ANIMAL AND PLANT NUTRITION, HEALTH, AND SAFETY

Evaluation of the Use of Microtracers™ in a Proficiency Testing Program

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Background: An effective proficiency testing program must utilize accurate splitting procedures to ensure that customers receive equivalent (by some measure) test items. When test items are not equivalent, it becomes impossible to separate variation among laboratories from variation among test items, and the program cannot achieve its objectives. Therefore, there is a critical need to validate the splitting process used to manufacture test items. _Objective_: The incorporation of Microtracers™ was investigated for validating the splitting process used in the Association of American Feed Control Officials Proficiency Testing Program and as a potential quality control event for a production run. _Methods_: Microtracers were incorporated into six production runs. From each run, 12 test items were randomly selected for evaluation. Proficiency test materials were prepared from commercially available feedstuffs using base animal feeds and feed additives. The tracers were incorporated into base feed with other additives, recovered, and counted from the randomly selected test items. _Results_: Uniformity of test items was evaluated with the following two statistical methods: (_1_) relative standard deviation (RSD) of particle counts according to a Poisson distribution and (_2_) a Pearson's Chi-square test. RSDs for counts (per mass basis) ranged from 2.49 to 4.13%, and Chi-square _P_ values ranged from 0.0097 to 0.3740 over the six sets. _Conclusions_: Microtracers were determined to be a potential tool for validating the splitting process used to manufacture proficiency test items and a tool for incorporating quality control events into the manufacture of proficiency test items. _Highlights_: The authors offer suggestions for proficiency testing programs.

Proficiency testing is a quality assurance tool that enables laboratories to compare their results on test methods to results from other laboratories that participate in the same program. Test items are periodically sent to participating laboratories for testing, with each laboratory's results assessed against those of its peers. Proficiency testing is widely considered a very critical component of a laboratory's quality assurance program. Proficiency test (PT) items are created from a starting material using a splitting protocol designed to generate test items as identical as possible (within the capabilities of random sampling). One such program is the Association of American Feed Control Officials (AAFCO) Proficiency Testing Program (https://www.aafco.org/Laboratory/Proficiency-Testing-Program).

The basic procedure for creating PT items is to divide a large amount of material into the desired number of test items, preferably with a rotary splitting device. The preference for use of a rotary splitter to divide the material is due to the excellent control of errors associated with the splitting process. These errors include grouping and segregation error controlled through the selection of a large number (hundreds) of increments, sample correctness controlled through proper rotary splitter design, and fundamental sampling error controlled through sufficient test item mass (1, 2). Each proficiency testing program should have established confidence that the test items shipped to participants are equivalent (i.e., they have no more between-item variation than would be expected by the chosen random probability distribution). This variation can only be accomplished by control of the three types of error previously mentioned. If the test items are not equivalent, it becomes impossible to separate variation among laboratories from variation among test items, and the PT is ineffective.

Microtracers™ have been reported as a tool for evaluating mixer performance and uniformity in feed materials (3–6). Microtracers FS tracers consist of stainless-steel particles colored with food-grade water soluble or water insoluble (lake)

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food dyes and are designed to be used in premixes and complete feeds. They can be retrieved from feed materials via magnetic separation. The particle count is approximately 50 000/g and the particle size range is 125–300 micron.

When analyzing particle count data, it is standard to assume an underlying Poisson distribution (7, 8; Bernatos, D., Department of Economics, University of California, San Diego, personal communication, 2012), with the shape and location of the distribution described by a single parameter, λ. In this study, the focal data are counts of Microtracers particles in individually packaged PT items that may or may not have been produced uniformly. If the PT items are sufficiently uniform, then repeated items in the production run will reflect a Poisson distribution around a mean value (λ) that is the count of tracer particles that were originally added per PT item, scaled to mass.

