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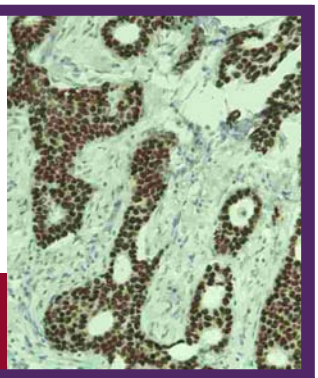

Gut Check #9 - Breath Testing For GI Disease

Hydrogen Breath Tests For Malabsorption

Hydrogen breath tests are now available in the Rex Endoscopy Suite. These tests are helpful in evaluating selected causes of small intestinal malabsorption, are superior to more traditional laboratory tests used in the past, and don't require a needlestick to obtain a specimen. The tests exploit the release of hydrogen gas (H2) when various sugars are digested by normal (anaerobic) colonic bacterial flora or in the setting of (abnormal) small intestinal bacterial overgrowth. Normally only a small amount of hydrogen is produced by as these sugars are generally digested and completely absorbed prior to reaching the colon. However, in certain malabsorption syndromes, the sugars may reach the colon, resulting in bacterial digestion, which may produce substances provoking increased water secretion and diarrhea, in addition to producing increased amounts of H2. The H2 is partially absorbed into the bloodstream, where it is released in the lungs and can be measured in the breath.

Currently at Rex, hydrogen breath tests can be used to look for either lactase deficiency or fructase deficiency. Lactose and fructose are disaccharides that are very common in the diet. Lactose is found in all dairy products, while fructose is present in many fruit juices or sweeteners. Deficiency of either lactase or fructase results in malabsorption of corresponding substrate and can produce abdominal pain, flatulence and diarrhea. The diagnosis can often be suspected and/or confirmed clinically by a careful dietary history, followed by resolution of symptoms when the offending sugar is eliminated from the diet. In problematic cases, the appropriate breath test can be employed. For example, a lactase deficient patient will exhale increased H2 following ingestion of a lactose load. (In the opinion of both authors, the lactose breath test is superior to the lactose tolerance test in evaluating the possibility of this disorder, both in terms of patient convenience as well as diagnostic power.)

Hydrogen breath testing can also be used to evaluate for small intestinal bacterial overgrowth. Normally there are few H2-producing anaerobic bacteria in the small intestine. If large numbers of such bacteria take up residence in the small intestine, the bacteria may begin to digest the sugars before the intestinal epithelial cells, producing increased H2 in addition to bloating and diarrhea, similar to that observed with a disaccharidase deficiency. Formerly bacterial overgrowth was believed a relatively rare condition observed in the setting of small intestinal diverticulosis, post-surgical blind loop syndrome, or severe motility disorders (e.g. scleroderma). It has recently been suggested that many cases of "irritable bowel syndrome" (with an estimated prevalence of up to 25%) may be due to bacterial overgrowth and symptoms may respond to appropriate antibiotic therapy. For this evaluation, the sugar lactulose is employed, as it is not digested by human epithelial cells but can be digested by bacteria. In a normal individual, H2 production will occur once the lactulose load reaches the colon. In bacterial overgrowth, there will be two peaks of H2 production. The first will occur as the lactulose passes through the small intestine, while the second occurs as it reaches the colon. This test may also be used to determine if the transit time through the small intestine is decreased, by comparing the time from ingestion to the detection of H2 in an individual patient to that observed in normal control subjects.

Pitfalls of Hydrogen Breath Testing

Some patients do not harbor H2 producing bacteria in their gut. Those of you who were attracted to college social fraternities no doubt recall that some individuals are colonized by methane producing bacteria instead of (or in addition to) H2 producers. Patients hosting methane producing bacteria will not benefit from H2 breath testing. While methane breath testing is theoretically possible, it is not widely available.

Other causes of malabsorption may result in false positive breath tests. Pancreatic exocrine insufficiency can impair sugar metabolism due to decreased digestive enzyme secretion. Conditions which interfere with enterocyte function (e.g. celiac disease) can likewise serve up an

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increased carbohydrate load to H2 producing bacteria in the distal gut with resultant increased H2 detected in breath testing. Clinical correlation is necessary to exclude these scenarios as causes of increased H2 excretion.

