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Fig. 1. Estrogen receptor IHC staining in human breast following combined deparaffinization and antigen retrieval with Dawn® dishwashing detergent. 400X

A Novel Approach to Combined Deparaffinization and Antigen Retrieval for IHC

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Abstract

The benefits of antigen retrieval (AR) techniques for formalin-fixed paraffin-embedded tissues prior to immunohistochemical (IHC) staining have been well documented,[1] although the actual chemical mechanisms are not completely understood. Traditional methods of AR are performed on tissues that have been deparaffinized with xylene (or some other paraffin solvent), and hydrated to water in graded alcohols, after which they are heated to high temperature in a solution of metallic salts or ionic buffer. Various heating sources may be used, some adapted from household kitchen devices, including the microwave oven, vegetable steamer, or pressure cooker. Even an autoclave may be used. A number of proprietary products now combine deparaffinization and heat-induced epitope retrieval (HIER) in a single solution without the use of organic solvents, offering environmental and work efficiency advantages. The primary drawback to these commercial dewaxing and AR solutions is their expense and relatively narrow range of applications. Our laboratory has discovered a novel means for completing dewaxing and heat-induced AR in one step using an inexpensive and readily available household product that may be used as an additive to any traditional AR solution.

Introduction

The use of proprietary combination dewaxing and AR solutions can become costly when one considers that most formalin-fixed tissues require epitope retrieval prior to immunohistochemical staining for many antigens. While traditional dewaxing and hydrating solvents are relatively inexpensive to buy, they pose a potential safety hazard for staff and must be disposed as hazardous waste that requires compliance with federal and local laws; this can also become quite costly.

Our laboratory discovered that a readily available household dishwashing liquid may be added to commonly used antigen retrieval buffers to create an effective and inexpensive solution that achieves simultaneous dewaxing and antigen retrieval of paraffin sections. The additive is prepared by mixing 5.0 mL of Dawn® dishwashing detergent into 1.0 L of deionized

water (0.5%) to create a stock diluent for AR buffers. In our laboratory, we utilize 1 part Dawn base diluent (0.5%) and 4 parts buffer to prepare our combination dewaxing/antigen retrieval solution. This combination solution is used exactly as one would any antigen retrieval solution, except that slides are placed into the combination solution prior to dewaxing. Immediately following the heating process, sections are placed into preheated water (which may be heated along with the AR solution), then allowed to cool. The dewaxing by-products remain in the retrieval solution and are discarded.

The combinations of buffer and additive solutions are limitless and can be tailored to meet the antigen retrieval needs of each individual laboratory. The specific applications of the AR solution providing the greatest performance with each antibody must be validated by individual labs and will depend on tissue fixation and staining methods employed. Utilizing a countertop electric pressure cooker, the following IHC applications have been validated in our lab using the Dawn (0.5%) additive with Citra® buffer (pH=6.0), or with solutions of Dawn and water only: Calretinin, Ki-67 (Mib-1), ER (6F11), PR(PR16), *Helicobacter pylori,* Her-2-neu, Pan Melanoma cocktail (Mart-1, HMB-45, Tyrosinase), LCA, CD 30 (Ber-H2),CD15 (Leu-M1), CD-10, Chromogranin A, Cytokeratin 34bE12, S-100, and Vimentin. The resulting stain intensities and tissue preservation characteristics rival the best-performing AR solutions and methods previously used in our laboratory.

Methods
In order to compare the effectiveness of our newly discovered AR solution, IHC stains were performed on normal and tumor tissue samples in triplicate with all of the various procedural parameters held constant except for the AR solution used. All of the AR procedures were carried out using a benchtop electric pressure cooker. The Citra

and Citra Plus reagents from Biogenex were received in concentrate form and diluted with deionized water to a 10X dilution factor before use. The Borg® and Reveal® products from Biocare were received in ready-to-use form. The Dawn base diluent was prepared by adding 5.0 mL to one (1) liter of deionized water. To prepare a working solution of Dawn, one part base diluent was added to 4 parts deionized water. Up to 24 thoroughly dried slides were placed into a single plastic Tissue-Tek® slide carrier holding approximately 250 mL of retrieval solution. This was placed into the pressure cooker reservoir along with an equal volume of deionized water in a separate dish, surrounded by 500 mL of deionized water. Retrieval time was consistently 3 minutes at 120°C at approximately 20 psi pressure, followed by cooling to 80°-85°C for 15 minutes and rinsing with tap water prior to loading onto the staining instrument. All of the stains were performed using the Ventana NEXES® automated IHC stainer with Ventana Enhanced DAB detection reagents. Counterstaining utilized hematoxylin. Other specific protocol details are described in the table below.

Results
The representative stains from each group of AR solutions were evaluated for tissue preservation,

cell morphology preservation, staining appropriateness with normal and tumor tissue elements, and signal to background noise ratio. The photomicrographs illustrate a comparable performance with each of the antibody/tissue combinations challenged.

Conclusions
As with all antigen retrieval methods currently being employed in IHC laboratories, the actual chemical mechanisms at work can only be theorized. The replacement of organic solvents by detergents is an easily understood effect, with surfactants acting on liquefied paraffin. The excellent performance of this detergent solution in the unmasking or enhancing of tissue antigens may be indicative of the strong effects of the heating process in the absence of any particular buffer environment. However, the effects of the detergent on the antigen sites independent of temperature cannot be ruled out. In either instance, the use of a Dawn dishwashing detergent solution can provide an effective and inexpensive means for combined deparaffinization and antigen retrieval in one step when properly validated in the individual IHC laboratory setting.

Dawn® is a registered trademark of Procter & Gamble.

Reference
1. Shi S-R,Taylor CR, Gu J. *Antigen Retrieval Techniques: Immunohistochemistry and Molecular Morphology.* Eaton Publishing; 2000.

Antibody Name	Manufacturer	Primary Incubation time (minutes)
Estrogen Receptor	Ventana Medical Systems	26
Ki-67 (mib-1)	Dako	28
Her-2-neu	Dako	32
Pan Melanoma	Biocare	28
CD 3 (T-cell)	Cell Marque	24
S-100	Ventana Medical Systems	4
CD 30 (Ber-H2)	Dako	24
Cytokeratin 34bE12	Dako	32
Chromogranin A	Ventana Medical Systems	28
Calretinin	Zymed	8
Vimentin	Ventana Medical Systems	20
Helicobacter pylori	Cell Marque	32

Troubleshooting Techniques for Bielschowsky Silver Stain for Senile Plaques and Neurofibrillary Tangles

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Abstract
With the increase in research dealing with Alzheimer's disease, the Bielschowsky silver stain has become a routine stain used in many laboratories. Bielschowsky originally introduced the stain in 1902 and its use remains widespread today. Silver staining techniques require very careful attention to detail for a successful outcome. Because of this, it can be difficult to obtain consistent, reproducible results for one stain from run to run. In this study, we will show some of the common problems that may cause staining inconsistencies when using the Bielschowsky silver stain method.

Introduction
Alzheimer's disease is the most common cause of dementia, accounting for approximately 60% of known dementia cases. Dementia is a devastating condition not only for the patient, but also the patient's family and caregivers. Other diseases can cause dementia, and because there is no antemortem test that can be used to differentiate the causes, Alzheimer's disease must be confirmed by postmortem examination. The Bielschowsky silver stain is the most common stain used to show the senile plaques and neurofibrillary tangles which differentiate Alzheimer's disease from other causes of dementia. Silver stains can be costly and time-consuming to run. Many

histology technologists become frustrated with what appears to be significant batch-to-batch variability. Silver stains require great attention to detail and conditions, thus making it difficult to get consistent, reproducible results from run to run.

Materials and Methods
Modified Bielschowsky Stain.
Purpose:
To demonstrate nerve fibers and the presence of neurofibrillary tangles and senile plaques to aid in the diagnosis of Alzheimer's disease.
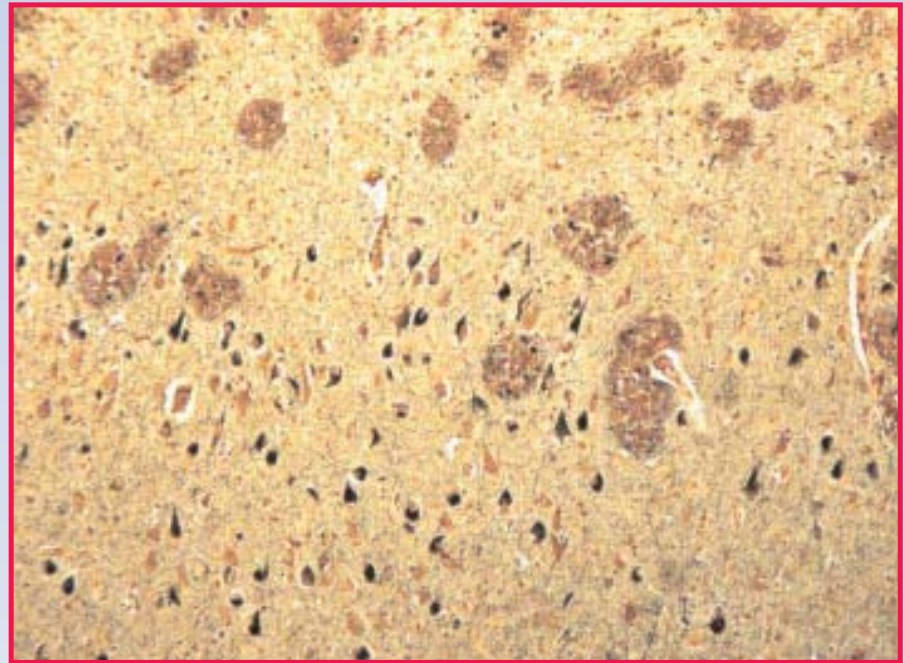

