ChronoBiology LETTER

DiagnosTechs™ THE LEADING LAB IN SALIVA TESTING SINCE 1989

Summer 2012 | 12th Edition

Diagnos-Techs™
Clinical & Research Laboratory
Quarterly Newsletter

OTHER ARTICLES IN THIS EDITION

Find Diagnos-Techs™ Physicians

John J. White, MD, CM

Often, Diagnos-Techs™ testing may indicate a need for pharmaceutical intervention. For practitioners without prescription-writing privileges, this means finding an allopathic physician or other healthcare practitioner who's experienced in interpreting Diagnos-Techs™ saliva test results and comfortable prescribing medications based on those results.

A comprehensive resource for finding prescription-writing healthcare practitioners who are familiar with Diagnos-Techs™ and the results of our suite of tests is as close as your computer.

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The Problem with One-Day Testing for Cycling Women

John J. White, MD, CM

Can a cycling woman's hormones be assessed accurately with just a single day's testing? A newly available one-day, cycling female hormone saliva test certainly sounds like a good -- or at least convenient -- alternative to testing throughout the cycle. However, the reality is that an individual womans menstrual cycle in no way compares to anothers, and a female hormone testing protocol that places mere expedience over accuracy is not of value for any woman.

Diagnos-Techs™ Whole-Cycle Testing Vs. a One-Day Snapshot

For many years, Diagnos-Techs™ has offered female hormone panels specifically designed to deliver an accurate picture of a woman's cycling hormones, without burdening her with unrealistic testing schedules and methods. Our Female Hormone Panel™, for instance, takes a snapshot at 11 discrete points in the menstrual cycle, providing cycle-wide data. Women simply collect saliva at approximately two- to three-day intervals through their cycles, a design that's been well accepted over the years by patients and widely praised by healthcare practitioners for its accuracy and ease-of-collection.

Some labs have introduced a one day menstrual cycle test which involves collecting four vials of saliva on the 21st day of a woman's cycle. The test consists of a circadian cortisol rhythm assessment, plus pooled testosterone, estradiol, and progesterone values. The latter two values are processed with a mathematical formula, purportedly to diagnose luteal phase failure and estrogen dominance.

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Diagnos-Techs™ has gone Paperless

In an effort to be more mindful of the environment, we will now be distributing an electronic version of our quarterly newsletter. If you do not have an email address listed on your account, please call **Client Services at 1.800.878.3787** to add your email address.

If you prefer to receive a printed copy of our ChronoBiology Letter, please email us at diagnos@diagnostechs.com.

7 Reasons Why a One-Day Female Hormone Test Doesn't Make the Grade

Without doubt, such a one-day hormone test promotes patient compliance and fits in nicely with a busy schedule. But the relevant issue is whether the data gathered by this test gives an accurate account of a woman's entire cycle. We don't think so, and here's why:

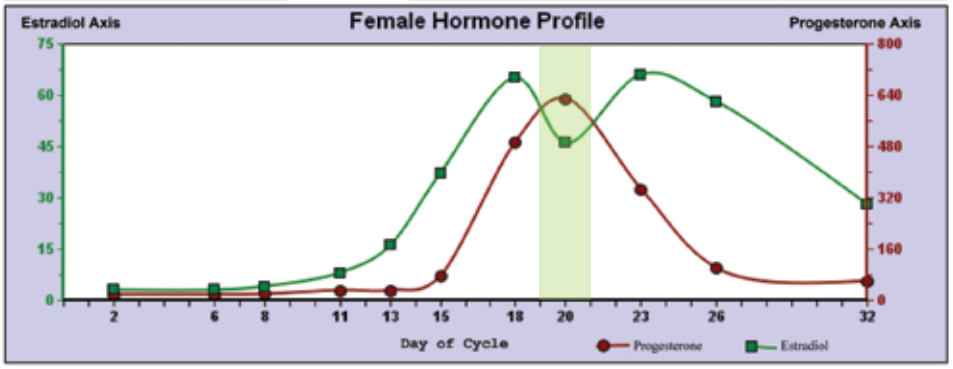
1. Every woman's cycle is unique. Duration, ovulatory timing, luteal phase length, and estradiol and progesterone patterns vary so much that a single-day sample, no matter how "complete," can't compare to the detailed data offered by a multi-sample test. In sports terms, a one-day test is like evaluating a quarterback's performance during a game using four photographs taken in the third quarter.
2. The medical staff at Diagnos-Techs™ has long recognized that hormones follow a circadian rhythm. To reflect this rhythm accurately, specimens are collected throughout the cycle at the same time of day, determined by the patient's convenience.
3. A single-day snippet of a woman's month-long cycle may or may not correctly identify luteal phase failure or estrogen dominance. Though the one-day saliva test in question has been compared to our 11-vial FHP™, it doesn't offer the same detailed picture of hormonal changes over the cycle, necessary for a confident diagnosis of either luteal phase failure or estrogen dominance.
4. One size does not fit all when testing female hormones. For peri- and post-menopausal women, Diagnos-Techs™ offers the PeriM™ (two vials) and PostM™ (one vial) panels. These tests are available in standard versions and expanded panels that test FSH and LH pituitary hormones to provide precisely tailored data without unduly burdensome sample collection. The information gathered by these targeted tests is more pertinent to a woman's particular phase in her cycling life.

