Memorandum

To: Eric Hauck, Martin Nielsen

From: Paul Slusarewicz

Date: August 1, 2018

Subject: Modified McMaster

Dear Eric,

As requested, here is a summary of what we discovered over the past day or two during your visit to ▮▮▮ in ▮▮▮▮▮▮.

It became apparent that ▮▮▮ is not using the AAEP recommend McMaster test, but rather a "modified McMaster" method sold as a kit by the Chalex Corporation under the tradename "Paracount". The major difference in the two methods is Paracount's elimination of a critical filtration step from the AAEP method.

The purpose of the filtration is to remove the bulk of the large fecal debris to make both sample pipetting and counting easier. Filtration has been used since the inception of the McMaster over 80 years ago, but it is cumbersome and dirty to perform, which is perhaps why Chalex markets Paracount as being "convenient".

However, convenience does come at a cost which, *en face*, is the extra time taken to dispense the samples into a McMaster slide due to pipette clogging, and the extra time taken to count the slide (because of the murkiness caused by the extra debris, see below). More seriously, however, are the inherent possible unknown effects that any modification may have on both assay performance and final results. To our knowledge, the Paracount (nor, for that matter, any other "modified McMaster" method) has never been validated against the AAEP method (which *is* the actual McMaster method) by either the manufacturer or ▮▮▮. This is not surprising because, in our experience, it is common practice in the field to make changes to the method for convenience, speed, cost, etc. and to merely assume on pure faith that the change has not affected the results. Therefore no one, anywhere, has any basis to claim that their modified method is in any way comparable or superior to the AAEP method except, of course, Parasight.

Yesterday, Paracount returned a value of 2000 EPG on a sample at ▮▮▮ when the Parasight system was giving 600 EPG, a difference of 3.3-fold. Concurrently, I ran a sample at the Gluck that had a count of 2000 EPG by both the AAEP McMaster and the Parasight system using the

Paracount method. Paracount produced an egg count of 5125, an increase of 2.5-fold, which was perfectly consistent to the differences being seen at ▮▮ between Paracount and Parasight. In addition, the Paracount method took substantially longer to analyze the slide. After over four years counting slides almost every day, I can analyze two grids of even a high-count sample in two to three minutes under 40x magnification. In the case of the Paracount method, the murkiness of the sample forced me to move through the slide very slowly to ensure that I did not miss eggs, and it took me over 5 minutes to properly count just a single grid. Clearly, the time saved in obviating filtration comes at the cost of having to take even more time in the downstream counting process, at least if one is careful about what one is doing. In our validation study, analysts in Martin Nielsen's lab could process samples twice as fast with Parasight than with the AAEP McMaster. I would not be surprised if this had jumped to 3-4 times faster had they been using the Paracount system because of the huge increase in counting time.

These, albeit preliminary, data suggest that filtration removes a large number of eggs and implies that Paracount consistently overcounts eggs relative to the AAEP McMaster. This would result in relative overuse of anthelminthics compared to a practice that is using the actual AAEP method, because, for example, a sample with a 100 EPG egg count by the AAEP method, which is below the AAEP treatment threshold, may give a result of 250-300 with Paracount, which would result in treatment if following the AAEP guidelines. It is interesting to note that Rood and Riddle, who use the full AAEP method, have found good agreement between their McMaster results and Parasight and have now adopted Parasight as their primary egg counting method because of the huge time savings afforded to their analysts.

In addition to accuracy, the effects of method modifications on other assay performance parameters such as precision are unknowable without conducting a formal study. This has been done for the Parasight system, which has been shown to be more precise than the AAEP McMaster (meaning that any given two measurements on the same sample are more likely to be closer together using Parasight than the AAEP method). Without carrying out a full validation, it is impossible to know how well the Paracount system compares in this respect, and so how reliable its results are from count to count, nor how much more or less prone it is to inter- and intra-user variability. Thus, in the case of a discrepancy between an ▮▮ McMaster and a Parasight result, it is impossible to know with any confidence which is more likely to be the "correct one". However, since the Parasight method *has* been validated, and Paracount has not, it would be unwise at this stage to merely assume that Paracount result is correct and that the Parasight one is in error.

This general blasé attitude to modifying the McMaster has been a pet peeve of Martin Nielsen's ever since I have known him. It appears that far too many practices/owners are using their own

variants of the McMaster that have in no way of knowing whether their results are comparable to the AAEP for any of several performance parameters. As a result, we are now in a position of having people out there using dozens, if not hundreds, of versions of the McMaster, with each user convinced that their particular version gives the "correct" answer despite the lack of even the smallest shred of scientific evidence to support that belief. However, the Parasight system *has* been validated and calibrated to give results equivalent to those obtained by trained analysts at the Gluck Equine Research Center, and in addition shown to be superior in a number of other ways. Thus, it would seem unwise in the extreme to assume that any difference found between a Parasight count and one from any modified McMaster is due to a problem with Parasight.

In summary, the only way, at present, that anyone would have to be scientifically confident in their results if they are *not* using the AAEP or the Parasight systems is to conduct their own validation study. It is our experience that this is never been the case.

I hope that this covers everything, but please feel free to let me know if you have any questions.

Paul.