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## Comparative Testing Report on the Detection and Quantification of Maize Events GA21, TC1507 and MIR604

Comparative testing round:  
ILC-EURL-GMFF-CT-02/11  
Preliminary Report

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# **Comparative Testing Report on the Detection and Quantification of Maize Events GA21, TC1507 and MIR604**

**Comparative testing round: ILC-EURL-GMFF-CT-02/11**

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**Joint Research Centre**

**Institute for Health and Consumer Protection  
Molecular Biology and Genomics Unit**

**Report number: EURL-CT-02/11 PreICTR**

**Status: Preliminary report**

**Confidentiality statement: The laboratory codes assigned to each participant in this comparative testing round are confidential. However, the EURL-GMFF will disclose details of the National Reference Laboratories that have been appointed under Regulation (EC) No 882/2004 and Regulation (EC) No 1981/2006 to DG SANCO for the purpose of an assessment of their performance.**

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## Executive Summary

The Joint Research Centre as European Union Reference Laboratory for Genetically Modified Food and Feed, established by Regulation (EC) No 1829/2003<sup>(1)</sup>, organised a comparative testing round for National Reference Laboratories nominated under Regulation (EC) No 882/2004<sup>(2)</sup> and Regulation (EC) No 1981/2006<sup>(3)</sup>, for Official control laboratories only and for laboratories from third countries that volunteered to participate.

In accordance with Article 32 of Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, the European Union Reference Laboratory for Genetically Modified Food and Feed shall organise comparative testing and shall ensure an appropriate follow-up of such testing.

The design and execution of the comparative testing round was in accordance with the ISO 17043 standard<sup>(4)</sup>. The European Union Reference Laboratory for Genetically Modified Food and Feed is accredited according to the ISO 17043 Standard regarding 'General requirements for proficiency testing'<sup>(4)</sup>.

The test items used in the comparative testing round ILC-EURL-GMFF-CT-02/11 were produced in-house. Pioneer Overseas Corporation provided a coarsely ground powder of TC1507 (unique identifier DAS-Ø15Ø7-1) seeds. Syngenta Biotechnology, Inc. provided devitalised seeds of maize events MIR604 (unique identifier SYN-IR6Ø4-5) and GA21 (unique identifier MON-ØØØ21-9). Participants had to screen two test items denoted maize powder levels 1 and 2, for the presence of maize events 3272, Bt11, Bt176, 59122, GA21, MIR604, MON 810, MON 863, NK603 and TC1507. In case of detection of the event its genetically modified (GM) content had to be determined. In September 2011, a total of 155 laboratories were invited to participate in ILC-EURL-GMFF-CT-02/11. Test items were shipped to participants at the end of October 2011 in plastic containers containing approximately 5 g of flour. The Food Safety and Quality Unit of IRMM managed the on-line registration and submission of results.

Participants could report the results in either mass/mass % or copy/copy %. The European Union Reference Laboratory for Genetically Modified Food and Feed calculated the robust means ( $\mu_R$ ) of the maize powder levels 1 and 2 test items in mass/mass % and in copy/copy %. All data were log-transformed and then robust statistics were applied to obtain a robust mean<sup>(5, 6, 7)</sup>.



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*Authorisation to publish:*

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## 1. Introduction

The aim of this preliminary report is to inform participants about the outcome of this comparative testing round. Participants that have carried out quantitative analyses will be informed about the z-scores obtained for the quantification of those GM events that were added by the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF) to the test items namely GA21, TC1507 and MIR604. Participants having obtained a z-score(s) outside the working range of -2 to +2 will be asked to repeat the experimental work. Those participants are not informed about the exact value of their z-score(s). A final report will be issued when the follow up of laboratories with a z-score(s) outside working range of -2 to +2 has been completed. The follow up of laboratories may involve an on-site visit to provide assistance.

## 2. Participants' results

The assignment of a laboratory number to each participant and the submission of results were managed by the FSQ Unit of IRMM. Results had to be reported on-line using a form for which each participant received an individual access code. A questionnaire was attached to the on-line reporting form to provide details of the analytical methods used.

