Technical Committee and Subcommittee Reports

2011–2012 Report of the Technical Committee

Committee members: C. Powell, *chair*; D. Sedin, C. Benedict; S. Brendecke; L. Chadwick; J. Cornell; M. Eurich; A. Fritsch; R. Jennings; K. Lakenburges; A. Porter; A MacLeod; C Pachello, J Palausky; Dave Maradyn and B. Foster (*senior advisor*)

Activity in 20 subcommittees was conducted by the ASBC Technical Committee and subcommittee chairs during 2011–2012. As a result, 4 methods are being recommended for inclusion in the ASBC *Methods of Analysis* (MOA):

- Viscosity of Wort by Rolling Ball Viscometer, chaired by Aaron Macleod (Canadian Grain Commission)
- Headspace Gas Chromatography-Flame Ionization Detector Analysis for Beer Volatiles, chaired by Joe Palausky (Boulevard Brewing Co.)
- Alpha-Amylase in Malt by Segmented Flow Analysis Using Potassium Ferricyanide, chaired by Theresa Chicos (Rahr Malting Co.)
- X-Alpha-Gal for Differentiation of Ale/Lager Yeast Strains, chaired by Wendy Box (University of Nottingham)

One method has been accepted as a provisional method within MOA:

- Headspace Gas Chromatography-Flame Ionization Detector Analysis for Beer Volatiles, chaired by Joe Palausky (Boulevard Brewing Co.)

One method was not recommended for inclusion in MOA. Additional time is required to evaluate methodology along with manufacturers input at this time:

- Rapid Immunoassay for Deoxynivalenol in Barley and Malted Barley, chaired by Andrea Stern (Malteurop)

The following methods will continue for another year of collaborative study:

- Wort Amino Acids by HPLC. This committee currently requires a chair person. Interested parties should contact Chris Powell or Aaron MacLeod for further details.
- Isomerized Alpha Acids in Beer by Solid Phase Extraction, and Subsequent Spectrophotometric Measurement, chaired by Tom Shellhammer and Philip Wietstock (Oregon State University)

The review of one section of the MOA will also be continued:

- Beer, chaired by Karl Lakenburges (Anheuser-Busch InBev) and Mark Eurich (MillerCoors)

The following subcommittees are being recommended for collaborative study in 2012–2013:

- Analysis for Total Vicinal Diketones (VDKs) in Beer by GC/ECD, chaired by Grant Ruehle (New Belgium Brewing Co.)
- Determination of Beta Glucan in Beer, chaired by Aaron MacLeod (Canadian Grain Commission)
- Determination of Gluten in Beer, chaired by Lindsay Guerdrum (New Belgium Brewing Co.)
- Statistical Analysis of Samples, chaired by Aaron MacLeod (Canadian Grain Commission)

As in previous years, the following eight standing subcommittees continue:

- Soluble Starch, chaired by Rebecca Jennings (Rahr Malting Co.)
- Check Services, chaired by Rebecca Jennings (Rahr Malting Co.), with Jim Munroe (retired Anheuser-Busch), Sue Casey (ASBC), Stephen Kenny (Washington State University IAREC), and John Barr (North Dakota State University)
- Coordination of New and Alternate Methods of Analysis, chaired by Joe Palausky (Boulevard Brewing Co.)
- International Methods, chaired by Chris Powell (University of Nottingham)
- Craft Brewers, chaired by Luke Chadwick (Bell's Brewery)
- Sensory Science, chaired by Annette Fritsch (Boston Brewing Company)
- International Hop Standards, chaired by Bob Foster (MillerCoors)
- Packaging Methods, chaired by Scott Brendecke (Ball Corporation) and Chaz Benedict (Hach Ultra Analytics)

Jim Munroe (retired member, formerly of Anheuser-Busch) continues to provide statistical input and recommendations to the Check Services program. Sue Casey, Stephen Kenny, and John Barr continue in their roles as Check Service managers for Beer Analysis, Hop Analysis, and Malt and Barley Analyses, respectively. Rebecca Jennings continues to work with the Check Services Committee, overseeing updates to the services provided and ensuring their relevancy to brewing sample analysis. As always, their hard work and dedication is greatly appreciated.

In 2010 the ASBC Board of Directors initiated a grant program to be administered by the ASBC Technical Committee for the development of methods or value products for inclusion in *Methods of Analysis*. This will be continued for 2012-13 with Mark Zunkel (Weihenstephan, hop flavor wheel development), Philip Wietstock (Oregon State University, new method for the analysis of IBU in beer and wort), and Alex Mott (University of Nottingham, method video development) receiving grants.

The Coordination of New and Alternate Methods of Analysis Subcommittee submitted a survey to members on April 24, 2012. Karl Lakenburges (subcommittee chair) worked closely with the Technical Committee to design the questions, and a number of topics were polled for interest in future subcommittees. The results were presented at the 2012 World Brewing Congress in Portland, OR. Based on the polling results and feedback at this

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meeting, multiple methods have been recommended for collaborative study in 2012–2013. As Karl has now completed a 3 year term of office, Joe Palausky has graciously accepted to continue his excellent work, although Karl will remain as an advisor for the coming year.

We would like to thank the current subcommittee chairs for their hard work and dedication in conducting their respective collaborative studies during the past year. Furthermore we would like to recognize the many subcommittee members who have participated over the past year. We would also like to recognize the dedication and hard work put forth by the Technical Committee.

We would also like to thank Ecolab and in particular Joe Dirksen for their hospitality and assistance in domestic arrangements during the ASBC Technical Committee fall meeting in St. Paul on October 9.

Finally, a special mention must be made to the previous chair of the Technical Committee; Dana Sedin has performed a simply outstanding job in pushing the committee forward to its current standing. His desire, commitment and leadership have been instrumental in building a proactive team of talented individuals, and ensuring a regular stream of methods for publication in *Methods of Analysis* over the previous 3 years.

Coordination of New and Alternate Methods of Analysis
(*Joe Palausky, j.palausky@boulevard.com*)

This is a standing subcommittee whose function is to collect, from various sources including polling members, new and alternate methods of analysis that may be useful for the industries our society serves. These methods are reviewed to establish their merit and utility prior to evaluation.

Soluble Starch
(*Rebecca Jennings, rjennings@rahr.com*)

This is a standing subcommittee whose goal is to coordinate a testing program for soluble starch that will ensure a consistent supply of quality soluble starch for the society. To further this goal, the subcommittee monitors process methodology utilized in the production of starch, investigates improved methods for starch quality testing, and evaluates potential new suppliers of starch.

Craft Brew
(*Luke Chadwick, lchadwick@bellsbeer.com*)

The mandate of this subcommittee is to engage the craft brewing members of ASBC and explore opportunities to make the society more relevant to these individuals. Additionally, the subcommittee aims to explore opportunities and pursue strategies to bring craft brewers who are not members of the society into ASBC.

Sensory Science
(*Annette Fritsch, annette@fritschsensory.com*)

This is a standing subcommittee. It was formed on the recommendation of the Technical Committee to bring more focus to sensory science in ASBC and provide a forum for sensory scientists in the brewing industry to share and discuss current methodologies and propose new methodologies for collaborative testing. The current focus is on updating the beer flavor wheel(s), methods for shelf-life testing, and decision trees for sensory evaluation.

International Hop Standards
(*Bob Foster, Robert.Foster@millercoors.com*)

This subcommittee has existed for 14 years as the International Subcommittee for Isomerized Hop Alpha-Acids Standards (ISIHAS) and is a standing subcommittee whose goal is to produce, purify, and verify isomerized and unisomerized hop standards for the brewing, hops, and related industries.

Packaging Methods
(*Scott Brendecke, sbrendec@ball.com*)

This is a standing subcommittee. It was formed to evaluate packaging methodology, review packaging methods within the MOA, and act as a liaison between ASBC and other packaging-related organizations.

International Methods
(*Chris Powell, chris.powell@nottingham.ac.uk*)

The function of this standing subcommittee is to encourage collaboration between ASBC and international brewing organizations. The primary focus is shared method collaboration with both BCOJ and EBC.

Wort Amino Acids by HPLC
(*Aaron MacLeod, aaron.macleod@grainscanada.gc.ca*)

Based on interest from polling in 2011, this subcommittee will evaluate high-performance liquid chromatography for the measurement of amino acids in wort.

Microbiological Methods in Brewing
(*Caroline Pachello, caroline.pachello@millercoors.com)*

This new subcommittee will evaluate novel methods for analysis of microbiological samples in brewing, including yeast- and bacteria-related assays. During the coming year information on innovative methodology and techniques will be collected and assessed. Individuals interested in contributing and/or participating in collaborative work are encouraged to contact Caroline directly.

Isomerized Alpha Acids in Beer by Solid Phase Extraction, and Subsequent Spectrophotometric Measurement
(*Tom Shellhammer, tom.shellhammer@oregonstate.edu*)

This collaborative is based on a method developed at Oregon State University in Tom Shellhammer's lab. The method utilizes solid-phase extraction followed by spectrophotometric detection for rapid and accurate bitterness analysis. The method correlates well with the IAA method using HPLC, thus providing a more accurate assessment of bitterness compared with the standard spectrophotometric method. The method utilizes methanol and water as solvents and can be run by any laboratory with a spectrophotometer.

Analysis for Total Vicinal Diketones (VDKs) in Beer by GC/ECD
(*Grant Ruehle, gruehle@newbelgium.com*)

This subcommittee was initiated on the recommendation of the subcommittee for Coordination of New and Alternate Methods and enters its first year. The subcommittee will evaluate the use of the headspace/gas chromatography/electron capture detection (GC/ECD) for measuring total viscinal diketones (VDKs) in beer.

Determination of Gluten in Beer
(*Lindsay Guerdrum, lguerdrum@newbelgium.com*)

Celiac disease is an inherited immune-mediated enteropathy that damages the small intestine, interfering with nutrient absorption upon consumption of gluten. It has previously been assumed that because beer is derived from material containing the toxic

prolamin fraction, it is unsuitable for celiac sufferers to drink. However, this assumption has been challenged as a considerable amount of protein modification and precipitation occurs during the malting, brewing, and fermenting processes. As a result, it is essential that the level of gluten in beer can be accurately determined for the purpose of labeling and marketing to the gluten-sensitive population. In this study, the R5 Competitive ELISA method from R-Biopharm will be evaluated as a tool for determination of gluten in beer.

Determination of Beta Glucan in Beer

(*Aaron MacLeod, aaron.macleod@grainscanada.gc.ca*)

This subcommittee was initiated based on the recommendation of the subcommittee for Coordination of New and Alternate Methods. It is anticipated that this subcommittee will evaluate the use of test kits for the determination of beta glucan in beer.