Fig. 1. Proper Bielschowsky staining—crisp golden background, nicely stained plaques and tangles. Normal neurons show no staining. Human brain, 100X
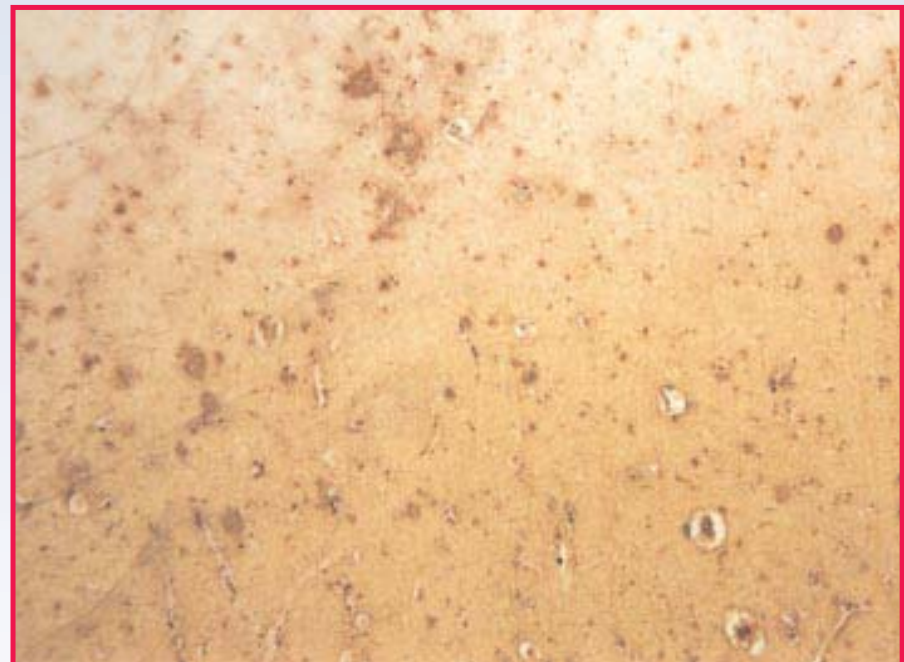

Fig. 2. Loss of staining and faded background are due to the age of this section (greater than 6 months old). Human brain, 100X

Principle:

A preliminary silver impregnation with silver nitrate is followed by an ammoniacal silver treatment. Silver is deposited on the neurofibrillary tangles and senile plaques and is then reduced to visible metallic silver (black) by the action of the formaldehyde reducing agent. Sections are not toned with gold chloride, thus a golden background remains.

Fixation:

Whole brains are fixed in 10% neutral buffered formalin for 2 weeks before sectioning.
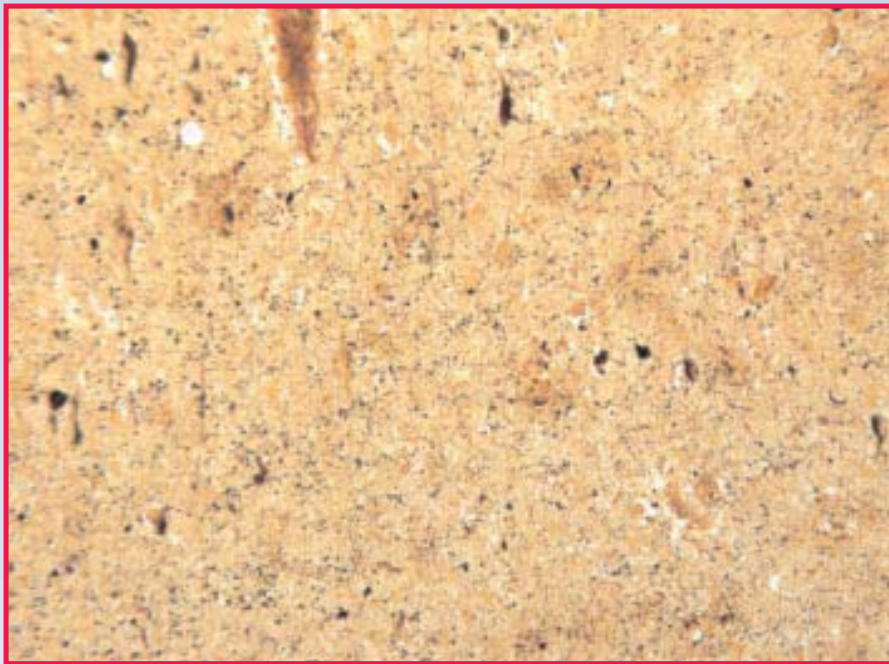

Fig. 3. The peppering effect in this section is due to silver precipitate and failure of plaques to stain properly with silver resulting from dirty glassware. Human brain, 160X
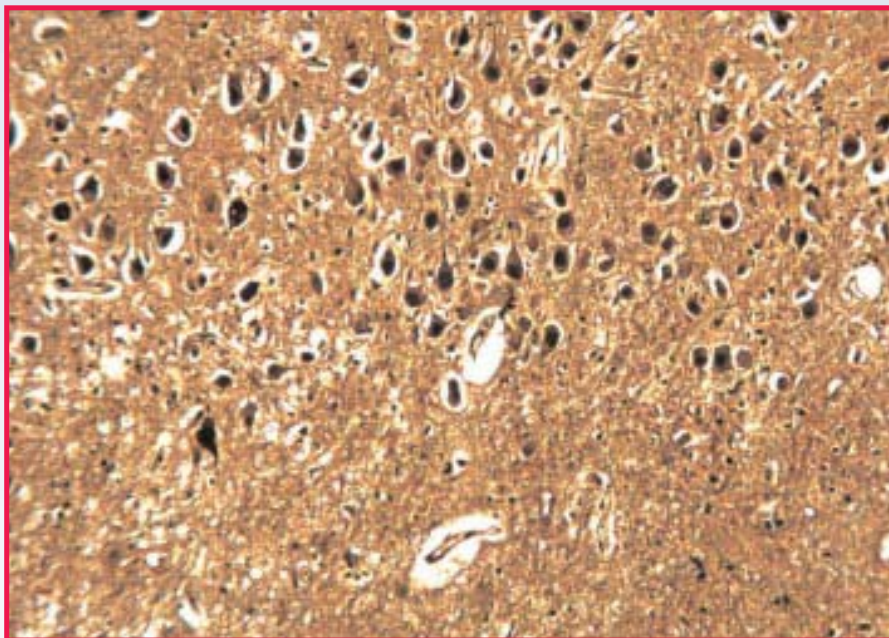

Fig. 4. Overdevelopment of the Bielschowsky stain, which makes it difficult to distinguish tangles from normal neurons. Human brain, 16X

Tissue handling:

After processing, paraffin sections are cut at 6 microns. DO NOT use charged or coated slides; instead, add about 0.05 g gelatin to water bath, fill with distilled water, and heat to 40°C. (Adding the gelatin first and then filling with water prevents gelatin from floating on the top of the water bath and depositing on slides.) If soaking blocks prior to sectioning, do not use ammonia to soak. Use plain water only. Allow slides to air dry overnight before staining. Use only glass or plastic slide holders, acid-washed glass coplin jars (see discussion), and nonmetallic forceps. Run no more than 10 slides per batch.

Solutions:

20% Aqueous Silver Nitrate
Silver nitrate
(Spectrum Chemicals S1085) …10 g
Distilled water   ………………50 ml

Make up just prior to use in a clean 250 ml flask. This solution should be clear. A cloudy solution is indicative of a glassware problem. Do not use if cloudy.

If solution is not to be used immediately, store in the dark. Use for initial staining and then retain solution.

Ammoniacal Silver
To 50 ml of the silver nitrate staining solution, add FRESH concentrated ammonium hydroxide (Fisher #A669500) drop by drop until precipitate just disappears. Do not add excess ammonia! It is better to have a few grains of silver left at the bottom of the flask.

Ammonia Water
Add 2 drops of concentrated ammonium hydroxide to a coplin jar of distilled water.

Developer
Formaldehyde (37%-40%) …20 ml
Distilled water ………………100 ml
Conc. nitric acid ……………1 drop
Citric acid   …………………0.5 g

Procedure:

1. Deparaffinize and hydrate sections. Rinse all glassware well with distilled water before staining begins.
2. Stain in 20% silver nitrate for 15 minutes in the dark. Save solution.
3. Put slides into a coplin jar of distilled water while doing step 4.
4. Pour silver nitrate back into flask and add ammonium hydroxide drop by drop. Mix by swirling flask until precipitate just disappears. (A brown precipitate will form and will disappear with the addition of ammonia.) Be careful not to add excess ammonia. It is better to have a small amount of silver left in the flask than to use excess ammonia. Solution should be clear, not cloudy.
5. Stain slides for 10 minutes in the dark.
6. Remove slides to second coplin jar containing ammonia water.
7. Add 2 drops of developer to the ammoniated silver and stir. Put slides into solution and begin checking by the microscope when slides begin to turn brown. Rinse slides in ammonia water and check staining under the microscope, watching for plaque development. Tangles and neurofibrils develop before plaques. Rinse in ammonia water and place back into ammoniated silver. Continue checking each case every minute or so. Timing of development will vary for each case, so it is important to check each one separately.

   When plaques are nicely developed, place slides into coplin jar of ammonia water. Do not overdevelop or a black precipitate will form over the slides and the tissue. Overdevelopment also makes it difficult to distinguish tangles from normal neurons.
8. Rinse slides in ammonia water, followed by a rinse in distilled water.
9. Dehydrate, clear, and coverslip immediately. Do not leave slides in xylene. This method uses two changes of fresh xylene, 30 dips each. Coverslip immediately.

