LIMING BIO

Herpes Simplex Virus 1 and 2 Antigen Rapid Test Device

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For professional *In vitro* diagnostic use only.

INTENDED USE

The *StrongStep*® HSV 1 and 2 antigen Rapid Test Device is a rapid visual immunoassay for the qualitative presumptive detection of *HSV 1 and 2 antigen* in cutaneous specimens. *This kit is intended to be used as an aid in the diagnosis of HSV infection.*

INTRODUCTION

HSV is an envelop, DNA-comtaining virus morphologically similar to the other members of the genus Herpesviridae.Two antigenically distinct types are recognized, designated type 1 and type 2.
HSV type 1 and 2 are frequently implicated in superficial infections of the oral cavity, the skin, the eye and the genitalia, Infections of the central nervous system (meningoencephalitis)and severe generalized infection in the neonate of immunocompromised patient are also seen, though more rarely. After the primary infection been resolved, the virus may exist in a latent form in nervous tissue, from where it may re-emerge, under certain conditions, to cause a recurrence of the symptoms.
The classical clinical presentation of genital herpes starts with widespread multiple painful macules and papules, which then mature into clusters of clear, fluid-filled vesicles and pustules. The vesicles rupture and form ulcers. Skin ulcers crust, whereas lesions on mucous membranes heal without crusting. In women, the ulcers occur at the introitus, labia, perineum, or perianal area. Men usually develop lesions on the penial shaft or glans. The patient usually develops tender inguinal adenopathy. Perianal infections are also common in MSM. Pharyngitis may develop with oral exposure.
Serology studies suggest that 50 million people in the United States have genital HSV infection. In Europe, HSV-2 is found in 8-15% of the general population. In Africa, the prevalence rates are 40-50% in 20-year-olds. HSV is the leading cause of genital ulcers. HSV-2 infections at least doubles the risk of sexual acquisition of human immunodeficiency virus (HIV) and also increases transmission.
Until recently, viral isolation in cell culture and determination of the type of HSV with fluorescent staining has been the mainstay of herpes testing in patients presenting with characteristic genital lesions. Besides PCR assay for HSV DNA has been shown to be more sensitive than viral culture and has a specificity that exceeds 99.9%. But these methods in clinical practice are currently limited, because the cost of the test and the requirement for experienced, trained technical staff to perform the testing restrict their use.
There are also commercially available blood tests used for detecting Type Specific HSV antibodies, but these serological testing cannot detect primary infection so they can be used only to rule out recurrent infections.
This novel antigen test can differentiate other genital ulcer diseases with genital herpes, such as syphilis and chancroid, to help the early diagnosis and therapy of HSV infection.

PRINCIPLE

The HSV antigen Rapid Test Device has been designed to detect *HSV antigen* through visual interpretation of color development in the internal strip. The membrane was immobilized with anti Herpes simplex virus monoclonal antibody on the test region. During the test, the specimen is allowed to react with colored monoclonal anti-HSV antibody colored particals conjugates, which were precoated on the sample pad of the test. The mixture then moves on the membrane by capillary action, and interacts with reagents on the membrane. If there were enough HSV antigens in specimens, a colored band will form at the test region of the membrane. Presence of this colored band indicates a positive result, while its absence indicates a negative result. Appearance of a colored band at the control region serves as a procedural control. This indicates that proper volume of specimen has been added and membrane wicking has occurred.

KIT COMPONENTS

20 Individually packed test devices	Each device contains a strip with colored conjugates and reactive reagents pre-coated at the corresponding regions.
2 bottle of Extraction Buffer - 10ml	0.1 M Phosphate buffered saline (PBS) and 0.02% sodium azide.
20 Extraction tubes	For specimens preparation use.
2 Workstation	Place for holding buffer vials and tubes.
1 Package Insert	For operation instruction.
1 Positive control swab (on request only)	Contain inactived HSV type II and sodium azide. For external control.
1 Negative control swab (on request only)	Not contain HSV type I&II. For external control.