Statistical Analysis of Samples

(*Aaron MacLeod, aaron.macleod@grainscanada.gc.ca*)

This subcommittee has been initiated to provide guidelines for the statistical analysis of data related to brewery samples. The subcommittee will focus on comparison and validation of analytical methods through single and multi-laboratory studies. It will address topics such as identifying the appropriate statistical test to apply, dealing with outliers, and interpreting results. The primary goal is to prepare a set of methods and guidelines to assist the nonexpert in correctly analyzing data.

Alpha-Amylase in Malt by Segmented Flow Analysis Using Potassium Ferricyanide

Subcommittee members: T. Chicos, *chair;* J. Andrews; S. Arndt; J. Barr; K. French; D. Frey; T. Henderson; M. Johnson; R. Joy; A. MacLeod; C. Martens; M. Schmitt (EBC); K. Stainbrook; A. Stern; and R. Jennings (*ex officio*).

Keywords: SFA

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the determination of alpha-amylase by segmented flow analysis (SFA) using potassium ferricyanide ranged from 1.0 to 3.6% and 3.0 to 5.0%, respectively, and were judged acceptable.
2. Based on the *t*-Test assuming unequal variances, no statistically significant differences were found between Malt-7C and SFA using potassium ferricyanide in the determination of alpha-amylase.

RECOMMENDATIONS

1. The subcommittee recommends that the method for alpha-amylase in malt by SFA using potassium ferricyanide be included in *Methods of Analysis*.
2. Discharge the subcommittee.

This was the third year of this subcommittee's evaluation of an alternative method for determining alpha-amylase activities in malt by segmented flow analysis (SFA) utilizing potassium ferricyanide (KFCN) for detection. Some members had expressed concern that the current approved method (Malt-7C) for automated flow analysis (1), which uses β-limit dextrin and iodine for detection, might not have the dynamic range to accurately cover a broader spectrum of malts.

In the first year, determination of alpha-amylase by segmented flow analysis using potassium ferricyanide produced acceptable repeatability coefficients of variation and unacceptable reproducibility coefficients of variation when compared to Malt-7A or -7B using glucose as the standard (4). The subcommittee recommended that the collaborative testing be repeated with a focus on Malt-7C as a comparison using the Megazyme Malt Amylase standard (E-MAST). In the second year, determination of alpha-amylase by segmented flow analysis using potassium ferricyanide produced acceptable repeatability coefficients of variation and unacceptable reproducibility coefficients of variation when compared to Malt-7C (5). It was recommended to use normalized data to avoid errors associated with the making of the Malt Amylase standard (E-Mast).

Previous attempts to establish alpha-amylase activity by segmented flow analysis had experienced high reproducibility coefficients of variation (2). Since most analytical labs were performing segmented flow analyses and the number of labs routinely using the standard reference method were decreasing, an effort was made to include only collaborators using the most prevalent methodology (SFA with iodine for detection). Satisfactory results from this collaborative resulted in approval of Malt-7C as a method for determination of alpha-amylase (3).

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PROCEDURE

A total of eight malted barley samples, four sample pairs (A/B-G/H) using different commercial varieties, similar but distinct, were malted on separate dates representing a wide range of alpha amylase levels. Samples A/B were 2010 Tradition malted on different production dates. Samples C/D were 2010 Lacey malted on different production dates. Samples E/F were 2010 Metcalfe malted on different production dates. Samples G/H were 2009 Conrad malted on different production dates. These samples were ground on a Buhler DLFU Disk Mill, according to Malt-4 (1) for fine grind. Calibration of the method used Megazyme's Malt Amylase standard as described in Malt-7C. Results were evaluated using the Youden unit block design (1).

RESULTS AND DISCUSSION

Results from eight collaborators using potassium ferricyanide for detection were received for the four sample pairs. Results from one collaborator were excluded prior to statistical analysis because of known deviations from the prescribed experimental protocol. The results are presented in Table I. The sample pairs were also analyzed by six collaborators using Malt-7C and these

TABLE I
Alpha-amylase Activity 20° DU (as is) by Segmented Flow Analysis using Potassium Ferricyanide for Detection

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	69.2	66.7	62.6	59.5	69.1	69.9	75.2	72.3
2	…	…	…	…	…	…	…	…
3	63.9	65.9	60.5	58.9	66.9	68.7	74.2	73.8
4	60.5	62.0	55.9	53.4	65.5	65.2	69.4	70.9
5	63.2	70.7	64.3	56.5	68.8	69.2	75.0	72.6
6	68.5	68.0	62.5	61.5	70.5	70.0	73.0	74.0
7	61.4	62.2	58.3	57.2	65.4	64.7	69.8	68.4
8	65.6	64.1	59.6	57.6	66.8	68.1	69.3	72.4
Mean	64.61	65.66	60.52	57.80	67.57	67.96	72.27	72.06
Grand mean	65.13		59.16		67.77		72.16	

TABLE II
Alpha-amylase Activity 20° DU (as is) by Malt-7C

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
9	67.0	66.0	67.0	67.0	72.0	70.0	78.9	82.9
10	69.1	63.6	68.5	67.0	76.6	74.9	81.1	88.1
11	44.0	43.9	42.4	42.0	48.8	48.4	85.0	88.1
12	57.4	60.8	57.5	56.4	67.8	64.0	90.4	99.7
13	50.7	49.9	50.0	48.8	51.8	50.9	…	…
8	37.6	38.8	38.8	39.9	43.5	43.4	86.1	90.4
Mean	54.30	53.83	54.03	53.52	60.08	58.60	84.30	89.78
Grand mean	54.07		53.78		59.34		87.04	

TABLE III
Statistical Summary of Results[a]

			Repeatability			Reproducibility		
Sample Pair	**No. of Labs**	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
KFCN								
A/B	7	65.13	2.32	3.6	6.51	3.25	5.0	9.10
C/D	7	59.16	1.66	2.8	4.64	2.72	4.6	7.61
E/F	7	67.77	0.67	1.0	1.89	2.06	3.0	5.76
G/H	7	72.27	1.55	2.1	4.33	2.33	3.2	6.54
Malt-7C								
A/B	6	54.07	2.09	3.9	5.85	11.92	22.0	33.36
C/D	6	53.78	0.68	1.3	0.91	12.19	22.7	34.12
E/F	6	59.34	0.95	1.6	2.67	13.28	22.4	37.19
G/H	5	78.26	1.82	2.6	5.08	5.40	7.7	15.11

[a] All calculations were made based on Tables I and II.

results are presented in Table II. Potential Outliers were identified using Dixon's ratio test (1), no data was excluded from statistical analysis as no deviations from experimental protocol were noted.

The statistical summary for the determination of alpha amylase by SFA using potassium ferricyanide and Malt-7C are presented in Table III. The repeatability and reproducibility coefficients of variation for the determination of alpha-amylase by SFA using potassium ferricyanide ranged from 1.0 to 3.6% and 3.0 to 5.0%, respectively, and were judged acceptable. Normalized data was not used as suggested from the second year of study (5) as the results were acceptable prior to normalization. The repeatability and reproducibility coefficients of variation for the determination of alpha-amylase by Malt-7C ranged from 1.3 to 3.9% and 6.2 to 22.7%, respectively. The results of the *t*-Test assuming unequal variances comparing alpha-amylase activity by SFA using potassium ferricyanide for detection and Malt-7C are presented in Table VII and there was no statistical difference.

TABLE IV
Comparison of Alpha-amylase Activity by Segmented Flow Analysis using Potassium Ferricyanide for Detection and Malt-7C Using the *t*-Test Assuming Unequal Variances[a]

Statistical Parameter	**Alpha Amylase**
Number of sample pairs, N	56
Mean of differences, D	3.52
Standard error of differences, S_D	2.60
Calculated t	1.35[b]
$t_{0.05}$	2.004

[a] Calculations based on (1).
[b] Not significant at the 95% confidence level.

LITERATURE CITED

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Differentiation of Ale and Lager Yeast Strains by Rapid X-α-Gal Analysis

Subcommittee members: W. Box, *chair*; D. Bendiak; L. Castonguay; S. Feliciano; T. Fischborn; B. Gibson; M Kuenker; B. Lodolo; M. Miller; S. Nicholls; F. Thiele; L. White and C. Powell (*ex officio*).

CONCLUSIONS

1. Of the three ale yeast strains tested, all were correctly identified by each collaborator after 0.5 h and results were confirmed after 24 h of incubation.
2. Of the three lager yeast strains analyzed, two were correctly identified by all collaborators after 0.5 h, and all three were correctly identified after 24 h of incubation by all collaborators except one.
3. All four of the in-house ale production strains analyzed were correctly identified as ale yeasts and gave no color change after 0.5 h of incubation or after 24 h of incubation.
4. Of the 19 in-house production lager strains analyzed, 17 were identified correctly after 0.5 h. The remaining two strains required longer than 0.5 h to give a positive reaction but were confirmed as lager yeast after 24 h of incubation.

RECOMMENDATIONS

1. The method evaluated can be used for the rapid differentiation of ale and lager yeast. For most yeast strains, an incubation time of 0.5 h is sufficient; however, a longer period may be required for certain lager strains in order to provide a definitive result.
2. It is proposed that the rapid X-α-gal technique be approved for inclusion in *Methods of Analysis* for the identification and differentiation of lager and ale strains.

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This was the first year of the subcommittee, formed to evaluate the use of the rapid X-α-gal technique for the differentiation of ale and lager yeast. Currently there are several means of differentiating between lager and ale yeast strains. These methods typically involve differentiation based on growth capacity under defined conditions, which is time consuming, or utilize molecular technology, which can be expensive and require a high level of expertise. The rapid X-α-gal method requires only general laboratory skills and aims to provide a result from growth in liquid media or from brewery samples after 0.5 h rather than 3–6 days on solid media as described previously (1). The rapid X-α-gal method is used to determine the capacity of yeast to cleave the melibiose homolog X-α-gal (5-bromo-4-chloro-3-indolyl-α-D-galactopyranoside), resulting in the development of a blue/green coloration derived from indol. Consequently, this method differentiates *Saccharomyces pastorianus* (lager) yeast, which are able to assimilate melibiose, from *S. cerevisiae* (ale) yeast, which cannot.

PROCEDURE

Six unknown yeast strains (three lager and three ale) and two known control strains (positive and negative) were sent to each collaborator. Collaborators were also provided with a sample of X-α-gal with which they were required to prepare a solution using the supplied 75% 1,2 propanediol. The prepared solution was stored in a light protected bottle at –20°C and allowed to attemperate to room temperature prior to use.

TABLE I
Lager/Ale Differentiation using the Rapid X-α-gal Technique after 0.5 h and 24 h of Incubation

				Test Yeast Strain					
Collaborator	**Incubation Time (h)**	**Positive Control**	**Negative Control**	**A**	**B**	**C**	**D**	**E**	**F**
1	0.5	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Ale
	24	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Lager
2	0.5	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Ale
	24	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Lager
3	0.5	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Ale
	24	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Lager
4	0.5	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Ale
	24	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Lager
5	0.5	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Ale
	24	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Lager
6	0.5	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Lager[a]
	24	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Lager
7	0.5	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Ale
	24	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Ale
8	0.5	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Ale
	24	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Lager
9	0.5	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Ale
	24	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Lager
10	0.5	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Lager
	24	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Lager
11	0.5	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Lager[a]
	24	Lager	Ale	Ale	Ale	Lager	Ale	Lager	Lager

[a] Noted as appearing very pale blue.