Results

Axons …………………………black
Neurofibrils …………………black
Plaques ……………brown to black

Discussion

It has been found that humidity plays a significant role in the consistency of this stain. The ammonium hydroxide used in step 4 must be fresh and free of moisture. When using old ammonium hydroxide which has
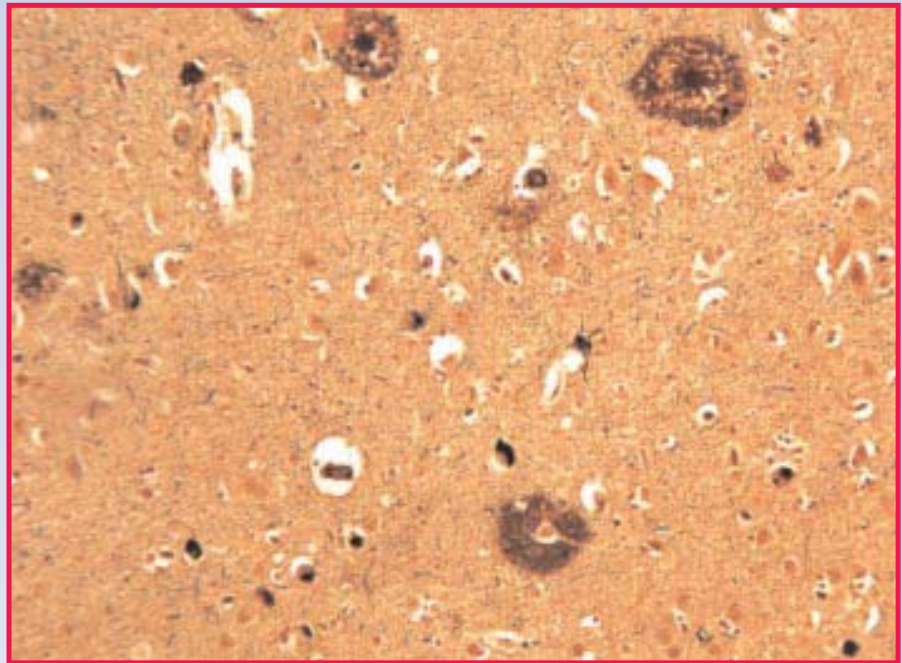

Fig. 5. Brown, muddy background staining can result from high humidity, old ammonium hydroxide, or when sections are left sitting in xylene too long before coverslipping. Human brain, 16X
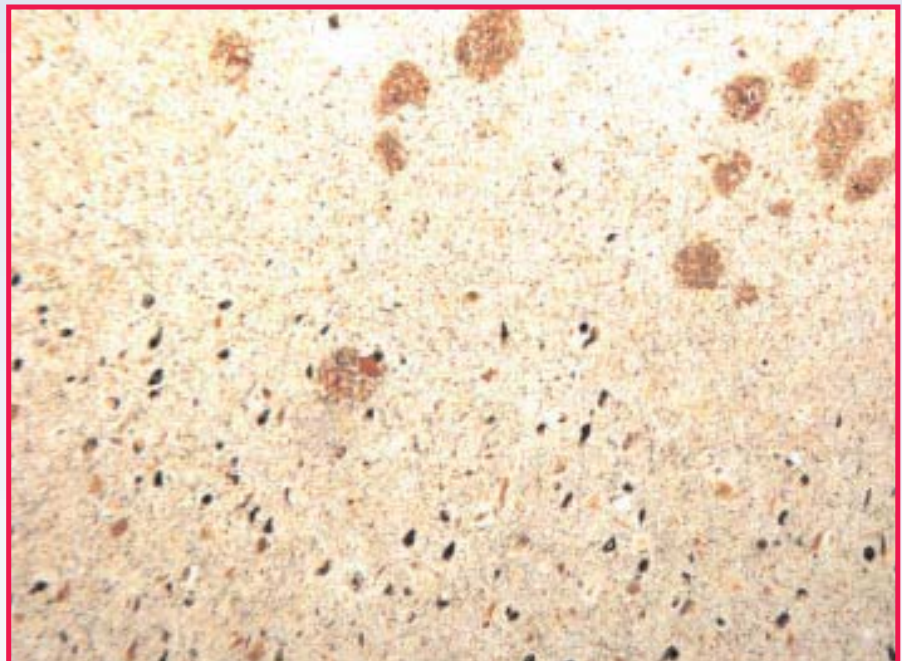

Fig. 6. Poor Bielschowsky staining due to high humidity. Note loss of background staining and amyloid plaques that do not pick up the silver stain. Human brain, 100X

absorbed atmospheric moisture, one will note that it is necessary to add more at step 4 in order to clear the precipitate. At the development step, the silver will tend to precipitate onto the tissue before the plaques develop, causing a "peppering" effect. The background often turns a dull brown or greenish color instead of golden. Humidity can also cause uneven staining within a section or batch of slides. These things can lead to interpretation problems. Changing to a new bottle of ammonium hydroxide will resolve these problems. It is suggested that labs using this stain order fresh ammonium hydroxide often, every 1-3 months depending on humidity levels. Store the bottle in a desiccator to help keep moisture out.

Development is another critical issue with this stain. Individual slides will develop at different times, making it necessary to frequently check the slides microscopically during development. Tangles will appear before plaques in most cases. It takes a little practice to get comfortable with development, so it is recommended to start with a known positive control and run the stain several times using that control. This will give the histotech a feel for when and how plaques develop, and when to stop development. Running too large a batch can lead to overstaining of some slides; that is why a limit of no more than 10 slides per run is suggested. Overdeveloping the slides can cause too dark a background, which makes it difficult to distinguish normal neurons from tangles.

Another problem area with all silver stains is glassware. All glassware used should be acid cleaned frequently. Fill coplin jars and flasks used to make up reagents with 50% nitric acid and allow to sit for at least 5 minutes, followed by a long wash in distilled water. In between acid washings, use full strength bleach and allow to sit overnight. Wash and rinse well. Dirty glassware will wreak havoc with this stain, mainly by causing silver to precipitate everywhere. Keep two sets of glassware, consisting of two square coplin jars and a 250 ml flask, to use for Bielschowsky staining exclusively. The use of charged or coated slides will cause the silver to precipitate out, leaving a poorly stained section, but a nicely mirrored slide.

Silver nitrate is light sensitive. Store tightly closed bottle in the refrigerator in the dark. Retain the cardboard sleeve which comes with the silver nitrate and store opened bottles in there. Because the dehydration and clearing steps need to be done rapidly, use fresh alcohols and xylenes each day. Coverslip immediately—do not leave in xylene.

While all of this may seem tedious initially, it becomes easier with practice. You will begin to recognize how much ammonium hydroxide is needed in step 4, when the ammonium hydroxide needs to be changed, proper development timing, as well as other nuances involved with silver staining. With practice, this staining technique will becomes less problematic to perform and should take about 30-40 minutes from start to finish. By paying close attention to detail, the Bielschowsky silver stain can be a consistent and reliable method to aid in the diagnosis of Alzheimer's disease.

References
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Controls for the Immunohistochemical Staining of Cytology Samples

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Introduction
There seems to be an increasing need to stain cytology samples such as fine needle aspirates (FNA) and non-GYNs with special stains, immunohistochemistry (IHC), and even in situ hybridization (ISH). This poses staining challenges for the laboratory in several ways. First and foremost, these cytology samples are not fixed in routine histological fixatives such as formalin. Secondly, they are not processed into paraffin on a tissue processor. In this respect cytology preps are unique in that they are excluded from routine tissue preparation techniques. As a result, the use of formalin-fixed, paraffin-embedded (FFPE) tissue controls for the IHC staining of cytology samples does not in fact control for the manner in which those samples are prepared, setting the stage for erroneous staining results. Yet for many labs, FFPE tissues may be the only controls available to validate staining runs. With a little ingenuity, this can be overcome. To begin, one must become acquainted with what goes on in the cytology prepping area.

Cytology
Cytology preps are often described as GYNs (the cervical/vaginal PAP), non-GYNs (most body fluids, effusions, and expectorants), and FNAs (fine needle aspirates of lesions). GYNs may be the traditional PAP smear, or the ever-increasing liquid phase PAP. The latter are generally collected in a fixative solution that has been formulated to work with specific

prepping instruments such as the ThinPrep® (Cytyc Corp., Boxborough, MA) or the AutoCyte® (TriPath Imaging Inc., Burlington, NC). These instruments prepare a uniform monolayer of cells that facilitates slide interpretation under the microscope. Non-GYNs are body fluids from outside the female gynecologic tract. They include bladder washes, spinal fluid, urine, pleural fluid, thoracenteses, bronchial lavages, etc. These samples are handled in a variety of ways, and may differ substantially from one lab to another. Samples of body fluids are often spun down in a centrifuge, after which the supernatant is decanted and the button of concentrated cells is used to make smears. Specimen preparation may be carried out either on one of these instruments previously mentioned, or by centrifugation, or they may simply be smeared onto a glass slide. Sputum samples require unique handling due to their viscous nature; this comes from the presence of mucous, which is often dissolved before smears are made.

FNAs are tissue-like samples extracted through a thin (20, 23, 25, 26 gauge) hollow needle attached to a 10 ml disposable syringe. A Cameco syringe holder allows for enough vacuum to extract the desired cells. The needle is guided into a lesion with radiologic guidance (such as MRI, CATT, or ultrasound).

Palpable, superficial lesions may be sampled directly without the need for radiologic guidance. The needle is inserted in several directions in order to fill the core of the needle with cells, which are then expelled onto glass slides to make smears. Afterward, the needle is rinsed in cytology fixative, typically 95% alcohol.

As in histology, fixing the cells is essential to preserving morphology.

However, fixation methods in cytology do not necessarily mimic those used in histology. There are about four general methods used to fix cytology preps: wet fixation, wet fixation followed by air drying, spray fixation, and postfixation after air drying.[1] Cytology fixatives include a variety of alcohol solutions (reagent, isopropanol, and ethanol), carbowax, cytology sprays and hair spray, and fixatives (e.g., Saccomanno) that dissolve the mucoid substance in sputum samples.[2] A variety of proprietary cytological fixatives are commercially available.