Participants had to screen two test items denoted maize powder levels 1 and 2, for the presence of maize events 3272, Bt11, Bt176, 59122, GA21, MIR604, MON 810, MON 863, NK603 and TC1507. In case of detection of the event its genetically modified (GM) content had to be determined. Participants could report the quantitative results in either mass/mass % (m/m %) or copy/copy % (cp/cp %). The expression of measurement results in cp/cp % follows the Recommendation (EC) No 2004/787<sup>(8)</sup>, where it is recommended that the results of quantitative analyses are expressed as GM DNA copy numbers in relation to target taxon-specific copy numbers calculated in terms of haploid genomes.

Participants were instructed to apply the formulas described below when reporting their results.

$$\text{m/m \%} = \frac{\text{mass GM [g]}}{\text{Total mass [g]}} \times 100 \% \quad (1)$$

$$\text{cp/cp \%} = \frac{\text{GM DNA copy numbers [cp]}}{\text{Target taxon-specific DNA copy numbers [cp]}} \times 100 \% \quad (2)$$



## 2.1 Qualitative results

Table 1 lists the GM events present in maize powder levels 1 and 2. Different quantities of the maize events GA21, TC1507 and MIR604 were added to both test items. Purity tests conducted in the EURL-GMFF showed the adventitious presence of maize events NK603 (0.01 %), MON 810 (below Limit of Quantification LOQ) and 59122 (below LOQ).

**Table 1:** Overview of GM events present in maize powder levels 1 and 2.

<b>GM event</b>	<b>Maize powder level 1</b>	<b>Maize powder level 2</b>
GA21	+	+
TC1507	+	+
MIR 604	+	+
NK603	Adv	Adv
59122	Adv	Adv
MON 810	Adv	Adv
MON 863	-	-
3272	-	-
Bt11	-	-
Bt176	-	-

+ indicates that the GM event was added to the test items, - indicates that the GM event was not added to the test items, Adv: adventitious presence

About 88 % of registered laboratories reported quantitative results for events GA21 and TC1507, whereas 77 % of registered laboratories reported quantitative results for event MIR604. It must be noted that some registered laboratories only performed qualitative analyses. With respect to the adventitious GM events about 70 % of registered participants quantified the NK603 content in the test items of which 95 % reported a low GM percentage. Some participants reported quantitative results for the events 59122 and MON 810 (11 % and about 14 %, respectively). Less than 14 % of registered participants reported quantitative results for the GM events MON 863, 3272, Bt11 and Bt176 which were not detected during the in-house purity tests.

## 2.2 Consensus value from participants

The consensus value ( $\mu_R$ ) from participants in the comparative testing round was calculated using robust statistics<sup>(9)</sup>. This approach minimises the influence of outlying values. All results were log-transformed prior to the calculation of the robust mean to establish a near-normal distribution allowing the interpretation of results on the basis of a normal distribution<sup>(6)</sup>. Robust means ( $\mu_R$ ) were calculated on the basis of the results reported in m/m % and cp/cp %, respectively.

### 2.3 Statistical data and summaries

The aim of a performance statistic is to provide participants with a meaningful result that can be easily interpreted. The procedure followed for the evaluation of participants' performance was agreed by the Members of the Advisory Board and relies on the calculation of z-scores on the robust means of the participants' results<sup>(10)</sup>.

Laboratories are compared on the basis of z-scores calculated from log-transformed data<sup>(6)</sup>. Z-scores are based on the robust means ( $\mu_R$ ) of the submitted results (Tables 2 to 13).