The interpretation of lactulose hydrogen breath testing is more complex than evaluation for a disaccharidase deficiency. Depending on the diagnostic criteria employed, the sensitivity of the test may vary. It may be difficult to discriminate between the early small intestinal peak and the later colonic peak. In other individuals, bacterial overgrowth may be present, but an underlying abnormality may be responsible for the clinical symptoms. A therapeutic trial of antimicrobial therapy should help in determining whether the underlying abnormality or bacterial overgrowth is producing the symptoms. Repeat breath testing can be used to determine if the antibiotics cleared the bacterial overgrowth.

Hydrogen Breath Testing at Rex

Testing for fructose intolerance, lactose intolerance, or small intestinal bacterial overgrowth is performed at the Rex Endoscopy Center. The desired test(s) can be ordered through Rex Surgical Scheduling (919) 784-3197. The patient should fast for at least 12 hours prior to testing. A baseline breath sample will be obtained prior to administering the appropriate carbohydrate load. Additional samples will be obtained every 15 minutes for up to several hours. Patients intolerant to the above sugars may experience bloating, abdominal pain and diarrhea during the test procedure. Hydrogen gas content is measured and an interpretive report is issued by the attending gastroenterologist.

Carbon 13 Breath Test for Helicobacter pylori Gastritis

For several years breath testing for H. pylori gastritis has been available at Rex Pathology Laboratory through Mayo Medical Laboratory. The test offers a sensitive and specific noninvasive test for confirming active H. pylori infection, as well as determining success of bacterial eradication following antibiotic therapy. As such, it serves as an adjunct to H. pylori serology and offers an alternative to gastroscopy. (The H. pylori fecal antigen test served an identical role with virtually identical sensitivity and specificity but was rarely ordered, and thus was recently discontinued.[2]) The test is based on the ability of gastric H. pylori producing a urease capable of digesting ^{13}C-urea into ^{13}CO2 and NH4. (^{13}C is a stable, nonradioactive, naturally occurring carbon isotope.) The ^{13}CO2 is absorbed into the blood and exhaled into the breath. The breath specimen is analyzed by infrared spectroscopy to compare the ratio of ^{13}CO2 to ^{12}CO2 following ingestion of a 75 mg specimen load of ^{13}C-urea (3 g of reconstituted Pranactin®-Citric solution).

The test should not be used for screening in the absence of symptoms or in patients with clear clinical indications for endoscopy. False positive tests may occur in humans harboring other gastric spiral bacteria (e.g. H. heilmannii) or in the setting of achlorhydria. Antibiotics, bismuth, and proton pump inhibitors may cause false negative results as these drugs inhibit H. pylori. Accordingly, these drugs should be discontinued two weeks prior to the test. If the test is being performed to assess eradication of H. pylori gastritis, a minimum of four weeks should intervene between the cessation of therapy and the breath test. Testing for eradication is not indicated in all cases of H. pylori gastritis, but should be considered in cases of gastric MALT-type lymphoma, early gastric carcinoma, or complicated peptic ulcer disease.

H. pylori breath testing at Rex

The test is performed in the Pathology Laboratory. The patient should fast at least one hour prior to testing. A baseline breath sample is obtained. The Pranactin®-Citric solution is prepared and administered to the patient. (As the 13C-urea solution contains 75 mg of phenylalanine, the test should be ordered with caution in patients with phenylketonuria.) After 15 minutes, a second breath specimen is collected. Both samples are forwarded to Mayo Medical Laboratories for analysis. The test is available Monday - Friday from 0900 - 1100. Call (919) 784-7276 to schedule.

Rex Healthcare Breath Testing

Test	Disease	Patient Fast	Test Performed In
Fructose	Fructase Deficiency	12 hr	Endoscopy*
Lactose	Lactase Deficiency	12 hr	Endoscopy
Lactulose	Small intestinal bacterial overgrowth	12 hr	Endoscopy
H. pylori breath test	H. pylori gastritis	1 hr	Laboratory**

* Call (919) 784-3197 to schedule
** Call (919) 784-7276 to schedule

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External Examination Of The Stillborn Fetus To Estimate Time Of Intrauterine Fetal Demise

Intrauterine fetal demise (IUFD) is an event that poses consternation not only for the family of the stillborn but also for the obstetrician. In many cases a probable cause of death can be determined from careful review of the obstetric records in conjunction with thorough placental examination. Postmortem examination of the fetus yields additional cause of death information in a minority of cases.

