Therapeutic areas & Diseases

Laboratory parameters analysed for Clinical trials

Module 4 Topic 3

Why are laboratory tests ordered

- Diagnosis
- Monitor progression of disease
- Monitor effectiveness of treatment
- To identify complications of treatment
- Screening population for diseases

Common Laboratory Tests

Most commonly ordered lab tests

- CBC (Complete Blood Count)
- BMP (Basic Metabolic Panel)
- CMP (Comprehensive Metabolic Panel)

CBC

Complete blood count

- With or without differential
- Peripheral venous blood is collected in a tube containing the anticoagulant EDTA and should be thoroughly mixed
- Methodology of testing:
  - Whole blood analyzer

CBC

- Red blood cell data
  - Total red blood cell count (RBC)
  - Hemoglobin (Hgb)
  - Hematocrit (Hct)
  - Mean corpuscular volume (MCV)
  - Red blood cell distribution width (RDW)
- White blood cell data
  - Total white blood cell (leukocyte) count (WBC)
  - A white blood cell count differential may also be ordered
- Platelet Count (PLT)

Red Blood Cell Count

- Count of the number of circulating red blood cells in $1mm^3$ of peripheral venous blood

Hematocrit

- Hematocrit is a measure of the percentage of the total blood volume that is made up by the red blood cells
  - Normal Hct in adult males - 40-54%
  - Normal Hct in adult females - 34-51%
- Hct is calculated from the RBC and MCV
  - Hematocrit % = RBC (cells/liter) x MCV (liter/cell)

Mean Corpuscular Volume

- The MCV is a measure of the average volume, or size, of an RBC
- The MCV is important in classifying anemias
  - Normal MCV = normocytic anemia
  - Decreased MCV = microcytic anemia
  - Increased MCV = macrocytic anemia

White Blood Cell Count

- A count of the **total** WBC, or leukocyte, count in $1mm^3$ of peripheral blood
- A decrease in the number of WBCs
  - Leukopenia
- An increase in the number of WBCs
  - Leukocytosis

WBC Differential Count

Types of leukocytes

- Neutrophils (includes bands)
- Lymphocytes
- Monocytes
- Eosinophils
- Basophils

Platelet Count

- A count of the number of platelets (thrombocytes) per cubic milliliter of blood
  - A decreased number of platelets
    - Thrombocytopenia
  - An increased number of platelets
    - Thrombocytosis

CBC as reported by Lab

Component	Value	Flag	Low	High	Units
WBC	9.4		4.0	10.0	K/UL
RBC	4.81		3.60	5.50	M/UL
HGB	13.7		12.0	16.0	GM/DL
HCT	41.1		34.0	51.0	%
MCV	85.4		85	95	FL
MCH	28.6		28.0	32.0	PG
MCHC	33.4		32.0	36.0	GM/DL
RDW	14.3		11.0	15.0	%
PLT CNT	220		150	400	K/UL

CBC as reported by Lab

Component	Value	Flag	Low	High	Units
DIFF TYPE AUTOMATED					
LYMPH #	3.6		1.0	4.0	K/MM3
MONO #	0.6		0.0	1.0	K/MM3
GRAN #	5.1		2.0	7.0	K/MM3
EO #	0.0		0.0	0.7	K/MM3
BASO #	0.0		0.0	0.2	K/MM3
LYMPH	39		20	45	%
MONO	6		0	10	%
GRAN	55		45	70	%
EO	0		0	7	%
BASO	0		0	2	%

Basic Metabolic Panel

Tests of electrolytes and kidney function:

- Sodium (Na)
- Potassium (K)
- Chloride (Cl)
- Carbon Dioxide Content (CO2)
- Blood Urea Nitrogen (BUN)
- Serum Creatinine (Cr)
- Serum glucose (Glu)
- Total Calcium (Calcium)

Sodium

- Sodium is the major cation in the extracellular space where serum levels of approximately 140mmol/L exist
  - Sodium salts are major determinants of extracellular osmolality.
  - Increased serum sodium level - Hypernatremia
  - Decreased serum sodium level - Hyponatremia

Potassium

Potassium is the major intracellular cation with levels of ~ 4 mmol/L found in serum

- Elevated serum potassium level - Hyperkalemia
- Decreased serum potassium level - Hypokalemia

Blood Urea Nitrogen

The BUN measures the amount of urea nitrogen in the blood.

