

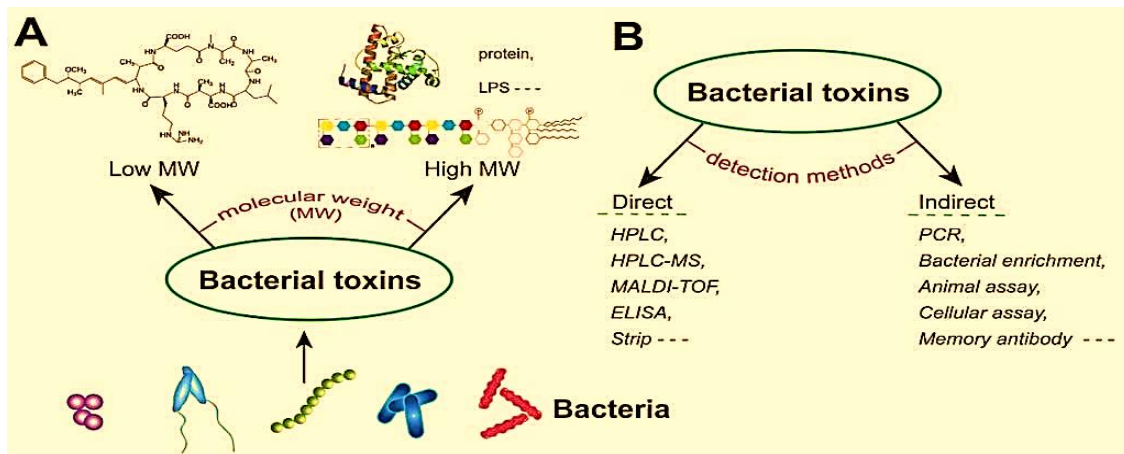
## Bacterial Toxin Methods Detection

### Diagnosis bacterial toxin

There are many methods to detection bacterial toxin including:

1-**Direct detection:** ex. Strip & enzyme-linked immunosorbent assay (**ELISA**) and high-performance-liquid-chromatography (**HPLC**).

2-**Indirect detection:** ex. polymerase chain reaction (**PCR**), Animal assay, Cellular assay.



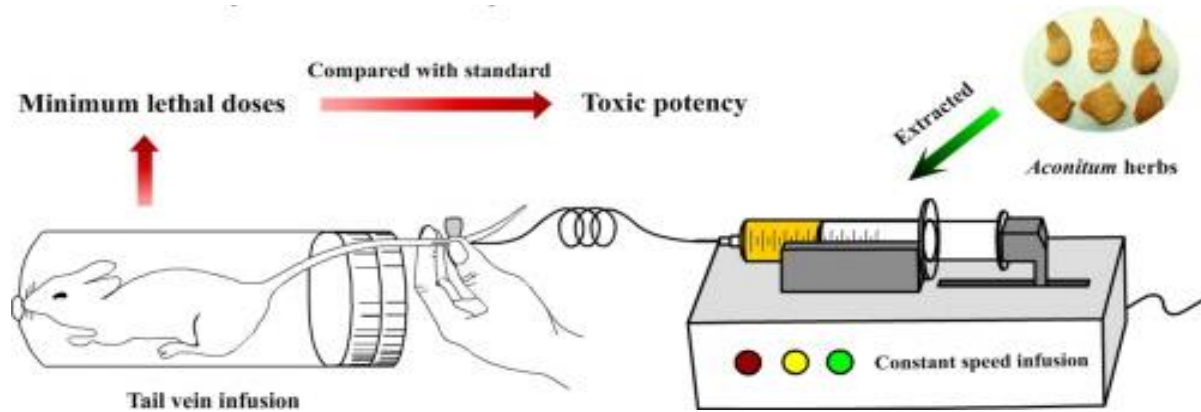
### Bacterial Toxin Assay Techniques

1. Biological assay
  - 1- Nucleic acid probes and polymerase chain reaction
  - 2- Immunological assay

**A-Biological assay:** are **rapid** and a **convenient** means of testing for bacterial toxins, they do not provide information on the biological activity of the toxin but these tests remain the **choice method** for some bacterial toxins (e.g. **botulinum toxins**), and including:

