

Dear bioPRYN Customer:

This letter explains the bioPRYN<sup>®</sup> Pregnancy Report and how you and your veterinarian 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 cow's blood when a fetus is present.

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. The OD of each sample and eight assay controls (standards) are measured. The standards are used to calculate a **Cutoff** value, and for comparison to the OD values for samples. Each assay plate has a unique cutoff because plates do not perform the same even if they are processed in parallel. Usually, the **Cutoff** is between 0.100 and 0.150.

Based on OD readings, samples are categorized in four PSPB Ranges that are typical for cows: **Open, Open Recheck, Pregnant Recheck,** or **Pregnant**. The OD values for the ranges (as calculated from the standards) 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 the cutoff, 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 the cutoff OD, the animal has a level of PSPB that is significantly different from the average pregnant cow and is in the range of **Open** (Not Pregnant) animals. The assay is NOT quantitative and cannot tell you how many days the cow is in gestation. However, generally speaking, a cow that is in the last trimester of pregnancy will read higher (often near 0.700 to 0.800) than a cow at 30 days (OD value of 0.200 to 0.300). We categorize **Pregnant Recheck** when the **Response in Test, OD** is between 100% and 115% of the **Cutoff** OD value and **Open Recheck** if the **Response in Test, OD** is between 90% and 100% of the **Cutoff** OD value. A **Recheck** categorization means the animal has a serum level of PSPB that does not allow categorization in the Open or Pregnant ranges. Three causes of **Recheck** status include: 1) the sample was taken too early in gestation (prior to 30 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 between 30 and 75 days of gestation); 3) the sample was collected earlier than 90 days 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. A follow up confirmatory test (within a week following the original sampling) will allow **Recheck** cows to attain a level of PSPB that provides a definitive categorization.

If an animal is categorized by bioPRYN in the **Open** range, 99.9% of these animals are open according to other methods of testing. The remaining cows are categorized in the **Pregnant, Pregnant Recheck** or **Open Recheck** ranges; a small percentage of cows in these ranges are actually open in follow up testing because some cows have a low amount of residual PSPB from either a previous pregnancy (postpartum less than 90 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. If an animal is categorized **Pregnant Recheck**, approximately 20% are categorized **Pregnant** in follow up testing. If an animal is categorized **Open Recheck**, approximately 7% are categorized **Pregnant** in follow up testing.

The OD information is valuable in your herd management. Unexpected OD values (PSPB levels) can be an added clinical sign of trouble with the pregnancy. For example, if you send us a sample from a cow near dry-off time and the OD reading is around 0.700 to 0.800, the cow is in a normal range for that stage of gestation. However, if the OD reading was 0.250, while she still exceeds the cutoff and bioPRYN would categorize her in the **Pregnant** range, the OD value is not within the normal range for that stage of gestation. A follow up check with this animal will help clarify her status. Additionally, an exceedingly high OD above the expected OD value of 0.200 to 0.300 in early pregnancy (between days 30 to 75) may indicate that the animal is carrying twins. The OD value, together with your knowledge of breeding dates and previous experience with a cow, 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 cow is a choice.

Thank you for your samples, and if you have any additional questions about the report, please don't hesitate to contact us. Our email address is [biotracking@biotracking.com](mailto:biotracking@biotracking.com) or you can call us at 208.882.9736.

Thanks,

R. Garth Sasser PhD, President