Citizen Petition

June 7, 2022 Date: The undersigned submits this petition under Section 505(j)(2)(C) of the Federal Food, Drug and Cosmetic Act ("FDCA") and 21 C.F.R. Sections 10.20, 10.30 and 314.93 or the Public Health Service Act or any other statutory provision for which authority has been delegated to the Commissioner of Food and Drugs) to request the Commissioner of Food and Drugs to__ (issue, amend, or revoke a regulation or order or take or refrain from taking any other form of administrative action).

A. Action Requested

The petition requests the Commissioner to issue, a regulation, to ban the use of glycogen assays that employ centrifugation of homogenates of patient specimens prior to the amyloglucosidase degradation of glycogen.,

B. Statement of Grounds

Problem with commercial Glycogen Assay Kits.

While doing the literature survey for a recent paper (Murray, 2021). I became aware of a problem with glycogen determinations in publications. One issue is with the glycogen determination on various samples of tissue homogenates. Glycogen is a polysaccharide consisting of different sizes of particles. The typical assay procedure involves degradation of the glycogen particles with an enzyme, amyloglucosidase, to release the glucose followed by the quantification of the glucose released. The sample homogenate is typically boiled to inactivate endogenous enzymes before the amyloglucosidase digestion. If the sample homogenate is centrifuged before the amyloglucosidase digestion and the supernatant used for the glycogen determination, it is likely that glycogen may be precipitated along with proteins and not included. Given the nature of glycogen and the possibility of boiling extracts before centrifugation precipitating glycogen, results in ambiguity about the quantification of glycogen, as mentioned by Brown et. al., (1978),. **The problem with the commercial kits is that their protocol involves the centrifugation of the boiled homogenate, usually up to 18,000 x g, for 5 to 10 minutes and the supernatant is used for the amyloglucosidase incubation.** Centrifugation after the amyloglucosidase degradation is not a problem. Calder and Geddes (1989) centrifuged homogenates at 16,000 x g four times and then combined the precipitates for their glycogen which is obviously the opposite of what the kits specify. The description of glycogen particles is described and illustrated by Prats, et. al. (2018).

The genesis of the problem with the commercial kits is the use of 96 well plates AND the fact that they conduct the amyloglucosidase degradation and the glucose determination by two different methods in the same well of the plates by the addition of different reagents and enzymes at different times. They specify centrifugation with the supernatant added to the reaction well . The reason for this is obvious, since particulate matter would sink to

the bottom and interfere with the light path for the optical determination of the resultant component in the well. However, as mentioned above, the centrifugation removes a significant amount of glycogen resulting in an inaccurate determination of glycogen. In one publication, when boiled tissue homogenates were centrifuged before amyloglucosidase degradation and only the supernatant was treated with the amyloglucosidase, the glycogen in the supernatant was approximately one third to one half of the glycogen in the total homogenate (Carr and Neff, 1984). They also made a similar observation with their rabbit liver glycogen standard.

The different manufacturers specify different temperatures and time for the amyloglucosidase degradation and they supply glycogen to be used as a standard. The problem with this is that commercially available glycogens are significantly degraded and as a result are degraded by enzymes faster than native glycogen (Murray, 2021) so this could be a potential problem. There is a published procedure for a suitable glycogen determination in 96 well plates. which is a two step procedure with a centrifugation after the amyloglucosidase degradation in between the two steps (Gómez-Lechón, et. al.,1996).

I am surprised that investigators and journal reviewers are not aware of the problem since it is no secret that glycogen is particulate and sparingly soluble. The result is that we now have several hundred published papers with inaccurate glycogen data. Many of these are in areas such as diabetes research, hormone research and other areas which might lead to unwarranted conclusions. These papers have been published from the late 90s to the present day and clearly indicate a failure of the peer review system. The kits have been used on human muscle samples (Nieman, et. al., 2015a,b).

There are at least 12 companies that are selling such kits and the stock market analysts project significant growth for the market through 2028. The centrifugation steps for most of these kits are listed in Appendix 1. I believe that something should be done to remove such defective assay kits from the marketplace.

References

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Appendix 1. Glycogen Assay Kits on the market.

SOURCE	KIT	CENTRIFUGATION
Abcam	A65620	18,000 x g, 10 min
Abcam	AB169558	18,000 x g, 10 min
Abnova	KA0861	18,000 x g, 10 min
Bio Vision	K960	18,000 x g, 10 min
BioAssay Systems	E2GN	14,000 x g, 5 min
Cayman Chemical Co.	700480	800 x g, 5 min
Cell BioLabs	MET-5022	10,000 x g, 10 min
LS Bio	K151	18,000 rpm, 10 min
MyBiosource	MBS841523	18,000 rpm, 10 min
Novus Biologicals USA	KA0861	18,000 rpm, 10 min
Sigma-Aldrich	MAK016	13,000 x g, 5 min

Elab Science sells a kit but it does not utilize amyloglucosidase or centrifugation. It is a sulfuric acid based test that hydrolyzes the total homogenate.

BioCat GmbH sells the Bio Vision K 960 kit.

Nanjing Yixun Biological Technology and Shanghai Xinfan Biological Technology are also reported to sell kits.

C. Environmental Impact

"We claim categorical exclusion under 25.30, 25.31, 25.32, 25.33, or 25.34 of this chapter or an environmental assessment under 25.40 of this chapter."

D. Economic Impact

Economic impact information will be submitted upon request of the commissioner.

E. Certification

The undersigned certifies, that, to the best knowledge and belief of the undersigned, this petition includes all information and views on which the petition relies, and that it includes representative data and information known to the petitioner which are unfavorable to the petition.

Allen K. Murray, Ph.D.

Glycan Technologies, Inc.

PO Box 17993

Irvine, CA 92623

(949)689-9664

amurray1@glycantecnologies.com