TECHNOLOGY NETWORKS

Introducing the ANTIBODY

Antibodies are a vital part of the immune system, playing a key role in our ability to fight infection and in the efficacy of vaccinations. However, that's not where their utility ends. Antibodies can be used as a biopharmaceutical product, in diagnostic testing *and* as an analytical tool for detection. **In this infographic we will take a closer look at what antibodies are, where they come from, how they function and how science is putting them to work in the laboratory.**

A brief history of the antibody

1890
First mention of antibodies by Emil von Behring and Shibasabura Kitasato in the study of diphtheria

1900
Paul Ehrlich proposed the **structure of antibodies**

1945
Antibodies first used as an analytical tool in the Coombs test to detect antibodies to rhesus factor in blood

1948
Involvement of plasma B cells proposed in generation of antibodies by Astrid Fagraeus

1957
Clonal selection theory of antibody generation proposed by Frank Burnet and David Talmage

1959
Molecular structure independently published by Gerald Edelman and Rodney Porter which wins them the Nobel Prize in 1972

1973
First atomic structure of an antibody fragment published

1975
Georges Köhler and César Milstein invent **monoclonal antibodies**

1977
First 3D structure of an antibody produced by Enid W. Silverton, Manuel A. Navia and David R. Davies

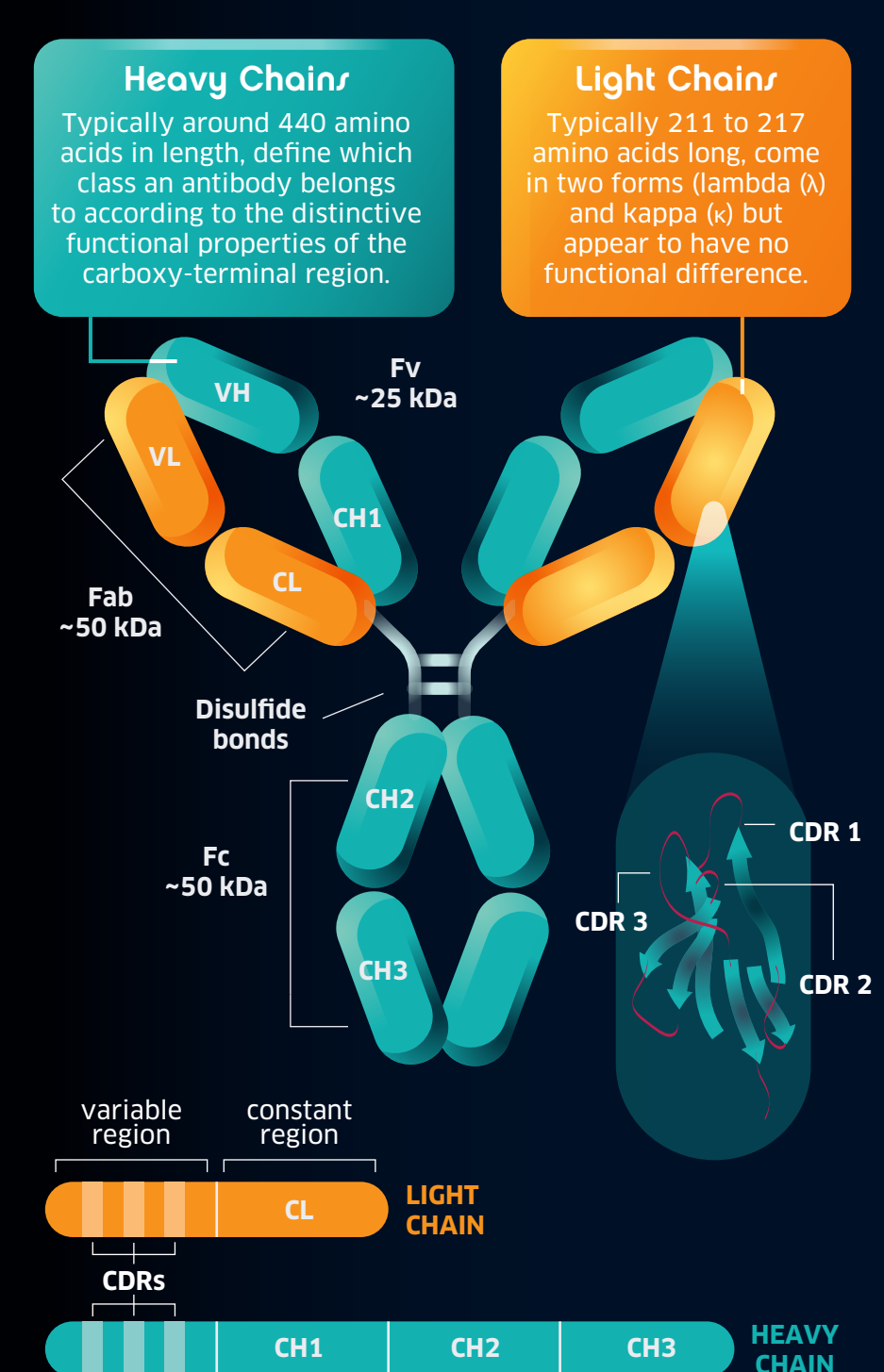
1985
First monoclonal antibody approved for use in humans to **prevent transplant rejection**