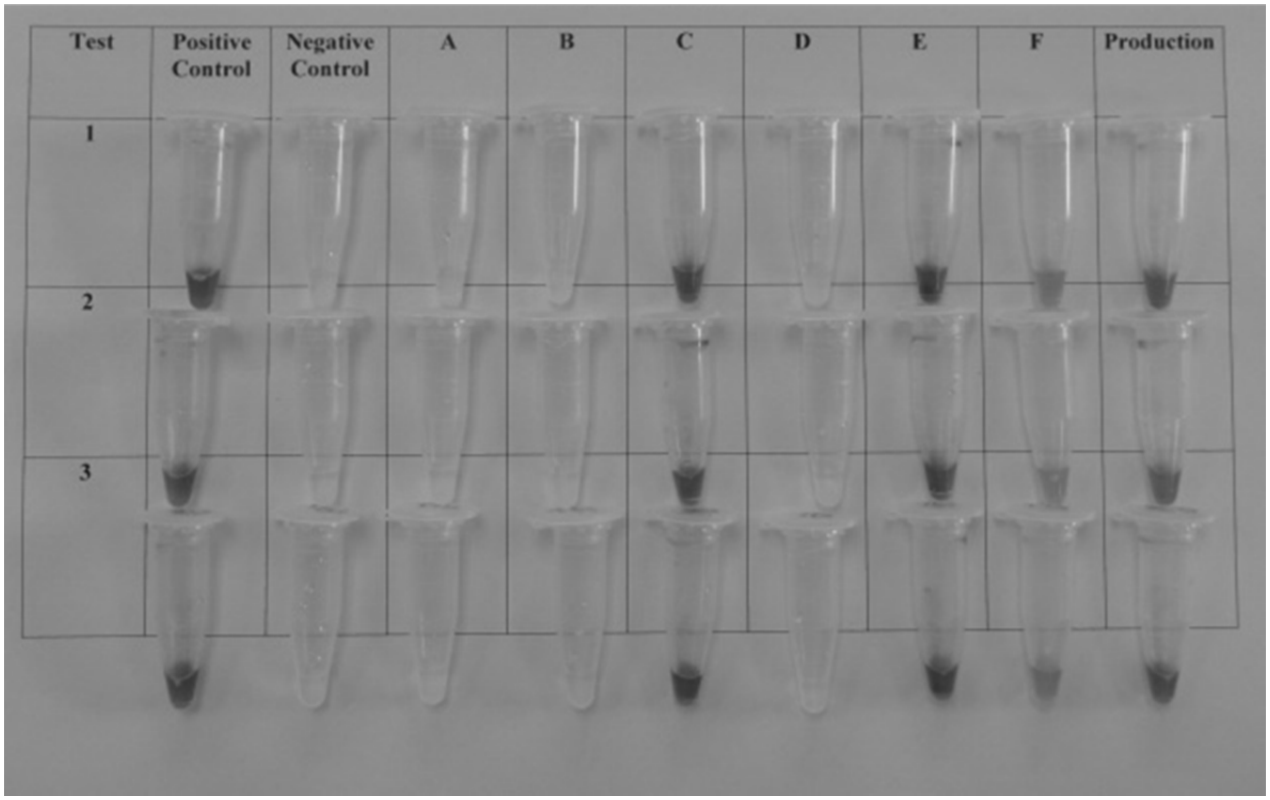

Fig. 1. Appearance of control samples, test strains, and production yeast after 3 hours of incubation at 27°C. Kindly provided by collaborator 4.

Each of the yeast cultures (control and test samples) was cultivated in liquid YPD growth medium at 27°C for 3 days. After incubation, the yeast was recovered and re-suspended in sterile distilled water at a final concentration of approximately 5×10^7 cells/mL, although a precise cell number was not required. This yeast suspension was used for the rapid X-α-gal test, which was performed by adding 100 µl of yeast suspension to each of three Eppendorf tubes containing X-α-gal solution. The tubes were vortexed to mix thoroughly and the samples were incubated at 27°C. For the negative control sample, 100 µl of yeast suspension was added to each of three empty Eppendorf tubes, and the tubes were vortexed and incubated at 27°C.

After 0.5 h of incubation, all tubes were inspected for color change. The appearance of a blue/green coloration indicated the presence of lager yeast, while no change in color (appearance remained cream/white) indicated ale yeast. All collaborators were required to score each of the unknown strains as lager/ale after 0.5 h and to confirm the results by observing each sample again after 24 h. In addition, each collaborator was encouraged to analyze an in-house production strain (ale, lager, or both) and to share the results obtained.

RESULTS AND DISCUSSION

The identities of the unknown test strains supplied were as follows: A, B, and D were ale yeasts while C, E, and F were lager yeasts. The positive control was a lager yeast strain and the negative control was an ale yeast strain.

Results were obtained from 11 collaborators. All 11 collaborators observed a color change (from cream/white to blue/green) after 0.5 h for the positive control, and the absence of a color change after 0.5 h for the negative control. The results for the negative control were confirmed by the absence of any color change after 24 h, while the positive control remained blue/green

TABLE II
Number of Collaborators Reporting Correct Strain Identification Using the Rapid X-α-gal Technique after 0.5 h and 24 h of Incubation

Sample	Number Tested	0.5 h	24 h
Positive control	11	11	11
Negative control	11	11	11
A (Ale)	11	11	11
B (Ale)	11	11	11
C (Lager)	11	11	11
D (Ale)	11	11	11
E (lager)	11	11	11
F (Lager)	11	3	10
Total	66	58	65

(Table I). Each of the 11 collaborators correctly identified the unknown test strains A, B, and D as ale yeast after 0.5 h and this was confirmed by the absence of a color change after 24 h (Table I). Test strains C and E were correctly identified as lager yeasts after 0.5 h by all 11 collaborators because of the formation of a blue/green coloration, which was observed to remain after 24 h (Table I). Test strain F was correctly identified as a lager yeast after 0.5 h by three of the collaborators (6, 10, and 11); however, for the majority of collaborators, it required longer than 0.5 h to achieve the correct identification (Table I). Furthermore, collaborator 7 did not correctly identify strain F as lager, even after 24 h. Interestingly, collaborators 6 and 11 reported that the blue/green coloration observed in strain F after 0.5 h was much paler than both the positive control and the other two unknown lager strains, C and E. This ambiguity with regard to color intensity may have led strain F to be incorrectly scored in several instances. It should be noted that collaborator 4 provided photographic evidence that in the case of strain F, the blue green coloration was not present after 0.5 h, but was observed after 3 h of incubation (Fig. 1). Collaborator 1 also conducted additional tests on each yeast strain by

TABLE III
Analysis of In-House Ale Production Strains by Collaborators Using the Rapid X-α-gal Technique

Collaborator	Number Tested	Correctly identified after 0.5 h	Correctly identified after 24 h
1	1	1	1
3	1	1	1
5	1	1	1
7	1	1	1
Total	4	4	4

TABLE IV
Analysis of In-House Lager Production Strains by Collaborators using the Rapid X-α-gal Technique

Collaborator	Number Tested	Correctly identified after 0.5 h	Correctly identified after 24 h
1	1	1	1
2	1	1	1
3	10	8[a]	10
4	1	1	1
5	1	1	1
6	1	1	1
8	1	1	1
10	2	2	2
11	1	1	1
Total	19	17	19

[a] Includes two samples of the same strain: one derived from agar slope and one after rehydration of an active dried yeast sample.

cultivation at 37°C. Strain F did not grow at this temperature and was therefore confirmed to be a lager yeast according to this method of differentiation. Irrespective, the data indicated that in the majority of instances (65 out of 66 analyses), unknown yeast strains were correctly identified as either ale or lager yeast (Table II).

In addition to the test cultures and the control strains supplied, each participant was encouraged to analyze an in-house production strain and to include this as experimental data. Ale production yeasts were analyzed by 4 collaborators only. In each instance these strains were indicated to be ale yeasts after 0.5 h, and were confirmed by the results obtained at 24 h (Table III). A total of 19 production lager yeasts were analyzed by 10 different collaborators. Of these 19 strains, 17 were confirmed as lager yeasts after 0.5 h and all 19 were confirmed as lager yeast after 24 h (Table IV). Interestingly, the lager production yeasts tested by collaborator 3 included two samples of the same strain: one sample from agar slope and one from rehydrated active dried yeast. The sample obtained from the agar slope was confirmed as lager yeast after 0.5 h whereas the rehydrated dried sample required a longer period of time, indicating that the physiological condition of a culture may influence the time required to observe a definitive color change.

When considering data from all of the strains examined (both test and production strains), each of the ale yeasts yielded a negative reaction that remained until at least 24 h of incubation. The majority of lager yeast samples examined (19 out of a total of 22 test and production strains) produced a positive reaction (a blue/green coloration) after 0.5 h and all except test strain F produced a positive reaction after 24 h of incubation. Although most lager yeasts in the trial were identified after 0.5 h of incubation, the data obtained indicated that for some strains, a longer period of incubation may be required for a definitive differentiation of ale and lager yeast. It is suggested that prior to routine use, brewing laboratories should test in-house cultures to determine if 0.5 h is sufficient for analysis of individual production strains or if a longer incubation time is required.

LITERATURE CITED

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Rapid Immunoassay Method for Deoxynivalenol Analysis in Barley and Malted Barley

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Keywords: DON, ELISA, HEIA, Vomitoxin

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the determination of deoxynivalenol (DON) in barley using the Diagnostix EZ-Tox HEIA kit for the study completed in 2011 ranged from 8.2 to 24.0% and 14.4 to 30.2%, respectively, and were judged acceptable.
2. Repeatability and reproducibility coefficients of variation for the determination of DON in barley using the Neogen Veratox ELISA kit for the study completed in 2011 ranged from 3.7 to 7.5% and 8.9 to 10.1%, respectively, and were judged acceptable.
3. Repeatability and reproducibility coefficients of variation for the determination of DON in malted barley using the Diagnostix EZ-Tox HEIA kit for the study completed in 2011 ranged from 8.7 to 16.8% and 15.8 to 26.4%, respectively, and were judged unacceptable.
4. Repeatability and reproducibility coefficients of variation for the determination of DON in malted barley using the Neogen Veratox ELISA kit for the study completed in 2011 ranged from 6.0 to 16.7% and 18.9 to 23.8%, respectively, and were judged unacceptable.
5. Based on the *t*-Test assuming unequal variances, Malt-13 and the Diagnostix EZ-Tox HEIA kit were not significantly different at the 95% confidence level for barley but were significantly different for malted barley in the study completed in 2011.
6. Based on the *t*-Test assuming unequal variances, Malt-13 and the Neogen Veratox ELISA kit were not significantly different at the 95% confidence level for barley but were significantly different for malted barley in the study completed in 2011.
7. Repeatability and reproducibility coefficients of variation for the determination of DON in malted barley using the Diagnostix EZ-Tox HEIA kit for the study completed in 2012 ranged from 8.1 to 27.4% and 7.9 to 27.2%, respectively, and were judged unacceptable.
8. Repeatability and reproducibility coefficients of variation for the determination of DON in malted barley using the Neogen Veratox ELISA kit for the study completed in 2012 ranged from 3.6 to 22.7% and 7.5 to 18.1%, respectively, and were judged unacceptable.
9. Based on the *t*-Test assuming unequal variances, Malt-13 and the Diagnostix EZ-Tox HEIA kit were significantly different at the 95% confidence level for the study completed in 2012.
10. Based on the *t*-Test assuming unequal variances, Malt-13 and the Neogen Veratox ELISA kit were significantly different at the 95% confidence level for the study completed in 2012.