The Poisson distribution model describes the mean of counts, and their variance are both equal to λ, with the SD equal to the square root of λ (or λ½). For example, if 400 particles (or in the case of Microtracers, colored spots) are counted an infinite number of times, it is expected to obtain a SD of 20 and a relative standard deviation (RSD) of 20/400 or 5%. The higher number of tracers counted, the lower the expected RSD (Table 1). The theoretical variation (due solely to that expected from the Poisson probability distribution) in counts among test items would approach variations close to those listed in Table 1. Because no splitting process will achieve perfection, the theoretical variations can be used as a basis for evaluating observed variations. A suggested maximum acceptable RSD of counts may be established to identify if test items are considered adequately uniform (Table 1). If the actual RSD value is higher than the upper limit of the 95% confidence interval, it could indicate unacceptable uniformity in the absence of other quality control data.

A goodness of fit test such as the Pearson's Chi-square test is an appropriate tool to evaluate Microtracer counts by testing if observed count distribution is significantly different from what may be expected from a truly random Poisson distribution (Bernotas, D., Department of Economics, University of California, San Diego, personal communication, 2012). The *P* value result of the Chi-square test estimates the probability that the set of observations were drawn from a uniform population and that the variation observed between PT items is solely due to random Poisson variation. The *P* value result of the Chi-square test expressed as a percentage has been defined in Microtracers literature, and suggested *P* values have been used by Good Manufacturing Processes (GMP) and GMP+ certification schemes to evaluate animal feed uniformity (8). Results of $P \leq 0.01$ reflect insufficient uniformity (Table 1), results of $P \geq 0.05$ reflect good uniformity, and results of $0.01 \leq P \leq 0.05$ suggest marginal uniformity, which may be suspect, and further investigation is warranted (8).

Materials composed of particles with discernible differences in physical properties (e.g., particle size, density, rigidity, or surface properties) have a tendency for segregation of particles (4). Uniformity in particle size and limited particle size range are especially critical in limiting segregation. Thus, Microtracers particle size distribution should be comparable to the materials to which it is incorporated to achieve uniform incorporation. Particle size distribution was evaluated for the feed materials used in this study and compared with that of the Microtracers.

Table 1. Correlation between the number of particles, RSD expected from perfect splits, maximum RSD values for acceptable uniformity, and suggested Chi-square *P* value limits

No. of particles	Average long-term RSD, % expected when splitting error is zero[a]	Maximum RSD, %, for any single PT material[b]	Chi-square *P* value limit for any single PT material
200	7.0	10	>0.01
300	5.8	8.2	>0.01
400	5.0	7.0	>0.01
600	4.1	5.8	>0.01
800	3.5	5.0	>0.01
1100	3.0	4.3	>0.01
1600	2.5	3.5	>0.01

[a] Poisson.

[b] Based on 95% confidence interval, n = 12, based on parametric bootstrapping.

This study was undertaken to investigate the potential use of Microtracers FS as a quality control tool to estimate the variability in the process used to manufacture PT items. Variability of test items was evaluated based on distribution of incorporated tracers. If the tracers are found to be a useful tool in evaluating uniformity of PT items, they may be suitable as a routine quality control tool for proficiency testing programs and for splitting processes for other applications.

Experimental

Materials

(**a**) *Microtracer FS Red No. 40 (AA-797)*.—Microtracers, Inc., San Francisco, CA.

(**b**) *Microtracer FS Blue No. 1 (A-13420)*.—Microtracers, Inc.

(**c**) *Base feed materials*.—Poultry feed, milk replacer, medicated beef feed, medicated swine feed, dairy feed, and poultry feed. The medicated beef feed and dairy feed were prepared from the same materials at different times and can be considered as replicates.

Apparatus

(**a**) *Equipment for preparation of proficiency testing materials*.—(*1*) *Hosokawa Micron Powder Systems Mikro Bantam Hammer and Screen Mill*.

(*2*) *Sepor rotary sample splitter*.—24 splits from Sepor, Inc. (Wilmington, CA).

(**b**) *Equipment for Microtracers recovery and counting.—Scale*.—OHAUS, CS200 series.

