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Issue No.	Revision Details	Effective Date
1.01	Addition of Malaria & D-dimer tests. Updated issue number format. Added reference to consultant. Issue number format updated.	25/10/2012
1.02	Update of DDimer preparation section.	14/12/2012
1.03	Update Accreditation for FBC & ADLC, Blood parasite. Addition of revision table.	25/02/2013
1.04	Updated TAT for Blood parasitology.	25/04/2013
1.05	Addition of reference range sources to all tests as per ISO15189:2012. Change of Accusay Malaria kit to OptiMAL kits.	08/04/2014
1.06	Coagulation updated from 4 hours stability to 12 hours as per revised WHO guidelines. D-dimer instrument updated to Roche Cobas h232 Point of Care meter.	23/04/15
1.07	Addition of Dr Patrick Hayden as haematology consultant Addition of Sat to running ESR Change of MS kit from Clearview IM (Inverness Medical) to Clearview IM II (Alere)	26/08/15
1.08	Removal of Blood Groups. Update for Blood parasitology stability. Change FBC stability from 3 days to 2 days.	19/04/17
2.01	Eurofins Biomnis rebranding	24/05/17
2.02	Minor discrepancy corrections	28/06/17
2.03	Activated partial thromboplastin time. Update transport & storage. Reference (SIEMENS Kit Insert & CSLI H21-A5). Update % parasitemia for Plasmodium knowlesi. Addition ideal procedure of avoidance of collecting blood through intravenous lines that have been previously flushed with heparin.	08/08/17
2.04	Travel history for parasitology & contact lab for symptomatic patients.	09/05/18

CONTENTS

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FULL BLOOD COUNT (FBC)

The Full Blood Count (FBC) is one of the most commonly requested laboratory tests and is a very useful indicator of potential problems that require further investigation. Common causes of an abnormal RCC & haematocrit include anaemia, acute blood loss & polycythaemia. Depressed WCC may be seen in AIDS, cancer and overwhelming infections. High WCCs are commonly observed in leukaemia and infections. A high neutrophil count often indicates an infection. A low lymphocyte count can be seen in AIDS patients. A high monocyte count can indicate a bacterial infection and a high eosinophil count indicates an allergic reaction or a parasitic infection. Common causes of a low platelet count include immune system disorders, some leukaemias and patients undergoing cancer treatments.

Preparation of patients: There is no physical preparation for the FBC test.
Precautions: Frozen, clotted, or grossly haemolysed samples can not be analysed.

Accredited	Yes
Method	Haematology – Sysmex XE2100D SOP: H49
Sample Requirements	Tube Type: Whole Blood EDTA (Lavender cap) Temperature: + 4ºC Miscellanous: Non fasting
Turn Around Time – Setup Schedule	24h

Mon	Tue	Wed	Thu	Fri	Sat
✓	✓	✓	✓	✓	✓

Stability	2 days @ + 4ºC

Units - Reference Ranges

Analyte	Adult Reference Range Male	Adult Reference Range Female	Units Of Measurement
WBC	3.88-10.49	3.88-10.49	10^9/L
RBC	4.28-5.59	3.73-5.02	10^12/L
HB	13.5-17.2	11.3-15.2	g/dL
HCT	0.381-0.499	0.323-0.462	Ratio
MCV	83.1-99.1	83.1-99.1	fL
MCH	28.3-33.9	28.3-33.9	pg
MCHC	32.1-36.6	32.1-36.6	g/dL
PLT	164-382	164-382	10^9/L
RDW	11.5-14.5	11.5-14.5	%
#Neut	1.56-6.52	1.56-6.52	10^9/L
#Lymph	1.01-3.13	1.01-3.13	10^9/L
#Mono	0.23-0.88	0.23-0.88	10^9/L
#Eos	0.05-0.51	0.05-0.51	10^9/L
#Baso	0.02-0.15	0.02-0.15	10^9/L

FULL BLOOD COUNT (FBC)	
Source	Reference ranges for FBC testing are derived from in house testing on corporate health screen clients (apparently healthy individuals). Data (n > 1000 all parameters, Male and Female subgroups n > 1000) was analysed according to the NCCLS C-28A guidelines, using the EP Evaluator software. Reference ranges were established using parametric, transformed parametric, or non-parametric methods as appropriate. Additionally, the in-house derived reference ranges are used in the moving-average calculations on the SYSMEX PC software. This is used to monitor instrument drift independent of patient population tested due to randomisation.

