Talc Roundtable Panel Discussion Questions

(Talc Methods Expert Panel Discussions)

1. **What work has been done to evaluate the overall accuracy and precision of the method?**

The Round Robin attempts to address both precision and accuracy according to USP <1225> VALIDATION OF COMPENDIAL PROCEDURES.

To determine precision, we quantitatively created spiked samples with known amounts of analyte at 5 different levels:

- 0%
- 0.0001% = 1 ppm
- 0.001% = 10 ppm
- 0.01% = 100 ppm
- 0.1% = 1000 ppm

Although some labs detected down to 0.001% (= 10 ppm), this was not shown to be reproducible so while a result can be said to be present at this level, a negative does not necessarily mean asbestos is not present at levels below this. Because the majority of labs detected the 0.01% (= 100 ppm), this was determined to be the method detection limit for a presence/absence type of test even though detection was achievable below this limit. Note that this test will not attempt to quantify results but will list presence or absence at a level of 0.01% (=100 ppm).

To determine accuracy (i.e. is the analyte identified accurately), spiked standards were created with three different types of analyte, two asbestiform and one non-asbestiform (chrysotile, tremolite asbestos, and non-asbestiform tremolite, respectively). We found that the labs showed 100% accuracy in distinguishing asbestos from non-asbestiform materials. Chrysotile was accurately identified in all cases as well. For amphibole asbestos, one lab identified anthophyllite asbestos instead of tremolite asbestos (likely due to anomalous extinction angles displayed by asbestos). In this case, amphibole asbestos was accurately identified, but the type of amphibole asbestos was not correctly identified. To resolve this, the USP test will not require speciation of amphibole, just presence of amphibole asbestos.

2. **Do the Panel members agree that the three proposed methods will quantify zero asbestos in pharmaceutical excipients?**

No analytical test can prove a negative. The best we can do is design a method that has a detection limit as defined in EPA 600/R-93/116: “The smallest concentration/amount of some component of interest that can be measured by a single measurement with a stated level of confidence.” Confidence in the measurement has to do with three things: 1) reproducibility, 2) statistical significance (i.e. not contamination or “background,” and 3) accurate interpretation. At diminishing levels of occurrence, all three of these factors become more substantial. The data collected from the Round Robin indicate that the detection level of the USP Phase I proposal is 0.01% (= 100 ppm).

3. **Can the Panel explain the logic underlying the two-phase proposal?**

The two phase approach deals with analysis of two different size ranges of the material: 1) the >5 µm size range (Phase I), and 2) the finer size range (Phase II). These are considered complementary

protocols. The >5 µm size fraction covers the bulk of a talc product (the majority of the particle size range of a cosmetic grade talc product and at least half of the particle size range of the finest grades of talc).

There is no reason to believe that asbestos would occur only in the <5µm size fraction of a natural milled talc/asbestos mixture; in fact, some research shows it is more prevalent in the largest size ranges, even when present in trace quantities. In addition to this, results from the Phase I protocol are more definitive for identification (have less interference from non-asbestos particles). It was also shown that the larger particles used for identification exist in an aggressively milled talc/asbestos mixture, even for trace quantities of asbestos as low as 0.001% (10 ppm).

Phase II protocol will deal with techniques to analyze the finest size fraction of a talc product and will include electron microscopy. Although there are differences in the size ranges for the different methods employed in the two phases, each method has its strengths and weaknesses. That is why a complementary approach is recommended. At present, the method detection level will be stated as 0.01% (= 100 ppm). If Phase II evaluation indicates a difference in detection level is warranted, an additional recommendation will take place. More details about the Phased approach can be found in the PLM presentation.

4. **Further, does the Panel agree that the two-phase proposal for excipients can be used for analyzing cosmetic products and cosmetic talc in particular?**

Talc ores may be used in personal care, pharmaceutical and industrial applications. However, this Talc panel focuses on developing and validating a method for talc used for pharmaceutical products. Talc products used for cosmetic applications are coarser than talc products used for other applications (plastics, paint, paper, rubber, ceramics, etc.). An example in the round robin study showed successful analysis of the finest of grades of talc using technologies in Phase I and Phase II protocol, a natural ore that contained a trace level of asbestos. In this analysis, it was shown that both Phase I and Phase II protocols indicated asbestos was present. However, as per 21 CFR 211.194(a)(2) of the current Good Manufacturing Practice regulations, it states that the "suitability of all testing methods used shall be verified under actual conditions of use."