(*2*) *Rotary Detector™ magnetic separator*.—Used for isolating the tracer from samples. Micro Tracers Services Europe German-made model with rare-earth magnet.

(*3*) *Spray bottle containing 50% ethanol solution.*

(**c**) *Equipment for particle size testing.—Horizontal sieve shaker*.—Humboldt Manufacturing Co. (Elgin, IL).

(*2*) *Woven wire test sieves*.—Advantech Manufacturing (New Berlin, WI), serialized to meet American Society for Testing and Materials (ASTM) E-11.

Methods

Preparation of Proficiency Testing Materials

Six AAFCO PT materials were prepared from commercially available feedstuffs using a base animal feed (as indicated in Table 2), incorporating one or more specific additives (e.g., vitamin premix, veterinary drugs, minerals) to obtain the desired variety and concentrations of analytes in the final PT material. Microtracers FS were incorporated into the PT materials with the other additives. The target mass of tracers was calculated to yield a minimum of 400 particles (of any type/color) per test item. Details for type and mass of base feed material, type and mass of Microtracers, average calculated test item mass, and estimated number of tracers particles added to the bulk and added per item are provided in Table 2.

Materials A, C, D, and E were composed of a base feed material and one or more smaller mass additives. The prescribed weight of each of the Microtracers FS Red No. 40 and Microtracers FS Blue No. 1 was initially blended into one of the additives. The PT materials were then prepared by proportionally blending the additives into the base feed material using the Sepor splitter. The entire mass of the blended PT item was then comminuted to reduce particle sizes using a Hosokawa Micron Powder Systems Mikro Bantam Hammer and Screen Mill. The ground material was processed through the Sepor Rotary Sample Splitter to produce the final test items.

Material B was blended with additives and Microtracers FS Red No. 40 and Microtracers FS Blue No. 1 in a similar manner; however, this blended material was not comminuted before the final processing into test items because comminution was not deemed necessary to control fundamental sampling error.

To generate Material F, Microtracers FS Red No. 40 and Microtracers FS Blue No. 1 were blended into an estimated 0.5 kg portion of the base material. The portion containing the tracers was then added back to the bulk poultry feed. The entire mass was processed through the Sepor Rotary Sample Splitter two times, recombining the splits each time, before the final processing into test items. Material F was of a sufficiently small particle size material for which no comminution was required to control fundamental sampling error.

The final bulk materials (blended ground or blended unground) were portioned using the Sepor Rotary Sample Splitter. The entire mass of material was processed through the splitter in multiple passes as necessary to process the entire mass of bulk material and produce the desired number of true subsample test items. The test items were transferred to labeled polyethylene bags and heat sealed.

During the final splitting process for each of the six materials, a test item was randomly selected at roughly regular intervals to obtain the 12 test items for tracer counts. The selected test items were labeled, packaged, and shipped to Microtracers, Inc., in San Francisco, CA.

Tracer Recovery and Counting

Upon receipt at Microtracers, Inc., the test items were individually weighed, and tracer particles were recovered and counted. Microtracers FS particles were isolated from the six feed materials magnetically using a Rotary Detector magnetic separator (MTSE German-made model). Recovered tracers were placed on large filter papers wetted with 50% ethanol solution to dissolve the dyes and yield colored spots. Individual tracer particles produced colored spots on the filter papers, which were dried and the spots counted. When the total number of counts exceeded 200, they exceed the number that can be reasonably counted on one test filter paper; therefore, counts were obtained from three subsamples, and the counts were numerically summed to obtain the total for each test item.