The unique preparation procedures employed for cytology samples warrant the use of known positive control cells that have been prepared in the same manner as the patient samples for IHC staining, in order to have valid interpretation of staining results. In the absence of this, the stage may be set for misinterpretation. IHC laboratories typically employ procedures customized to compensate for the effects of formalin and paraffin processing which may have otherwise masked tissue antigens, yet it cannot be

assumed that carrying out these same strategies for epitope retrieval in cytology samples will yield valid staining results.

Controls
Ideally, cytology controls should be readily available in the IHC staining laboratory, although this is typically not the case. Stain protocols must be tailored through trial and error to achieve optimal results.

It goes without saying that the best kind of control material would come directly out of the cytology lab, fixed and prepped in the same manner as any patient cytology sample. In spite of the volume of many of the fluids that come into the cytology lab, cellularity is often scant and unpredictable. The fluid might be centrifuged or used in a monolayering instrument to make the initial PAP-stained slides for screening; after this, there may be little cellular material remaining. Going back to the original sample may not produce enough cells of interest to provide even a few more slides, and certainly not the large number needed for controls. Additionally, if the sample is rather cellular, it is often submitted for a

Fig. 1. Slides of cell lines fixed in various cytology fixatives.

Fig. 2A, 2B. Cells scraped from a lymphoma placed into various cytological fixatives.

slide. For non-GYNs, fluids of the same type may be pooled as well. For example, sputums known to be positive for AFB or *Pneumocystis carinii* may be pooled in order to make more slides. This collection of cells may be stored in cytological fixative for a limited time depending on which cytological fixative is employed. This will only succeed if the cytology staff is vigilant in keeping an eye out for suitable cases.

2. Cell cultures: Cell lines of specific tumor types may be acquired through the American Type Culture Collection (ATCC). These are received as frozen pellets and need to be grown up in a specific culture medium. To take advantage of this particular opportunity, one needs to have either the expertise to grow cells in culture or access to those who do. With the right expertise, a nearly inexhaustible supply of cells can be maintained. Without it, the cells may starve, be grown in the wrong medium, or become overcrowded and die. When the cells are ready to harvest, they can be introduced into an electrolyte solution for transport to the histology lab or put directly into the cytology fixative to be used. However, this approach will be limited by the available resources for growing up and maintaining cell populations. It is imperative to always replace some cells for future needs (see Fig. 1).

3. Solid tissue: Solid tissue presents a great opportunity for collecting cells to use as cytology controls. An arrangement can be made with the OR suite to pick up unfixed tissues for this application. Immediately begin grossing the sample and, during this process, cells may be collected from unfixed solid tumors and organs by scraping very lightly across the surface of the tissue with the edge of a scalpel blade. The cells are rinsed off the blade's edge into an appropriate cytological fixative or electrolyte solution. A light touch

cell block, thereby consuming the remainder of the sample. The very nature of cytology samples and how they are used for evaluation usually eliminates the opportunity for further use in the making of control slides.

Making Cytology Controls
There are several approaches this author has taken that have provided satisfactory results. No matter which approach has been used, there is a fair amount of discovery work required until the procedure is ready to be implemented for routine use.

Collecting cells to use as controls:
1. Body fluids, FNAs, and liquid-based PAPs: These cytology samples may be pooled to create a batch of cells. For example, liquid phase PAPs known to be positive for either high risk or low risk Human Papilloma Virus (HPV) may be pooled to create a stock of cells to use in making more ThinPreps. This will be effective only if there are samples of adequate cellularity to permit making extra slides to be held for use as controls. Even then, it is impossible to ensure that there will be positive cells on every

is required to prevent scraping off large sheets of cohesive cells. Another approach is to collect cells by using the same kind of needle that is used to perform FNAs, expelling the contents of the needle into the appropriate cytological fixative. Slides are then made from this collection of cells. This latter method is especially suitable for FNAs as these samples tend to be cohesive cells (see Fig. 2A, 2B).

Fixing cells to use for controls:
1. An electrolyte solution may be used to collect the cells prior to sorting them into the cytology fixatives. This is especially helpful if more than one application is intended for these cells.

2. It is most important to use the exact fixatives that are being used in one's cytology department. Many use 95% ethanol for FNAs, and then proprietary fixatives for ThinPreps, air-dried slides, etc. Controls should be fixed accordingly. Any deviation from the way it is done in the cytology lab may result in a difference in IHC staining, compromising the effectiveness of the control.

3. Once the cells are in fixative, preps can be made.

Preparing control slides:
1. Again, it is important to mimic what is done in the cytology lab. If it is possible to use the cytology instruments to make the preps, all the better. A number of control slides can be made from the same sample of cells and then fixed in a variety of ways (see Fig. 3).

2. A small aliquot may be taken from this pooling of cells when a control is needed. Or, controls can be made in advance and stored in the appropriate cytological fixative. If the cytology lab makes slides with a specific fixative followed by air drying, one should do the same. Alternatively, if the slides are

sprayed after drying, this too should be copied. Air-dried and sprayed slides store well in a desiccator or a box with desiccant in it.

3. Charged glass slides may be useful in keeping cells adhered to the glass.

Protocols
Cytology samples that are sprayed with hair spray or any of the many cytological spray fixatives may need to be soaked in 95% alcohol prior to staining to remove the spray fixative. Air-dried samples may or may not be placed in PBS or similar buffer prior to IHC staining. Much of the handling of these prepared slides is going to be dictated by the protocols to be followed. It is likely that some effort will have to go into discovering the best way to handle the slides.

Protocols often need to be adjusted for the fixatives being used in the cytology preps, with IHC and ISH being the most affected. These protocols include proteolytic enzyme digestions, some antigen unmasking processes, incubation times, and blocking steps. In most applications, an enzyme digestion step needed for IHC on paraffin may be eliminated for the cytology sample. ISH, however, will most likely require it, but may be less than its paraffin counterpart. Endogenous biotin may be higher in some cytology samples, particularly with bladder washes, urines, FNAs of kidney, and sometimes retroperitoneal masses. However, this is only an issue if a biotin-streptavidin detection method is used. A biotin blocking kit will easily remedy this. Endogenous peroxidase may be an issue with bloody samples when using a horseradish peroxidase detection method. Block with 3% hydrogen peroxide. Endogenous alkaline phosphatase may be troublesome with kidney FNAs, but only if using an alkaline phosphatase detection method. This can be easily blocked with levamisole. Hormone receptors may still require the same antigen unmasking that is required

Fig. 3. A cytocentrifuge (like the Sakura Cyto-Tek®) is used to concentrate cells from body fluids when preparing IHC control samples..

for other markers in fixed, paraffin-embedded material such as Ki67 and P53. In some instances, a clone that works well for paraffins may not prove to be suitable for alcohol-based fixatives. Whatever changes will be needed to accommodate cytology samples are mostly dependent upon the choice of cytology fixative and the staining protocol to be followed. Care must also be exercised while staining, since cells tend to shed from the slides and fall into solutions while performing IHC staining of cytology slides. This is not new to cytology but is something the histology section usually does not encounter. Reusing solutions should be done only if they are filtered after use.

Antigen Retention:
Most techs who routinely perform IHC are familiar with the concept of loss of antigenicity. Although the causes are not clearly understood, experience has taught us that it exists. This also applies to cytology controls. It has been this author's experience that preps fixed in a methanol-based cytology fixative versus those fixed in an ethanol-based fixative do not necessarily retain the same degree of antigenicity for the same period of time. When keeping a cache of cytology slides for IHC purposes, they should be monitored for antigen retention. Once the viable time frame has been determined for a given set of antibodies, toss out old slides in favor of new ones. Cytology fixatives also have their own

influences on antigens, so look at all of the fixatives for their separate impact on antigenicity. Air drying, compared to being kept wet in a cytological fixative, may prove to be satisfactory for some applications but not for others. That was our experience. Estrogen receptors (ER) and progesterone receptors (PR) did not perform reliably on slides that were air dried following fixation in 95% alcohol, but did fine on slides that were prevented from drying and stored in 95% alcohol. Most cluster differentiation (CD) markers did well on air-dried slides. Each lab's experience may prove to be different.

RNA:
RNA is not robust enough to withstand the test of time or a number of handling techniques. Personal experience has shown that RNA was not detectable after air drying slides, while fixed slides that were kept wet remained usable.

Conclusion
It is important to remember that the intended use of a control slide is to provide evidence that a procedure was carried out in a precise manner. For this reason, a control slide verifies the conditions that were used to run the patient samples. These conditions should mimic the same handling, fixing, and preparation techniques in order to give one confidence that the procedure is optimized. Staining protocols that are already in place for routine histology tissues are not necessarily optimized to facilitate the same results with a cytology sample. Fixation, just as with histology tissues, is a key player in the preservation of cell morphology and antigenicity. But fixatives go about the process of denaturing and preserving cells in different ways. This, along with the methods used in slide preparation, exerts influence on final staining outcomes. The demand for extra staining techniques to be applied to cytological samples is on the rise. HPV testing and FNAs are leading the list, followed by non-GYNs for

tumor markers and hormone receptors. Since cytology offers a less invasive means of gathering cells to determine pathology, it makes sense to make the most of these when appropriate. In the long run, the focus is always on patient care.

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The Gram-Twort Method: A Superior Stain for Bacteria

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Abstract
The Gram's stain remains the most common means of identifying gram-positive bacteria in tissue sections. The quality of the stain is frequently dependent upon the degree of expertise of the technologist; poor judgment in the differentiation step can result in over- or under-differentiation of both gram-positive and gram-negative bacteria. Many solvents used in Gram's stain procedures are difficult for small laboratories to store or to dispose of safely. The Gram-Twort method is quick and simple to perform and yields results far superior to other more commonly performed bacterial stains.

