Summary of comparison of advantages and disadvantages of the methods used for the detection of animal proteins in feed.

OFFICIAL METHODS

	Light microscopy	PCR
Advantage	• Low levels of contamination are detectable (<0.1%) • Good sensitivity and specificity • Almost universal basic lab equipment • Inexpensive (few reagents, limited investment) • Qualitative disclosure of contamination (it allows to differentiate prohibited material such as MBM vs authorised • Easiness of implementation • Rapid method • Well documented field of science (books, manuals, image galleries, etc)	• Identification of species and taxonomic groups (e.g. ruminant, pig) • Low levels of contamination are detectable (<0.1%) • Perfect specificity • Rapid method • At lab level, needs good practice but reduced human expertise
Disadvantage	• Needs experienced microscopists • Continuous training to keep skills at the top but also because of the emergence of new feed compounds and animal by-products • No species identification: e.g. the method is unable determine if terrestrial bones originate from bovines, pigs, goats... • Based on particle detection only, some contaminants are not always visible because of manufacturing processes • No quantification possible	• Indirect method: the DNA from the animal product is detected, not the proteins • Inability to distinguish between authorized and prohibited products from a species : a positive signal for bovine can originate from PAPs but also from dairy products • Restrictions in the choice of suitable DNA target sequence: have to be short enough (<100bp) and to originate from multicopy sequences (e.g. mitochondrial DNA) to reach the required sensitivity • Cut-off value specific of the PCR platform (combination of equipment and reagents) and of the target • Needs calibrants • Quantification of the amount (in mass fraction) of animal proteins is impossible

ALTERNATIVE METHODS

	IMMUNO ASSAYS	NIRM	MASS SPECTROMETRY
Advantage	• Commercial kits ready- and easy-to-use • Possibility of automatic readers for results • Field or industry use (quality control) • Detection of proteins	• Method is free from interpretation by an operator: no skills are requested • Non-destructive method : other analysis can be performed on recovered particles for further identification • Low levels of detection (<0.1%) • Sediment from light microscopic method can also be analysed by NIRM • Potential for quantification • Automation possible of all spectral data treatment	• Good sensitivity • High specificity regarding the species AND tissue • Good resistance of the primary structure (amino acid sequence) to heat treatment • Simultaneous analyses for several peptide biomarkers (multitarget method) • Need good practice but reduced human expertise (for targeted approach)
Disadvantage	• ExpensiveLimit of detection not low enough • Screening method, no confirmatory test • False positive and false negative results • No quantification possible	• Price of equipment • Spectral database development or subscription • Use of complexes statistical discriminant models • Time consuming method (large number of spectra required before analysis) • No animal species identification	• Time consuming, in particular for the sample preparation • Expensive (equipment, reagent, standard,..) • Chemical consuming • Need previous selection of specific peptide biomarkers