What is an antibody?

Antibodies in their basic form are Y shaped glycoproteins, consisting of four polypeptide chains, two identical heavy chains and two identical light chains, joined by disulfide bonds.

Heavy Chains
Typically around 440 amino acids in length, define which class an antibody belongs to according to the distinctive functional properties of the carboxy-terminal region.

Light Chains
Typically 211 to 217 amino acids long, come in two forms (lambda (λ) and kappa (κ) but appear to have no functional difference.



Antibodies perform two main functions with the different regions of their structure:

The **crystallizable fragment (Fc)** region interacts with the immune system (e.g. phagocytes and the compliment system) to facilitate antigen removal.

- **The Fc region consists of two constant domains (CH2 and CH3),** enabling it to interact and perform its function consistently.

The **antigen binding fragment (Fab)** recognizes antigens.

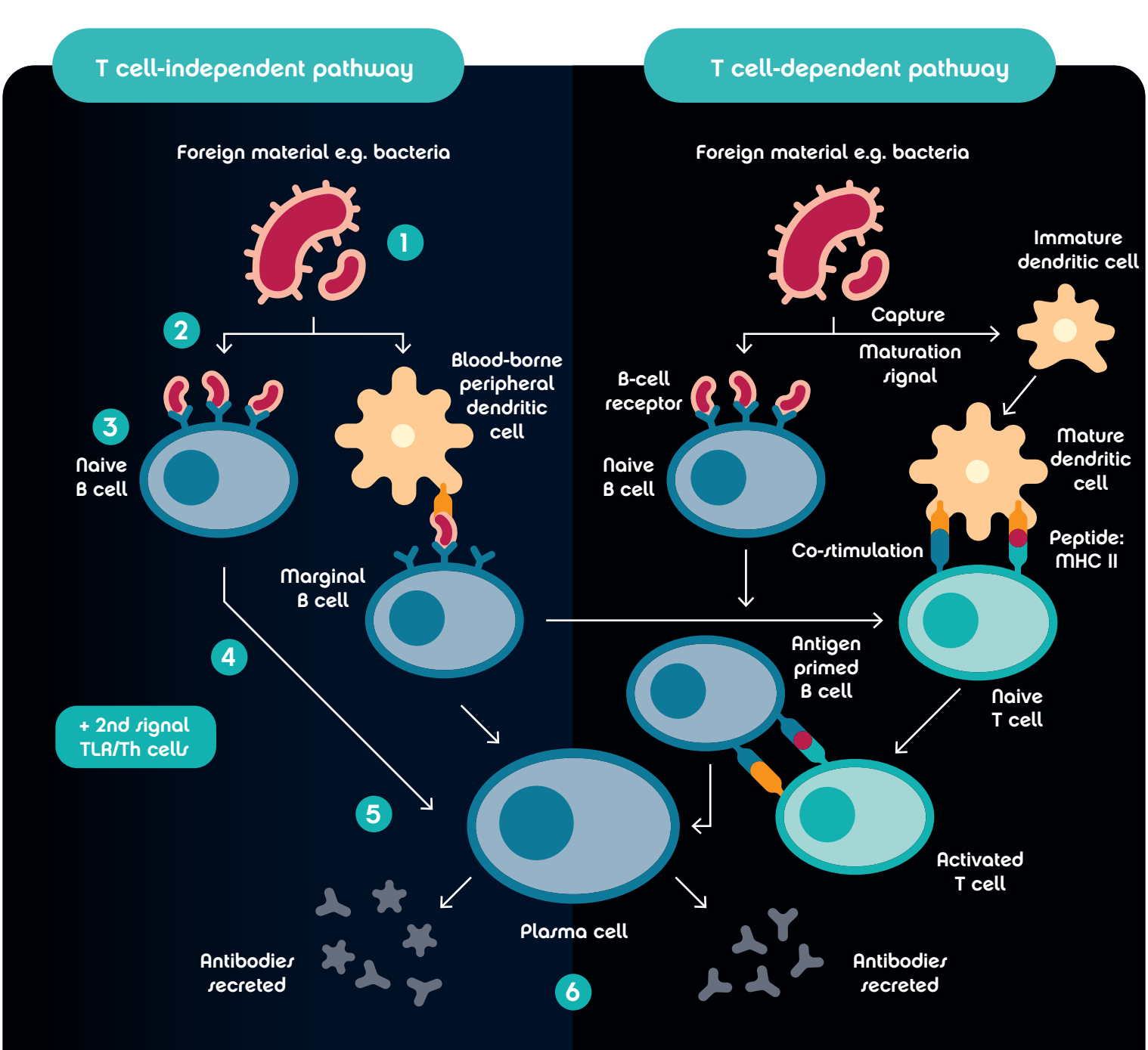
The Fab contains four domains:

