Reading your First Report for Testing Sheep

This letter explains the BioPRYN® Pregnancy Report and how you can use the information to interpret the results. The BioPRYN test measures the level of placental Pregnancy-Specific Protein B (PSPB) in serum. PSPB is from the placenta and enters the Sheep's blood when a fetus is present. The BioPRYN assay can be reliably applied to detect pregnancy status in ewes 30 days post breeding (dpb) and 73 days since lambing (dsl).

Information is displayed in five columns: **Tube Number; Animal ID; Response in Test, OD; PSPB Range;** and **Days Post Breeding**. The **Response in Test, OD** is a measure of optical density (how much light is absorbed by the sample). The OD signal is correlated to the amount of PSPB in the sample. In each assay, the OD of each sample and eight assay controls (standards) are measured. The standards are used to normalize the OD value of each sample to give a standardized OD output reported in the **Response in Test, OD** column.

The **Response in Test, OD** is compared with previously established ranges for determination of pregnancy category. Samples are categorized in four PSPB Ranges that are typical for sheep: **Not Detectably Pregnant, Low Recheck, High Recheck,** or **Pregnant**. The OD values for the ranges can be found just above the **Tube** and **Animal Numbers**. These PSPB ranges have been shown to be highly consistent with pregnancy status of ruminants when sampling is performed according to the prescribed protocol.

If the **Response in Test, OD** is higher than 0.210, the animal has a level of PSPB that is significantly different from the average non-pregnant animal and is in the range of **Pregnant** animals**;** if **Response in Test, OD** value is lower than 0.135, the animal has a level of PSPB that is significantly different from the average pregnant sheep and is in the range of **Not Detectably Pregnant** animals. The assay is semi-quantitative in a limited range but cannot tell you how many days the sheep is in gestation due to a large amount of sheep to sheep variation in production of PSPB over the pregnancy. However, generally speaking, a sheep that is in the last trimester of pregnancy will read higher (often greater than 0.8) than a sheep at 28-60 days (OD value of 0.210 to 0.600). We categorize **High Recheck** when the **Response in Test, OD** is between 0.150- 0.210 and **Low Recheck** if the **Response in Test, OD** is between 0.135-0.150. A **Recheck** categorization means the animal has a serum level of PSPB that does not allow categorization in the Not Detectably Pregnant or Pregnant ranges. A **High Recheck** animal has a level of PSPB that is slightly greater than a **Low Recheck** animal. Three causes of **Recheck** status include: 1) the sample was taken too early in gestation (prior to 28 days), not allowing a high enough level of PSPB for a **Pregnant** categorization; 2) early embryonic death with decrease in PSPB that has not cleared from the blood at the time of sampling (PSPB clears within 4 days of embryo death when initially categorized in the **Recheck** range); 3) the sample was collected earlier than 73days postpartum and residual PSPB from the previous pregnancy has not cleared from the system. Less than 5% of total samples are categorized in the **Recheck** range when tested according to the prescribed protocol. A follow up confirmatory test (within a week following the original sampling) will allow **Recheck** sheep to attain a level of PSPB that provides a definitive categorization.

If an animal is categorized by BioPRYN in the **Not Detectably Pregnant** range, 99.9% of these animals are Not Pregnant according to other methods of testing when the initial sample is taken at 28 days post breeding or later. The remaining sheep are categorized in the

Pregnant, High Recheck or **Low Recheck** ranges; a small percentage of sheep in these ranges are actually Not Pregnant in follow up testing due to a low amount of residual PSPB from either a previous pregnancy (postpartum less than 73 days) or a new embryo that has recently died. If an animal is categorized **Pregnant,** 93 to 95% are categorized **Pregnant** in follow up testing. As indicated above, an animal in the **High Recheck** or **Low Recheck** ranges can be definitively categorized when sampled a week following the initial test. The **Recheck** categories can assist your herd management by identifying animals with abnormal pregnancy results that otherwise would not be indicated.

We have tuned the BioPRYN assay so the optimum OD response to PSPB concentration is in the range of 0.2 nanograms of PSPB per milliliter (ng/ml) of serum (approximately 0.1 OD units) to 8 ng/ ml of serum (approximately 0.8 OD units). This provides the most accurate pregnancy categorization at 28 days post breeding while providing some information on PSPB levels beyond 70 days post breeding. Above 0.8 OD units (8ng/ml PSPB) the samples are near the maximum value of the assay. As a result, the most consistent information for the relationship between OD and PSPB concentration is between 0.1 OD units and 0.8 OD units. The maximum OD value of different assays will vary slightly, but every sheep that achieves the maximum value within a single assay will be given the same OD value (for example 0.987). **If you are trying to determine the differences in PSPB concentration based on Response in Test, OD, every sheep with a value greater than 0.8 OD units should be considered to have the same OD value.** Due to sheep to sheep differences some sheep achieve this value closer to 70 days in gestation while some do not reach this level until closer to 100 days.

The OD information can still be valuable in your herd management if you keep this concept in mind. Unexpected OD values (PSPB levels) can be an added clinical sign of trouble with the pregnancy. For example, if you send a sample from a sheep near dry-off time and the OD reading is greater than 0.800, the sheep has the expected OD for that stage of gestation. However, if the OD reading is 0.250, while she still exceeds the cutoff and BioPRYN would categorize her in the **Pregnant** range, the OD value is not expected for that stage of gestation. A follow up check with this animal will help clarify her status. The OD value, together with your knowledge of breeding dates and previous experience with a sheep, may be a valuable tool. If the OD is not as expected for a given breeding date, then a follow up check and re-test of the sheep is a choice.

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