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RECOMMENDATIONS

1. From the study completed in 2011 the subcommittee recommended repeating the malted barley portion of this study for a third year with more homogeneous malt samples that possess DON levels in the desirable range.
2. From the study completed in 2011 the subcommittee recommended that the method for DON in barley for both Diagnostix EZ-Tox HEIA kit and Neogen Veratox ELISA kit no further testing was recommended.
3. From the study completed in 2012 the subcommittee recommends taking time to evaluate the methods with the manufacturers before pursuing another year of study.
4. The subcommittee does not recommend the use of this method for either barley or malted barley at this time.

This was the third year of this subcommittee's evaluation of the use of rapid immunoassay methods for determining deoxynivalenol (DON) in malted barley. This report will cover the subcommittee's evaluation for year 2 and year 3 of the rapid immunoassay methods for DON analysis in barley and malted barley. In its first year, the method produced unacceptable repeatability and reproducibility coefficients of variation for both the Diagnostix EZ-Tox homogeneous enzyme immunoassay (HEIA) kit and Neogen Veratox enzyme-linked immunosorbent assay (ELISA) kit (2). In the first year there were not enough Malt-13 collaborators to do a *t*-Test comparison of the methods with the standard reference method (1). The subcommittee recommended that the collaborative be repeated with additional collaborators and to add barley to the collaborative. It was also recommended that the collaborative be repeated with samples that were more in the range of what is typically seen in the industry.

In the second year of study, the method produced unacceptable repeatability and reproducibility coefficients of variation for both the Diagnostix EZ-Tox HEIA kit and the Neogen Veratox ELISA kit for malted barley. However, at the time of study both kits produced acceptable repeatability and reproducibility coefficients of variation for barley. The subcommittee recommended that the malted barley portion of the study be repeated with more homogenous malt samples that possess DON levels in the desirable range. No further testing was recommended for the barley portion of the study. However, in light of the results for the 2012 study on malted barley, it was recommended that barley not be accepted until further testing could be done and the methods could be evaluated with the manufacturers.

PROCEDURE

For the study completed in 2011, collaborators were provided a total of 12 samples for testing, six samples of barley representing sample pairs 1/2 through 5/6 and six samples of malted barley representing sample pairs A/B through E/F. Sample pairs were chosen to represent barley and malted barley with varying levels of DON, ideally ranging from 0.5 to 2.0 mg/L. Barley samples were prepared using a FOSS Cyclotec 1093 (Barley-4) and malt samples were prepared using a Buhler DFLU disc mill with settings determined by method Malt-4 for fine grind. Ground samples were sealed to prevent moisture gain and sent to each collab-

TABLE I
Diagnostix EZ-Tox Homogeneous Enzyme Immunoassay Method for Detecting Deoxynivalenol (mg/L) in Barley 2011

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**1**	**2**	**3**	**4**	**5**	**6**
D1	3.68	3.42	0.35	0.31	1.03	1.16
D2	3.37	3.37	0.43	0.47	0.93	1.04
D3	3.60	2.86	0.34	0.54	1.07	1.13
D4	3.00	3.00	0.44	0.97	1.26	1.30
D5	2.29	2.22	0.21	0.48	0.87	0.84
D6	4.04	3.05	0.29	0.47	0.87	1.01
D7	3.97	3.38	0.40	0.55	0.65	1.04
D8	3.21	3.31	0.42	0.61	1.03	1.07
D9	3.67	3.29	0.45	0.65	0.93	1.05
Mean	3.424	3.097	0.368	0.559	0.958	1.069
Grand mean	3.261		0.464		1.014	

TABLE II
Neogen Veratox Enzyme-Linked Immunosorbent Assay Method for Detecting Deoxynivalenol (mg/L) in Barley 2011

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**1**	**2**	**3**	**4**	**5**	**6**
N1	3.58	2.97	0.43	0.63	1.12	1.25
N2	3.04	2.67	0.37	0.49	0.92	1.09
N3	2.75	2.50	0.45	0.60	1.00	1.20
N4	3.45	2.70	0.40	0.50	0.95	1.05
N5	3.50	2.65	0.40	0.55	0.90	1.00
N6	3.00	2.25	0.45	0.50	0.95	1.00
Mean	3.220	2.622	0.416	0.545	0.974	1.098
Grand mean	2.921		0.481		1.036	

TABLE III
GC Assay Method (Malt-13) for Detecting Deoxynivalenol (mg/L) in Barley 2011

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**1**	**2**	**3**	**4**	**5**	**6**
G1	2.55	1.80	0.39	0.43	0.84	0.82
G2	2.45	1.80	0.38	0.43	0.86	0.76
G3	2.86	2.80	0.29	0.50	0.98	0.96
G4	3.15	2.54	0.44	0.67	1.13	1.22
G5	3.50	2.80	0.40	0.59	0.98	1.03
Mean	2.902	2.347	0.379	0.522	0.956	0.957
Grand mean	2.625		0.451		0.957	

TABLE IV
Diagnostix EZ-Tox Homogeneous Enzyme Immunoassay Method for Detecting Deoxynivalenol (mg/L) in Malted Barley 2011

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
D1	0.11	0.26	0.51	0.35	1.64	0.96
D2	0.29	0.34	0.51	0.47	1.42	1.04
D3	0.22	0.37	0.60	0.50	1.66	1.10
D4	0.15	0.32	0.78	0.43	1.95	0.93
D5	0.19	0.30	0.39	0.46	1.16	0.74
D6	0.15	0.28	0.60	0.49	1.79	1.21
D7	0.29	0.38	0.59	0.35	1.62	1.05
D8	0.18	0.31	0.60	0.42	1.25	0.93
D9	0.34	0.49	0.62	0.55	1.67	0.98
Mean	0.211	0.337	0.575	0.444	1.572	0.991
Grand mean	0.274		0.509		1.281	

TABLE V
Neogen Veratox Enzyme-Linked Immunosorbent Assay Method for Detecting Deoxynivalenol (mg/L) in Malted Barley 2011

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
N1	0.37	0.51	0.91	0.72	2.17	1.60
N2	0.36	0.39	0.66	0.53	1.71	1.47
N3	0.40	0.55	0.95	0.80	1.85	2.25
N4	0.30	0.30	0.80	0.50	2.00	1.35
N5	0.35	0.55	0.85	0.70	2.05	1.45
N6	0.25	0.30	0.65	0.45	1.55	1.00
Mean	0.337	0.433	0.803	0.616	1.888	1.519
Grand mean	0.385		0.709		1.704	

TABLE VI
GC Assay Method (Malt-13) for Detecting Deoxynivalenol (mg/L) in Malted Barley 2011

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
G1	0.26	0.33	0.65	0.52	0.96	0.76
G2	0.25	0.32	0.59	0.54	0.97	0.75
G3	0.18	0.22	0.32	0.52	0.91	0.64
G4	0.15	0.22	0.40	0.36	0.93	0.64
G5	0.18	0.27	0.39	0.35	0.93	0.70
Mean	0.201	0.269	0.469	0.457	0.939	0.696
Grand mean	0.235		0.463		0.818	

orator in January 2011. Barley sample pair 1/2 was obtained from 6-row barley from different lots, variety tradition. Sample pair 3/4 was obtained from 2-row barley and sample pair 5/6 was obtained from 6-row barley. Due to the extreme difficulty in obtaining malted barley with detectable DON levels this crop year, it was necessary to blend 2-row malted barley with higher DON content into 2-row malted barley with lower DON content in order to obtain each of the 3 pairs of malted barley samples; A/B, C/D and E/F .

For the study completed 2012 collaborators were provided a total of 10 samples for testing, samples of malted barley representing sample pairs 1/2 through 9/10. Sample pairs were chosen to represent malted barley with varying levels of DON, ideally ranging from 0.5 to 3.0 mg/L. Malted barley samples were prepared using a Buhler DFLU disc mill with settings determined by method Malt-4 (1) for fine grind. Ground samples were sealed to prevent moisture gain and sent to each collaborator in January 2012. All five sample pairs were obtained from 6-row malted barley. Sample pairs 1/2 and 7/8 were obtained from different lots of 6-row Lacey malted barley from the 2011 crop year. Sample pairs 3/4, 5/6, and 9/10 were obtained from different lots of Tradition malted barley from the 2010 and 2011 crop. Results were evaluated using the Youden unit block design (1) and the *t*-Test assuming unequal variances at the 95% confidence level.

RESULTS AND DISCUSSION

In the study completed in 2011, results for the Diagnostix EZ-Tox HEIA kit were received from nine collaborators for sample pairs 1/2, 3/4, 5/6, A/B, C/D, and E/F; six collaborators reported results for the Neogen Veratox ELISA kit; and five collaborators reported results for the Barley-11 and Malt-13 methods. Results for one Neogen participant were found to be significantly different from other participants and the cause was isolated. The lab used a higher sample to water ratio than the other Neogen collaborators and upon consideration, it was deemed acceptable to factor the dilution into those results for further evaluation. The results for the EZ-Tox, Veratox and GC methods are summarized for barley in Tables I, II and III, respectively and for malted barley in tables IV, V and VI, respectively. Outliers were determined using Dixon's ratio test (1); however, no outliers were removed from statistical analysis. Outliers were not excluded because of

TABLE VII
Statistical Summary of Results for Rapid Immunoassay Methods 2011– Barley[a]

			Repeatability			Reproducibility		
Sample Pair	**# of Labs**	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
EZ-Tox								
1/2	9	3.261	0.268	8.2	0.751	0.470	14.4	1.316
3/4	9	0.464	0.111	24.0	0.311	0.140	30.2	0.392
5/6	9	1.014	0.084	8.2	0.234	0.149	14.7	0.418
Neogen								
1/2	6	2.851	0.187	6.6	0.524	0.261	9.2	0.731
3/4	6	0.481	0.036	7.5	0.101	0.048	10.1	0.136
5/6	6	1.036	0.038	3.7	0.107	0.093	8.9	0.259
GC								
1/2	5	2.625	0.199	7.6	0.557	0.473	18.0	1.326
3/4	5	0.451	0.064	14.2	0.179	0.083	18.5	0.233
5/6	5	0.957	0.052	5.4	0.144	0.154	16.1	0.432

[a] All calculations were made based on Tables I-III.