ERYTHROCYTE SEDIMENTATION RATE (ESR)

The Erythrocyte Sedimentation Rate (ESR) is a traditional test used in Haematology. It is affected by the numbers of erythrocytes present in the blood, changes in plasma protein pattern, age and sex of the patient. Three phases can be distinguished in the sedimentation process: the lag phase (reflects the period in which the individual erythrocytes form rouleaux), the decantation or precipitation phase (the plasma-red cell interface falls more rapidly), and the final packing phase (the red cells aggregates pile up on the bottom of container). The ESR is dependent on the presence of agglomers, such as fibrinogen, IgM, alpha2-macroglobulin and other acute phase proteins. It is a completely non-specific test. It is a measure of the presence and severity of pathological processes. In general, the ESR is elevated in all acute, general infections, in localized, acute, inflammatory conditions, variations in the ESR depend on the nature and severity of the process. Additionally, the ESR is an important screen for occult disease. The ESR is also useful to differentiate organic disease from functional disorders, or as a guide to the progress of diseases such as rheumatic carditis, rheumatoid arthritis, and certain malignancies, including Hodgkin's disease, and is diagnostic for Temporal Arteritis.

Preparation of patients: There is no physical preparation for the ESR test.

Precautions: The ESR should not be used to screen healthy persons for disease.

Accredited	Yes
Method	Haematology – Capillary photometric-kinetic technology SOP: H9
Sample Requirements	Tube Type: Whole Blood (Lavender cap) Temperature: + 4ºC Miscellanous: Non fasting
Turn Around Time – Setup Schedule	24h
Stability	2 days @ + 4ºC
Units - Reference Ranges	mm/hr
Source	Reference ranges for the ESR assay are derived in house. Data was obtained from a clinical normal population and statistics generated using the Graph Pad statistics module. Data was analysed for Gaussian distribution and reference ranges derived using either parametric or non-parametric statistics. A copy of the data is kept in the QA department.

Mon	Tue	Wed	Thu	Fri	Sat
✓	✓	✓	✓	✓	✓

ESR Ref Ranges	Male	Female
>50 Years	0-12	0-15
<50 Years	0-8	0-10

INFECTIOUS MONONUCLEOSIS

Infectious mononucleosis (glandular fever) is an acute infectious disease caused by the Epstein-Barr virus and primarily affects lymphoid tissue. It is characterized by the appearance of enlarged and often tender lymph nodes, enlarged spleen, and abnormal lymphocytes in the blood. Patients usually, but not always, develop a transient heterophile antibody response.
The detection of heterophile antibodies of Infectious Mononucleosis by the agglutination of sheep red cells was first reported by Paul and Bunnel. Subsequent work identified the need for differential absorption of sera to remove non-infectious mononucleosis heterophile antibodies. Fetcher and Woolfolk showed that antigens obtained from bovine erythrocytes were more effective than those antigens obtained from either sheep or horse erythrocytes.

Preparation of patients: There is no physical preparation for the infectious mononucleosis test.

Precautions: IgG and IgM values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported IgG or IgM level cannot be correlated to an endpoint titre.

Accredited	No
Method	Haematology- Immunoassay SOP: H20
Sample Requirements	Tube Type: Serum (gold or red cap) Plasma (Green and lavender cap) Temperature: + 4ºC Miscellaneous: Non fasting
Turn Around Time – Setup Schedule	24h
Stability	3 days @ + 4ºC
Result	Positive or negative
Source	'Clearview IM II' Kit Insert, Alere group.

Mon	Tue	Wed	Thu	Fri
✓	✓	✓	✓	✓

SAMPLE REQUIREMENTS FOR COAGULATION TESTS

PROCEDURE

Sample Requirements and Collection

- Patients should be relaxed pre-venepuncture. Excessive stress and exercise will increase FVIII, vWF antigen and fibrinolysis. Venous occlusion should be avoided.
- Difficult venepuncture with trauma may lead to platelet activation with release of PF4 from alpha granules.
- Venous blood should be collected into coagulation tubes containing Sodium Citrate 3.2%, 0.105M, 3ml.
- Specimens must be mixed immediately post venepuncture to avoid clot activation, by GENTLY inverting the tubes 5 to 10 times.
- The ratio of whole blood to anticoagulant is crucial. Under-filled specimens will not be processed as over- or under-filled tubes can adversely affect results.
- Any warfarin treatment should be mentioned on the request form.