- Urea is formed in the liver as the end product of protein metabolism and is transported to the kidneys for excretion.
- Nearly all renal diseases can cause an inadequate excretion of urea, which causes the blood concentration to rise above normal.
- The BUN is interpreted in conjunction with the creatinine test - these tests are referred to as “renal function studies”

Creatinine

The creatinine test measures the amount of creatinine in the blood.

- Creatinine is a catabolic product of creatine phosphate used in skeletal muscle contraction.
- Creatinine, as with blood urea nitrogen, is excreted entirely by the kidneys and blood levels are therefore proportional to renal excretory function

Uric Acid

- Formed from breakdown of nucleic acids and excreted as a waste product by kidneys
- Increased in kidney disease, but most often used to diagnosis gout (pain in joints, mainly big toe, due to precipitated uric acid crystals)
- Also increased in increased cell destruction, such as after massive radiation or chemotherapy

Glucose

- Plasma glucose levels should be evaluated in relation to a patient's meal
  - i.e., postprandial vs fasting
- Elevated glucose levels may also be indicative of diabetes mellitus
- The criteria for the diagnosis of diabetes:
  - Fasting Plasma Glucose ≥126 mg/dL
  - 2 hour Post-Prandial Glucose ≥200 mg/dl
  - Random Plasma Glucose >200 mg/dL in the presence of symptoms

BMP as reported by Lab

Component	Value	Flag	Low	High	Units
Sodium	142		136	144	mm/l
Potassium	3.9		3.3	5.1	mm/l
Chloride	107		98	108	mm/l
Co2	27		20	32	mm/l
Bun	10		7	22	mg/dl
Creatinine	0.80		0.7	1.5	mg/dl
Glucose	100		70	100	mg/dl
Calcium	8.5	I	8.9	10.3	mg/dl

Complete Metabolic Panel

More extensive laboratory evaluation of organ dysfunction and includes:

- Sodium
- Potassium
- Chloride
- Carbon Dioxide Content
- Albumin
- Total Bilirubin
- Total Calcium
- Glucose
- Alkaline Phosphatase
- Total Protein
- Aspartate Aminotransferase
- Blood Urea Nitrogen
- Creatinine
- Lipid profile

Total Protein

- Albumin and globulin constitute most of the protein within the body and are measured in the total protein test
- Albumin comprises ~ 60% of the total protein within the extracellular portion of the blood
- Albumin's major effect within the blood is
  - to maintain colloid osmotic pressure
  - To transport many important blood constituents e.g. drugs, hormones, enzymes
- Albumin is synthesized in the liver and therefore is a measure of hepatic function

Alkaline Phosphatase

- Alkaline phosphatase is an enzyme present in a number of tissues, including liver, bone, kidney, intestine, and placenta, each of which contains distinct isoenzyme forms
- The two major circulating alkaline phosphatase isoenzymes are bone and liver
- Elevation in serum alkaline phosphatase is most commonly a reflection of liver or bone disorders.
- Levels of alk phos are increased in both extrahepatic and intrahepatic obstructive biliary disease

Total Bilirubin

- The total serum bilirubin level is the sum of the conjugated (direct) and unconjugated (indirect) bilirubin.
- Normally the unconjugated bilirubin makes up 70-85% of the total bilirubin
- Bilirubin metabolism begins with the breakdown of red blood cells in the reticuloendothelial system and bilirubin metabolism continues in the liver
- Elevation in total bilirubin reflects any aberrations in bilirubin metabolism or increased hemolysis

Aspartate Aminotransferase (AST)

- Formerly called SGOT
- AST is an enzyme that is present in hepatocytes and myocytes (both skeletal muscle and cardiac)
- Elevations in AST are most commonly a reflection of hepatocellular injury
- It may also be elevated in myocardial or skeletal muscle injury

Alanine Aminotransferase (AST)

- Formerly called SGPT
- Increases up to 10x in cirrhosis, infections or tumors and up to 100x in viral or toxic hepatitis

Lipid Metabolism Tests

- Cholesterol
  - Present in all tissues
  - Serves as the skeleton for many hormones
  - Recommended value - less than 200 mg/dL in adults)
  - LDL = “bad” cholesterol
  - HDL = “good” cholesterol