**Table 2.** Z-scores for event GA21 maize powder level 1 for results reported in m/m %

<b>Maize event GA21</b>			
<b>Laboratory number</b>	<b>z-score</b>	<b>Laboratory number</b>	<b>z-score</b>
L002	-0.04	L042	-0.49
L003	-0.41	L044	-0.77
L004	-0.10	L045	0.30
L005	1.61	L047	-0.33
L006	0.09	L050	0.95
L007	1.23	L051	-0.10
L008	1.61	L055	1.27
L009	*	L056	-0.42
L011	-1.70	L058	0.32
L012	1.36	L059	0.38
L013	-0.10	L060	-0.18
L015	*	L061	-0.49
L016	0.03	L062	-0.25
L017	0.09	L063	-0.04
L018	-0.77	L069	0.15
L019	-0.77	L070	-0.49
L020	-0.88	L071	-0.99
L021	-0.68	L073	*
L023	-0.41	L076	-0.25
L024	-0.49	L077	-0.25
L025	0.48	L078	1.39
L026	1.23	L079	-0.49
L027	-0.41	L081	0.38
L029	0.53	L082	-0.25
L030	-0.88	L083	-0.88
L031	-0.04	L085	-0.68
L033	0.27	L087	1.39
L034	-0.10	L088	0.95
L035	-1.24	L096	0.62
L036	1.85	L097	-0.41
L037	-0.04	L100	0.27
L038	0.09	L101	-0.49
L040	-0.18	L105	-0.18
L041	-0.49		

Z-scores are calculated on the basis of the robust mean, \* z-score outside the working range of -2 to +2

**Table 3.** Z-scores for event GA21 maize powder level 2 for results reported in m/m %

<b>Maize event GA21</b>			
<b>Laboratory number</b>	<b>z-score</b>	<b>Laboratory number</b>	<b>z-score</b>
L002	-1.44	L042	-0.32
L003	-0.61	L044	-0.15
L004	0.22	L045	0.63
L005	1.69	L047	-0.32
L006	-0.79	L050	*
L007	0.33	L051	-0.09
L008	*	L055	1.01
L009	*	L056	-0.54
L011	-0.59	L058	0.41
L012	*	L059	0.54
L013	-0.13	L060	-0.43
L015	-0.45	L061	-0.32
L016	-0.24	L062	-0.28
L017	-0.22	L063	-0.44
L018	-0.36	L069	0.14
L019	-0.69	L070	-0.43
L020	-0.78	L071	-0.02
L021	-0.55	L073	*
L023	-0.15	L076	-0.20
L024	-1.36	L077	-0.34
L025	1.48	L078	0.86
L026	1.18	L079	-0.11
L027	-0.33	L081	0.65
L029	-0.11	L082	-0.40
L030	-0.10	L083	-0.64
L031	0.48	L085	-0.39
L033	0.17	L087	1.91
L034	-0.21	L088	0.49
L035	-0.23	L096	0.94
L036	*	L097	-0.01
L037	0.16	L100	0.22
L038	-0.10	L101	-0.11
L040	0.00	L105	-0.13
L041	-0.78		

Z-scores are calculated on the basis of the robust mean, \* z-score outside the working range of -2 to +2

**Table 4.** Z-scores for event GA21 maize powder level 1 for results reported in cp/cp %

<b>Maize event GA21</b>			
<b>Laboratory number</b>	<b>z-score</b>	<b>Laboratory number</b>	<b>z-score</b>
L005	1.64	L054	-0.37
L009	1.03	L060	-1.34
L010	1.14	L065	*
L024	-1.66	L066	*
L026	1.27	L067	1.14
L028	-1.66	L068	*
L029	0.36	L074	-0.84
L030	*	L080	0.25
L032	-1.66	L083	-0.84
L039	-1.20	L089	*
L043	-0.21	L095	*
L046	*	L098	*
L049	-0.46	L099	-0.07
L052	*		

Z-scores are calculated on the basis of the robust mean, \* z-score outside the working range of -2 to +2

**Table 5.** Z-scores for event GA21 maize powder level 2 for results reported in cp/cp %

<b>Maize event GA21</b>			
<b>Laboratory number</b>	<b>z-score</b>	<b>Laboratory number</b>	<b>z-score</b>
L005	1.98	L054	0.44
L009	1.47	L060	-1.35
L010	1.94	L065	*
L024	*	L066	*
L026	1.46	L067	1.37
L028	-1.47	L068	*
L029	0.18	L074	-0.94
L030	-1.02	L080	0.07
L032	-1.24	L083	-0.36
L039	0.41	L089	*
L043	0.24	L095	*
L046	*	L098	*
L049	-0.38	L099	0.24
L052	1.93		

Z-scores are calculated on the basis of the robust mean, \* z-score outside the working range of -2 to +2