- Two variable domains **(VH and VL),** which are collectively called the **variable fragment (Fv)**, providing antigen specificity.
- Two constant domains **(CL and CH1)** that provide a structural framework.

Each variable domain contains three hypervariable loops called **complementarity determining regions (CDR1, CDR2 and CDR3).** Their hypervariable nature enables antibodies to recognize an almost unlimited number of antigens.

Where do antibodies come from?

1. When a foreign material meets our immune system, it is broken down into pieces – antigens – by specialized phagocytic cells including dendritic cells and macrophages.
2. These cells present the antigen (earning them their collective name of antigen presenting cells) to **B cells**. B cells may also bind some antigens if they encounter them directly.
3. B cells then begin to synthesize membrane-bound versions of antibodies (**immunoglobulins**), each B cell will produce many copies (~10^5 on the surface of each B cell) of one unique antibody that binds one specific epitope.



Some antigens, including foreign polysaccharides and unmethylated CpG DNA, can activate B cells without help from T cells; this is called T-cell-independent activation.

However, other antigens and foreign proteins require interaction with T helper cells to provide a co-stimulatory factor to induce B-cell activation. Consequently, antibodies produced through T cell-independent activation are typically produced more quickly but are of lower affinity, whereas antibodies produced via T cell-dependent activation take longer but are higher affinity.

4. When the immunoglobulins on a naïve or memory B cell successfully bind their target antigen, they proliferate and differentiate into **antibody** secreting B cells.
5. These secreting B cells mature into large **plasma cells** that continuously secrete antibodies at around 2000 molecules per second.
6. In circulation, plasma cells last for a few days but can persist in bone marrow for months or even years.

Immunoglobulins and antibodies are very similar.
However, immunoglobulins have a transmembrane domain and are attached to the surface of B cells, whilst antibodies lack this domain and so are released into the circulation.

How do antibodies work?

