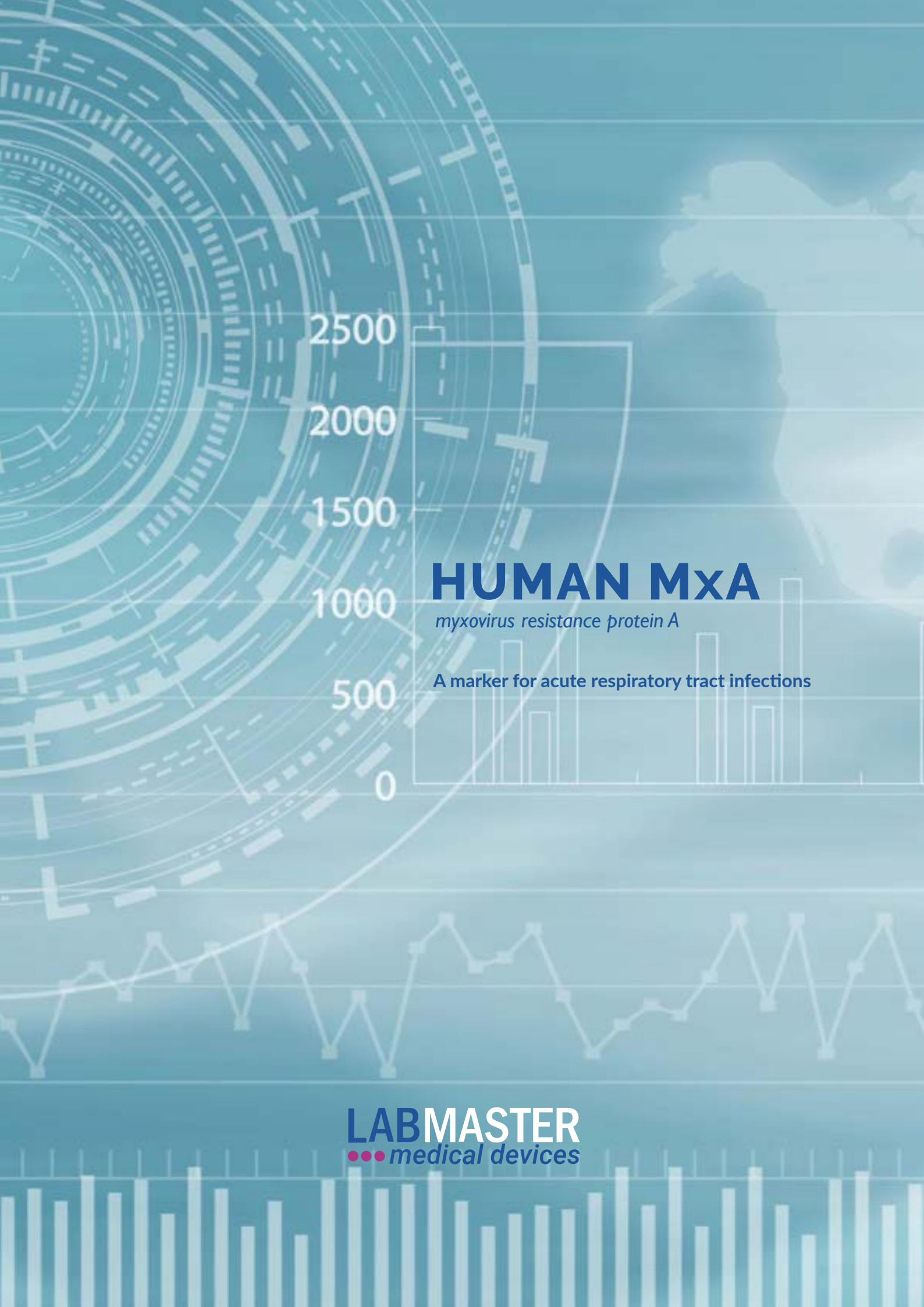

HUMAN MxA

myxovirus resistance protein A

A marker for acute respiratory tract infections

LABMASTER
medical devices

ORIGINALLY, MxA LEVELS IN WHOLE BLOOD WERE SHOWN TO DETECT VIRAL ILLNESS AND, IN PARTICULAR, RESPIRATORY VIRUS INFECTIONS.