**Table 6.** Z-scores for event TC1507 maize powder level 1 for results reported in m/m %

<b>Maize event TC1507</b>			
<b>Laboratory number</b>	<b>z-score</b>	<b>Laboratory number</b>	<b>z-score</b>
L001	0.01	L038	0.01
L002	-0.23	L040	-0.29
L003	0.01	L041	-0.29
L004	0.74	L042	-1.38
L005	0.33	L044	0.38
L006	1.11	L045	1.13
L007	1.63	L047	-1.08
L008	0.13	L050	*
L009	*	L051	0.43
L011	0.70	L055	*
L012	1.49	L056	-0.28
L013	0.52	L058	-0.29
L015	-0.10	L059	-0.50
L017	-0.43	L060	0.13
L018	-0.04	L061	-0.50
L019	0.38	L062	-0.10
L020	-0.57	L069	-0.43
L021	0.33	L070	-0.50
L022	0.61	L071	-0.50
L023	0.07	L073	*
L024	-0.98	L077	-0.57
L025	-1.61	L078	0.28
L026	0.13	L079	-0.50
L027	-0.16	L081	0.61
L029	1.21	L082	0.90
L030	-0.36	L083	-1.73
L031	0.61	L085	-0.65
L033	-0.04	L087	-1.17
L034	-0.36	L096	0.38
L035	0.01	L097	1.49
L036	-1.17	L100	0.28
L037	0.28	L101	0.38

Z-scores are calculated on the basis of the robust mean, \* z-score outside the working range of -2 to +2

**Table 7.** Z-scores for event TC1507 maize powder level 2 for results reported in m/m %

<b>Maize event TC1507</b>			
<b>Laboratory number</b>	<b>z-score</b>	<b>Laboratory number</b>	<b>z-score</b>
L001	-0.27	L040	-0.27
L002	-0.25	L041	-0.18
L003	-0.32	L042	1.67
L004	0.56	L044	0.48
L005	0.14	L045	1.31
L006	-0.27	L047	-0.65
L007	0.05	L050	-1.67
L008	-0.01	L051	0.14
L009	*	L055	*
L011	0.67	L056	0.16
L012	1.52	L058	0.20
L013	0.73	L059	-0.23
L015	0.05	L060	0.07
L017	0.22	L061	-0.37
L018	-0.42	L062	0.12
L019	-0.77	L069	-0.23
L020	-0.27	L070	0.51
L021	0.84	L071	-0.37
L023	0.24	L073	*
L024	-0.98	L077	-0.44
L025	-0.77	L078	0.71
L026	0.03	L079	-0.63
L027	-0.14	L081	0.64
L029	1.06	L082	-0.37
L030	-0.18	L083	-0.98
L031	0.22	L085	-0.18
L033	-0.23	L087	-0.32
L034	-0.30	L096	0.43
L035	-0.37	L097	1.34
L036	-0.57	L100	-1.60
L037	-0.08	L101	0.26
L038	0.22		

Z-scores are calculated on the basis of the robust mean, \* z-score outside the working range of -2 to +2

**Table 8.** Z-scores for event TC1507 maize powder level 1 for results reported in cp/cp %

<b>Maize event TC1507</b>			
<b>Laboratory number</b>	<b>z-score</b>	<b>Laboratory number</b>	<b>z-score</b>
L005	1.20	L063	0.64
L009	*	L065	*
L024	-1.62	L066	-1.28
L026	0.99	L067	0.70
L028	-1.28	L068	0.77
L029	1.80	L074	-1.14
L030	-0.99	L076	-0.30
L032	0.37	L077	0.22
L039	-0.21	L080	0.37
L043	*	L083	-0.86
L046	*	L088	*
L049	*	L089	-1.62
L052	0.51	L095	-0.51
L054	0.83	L098	*
L060	-0.51	L099	-0.62

Z-scores are calculated on the basis of the robust mean, \* z-score outside the working range of -2 to +2

