

# An Alternative Procedure for Detecting Pregerminated Grains in Barley

Submitted on behalf of the UK Malting Industry

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## 1 Introduction

The Analytica-EBC methods for detecting pregerminated grains in barley viz. 3.8.1 Fluorescein Dibutyrate (FDB) and 3.8.2 Methylene Blue have not been considered suitable for intake use in the UK. Each imposes an extra test procedure, involving additional resources, on the range of tests already being performed on grain at delivery to maltings.

It is possible to detect pregermination by the 2,3,5-triphenyl tetrazolium chloride rapid stain test for viability (Analytica-EBC method 3.5.1) which is performed routinely on grain presented at UK malting barley intakes. Using modest magnification, movement of root- and/or shoot-forming tissue within the stained, bisected embryo can readily be detected. Such movement is indicative of pregermination and, in the hands of an experienced operator, the extent of movement can be used to decide whether grain is acceptable for malting. Detecting pregermination in this way requires minimal extra resources.



## 2 Objectives

Funded by the UK Home Grown Cereals Authority (HGCA), the Maltsters Association of Great Britain (MAGB) organised a workshop in January 2004 to examine how pregermination, if it were to occur in UK malting barley, could be assessed in a uniform manner across all sectors of the malting barley industry.

Arising from the workshop a sub-group was formed with a brief to find a suitable sample of pregerminated barley, bisect a number of corns and stain the matching halves using either 2,3,5-triphenyl tetrazolium chloride or fluorescein dibutyrate. Photographs of the matching half corns would be taken and the effectiveness of the tetrazolium technique assessed against the reference technique.



## 3 Procedure

Approximately 100 corns were bisected and stained. Before proceeding further it was important to establish that the levels of pregermination as seen by tetrazolium staining agreed with those as seen by the reference fluorescein dibutyrate technique. This criterion was met in full. 30 pairs of half corns were then selected with differing degrees of pregermination, from none to severe.

The paired tetrazolium and FDB stained half corns were given appropriate identification numbers and then photographed individually; tetrazolium stained half corns were photographed in natural light, FDB stained half corns in UV light. Example photographs are shown in Panel 6a.

An expert panel drawn from the industry then selected a final series of 9 pairs of photographs depicting progressive stages of pregermination. The photographs of the tetrazolium stained halves were used to produce a definitive pictorial reference guide for the UK grain industry (Panel 6b).



## 4 Discussion

The assessment of pregermination by staining is subjective in nature, relying on the expertise of the technician to interpret the stain and relate that to the degree of pregermination. Unless this is done carefully grain affected by pregermination may be accepted or rejected for malting when it should not have been. One important objective of the work was to improve the uniformity of interpretation when assessing pregermination but *not* to set limits on acceptability for intake; setting limits must always be a decision for an individual company. The objective was met.

By staining matching halves of corns with one or other of two staining reagents it was clearly demonstrated that the extension of a technique in regular use for examining the germinative capacity of barley could give similar results to the reference technique when assessing pregermination. Selection of appropriate photographs of stained half corns and compiling them into a poster on CD has allowed a definitive reference document to be made available across the UK malting barley industry.



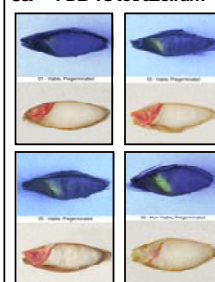
## 5 Summary

1. Analytica-EBC Methods 3.8.1 Fluorescein Dibutyrate and 3.8.2 Methylene Blue for assessing pregermination in malting barley have not been considered suitable for intake use in the UK.
2. A simple extension of the 2,3,5-triphenyl tetrazolium chloride rapid stain test for germinative capacity can be used to detect pregermination and is easy and quick to apply.
3. Paired halves of bisected corns stained with tetrazolium chloride or fluorescein dibutyrate show excellent agreement in the detection of pregermination.
4. A CD with a definitive pictorial guide of tetrazolium stained corns has been prepared for use throughout the UK malting barley industry. This guide can also be found on the MAGB website at [www.ukmalt.com](http://www.ukmalt.com)
5. The procedure is to be included in Analytica-EBC Method 3.5.1.

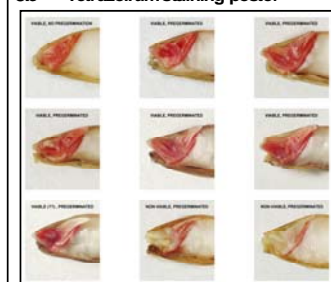


## 6 Stages of Pregermination

6a FDB vs tetrazolium



6b Tetrazolium staining poster



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UK Home Grown Cereals Authority (HGCA)

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