Lipid Metabolism Tests

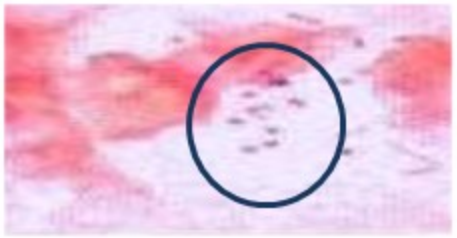
- Triglycerides
  - Main storage form of lipids, comprising 95% of fat tissue
  - Hypertriglyceridemia - having high blood levels of triglycerides - may increase risk of heart attack

CMP from a patient with Congestive Heart Failure

Glucose	112	H	[70 – 100]	mg/dl
Blood Urea Nitrogen	39	H	[7 - 22]	mg/dl
Creatinine	1.6	H	[0.7 - 1.5]	mg/dl
Calcium	8.9		[8.5 - 10.5]	mg/dl
Sodium	132	L	[136 - 146]	mmol/L
Potassium	4.0		[3.5 - 5.3]	mmol/L
Chloride	93	L	[98 - 108]	mmol/L
Carbon Dioxide	23		[20 - 32]	mmol/L
Albumin	3.1	L	[3.6 - 5.0]	gm/dl
Protein, Total	5.8	L	[6.2 - 8.0]	gm/dl
Alkaline Phosphatase	200		[25 - 215]	IU/L
AST	35		[5 - 40]	IU/L
Bilirubin, Total	1.9	H	[0.2 - 1.4]	mg/dl

Detection Tests

- Microscopy
- Direct examination of a specimen (or may use stains) to detect the presence of organisms
- Pros:
  - Quick and easy
  - Preliminary results
- Cons:
  - Not specific



Gram negative diplococci

Detection Tests

Culture

- The process of growing and propagating organisms in a media that is conducive for their growth
- Pros:
  - Confirm the organism
  - Reproduce the organism
  - Use for additional testing
- Cons:
  - Delay in confirmation
  - Require viable organism
  - Difficult for fastidious organisms

S. pneumoniae on blood agar plate

Detection Tests

Antigen Test

Technique	Principle
Agglutination	Known antiserum causes bacteria or other particulate antigens to clump together or agglutinate
Complement fixation	Known antiserum mixed with the test antigen and complement is added. Sheep red blood cells and hemolysins are then added. Positive test: no hemolysis, negative test: hemolysis
Enzyme-linked immunosorbant assay (ELISA) ; Enzyme immunoassay (EIA)	A rapid test where an antibody or antigen is linked to an enzyme as a means of detecting a match between the antibody and antigen.
Fluorescent antibody	Fluorescent dye is attached to known antibodies. When the fluorescent antibody reacts with the antigen, the antigen will fluoresce when viewed with a fluorescent microscope.

Identification Methods

Polymerase Chain Reaction (PCR)

- Method used to amplify a specific region of a DNA strand
- Pros:
  - Simple process, eliminates tedious work,
  - Results available within a day
  - Does not require a viable organism as only a strand of DNA is needed
  - Sensitive test
- Cons:
  - Sensitive – can pick up environmental contaminants
  - Unable to distinguish between certain species

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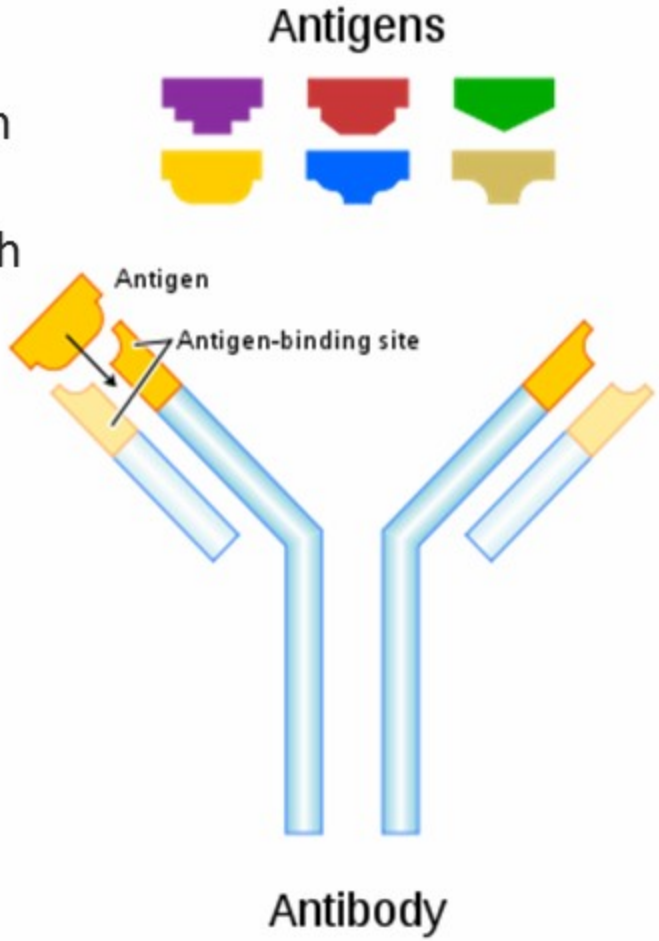
Identification Methods