TABLE VIII
Statistical Summary of Results for Rapid Immunoassay Methods 2011– Malted Barley[a]

			Repeatability			Reproducibility		
Sample Pair	**# of Labs**	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
EZ-Tox								
A/B	9	0.274	0.024	8.7	0.067	0.072	26.4	0.202
C/D	9	0.509	0.086	16.8	0.240	0.089	17.5	0.249
E/F	9	1.281	0.148	11.6	0.415	0.202	15.8	0.565
Neogen								
A/B	6	0.385	0.055	14.4	0.155	0.092	23.8	0.257
C/D	6	0.709	0.043	6.0	0.120	0.134	18.9	0.375
E/F	6	1.704	0.285	16.7	0.798	0.333	19.6	0.934
GC								
A/B	5	0.235	0.013	5.4	0.035	0.049	21.0	0.138
C/D	5	0.463	0.088	19.0	0.246	0.121	26.2	0.340
E/F	5	0.818	0.025	3.1	0.071	0.044	5.4	0.124

[a] All calculations were made based on Tables IV-VI.

TABLE IX
Comparison of Diagnostix EZ-Tox HEIA and Gas Chromatography for the Determination of Deoxynivalenol (mg/L) 2011. Using the *t*-Test Assuming Unequal Variances[a]

Statistical Parameter	**Barley**	**Malted Barley**
Number of results, *N*	84	84
Calculated *t*	0.946[b]	2.248[c]
$t_{0.05}$	1.993	2.026

[a] All calculations were made based on (1).
[b] Not significant at the 95% confidence level.
[c] Significant at the 95% confidence level.

TABLE X
Comparison of Diagnostix EZ-Tox HEIA and Gas Chromatography for the Determination of Deoxynivalenol (mg/L) 2011. Using the *t*-Test Assuming Unequal Variances[a]

Statistical Parameter	**Barley**	**Malted Barley**
Number of results, *N*	66	66
Calculated *t*	0.524[b]	3.760[c]
$T_{0.05}$	1.998	2.011

[a] All calculations were made based on (1).
[b] Not significant at the 95% confidence level.
[c] Significant at the 95% confidence level.

the low values that were observed and no known deviations from protocols were noted.

The statistical summary of the DON data for the Diagnostix HEIA kit, Veratox ELISA kit and GC methods are represented in Table VII for barley and Table VIII for malt. In the three barley pairs, there were increased coefficients of variation in both repeatability and reproducibility for the lowest concentration sample pair. The three malted barley pairs had higher coefficients of variation which may have been the result of the sample preparation for the malted barley samples in this study. For future study it is recommended that malted barley samples be obtained at the necessary concentrations rather than blending malted barley with extreme concentration differences.

The results of the *t*-Test assuming unequal variances are presented in Tables IX for barley and Tables X for malted barley. The *t*-Test assuming unequal variances was used for comparison of the different methods due to the different number of collaborators for each method. Based on the *t*-Test assuming unequal variances, Barley-11 and the Diagnostix EZ-Tox HEIA kit were not significantly different at the 95% confidence level. Based on the *t*-Test assuming unequal variances, Barley-11 and the Neogen Veratox ELISA kit were not significantly different at the 95% confidence level. Based on the *t*-Test assuming unequal variances, Malt-13 and the Diagnostix EZ-Tox HEIA kit were significantly different at the 95% confidence level. Based on the *t*-Test assuming unequal variances, Malt-13 and the Neogen Veratox ELISA kit were significantly different at the 95% confidence level. It was recommended that the malted barley portion of this study be repeated for a third year with more homogeneous malted barley samples that possess DON levels in the desirable 0.5 to 2.0 mg/L range.

For the study completed in 2012, results for the Diagnostix EZ-Tox HEIA kit were received from nine collaborators for sample

TABLE XI
Deoxynivalenol (mg/L) in Malted Barley by Diagnostix Ez-tox Homogeneous Enzyme Immunosassay 2012

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
D1	1.59	0.84	4.82	2.69	1.70	1.32	0.56	0.50	0.79	0.98
D2	1.40	0.90	4.00	4.10	3.50	1.00	0.50	0.60	0.80	1.00
D3	1.31	1.37	3.21	3.58	2.77	1.20	0.54	0.46	0.80	0.91
D4	1.21	1.02	3.00	3.00	1.68	1.15	0.58	0.51	0.69	0.74
D5	1.34	0.79	3.04	2.57	1.94	1.11	0.56	0.54	0.81	0.75
D6	1.13	0.98	3.68	4.15	2.54	2.38	0.50	0.53	0.86	0.78
D7	1.50	0.92	3.67	3.00	2.33	1.10	0.49	0.51	0.91	0.71
D8	1.32	0.89	3.27	3.63	2.48	1.08	0.51	0.45	0.81	0.67
D9	2.20	1.10	4.42	4.39	2.60	1.40	1.29	1.02	0.96	0.87
Mean	1.444	0.979	3.682	3.457	2.393	1.304	0.614	0.569	0.826	0.822
Grand mean	1.212		3.569		1.849		0.592		0.821	

TABLE XII
Deoxynivalenol (mg/L) in Malted Barley by Neogen Veratox Enzyme-linked Immunosorbent Assay 2012

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
N1	1.90	1.45	4.63	4.53	4.25	1.96	1.04	0.92	1.41	1.50
N2	2.20	1.40	4.60	4.70	3.50	1.90	1.20	1.00	1.40	1.50
N3	1.90	1.40	4.70	4.50	3.80	1.70	0.80	0.90	1.30	1.60
N4	1.88	1.19	4.84	4.31	2.70	1.76	0.92	0.74	1.25	0.88
N5	2.80	1.00	4.00	3.80	3.40	1.60	0.80	1.00	1.40	1.60
Mean	2.136	1.288	4.554	4.368	3.530	1.784	0.952	0.912	1.352	1.416
Grand mean	1.712		4.461		2.657		0.932		1.384	

TABLE XIII
Deoxynivalenol (mg/L) in Malted Barley by Gas Chromatography 2012

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
G1	0.75	0.53	2.13	1.95	1.68	0.68	0.41	0.39	0.47	0.54
G2	0.85	0.72	2.00	2.94	2.07	0.70	0.46	0.39	0.61	0.59
G3	0.78	0.73	2.21	2.88	1.46	0.55	0.40	0.32	0.46	0.58
G4	0.89	0.58	2.13	2.35	1.70	0.80	0.38	0.33	0.51	0.49
Mean	0.818	0.640	2.118	2.530	1.728	0.683	0.413	0.358	0.513	0.550
Grand mean	0.729		2.324		1.206		0.386		0.532	

TABLE XIV
Statistical Summary of Results for Rapid Immunoassay Methods 2012[a]

			Repeatability			Reproducibility		
Sample Pair	**# of Labs**	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
EZ-Tox								
1 & 2	9	1.212	0.245	20.2	0.685	0.255	21.0	0.713
3 & 4	9	3.569	0.572	16.0	1.600	0.651	18.2	1.823
5 & 6	9	1.849	0.506	27.4	1.417	0.504	27.2	1.411
7 & 8	9	0.592	0.073	12.3	0.203	0.219	37.0	0.613
9 & 10	9	0.824	0.103	12.5	0.289	0.101	12.3	0.283
Neogen								
1 & 2	5	1.712	0.389	22.7	1.090	0.309	18.1	0.866
3 & 4	5	4.461	0.161	3.6	0.451	0.335	7.5	0.938
5 & 6	5	2.657	0.370	13.9	1.036	0.416	15.7	1.165
7 & 8	5	0.932	0.127	13.6	0.355	0.142	15.3	0.398
9 & 10	5	1.384	0.182	13.1	0.509	0.221	16.0	0.618
GC								
1 & 2	4	0.729	0.079	10.9	0.222	0.084	11.5	0.236
3 & 4	4	2.324	0.349	15.0	0.978	0.337	14.5	0.944
5 & 6	4	1.206	0.156	13.0	0.438	0.193	16.0	0.540
7 & 8	4	0.386	0.019	4.9	0.052	0.036	9.3	0.101
9 & 10	4	0.532	0.049	9.2	0.138	0.058	10.9	0.163

[a] All calculations were made based on Tables XI–XIII.

TABLE XV
Comparison of Diagnostix EZ-Tox HEIA and Gas Chromatography for the Determination of Deoxynivalenol (mg/L) in Malted Barley 2012. Using the *t*-Test Assuming Unequal Variances[a]

Statistical Parameter	Diagnostix HEIA
Number of results, *N*	90
Calculated *t*	3.340[b]
$t_{0.05}$	1.986

[a] All calculations were made based on (1).
[b] Significant at the 95% confidence level.

TABLE XVI
Comparison of Neogen Veratox ELISA and Gas Chromatography for the Determination of Deoxynivalenol (mg/L) in Malt 2012. Using the *t*-Test Assuming Unequal Variances[a]

Statistical Parameter	Neogen Veratox ELISA
Number of results, *N*	50
Calculated *t*	4.200[b]
$T_{0.05}$	2.008

[a] All calculations were made based on (1).
[b] Significant at the 95% confidence level.

pairs 1/2, 3/4, 5/6, 7/8, 9/10; five collaborators reported results for the Neogen Veratox ELISA kit; and four collaborators reported results for the Malt-13 method. The results for EZ-Tox, Veratox and GC methods are summarized in Tables XI, XII, and XIII, respectively. Outliers were determined using Dixon's ratio test (1); however, no outliers were removed from statistical analysis. Outliers were not excluded because of the low values that were observed and no known deviations from protocols were noted.

The statistical summary of the DON data for the Diagnostix HEIA kit, Neogen Veratox ELISA kit and GC methods are represented in Table XIV.

The results of the *t*-Test assuming unequal variances are presented in Table XV and XVI. The *t*-Test assuming unequal variances was used for comparison of the different methods due to the different number of collaborators for each method. Based on the *t*-Test assuming unequal variances, Malt-13 and the Diagnostix EZ-Tox HEIA kit were significantly different at the 95% confidence level. Based on the *t*-Test assuming unequal variances, Malt-13 and the Neogen Veratox ELISA kit were significantly different at the 95% confidence level.

The subcommittee recommended that the rapid immunoassay method for DON in barley and malted barley for both Diagnostix EZ-Tox HEIA and Neogen Veratox ELISA be put on hold to further evaluate the methods with the manufacturers. It is recommended that the rapid immunoassay methods for DON not be used in either barley or malted barley analysis.

