

ImmcoStripe™ hsp-70 Antibody LIA

New Line Immunoassay for Detecting Antibodies Associated with Autoimmune Hearing Loss

ImmcoStripe™ hsp-70 assay enables detection of 68kD antibodies to a 68kD heat shock protein associated with autoimmune hearing loss. The addition of serum, conjugate, and cutoff lines provide robust internal controls which are absent in Western Blot assays. The LIA is easier to read, track and record results.

INTRODUCTION

Sensorineural hearing loss (SNHL) is a debilitating condition that affects approximately 15,000 individuals per year, with ~4000 cases reported annually in the United States. SNHL may be caused by a variety of factors. Only 10-15% of cases are linked to a specific cause and the majority of cases elude definitive diagnosis.

DISEASE

Cases with no defined cause are referred to as idiopathic SNHL. Suggested causes of idiopathic SNHL include viral infections, vascular compromise, and intracochlear membrane breaks as well as autoimmunity.

Autoimmune hearing loss is a subset of SNHL in which there is a sudden onset, rapidly progressing or fluctuating hearing loss that can be unilateral and often progresses to become bilateral.

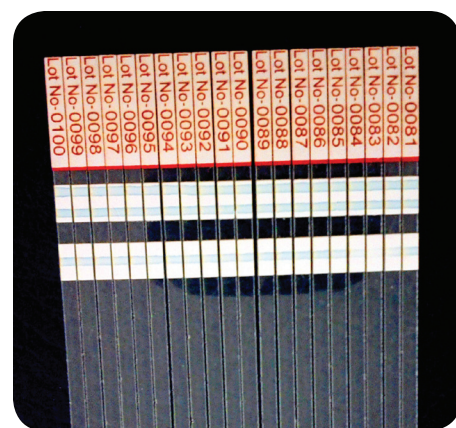
DIAGNOSIS

Autoimmunity is one of the few causes of hearing loss in which prompt detection of autoantibodies and early intervention may prevent progression of the hearing loss. There are number of other disorders associated with hearing loss with symptoms similar to autoimmune hearing loss. This makes the diagnosis of autoimmune hearing loss difficult based on clinical presentation alone. Autoimmune hearing loss may occur as the primary or only manifestation of disease. It may be associated with other systemic autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, granulomatosis with polyangiitis (GPA), etc. Autoantibodies identified in patients with idiopathic hearing loss include:

- 68kD (hsp-70) Antibody
- PO Antibody
- Phospholipid Antibody
- Antinuclear Antibody (ANA)

68kD ANTIBODY

Harris and Sharp in 1990 demonstrated that approximately 58% serum samples of patients with sudden SNHL recognized a 68kD protein in bovine inner ear extract. This 68kD protein was later identified as hsp-70. Since then, hsp-70 antibodies have been recognized as markers supporting diagnosis of autoimmune hearing loss. As levels of anti-hsp-70 antibody fluctuate with disease activity, the test may also be useful to follow patient response to treatment. Immco Diagnostics, in collaboration with the University of California, San Diego, has standardized this assay to aid in diagnosis of autoimmune hearing loss (Patent No. US005422282).



68kD (hsp-70) ANTIBODY INCIDENCE IN SNHL

Study Group	Sensitivity	Specificity
Moscicki et al 1994	58%	96%
Hirose et al 1999	42%	91%
Munari et al 2003	84%	93%
Park et al 2006	76%	91%
Bonaguri et al 2007	60%	92%

WHAT THE EXPERTS SAY ABOUT ANTI-68kD (hsp-70) ANTIBODIES

Hirose 1999: "Hsp-70 is the best test for predicting cortico-steroid responsiveness." *Laryngoscope*. 1999.

Park et al 2006: "...serum hsp-70 levels might have a clinical role for predicting prognosis of hearing loss in patients with SNHL." *Laryngoscope*. 116:121-125; 2006.

Bonaguri et al 2007: "anti-hsp-70...is still the only diagnostic marker that identifies an autoimmune origin of hearing loss." *Autoimmunity*. 40:73-78; 2007.

Prof. Nicola Quaranta: "...correlation between presence of anti-hsp and hearing recovery are novel and interesting findings." *ENT News* 2008.

NEW ImmcoStripe™ hsp-70 LINE IMMUNOASSAY (LIA)

Immco Diagnostics has developed a new LIA highly specific for the detection of antibodies associated with autoimmune hearing loss that enables accurate visual interpretation. The new ImmcoStripe™ hsp-70 LIA is flexible, simple to use, and easier to interpret compared to traditional Western Blot (WB) assays. ImmcoStripe™ hsp-70 uses purified recombinant inducible hsp-70 antigen. The hsp-70 antigen is immobilized onto nitrocellulose membrane strips along with conjugate, serum and cut-off control lines.