Discussion
First published in 1884 by Hans Christian Joachim Gram, the original Gram's stain used aniline gentian violet combined with iodine-potassium iodide to stain gram-positive bacteria. Alcohol was used to differentiate the organisms. This procedure enabled Gram to recognize two distinct types of bacteria. Subsequent methods varied largely in the solvent used for differentiation. Carl Weigert, for example, proposed the use of aniline oil in this role. Although aniline oil differentiates slowly, it is an extremely hazardous chemical with which to work. Brown and Brenn used ethyl ether-acetone to differentiate the gram-positive bacteria followed by picric acid-acetone to differentiate gram-negative bacteria. In a method still popular today, underdifferentiation with ethyl ether-acetone makes it difficult to discern gram-positive bacteria from surrounding tissue elements, or to stain the gram-negative bacteria. If over-differentiated with picric acid-acetone, the dye is removed from the gram-negative bacteria; underdifferentiation leaves the background too busy to make ready identification of gram-negative bacteria possible. Other solvents used for differentiation are acetone, propanol, methanol, butanol, and dioxane. Methanol was found to work too quickly; butanol too slowly.

A true Gram's stain is one that uses iodine which forms a precipitate with crystal violet in the wall of gram-positive bacteria. The resulting dye precipitate formed is insoluble in water. In gram-positive bacteria, the peptidoglycan layer of the cell membrane is thicker than in gram-negative bacteria. This thicker layer retards extraction of the dye-iodine complex. The thinner peptidoglycan layer of the gram-negative bacteria is unable to retain the crystal violet-iodine complex when exposed to solvents.[1] This results in slow decolorization of this group of organisms.[2] A 2% solution of crystal violet has been found to be the ideal concentration for dependable Gram's stain differentiation. Lower concentrations typically result in poor differentiation. The dye will be replaced with the counterstain in
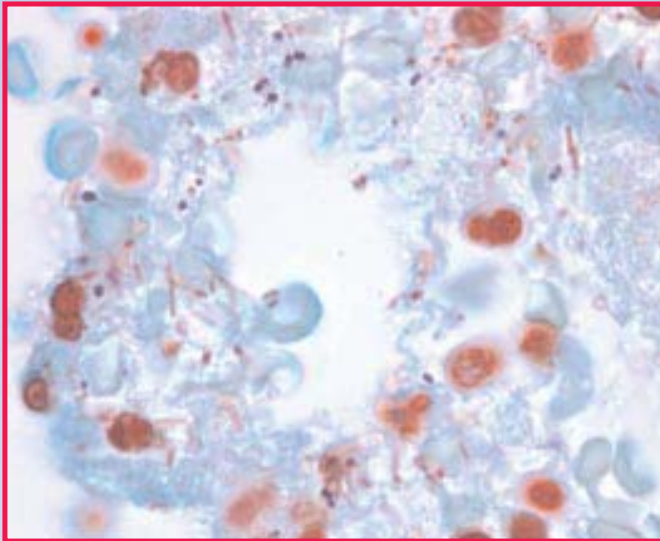
Fig. 1. The fast green counterstain provides a lighter background, allowing good contrast with both gram-positive and gram-negative bacteria. Gram-Twort stain, bacteria control tissue. 1000X
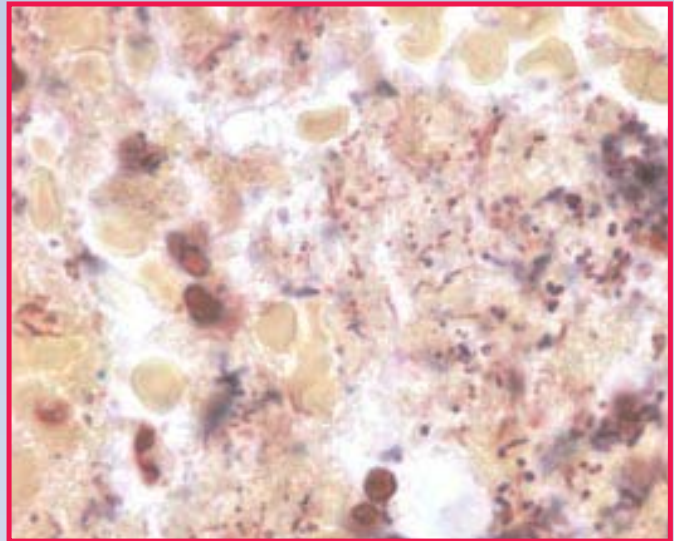

Fig. 2. The dark background staining from the Brown and Brenn technique makes it difficult to discern gram-positive and gram-negative bacteria. Brown and Brenn stain, bacteria control tissue. 1000X

gram-negative bacteria, but at a slower rate.[3] Prolonged washes in water should be avoided.

The Gram-Twort stain is both quick and easy to perform. Neutral red gives a lighter stain to the nuclei than most counterstains employed.[4] The use of fast green as a secondary counterstain for the background elements provides a good contrast for both the crystal violet and fast red; acetone is less likely to overdifferentiate the gram-negative bacteria as may occur with other solvents. Gram-negative bacteria are easily visible (Fig. 1). In contrast, a Brown and Brenn stain (Fig. 2) makes it difficult to discern gram-negative bacteria stained red with basic fuchsin against the intense red of the nuclei and background.

GRAM-TWORT STAIN[5]

Fixation: 10% neutral buffered formalin

Sections: 3-5 microns

Solutions:
Lillie's Crystal Violet

Crystal violet ....10 g
95% ethanol ....100 ml

Dissolve the crystal violet in the 95% ethanol.

Ammonium oxalate ....4 g
Distilled water.................400 ml

Dissolve the ammonium oxalate in the distilled water. Combine the crystal violet solution and the ammonium oxalate solution, stir for at least 3 hours, then filter.

Lugol's Iodine

Iodine ....1 g
Potassium iodide ....2 g
Distilled water.................100 ml

Neutral Red/Fast Green Stock Solution

0.2% Fast Green:
Fast green ....0.1 g
95% ethanol ....50 ml

0.2% Neutral Red:
Neutral Red ....0.9 g
95% ethanol....................450 ml

Cover the 2 solutions and stir for several hours; leave standing overnight, then filter. Combine the two solutions.

Working Neutral Red/Fast Green Solution

Stock solution ....15 ml
Distilled water ....45 ml

Mix and use immediately in a closed coplin jar.

Procedure
1. Deparaffinize and hydrate to water.

2. Lay slide flat and filter Lillie's crystal violet directly onto slide. Stain for 1 minute.

3. Wash briefly in tap water.

4. Place in Lugol's iodine for 1 minute.

5. Drain the slide (do not allow to dry) and flood with acetone to differentiate until no more color washes off, 2-5 seconds.

6. Rinse in distilled water.

7. Counterstain with neutral red/fast green working solution in a closed coplin jar for 5 minutes.

8. Rinse briefly in distilled water.

9. Dehydrate quickly, clear, and mount.

Results
Gram-positive bacteria ....blue

Gram-negative bacteria ....red

Nuclei ....red

Collagen,   varying
red blood cells,   shades
cytoplasm ....of green

It is important that the slide not be permitted to dry before differentiation in acetone. If the slide dries, it will greatly lengthen the differentiation time. Prolonged periods in ethanol will remove the dyes from both gram-positive and gram-negative bacteria.

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Fig. 1. Diagrammatic representation showing that the main cellular location of the prion protein (PrP) is the cell membranes, depicted here as a rail-like structure; PrP is attached to the membrane by the GPI (glycosylphosphatidyl inositol) anchor and hangs from the cell into the extracellular space; glycans (••) and specialized regions of the prion protein (depicted in yellow and red) are indicated.

Prion Diseases and Safety in the Histology Laboratory

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Prion diseases, or transmissible spongiform encephalopathies (TSEs), are fatal neurodegenerative diseases found in humans and animals. TSEs have raised concerns about public health safety as well as biosafety in operating rooms, autopsy services, and laboratories, especially the histology laboratory, because of the difficulty in neutralizing their infectious agents, called prions. Formaldehyde processing is known to kill virtually all infectious organisms but, due to their unique mechanism, prions are resistant to conventional fixatives and decontamination methods, posing a serious challenge to the histologist.

It came as a surprise to the scientific community when it was discovered that prions, the infectious agent of TSEs, are just proteins which, unlike bacteria and viruses, lack nucleic acid.[1] The process of normal protein manufacturing actually begins in the nucleus of the cell, where the genomic DNA, the blueprint for all proteins, is stored. Messenger RNA, a copy of the DNA blueprint, travels from the nucleus to the cell cytoplasm where it assembles the amino acids needed to build the various proteins. Normal or cellular prion protein (PrP^C) is made in this way. However, PrP^C is destined to be transported to the surface of the cell. Thus, as with all proteins that are associated with cell membranes, PrP^C is actually assembled and undergoes initial modifications in the endoplasmic reticulum (ER), a cell organelle made of elongated membranes. While it is being assembled, PrP^C remains attached to the membranes of the ER by a complex molecule made of sugars and fat called the glycol-lipid anchor. During assembly, sugars are also attached to PrP^C molecules, as PrP^C is a glycoprotein. The presence of sugars generates three major forms, called glycoforms, of PrP^C: highly glycosylated (with two sugars attached), intermediate (with one sugar), and unglycosylated (lacking sugars). Next, PrP^C is transferred to the Golgi apparatus, another membranous organelle similar to the ER, where the sugars are modified to take on their final configuration, and PrP^C is ready for the second leg of its journey in the cell. Packed in vesicles formed from the Golgi apparatus, PrP^C is carried to the cell membrane where it is positioned so that it hangs by the anchor outside of the cell. This critical location exposes PrP^C to any harmful agents that may be present in the extracellular space[1] (see Fig. 1).