Nakabayashi et al., 2006; Toivonen et al., 2015

INTRODUCTION

Human myxovirus resistance protein 1 (MxA) is a key mediator of the interferon-induced antiviral response against a wide range of viral infections of both negative- and positive-sense RNA viruses, including orthomyxoviruses and paramyxoviruses, bunyaviruses, picornaviruses, rhabdoviruses and togaviruses (Haller and Kochs, 2011). Initially, MxA has been most fully described for the orthomyxoviruses, hence the name, and in particular for influenza A virus (Mitchell et al., 2013). Nucleocapsid Protein (NP) is the primary viral protein underlying susceptibility to MxA. The basal concentration of MxA is visibly different in asymptomatic adults and children, rises differently during bacterial or viral infections to different respective values, and subsequently decreases to the original values following the elimination of the infection. Such full scale differentiation allows not only use MxA specificity for viral recognition, but also separate effects of bacterial and viral infection on human organism.

MxA IS A DIAGNOSTIC MARKER

A number of scientific publications support the use of MxA as a marker for (1) acute viral respiratory infection; (2) differentiating bacterial from viral origin of similar symptoms; and (3) monitoring multiple sclerosis (MS). Originally, MxA levels in whole blood were shown to detect viral illness and, in particular, respiratory virus infections (Nakabayashi et al., 2006; Toivonen et al., 2015). Also, Ivaska et al. (2017) linked levels of MxA with existence of viral infection causing febrile pharyngitis. Zav'yalov et al. (2019) reviewed MxA and MxB as biomarkers for differentiating viral from bacterial infections. Similarly, Piri et al. (2022) described MxA as a marker of acute viral infection allowing to differentiate and to deal with various levels of viral-bacterial co-infection. Independently, MxA levels were found to be elevated in patients with MS, where the MxA protein assay was suggested for optimal monitoring of IFN-beta bioactivity in the treatment of MS patients (Vallittu et al. 2008). All in all, MxA is a potent multi-purpose biomarker for monitoring selected aspects of viral infection.

PROPERTIES OF MxA

Human myxovirus resistance protein 1 (Mx1 isoform A, or MxA) is an interferon (IFN)-induced dynamin-like GTPase that functions as an antiviral restriction factor against many viral pathogens (Gao et al., 2011). Antiviral restriction factors are such specific first line defense host cellular proteins that block viral replication and propagation, and also trigger innate responses against infections. MxA is induced by type I (alpha and beta) and type III (lambda) IFNs, and thus is widely used as a reliable marker for IFN bioactivity (Holzinger et al., 2007).

Historically, the Mx1 gene was first identified in mice, which were resistant to the mouse-adapted influenza virus (Mitchell et al., 2013). The human orthologue was identified later. Crystal structure of the MxA protein has been deposited in the Protein Data Bank with accession code 3SZR (Gao et al., 2011). The MxA monomer shows an extended three domain architecture: (1) The N-terminal GTPase domain; (2) the central Bundle Signaling Element (BSE) domain; and (3) the C-terminal Stalk domain. Two MxA monomers form homo-dimer structure, interacting with their all-alpha Stalk domains. The length of a MxA monomer is 662 amino acids, and the molecular weight is 78 kDa. The monomers of human MxA and mouse Mx1 share 67% of sequence identity and 87% of sequence similarity with the short segments of sequence differences spread along the entire length of monomers.

DEVELOPING A MxA IMMUNOASSAY

Standard sandwich type immunoassays for the detection of human MxA are based on four key components: (1) specific anti-human monoclonal capture antibody (the capture MxA MAb); (2) labeled monoclonal tracer antibody (the tracer MxA MAb); (3) a recombinant MxA protein standard; and (4) a buffer with cell lysis ability for the whole blood sample dilution and MxA release. The recombinant MxA protein can be used as a calibrator in human MxA immunoassays. Labmaster Ltd uses following immunoassay components:

(1) Monoclonal capture anti-human MxA antibodies (capture MxA MAb), acquired from the University of Turku, Turku, Finland;

(2) Labeled tracer monoclonal anti-human MxA antibodies (tracer MxA MAb), acquired from the University of Turku;

(3) A purified recombinant MxA protein, acquired from the University of Turku;

(4) Lysis buffer for the whole blood sample dilution and handling, created by standard techniques.