MATERIALS REQUIRED BUT NOT PROVIDED

Timer	For timing use.

PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after expiration date indicated on the package. Do not use the test if its foil pouch is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to performing any tests.
- Do not eat, drink or smoke in the area where the specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Do not interchange or mix reagents from different lots. Do not mix solution bottle caps.
- Humidity and temperature can adversely affect results.
- When the assay procedure is completed, dispose the swabs carefully after autoclaving them at 121°C for at least 20 minutes. Alternatively, they can be treated with 0.5% sodium hypochloride (or house-hold bleach) for one hour before disposal. The used testing materials should be discarded in accordance with local, state and/or federal regulations.
- **Do not use cytology brushes with pregnant patients.**

STORAGE AND STABILITY

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Cares should be taken to protect components in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipments, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND STORAGE

- **Specimens collection**
- A good sampling technique is essential for the optimal detection of HSV in clinical specimens. Specimens from a clinical lesion should be collected by qualified individuals using a sterile swab. Specimens must be collected so as to contain as much infected material as possible. Polyester or Dacron tipped sterile swabs with plastic or stainless steel stems are recommended.
- Creams, ointments, lotions, ice, alcohol, betadine solution, zinc, or a recent sitz bath all reduce viral yield significantly. Use of such remedies should be avoided, if possible, prior to specimen collection or be reported to the physician when the lesion is sampled.
- **Clinical specimens**
- Ulcers should be firmly rubbed with the swab in order to pick up infected cells and exudates from the base of the ulcer. Vesicles should be carefully opened, absorb the fluid on the swab. The base of the lesion should be rubbed with the swab. Pustular lesions should be treated as for vesicles; the crusts may sent dry. Place the swab in a clean screw-capped vial. Specimens may be stored for 24 hours at room temperature (15-30°C) or 1 week at 4°C or no more than 6 month at -20°C.
- **Cervical specimens in symptomatic patients**
- Firmly swab any visible lesions, otherwise swab cervix. Withdraw the swab without touching the vaginal surface and place in a screw-capped vial. Specimens may be stored 24 hours at room temperature (15-30°C) or 1 week at 4°C or no more than 6 month at -20°C.

PROCEDURE

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

1. **Prepare swab specimens:**
   - Place a clean extraction tube in the designated area of the workstation. Add 15 drops of Extraction Buffer to the extraction tube.
   - Immerse the patients swab into the Extraction tube and extract 2 minutes at room temperature. During extraction, use a circular motion to roll the swab against the side of the extraction tube so that the liquid is expressed from the swab and can reabsorb. Discard the swab following guidelines for handling infectious agents.
   - The specimens extracted can retain at room temperature for 60 minutes without affecting the result of the HSV test.
2. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. To obtain a best result, the assay should be performed within one hour.
3. Add 3 drops (approximately 100 µl) of extracted sample from the Extraction Tube to the sample well on the test cassette.
   Avoid trapping air bubbles in the specimen well (S), and do not drop any solution in observation window.
   As the test begins to work, you will see color move across the membrane.
4. Wait for the colored band(s) to appear. The result should be read at 15 minutes. Do not interpret the result after 20 minutes.

INTERPRETATION OF RESULTS

POSITIVE RESULT: 	Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).
NEGATIVE RESULT: 	Only one colored band appears in the control region (C). No apparent colored band appears in the test region (T).
INVALID RESULT: 	Control band fails to appear. Results from any test which has not produced a control band at the specified reading time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of the color in test region (T) may vary depending on the concentration of aimed substances present in the specimen. But the substances level can not be determined by this qualitative test.
2. Insufficient specimen volume, incorrect operation procedure, or performing expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

- Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered as an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.
- External procedural controls may provided(on request only) in the kits to ensure that the tests are functioning properly. Also, the Controls may be used to demonstrate proper performance by the test operator. To perform a positive or negative control test, complete the steps in the Test Procedure section treating the control swab in the same manner as a specimen swab.