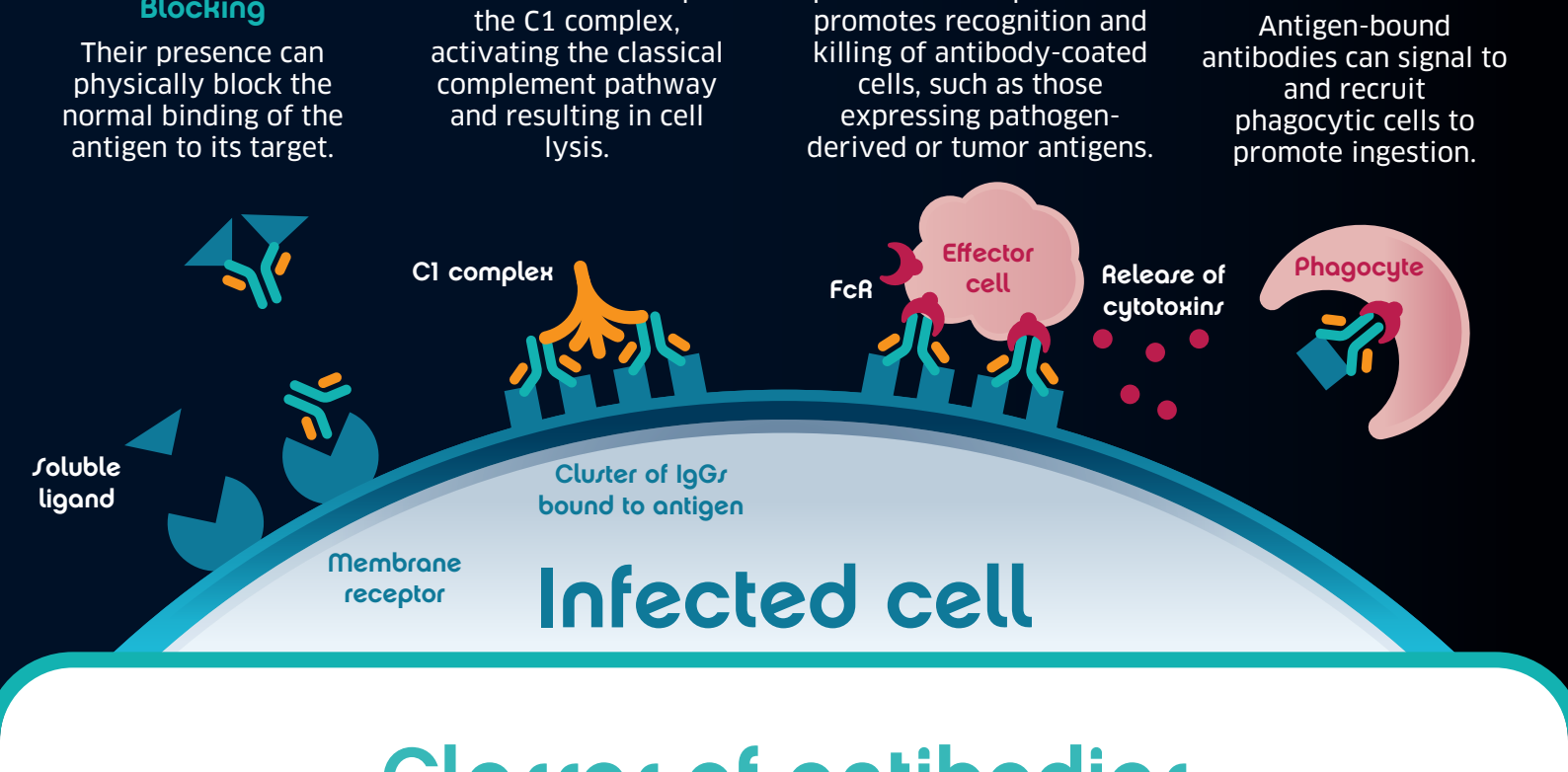
Antibodies bind to their target antigen via the Fab region, from which they can act through several different mechanisms.

Blocking
Their presence can physically block the normal binding of the antigen to its target.

Complement dependent cytotoxicity (CDC)
When the Fab region is bound to its target antigen, it may promote interaction with C1q on the C1 complex, activating the classical complement pathway and resulting in cell lysis.

Antibody-dependent cellular cytotoxicity (ADCC)
The Fc region of bound antibodies may interact with effector cells that possess Fc receptors. This promotes recognition and killing of antibody-coated cells, such as those expressing pathogen-derived or tumor antigens.

Opsonization and phagocytosis
Antigen-bound antibodies can signal to and recruit phagocytic cells to promote ingestion.