Expanded Female Hormone Panel — *Figure 1*

Day of Cycle	Day	2	6	8	11	13	15	18	20	23	26	32
Estradiol	pg/ml	3	3	4	8	16	37	65	46	66	58	28
Progesterone	pg/ml	18	18	19	31	31	75	494	629	345	100	60

Cycle Information	
Start	02/11/2012
End	03/13/2012
Length	31

Ranges	*Phase*	*Estradiol*	*Progesterone*
	Follicular	2 - 10 pg/ml	20 - 100 pg/ml
	Preovulatory	**7 - 25 pg/ml**	
	Luteal	3 - 16 pg/ml	65 - 500 pg/ml



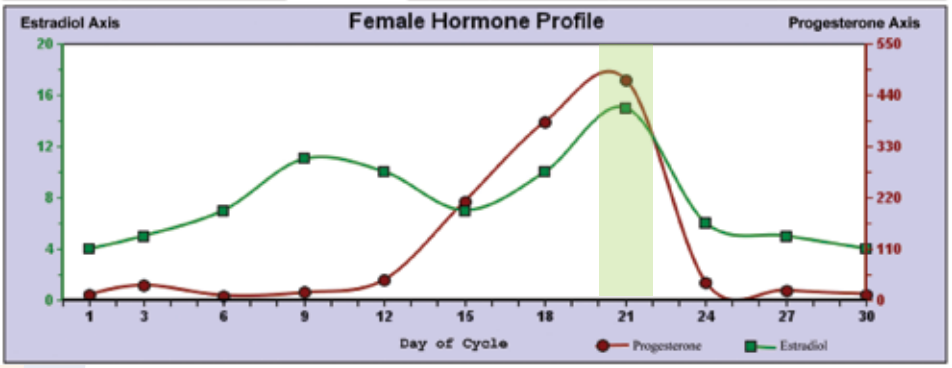
In this 35-year-old woman with acne and premenstrual problems, a Day 21 analysis (green) would miss significantly elevated late-luteal phase estradiol levels producing estrogen dominance and the dramatic drop in progesterone. In view of the low follicular phase progesterone level, biphasic progesterone supplementation (fixed low-dosage in the follicular phase, plus timed graduated dosing in the luteal phase) seems indicated. This biphasic progesterone deficiency would not be identified using a single Day 21 sample.

Expanded Female Hormone Panel — *Figure 2*

Day of Cycle	Day	1	3	6	9	12	15	18	21	24	27	30
Estradiol	pg/ml	4	5	7	11	10	7	10	15	6	5	4
Progesterone	pg/ml	12	32	10	16	44	212	383	473	37	21	13

Cycle Information	
Start	03/17/2011
End	04/15/2011
Length	29
Average	30

Ranges	*Phase*	*Estradiol*	*Progesterone*
	Follicular	2 - 10 pg/ml	20 - 100 pg/ml
	Preovulatory	**7 - 25 pg/ml**	
	Luteal	3 - 16 pg/ml	65 - 500 pg/ml



This 37-year-old woman complained of premenstrual problems and infertility. A single-day assay (green) would miss both estrogen dominance in the last part of the luteal phase and very low progesterone. There is evident need for supplementary progesterone to build up a uterine mucosa sufficient to support a fertilized ovum. Biphasic progesterone supplementation in the follicular phase is warranted in this case, as well.

