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Original Article

# Immunochemical assay with monoclonal antibodies for detection of staphylococcal enterotoxin H

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ABSTRACT

Staphylococcal enterotoxins cause food poisoning of various degrees of severity. For milk and meat products, there is a high probability of contamination with staphylococcal enterotoxin H (SEH). In this regard specific and sensitive methods are required to be developed for its detection and monitoring. In this work, the gene *seh* was expressed and a preparation of recombinant toxin was obtained. Using hybridoma technology, a panel of high-affinity monoclonal antibodies (mAbs) to SEH was produced. The antibodies were characterized and shown to have no cross-reactivity towards the main staphylococcal enterotoxins (A, B, C1, D, E, G and I). Based on these mAbs, a method for specific and quantitative detection of SEH was developed in the format of sandwich enzyme immunoassay (linear range, 0.2–3 ng/ml). All the mAbs produced revealed SEH by immunoblotting. Immunochemical analysis of the culture fluids of staphylococcal isolates obtained from the milk of mastitis-infected cows by immunoblotting and sandwich enzyme immunoassay demonstrated the conformity of these methods. Using the developed method, the toxin was revealed in blood serum and liquid food products practically to 100%. From non-liquid foods, it was shown to be extracted to a maximum with a buffer of pH 4.0–4.5.



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## 1. Introduction

Staphylococci are widespread in nature: about 30% of the planet's population are carriers of *Staphylococcus aureus* [1]. The pathogenic strains of *S. aureus* produce toxins (staphylococcal enterotoxins, SEs) and cause diseases of varying degree of severity, from mild ailments to septic shock [2,3]. According to the estimates of the US Centers for Disease Control and Prevention, 240,000 cases of staphylococcal food poisoning (SFP) occur each year in the US, leading to hospitalization in 1000 cases and to six deaths. In the European Union, the number of SFP outbreaks is rising, with 386 SFP outbreaks reported in 2013 [4]. Enterotoxins, the main factors of staphylococcal pathogenicity, are globular proteins with a molecular mass of 27–30 kDa, soluble in water and saline solutions. They are rich in lysine, aspartic acid, glutamic acid and tyrosine residues. Most of them possess a cystine loop required for proper conformation and, probably, involved in emetic activity. They are highly stable, are persistent to most proteolytic enzymes, such as pepsin or trypsin, thus preserving their activity in the digestive tract after ingestion, and are resistant to low pH values. Staphylococcal enterotoxins are also highly heat stable; they are thought to be more heat stable in foodstuffs than in a laboratory culture medium [5,6].

The toxic effect of SEs in primates manifests itself in enteric disorders and vomiting. In the infected organism, they act as superantigens, interacting simultaneously with T-cell receptors (TCR) and molecules of MHC class II (MHC-II) antigens on antigen presenting cells, which leads to the suppression of adaptive immune response and nonspecific activation of T-lymphocytes that massively release cytokines; this, in turn, results in a systemic shock and immune system imbalance. Superantigenic and emetic activities of SEs are two separate functions localized on separate domains of the protein [5].

SEH was found relatively recently. Structurally SEH is close to other SEs; it has about a 30% homology of the amino acid sequence with each of them. In contrast with other SEs (of pI 7.0–8.6), it is an acidic protein with an isoelectric point of 5.7 [7]. Most SEs stimulate the Vβ region of the T-cell receptor; SEH interacts with the T-cell receptor Vα region [8].

There is a sufficient amount of data on its participation in staphylococcal food poisoning. Shown that SEH-producing *S. aureus* isolates are of high prevalence in staphylococcal food poisoning cases [9]. *S. aureus* is the main cause of mastitis [10]. The milk of mastitis-infected animals and the products made from this milk can contain staphylococci and SEs. The *seh* gene was found in *S. aureus* isolates from milk and dairy products in South Italy [11], Japan [12] and Norway [13]. The *seh* gene was found in isolates *S. aureus* not only of cattle, but also of small ruminants. Studies conducted in Italy showed that the *seh* gene was the most frequent – 33.3% [14]. PCR technology showed the presence of the *seh* gene in dried fat-free milk during the large-scale milk-product poisoning of more than 13,000 people in Japan in July 2000. Wherein *seh* was detected more often than other SEs genes in *S. aureus* isolates from milk of cows with mastitis [12]. SEH is capable of inducing the apoptosis of epithelial cells of staphylococcus-infected cow mammary glands, which may be an important factor of staphylococcal pathogenicity of mastitis [15]. Facts that indicate the risk of contamination of milk and milk products SEH make necessary a highly sensitive detection of toxin.

SEH can be assayed by molecular biological methods (by detection of the *seh* gene [16] and by immunochemical methods (by detection of the protein as a gene product evaluation), using polyclonal antibodies specific for SEH itself [17]. However, the use of polyclonal antibodies has a number of disadvantages (such as cross-reactivity), and the reproducibility of results often depends on the antibody batch. While PCR is one of the techniques commonly used to screen for SE genes, due to its simplicity and low cost, variations in observed SEH concentrations [18] suggest that genotyping alone is insufficient to assess the risk associated with a particular contamination. The spread of cases of SEH-caused food poisoning makes it necessary to develop selective and highly sensitive methods of detecting this enterotoxin in food products. Currently, many different formats for the immunochemical determination of the analyte can be developed on the basis of monoclonal antibodies: various versions of Enzyme-Linked Immuno Sorbent Assay, the use of fluorescent and fluorescent labels, including fluorescence-polarization and fluorescence resonance energy transfer. Monoclonal antibodies are also successfully used in the creation of biosensors that detect the sought-for substances in situ in real time, in which the binding of the antibody to the ligand in the biosensor leads to a detectable signal [19]. However, the success of any immunochemical platform is primarily determined by the quality of the antibodies used. The objective of this work was to obtain selective reagents – monoclonal antibodies to SEH, and to use them for developing methods of immunochemical assay SEH in biological fluids and food products.

## 2. Materials and methods

### 2.1. *Isolation and cloning of the* seh *gene*

*S. aureus* strain MRSA2308 provided by N.F. Gamaleya Federal Research Center for Epidemiology & Microbiology was used as a bacterial strain for the PCR amplification of the whole staphylococcal *seh* gene. The PCR was performed using the specific primers P1 (5′-TTTCCATGGAAGATTTACACGATAAAAGTGAGTTAAC-3′) and P2 (5′-TTTGCGGCCGCTACTTTTTTCTTAGTATATAGATTTAC-3′). The primers were designed according to the *seh* sequence (GenBank Accession Number AY345144.1). The forward primer (P1) contained an engineered NcoI site (underlined), and the reverse primer (P2) incorporated an engineered NotI site (underlined). The optimized PCR conditions were as follows: initial denaturation at 95 °C for 2 min followed by 30 cycles of denaturation (95 °C, 1 min), annealing (50 °C, 30 s), extension (72 °C, 1 min), and final extension at 72 °C for 5 min. The amplified product was isolated from the agarose gel with a Cleanup Mini kit (Eurogen, Moscow) and cloned into the expression vector pET28b containing 6 × His tag as a NcoI–NotI fragment. The ligated product was transformed in the expression host Rosetta-gami *Escherichia coli* (DE3). Recombinant clones were screened by the PCR for the presence of toxin gene. The sequence of the

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