The basic event that triggers prion diseases is the change in shape or conformation of PrP^C. This protein, which is normally flexible and soluble, is converted into a form, called scrapie PrP or PrP^{Sc}, which is rigid and tends to form large aggregates. Because of this aggregate-forming property, PrP^{Sc} captures nearby PrP^C molecules,

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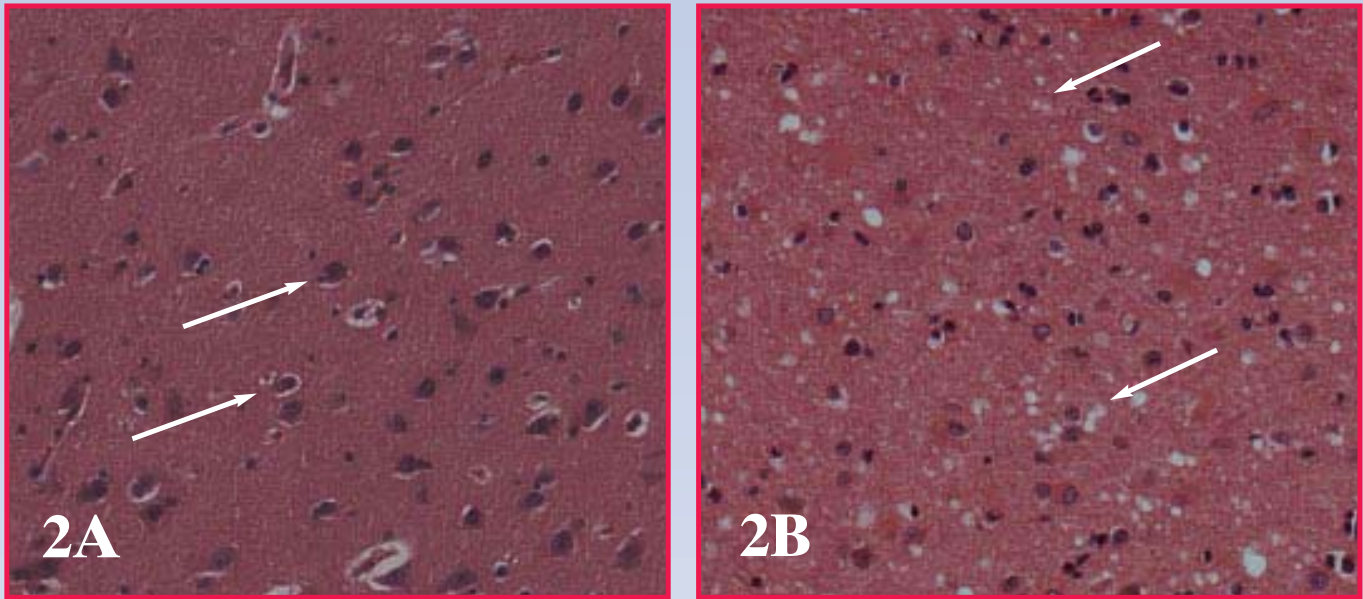
Fig. 2. Histological sections: (A) normal brain tissue; neuronal cell bodies (arrow) and other cells and vessels are separated by neuropil, ie, tissue-containing cell processes; (B) brain tissue from a patient with sporadic Creutzfeldt-Jakob disease (sCJD) (H&E); the tissue shows moderate spongiform degeneration which appears as multiple vacuoles in the neuropil (arrows). 20X

transforming them into copies of themselves. Through this process, PrP^{Sc} increases in number until it causes the disease. The mechanism is shared by all prion diseases.

Human prion diseases have a prevalence of one case per 1 million of the general population.

They exist in three forms: sporadic, familial (or inherited), and acquired by infection. Sporadic prion diseases are by far the most common, accounting for 85% of all prion diseases. In the sporadic form, the PrP^{C} to PrP^{Sc} conversion occurs spontaneously as a mishap during protein manufacturing, which often happens late in life. The familial form, which accounts for about 15% of prion diseases, is due to the presence of a defect or mutation in the gene encoding PrP^{C}. The mutated PrP is unstable and, with time, converts into PrP^{Sc}, triggering the disease. In the form acquired by infection, PrP^{Sc} comes from the outside, generally from contaminated food or as a consequence of medical or surgical interventions. The iatrogenic form can be transmitted through the use of contaminated surgical instruments, human-derived pituitary hormone therapy, and transplants of dura mater (the outer brain covering) and cornea. Approximately 270 prion cases of the iatrogenic form have been documented worldwide[2] (see Tables 1 & 2).

Most human prion diseases begin clinically at the age of 50-60 years with progressive mental deterioration that soon becomes associated with progressive unsteadiness and clumsiness, visual deterioration, and myoclonus (muscle twitching). The patient is usually mute and immobile in the terminal stages, and in most cases death occurs within months. The

Table 1: Classification of Human Prion Disease

Sporadic	Creutzfeldt-Jakob Disease (CJD) Fatal insomnia
Familial (inherited)	Creutzfeldt-Jakob Disease Fatal familial insomnia (FFI) Gerstmann-Straussler-Scheinker disease
Acquired by infection	Iatrogenic (CJD) Kuru variant CJD

Table 2: Classification of Animal Prion Disease

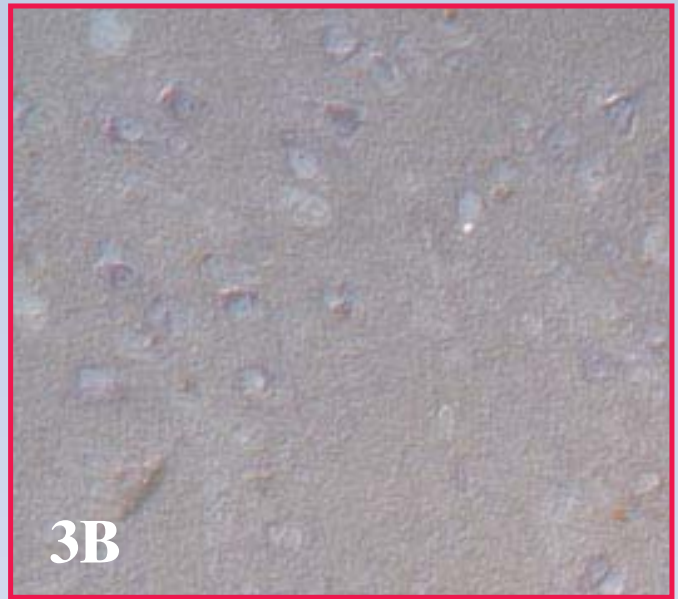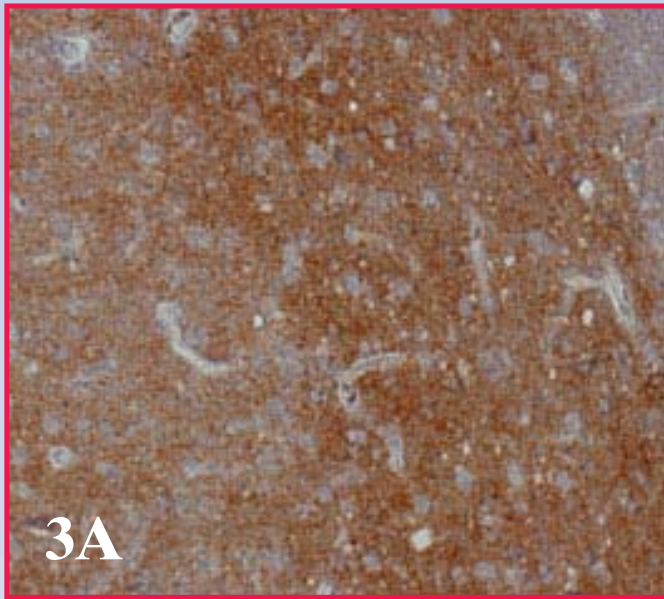|	
Probably all acquired by infection	Scrapie Bovine spongiform encephalopathy Chronic wasting disease Transmissible mink encephalopathy Transmissible spongiform encephalopathy of domestic and captive animals

Fig. 3. Immunostaining with a monoclonal antibody to PrP: (A) normal brain; normal PrP is undetectable in normal brain; (B) sCJD brain; there is a fine punctate immunostaining corresponding to small deposits of putative scrapie PrP. 20X. Courtesy of Diane Kofskey.

histopathological hallmark of prion diseases is spongiform degeneration that leaves the brain riddled with many microscopic vacuoles and is typically associated with varying degrees of astrogliosis and loss of neurons. In some rarer prion diseases, like fatal familial insomnia (FFI), spongiform degeneration may be lacking while selective neuronal loss and gliosis are prominent. In other diseases, like Gerstmann-Straussler-Scheinker disease (GSS) and variant Creutzfeldt-Jakob disease (vCJD), amyloid plaques containing abnormal PrP are prominent[1,2] (see Figs. 2 & 3).

It was known for many years that abnormal prion protein affected animals, but animal prion diseases were not considered harmful to humans. In the 1980s, a prion disease called bovine spongiform encephalopathy, or BSE, affected cattle in the United Kingdom (UK). Quickly BSE became an epidemic. More than 180,000 heads of cattle were proven to have BSE, but an estimated one to two million infected animals unwittingly entered the market and were consumed. The BSE epidemic was apparently triggered and sustained

by the practice of feeding cattle with diets enriched in proteins originating from other affected animals, most likely cattle and sheep. (Sheep have been known to carry another prion disease, scrapie, which apparently is not transmissible to humans.) When the high protein diets were discontinued, BSE receded.

However, in the mid 1990s, a new prion disease, called variant CJD (vCJD), appeared in the UK. Variant CJD affects humans and appears to be acquired from eating contaminated beef[1,2] (see Fig. 4).