5. The pattern of progesterone deficiency (follicular and/or early, middle, or late luteal phase), which may produce menopausal or menstrual problems, must be evaluated completely. This pattern then points the way toward an appropriately designed treatment program, which may include biphasic progesterone supplementation (Figure 1).
6. A one-day assay is meaningless for evaluating women with fertility problems. This single test does not provide invaluable sequential FSH and LH measurements, nor does it address multiple issues that may be interacting to cause infertility (Figure 2).
7. Finally, there's the issue of cost. Our comprehensive cycling Female Hormone Panel, which includes 24 individual hormone assays, actually costs $25 less than the incomplete one-day alternative, which includes only seven assays.

Effective, Lifelong Cycle Management Takes More than a Single-Serving Test

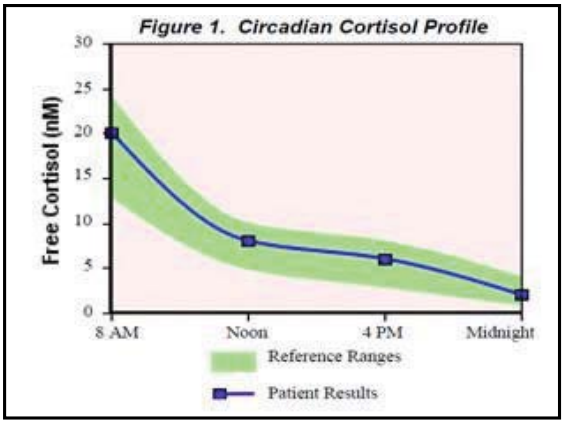
Can a cycling woman's complex hormonal picture be accurately assessed with a one-day test? Years of work with samples collected from women throughout the cycling years tells us that, while convenient, this test lacks the accuracy and cycle-wide insight needed to analyze a woman's hormonal health and well-being. Every woman is one-of-a-kind, and a one-day assessment is not sufficient to provide a complete hormonal picture for her and her alone. In female hormone testing, like clothing, one size does not fit all.

Timing TAP™ Collections for Shift Workers

John J. White, MD, CM

The Temporal Free Cortisol Rhythm Panel (TAP™) highlights cortisol levels across a patient's circadian cycle using four cortisol measurements taken throughout the waking hours. The TAP lies at the heart of the expanded Adrenal Stress Index™ (ASI™), which also measures pooled DHEA/S, 17-hydroxy progesterone, and salivary secratory IgA (SIgA), along with two insulin levels (fasting and random/post-stimulation) and SIgA gliadin levels (gluten sensitivity is a common and often unrecognized stressor).

But what about workers whose schedules are out of sync with the usual waking circadian pattern? We are often questioned about the applicability of circadian cortisol testing for patients whose days are not routine, such as swing shift and nightshift workers. A routine morning-to-midnight collection schedule not only fails to represent accurately their lifestyles and schedules, but would require, at times, waking up to test. This in itself is stressful and most certainly would provoke unnatural cortisol levels.



Analysis of circadian cortisol rhythm patterns underscores the importance of a patient's unperturbed sleep cycle in rejuvenating the adrenals and providing the elevated cortisol levels seen on awakening. Sleep, at whatever time of day, appears central to a regular cortisol rhythm. In light of this, specimen collection times can be adjusted for shift workers to reflect normal "day" centered on their sleep patterns.

To accomplish this, the first cortisol specimen (fasting) should be collected on awakening. The fourth specimen (bedtime) should be collected prior to sleep. The second specimen can be collected around "lunchtime," with the third collected just before "supper." This schedule provides the closest approximation to that of a patient leading a "regular" lifestyle.

Although there is little published experience with data gathered in this way, this collection schedule mimics the standard collection schedule, providing a comparable circadian cortisol rhythm assessment for patients who have adjusted to a nonstandard sleep/wake cycle. Sleep, the major factor in adrenal rejuvenation, is central to all collection schedules, and data derived in this way definitely has value in assessing cortisol circadian rhythm and any effects of stress on shift workers and others who keep unusual hours.