DESCRIPTION AND AVAILABILITY OF REQUIRED ANTIBODIES

For use in sandwich immunoassays, a pair of monoclonal antibodies that are specific to human MxA is best to order directly from the companies doing recombinant protein production and antibody production. For example, InVivo BioTech Services GmbH (http://www.invivo.de) is the University of Turku provider.

LABMASTER LUCIA™ MxA IMMUNOASSAY

Labmaster LUCIA™ human MxA quantitative immunoassay is a standard sandwich type immunoassay for the whole blood samples, with monoclonal anti-human MxA capture and tracer antibodies, supplied by the University of Turku, measuring time is 11 min, detection range: 50-1000 ng/mL, diagnostic specificity 98%, and diagnostic sensitivity 91%. The calibration curve of the Labmaster MxA immunoassay with the assay-specific antibodies is shown in Figure 1:

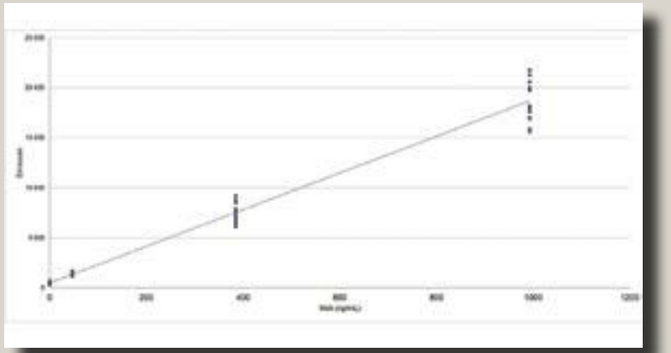

Figure 1. Calibration curve of human MxA in a Labmaster sandwich immunoassay.

The Labmaster MxA Kit does maintain its performance for 764 days with the shelf-life stability claim of 734 days (Figure 2).

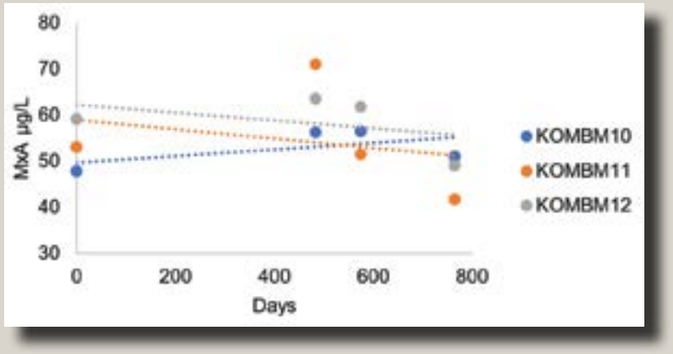

Figure 2. MxA concentration of calibrator 50 ng/mL measured at 0, 483, 574 and 764 days for the Labmaster MxA stability test.

The following substances, when tested in whole blood at low and high MxA concentrations, were found not to interfere at the concentrations indicated in the last column of Table 1. A deviation exceeding 20% is considered a significant interference

Interfering Substance	Highest Tested Concentration (CLSI EP07)	No Interference Found Up To the Following Concentration
Unconjugated Bilirubin	20 mg/dL	20 mg/dL
Conjugated Bilirubin	33 mg/dL	33 mg/dL
Triglycerides	3300 mg/dL	3300 mg/dL
Cholesterol	5 g/L	1.5 g/L
Ascorbic Acid	60 mg/L	60 mg/L
EDTA	5 mg/L	1.25 mg/L
Heparin	15 000 U/L	10 000 U/L

Table 1. Interfering substances tested with the Labmaster MxA test. Column 1 shows tested substances; column 2 shows highest tested concentration; column 3 shows highest concentration, up to which no interference has been detected.

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