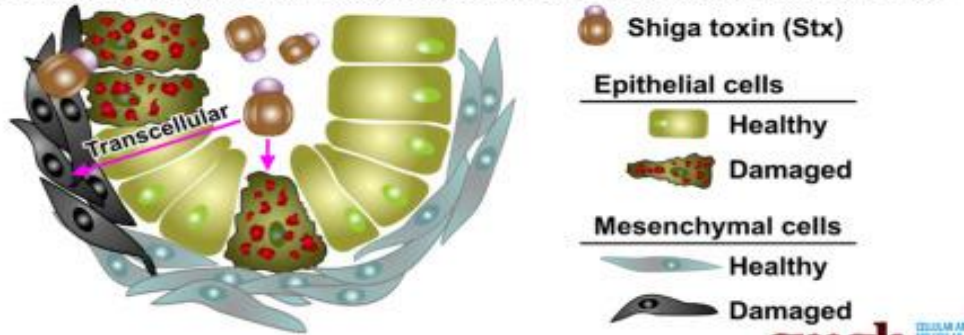
- 1-In vivo (animal Assay tests):e.g. the mouse lethality test, monkey emesis tests.
- 2-In vitro tests (tissue culture): e.g. Chinese hamster ovary.

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### \*Animal Assay tests

#### Human Intestinal Epithelial and Mesenchymal Cells Are Sensitive to Shiga Toxin



### \* Tissue effect with shiga- toxin

**B- Nucleic acid probes and polymerase chain reaction:** is a highly **specific** and **sensitive** method for **amplifying** nucleic acid sequences exponentially.

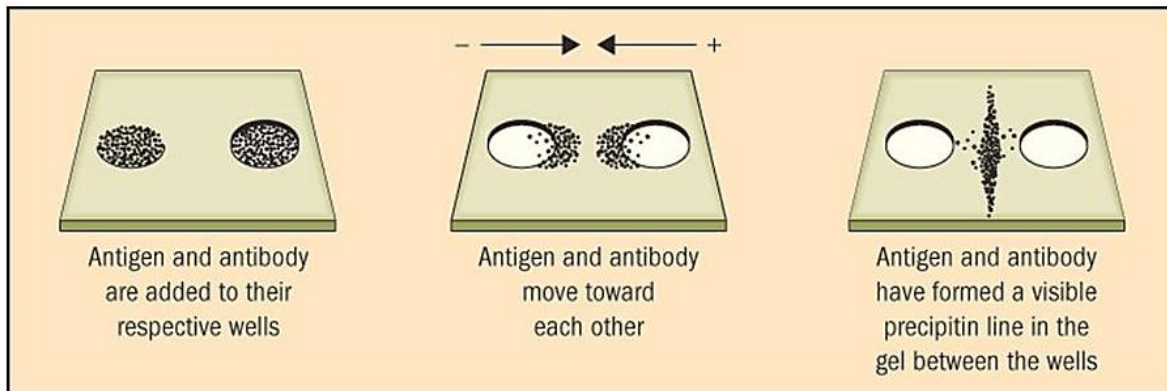
**Nucleic acid probes:** are based on the detection of unique nucleotide sequences within the DNA or RNA of a microorganism; these unique nucleotide is code of toxin substance e. x. **DNA probes for shiga-like toxin.**

**C- Immunological assay:** are much **simpler** and **cheaper** than biological assays and have therefore been widely used, were developed based on techniques such as:

**1-Gel diffusion:** under the influence of an electric current, the toxin migrates into **agarose gel containing antibody**, this test requires **only 20 ml** of sample. If the

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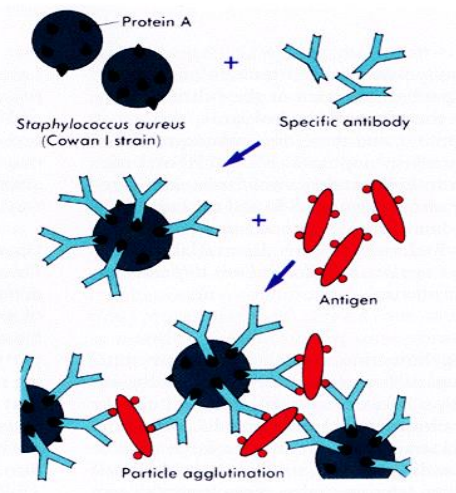
concentration of the enterotoxin is **too high**, the formation of precipitation line is **inhibited**, so it is essential to use a range of dilutions of the test sample, this used to quantify staphylococcal enterotoxins.



### \*Gel diffusion

**2- Haem-agglutination:** unlike the gel diffusion methods they do not require the antigen to be in a precipitation form. In haem-agglutination test **dilutions of sample** are reacted with a **constant amount of antibody**. Toxin coated erythrocytes are then added and these agglutinate. The result appearance as **single large solid mass**, like sensitized *S. aureus* cells are mixed with the test sample on a slide and agglutinate within 10min if the target toxin is present.