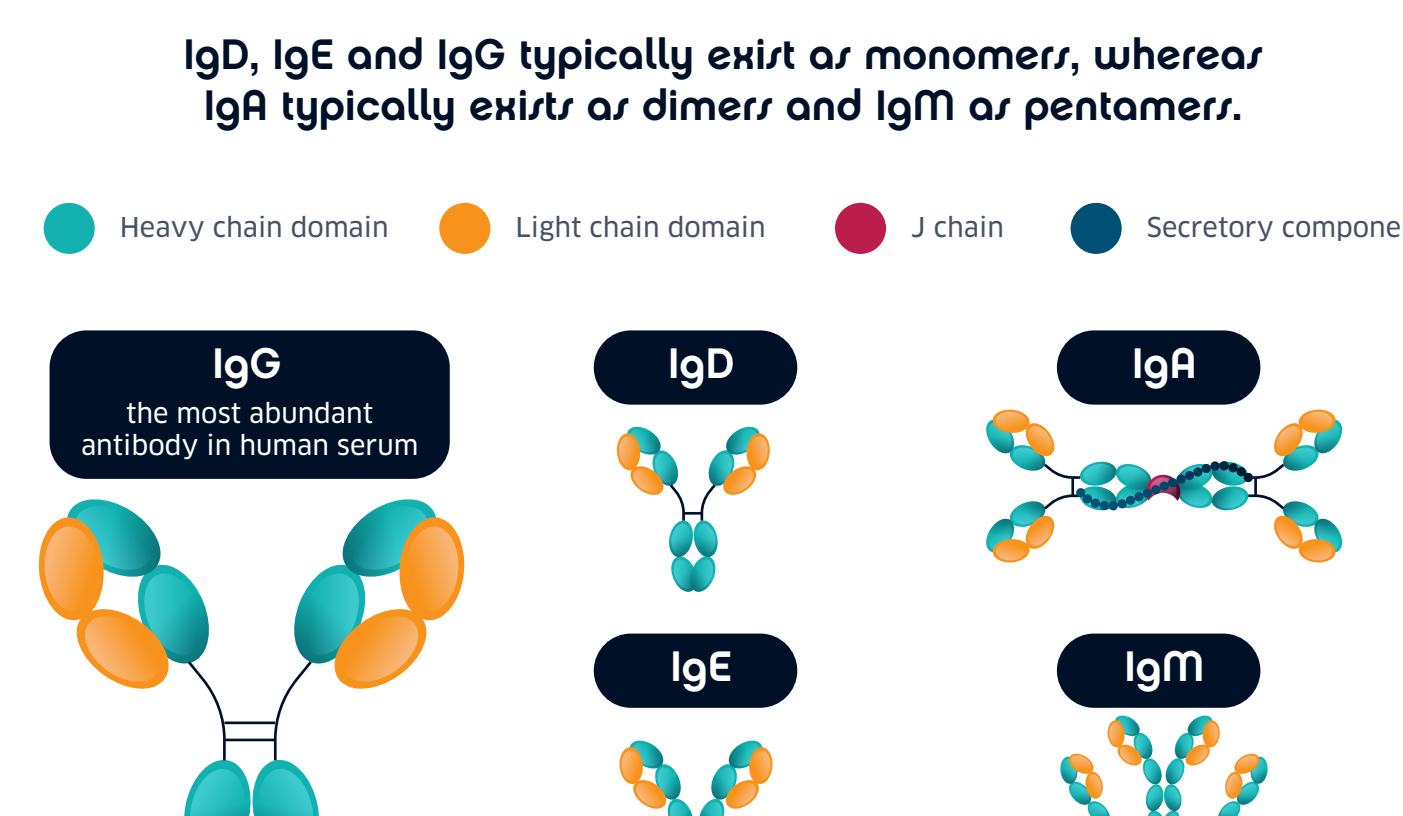


Classes of antibodies

There are five classes of antibodies, also called isotypes, found in human serum that are distinguished by the heavy chain they possess. These are summarized in the table below.

	IgA	IgD	IgE	IgG	IgM
Heavy chain	α	∂	ε	γ	μ
Location	Intravascular and secretions	B cell surface	Basophils and mast cells in saliva and nasal secretions	Intra- and extravascular	Mostly intravascular
Function	Protect mucus membranes	Unknown	Protect against parasites and associated with allergic response	Secondary immune response	Primary immune response
Subclasses	2 (IgA1, which accounts for ~ 80% of IgA in serum, and IgA2, the major subclass in secretions like milk)	1	1	4 (IgG1, IgG2, IgG3 and IgG4. IgG subclasses are highly conserved, differing in their constant region, particularly their hinges and upper CH2 domains)	1
Molecular weight (kDa)	320	180	200	150	900
% of antibodies in serum	5 - 15%	~ 0.2%	~ 0.002%	70 - 85%	5 - 10%
Crosses the placenta	x	x	x	✓	x
Fixes compliment	x	x	x	✓	✓
Fc regions bind to	Myeloid cells	B cells	Mast cells and basophils	Phagocytes	B, T, and NK cells

IgD, IgE and IgG typically exist as monomers, whereas IgA typically exists as dimers and IgM as pentamers.