**Table 9.** Z-scores for event TC1507 maize powder level 2 for results reported in cp/cp %

<b>Maize event TC1507</b>			
<b>Laboratory number</b>	<b>z-score</b>	<b>Laboratory number</b>	<b>z-score</b>
L005	1.02	L063	0.53
L009	*	L065	-0.45
L024	-1.61	L066	-1.04
L026	0.90	L067	0.74
L028	-1.37	L068	0.08
L029	1.70	L074	-1.10
L030	-0.81	L076	0.11
L032	0.25	L077	0.67
L039	0.28	L080	0.74
L043	*	L083	-0.10
L046	-1.07	L088	*
L049	*	L089	-0.41
L052	0.33	L095	-0.77
L054	0.74	L098	*
L060	-0.56	L099	-0.60

Z-scores are calculated on the basis of the robust mean, \* z-score outside the working range of -2 to +2

**Table 10.** Z-scores for event MIR604 maize powder level 1 for results reported in m/m %

<b>Maize event MIR604</b>			
<b>Laboratory number</b>	<b>z-score</b>	<b>Laboratory number</b>	<b>z-score</b>
L002	-0.14	L041	-0.42
L003	-0.34	L044	-0.33
L005	0.93	L045	0.53
L006	0.96	L047	-0.45
L007	1.64	L050	0.22
L008	-0.87	L051	-0.13
L009	*	L055	*
L011	0.41	L056	-0.12
L012	*	L058	-0.26
L013	-0.43	L059	-0.03
L015	-0.16	L060	-0.02
L017	-0.44	L061	-0.11
L018	*	L062	-0.28
L019	*	L063	-0.11
L020	0.36	L069	0.14
L021	*	L070	0.41
L023	0.17	L071	-0.50
L024	0.18	L077	0.56
L025	-0.33	L078	-1.12
L026	-0.96	L079	-0.33
L030	-0.32	L081	-0.03
L031	0.43	L082	0.11
L033	-0.02	L083	0.46
L034	-0.48	L085	-0.26
L035	0.10	L087	0.47
L036	*	L096	-0.42
L037	0.44	L097	0.67
L038	0.01	L100	0.21
L040	-0.24	L101	-0.03

Z-scores are calculated on the basis of the robust mean, \* z-score outside the working range of -2 to +2



**Table 11.** Z-scores for event MIR604 maize powder level 2 for results reported in m/m %

<b>Maize event MIR604</b>			
<b>Laboratory number</b>	<b>z-score</b>	<b>Laboratory number</b>	<b>z-score</b>
L002	0.63	L041	-0.42
L003	-0.34	L044	-0.39
L005	0.90	L045	0.69
L006	-0.34	L047	*
L007	0.37	L050	-0.06
L008	-0.80	L051	0.11
L009	*	L055	*
L011	0.99	L056	-0.42
L012	*	L058	0.02
L013	-0.20	L059	0.02
L015	-0.31	L060	-0.03
L017	-0.54	L061	-0.03
L018	*	L062	-0.34
L019	*	L063	0.13
L020	-0.03	L069	0.27
L021	*	L070	0.41
L023	0.04	L071	-0.57
L024	0.16	L077	0.58
L025	-0.91	L078	-1.35
L026	-0.91	L079	-0.03
L030	-0.08	L081	-0.51
L031	-0.03	L082	0.02
L033	0.07	L083	0.35
L034	-0.28	L085	-0.13
L035	0.93	L087	0.24
L036	*	L096	-0.57
L037	0.65	L097	0.09
L038	0.02	L100	-0.23
L040	-0.20	L101	0.16

Z-scores are calculated on the basis of the robust mean, \* z-score outside the working range of -2 to +2

**Table 12.** Z-scores for event MIR604 maize powder level 1 for results reported in cp/cp %

<b>Maize event MIR604</b>			
<b>Laboratory number</b>	<b>z-score</b>	<b>Laboratory number</b>	<b>z-score</b>
L005	1.49	L065	*
L009	*	L066	-1.97
L024	-0.77	L067	0.45
L026	-0.41	L068	0.87
L028	*	L074	-0.66
L030	-1.27	L076	0.60
L032	-1.36	L080	0.72
L043	1.82	L083	1.02
L046	-1.54	L088	0.90
L052	-0.89	L089	*
L054	0.95	L095	*
L060	-0.97	L098	*

Z-scores are calculated on the basis of the robust mean, \* z-score outside the working range of -2 to +2