Transportation and Storage

- PT/INR specimens should ideally be analysed within 12 hours of collection and transported to the laboratory at room temperature.
- APTT and Fibrinogen should ideally be analysed within 4 hours of collection. Where this is not possible please centrifuge at room temperature (RT) @3000rpm (1500g) for at least 15 minutes, and then carefully remove the plasma from the cells, transfer to a fresh plastic plain tube and freeze at -20^{o}C.
- Non-frozen coagulation specimens should be transported at RT ASAP to avoid deterioration of labile factors V and VIII.
- Collection of blood through intravenous lines that have been previously flushed with heparin should be avoided. In the event blood is drawn from an indwelling catheter, the line should be flushed with 5ml of saline, and the first 5ml of blood or 6 times the line volume be drawn off and discarded before coagulation tube is filled.

Plasma Sample Stability (CLSI H21-A5)

- PT 24 hours @ RT or 2 weeks @-20^{o}C
- APTT 4 hours @ RT or 2 weeks @-20^{o}C & 12 Months @-70^{o}C
- Fibrinogen- 4 hours @ RT

FIBRINOGEN (CLAUSS METHOD)

Clauss developed a simple method for the quantitative determination of Fibrinogen by measuring the clotting time of dilute plasma after the addition of Thrombin. The clotting time is inversely proportional to the Fibrinogen concentration. The clotting time obtained in this manner is then compared with that of a standardised Fibrinogen preparation. The plasma must be diluted to provide a low level of potential inhibitors, e.g. FDP's and Heparin. A strong Thrombin solution is utilised in order to ensure that the clotting time is independent of Thrombin concentration over a broad range.

Preparation of patient: Patients should be relaxed pre-venepuncture. Excessive stress and exercise will increase Factor VIII, vWF antigen and fibrinolysis. Veno-occlusion should be avoided.

Precautions: This test is not recommended for patients with active bleeding, acute infection or illness, or in those patients who have received blood transfusions within four weeks. Drugs that may increase Fibrinogen levels include Oestrogens and oral contraceptives. Drugs that may cause decreased levels include anabolic steroids, androgens, Phenobarbital, Urokinase, Streptokinase, and Valproic acid.

Accredited	Yes
Method	Haematology- Coagulation SOP: H35
Sample Requirements	Tube Type: Sodium Citrate Plasma (Light blue cap) **Temperature**: 4 hours Room temperature or 2 weeks @ -20ºC. If an expected delay in transporting samples to the laboratory samples should be centrifuge, separated & send as frozen within 4 hours of blood collection. Miscellaneous: Non fasting **Collection: Cf. Special requirement for Coagulation test**
Turn Around Time – Setup Schedule	24h Mon ✓ Tue ✓ Wed ✓ Thu ✓ Fri ✓
Stability	Reality: Within 4 hours, unless centrifuged at room temperature for 3000rpm (1500g) separated and frozen can only be thawed once. If an expected delay from collection time to receipt in the laboratory, suggest send frozen sample. (SIEMENS Kit Insert & CSLI H21-A5)
Units - Reference Ranges	1.5-4.0 g/l
Source	The RR was derived from the Adelaide & Meath Hospital CA-6000 Analyser. This is based on the correlation study during validation and compatible platforms, reagents, calibrators and controls.

PROTHROMBIN TIME (PT)

The PT test (scientific name- tissue activated induced coagulation time) has been in clinical practice for over 60 years. The first standardised one-stage PT test was devolved by Dr. Armand Quick in 1932. It has now become the basic coagulation screening test for the diagnosis of acquired and congenital deficiencies of clotting factors in the Extrinsic pathway. The assay was designed to measure a coagulation defect before the introduction of oral anticoagulants, and later adapted for monitoring their dosage. The PT reflects changes in the Extrinsic factors II, VII and X, three of the principle clotting factors depressed by Coumarin drugs, and FV, not reduced by oral anticoagulation. It can also be used to assess the protein synthesis capability of the liver in chronic or acute hepatic disorders. The test depends on the activation of Factor X in the presence of Factor VII by Tissue Factor (TF) and bypassing of the Intrinsic clotting pathway. The speed of the reaction and the responsiveness of the PT to deficiencies of clotting factors depend upon the properties and concentration of the TF as well as on the clotting factor concentrations.

Preparation of patient: Patients should be relaxed pre-venepuncture. Excessive stress and exercise will increase Factor VIII, vWF antigen and fibrinolysis. Veno-occlusion should be avoided.

Precautions: The doctor should check to see if the patient is taking any medications that may affect test results. This precaution is particularly important if the patient is taking Warfarin, because there are a number of medications that can interact with Warfarin to increase or decrease the PT time.

