## 4.1.5 Dilution of Preservative Studies

Historically, the information regarding media volumes used for sterility testing in Section V.A. of the Outline of Production was collected on inspection and provided to the laboratory. Firms would group like products and conduct the studies on representative serials or mock ups.

Currently, studies patterned after SAM 903 are submitted during the licensure process. Firms tend to submit data using serials of the specific product. The amount of media used for the test and the date that data to support the volume was accepted are now noted in the filed Outline of Production.

While firms may be allowed to add up to a certain level of antibiotic within a specified range, serials may not represent that maximum amount. Issues of the dilution of preservative not yielding adequate media amounts have been detected pre and post licensure. Therefore, the dilution of preservative studies should be conducted with serials at the maximum antibiotic level allowed in the range specified in the Outline of Production . This may require spiking the prelicensing serials or adjusting the maximum antibiotic concentration level cited in the Outline of Production.

During the licensure process, the antibiotic concentration of the prelicensing serials should be compared to the maximum amount allowed in the outline and the concentration of the serial(s) used for the dilution of preservative study.

During the confirmatory testing of the prelicensing serials and check tests, an 11$^{th}$ vessel screening test is being conducted. The screening test is described in the following notices:

- CVB Notice 09-02 - entitled "Dilution of Preservative Screening for Broth-based Sterility Tests", dated January 22, 2009.

- CVB Notice 09-25 - entitled "Dilution of Preservative Screening for Plate-Based Sterility Tests", dated December 31, 2009.

This is basically an extra tube or plate that receives the product and the indicator organism. If the 11$^{th}$ vessel shows no growth, the lab conducts a dilution of preservative test in accordance with SAM 903 and 9 CFR 113.25. Therefore, a prelicensing serial or other serial picked for testing may be reported as unsatisfactory for dilution of preservative and a no test for sterility testing conducted in accordance with 9 CFR 113.26/27.

Because of differing opinions on whether dilution of preservative should be conducted for live bacterial products, the following notice was published:

> CVB Notice 12-21 – entitled "Conducting Dilution of Preservative Studies for Live Bacterial Vaccines", dated October 15, 2012.

It indicates: For live bacterial products with antibiotics added, dilutions of preservative studies are required in accordance with 9 CFR 113.25(d). For live bacterial products that lack antibiotics at formulation, dilution of preservative studies will not be required at this time. However, in an effort to evaluate this situation, the CVB laboratory has begun inoculating an extra media vessel for all live bacterial products utilizing the purity test in accordance with 9 CFR 113.27(b). If a lack of indicator organism growth in the extra tube is seen in check or confirmatory testing, this policy will be re-evaluated.

In the case of live *E. coli* products (and potentially others), the *E. .coli* will outgrow the indicator organisms and interfere with their growth almost completely. The use of larger amounts of media does not circumvent this situation. In this case one might view the *E. coli* itself as an indicator organism for validating the media volume.