LIMITATIONS OF THE TEST

1. The HSV antigen Rapid Test Device is for professional *in vitro* diagnostic use, and should be used for the qualitative detection of *HSV* only. There is no meaning attributed to line color intensity or width.
2. Detection of HSV antigen is dependent on the number of organisms present in the specimen. This may be affected by specimen collection methods and patient factors such as age, history of STD, presence of symptoms, etc.
3. The likelihood of detecting HSV decreases with time following the oneset of disease and the development of lesions. The probability of viral isolation decreases as the leison ulcerates, crusts and heals. Specimens should be collected as soon as possible after the appearance of leisons. It is reported that there is a 90% chance of obtaining a positive culture when the specimen is obtained from the base of a freshly unroofed vesicle or pustule, but that sensitivity decreases to 70% when the specimen is obtained from an existing herpes ulcer and drops to only 27% when a crusted lesion is used as a specimen source.
4. The tests have only been evaluated with cutaneous specimens and genital swabs,other specimen such as cerebrospinal fluids, eye swabs, urines, respiratory specimens do not have clinical data yet.
5. As with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

Table: HSV antigen Rapid Test vs. PCR

Clinical Specimens

Relative Sensitivity: 89.1% (77.7%-95.9%)* **Relative Specificity:** 95.9% (89.9%-98.9%)* **Overall Agreement:** 93.5% (88.3%-96.8%)* ***95% Confidence Interval**			PCR		
			+	-	**Total**
	***StrongStep®* HSV Test**	+	49	4	53
		-	6	94	100
			55	98	153

The antibody used in the HSV test has been shown to detect all HSV serovars. Cross reactivity with other organisms has been studied using suspensions of 10^7 org/ml. The following organisms were not detected using the test:

Acholeplasma laidlawii	*Mycoplasma spp*
Achinetobacter spp	*Neisseria gonorrhoeae*
Aeromonas spp	*Peptococcus spp*
Bacteroides spp	*Peptostreptococcus spp*
Campylobacter spp	*Proteus spp*
Candida spp	*Pseudomonas spp*
Citrobacter spp	*Salmonella spp*
Chlamydia trachomatis	*Serratia spp*
Clostridium spp	*Shigella spp*
Cytomegalovirus	*Staphylococcus aureus(cowan 1 strain)*
Enterobacter spp	*Staphylococcus spp(coag.neg)*
Epstein Barr Virus	*Staphylococcus spp(coag.pos)*
Escherichia coli	*Streptococcus spp*
Gardnerella spp	*Trichomonas spp*
Haemophilus influenzae	*Ureaplasma urealyticum*
Klebsiella spp	*Varicella zoster virus*
Lactobacillus spp	*Veillonella spp*
Listeria spp	

LITERATURE REFERENCES

1. Adam E. Glaser R, ed. Marcel Dekker Inc New York pp 1-55
2. Nahmias AJ and Josey WE(1976)Evans AS, ed J Wliiley and Sons, London pp253-271
3. CDC. Morbid.Mortal. Weekly Rep. 34(suppl 3-s): 535, 1985.
4. "The (herpes) helper(1984)" Palo Alto, CA 94302. Fall 1984 pp 7.
5. Waggoner-Fountain LA, Grossman LB. Herpes simplex virus. Pediatr Rev 2004; 25:86 – 93.
6. Sexually Transimitted Diseases, A Practical Guide for Primary Care. Anita L. Nelson, MD. Humana Press, 2006.

GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	In vitro diagnostic medical device		Use by
	Manufacturer	Σ	Contains sufficient for <n> tests
	Do not reuse	EC REP	Authorized representative in the European Community
CE	CE marked according to IVD Medical Devices Directive 98/79/EC		

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