Accredited	Yes
Method	Haematology- Coagulation SOP: H33
Sample Requirements	Tube Type: Sodium Citrate Plasma (Light blue cap) **Temperature**:12 hours Room Temperature or 2 weeks @ -20ºC Miscellaneous: Non fasting. **Collection: Cf. Special requirement for Coagulation test**
Turn Around Time – Setup Schedule	24h Mon ✓ Tue ✓ Wed ✓ Thu ✓ Fri ✓
Stability	Whole blood: 12 hours at room temperature. If a longer delay is expected in transport to the laboratory, centrifuge @ 3000rpm (1500g) for at least 15 minutes, separate, and freeze plasma. Can only be thawed once. (SIEMENS Kit Insert & CSLI H21-A5)
Units - Reference Ranges	PT 10 - 13 Seconds INR 0.9 - 1.3 INRW 2.0-4.0
Source	The RR was derived from the Adelaide & Meath Hospital CA-6000 Analyser. This is based on the correlation study during validation and compatible platforms, reagents, calibrators and controls.

ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

This test is also known as the Partial Thromboplastin Time with Kaolin (PTTK) and the Kaolin Cephalin Clotting Time (KCCT) reflecting the methods used to perform the test. The APTT is the main screening test for the Intrinsic clotting system and is the second most common coagulation test being exceeded in frequency only by the prothrombin time.

The Partial Thromboplastin of the APTT is a phospholipid extract of animal tissue or from vegetable sources. The phospholipids act as a platelet substitute in the Intrinsic system. The test incorporates an activator, in the absence of added Thromboplastin, which shortens the test and increases the precision and reproducibility by eliminating the variable effects of contact from glass surfaces and by effecting maximum activation. The activator is used to stimulate the production of FXIIa by providing a surface for the function of high molecular weight Kininogen, Kallikrein and FXIIa. The contact activation occurs for a time at 37ºC. Calcium is then added to trigger further reactions and the time required for clot formation measured. Standardised Phospholipids are required to form complexes, which activate FX and Prothrombin, which allows the test to be conducted in patient Platelet poor plasma (PPP).

The test depends not only on the contact factors and factors VIII and IX, but also on the reactions with factors X, V, Prothrombin and Fibrinogen. It is also sensitive to the presence of circulating anticoagulants (inhibitors) and Heparin.

Preparation of patient: Patients should be relaxed pre-venepuncture. Excessive stress and exercise will increase Factor VIII, vWF antigen and fibrinolysis. Veno-occlusion should be avoided.

Precautions: APTT results may be affected by many commonly administered drugs and further studies should be made to determine the source of unexpected abnormal results. Oral contraceptive and Oestrogen therapy in males have been found to decrease APTT in vivo. Conversely, Heparin, Warfarin, Lupus anticoagulant and radio therapy have been found to increase APTT in vivo.

Accredited	Yes
Method	Haematology – Coagulation SOP: H34
Sample Requirements	Tube Type: Sodium Citrate Plasma (Light blue cap) **Temperature:** 4 hours Room temperature or 2 weeks -20ºC Miscellaneous: Non fasting **Collection: Cf. Special requirement for Coagulation test**
Turn Around Time – Setup Schedule	24h Mon ✓ Tue ✓ Wed ✓ Thu ✓ Fri ✓
Stability	Whole blood: 4 hours at room temperature. If a longer delay is expected in transport to the laboratory, centrifuge @ 3000rpm (1500g) for at least 15 minutes, separate, and freeze plasma. Can only be thawed once. (SIEMENS Kit Insert & CSLI H21-A5)
Units - Reference Ranges	22.6-29.6 seconds.
Source	The RR was derived from the Adelaide & Meath Hospital CA-6000 Analyser. This is based on the correlation study during validation and compatible platforms, reagents, calibrators and controls.

BLOOD PARASITOLOGY (MALARIA, MICROFILARIAE & TRYPANOSOMES)