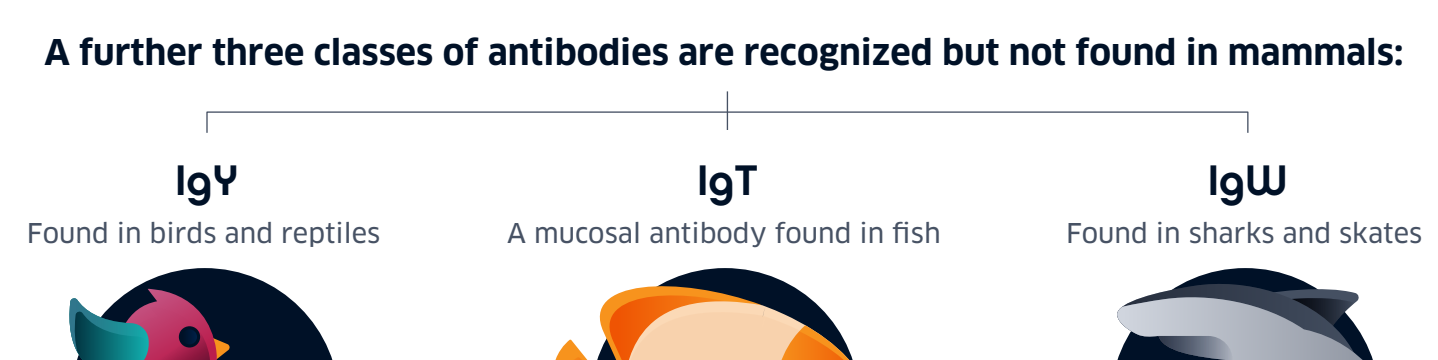


A further three classes of antibodies are recognized but not found in mammals:

IgY
Found in birds and reptiles

IgT
A mucosal antibody found in fish

IgW
Found in sharks and skates



Allelic variation, sometimes associated with certain ethnic groups, may exist even within antibody subtypes. These are called allotypes.

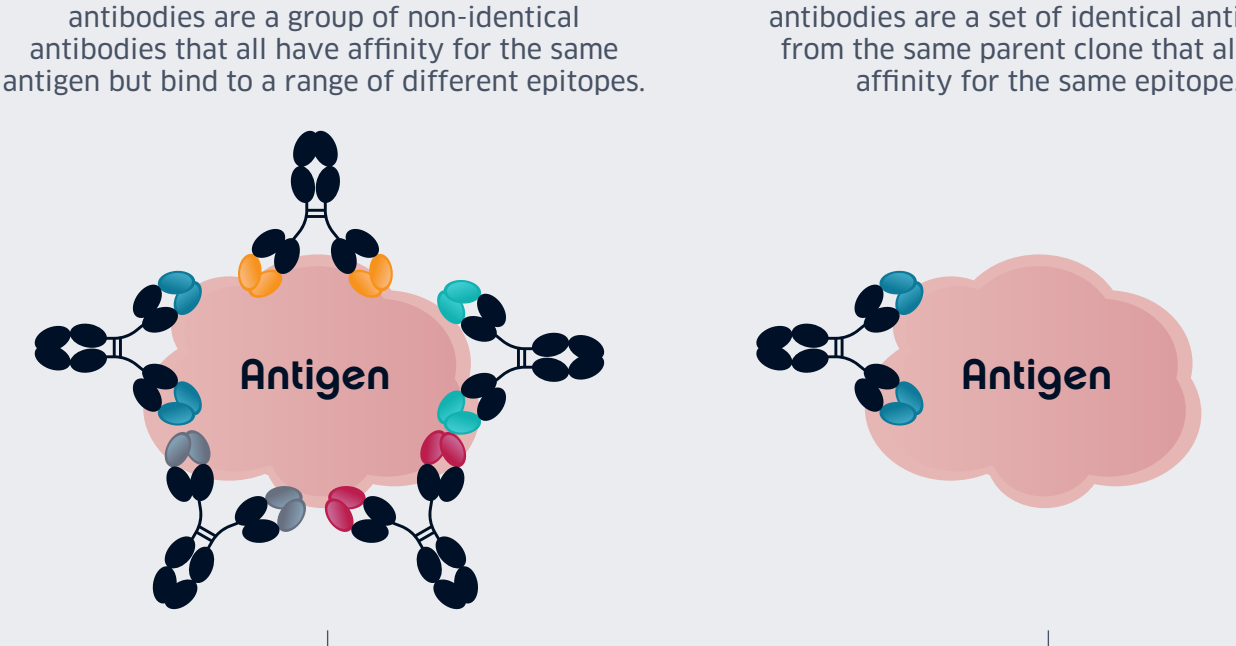
Example

Ig (Antibody) → IgG (Isotype) → IgG1 (Subclass) → IgG1m17 (Allotype)

Monoclonal vs polyclonal

Polyclonal antibodies are a group of non-identical antibodies that all have affinity for the same antigen but bind to a range of different epitopes.

Monoclonal antibodies are a set of identical antibodies from the same parent clone that all have affinity for the same epitope.