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Headspace Gas Chromatography-Flame Ionization Detector Analysis for Beer Volatiles

Subcommittee members: J. Palausky, *chair*; F. Castañé (EBC); M. Christopherson; A. Froeschner; J. Olšovská; M. Qian; L. Torres; and K. Lakenburges (*ex officio*).

Keywords: Alcohol, Aldehyde, Ester, FID, GC

CONCLUSIONS

1. Repeatability coefficients of variation for the determination of acetaldehyde by headspace GC-FID ranged from 4.9 to 17% and were judged acceptable.
2. Reproducibility coefficients of variation for the determination of acetaldehyde by headspace GC-FID ranged from 7.3 to 22% and were judged unacceptable.
3. Repeatability and reproducibility coefficients of variation for the determination of ethyl acetate by headspace GC-FID ranged from 3.6 to 5.4% and from 8.0 to 10%, respectively, and were judged acceptable.
4. Repeatability coefficients of variation for the determination of isoamyl acetate by headspace GC-FID ranged from 3.5 to 7.3% and were judged acceptable.
5. Reproducibility coefficients of variation for the determination of isoamyl acetate by headspace GC-FID ranged from 11 to 24% and were judged unacceptable.
6. Repeatability and reproducibility coefficients of variation for the determination of isoamyl alcohol by headspace GC-FID ranged from 2.6 to 4.2% and from 6.9 to 8.1%, respectively, and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends accepting the method as a provisional method based on the acceptable repeatability but unacceptable reproducibility and include in the provisional section of *Methods of Analysis*.
2. Discharge the subcommittee.

http://dx.doi.org/10.1094/ASBCJ-2012-1101-05

This was the third year of this subcommittee's existence. Based on polling by the subcommittee for Coordination of New and Alternative Methods of Analysis (1), this subcommittee was formed to evaluate the applicability of headspace gas chromatography-flame ionization detector (GC-FID) analysis for the determination of volatile organic compounds in beer. In the first year,

TABLE I
Acetaldehyde (mg/L) in Beer by Headspace Gas Chromatography- Flame Ionization Detector

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
1	2.5	2.1	4.2	4.8	2.2	2.3
2	2.7	2.1	4.5	5.1	1.6	2.9
3	3.5[a]	3.1[a]	4.9[a]	5.6[a]	2.6[a]	4.4[a]
4	2.4	2.2	3.5	3.6	2.3	3.2
5	2.4	2.1	3.5	4.4	2.4	4.1
6	2.6	2.3	3.7	4.2	1.6	2.8
7	2.7	2.6	4.1	5.0	1.3	3.0
8[b]	…	…	…	…	…	…
Mean[c]	2.55	2.24	3.90	4.52	1.92	3.06
Grand mean[c]	2.39		4.21		2.49	

[a] Outlier at $P \leq 0.05$ based on totals and/or differences (1).
[b] Data excluded due to known deviation from protocol.
[c] Calculated excluding outliers.

TABLE II
Ethyl Acetate (mg/L) in Beer by Headspace Gas Chromatography- Flame Ionization Detector

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
1	20.3	18.8	34.0[a]	33.6[a]	12.6	15.7
2	24.0[a]	24.9[a]	35.7[a]	35.0[a]	16.3[a]	19.0[a]
3	18.9	19.5	27.1	26.5	12.7	14.9
4	17.2	16.7	21.2	20.0	10.7	12.4
5	16.9	16.5	23.7	24.8	11.1	13.5
6	17.4	17.1	23.7	24.1	11.4	13.1
7	15.9	18.4	24.8	26.7	13.0	14.0
8[b]	…	…	…	…	…	…
Mean[c]	17.77	17.82	24.10	24.41	11.92	13.92
Grand mean[c]	17.80		24.25		12.92	

[a] Outlier at $P \leq 0.05$ based on totals and/or differences (1).
[b] Data excluded due to known deviation from protocol.
[c] Calculated excluding outliers.

TABLE III
Isoamyl Acetate (mg/L) in Beer by Headspace Gas Chromatography- Flame Ionization Detector

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
1	2.2	2.1	4.2[a]	4.4[a]	1.0	1.4
2	2.3	2.5	3.6	3.7	1.1	1.4
3	2.1	2.4	3.3	3.5	1.1	1.3
4	1.9	1.9	2.5	2.5	0.9	1.0
5	1.3[a]	1.4[a]	2.0	2.2	0.7[a]	0.8[a]
6	1.4[a]	1.5[a]	2.0	2.2	0.7[a]	0.8[a]
7	1.7	2.1	2.8	3.2	1.0	1.1
8[b]	…	…	…	…	…	…
Mean[c]	2.05	2.21	2.70	2.87	1.02	1.24
Grand mean[c]	2.13		2.79		1.13	

[a] Outlier at $P \leq 0.05$ based on totals and/or differences (1).
[b] Data excluded due to known deviation from protocol.
[c] Calculated excluding outliers.

TABLE IV
Isoamyl Alcohol (mg/L) in Beer by Headspace Gas Chromatography- Flame Ionization Detector

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
1	49.1	48.0	58.2	60.0	48.9	56.7
2	57.3	60.1	61.0	62.3	60.4	65.4
3	54.5	58.4	58.7	61.4	61.1	65.3
4	51.9	51.0	49.2	49.6	53.2	58.0
5	56.5	55.5	56.9	62.0	58.0	63.3
6	54.7	53.6	54.6	57.3	56.3	59.6
7	51.7	58.6	57.7	64.0	66.3	66.4
8[a]	…	…	…	…	…	…
Mean	53.68	55.02	56.62	59.52	57.82	62.10
Grand mean	54.35		58.07		59.96	

[a] Data excluded due to known deviation from protocol.

collaborative analysis showed unacceptable repeatability for two of four compounds tested and unacceptable reproducibility for three of four compounds tested (2). Following ruggedness analysis, minor modifications were made to the sampling portion of the collaborative protocol. In the second year, collaborative analysis showed acceptable repeatability for all compounds tested but unacceptable reproducibility for three of four compounds tested (3). In the third year, a check sample containing known concentrations of the four tested compounds was provided along with the beer samples to provide additional information on potential bias due to calibration standard preparation.

PROCEDURE

Three sample pairs of commercial beers were sent to each collaborator. Each pair was of the same brand but from different production times. All sample pairs were commercially available lager beers selected to cover a range of volatile concentrations. Calibration was accomplished by standard additions of volatiles with 1-butanol as an internal standard. Results were evaluated using the Youden unit block design (4).

RESULTS AND DISCUSSION

Results from eight collaborators were received for the three sample pairs. Results for one collaborator were excluded prior to statistical analyses because of known deviations from the prescribed experimental protocol. Therefore, seven data sets were used for statistical analysis. Data for acetaldehyde, ethyl acetate, isoamyl acetate, and isoamyl alcohol are presented in Tables I through IV, respectively. Outliers were identified using Dixon's ratio test (4). The statistical summary of the volatile data are shown in Table V.

The repeatability coefficients of variation were judged acceptable for all compounds tested with the exception of acetaldehyde in sample set E/F. With inclusion of sample set (E/F) for acetaldehyde, the repeatability coefficient was 4.9 to 17%. The repeatability coefficients of variation for ethyl acetate, isoamyl acetate, and isoamyl alcohol ranged from 3.6 to 5.4%, 3.5 to 7.3%, and 2.6 to 4.2%, respectively.

The reproducibility coefficients of variation were judged acceptable for two of four compounds tested. The reproducibility coefficients of variation for ethyl acetate and isoamyl alcohol ranged from 8.0 to 10%, and 6.9 to 8.1%, respectively and were judged acceptable. The reproducibility coefficients of variation for acetaldehyde and isoamyl acetate ranged from 7.3 to 22% and 11 to 24%, respectively and were judged unacceptable.

A check sample prepared at known concentrations for all target compounds in a 5% by volume ethanol/water solution was provided with collaborative samples. The data for the check sample (see Table VI) was evaluated to look for differences in collaborators calibration curves for each compound. For example, the results for ethyl acetate from Collaborator 2 are all biased high in relation to the rest of the data set indicating that the prepared standard and/or calibration curve used for quantification of beer samples may have been biased. The check standard result for ethyl acetate from Collaborator 2 also shows a high bias (121%) which confirms the potential bias.

The current data shows acceptable repeatability and marginal reproducibility. The difference in the inter-laboratory results is

TABLE V
Statistical Summary of Results[a]

				Repeatability			Reproducibility		
Compound	**Sample Pair**	**# of Labs**	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Acetaldehyde									
	A–B	6	2.39	0.12	4.9	0.33	0.17	7.3	0.49
	C–D	6	4.21	0.21	5.1	0.60	0.52	12	1.44
	E–F	6	2.49	0.42	17	1.17	0.54	22	1.50
Ethyl acetate									
	A–B	6	17.80	0.97	5.4	2.71	1.42	8.0	3.97
	C–D	5	24.25	0.88	3.6	2.46	2.43	10	6.81
	E–F	6	12.92	0.50	3.9	1.39	1.09	8.4	3.10
Isoamyl acetate									
	A–B	5	2.13	0.16	7.3	0.44	0.25	12	0.70
	C–D	6	2.79	0.10	3.5	0.27	0.67	24	1.87
	E–F	5	1.13	0.05	4.5	0.14	0.13	11	0.36
Isoamyl alcohol									
	A–B	7	54.35	2.26	4.2	6.32	3.75	6.9	10.49
	C–D	7	58.07	1.48	2.6	4.16	4.35	7.5	12.19
	E–F	7	59.96	1.70	2.8	4.77	4.88	8.1	13.66

[a] All calculations were made based on Tables I through IV.

TABLE VI
Check Sample Results (Known Solution)

Collaborator	**Acetaldehyde (mg/L)**	**Ethyl Acetate (mg/L)**	**Isoamyl Acetate (mg/L)**	**Isoamyl Alcohol (mg/L)**
1	4.3	8.3	0.84	35.4
2	4.8	9.7	0.79	37.7
3	5.4	8.5	0.86	40.0
4	3.7	7.7	0.76	38.6
5	4.2	8.7	0.70	40.9
6	4.0	7.3	0.57	35.9
7	5.3	8.4	0.84	43.0
Certified concentration	4.0	8.0	0.81	40.0

most likely attributable to difficulties in the preparation of calibration standards or unfamiliarity in handling volatile chemicals. Analysts must use care in dealing with the volatile chemicals as the compounds can evaporate (i.e., volatilize) quickly when opened. Standard preparations should be performed by a trained analyst and be performed both accurately and quickly to reduce potential errors. It is recommended to include the use of a second-source or certified reference material check standard to establish the accuracy of the calibration curve for each compound prior to analysis and validate the standard preparation.

ACKNOWLEDGMENTS

This is the third year of this subcommittee and many individuals and organizations have donated both time and resources toward the collaborative analyses performed over the years. The Chair would like to acknowledge the assistance of those that could not participate this year but have provided valuable input for the subcommittee including: M. Aistrope, C. Brodie, L. Chadwick, V. Kellner, R. Ortiz, and K. Taylor.