Particle Size Distribution

Particle size distribution was determined at Microtracers, Inc. for one test item of each of the six PT materials following Standard Test Method for Sieve Analysis (ASTM C136 – 06). Approximately 30 g of materials A, B, C, D, and E and approximately 40 g of material F were sieved using five sieves

Table 2. Proficiency testing materials

AAFCO round	Type of material	Total mass, kg	Microtracer type	Microtracer, g	Est. total particles added	Avg. calculated mass, g	Approx. particles/pkg	Point of addition[a]
A	Poultry feed	118	FS Red No. 40	3	150 000	308	390	1
			FS Blue No. 1	3	150 000	308	390	1
B	Milk replacer	95	FS Red No. 40	3	150 000	305	480	2
			FS Blue No. 1	3	150 000	305	480	2
C	Beef feed, medicated	90.8	FS Red No. 40	3	150 000	297	490	1
			FS Blue No. 1	3	150 000	297	490	1
D	Swine feed, medicated	116	FS Red No. 40	3	150 000	303	390	1
			FS Blue No. 1	3	150 000	303	390	1
E	Dairy beef feed	90.8	FS Red No. 40	3	150 000	297	490	1
			FS Blue No. 1	3	150 000	297	490	1
F	Poultry feed	7.2	FS Red No. 40	0.65	32 500	100	450	3
			FS Blue No. 1	0.65	32 500	100	450	3

[a] 1 = Added with additives before milling; 2 = Added with additives before splitting, not milled; and 3 = Added with minerals spikes before splitting, not milled.

and a receiving pan. Sieves used were ASTM 11 Standard Sieve Numbers 40, 100, 120, 140, and 200. The sieves were assembled in top of each other in ascending order, and the material (test portion) was added to the top sieve (Standard Sieve Number 40).

Sieves were shaken using a horizontal sieve shaker (Humboldt Manufacturing Co.) with a uniform, horizontal circular motion (120 RPM) for 20 min. After shaking, the contents of each pan were weighed and the mass expressed as percent of the test portion mass.

Statistical Calculations and Treatments

Raw counts per test item for Microtracers FS Red No. 40 and Microtracers FS Blue No. 1 were performed, and an average and RSD of counts were calculated. The two colors of tracers were initially incorporated as quality control duplicates. However, to increase the confidence in the counting (i.e., higher counts = higher precision), red and blue counts were also combined, and an average and RSD of total counts were calculated. Counts were expressed as a "concentration" by expressing results on a count-per-mass basis.

In addition to the average and RSD, the Pearson's Chi-square *P* value was calculated for total counts using 10 degrees of freedom (12-2), sample counts, X_i, counts average, $\bar{X}$, and the table of Pearson's Chi-squared probabilities (Equation 1):

$$\sum_{i=1}^{N}\frac{\left(X_i-\bar{X}\right)^2}{\bar{X}} \quad (1)$$

Results

The test item mass, raw counts for Microtracers FS Red No. 40, Microtracers FS Blue No. 1, and total raw counts (red and blue combined), tracer counts expressed on mass basis for Microtracers FS Red No. 40, Microtracers FS Blue No. 1, and total tracer counts (red and blue combined) are reported for each PT item tested as follows: in Table 3 for Material A, poultry feed; in Table 4 for Material B, milk replacer; in Table 5 for Material C, beef feed; in Table 6 for Material D, swine feed; in Table 7 for dairy beef feed; and in Table 8 for poultry feed. The respective averages, SDs, and RSDs for each of these metrics are also reported in Tables 3–8.

A summary of the average test item mass, test item mass RSD, average total tracer count, tracer count RSD, and Chi-square *P* value are reported for the six materials in Table 9.

Particle Size Distribution

Results of particle size distribution (sieve testing) for each of the six materials is provided in Table 10. The Microtracers were predominately captured between the size 100 and 40 sieves (in the range of 0.149–0.420 mm) as expected. Materials A, C, D, E, and F were predominately captured between the size 120 and 40 sieves (in the range of 0.125–0.420 mm). Material B demonstrated the widest spread in particle size range, with a fairly even spread over the entire range and the largest percentage of both coarser particles and finer particles.

Discussion

Effect of Material Particle Size Distribution

Comparison of particle size distribution for two Microtracers and six PT materials (see Figure 1), as presented in Table 10, suggest segregation between the components of the mixture should be relatively moderate and are consistent with low RSDs for counts reported in Table 9. It is interesting to note that the highest RSD (4.1%) for tracer counts and lowest *P* value (0.0097) was for Material B, Milk Replacer, which also had the highest percentage of particles >0.420 mm and the widest spread in particle size distribution. These "less uniform" findings could be partially explained by some minor signs of segregation for Material B: the material has a relatively high contribution of coarse fraction + 40 mesh (larger than 0.42 mm).