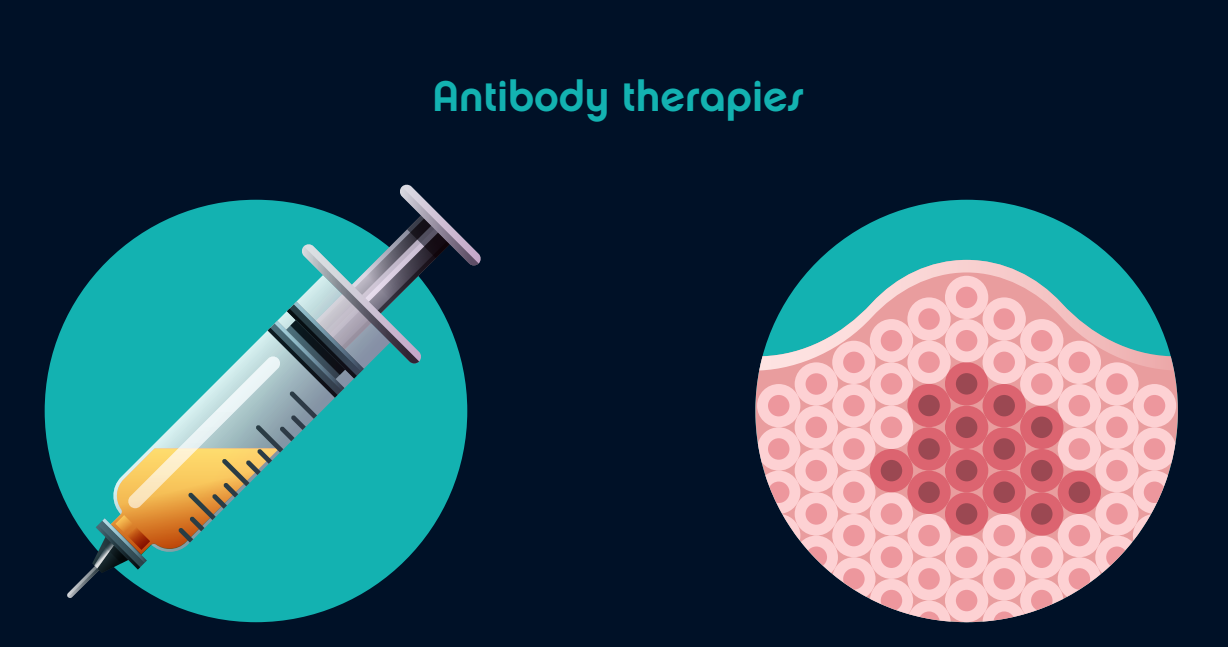


We naturally produce polyclonal antibodies in response to an invader and these are often well suited to many research applications. However monoclonal antibodies can be produced *in vitro* for applications that require them, e.g. for therapeutic use.

Applications of antibodies

Whilst antibodies are an incredibly important product of our immune systems, they can also be utilized in effective therapies and provide useful tools for a wide variety of assays in the laboratory.

Antibody therapies



Therapeutic antibodies, most of which are monoclonal antibodies, are now the most rapidly growing class of approved biopharmaceutical drugs, used to treat conditions including cancer, autoimmune and inflammatory diseases and Alzheimer's Disease.

Antibodies are selected that target specific problematic antigens in the body, blocking their normal action and thus reducing or preventing the condition they cause.

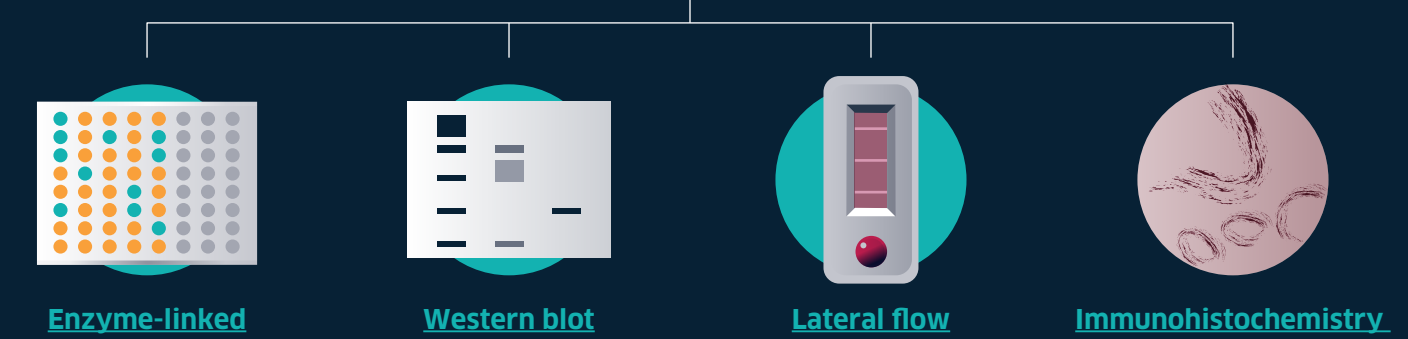
Typically, monoclonal antibodies are used in therapeutics because there is little batch-to-batch variation, they can be produced in large quantities and are very specific yielding little cross-reactive binding. However, they are expensive and time consuming to produce.