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Viscosity of Wort by Rolling Ball Viscometer

Subcommittee members: A. MacLeod, *chair*; A. Caruso; M. Fogarty; S. Harasymow; T. Henderson; R. Joy; C. Martens; A. Miller, G. Olscamp; A. Price; K. Price ; B. Stephans; A. Stern; and R. Jennings (*ex officio*).

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for viscosity of wort by rolling ball viscometer ranged from 0.4 to 1.3% and 1.4 to 4.1%, respectively, and were judged acceptable.
2. Based on the F-test for variances, no significant difference was found in reproducibility between the rolling ball and reference method at the 95% confidence level at viscosity levels normally encountered in routine malt quality testing.
3. Based on analysis of variance, the rolling ball method was not significantly different from the reference method at the 95% confidence level.

RECOMMENDATIONS

1. The subcommittee recommends viscosity of wort by rolling ball viscometer be included in *Methods of Analysis.*
2. Discharge the subcommittee.

This is the subcommittee's first year of existence started on the recommendation of the subcommittee for *Methods of Analysis* Wort Review (2). On the basis of polling by the subcommittee for Coordination of New and Alternate Methods of Analysis it was found that many malt labs employ an automated instrument using the rolling ball principle for determination of wort viscosity, rather than the traditional glass capillary tube, and there was sufficient interest for collaborative testing. A collaborative test was required to determine repeatability and reproducibility coefficients of variation for the new method prior to inclusion in the ASBC *Methods of Analysis*.

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PROCEDURE

A total of six malted barley samples (three sample pairs) were sent to each collaborator with a range of wort viscosity levels. Sample pairs represented three different lots of two rowed barley which were malted on separate days. For each malt sample, collaborators were instructed to prepare a congress wort using their own mashing apparatus and determine wort density by Malt-4. Wort viscosity was determined using a suitable rolling ball viscometer and by the reference method, Wort-13 (1), using a suitable Cannon-Fenske or Ubbelohde type glass capillary tube if available. Results were evaluated using the Youden unit block design (1) and means were compared using the analysis of variance procedure of SAS 9.1 (SAS Institute) statistical software package.

RESULTS AND DISCUSSION

Thirteen collaborators submitted results using an automated rolling ball viscometer for all three sample pairs; A/B, C/D, and E/F. Collaborators were asked to provide details of their measuring systems. Nine collaborators reported using a 1.60 mm capillary, and four used a 1.70 mm capillary. All results were analyzed together for statistical purpose. Six collaborators also submitted results using the standard reference method. Results from individual collaborators are presented in Tables I and II respectively. No outliers were identified according to Dixon's ratio test (1).

The statistical summary for the determination of viscosity by rolling ball viscometer and Wort-13 are presented in Table III. Repeatability and reproducibility coefficients of variation for the rolling ball method ranged from 0.4 to 1.3% and 1.4 to 4.0%, respectively, and were judged acceptable. It is important to note that these results represent the variation in the entire method, which includes the variation associated with preparation of the laboratory wort. This explains why the results of this study show higher variation than in previous collaborative studies where wort samples were sent directly to collaborators (3). Also, higher variation in viscosity results between labs was obtained at the higher viscosity level using the automated method, which confirms results seen previously (3). A similar relationship was not seen in the results for the reference method, where reproducibility was independent of the magnitude.

The F-test was used to detect differences in the precision between the two methods at viscosity levels normally encountered

TABLE I
Wort Viscosity (cP) by Rolling Ball Viscometer

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
1	1.45	1.45	1.53	1.53	2.13	2.13
2	1.46	1.46	1.56	1.56	2.30	2.28
3	1.44	1.44	1.52	1.53	2.02	2.05
4	1.47	1.45	1.56	1.59	2.19	2.17
5	1.43	1.45	1.54	1.54	2.26	2.21
6	1.45	1.45	1.54	1.54	2.11	2.11
7	1.47	1.46	1.55	1.56	2.21	2.19
8	1.43	1.43	1.54	1.52	2.11	2.09
9	1.50	1.51	1.60	1.61	2.35	2.34
10	1.47	1.47	1.56	1.55	2.25	2.15
11	1.47	1.47	1.56	1.55	2.24	2.12
12	1.47	1.47	1.56	1.57	2.28	2.27
13	1.45	1.44	1.52	1.51	2.15	2.11
Mean	1.458	1.456	1.549	1.551	2.200	2.171
Grand mean	1.457		1.550		2.186	

TABLE II
Wort Viscosity (cP) by ASBC Wort-13

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
4	1.45	1.45	1.54	1.52	2.15	2.13
12	1.43	1.43	1.49	1.49	2.22	2.21
14	1.46	1.46	1.55	1.55	2.16	2.14
15	1.45	1.43	1.53	1.51	2.17	2.15
16	1.46	1.49	1.56	1.55	2.13	2.15
17	1.46	1.48	1.57	1.56	2.14	2.16
Mean	1.450	1.455	1.542	1.530	2.162	2.156
Grand mean	1.453		1.536		2.159	

TABLE III
Statistical Summary of Results[a]

			Repeatability			Reproducibility		
Sample Pair	**No. of Labs**	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Rolling ball viscometer								
A/B	13	1.457	0.006	0.4	0.017	0.020	1.4	0.055
C/D	13	1.550	0.010	0.6	0.027	0.025	1.6	0.069
E/F	13	2.186	0.029	1.3	0.081	0.089	4.1	0.248
Reference Method (Wort-13)								
A/B	6	1.453	0.012	0.8	0.033	0.018	1.3	0.052
C/D	6	1.536	0.007	0.5	0.020	0.028	1.8	0.078
E/F	6	2.159	0.013	0.6	0.037	0.030	1.4	0.085

[a] All calculations were made based on Tables I and II.

TABLE IV
Comparison of Laboratory Variances (F-Test)[a]

Method	**Sample Pair**	**Number of Labs**	**Reproducibility Error**	**Pooled Variance**	**df**	**F**
Rolling ball	A/B	13	0.020	0.00055	24	1.21
	C/D	13	0.025			
Wort-13	A/B	6	0.018	0.00067	10	
	C/D	6	0.028			

[a] Critical value for F < 0.025 (two-tailed test) = 2.64.

TABLE V
Comparison of Means by Analysis of Variance (ANOVA)

Source of Variation	**Sum of Squares**	**df**	**Mean Square**	**F**	**P-value**
Method	0.0055	1	0.0055	2.52	0.11
Sample	11.73	5	2.35	1060.65	<0.0001
Error	0.237	107	36		
Total	4658	113			

in the routine malt quality lab, for this purpose the highest viscosity sample pair was excluded. The data is presented in Table IV. As the calculated F of 1.21 was less than the critical value of 2.64, no statistically significant difference in precision between the methods was found.

The method means were compared using analysis of variance (ANOVA). A paired t-test could not be used, as the automated and reference methods were not evaluated in the same laboratories. Results can be found in Table V. While the rolling ball method produced slightly higher results, the difference was not significant at the 95% confidence level.

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Coordination of New and Alternate Methods of Analysis

Subcommittee members: K. Lakenburges, *chair*; C. Benedict; S. Brendecke; L. Chadwick; J. Cornell; M. Eurich; R. Foster; A. Fritsch; R. Jennings; A. Macleod; A. Porter; C. Powell; and D. Sedin (*ex officio*).
Associate members: J. Masschelin (TTB)
Corresponding members: E. Welten (EBC); and S. Furusho (BCOJ).

RECOMMENDATIONS

1. Conduct on-line polling to obtain input on new and alternative methods.

The function of this subcommittee is to collect, from various sources, new and alternate methods of analysis that may be useful to the industries our Society serves. These methods are reviewed to establish their merit and usefulness, and a recommendation regarding collaborative testing is made to the Technical Committee. The subcommittee tracks and records the disposition of each method considered. The subcommittee is also charged with the responsibility of periodically reviewing existing methods for accuracy and usefulness.

STATUS OF SUBCOMMITTEE

Membership and Meetings

Given the very close tie this subcommittee has with the Technical Committee, it has been decided to make the New & Alternate Methods subcommittee an integral part of the Technical Committee's activities and align membership of the two groups. Additional subject matter experts will be added to this subcommittee, or consulted with on an as needed basis.

The subcommittee held a meeting at the 2012 World Brewing Congress in Portland, OR. Topics of interest and discussion included:

- Standardized guidelines for yeast storage, handling, and propagation (Derek Stepanski). Derek had a poster presentation at the WBC and has taken some videos that could be used to help create guidelines. The Craft Brew Subcommittee plans to cover this topic in the future.
- Method for measuring gluten in beer (Dirk Bendiak). There was some discussion on a method used by Canadian breweries. Could be a possibility for collaborative study.
- Rapid method for determination of the color of malts utilizing a small amount of grain and a microwave. There was a poster presentation at the WBC by Yin Lee on the method.
- A simple method for the determination of beta-glucans in wort (Aaron Macleod).
- There was a general inquiry for a fast and easy method to test for propylene glycol in beer. No response was given from those attending the meeting.

Topics for Polling

Polling questions were developed for on-line polling to gather information on potential new methods for collaborative study. These questions were formatted into a web-based survey with assistance and administration by ASBC staff. The topics in the online poll along with background information are described below. Results from the poll can be found in the Appendix of this report.

Input on New and Alternative Methods. This subcommittee and the Technical Committee receive input on potential new and alternative methods throughout the year. Much of the input comes through the ASBC Annual Meeting, but the poll is another valuable tool to gather additional information. This year's poll included questions concerning use of modifications of existing methods in *MOA*.

Hop Aroma Analysis. With the importance of hop aroma on the sensory perception of many beers, questions were developed to gather information on methods used to quantitate hop aroma compounds by gas chromatography and gauge interest in a collaborative study (Dana Sedin).

Post CIP Rinsing. There is great interest in the industry in water conservation and optimizing water use in beer production. However, process piping, vessels, and tanks must be effectively rinsed after CIP. Questions were developed to poll what methods and analytical tools are used to determine when proper rinsing is complete. (Chaz Benedict).

Purging Tanks with CO_2. In order to minimize oxygen in beer, filter beer tanks are purged with CO_2 prior to filling. Questions were developed to gather information on analytical tools used to determine when purging is complete (Chaz Benedict).

Packaging Materials. Questions were developed to gather information on how bottles and cans are rinsed prior to filling with product and how packaging materials are tested for contaminants. A question to gauge interest in guidelines for standard tests for package contaminants was also included (Scott Brendecke).

Mycotoxin Testing of Brewing Raw Materials. Questions were developed to determine if brewing raw materials were being tested for mycotoxins (Aaron Macleod).

Packaging Methods. In an effort to update the Packaging Methods section of *MOA*, the Technical Committee is seeking to evaluate and update packaging methods. Methods are being reviewed and questions are being developed to include in future polling.