Table 3. Material A: poultry

Sample ID	Actual test item mass, g	Raw count, red	Raw count, blue	Total raw count	Particle count, red[a]	Particle count, blue[a]	Particle count, total[a]
1	300	560	549	1109	572	560	1132
2	310	591	621	1212	584	613	1197
3	315	626	604	1230	609	587	1196
4	300	624	561	1185	637	573	1210
5	315	573	567	1140	557	551	1108
6	305	564	584	1148	566	586	1153
7	300	631	576	1207	644	588	1232
8	320	630	628	1258	603	601	1204
9	305	594	635	1229	596	638	1234
10	305	634	645	1279	637	648	1284
11	305	580	568	1148	582	570	1153
12	295	571	555	1126	593	576	1169
Average	306	598	591	1189	598	591	1189
SD	7.42	28.9	33.9	54.9	28.8	29.4	49.1
RSD, %	2.42	4.84	5.73	4.62	4.81	4.98	4.13

[a] Particle count = (hand count × average test item mass)/ test item mass.

Table 4. Material B: milk replacer

Sample ID	Actual test item mass, g	Raw count, red	Raw count, blue	Total raw count	Particle count, red[a]	Particle count, blue[a]	Particle count, total[a]
1	290	618	564	1182	647	590	1237
2	294	606	540	1146	626	558	1184
3	302	638	564	1202	642	568	1210
4	308	619	606	1225	611	598	1208
5	298	642	645	1287	655	658	1313
6	308	648	636	1284	638	626	1264
7	306	659	634	1293	655	630	1284
8	303	693	578	1271	694	578	1272
9	310	620	586	1206	607	574	1181
10	305	661	607	1268	658	604	1262
11	306	724	628	1352	720	624	1344
12	314	739	602	1341	714	581	1295
Average	303	656	599	1255	655	599	1254
SD	6.91	42.8	33.3	63.1	36.6	30.1	51.5
RSD, %	2.28	6.52	5.56	5.03	5.58	5.02	4.11

[a] Particle count = (hand count × average test item mass)/ test item mass.

Table 5. Material C: beef feed, medicated

Sample ID	Actual test item mass, g	Raw count, red	Raw count, blue	Total raw count	Particle count, red[a]	Particle count, blue[a]	Particle count, total[a]
1	294	614	591	1205	601	579	1180
2	292	606	574	1180	598	567	1165
3	285	594	575	1169	600	581	1181
4	290	567	551	1118	564	548	1112
5	283	584	551	1135	595	561	1156
6	278	566	556	1122	585	575	1160
7	283	575	531	1106	584	539	1123
8	299	618	588	1206	595	566	1162
9	288	627	576	1203	628	577	1205
10	287	617	592	1209	618	593	1212
11	288	644	579	1223	643	579	1222
12	288	637	573	1210	636	572	1208
Average	288	604	570	1174	604	570	1174
SD	5.39	26.70	18.72	42.4	23.21	14.82	34.48
RSD, %	1.87	4.42	3.29	3.61	3.84	2.60	2.94

[a] Particle count = (hand count × average test item mass)/ test item mass.

Application of Microtracers in Validating the Splitting Process

Incorporating tracers into multiple PT production runs of representative PT materials can be used to draw a general conclusion about the efficacy of the splitting process. The tracers were incorporated into six AAFCO production runs in a similar manner, but some of the materials were ground after the tracer incorporation and some were not. It might be argued that these preparations are not identical and should not be compared, but all preparations are representative of the process used to generate AAFCO PT materials. Six production runs are probably minimally sufficient replication, but the general process has been illustrated with this study.

