INTERNATIONAL STANDARD

ISO 13019

First edition
2018-06

Tissue-engineered medical products — Quantification of sulfated glycosaminoglycans (sGAG) for evaluation of chondrogenesis

Produits médicaux issus de l'ingénierie tissulaire — Quantification des glycosaminoglycanes sulfatés (sGAG) pour l'évaluation de la chondrogenèse

Reference number
ISO 13019:2018(E)

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Published in Switzerland

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Foreword

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This document was prepared by Technical Committee ISO/TC 150, *Implants for surgery,* Subcommittee SC 7, *Tissue-engineered medical products.*

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Tissue-engineered medical products — Quantification of sulfated glycosaminoglycans (sGAG) for evaluation of chondrogenesis

1 Scope

This document specifies test methods for the quantification of sulfated glycosaminoglycans (sGAG), one of the major extracellular matrix components in articular, meniscal, and elastic cartilages, and tissue-engineered cartilage constructs.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purpose of this document, the following definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
tissue-engineered cartilage
cultured construct obtained by combining living cells such as chondrocytes, or stem cells with or without scaffolds, or other types of biomaterials

3.2
sulfated glycosaminoglycans
long unbranched chains of repeating disacchararide units, which are sulfated at variable positions

EXAMPLE Chondroitin sulfate, keratan sulfate, dermatan sulfate.

3.3
test sample
piece of *tissue-engineered cartilage* (3.1) construct

3.4
parallel test sample
one of three samples divided from a *test sample* (3.3)

Note 1 to entry: The protocol provides a sGAG value from each corresponding parallel test sample.

3.5
cysteine
non-essential and sulfur containing amino acid, used for preparing digestion solutions

3.6
papain
cysteine protease enzyme, used for digesting extracellular matrices of diverse tissues

[SOURCE: Enzyme Commission (EC) Number 3.4.22.2]

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3.7
1,9-dimethylmethylene blue
dye that specifically binds to a range of sulfated glyocosaminoglycans (3.2), whose absorbance after binding correlates linearly with the amount of bound sGAG

3.8
chondroitin 6-sulfate
one of the sulfated glycosaminoglycans (sGAG), an important structural component of cartilage and connective tissues

4 General and principle

The quantification of sulfated glycosaminoglycans (sGAG) is ranked as one of the first steps in evaluating chondrogenesis of tissue-engineered constructs, since they are one of the primary extracellular matrix consituents of native cartilage.

sGAG can be quantified by digesting test samples with enzymes to release sGAG into solution, incubating with a sGAG-binding dye, measuring the absorbance, and normalizing to the weight (wet or dry) or DNA content of the test sample.

This document applies to tissue-engineered cartilage constructs even if the amount of sGAG content might be low and offers a dynamic range of 3 µg/ml to 100 µg/ml using a standard curve.

Binding of 1,9-dimethylmethylene blue (DMMB) dye is widely-accepted for quantifying sGAG[21][22][29], and therefore was selected for this document.

The procedure to quantify sGAG in constructs comprises the following steps:

1) Pre-treatment;

2) Extraction of sGAG;

3) Measurement of sGAG content by the DMMB assay;

4) Normalization of sGAG content.

In order to realize the accurate quantification, careful attention should be paid to critical steps, such as measuring of weights and volumes, pipette manipulations, and drawing of standard curves. During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled water or water of equivalent purity (See Annex B). An example of a test report format for sGAG measurement is shown in Annex E.

5 Apparatus

5.1 Clean bench.

5.2 Sterilizer, e.g. autoclave.

5.3 Phase contrast microscope.

5.4 Cell culture incubator, capable of maintaining a humidified atmosphere containing 5 % CO_2 at 37 °C.

5.5 Cell counting apparatus, e.g. hematocytometer.

NOTE If applicable, refer to ISO 20391-1.

5.6 Centrifuge.

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