Antibody-based assays

Assays that utilize antibodies are primarily aimed at detection and can be broken into two groups:

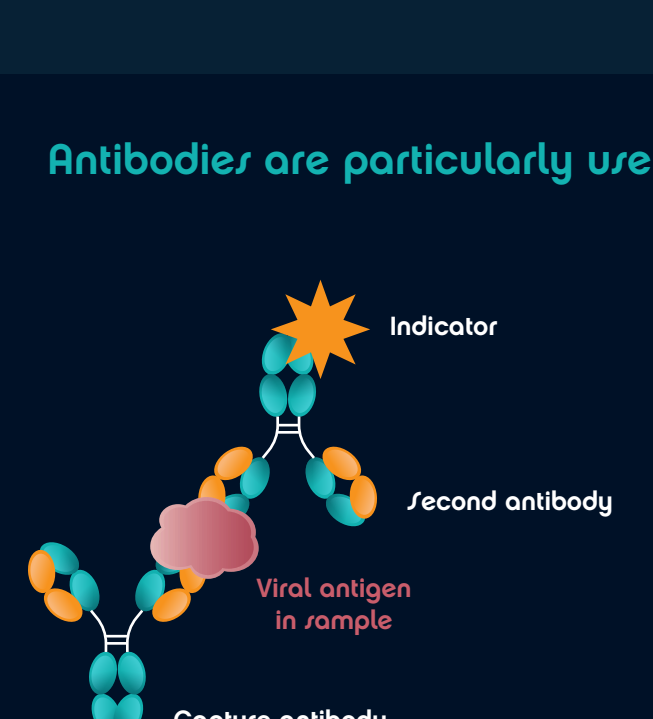
- **Assays that detect antibodies**
- **Assays that use antibodies to detect target molecules**

These include:

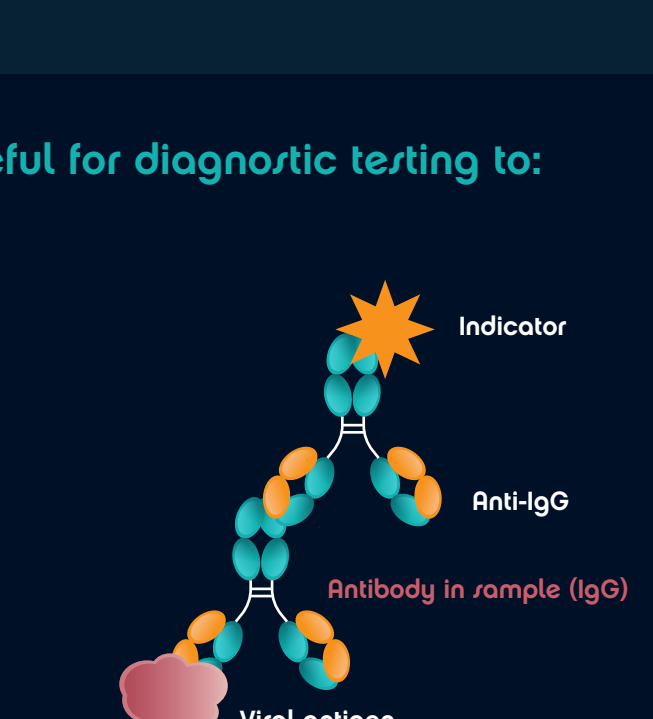


- Enzyme-linked immunosorbent assay (ELISA) – direct ELISA and sandwich ELISA
- Western blot
- Lateral flow assay (LFA)
- Immunohistochemistry – Labeling for microscopy

Antibodies are particularly useful for diagnostic testing to:



Determine if an antigen, such as a pathogen surface protein, is present in a sample by using a specific antibody to detect it - identifies active infection



Detect if someone is actively producing antibodies to a pathogen and therefore is or has been infected e.g. coronavirus antibody tests

Typically, polyclonal antibodies are used in assays as they are cheap and have high overall affinity and sensitivity from detecting multiple epitopes. However, there are issues with batch-to batch variation in commercially produced antibodies, and they can give increased background "noise" from cross-reactive binding.