Application of Microtracers as Quality Control Event for the Preparation of a Specific Test Item

When incorporating tracers as a quality control event to evaluate a specific production run of PT items, two independent statistical approaches (RSD of counts and *P* value) were evaluated to compare results from statistical approaches. Results

Table 6. Material D: swine feed, medicated

Sample ID	Actual test item mass, g	Raw count, red	Raw count, blue	Total raw count	Particle count, red[a]	Particle count, blue[a]	Particle count, total[a]
1	314	567	574	1141	538	544	1082
2	301	597	578	1175	590	571	1161
3	295	614	591	1205	619	595	1214
4	309	562	565	1127	540	543	1083
5	303	636	605	1241	623	593	1216
6	308	588	572	1160	568	553	1121
7	300	622	529	1151	617	525	1141
8	299	620	570	1190	617	567	1184
9	263	543	514	1057	615	582	1196
10	289	551	533	1084	566	548	1114
11	294	627	541	1168	633	547	1180
12	293	591	539	1130	600	547	1147
Average	297	593	559	1152	594	560	1153
SD	13.04	31.57	27.59	50.4	33.15	22.02	46.80
RSD, %	4.39	5.32	4.93	4.37	5.58	3.94	4.06

[a] Particle count = (hand count × average test item mass)/ test item mass.

Table 7. Material E: dairy beef feed

Sample ID	Actual test item mass, g	Raw count, red	Raw count, blue	Total raw count	Particle count, red[a]	Particle count, blue[a]	Particle count, total[a]
1	295	646	555	1201	636	546	1182
2	294	638	555	1193	629	547	1176
3	287	605	585	1190	613	592	1205
4	296	620	609	1229	608	597	1205
5	281	589	560	1149	609	579	1188
6	279	550	527	1077	572	548	1120
7	285	606	612	1218	617	623	1240
8	296	570	543	1113	558	532	1090
9	288	597	598	1195	601	602	1203
10	281	595	546	1141	614	563	1177
11	303	652	592	1244	625	567	1192
12	298	654	598	1252	638	583	1221
Average	290	610	573	1184	610	573	1183
SD	7.75	32.97	28.81	53.5	23.96	27.39	41.39
RSD, %	2.67	5.40	5.03	4.52	3.93	4.78	3.50

[a] Particle count = (hand count × average test item mass)/ test item mass.

for red and blue tracers individually are reported in Table 11, and results for red and blue tracers combined are reported in Table 12.

When tracer colors were counted separately, RSD tended to increase for both red and blue tracers as expected from the Poisson statistics (Table 1). Furthermore, experimental data for all particle counts obtained from this study corresponded well with RSD values predicted by Poisson statistics (Table 1).

Conclusions

Microtracers have the potential to be used to supplement analytical testing in establishing uniformity of PT items. They are inexpensive to incorporate. Recovery and counting can be completed very quickly, so results can be available to make decisions before shipment. Incorporation of tracers can be a first step to assure uniformity of splits, alerting the program to splitting problems. Microtracers incorporation is a challenging test of uniformity, so if the materials pass the uniformity test, a PT program can be reasonably sure that splitting processes were acceptable. The authors conclude that there is no need to incorporate two types of tracers, but that type(s) and number incorporated should be based on the program objectives or criteria.

The incorporation of Microtracers has two applications for proficiency testing programs. The first application, as presented here, is the validation of the splitting procedure used to generate test items. For this application, splitting variance over multiple

Table 8. Material F: poultry feed

Sample ID	Actual test item mass, g	Raw count, red	Raw count, blue	Total raw count	Particle count, red[a]	Particle count, blue[a]	Particle count, total[a]
1	103	833	702	1535	841	709	1550
2	105	892	701	1593	889	698	1587
3	107	905	770	1675	878	747	1625
4	101	855	721	1576	878	740	1618
5	107	864	747	1611	841	727	1569
6	108	883	769	1652	854	744	1598
7	100	802	750	1552	832	778	1611
8	107	868	749	1617	848	731	1579
9	107	817	706	1523	799	690	1489
10	101	844	713	1557	874	738	1613
11	101	843	733	1576	872	758	1631
12	104	884	694	1578	886	696	1582
Average	104	858	730	1587	858	730	1588
SD	2.87	31.15	27.04	45.5	26.76	27.01	39.47
RSD, %	2.76	3.63	3.71	2.86	3.12	3.70	2.49

[a] Particle count = (hand count × average test item mass)/ test item mass.