The Therapy Corner
Helicobacter pylori

Brandy Webb, ND

Helicobacter pylori is a small, curved, rod-shaped bacterium that inhabits the stomach and duodenum. Up to 40% of the U.S. population is infected with or colonized H. pylori; most colonization occurs during childhood.[1] *Colonization* refers to positive *H. pylori* status without symptoms, while infection is reserved for cases where symptoms like dyspepsia are present. Due to a three-to six-fold increased risk of stomach cancer, it is important to identify affected individuals and consider protocols for *H. pylori* eradication.[2]

Helicobacter pylori's behavior and structure make it particularly effective in colonizing the upper gastrointestinal tract. First, *H. pylori's* flagella propel it toward the mucosal lining of the stomach or duodenum. Then, its corkscrew-shaped body burrows deep into the gastrointestinal wall beneath the protective layer of mucus. The immune cells dispatched by the body, including killer T-cells, cannot effectively penetrate the mucous layer to reach *H. pylori*. Compounding the problem, the inflammatory effects of immune cell activity (such as the release of free radicals) may contribute to the development of ulcers and dysplasia.[3]

H. pylori releases enzymes that directly break down the mucous layer, which most likely further contributes to ulcer formation.[2] Once *H. pylori* has burrowed into the mucosal lining of the stomach or duodenum, it survives the acidic environment of the stomach by secreting an enzyme called *urease*, which hydrolyzes the surrounding urea into bicarbonate and ammonia, strong bases that neutralize stomach acids.[4]

Helicobacter pylori is spread via fecal-oral transmission (through consumption of contaminated food or water) and by oral-oral transmission (through intimate person-to-person contact). The vast majority of individuals with *H. pylori* never develop symptoms. Of those who do present with symptoms, dyspepsia is the most common. *Dyspepsia* is classified as gnawing or burning pain in the epigastric region, frequently accompanied by bloating, nausea, and abdominal fullness.[5] Ulcers may or may not be present initially, but there exists a strong association between *H. pylori* and peptic ulcer disease (PUD). *Helicobacter pylori* is implicated in the formation of 90% of duodenal ulcers and 80% of gastric ulcers.[6]

Why Eradication Is Important

Helicobacter pylori colonization is a major cause of gastric adenocarcinoma and increases the risk of gastric mucosa-associated lymphoid tissue (MALT) lymphoma.[7] In 1994, the International Agency for Research on Cancer formally classified *H. pylori* as a carcinogen in humans.[7] Furthermore, *H. pylori* is a significant factor in the pathophysiology of hypochlorhydria, gastritis, and peptic ulcer disease. For these reasons, eradicating *H. pylori* through conventional or natural methods may be warranted, regardless of symptom status.

Salivary IgG H. Pylori Testing

Measuring *Helicobacter pylori* IgG levels in saliva is a convenient alternative to other forms of *H. pylori* testing, such as serum IgG, urease breath test, stool antigen testing, and biopsy. Saliva IgG *H. pylori* testing has a sensitivity of 93% and specificity of 83% and is considered "a valuable alternative to invasive procedures" due to its simplicity and practicality.[8,9] Diagnos-Techs™ offers a convenient salivary IgG *H. pylori* test, which is available alone (lab code *GP8-S*) or as part of our comprehensive GI panels (lab codes *GI-1* and *GI-2*).

Conventional Treatment

The treatment goal in asymptomatic patients is eradication of the *Helicobacter pylori*; additional therapeutic considerations may be appropriate in symptomatic patients. The scope of this article is limited to adults (for guidelines on treating children, please see the *North American Society for Pediatric Gastroenterology and Nutrition Position Statement*).[10] There are a variety of conventional drug combination protocols available, the most effective involving a "triple-therapy" or "quadruple-therapy" employing bismuth, two antibiotics, and, frequently, a proton-pump inhibitor (PPI). Bismuth helps prevent drug resistance, while the PPI helps heal the damaged mucosa (some sources suggest that PPIs may increase drug resistance). Due to the significant risk of drug

resistance, most pharmacologic agents can be tried only once. If a patient tests positive after initial therapy, consider modifying the drug regimen. The antibiotics most frequently used for *H. pylori* include amoxicillin (*Amoxil®, Trimox®*), clarithromycin (*Biaxin®*), and metronidazole (*Flagyl®*).[5] Commonly prescribed triple- and quadruple- therapies typically last 10-14 days, although newer, shorter regimens are now available. One of the newer protocols, which has demonstrated an impressive eradication rate, involves a one-day quadruple-regimen:[5]

