

ASVCP Guidelines: Principles of Quality Assurance and Standards for Veterinary Clinical Pathology (version 3.0)

Section 10S Supplemental Information on Immunoassay Techniques

10S.1 Competitive versus non-competitive immunoassays There are numerous methods for IA which can be divided into two main categories: competitive and non-competitive. Both competitive and noncompetitive IA involve a capture antibody and a tracer. A tracer is a labeled molecule, either antigen or antibody. It carries the signal quantified by the analyzer (radioactivity, chemiluminescence, fluorescence, or enzymology). The capture antibody is specific for a hapten of the measured substance and is often attached to a support (inner wall of plastic tube or cupule, latex or magnetized beads). The tracer is a labelled antigen (Ag*) in competitive IA, whereas it is a labelled antibody (Ab*) in noncompetitive IA.¹

In these two methods, the nature of the tracer as well as the proportionality between the signal and the measured substance are opposite. Sometimes, competitive IA are called “indirect” while non-competitive IA are called “direct” (see 10.2.2.3).¹

Figure 1S schematizes the principle of these two types of IA. Competitive IA has the substance of interest competing with the tracer Ag* for the capture antibody, so that the signal is inversely proportional to the substance of interest concentration [**Figure 2**]. In this form of IA, the capture antibody is limiting. Noncompetitive IA have the capture antibody in excess, capturing all the substance of interest, which is then targeted by a second antibody (the tracer: Ab*) of different specificity. Thus, the signal is proportional to the concentration of the substance of interest (Figure 2). Radioactive, chemiluminescent, fluorescent, and enzymatic IA can all be used with competitive or noncompetitive formats but may be limited by the availability of kits or reagents.

10S.2 Direct or indirect immunoassays The terms “direct” and “indirect” may be misleading.

Typically, “direct IA” refers to noncompetitive IA and “indirect IA” refers to competitive IA.

However, the terms direct and indirect are sometimes used for other purposes, potentially leading to confusion. The terms are sometimes used to qualify the link of the tracer to the capture antibody. This is a direct link in competitive assay, and an indirect link (through the substance of interest) in noncompetitive IAs [Figure 1S]. The terms direct and indirect are also sometimes used to qualify the generation of luminescence or fluorescence. Indeed, luminescence and fluorescence can:

- be generated either directly by intrinsically luminescent or fluorescent tracers.
- or alternatively, can be generated indirectly by an enzymatic reaction, in which case (despite the nature of the final signal) these techniques belong to enzymology.

10S.3 Heterogeneous or homogeneous phase immunoassays When the free tracer (Ag^* or Ab^*) and the linked tracer ($\text{Ag}^*\text{-Ab}$ or Ag-Ab^*) have the same activity (meaning they emit a similar signal), a separation before reading the signal is needed to conserve only the linked tracer. Because of this separation step, the method is called heterogeneous phase. There are several means of separation:

- Most commonly by adsorption or covalent fixation of the capture antigen to the inner wall of a plastic tube or cupule (for which the separation is obtained by simple washing or aspiration) or to the surface of latex or magnetized beads (for which the separation is obtained by centrifugation or magnetization, respectively)

- Alternatively by precipitation, via a second antibody (anti-capture antibody, when the latter is not fixed to a support), via a polymer, or via a fixed protein binding to the Fc fragment of the capture antibody

On the other hand, when the free tracer and the linked tracer have different activities, there is no need for separation, which is called homogeneous phase IA.¹ Chemiluminescent, fluorescent, and enzymatic IA all exist in heterogeneous and homogeneous design. Radioactive IA are all heterogeneous.

10S.4 Revelation method or signal The revelation methods correspond to the type of signal carried by the tracer; more detailed IA methodology information is beyond the scope of this document. Of the four types of signal used for IA in endocrinology, only radiolabeled tracers have legal requirements.

Reference Section 10S, Supplemental on Immunoassay Techniques

1. Benoist JF, Biou D, Chevenne D. [Biological Marker Assessment by Immunological Analysis]. In: Beaudeau JL, Durand G, eds. Biochimie medicale marqueurs actuels et perspectives [Medical Biochemistry Current Markers and Perspectives]. 2nd ed. Peronnas, France: Lavoisier; 2011:9-38.

Figure 1S: Principle of competitive and noncompetitive immunoassays

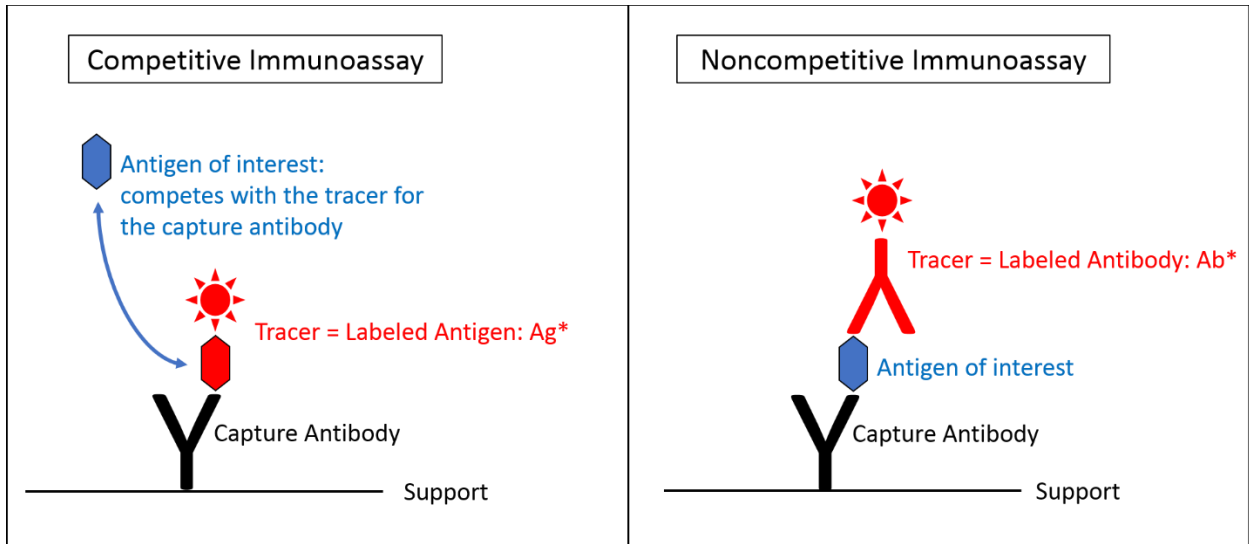


Figure 2S: Proportionality curves between the signal and the antigen of interest in competitive and noncompetitive immunoassays