Hemagglutination Test			
	Components	Interaction	Microtiter test results
A	RBC		No reaction
B	Toxin RBC		Hemagglutination
C	Toxin Antibody RBC		Hemagglutination inhibition



### - Haem-agglutination

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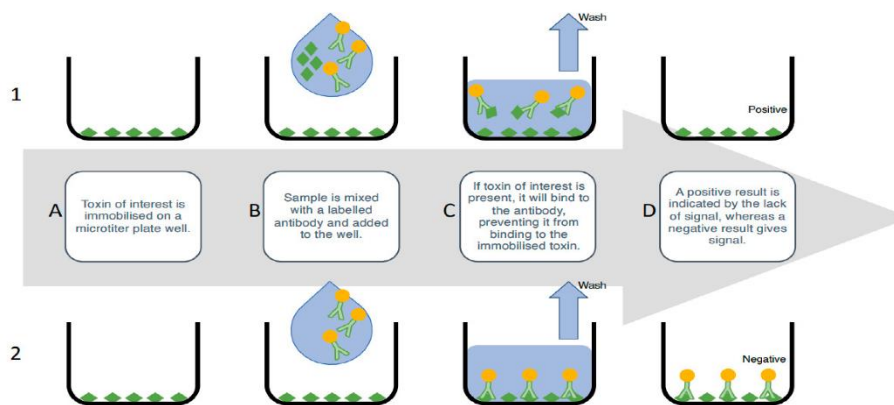
**3-Enzyme-linked immunosorbent assay:** is the quantitative assay (ELISA) method, contain 96-well plate. **In the direct antibody sandwich ELISA**, antigen-sample is captured to specific antibody-coated wells and the result detected by form a colored or fluorescent product. The amount hence **color** is directly proportional to the target **analytic concentration**.

If the toxin is present in the sample,

**Sample with Ag + AB labeled + immobilized toxin = Positive & lack of signal.**

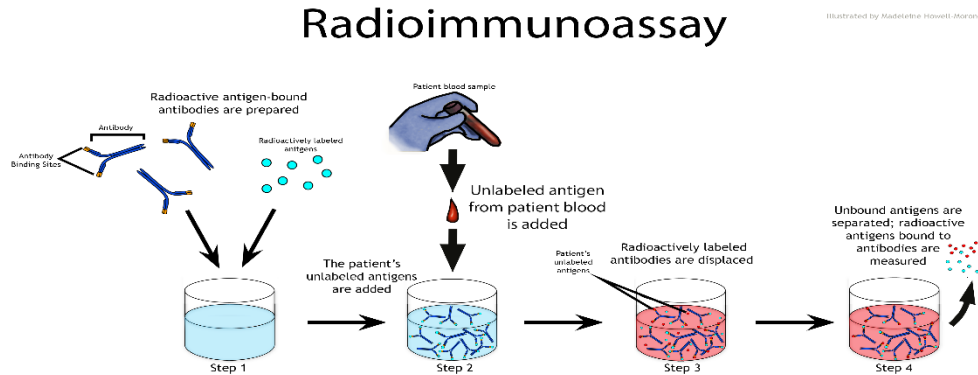
If the toxin is **absent** in the sample,

**Sample only + AB labeled + immobilized toxin = Negative & indicated by a signal,** because the labelled antibody has not been inhibited from binding to the immobilized toxin.



**4-Radioimmunoassay (RIA):** a radioactive label is coupled to the antigen and the antigen is reacted with the specific antibody. The amount of antigen combined with the antibody is determined with a **radioactivity counter**. This technique is more **sensitive** than gel diffusion methods, but has the **disadvantage** that special facilities are required to handle and dispose of radioactive materials.

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### Testing for Toxicity

**Limulus Amoebocyte assay (LAL):** is a test used to detect tiny amounts of endotoxin. LAL is an aqueous extract of blood cells (amoebocytes) from the crab, *Limulus*. LAL reagent reacts with bacterial endotoxin and lipopolysaccharide (LPS), which is a membrane constituent of Gram-negative bacteria. The Gel Clot assay is run in tubes that are placed in a water bath or in dry heated oven at 37°C. After a one-hour incubation period, the tubes are flipped 180°. A firm clot that stays in the bottom of the tube indicates a positive reaction.

**Complete blood count (CBC):** is a common blood test that evaluates the three major types of cells in the blood: red blood cells, white blood cells, and platelets and it used to indicate early toxin exposure.

**E-Map – tests:** test the body's subtle energy circuits to determine the presence of toxins. It can measure toxicity such as candida, heavy metals and environmental or food sensitivities.

**MELISA Testing** - this is using a newer technology to determine the reactivity of the immune system to chemical compounds. The more reactive the immune system is to a toxin, the more toxicity symptoms develop.

**Liver Detox Profile** - is a blood and urine test that helps measures the liver's detoxification pathways. It does not specifically measure toxins, but these pathways are critical.