Bismuth subcitrate or subsalicylate (*Kaopectate®, Pepto-Bismol®*)—240mg PO QID

Amoxicillin (*Amoxil®, Trimox®*)—2g PO QID

Metronidazole (*Flagyl®*)—500mg PO QID

Lansoprazole (*Prevacid®*)—60mg once

There is disagreement on whether longer regimens have significantly greater efficacy; some sources recommend longer courses for better eradication while others prefer shorter courses for better compliance, fewer side effects, and lower cost. A typical longer-course triple-therapy consists of the following (generally given for seven, 10, or 14 days):[11]

Amoxicillin (*Amoxil®, Trimox®*)—1g PO BID

Clarithromycin (*Biaxin®*)—500mg PO BID

Lansoprazole (*Prevacid®*)—30mg PO BID

This particular combination is available from some pharmaceutical companies as a kit of "daily administration cards," each of which contains all of the pills to be taken on that day. Bismuth subcitrate or subsalicylate may be prescribed separately to achieve a quadruple-therapy. Due to the rampant drug resistance of *Helicobacter pylori*, researchers are continuing to investigate alternative drug protocols. Novel drug regimens that have shown promising results include 1) a 14-day course of nitazoxanide (*Alinia®*) and sucralfate (*Carafate®*);[12] 2) a four-day course of dexlansoprazole (*Dexilant®, Kapidex®*), moxifloxacin (*Avelox®*), amoxicillin (*Amoxil®, Trimox®*), nitazoxanide (*Alinia®*), and doxycycline (*Adoxa®, Vibramycin®*);[13] and 3) a seven-day course of levofloxacin (*Levaquin®*), omeprazole (*Prilosec®*), nitazoxanide (*Alinia®*), and doxycycline (*Adoxa®, Vibramycin®*).[14]

It is important to rule out known drug allergies to individual medications before placing any patient on these drug regimens. Also, such protocols may not be suitable for pregnant women, due to the potential for fetotoxicity (there are concerns about using some of these drugs while breastfeeding, as well). Common side effects include nausea, vomiting, diarrhea, dark tongue, and dark stools. For this reason, consider giving adjunctive natural therapies to mitigate specific side effects. A potent probiotic can help minimize bowel disruption, and it can also prevent the fungal overgrowth that can result from antibiotic use. Consider dosing with 50-100 billion CFUs daily, starting on the first day of drug therapy and continuing for a minimum of three weeks after completing the drug course. Ginger, peppermint, and various homeopathic remedies have been demonstrated to relieve nausea and vomiting, while apple pectin can be helpful for diarrhea.

Natural Treatments

There are numerous studies demonstrating the efficacy of natural substances in eradicating *Helicobacter pylori in vitro*, but more human trials are needed to fully elucidate which natural agents are effective *in vivo*. The following recommendations may be prescribed alone or in combination with conventional treatment. In either case, repeat testing is recommended to confirm eradication, since many therapies are effective at suppressing infection in the short term but may not completely eradicate *H. pylori*. Natural treatment considerations may include:

Vaccinium macrocarpon (cranberry)—250ml (16oz) juice QD or BID

Allium sativum (garlic)—4mg in divided doses QD

Berberine—300mg TID

Grapefruit seed extract—500mg BID

Vitamin C—5g per day in divided doses

Probiotics—50-100 billion CFU daily per day for two weeks then 30-40 billion CFU per day

There are promising results indicating that *Vaccinium macrocarpon* reduces *H. pylori* adhesion to gastric epithelium and may inhibit enzymes that aid the survival of the bacterium.[15,16,17,18] Numerous *in vitro* studies demonstrate that *Allium sativum* inhibits *H. pylori* growth, although additional clinical trials are needed to demonstrate this affect *in vivo*.[19,20,21] Berberine is an alkaloid present in herbs like *Hydrastis canadensis* (goldenseal) and *Mahonia aquifolium* (Oregon grape). This compound possesses inhibitory effects against *H. pylori in vitro*.[22] Grapefruit seed extract shows antimicrobial activity against a wide