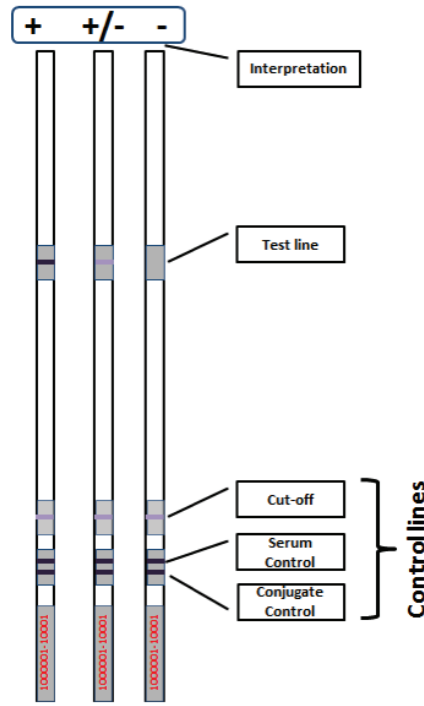
To perform the test, strips are incubated with diluted patient serum. In positive sera, antibodies specifically bind to the rhsp-70 protein on the strip. The strips are washed and the reconstituted conjugate is added. After incubation and wash steps, the ready to use substrate is added to the strips. During incubation, conjugate and substrate binding produce a chromogenic reaction — visible violet lines for serum, conjugate, and cut-off control lines. If the sample is positive for anti-hsp-70 antibodies, the test line will show a reaction more intense than the cut-off line. If the sample is negative, a visibly weaker reaction will show versus the cut-off line. Reactions are read visually and reported as positive, negative or equivocal.

Immco Diagnostics' LIAs are a robust and accurate alternative to WB and can be used either for screening or confirmation diagnosis.

FEATURES

Procedural Controls: The two procedure control lines ensure the addition of specimen, conjugate and substrate. The cut-off line provides a colorimetric standard for the evaluation of the reacted hsp-70 test line. Positive and Negative controls are available as optional components and may be run for additional quality control.

Interpretation: The test strip has three control lines: the cut-off line, the serum control line and the conjugate control line. The cut-off allows the technician to determine the test result as positive, negative or indeterminate (+/-).



SCHMATIC OF REACTED HSP-70 STRIPS

Traceability: ImmcoStripe™ hsp-70 uniquely provides complete traceability with a user friendly multisession report for recording results.

Sensitivity and Specificity: LIA is cleaner and devoid of background bands or signals. This increases the accuracy of results and makes their interpretation easier.

Reproducibility: The LIA manufacturing process eliminates lot to lot variations in line intensities and positions, a typical disadvantage when interpreting WBs.

CLINICAL STUDY

A recent study compared the performance of ImmcoStripe™ hsp-70 to the Immco Otoblot hsp-70 Western blot method for the detection of hsp-70 in well-characterized specimens from SNHL patients, idiopathic hearing loss patients, disease controls and “normal” human sera. The study showed 99% correlations between methods.

ACCURACY

The accuracy was assessed through method comparison between ImmcoStripe™ hsp-70 line blot assay and Immco Otoblot hsp-70 Western blot test. Precision was assessed through assaying of samples that were negative, equivocal and positive. They were performed to determine qualitative reproducibility from run to run and operator to operator. Results produced 100% qualitative agreement.

The ImmcoStripe™ hsp-70 Antibody Line Immunoassay compares well with Immco Otoblot hsp-70 Western blot assay in method correlation and has 100% agreement in results in manual and automated methods.

SELECTED REFERENCES

McCabe BF, Autoimmune sensorineural hearing loss. *Ann Otol.* 1979; 88:5852-589.

Moscicki RA, San Martin JE, et al. Serum antibody to inner ear proteins in patients with progressive hearing loss. Correlation with disease activity and response to corticosteroid treatment. *JAMA.* 1994;72:611-6.

Hirose K, Wener MH, Duckert LG. Utility of laboratory testing in autoimmune inner ear disease. *Laryngoscope* 1999;109:1749-54.

Park SN, Yeo SW, Park KH. Serum heat shock protein 70 and its clinical characteristics in patients with sudden sensorineural hearing loss. *Laryngoscope* 2006;116:121-25.

Bonaguri C Orsoni JG et al. Anti-68kDa antibodies in autoimmune sensorineural hearing loss *Autoimmunity* 2007;40:73-78.

Tebo AE Jaskowski TD et al. Comparison of immune assays for the detection of ant-hsp 70 antibodies in patients with idiopathic sensorineural hearing loss. *Clinica Chimica Acta* 2007;381:140-44.

Munari L, Charchat S, et al. An ELISA serum assay for autoantibodies to hsp-70 in immunemediated hearing loss. *J Immunol Methods.* 2003;283:155-61.

PRODUCT INFORMATION

CODE	DESCRIPTION	DETERMINATIONS
6001*	ImmcoStripe™ hsp-70 LIA * for research use only in USA	20

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