Topics to Archive

None

APPENDIX: SUMMARIZED RESULTS FROM 2012 ON-LINE POLLING

Top Line Results

- 89 responses were received.
- 12 respondents submitted information on new and alternative methods.
- 4 respondents submitted information on modifications of existing methods.
- 14 responded that hop aroma analysis was performed in their lab and an additional 16 were interested in the analysis but not currently performing it.
- 16 were interested in participating in a collaborative study to evaluate a method for hop aroma analysis in beer by GC.
- 15 thought it would be helpful to have guidelines for analytical tools to validate rinse after CIP.

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- 10 were interested in more information on analytical tools for purging tanks with CO_2.
- 32 answered that it would be helpful to have guidelines for standard tests for package contaminants.
- 15 responded that their brewing raw material supplier routinely provided mycotoxin data and 4 tested for mycotoxins within their organization.

Information Provided Regarding New or Alternative Methods

- Standardization for yeast storage, handling, and propagation (Derek Stepanski).
- Measuring gluten in beer (from 2 respondents) (Guerdrum and Bamforth 2012 ASBC Journal).
- Determination of diacetyl by HPLC (Scott Bruslind) (dx. doi.org/10.1021/jf3007163 J. Agric. Food Chem. 2012, 60, 3013-3019).
- Determining color of caramel malts, a rapid extraction and analysis of color using little equipment and 1/3 the time of a standard mash.
- Measuring gushing potential in beer, barley, and malt (Deckers et al., 2011. Dynamic Light Scattering (DLS) as a tool to detect CO_2-Hydrophobin structures and study the primary gushing potential of beer. Journal of ASBC 69 (3), 144-149).
- Measuring osmolarity of wort as a predictor of sugar production (from 2 respondents and discussed at last year's meeting) (JASBC 65:59-62, JASBC 69:28-38, JASBC 66: 151-161, JASBC 67:206-216).
- Measurement of beer foam stability (MEBAK).
- Measurement of purine content of an alcoholic beverage for the dietary care of gout and hyperuricemia ((www.interscience.wiley.com) DOI 10.1002/bmc.1197).
- Flavor
- NDMA
- Alcohol levels

Information Provided Regarding Modifications of Existing Methods

- Hops-6A Alpha and Beta-Acids in Hops - modified to decrease the total number of grams and solvent for raw hops and hop extract by half.
- Beer-10 Color, Beer-26 Formazin Turbidity Standards. Established a correction value for turbidity present in a sample using the ASBC standard of 700 nm absorbance to determine "free of turbidity" as well as ultra filtered samples spiked with formazin to establish a color contribution/turbidity correlation value.
- Beer-6 Calculated Values (OE, RDF, Carbs) for improved accuracy (J. Inst. Brew. 2009 115:318).
- Malt extract determination. Mass balance to improve precision.

Report of 2011-BCOJ Collaborative Work

Quantitative Analysis of Total Purine Content Using the HPLC-UV Method in Beer, Low-Malt Beer, and Third-Category Beer

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Keywords: Total purine content, HPLC

CONCLUSIONS

1. Relative repeatability standard deviation (RSD_r) and repeatability limit (r_{95}) for determination of total purine content using the HPLC-UV method ranged from 2.2 to 8.5% and from 2.9 to 20.6 mg/L, respectively, and were judged acceptable.
2. Relative reproducibility standard deviation (RSD_R) and reproducibility limit (R_{95}) for determination of total purine content using the HPLC-UV method ranged from 15.6 to 31.4 % and from 9.1 to 131.2 mg/L, respectively, and were judged unacceptable.

RECOMMENDATIONS

1. It was concluded that the HPLC-UV method failed because the RSD_R range was unacceptable.
2. The subcommittee recommends repeating this study for a second year with some improvement on HPLC conditions.

Although beer contains not so much amount of purine, consumption of large amounts of alcoholic beverages, particularly beer, is associated with an increasing risk of gout.

Recently, consumers have become concerned about the effects of the purine content in beer on health. A method is needed that will allow the accurate quantification of total purine content in beer (1). This subcommittee was charged with evaluating high performance liquid chromatography (HPLC) for quantification of total purine content in beer, low-malt beer and third-category beer.

PROCEDURE

The collaborative study was carried out by 11 collaborators. Collaborators were provided with eight sample pairs consisting of low purine content beer (ca. 10–30 mg/L, A/B, C/D, and E/F), moderate purine content beer (ca. 40–90 mg/L, G/H, I/J, and K/L), and high purine content beer (ca. 100–140 mg/L, M/N and O/P).

Each sample (4.5 mL) was degassed prior to analysis and be placed in test tube on ice and 70% perchloric acid (0.5 mL) was added, followed by heating at over 95°C for 60 min in boiling water with stirring. The hydrolyzed sample solution was neutralized with 8.0 mol/L KOH, followed by centrifugation (3,000 rpm, 10 min). At this time, if necessary, the collaborators could dilute the solution with the appropriate volume of Milli-Q water to facilitate the procedure. The supernatant of the solution was filtered with 0.45 μm hydrophilic filters, then injected into the HPLC system for analysis of adenine, guanine, hypoxanthine and xanthine. The total purine content was calculated by adding together the amounts of each purine using the standard addition method.

The standard curves were prepared from adenine, guanine, hypoxanthine, and xanthine reagents (>99% purity). This standard mixture solution was added to the samples at concentrations of 1.0, 2.5, 5.0, 10.0, 50.0, 100.0 mg/L.

HPLC was performed under the following conditions: instrument, HPLC-UV system without regard to manufacturer; column, Shodex Asahi Pak GF-310 HQ (7.5 mm i.d. and 300 mm length) or GS-320 HQ (7.5 mm i.d. and 300 mm length); mobile phase, 150 mM sodium phosphate buffer (titrating 150 mM sodium dihydrogenphosphate (Nacalai tesque, 98% purity) aqueous solu-

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TABLE I
Total Purine Content (mg/L) Determined Using HPLC-UV Method

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**
1[a]	15	15	19	19	38	38	64	65	91	89	76	77	128	131	155	159
2	50[b]	62[b]	6[b]	36[b]	138[b]	29[b]	196[b]	171[b]	175[b]	228[b]	18[b]	17[b]	40[b]	42[b]	92[c]	113[c]
3	11	11	14	14	26	27	59	60	73	73	70	70	121	122	158	155
4	13	14	18	17	30	29	60	60	84	83	73	72	116	115	136	135
5	13	13	18	18	29	29	60	59	83	82	71	71	116	113	130	129
6	18	17	14	17	29	29	56	58	78	101	70	73	101	111	128	132
7	21	17	16	17	33	37	80	83	68	66	108	113	128[b]	65[b]	403[b]	403[b]
8	14	15	21	21	50	46	63	56	98	91	88	90	143	121	152	146
9	22	23	33[b]	56[b]	36	39	80	87	121	100	60[b]	100[b]	165	168	180[b]	134[b]
10	16	15	9	11	28	28	73	74	93	98	85	88	143	154	263[c]	266[c]
11[a]	14	11	15	17	29	30	57	57	76	81	65	63	104	105	120	118
Mean	15.7	15.1	16.0	16.8	32.8	33.2	65.2	65.9	86.5	86.4	78.4	79.7	126.3	126.7	148.2	150.3
Grand mean	15.4		16.4		33.0		65.6		86.5		79.1		126.5		149.3	

[a] Collaborators who diluted sample solution with the appropriate volume of distilled Milli-Q water.
[b] Outliers identified by outlier test and excluded from the statistics analysis.
[c] Outliers identified by outlier test but included in the statistics analysis.

TABLE II
Statistical Summary of Results of HPLC-UV Method

	Sample Pair A/B	Sample Pair C/D	Sample Pair E/F	Sample Pair G/H	Sample Pair I/J	Sample Pair K/L	Sample Pair M/N	Sample Pair O/P
Number of laboratories	10	9	10	10	10	9	9	9
Grand mean (m)	15.4	16.4	33.0	65.6	86.5	79.1	126.5	149.3
Repeatability standard deviation (S_r)	1.2	1.0	1.5	2.4	7.3	1.7	6.4	5.4
Relative repeatability standard deviation (RSD_r %)	8.0	6.3	4.5	3.7	8.5	2.2	5.1	3.6
Repeatability limit (r_{95})	3.4	2.9	4.2	6.7	20.6	4.8	17.9	15.2
Predicted relative repeatability standard deviation ($PRSD_r$ %)	7.1	7.0	6.3	5.7	5.5	5.5	5.1	5.0
$HORRAT_r$ ($RSD_r/PRSD_r$)[a]	1.1	0.9	0.7	0.6	1.6	0.4[b]	1.0	0.7
Reproducibility standard deviation (S_R)	3.5	3.2	6.7	10.3	13.5	14.3	20.9	46.9
Relative reproducibility standard deviation (RSD_R %)	23.0	19.7	20.4	15.7	15.6	18.1	16.5	31.4
Reproducibility limit (R_{95})	9.9	9.1	18.9	28.8	37.8	40.0	58.4	131.2
Predicted relative reproducibility standard deviation ($PRSD_R$ %)	10.6	10.5	9.5	8.5	8.2	8.3	7.7	7.5
$HORRAT_R$ ($RSD_R/PRSD_R$)[a]	2.2	1.9	2.2	1.8	1.9	2.2	2.1	4.2

[a] According to AOAC International Guidelines, HORRAT values should be between 0.5 and 2.0 (4).
[b] Accurate result although $HORRAT_r$ values was under 0.5.

tion to pH 2.5 with phosphoric acid); flow rate, 0.6 mL/min; column temperature, 35°C; detector wavelength, 260 nm; injection volume, 20 µL. Measurement of adenine, guanine, hypoxanthine and xanthine was performed in duplicate.

The results were processed according to JIS Z 8401 guidelines (2) and statistical analysis for the processed data was performed according to JIS Z 8402-2 guidelines (3) and AOAC International Guidelines (4).

RESULTS AND DISCUSSION

Results from 11 collaborators who performed the HPLC-UV method were received for the eight sample pairs (A/B, C/D, E/F, G/H, I/J, K/L, M/N, and O/P). The results for total purine content are shown in Table I. All samples were checked for outliers using Mandel's *h* and *k* statistics, and Cochran and Grubbs outlier test.

According to the rule for finishing the outlier test before the number of outlier exceed 2/9 of the number of collaborators(4), only 2 outliers were excluded from the statistical analysis(3) in spite of the Cochran and Grubbs outlier test detected more than 3 outliers in the data set, the statistical summary of results is shown in Table II. RSD_r ranged from 2.2 to 8.5%; r_{95} ranged from 2.9 to 20.6 mg/L, respectively, and were judged acceptable. RSD_R ranged from 15.6 to 31.4%; R_{95} ranged from 9.1 to 131.2 mg/L, respectively, and were judged unacceptable.

It was concluded that the HPLC-UV method failed because the RSD_R range was unacceptable. The subcommittee recommends repeating this study for a second year with some improvement on HPLC conditions.

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