**Table 13.** Z-scores for event MIR604 maize powder level 2 for results reported in cp/cp %

<b>Maize event MIR604</b>			
<b>Laboratory number</b>	<b>z-score</b>	<b>Laboratory number</b>	<b>z-score</b>
L005	1.17	L065	*
L009	*	L066	-1.89
L024	-1.07	L067	0.47
L026	-0.63	L068	0.20
L028	*	L074	-0.95
L030	-1.31	L076	0.36
L032	-1.51	L080	0.68
L043	*	L083	0.62
L046	*	L088	1.09
L052	-1.93	L089	*
L054	0.52	L095	*
L060	-1.26	L098	*

Z-scores are calculated on the basis of the robust mean, \* z-score outside the working range of -2 to +2

### 3. Interpretation of z-scores

In general one assumes a normal distribution when calculating z-scores. In which case there is a 5 % probability that some z-scores will fall outside the working range of -2 to +2 and a 0.3 % probability that some z-scores will fall outside the working range of -3 to +3. A z-score outside the working range of -2 to +2 indicates that a participant is probably not performing according to specifications although this cannot be stated with 100 % certainty. The higher the value of the target standard deviation for proficiency assessment  $\hat{\sigma}$ , the more likely participants with a z-score outside the working range of -2 to +2 are underperforming. However, a higher  $\hat{\sigma}$  will also increase the probability of accepting unsatisfactory measurement results. Hence, a compromise should be made between the choice of the value of  $\hat{\sigma}$  and the attempt to assess the participants' performance. In any case a z-score outside the working range of -3 to +3 will quite clearly identify an underperforming participant and will require follow-up. It should be taken into consideration that a laboratory performing well has a 5 % probability of obtaining a z-score outside the working range of -2 to +2 by mere chance.

### 4. Conclusions

In this fourth comparative testing round participants were faced with a challenge because they were asked to screen two maize powder test items for ten maize GM events and to determine the GM content of those GM events that were detected. The majority of participants reported quantitative results for the GM events that had been added to the test items by the EURL-GMFF (i.e. GA21, TC1507 MIR604). A minority of participants reported quantitative results for the GM events which were not detected during the in-house purity tests (i.e. MON 863, 3272, Bt11 and Bt176).

The outcome of this fourth comparative testing round was positive in general. More than 86 % of participants that expressed the results in mass/mass % gained a z-score in the range of -2 to +2 for both maize powder levels 1 and 2. The share of z-scores in the range of -2 to +2 was lower for those participants expressing the results in cp/cp %, namely greater than 67 %.

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#### Abstract

In the frame of Regulation (EC) No 882/2004, the European Union Reference Laboratory for Genetically Modified Food and Feed has the duty to organise comparative testing rounds and to ensure an appropriate follow-up of these activities. This report describes the outcome of the fourth comparative testing round ILC-EURL-GMFF-CT-02/11. Participants were required to screen two test items denoted maize powder levels 1 and 2, for the presence of maize events 3272, Bt11, Bt176, 59122, GA21, MIR604, MON 810, MON 863, NK603 and TC1507. Any events detected were then to be quantified.

This comparative testing round was organised in collaboration with the Food Safety and Quality Unit of the Institute for Reference Materials and Measurements (Geel, BE). The maize test items were produced in-house. The Food Safety and Quality Unit managed the on-line registration and submission of results.

A total of 159 laboratories were invited to participate in ILC-EURL-GMFF-CT-02/11. Ninety-three laboratories from 40 countries returned results, of which 62 were National Reference Laboratories, seven were only members of the European Network of GMO Laboratories, eight were only Official control laboratories and 16 were laboratories from third countries. Nine laboratories including one National Reference Laboratory, one European Network of GMO Laboratory and seven laboratories from third countries did not submit results.

In this fourth comparative testing round greater than 86 % of participants gained a satisfactory z-score in the range of -2 to +2 for the results expressed in mass/mass % for both maize powder levels 1 and 2 regardless of the GM event. However, a lower percentage (43 – 86 %) of z-scores within the working range of -2 to +2 was calculated for those participants that expressed the results in copy/copy %.

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