item \_\_\_\_\_ was found to be typical of a (describe stopper or stopper component type) manufactured (or marketed) by \_\_\_\_\_.
  - c. Examination or the stopper in sample/item \_\_\_\_\_\_ found insufficient characteristics for characterization purposes.



FIG. 3—Stereoscopic light photomicrograph showing a rubber stopper with a single surface puncture hole (top) which reveals two puncture holes below the surface (bottom) when spread during analysis (scale = 1.0 mm).

- 2. Rubber Stopper or Rubber Stopper Component Puncture Examination
  - a. Visual and microscopic examination of the stopper in sample/item \_\_\_\_\_\_ was found to have NO defects or puncture sites.
  - b. Visual and microscopic examination of the stopper in sample/item \_\_\_\_\_\_ was found to have a MINIMUM of \_\_\_\_\_ puncture sites readily apparent on the top of the stopper and a MINIMUM of \_\_\_\_\_ puncture sites readily apparent on the bottom of the stopper.
  - c. Visual and microscopic examination of the stopper in sample/item \_\_\_\_\_\_ was found to have a MINIMUM of \_\_\_\_\_ puncture sites readily apparent in the top AND bottom of the stopper.

## **Results and Discussion**

Initial examination of any rubber stopper must include careful evaluation of the shape of any suspect puncture holes. Early stud-

ies at the FCC of syringe needle punctures in rubber pharmaceutical bottle/vial stoppers and intravenous (IV) bag, rubber port septa found the resultant puncture hole to usually have a characteristic shape resembling a "smiling mouth" or "bird in flight" (Fig. 4, bottom and top arrows, respectively) similar to a parenthesis "(" or a bracket "{", respectively, on a keyboard. SLM analysis of the puncture hole as the syringe needle was inserted showed that the trocar-shape of the needle tip (Fig. 5) is responsible for the unique hole shape. The hole begins from a central point (tip of the needle), which slightly pushes the rubber down until the two bevel-cut trocar edges actually cut through the rubber from the central point. The tip and trocar bevels are both located on one side of the syringe needle shaft at the tip. With the penetration hole cut by the bevels, the remainder of the shaft face slides down the bevel and follows the cut hole.

Examination of the top surface of a rubber stopper with known syringe needle holes showed that the characteristic "bird in flight" or "smiling mouth" shape were easily distinguishable and could be distinguished from most other objects used to cause a puncture hole. Figure 6 shows examples of four common puncturing objects [A—sewing needle, B—push pin, C—nail (finishing), and D—paper clip] and the resultant holes made by each in a rubber stopper. Frequently used pharmaceutical containers such as those in hospitals or those for individuals requiring routine injections (diabetics, etc.) may have many needle penetration holes, and most will be in the target area where frequent overlap may occur. It was necessary



FIG. 4—SLM photomicrograph of head of a rubber stopper showing characteristic "smiling mouth" (bottom) and "bird-in-flight" (top) puncture holes made by the same syringe needle. Scale in 0.1 mm increments.



FIG. 5—SLM of syringe needle tip penetrating rubber stopper (head surface) showing the cutting surfaces of the needle's trocar faces. Scale in 0.1 mm increments.

to carefully examine all top surfaces of the stopper and increase SLM magnification to view well into visible puncture holes to determine if the site is a single or multiple puncture site. Counting and confirming top and bottom holes was also most valuable in determining multiple puncture sites.

The design of rubber stoppers must be considered in the method of analysis. Figures 7 and 8a show the cross-sectional view of a rubber stopper with a syringe needle inserted through the target ring and into the central bore. Those figures further show the relationship of the target area to the central bore and bottle flanges. Figure 8, Sections *a-f* show a number of possible syringe needle penetration angles and locations on the stopper that will have puncture holes. The number of holes that would be detected in the stopper range from two holes (Fig. 8a-d), a rare but possible three holes (Fig. 8e) and four holes (Fig. 8f). The beveled angle of most commercially available syringe needles may also cause the needle to "track" at an angle toward the tip side of the needle shaft. This angled tracking may result in an "exit" hole on the bottom side of the stopper that does not appear to line up directly with the entrance hole. This condition is particularly true when the syringe needle passed through the long axis of the bottle corkage rubber (Fig. 8b).

Additional puncture hole characteristics are the apparent extrusion or pushing outward of a piece of stopper rubber on the corkage side as the needle is inserted called a "pushout" or occasionally on the head side as rubber material is slightly lifted out of the entrance hole upon withdrawal of the needle called a "pullout." Occasionally the inserted needle pushes a lip or piece of stopper rubber further into the hole upon insertion or pulls a lip or piece of stopper rubber further back into the penetration hole as the needle is withdrawn.

The condition of incomplete penetration or hesitation holes was noted on both suspect stoppers and test stoppers prepared in the laboratory. The hesitation hole appears to be created by the attempt of an individual to possibly aim for the target area on the stopper and touch the stopper prior to actually inserting the needle through the rubber stopper. The act of touching the rubber material with a fresh and very sharp needle tip results in a slight cut or partial hole in the rubber. When the actual insertion is performed, the needle may be slightly repositioned, resulting in what from the initial stopper head examination appears as two puncture sites, but is actually only one complete and one partial puncture. Only careful SLM and probe analysis of both sides of the stopper can determine the total or documented minimum number of complete needle penetrations.

The stopper worksheet is invaluable for documenting and tracking observed puncture holes on both sides of rubber stoppers. Mul-



FIG. 6—SLM photomicrographs showing puncture holes created by selected pointed objects on the head side of rubber stoppers. Scale in 0.1mm increments. (A) Sewing needle (0.69 mm diameter)—three punctures, (B) push pin (1.13 mm diameter)—three punctures, (C) finishing nail (1.81 mm diameter)—single puncture and (D) paper clip (0.71 mm diameter)—two punctures, respectively.

tiple puncture holes in a stopper created by both a large bore needle (such as those used to withdraw venous blood) and a small bore needle (such as those used for intra-muscular injection or diabetic/insulin injection) will produce holes of notably different sizes that are easily differentiated. However, the ability to determine accurate needle gauge size by measuring the resultant puncture hole may not be as easily accomplished. Whereas the difference(s) between a 16 and 27 gauge needle puncture hole may be easily noted, the difference between a 27 and 29 gauge needle puncture may not be determined with such confidence. The ability to examine a needle puncture hole in a rubber stopper and accurately determine the needle diameter (gauge size) is still questionable and will continue to be an area of research at the FCC.



FIG. 7—Cross-section illustration of rubber stopper with syringe needle correctly inserted through target ring into central bore. This configuration will result in two holes (one in the head side and one in the corkage side) of the stopper.



FIG. 8—A-F show a number of possible syringe needle penetration angles and locations on the stopper that will bear puncture holes. The number of holes detectable in the stopper range from two holes (A-D, one in the head side and one in the corkage side), a rare but possible three holes (E, one in the head side and two in the corkage side), and four holes (F, one in the head side and three in the corkage side).

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