5. **Does the Panel support the development of consensus standards, like ASTM D22.07, for analysis of talc in both pharmaceuticals and cosmetics?**

Yes. ASTM International is a consensus body whose Subcommittee D22.07 (Sampling, Analysis, Management of Asbestos, and Other Microscopic Particles) has more than 150 members from all stakeholder groups. For several years, D22.07 has been developing a suite of methods for analyzing asbestos in talc. This suite includes XRD, PLM, and TEM. SEM could be added at a later time. Several members of this USP Talc Methods Expert Panel are also ASTM D22.07 members. Nonetheless, some type of formalized collaboration would be necessary between the two organizations, but that decision would be made at higher levels in both USP and ASTM .

6. **Has the Panel established consensus definitions for asbestos and talc?**

Yes. The Panel voted early in its development to use existing definitions for how to recognize asbestiform in the light microscope. The agreed upon definition exists in the most widely used PLM

method by commercial labs analyzing bulk building materials for asbestos, that of the EPA 600/R-93-116 method.

7. **What reference materials can be established for asbestos and talc and how will they be made available?**

1) Under Expert Panel#2 -Phase I, there have not been detailed discussions on reference materials development
2) Expert Panel#3 will handle reference materials development for Phase I and Phase 2 methodologies

8. **How does the Panel envision the two-phase proposal will be used to sample bulk quantities of talc for asbestos?**

First, note that the proposed USP method is for *already ground* talc and does not address ore-type samples. However, the principles in these methods could be used for ore-type samples as well.

Second, for large quantities of already ground talc products sampling is an issue, as with any analytical method. This is especially a problem with methods that use smaller starting amounts that are intended to represent large quantities of material. The more material that is used for the analysis the more representative will be the analysis. The most material used for analysis is for XRD, followed by PLM, SEM, and TEM (in that order). Attempts should be made to avoid bias in the sampling. It is recommended for the PLM that 5 separate slide preparations be made in attempt to avoid bias (this will be between 5 and 10 mg of material analyzed). The sieve preparation method also increases the starting amount that is sampled for the PLM analysis. Starting material for the sieve method is 2 g. From that, only a small portion is retained on the 400 mesh sieve (~10 %), and from this several mg subsamples are taken for each slide prep.

9. **Regarding testing accuracy: What is the false positive rate? What is the false negative rate? Is there a way to guard against a false positive or a false negative?**

There were two false positives in the PLM Round Robin, each involved detection of a single particle of chrysotile in a sample that was not intentionally spiked with chrysotile. It was recognized that cross contamination and/or other background contamination (especially chrysotile) could occur. This is why laboratories have programs for analysis of blanks. For the USP Round Robin (0.01% level), all analysts noted multiple particles that were identified (between 4 and 8 particles/mg with typically 1 – 2 mg analyzed in total = 8 – 16 particles per analysis). Because of this and because the Expert Panel agreed that a positive result cannot be concluded based on a single particle (as it cannot be proven different than from contamination/background), there can be confidence that false positives are not likely.

Regarding false negatives, some of the labs detected asbestos at the 0.001% level but this was not as consistent as results for the 0.01% level. Therefore, the 0.01% level will be used as the method detection level to guard against false negatives. Because the method detection level is set higher than some individual tests, this is assurance that false negatives are unlikely.

10. **Is the overall design of the testing presumptive or confirmatory?**

An actual round robin was performed using materials spiked with known standard research materials at known concentrations that bracket the method detection level. Therefore, the conclusions obtained about the methods and analyses are based on actual data and are not presumptive. There were five participating labs performing the analysis in a double-blind study so these results and conclusions (at least for Phase I) should be considered confirmatory.

11. **Will there be guidance provided on how a laboratory should report the instance of a positive result on any single test?**

For XRD, if amphibole would be detected at > 0.2% or serpentine would be detected at > 0.2% or 1% (if chlorite present) on the single analysis, then the analyst would need to report accordingly.

However, based on QUESTION 13 **Should a single positive result be confirmed through replicate testing or testing by a second laboratory to confirm findings?**

It would be recommended to the analyst/laboratory to take two new sub-samples for evaluation to generate a total of three replicates. If two of three replicates have detectable amphibole or serpentine report accordingly at > 0.2% amphibole or serpentine (or >1% serpentine if chlorite present). If two or three replicates do not have detectable amphibole or serpentine then report none detected < 0.2% amphibole or serpentine (or none detected < 1% serpentine if chlorite present). The final decision for the acceptance criteria will be the responsibility of the Excipients Expert Committee.

12. **Will there be a minimum number of fibers required to report a positive finding?**

Note that the recommended protocol is to report all particles regardless of the final conclusion. See discussion of #11. More than one particle (with conclusive ID) is considered a positive finding. While one single particle can be a conclusive ID for asbestos, it does not constitute a positive finding (as not different than contamination or background).

If the ID is not conclusive (i.e. particles that do not show conclusive asbestiform characteristics), 5 (five) particles are needed to evaluate the population. If the result of that analysis complies with the definition of EPA 600/R-93-116), then it is a finding of asbestos present. All results below that are reported as <0.01% (=100 ppm).