Table 9. Summary of mass, particle count, RSD, Chi-square, and Poisson probability for AAFCO PT items

Round	Type of material	Actual, g	Test item mass, RSD, %	Avg. total tracer count	Total tracer count, RSD, %	Chi-square *P* value
A	Poultry feed	306	2.42	1189	4.13	0.0137
B	Milk replacer	304	2.28	1255	4.11	0.0097
C	Beef feed, medicated[a]	288	1.87	1173	2.94	0.3460
D	Swine feed, medicated	297	4.39	1153	4.06	0.0219
E	Dairy beef feed[a]	290	2.67	1183	3.50	0.1020
F	Poultry feed	104	2.76	1588	2.49	0.3740
Combined C and E	Dairy/beef feed	289	2.12	1178	3.15	0.2170

[a] Prepared from the same materials; can be considered replicates.

Table 10. Particle size distribution by material

	Sieve sizes					
Standard sieve number	+40	–40/+100	–100/+120	–120/+140	–140/+200	–200
Particle size range, mm	>0.420	0.149–0.420	0.125–0.149	0.105–0.125	0.074–0.105	<0.074
Material	Particle size distribution, % of total mass					
Microtracer A-13420 (FS-Blue No. 1)	0.00	90.57	6.35	2.67	0.37	0.04
Microtracer AA-797 (FS-Red No. 40)	0.06	81.64	12.70	4.41	1.19	0.00
A, Poultry feed	6.62	40.2	40.22	2.05	5.76	5.15
B, Milk replacer	21.15	24.50	21.00	3.32	14.16	15.87
C, Beef feed	6.45	42.24	9.98	4.16	8.37	27.8
D, Swine feed	3.39	32.33	27.42	6.63	13.83	16.39
E, Dairy beef feed	5.18	38.7	12.06	3.78	10.15	30.13
F, Poultry feed	6.71	40.8	38.28	1.32	2.22	10.67

production runs would be evaluated. The second application would be as a quality control application to make a decision on the acceptability of a specific production run before it is shipped to customers. For this application, either RSD and/or Chi-square statistics could be used (in addition to other data) to determine the acceptability of a newly prepared round of PT items.

Tracers do not have the same physical, chemical, microbiological, or radiological properties of an analyte, so

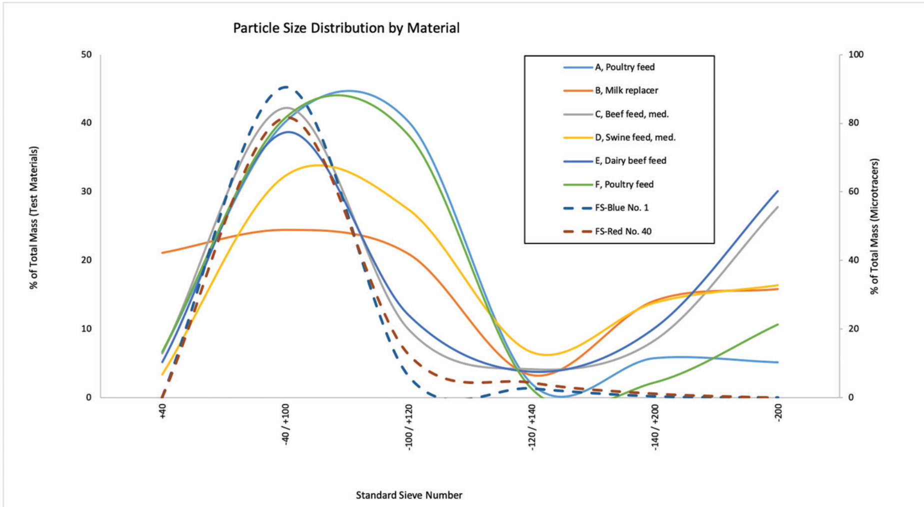

Figure 1. Particle size distribution for PT materials and Microtracer products.