Serology

- Study of blood serum, with emphasis on testing of antibodies in the serum
- **Antigen**: A 'foreign' substance which stimulates the body to produce antibody
- **Antibody**: A protein molecule produced by the body's immune system in response to a specific antigen. The antibody combines with the antigen and disables it.
  - Also called Immunoglobulins (e.g. IgG, IgM, IgA, IgE)
  - Referred to as **anti**-(name of antigen),
    e.g. anti-HCV, anti-HAV



Serology

Antibodies

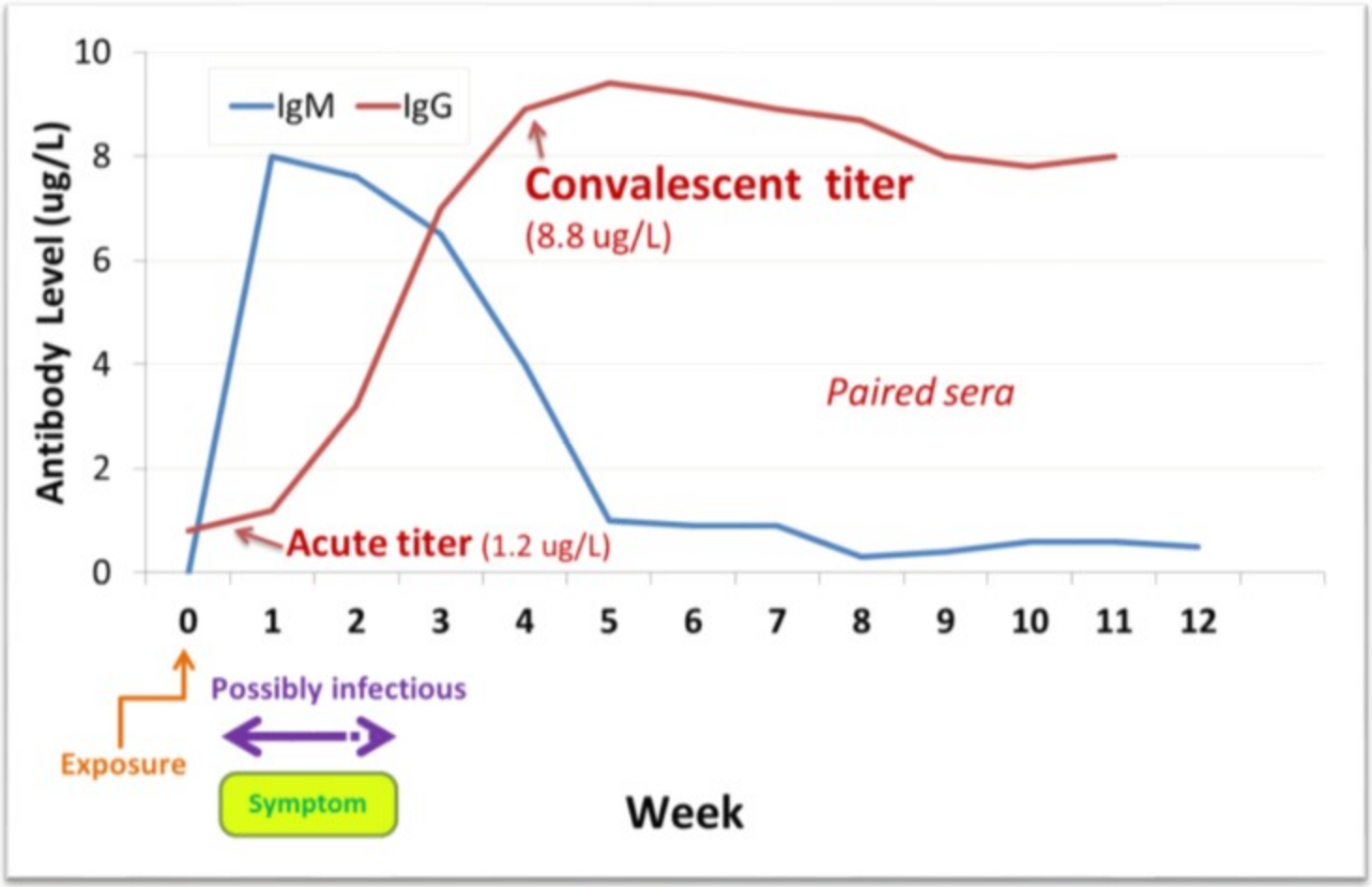
- **IgM**: type of antibody produced by the body, usually the first antibody to appear in response to a foreign substance exposure, then eliminates the organism in the early stages of immunity before there is sufficient IgG
- **IgG**: type of antibody that provides the majority of antibody-based immunity against invading organisms, the only antibody that crosses the placenta to provide immunity to the fetus

