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P.42.- The use of AGID technique to identify the specie purity in Spray Dried Porcine Plasma

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There is currently urgent interest in identifying the species of origin of the components of different animal by-products. In Europe, this interest is expected to increase with authorization of the re-introduction of these proteins into animal feed formulations. The number of validated methods to differentiate the species of origin for most of these products is limited. The objective of this study was to develop an easy method, inexpensive, reliable and accurate to detect the presence of bovine protein (IgG) in porcine plasma before and after spray-drying using the Agar Gel Immunodiffusion (AGID) technique.

Bovine blood and porcine blood were directly obtained from a jugular venipuncture of animals and placed in a container with 0.4% (w/v) sodium tri-poly-phosphate as anticoagulant. In the lab, the obtained bovine blood was mixed using calibrated pipettes with porcine blood at different inclusion levels, from 0 to 1% (v/v). These mixed samples were centrifuged to separate the cellular fraction. The liquid plasma obtained was analyzed before and after spray-drying using a lab spray-drier (Buchi 190 Mini Spray Drier) under the same technical conditions used at industrial level. Alternatively, contaminated plasma samples were obtained after the centrifugation of bovine and porcine blood. Pure bovine plasma was mixed with pure porcine plasma with the help of calibrated pipettes at different levels of inclusion, from 0 to 1% (v/v)

The liquid plasma samples were used directly without dilution. The spray dried plasma were diluted to obtain a 7% (w/v) of protein concentration (similar to liquid plasma) in PBS pH 7.2 buffer. Antibodies used against proteins present in the samples**:** anti-IgG bovine (B-8395 Sigma) and anti-IgG porcine (P-0916 Sigma). Positive and negative standards**:** pure bovine plasma (B-8392 Sigma) and pure porcine plasma (P-2891 Sigma). Detailed description of the AGID technique used can be found elsewhere (Polo et al., 2004. JAOAC). The presence of precipitating proteins indicate specific immune-reaction between the antibody and some proteins in the samples.

The technique developed was able to detect the presence of bovine IgG in porcine plasma at inclusion levels above 0.5% v/v in all cases. No differences were found when cross contamination was simulated before or after whole blood centrifugation.

The method described is reliable and inexpensive, the samples for the analyses are easy to prepare, and they require both minimal laboratory equipment and expertise to detect bovine IgG in porcine blood products at inclusion levels of > 0.5% v/v.

Keywords

Species identification, spray dried porcine plasma, blood products, AGID, bovine IgG identification.