Estimation of the time of intrauterine demise relative to time of delivery is often an additional important issue. Maternal documentation of fetal activity is an important indicator of fetal viability. However pinpointing the time at which fetal activity ceases can be quite subjective especially in primigravidas, or during periods in which the mother has been asleep, quite active or distracted. Since the placenta is nourished by maternal blood flow until the time of delivery, it is usually not helpful in estimating the time of fetal death. However, external examination of the stillborn fetus can provide useful clues as to the length of time from fetal death until delivery. Unfortunately there has been relatively little study or discussion of this topic in the literature.

Jonathan Wigglesworth in his *Perinatal Pathology* monograph provides somewhat terse details of his experience in estimating time of fetal death.[1] Wigglesworth utilized "skin slippage", defined as dermal-epidermal separation with the application of slight oblique pressure, for his determinations. He noted that "skin slippage" is initially noted at around six hours since intrauterine demise and is to be expected after 12 hours. The spontaneous formation of bullae indicates an interval of 24 hours or more from death to delivery. After five days of intrauterine demise he described separation of the cranial bones from the dura so that the fetal skull tended to collapse and/or be easily compressible.

Genest and Singer performed a detailed retrospective study aimed at providing useful parameters for estimating duration of fetal demise.[2] The study group comprised 86 cases of stillbirth, in which the time of death was well documented by serial ultrasound or Doppler examination. The gestational age of the fetus at the time of death, the interval between intrauterine death and delivery were recorded. Other information reviewed included important clinical or placental findings, the presence or absence of chorioamnionitis, and the presence or absence of fetal hydrops. Any internal findings at autopsy were not considered. All of the study cases had whole body anterior and posterior photographs available for review. In addition, 83% had lateral whole body photographs, while 21% had at least one additional close-up photograph.[2] While their study involved examination of postmortem photographs and not actual gross examination of the macerated fetuses important information was provided from these sources. Desquamation and/or "skin slippage" are parameters that were found to be reliable estimators of the time of fetal death. The authors determined that desquamation of at least one cm of skin correlated with at least six hours from death to delivery. Desquamation involving the face, back or abdomen indicated an interval of at least 12 hours with desquamation involving at least five percent of the total body surface area or at least two body regions suggesting at least an 18 hour interval. These authors also noted that a brown or red discoloration of the umbilical cord correlated with at least a six hour interval. Genest and Singer noted that mummification, which they defined as a dehydrated appearance with tan brown skin and compression or flattening of the fetus, was a good indicator of at least two weeks between fetal death and delivery. (See Table for Summary)

In summary, thorough external examination of a stillborn fetus can prove useful in the estimation of the interval from intrauterine demise to delivery, as well as screen for congenital anomalies that may not have been detected during the prenatal period. Familiarity with these features may help the obstetrician in the immediate assessment of the stillborn and in subsequent discussions with the decedent's family. This can be particularly useful in situations in which the family declines postmortem pathologic examination of the fetus. Another option in such situations is to offer the family the choice of an **autopsy limited to external examination only**, if the fetus is at least 20 weeks in gestational age. (Fetuses of less than 20 weeks gestational age are automatically submitted for routine gross and microscopic surgical pathology examination as a "Histology" specimen. However, if the family is averse to internal organ inspection with microscopic examination, they can sign a **permit for external evaluation only** of a less than 20 week fetus, which should be submitted along with the specimen when delivered to the Histology Laboratory.)

TABLE 1

External features useful in estimating the interval between intrauterine fetal demise and delivery.