Serology

- **Titer**: the amount of antibodies present in the blood, usually as a result of infection.
- **Acute titer and Convalescent titer**: At the acute stage of disease, serum is tested (acute phase), followed by another blood draw and testing about 3 weeks (convalescent phase) later; IgG levels are compared and a 4-fold increase between acute and convalescent samples usually indicate infection

Serology

Antibody Response to Infection



Antibody Testing

Pros:

- Screening tool
- Readily available
- Indicates response to antigen (even if antigen is not detectable) – may indicate infection or immunity

Antibody Testing

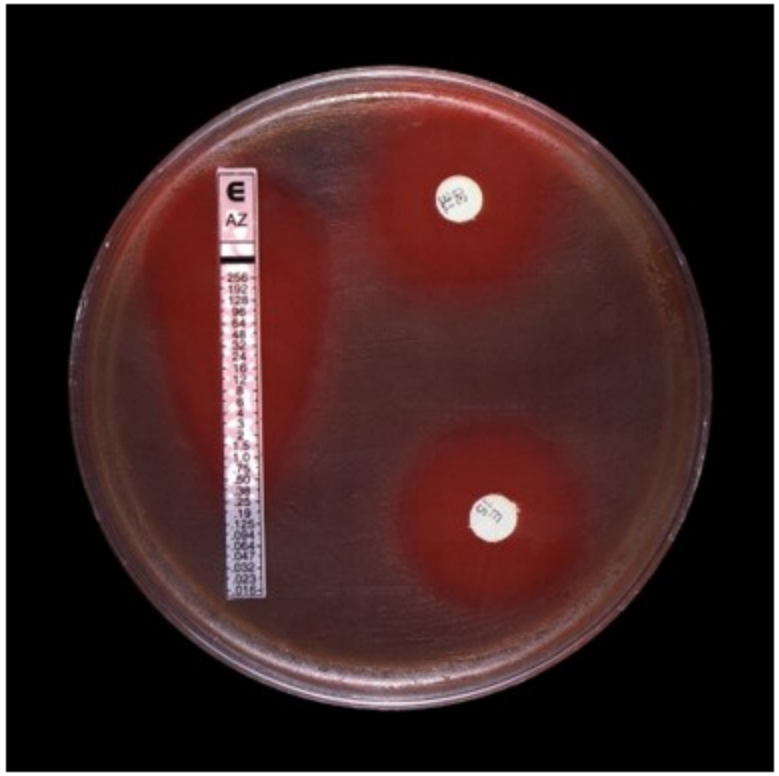
Cons:

- Paired testing necessary for some diseases - may take a while to get results, impact on patient management
- Unable to differentiate between immunity and disease
- Sensitivity and specificity:
  - False-negative result: compromised immune system
  - False-positive result: liver disease, low disease prevalence

Antimicrobial susceptibility

MIC (minimum inhibitory concentration)

- lowest concentration of antimicrobials that will inhibit the growth of organisms
- MICs are important to confirm resistance of organisms to an antimicrobial agent.



