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Dear BioPRYN Customer:

Thank you for your recent submission of samples for BioPRYN testing for Cattle. This letter explains the BioPRYN[®] 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. The BioPRYN assay can be reliably applied to detect pregnancy status in cows and heifers 28 days post breeding (DPB) and 73 days since calving (DSC).

In the report, the 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 cows: **Open, Low Recheck, High Recheck,** or **Pregnant**. The OD values for the ranges can be found just above the **Tube Numbers and Animal IDs, etc**. 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 cow and is in the range of **Open** (Not Pregnant) animals. The assay is semi-quantitative in a limited range but cannot tell you how many days the cow is in gestation due to a large amount of cow to cow variation in production of PSPB over the pregnancy. However, generally speaking, a cow that is in the last trimester of pregnancy will read higher (OD value often greater than 0.8) than a cow 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 Open 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 73 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 when tested according to the prescribed protocol. 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 when the initial sample is taken at 28 days post breeding or later. The remaining cows are categorized in the **Pregnant, High Recheck** or **Low Recheck** ranges; a small percentage of cows in these ranges are actually open 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.

The OD information can also be 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 a sample from a cow near dry-off time and the OD reading is greater than 0.800, the cow 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 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.

Again, thank you for submitting samples for testing. If you have any questions regarding your results, please contact us by email at testinglab@biotracking.com or by phone at 208.882.9736. We look forward to doing business with you in the future.

Thank you,

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