As of February 2003, 140 cases of vCJD have been reported—130 in the UK, 6 in France, and 1 each in
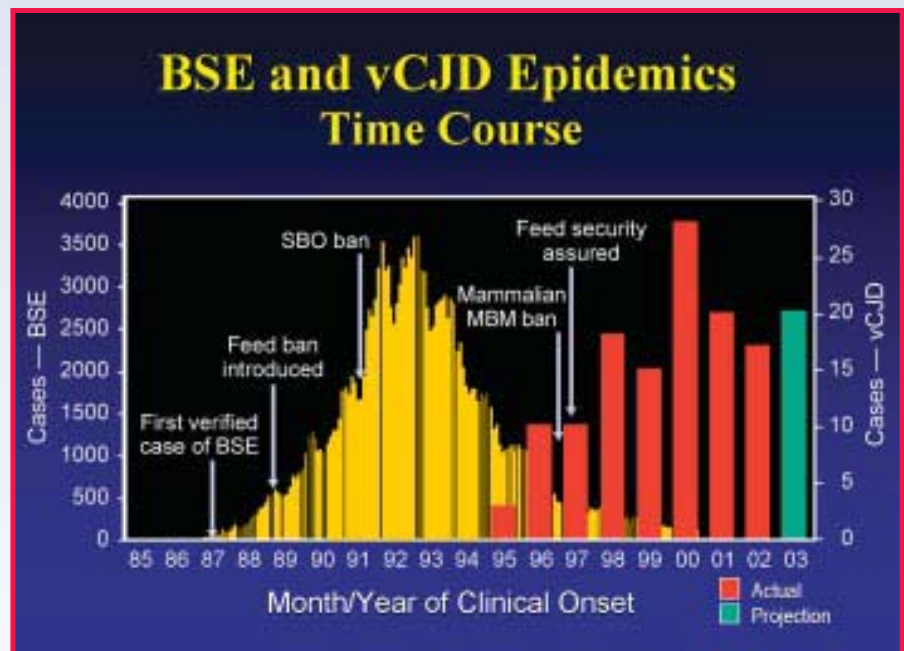
Fig. 4. Graph representing the time course of the BSE and vCJD epidemics. Note the bimodal incidence of the two diseases with 8-year separation of the two corresponding peaks. Courtesy of the International Life Sciences Institute.
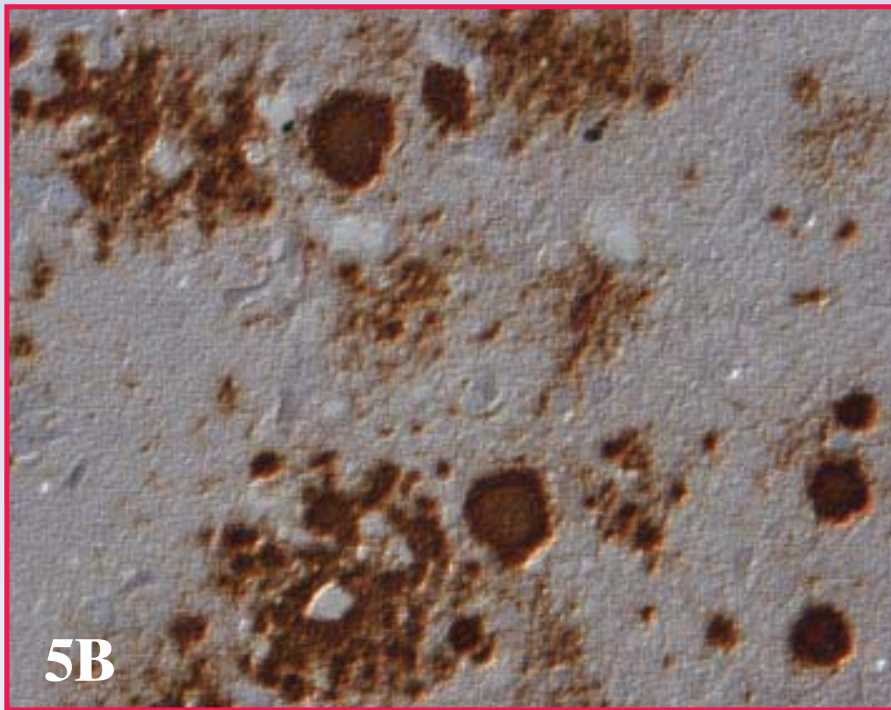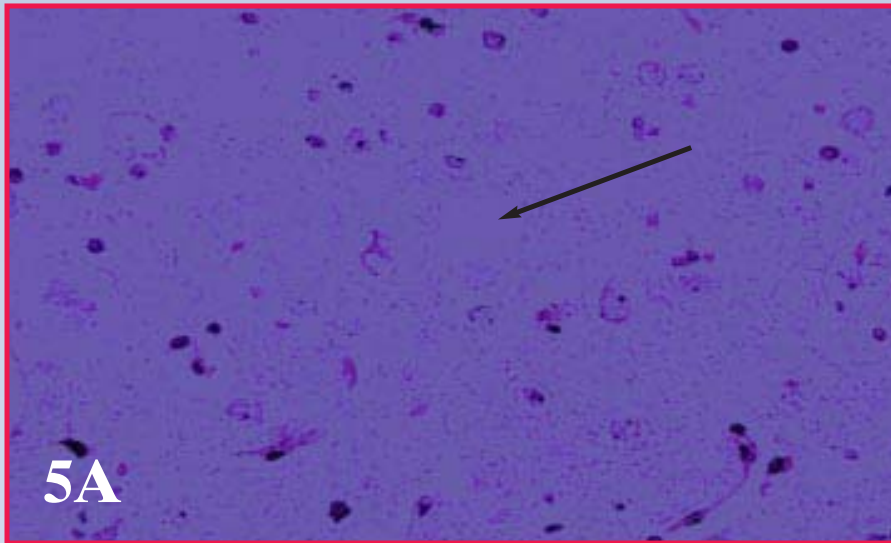
Fig. 5. Histological and immunostained section of brain from a patient with variant CJD (vCJD) (H&E).
(A) The hallmark of vCJD is the presence of florid plaques (arrow), ie, an amyloid plaque. (B) The immunostaining for PrP is very intense, especially in the florid plaques surrounded by vacuoles. 20X

at Case Western Reserve University, headed by Dr. Pierluigi Gambetti. The goal of the surveillance is to monitor, characterize, and store all cases of suspected and proven human prion disease which occur in the United States. More importantly, it serves as a center equipped with highly trained and experienced personnel accustomed to handling the demands of working with infectious material. It is highly recommended by the World Health Organization (WHO) to contact the center whenever there is a case of suspected human prion disease. Although one case has been reported in Canada, there is no evidence supporting the presence of BSE in the US, although the extensive testing that led to the discovery of the disease in other countries has not been carried out here. In contrast, chronic wasting disease (CWD), a disease of deer and elk first observed in the 1960s and recognized as a prion disease in 1978, is now known to be present in free range or captive deer and elk in Colorado, Wyoming, Nebraska, South Dakota, Wisconsin, New Mexico, Illinois, Minnesota, Montana, Oklahoma, and Kansas. Concern has been raised regarding the possibility that the prion associated with CWD might be transmitted to humans in a similar way. However, the examination of several cases of prion disease in hunters and regular consumers of deer and elk meat has yielded no evidence that CWD is transmitted to humans.

The special characteristics exhibited by prions require careful attention to facilities, equipment, policies, and procedures. Early detection of potential cases and having the essential engineering controls in place are critical to prion risk management.

Routine autopsies and processing of formalin-fixed tissue require biosafety level 2 precautions, ie, procedures must be conducted in a dedicated area, preferably

Italy, Ireland, Canada, and the US. However, in the Canadian and US cases, the diseases were most likely acquired in the UK. Variant CJD is different from the classical CJD in that it generally begins at a mean age of 27 years (range 16-74) with behavioral changes, pain, and abnormal sensations in the extremities. Only in later stages do CJD-like signs appear. The mean disease duration is 16 months (range 9-38). The

neuropathological changes are characterized by spongiosis and the presence of florid or daisy-like plaques—PrP-containing amyloid core surrounded, like petals, by vacuoles (see Fig. 5).

The finding of BSE, and even more the vCJD, prompted European countries to establish prion surveillance centers. In 1997, the National Disease Pathology Center was established in Cleveland, Ohio

inside a biosafety cabinet, and using absorbent pads to eliminate spread of fixative and other contaminated liquids.[3] During processing, personnel are required to wear disposable garments (nitrile gloves, laboratory coat, apron, and face shield). Formalin-fixed tissue blocks should be placed directly into cassettes, then postfixed with concentrated (96%-100%) formic acid for 1 hour followed by an additional 48 hours in 10% buffered formalin. This treatment does not significantly affect histology and immunohistochemistry. If a dedicated processor and stainer are not available, processing and staining are done by hand using disposable containers. During sectioning of the blocks, excess paraffin is collected in biohazard bags. Coverslipped slides are soaked for 2 hours in 2N NaOH. Each block is sealed with paraffin, labeled "CJD precaution," and filed separately from other cases.[3,4]

Decontamination requires robust use of sodium hydroxide. Dry waste (paper towels, disposable garments, excess paraffin) must be disposed in double biohazard bags for incineration. Liquid waste (formalin, alcohol, xylene) is placed in plastic containers with NaOH to a final concentration of 1N. In general, the use of 1N NaOH and/or autoclaving is recommended for the instruments that can tolerate such treatment. Instruments (forceps, knives, cutting boards, scissors) are soaked in 25% bleach (1:4 dilution of commercial bleach) immediately after use, autoclaved at 132°C for 4.5 hours, and rinsed in water. For equipment that can't be autoclaved: soak overnight or soak in 3 changes of 1N NaOH for 30 minutes each, then rinse copiously with water. Dispose the liquid as biohazard waste. For contaminated surfaces that can tolerate NaOH: apply 1N NaOH to the surface, allowing 15 minutes of contact, and wipe with 0.025M acetic acid, wash thoroughly with water and wipe surface dry. If a spill occurs or tissue comes in contact with a surface, cover the area with paper towels soaked in 1N NaOH, continuously kept moist, for 1-2 hours.[1-4]

The dreaded event, of course, is when tissue that has been processed under normal conditions is found to be prion positive. It is during these times that care must be taken to backtrack and begin the arduous task of decontamination. However, decontamination of large instruments (processors and stainers) is not an easy task. It requires a plan incorporating the Centers for Disease Control and Prevention (CDC) guidelines and manufacturer recommendations. Depending on the degree of contamination, equipment may have to be replaced.