Methods:

- Disk diffusion test
- Broth dilution test

Antimicrobial susceptibility

BLOOD CULTURE AER/ANA — Ô FINAL — 05/23/10 11:57

05/21/10 *** Culture ***

ORGANISM 1 Streptococcus pneumoniae 05/23/10 11:29

ATTENTION: REPORTABLE DISEASE-CONTACT LOCAL HEALTH DEPT!!
Inpatient reporting done by Clinical Epidemiology, Microbiology, and Virology Departments.

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The interpretation for Penicillin, Cefotaxime and Ceftriaxone for this isolate is based on MENINGITIS breakpoints.
The breakpoints for isolates from other sources (blood, respiratory tract, etc.) are:

Antibiotic	Cefotaxime	Ceftriaxone	Penicillin	Pen.(oral)
Susceptible	</= 1.0	</= 1.0	</= 2	</=0.06
Intermediate	2.0	2.0	4	.012-1
Resistant	>/= 4.0	>/=4.0	>/= 8	>/= 2

Please consult Infectious Disease specialist if you have any questions.
Ref:CLSI; M100-S20 2010.

ORGANISM / ANTIBIOTICS	S. pneumoniae MIC	Interp
Amox/K (Augmentin)	4/2	I
Ampicillin (Omnipen)	>4	
Cefepime (Maxipime)	2	I
Cefotaxime (Claforan)	2	R
Ceftriaxone (Rocephin)	2	R
Cefuroxime (Zinacef, Ceftin)	>2	R
Erythromycin	>0.5	R
Levofloxacin (Levoquin)	1	S
Penicillin	4	R
Tetracycline	>4	R
Trimeth/Sulf (Bactrim)	>2/38	R
Vancomycin (Vancocin)	0.5	S

S=Susceptible, I= Intermediate, MS= Moderately Susceptible, R=Resistant, IB= Inducible Beta-lactamases

Efficacy Endpoint

Surrogate Endpoints

- Phase 3 clinical trials evaluate the effect that new interventions have on the clinical outcomes of particular relevance to the patient
- A surrogate endpoint is a laboratory measure (or a physical sign) that is intended to be used as a substitute for a clinically meaningful endpoint
- Changes induced by a therapy on a surrogate endpoint are expected to reflect changes in a clinically meaningful endpoint

Surrogate Endpoint

Examples of Surrogate Endpoints

- Hypercholesterolemia
  - Cholesterol levels: surrogate for atherosclerotic disease
- HIV
  - CD4 count or viral load: surrogate for complications of HIV
- Diabetes Mellitus
  - Blood glucose / hemoglobin A1c: surrogate for complications

Surrogate Endpoint

- Surrogate endpoints also used in vaccine efficacy trials in terms of correlates of protective immunity, measured as antibody titres following vaccination
- Qin et al used data from a trial of a trivalent influenza vaccine carried out in 1943 to illustrate the validation of influenza-specific antibody titre as a surrogate of protection for hospitalization with influenza (the clinical endpoint), using the Prentice criteria
  - Vaccine efficacy (VE) of 73% was reported against hospitalization with influenza Weiss strain A (criterion i)
  - Antibody titres to Weiss strain A were higher in vaccinated compared to unvaccinated individuals (criterion ii)
  - The antibody titres were inversely associated with hospitalization for influenza (criterion iii)

Surrogate Endpoint

- Haemagglutination Inhibition (HI) antibody titre cut-off level for clinical protection against influenza infection using adjuvanted trivalent vaccine (Trombetta CM et al, *Vaccines* 2014, *2*, 707-734)

Protection Level	Antibody Cutoff Level
50%	1:110
70%	1:215
80%	1:330
90%	1:629

Monitoring for adverse effects

- Monitoring for adverse effects by repeated laboratory testing may have begun with the observation that chloramphenicol could cause bone-marrow toxicity in case of prolonged or repeated administration

Drugs	Monitoring requirement
ACE inhibitors or angiotensin-II receptor antagonists	Creatinine Potassium
Diuretics	Electrolytes

Monitoring for adverse effects

- Adverse events related to drug interactions e.g. oral anticoagulants, or exposure in vulnerable patients with disease states that predispose patients to NSAID toxicity, are common and may result in significant morbidity and mortality
- Following tests are carried out:
  - Serum electrolytes, including magnesium and phosphorus
  - Renal function studies
  - Liver function tests
  - Coagulation studies, including a prothrombin time with international normalized ratio (INR)
  - Complete blood count (CBC)