Feature	Interval
Desquamation of >1 cm	6 hours
Skin Slippage	6-12 hours
Umbilical cord red-brown discoloration	6 hours
Desquamation of face, back, abdomen	12 hours
Desquamation of >5% of body surface area	18 hours
Desquamation of > 2 areas on body	18 hours
Moderate or Severe Desquamation	24 hours
Bullae formation	24 hours
Cranial collapse or overlap of cranial bones	5 days
Mummification	2 weeks

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Estrogen And Progesterone Receptors In Breast Carcinoma Revisited

Two methods have been described for determining estrogen and progesterone receptor status in breast carcinomas. The original biochemical ligand assay provides a quantitative result, but is limited because the test requires frozen tumor tissue measuring at least 1.0 cm. in greatest dimension and there is no assurance that the tissue being assayed is indeed cancerous. The more recently developed immunohistochemical (IHC) assay provides a qualitative or semiquantitative result, but permits confirmation that the tissue being studied contains malignant cells, can be performed on formalin-fixed, paraffin embedded tissue, and can establish receptor activity on microscopic sized lesions. Multiple clinical trials using the original biochemical assay established that approximately 70% of invasive breast cancers will express estrogen receptor, 60-65% are progesterone positive and that the greatest benefit was observed when the estrogen level of greater than 100 fmol/mg protein[1]). However, despite only 85% concordance between the two assays and a paucity of published validated methods, the IHC method has largely replaced the ligand binding assay worldwide. The IHC method permits discrimination between invasive carcinoma, in situ carcinoma and adjacent benign breast tissue. The Rex Laboratory has used the DAKO IHC method for over 15 years with the well accepted and established clones ID5 for the estrogen receptor and PgR636 for the progesterone receptor.

The Rex Laboratory data from 2004 were reviewed in an earlier issue of the Rex Laboratory Bulletin[2]. This article updates this data and discusses some relevant issues in hormone receptor methodology and invasive breast cancer. First, the size of diagnostic biopsies has significantly decreased from open surgical biopsies to mammotome and stereotactic core biopsies. As the size of the tissue containing breast cancer grows smaller and smaller, several important details regarding IHC testing have to be kept in mind. Most of the time, there is no problem with the estrogen assay, since estrogen receptor positivity is generally an all (positive) or none (all negative) phenomenon[3]. Progesterone receptor staining reactions, on the other hand, typically demonstrate a more focal rather than diffuse type of staining pattern. This factor is not as critical, since the estrogen receptor status is the driver for therapeutic responsiveness to hormonal manipulation. For an invasive cancer to be labeled as estrogen receptor negative, an appropriate internal positive control (adjacent benign estrogen positive breast tissue) should be present. With smaller biopsies that are primarily cancer, adjacent normal breast tissue may not be present. In this situation, a case can be made for repeating"negative' hormone receptor assays on subsequent larger "biopsy" specimens.

Although semiquantitative scoring systems (e.g. Allred) exist, the College of American Pathologists currently recommends reporting hormone receptor status as the percentage of positive staining nuclei only. The guidelines for determining a positive receptor result are as follows[4]:

No nuclei staining	=	Negative
> 0-10% nuclei	=	Borderline or low positive
Greater than 10%	=	Positive

At Rex, we report both the nuclear intensity as well as the percentage of positive cells for those who find this helpful in patient management. (The masthead image illustrates "3+" nuclear staining in 100% of the tumor cell population.)

Table 1 below compares the Rex Laboratory estrogen and progesterone receptor data to the literature sources. The data remains consistent with acceptable performance, when compared to national sources[5].

John P. Sorge, M.D.

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Table 1: Rex Laboratory Receptor data in invasive breast cancers.

	2004 No. (%)	2006 No. (%)	Literature[5] No. (%)
ERA Positive Cases:	235 (77.8%)	222 (71.6%)	4100 (75%)
PRA Positive Cases:	207 (68.5%)	187 (60.3%)	3016 (55%)
ERA (+) PRA (+)	206 (68.2%)	187 (60.3%)	3016 (55%)
ERA (+) PRA (-)	29 (9.6%)	35 (11.3%)	1084 (20%)
ERA (-) PRA (+)	1 (0.3%)	0 (0%)	0 (0%)
ERA (-) PRA (-)	66 (21.9%)	88 (28.4%)	1397 (25.4%)
Total Cases Evaluated:	302	310	5497

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