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Therapy Corner *Continued.*

variety of gastrointestinal pathogens, including *H. pylori*.[23] Several studies demonstrate an association between vitamin C supplementation and *H. pylori* prevention and eradication.[24,25,26] *Bifidobacterium bifidum, Lactobacillus casei*, and *Saccharomyces boulardii* have been shown to be effective at inhibiting *Helicobacter pylori*, preventing *H. pylori*-associated ulcer formation and reducing *H. pylori* treatment-related symptoms, respectively.[27,28,29]

Retesting & Recurrence

Helicobacter pylori IgG titers can remain elevated for an extended period following eradication, so allow many months to pass before repeating salivary IgG testing. If earlier testing is desired, consider a stool antigen test or urease breath test. In these cases, allow at least four weeks after treatment before retesting to assess eradication accurately.

Many natural *Helicobacter pylori* treatment strategies can be used long term to protect the gastrointestinal tract against recolonization. Encourage patients to regularly consume cranberry juice, fresh garlic, and citrus fruits. Broccoli sprouts, green tea, and fermented foods are also associated with reduced incidence of *H. pylori*.[30] They appear to work by inhibiting *H. pylori* colonization, decreasing gastric inflammation, and repressing precancerous changes.[11,30] Dietary recommendations such as these can minimize the risk of *H. pylori* recurrence and related sequelae in treated individuals.

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DTI Free Webinars

Diagnos-Techs™ offers fast-paced, patient health-focused webinars on the 2nd and 4th Thursday of each month. Webinars are live and presented at 9:00 a.m. Pacific Time and again at 12:00 p.m. Pacific Time. We encourage questions during and after our webinars. To ensure participants get the most out of our webinars, our medical staff compiles all submitted questions, answers them, and emails the Q&A document to every webinar participant. For information on attending upcoming Diagnos-Techs™ webinars, please contact Client Services at **1-800-878-3787**. To view our previously recorded webinars, please visit the provider section of our website at **www.diagnostechs.com**.

Upcoming Webinars:

July 26th, 2012 – *Hidden Cuprits of Common GI Complaints, Part 2*

August 9th, 2012 – *Hormones and Pregnancy, Part 1*

August 23rd, 2012 – *Hormones and Pregnancy, Part 2*

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As a courtesy, we will submit claims to most insurance companies at our clients' and patients' requests. In addition, we are able to bill insurance carriers for all referring doctors, nurse practitioners, and registered nurses, and we can advise you on which services are billable. Although we are a non-contracted provider with all insurance companies (with the exception of Medicare), most insurance carriers cover our services and are billable. Depending on a patient's benefit plan, insurance companies usually cover our tests at the maximum allowable rates, so there are no out-of-pocket expenses.

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The *Health Insurance Portability and Accountability Act* of 1996 (**HIPAA**) requires us to protect and maintain the privacy of our patients' identifiable health information. The standards are meant to improve the efficiency and effectiveness of the nation's health care system. We are committed to implementing appropriate administrative, technical and physical safeguards to protect the privacy of protected health Information. For more information, go to **www.diagnostechs.com** and click *About Us/Notice of HIPAA Privacy Practices.*

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- We must have your **patient's name** and **date of birth** on all vials and order forms to process samples.
- **Please advise patients** to consult with you if they have questions regarding their medications or test results. We are unable to discuss these topics directly with patients. Medical support is available for provider questions about medications, results, treatment suggestions, and test recommendations at 1-800-878-3787.
- **Cotton Collection Tip:** To ensure adequate saturation of cotton rolls supplied in some of our test kits, instruct patients to wait until their mouths have refilled with the usual amount of saliva before removing and placing rolls in vials.

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Corporate Address
19110 66th Avenue S., Building G
Kent, WA 98032 USA

Customer Service
p 800-878-3787 **f** 425-656-2871
p 425-251-0596

Accounting
f 425-264-0612
email billing@diagnostechs.com

Shipping
p 800-878-3787 **f** 253-398-2449

Technical Services
p 800-878-3787 **f** 425-251-0637

Lab Address
Sample Processing
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- ☐ Include all samples, test form, and, if applicable, a check or a copy of the front and back of insurance card together inside the supplied box. Please be sure to seal the box with clear tape OR the UPS shipping label (US only).

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THE LEADING LAB IN SALIVA TESTING SINCE 1989

19110 66th Avenue South, Building G
Kent, Washington 98032 USA

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