The results of the labs participating in the Round Robin showed 100% agreement on whether a population was conclusive or not. All participating labs reported finding MULTIPLE asbestos particles in the 5 slide preps. But, if only one particle per 5 slide preps is noted, the particle is reported, just the result of the test for asbestos will be reported as below the LOD of <100ppm.

13. **Should a single positive result be confirmed through replicate testing or testing by a second laboratory to confirm findings?**

For XRD, replicates see Question 11

A single finding of 0.01% or greater carries with it the confidence that this is a reproducible result in agreement with the definition of “detection limit” from EPA 600/R-93-116. We emphasize that this

finding will be a result of approximately 8- 16 particles per analysis. Therefore, it is appropriate that this result can stand alone. Duplicate testing is always a good idea for good laboratory practices; however.

14. **Different test methods have different analytical sensitivity. How does USP intend to determine a detection limit for TEM?**

Analytical sensitivity is not the same as empirical detection limit evaluation. The USP Talc Expert Panel plans to conduct empirical studies to determine the detection limit of TEM when the Phase II evaluation takes place.

15. **How would the Panel address the reproducibility issue given the small sample sizes used for TEM testing?**

TEM was only a minor topic of discussion in Phase I where the focus was on XRD and PLM.

From a broad perspective, subsampling of particles is always a challenge because of possible inhomogeneity of the material being sampled. In general, as subsample size versus original sample size decreases, the probability of the subsample not matching the composition of the original sample size increases. A subsample that does not yield a result consistent with the larger (parent) sample can yield either an underestimation or an overestimation of actual asbestos present.

In the case of TEM's underestimation as drastic as "None detected", this would be no more consequential than the previous "None detected" from the preceding PLM analysis of that sample. In the case of overestimation by TEM, the conclusion would be "Asbestos present", which is consistent with the desired detection of asbestos in talc.

TEM sample size will almost certainly be thoroughly explored during Phase II.

16. **Why is USP considering TEM when no other bulk methods rely on TEM? Has the Panel considered the possible downsides of using TEM? Is it always a practical or even possible method?**

Actually, several bulk methods include TEM as an alternative or complementary method:

The 1993 EPA bulk method, which is the backbone of the proposed USP PLM method, has a section devoted to TEM analysis of bulk materials.

ISO 22262-1 (Sampling and qualitative determination of asbestos in commercial bulk materials) has TEM as an alternative to PLM.

ISO 22262-2 (Quantitative determination of asbestos by gravimetric and microscopical methods) also has TEM as an alternative to PLM.

NY ELAP 198.4 is a TEM method for analyzing non-friable organically bound bulk samples.

Concerning the last two parts of this question, it is expected that the downsides and practicality of TEM will be evaluated in Phase II.

17. **With the definitions of "asbestos" being so different in the U.S. and Europe, how does the Panel propose dealing with the harmonization issues?**

Harmonization is always a goal with USP. However, there are harmonized attributes and non-harmonized attributes that exist within the various monographs. The Expert Panel has provided recommendations for the United States Pharmacopeia. It is our hope that this would be adopted globally, but the conclusions we reached are independent of whether global harmonization is possible.

18. **What techniques are being used to ensure a distinction can be made in the testing of talc to ensure an identified amphibole is characterized as to whether it crystalized in the asbestiform habit? In other words, how will the Panel ensure an amphibole that did not crystalize in the asbestiform habit is not mistakenly determined to be asbestos?**

Microscopy methods do not give information about how a particular mineral crystallized; however, we can apply principles set forth in existing methods for how to recognize asbestiform particles in a light microscope. This definition exists in the EPA 600/R-93/116 method and versions of it exist in ISO 22262-1 as well as other methods. We have used these principles in the round robin testing and have shown 100% agreement with asbestiform vs. non-asbestiform populations.

19. **Is it the view of the Panel that a microscopist can determine a sample contains asbestos based on the examination of a single amphibole particle?**

See discussion of #10, #11, and #12. Summary from #12 reproduced here: Note this phase only includes XRD and PLM (not TEM). Note that the recommended protocol is to report all particles regardless of the final conclusion.

Summary: while one particle can be a conclusive ID for asbestos, it does not constitute a positive finding (as not different than contamination or background). More than one particle (with conclusive ID) is considered a positive finding. If the ID is not conclusive (i.e. particles that do not show conclusive asbestiform characteristics), a minimum of 5 particles are needed to qualify as a population as listed in EPA600/R-93/116. If the result of that analysis complies with the definition of EPA 600/R-93-116) then it is a finding of asbestos present. All results below that are reported as <0.01% (=100 ppm). The results of the labs participating in the Round Robin showed 100% agreement on whether a population of fibers was conclusively asbestiform or not.