Although prion diseases are much better understood and diagnosed today, strict adherence to the specific guidelines established by the CDC and the World Health Organization are imperative when working with prion-infected tissue. The National Pathology Disease Surveillance Center, available at: www.cjdsurveillance.com, under the guidance of the CDC, is available for prompt referral of these cases.

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Educational Opportunities in 2004

January 16   **University of Texas Health Sciences Ctr/ San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **"Basic Principles of Fixation"**
Speaker: Barry Rittman, PhD
     Univ of Texas Health Sciences Center at Houston

January 21   **National Society for Histotechnology Teleconference**
Title: **"New Innovations for Double and Triple IHC Stain Technology"**
Speaker: David Tacha, PhD, HT(ASCP)
     Biocare Medical
     Walnut Creek, CA
Contact: NSH office (301) 262-6221

February 18   **National Society for Histotechnology Teleconference**
Title: **"T&B Staining in Immunohistochemistry"**
Speaker: Alvin W. Martin, MD, & Sheron Lear, HTL(ASCP)
     University of Louisville
     Louisville, KY
Contact: NSH office (301) 262-6221

February 20   **University of Texas Health Sciences Ctr/ San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **"Apoptosis TUNEL IHC on Paraffin & Plastic Sections"**
Speaker: Frances Swain, HT(ASCP)
     University of Arkansas for Medical Sciences
     Little Rock, AR

March 6   **Arkansas Society for Histotechnology**
Site: Conway, AR
Contact: Donna Montague (501) 526-6147
     Carolyn Black (501) 686-6539
Email: dcmontague@uams.edu
     Blackcarolyn@uams.edu

March 17   **National Society for Histotechnology Teleconference**
Title: **"Sectioning Artifacts: Causes and Cures"**
Speaker: Peggy A. Wenk, BA, BS, HTL(ASCP)SLS
     William Beaumont Hospital
     Royal Oak, MI
Contact: NSH office (301) 262-6221

March 18-20   **Tennessee Society for Histotechnology**
Site: Marriott-Vanderbilt Hotel, Nashville, TN
Contact: Rhonda Schalk (865) 481-1172
Email: TSHpresident@juno.com

March 19   **University of Texas Health Sciences Ctr/ San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **"Tissue Engineering for the Histologist"**
Speaker: Linda Jenkins, HT(ASCP)
     Clemson University
     Clemson, SC

April 15-18   **Texas Society for Histotechnology**
Site: Holiday Inn Park Plaza, Lubbock, TX
Contact: Donna Willis (817) 878-5644
     Cliffa Vaughn (915) 690-6604
Email: donnawillistx2@aol.com
     Vychan1@prodigy.net

April 16   **University of Texas Health Sciences Ctr/ San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **"Grossing Procedures for the Histotech"**
Speaker: Michael LaFriniere PA, HT(ASCP)
     Memorial Hospital
     Chattanooga, TN

April 21   **National Society for Histotechnology Teleconference**
Title: **"Basic Methods for Producing High Quality Slides of Paraffin Embedded Bone"**
Speaker: Robert A. Skinner, BS, HTL(ASCP)
     University of Arkansas for Medical Sciences
     Little Rock, AR
Contact: NSH office (301) 262-6221

April 23-24   **Colorado Society for Histotechnology**
Site: Stanley Hotel, Estes Park, CO
Contact: John McGinley (970) 491-3041
Email: john.mcginley@colostate.edu

April 28-30   **Iowa, Minnesota, Wisconsin Tri-State Meeting**
Site: Holiday Inn, Dubuque, IA
Contact: Judi Stasko (515) 663-7445
     Pam Hesch (507) 284-4220
Email: jstasko@nadc.ars.usda.gov
     Hesch.pamela@mayo.edu

April 29 - May 1   **Histology Society of Ohio**
Site: Clarion Westgate, Toledo, OH
Contact: Susan Yoakam (419) 291-3702
Email: susan.yoakam@promedica.org

April 30 - May 1   **New York State Histotechnological Society**
Site: Holiday Inn, Saratoga Springs, NY
Contact: Judy LaDuc (518) 897-2247
Email: jaladuc@capital.net

April 30 - May 2   **Florida Society for Histotechnology**
Site: Embassy Suites, Deerfield Beach, FL
Contact: Jerry Santiago (800) 277-4613 x2874

May 1   **Massachusetts Society for Histotechnology Spring Symposium**
Site: Boston Symphony Hall
Contact: Jason Burrill (978) 658-6000 x1652
Email: jburrill@criver.com

May 1-2   **Washington State Histology Society**
Site: Richland, WA
Contact: Linda Cherepow (206) 667-1378
     Martha Pope (509) 375-2930
Email: lcherepo@thcrc.org
     Rmpope@charter.net

May 13-14   **Illinois Society of Histotechnologists**
Contact: Marge Horn (312) 791-5486

May 14   **ASCP Teleconference**
12:00 pm Central Time (800) 621-4142
Title: **"Microwave Technology in the Rapid Processing of Transplant Biopsies"**
Speaker: Lillian Antonio
     Mount Sinai Medical Center, New York City

May 19   **National Society for Histotechnology Teleconference**
Title: **"Staining and Demonstration of Microorganisms"**
Speaker: M. Lamar Jones, BS, HT(ASCP)
     LSU Health Sciences, Shreveport, LA
Contact: NSH office (301) 262-6221

May 20-22   **Michigan Society of Histotechnologists**
Site: Crown Plaza, Grand Rapids, MI
Contact: Paula Bober (313) 745-2540

May 20-24   **NSH Region III hosted by Alabama Society for Histotechnology**
Site: Wynfrey Hotel, Birmingham, AL
Contact: Rita Humphrey (205) 939-9639
     Michael LaFriniere (423) 495-6117
Email: rita.humphrey@chsys.org
     Mike_lafriniere@memorial.org

May 21   **University of Texas Health Sciences Ctr/ San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **"A Practical Approach to Image Analysis"**
Speaker: Donna Montague, MS
     University of Arkansas for Medical Sciences
     Little Rock, AR

June 3-5   **Arizona Society for Histotechnology**
Site: Grace Inn-Ahwatukee, Phoenix, AZ
Contact: Karen Lahti (480) 857-0977
Email: karen@gateslinger.com

June 3-5   **NSH Region II hosted by Maryland Society of Histotechnologists**
Site: Holiday Inn Select North, Timonium, MD
Contact: Terri Decarli (410) 787-4187
     Renate Jaacks (410) 879-9012
Email: terdec@northarundel.org
     r.jaacks@verizon.net

June 4-5   **Louisiana Society for Histotechnology**
Site: Holiday Inn Select, Kenner, LA
Contact: Jane Goodman (504) 897-8830

June 16   **National Society for Histotechnology Teleconference**
Title: **"Peripheral Nerve Biopsies: Anatomy, Useful Stains, Preparation and Case Examples"**
Speaker: Jon D. Wilson, MD
     William Beaumont Hospital, Royal Oak, MI
Contact: NSH office (301) 262-6221

June 18   **University of Texas Health Sciences Ctr/ San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **"Transporting Lab Samples—The New Regulations"**
Speaker: Linda Durbin
     EXAKT Technologies Inc.
     Oklahoma City, OK

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Educational Opportunities in 2004
(cont'd from page 43)

June 18 **ASCP Teleconference**
12:00 pm Central Time (800) 621-4142
Title: **"A Guide to Maintenance of Histology Equipment"**
Speaker: Dawn Truscott, HT(ASCP)
  Richard Allan Scientific Co.
  Kalamazoo, MI

July 16 **University of Texas Health Sciences Ctr/ San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **"Back to Basics: Troubleshooting the H&E Stain"**
Speaker: Joan Vesey, HT(ASCP)
  Richard Allan Scientific Co.
  Kalamazoo, MI

July 21 **National Society for Histotechnology Teleconference**
Title: **"Taking the ASCP Qualification in Immunohistochemistry (QIHC) Exam"**
Speaker: Patsy Ruegg, HT(ASCP)QIHC
  IHCtech, Inc.
  Aurora, CO
Contact: NSH office (301) 262-6221

August 20 **University of Texas Health Sciences Ctr/ San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **"Microwave Usage in Today's Histology Lab"**
Speaker: Donna Willis, HT/HTL(ASCP)
  Harris Methodist Hospital
  Ft. Worth, TX

September 17 **University of Texas Health Sciences Ctr/ San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **"Tissue Culture"**
Speaker: Gillian Rittman, O.N.C.
  University of Texas Health Science Center
  Houston, TX

September 18-23 **National Society for Histotechnology Symposium/Convention**
Site: Toronto, Canada
Contact: NSH office (301) 262-6221

October 15 **University of Texas Health Sciences Ctr/ San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **"*In-situ* Hybridization for HPV"**
Speaker: Jerry Santiago, BS, HTL(ASCP)QIHC
  Ventana Medical Systems

November 17 **National Society for Histotechnology Teleconference**
Title: **"The Necessity and Stages of Team Development"**
Speaker: H. Skip Brown, BS, HT(ASCP)
  Lab Management Consultants
  St. Louis, MO
Contact: NSH office (301) 262-6221

November 19 **University of Texas Health Sciences Ctr/ San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **"Optimal Handling Procedures for Skeletal Muscle Biopsies"**
Speaker: Vinnie Della Speranza, MS, HTL(ASCP)
  Medical University of South Carolina

December 15 **National Society for Histotechnology Teleconference**
Title: **"Bloodborne Pathogens: Are You Covered?"**
Speaker: Maureen Doran, BA, HTL(ASCP)
  SIU School of Medicine
  Carbondale, IL
Contact: NSH office (301) 262-6221

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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Vinnie Della Speranza, **HistoLogic**® Editor, 165 Ashley Avenue, Suite 309, Charleston, SC 29425. Articles, photographs, etc, will not be returned unless requested in writing when they are submitted.

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