Table 11. Evaluation by red and blue tracers by statistical approach

	Red tracers					Blue tracers				
Material	Avg. Count, red	Particle red counts, RSD, %	RSD pass/ fail based on Table 1	Chi-square *P* value	*P* value pass/ fail based on Table 1	Avg. count, blue	Particle blue counts, RSD, %	RSD pass/ fail based on Table 1	Chi-square *P* value	*P* value pass/fail
A, Poultry feed	598	4.81	Pass	0.1209	Pass	591	4.98	Pass	0.0909	Pass
B, Milk replacer	656	5.56	Pass	0.0122	Marginal	599	5.02	Pass	0.0841	Pass
C, Beef feed	604	3.84	Pass	0.4608	Pass	570	2.60	Pass	0.9397	Pass
D, Swine feed	594	5.58	Pass	0.0266	Marginal	560	3.94	Pass	0.4935	Pass
E, Dairy beef feed	610	3.93	Pass	0.4048	Pass	573	4.78	Pass	0.1551	Pass
F, Poultry feed	858	3.12	Pass	0.5136	Pass	730	3.70	Pass	0.3639	Pass
Combined C and E, beef/dairy beef feed	1214	3.22	Pass	0.1817	Pass	1143	2.12	Pass	0.8421	Pass

Table 12. Evaluation by material for combined counts by approach

Material	Avg. total raw count	Total particle count, RSD, %	RSD pass/fail	Chi-square *P* value	*P* value pass/fail
A, Poultry feed	1189	4.13	Pass	0.0137	Marginal
B, Milk replacer	1255	4.11	Pass	0.0097	Fail
C, Beef feed	1174	2.94	Pass	0.3460	Pass
D, Swine feed	1152	4.06	Pass	0.0219	Marginal
E, Dairy beef feed	1184	3.50	Pass	0.1020	Pass
F, Poultry feed	1588	2.49	Pass	0.3740	Pass
Combined C and E, beef/dairy beef feed	1178	3.15	Pass	0.2170	Pass

they may not behave the same as all the analytes of interest and will mimic some analytes more than others. However, each analyte has different characteristics as well, so testing one analyte cannot be extrapolated to others with different properties. If a few grams of tracers can be incorporated into 100 kg of material and successfully split into 400 test items, it is a good validation of the splitting protocol.

Programs must first establish that Microtracers are appropriate for their materials. If appropriate, the proper tracer needs to be selected, the proper statistics established, and specific pass/fail criteria established fit for the intended purpose.

Though Microtracers have been shown to be a good tool for evaluation of splitting performance, other techniques should not be abandoned. One other very simple but effective technique that could be used is evaluating the variance of test item masses. If masses are not consistent within predetermined acceptable limits, additional "homogeneity testing" or quality control is probably not warranted. The RSDs of AAFCO PT item masses varied between 1.87 and 2.76% with the exception of Test Item D, for which a RSD of 4.39% was observed. Masses should be collected for random items at every production run, and the PT program should establish limits on acceptable variation in item mass.

The authors offer the following suggestions for PT programs:

(*1*) Random weights should be recorded as a routine QC and evaluated for every production run, charted and assessed for acceptability, and evaluated for trends.

(*2*) Tracers, such as Microtracer FS tracers, have potential for process validation. They can be incorporated into materials used to prepare PT rounds and evaluated in a set of randomly selected PT items to make up the validation set, and counts can be used to draw conclusions on the general acceptability of the splitting process (process validation).

(*3*) Tracers, such as Microtracer FS tracers, may be incorporated as a routine quality control event to monitor the uniformity of splits within a production run. Evaluation of the current production run against historical production runs provide a mechanism to monitor for out-of-control processes and trends.

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