Human blood parasites include an eclectic collection of organisms ranging from protozoan intracellular pathogens to nematodes residing in the extracellular compartment.
Malaria is caused by a group of related intracellular protozoan pathogens of the genus Plasmodium. These species exhibit a complex life cycle reliant on a mammalian host and anopheles mosquito vector. In the human host they are obligate intracellular pathogens infecting initially the liver in the sporozoite form. In the liver the parasites replicate and develop into merozoites which are then released into the blood stream. These infect the erythrocyte and begin a restricted life cycle. Each parasite may develop into a schizont (a cluster of infectious units which may invade further erythrocytes) or a gametocyte (the sexual form which may be transferred to the anopheles mosquito upon taking a blood meal). Re-infection of the liver does not occur.
Suspected malaria is a medical emergency. Sampling and processing of the blood sample must not be delayed if malaria is suspected.
Four primary species of malaria have been identified in humans: P. falciparum, P. vivax, P. ovale and P. malariae. A fifth species, P. knowlesi has been recently identified. Their geographic distribution is unique for each species.
Blood should ideally be taken direct from the patient's finger or ear & the films prepared at the bedside or in the clinic. When this is not possible blood taken into anticoagulant (EDTA) can be used. Thick & thin blood films should be made as soon as possible, certainly less than 2 hours after the EDTA blood was drawn, to minimise morphological changes in the parasites. Parasite and red cell morphology can be seriously affected if the blood has been in anticoagulation for too long.
Where there is a strong clinical suspicion if the first films are negative, blood should be taken and films made and checked at least two times over the first 24 hours and further films examined every 12 hours after that if strongly clinically indicated.
Trypanosomes are kinetopastids of the genus Trypanosoma. Two species are known to infect humans: T. brucei, a species causing sleeping sickness found in sub-Saharan Africa and T. cruzi, a South American variant which causes Chagas disease. They are transferred by blood feeding invertebrate vectors.
Microfilaria are an early developmental stage of a collection of nematode parasites of the Onchocercidae family. These parasites are transmitted by blood feeding invertebrates in tropical regions. In the human host adult filaria reside in body cavities, mating to produce microfilaria which are released into the peripheral blood. Periodicity has been widely observed in some species.

Preparation of patients: Ensure patient is bled at appropriate period for microfilariae testing.
Travel history should be recorded and any clinical details recorded on the request form.
In symptomatic patients please phone the laboratory prior to sending the sample.

Precautions: Global distribution of malaria is restricted to areas endemic to the anopheles mosquito. Latent infections of some species may occur due to hypnozoites stored in the liver. P. knowlesi is morphologically indistinguishable from P. malariae on blood film preparations.
The presence of microfilariae in the peripheral blood exhibits periodicity. It is important that the patient is bled at the appropriate period for the suspected species.

Accredited	Yes (For
Method	Haematology – Thick & Thin Blood film & OptiMAL Malaria antigen kit (Kit Insert BIO-RAD OptiMAL- 01/2010, code:881056) SOP: H47
Sample Requirements	Tube Type: Whole Blood K2/K3 EDTA (Lavender cap) Thick & Thin blood films taken less than 2 hours after blood drawn. Temperature: + 4ºC Miscellanous: Non fasting Observe periodicity where applicable.

BLOOD PARASITOLOGY (MALARIA, MICROFILARIAE & TRYPANOSOMES)	
Turn Around Time – Setup Schedule	24h Mon ✓ | Tue ✓ | Wed ✓ | Thu ✓ | Fri ✓
Stability	Blood films Thick & thin need to be made less than 2 hours after the blood EDTA was drawn. 2 days @ + 4ºC (Opti-Mal Malaria Antigen Kit)
Units - Reference Ranges	% parasitaemia (applicable to P. *falciparum* & P. *knowlesi* infection) No reference ranges applicable. Genus and species reportable.
Source	WHO Guideline: 'The Laboratory Diagnosis of Malaria'. J.W Bailey, B.J Bain, J Parker-Williams and P.Chiodini for the General Haematology Task Force of the British Committee for Standards in Haematology. http://www.bcshguides.com/documents/malaria-bcsh.2005.pdf Malaria Reference Laboratory. www.malaria-reference.co.uk

D-DIMER

D-dimer is a degradation product of cross-linked fibrin. The D-dimer concentration is a measure of the fibrinolytic activity of plasmin in the vascular system. Elevated concentrations of D-dimer indicate increased coagulatory and fibrinolytic activity. With a normal D-dimer value, acute deep vein thrombosis and pulmonary embolisms may be ruled out with high reliability.

Preparation of patients: There is no physical preparation for the D-Dimer test.

Precautions: No modifications to change diet, medications, or activity required before this test. Phlebotomists should enquire about any blood thinners or anticoagulant medication any diseases like liver disease and rheumatoid arthritis. There are some herbs that are also able to replicate the effects of blood thinning medication.

Accredited	No
Method	Haematology – Roche Cobas h232 Point of Care meter SOP: H46
Sample Requirements	Tube Type: Whole blood Lithium Heparin (green cap & Orange cap for Sarstedt tubes) Temperature: 15-25°C (Do not refrigerate or freeze) Miscellanous: Non-fasting
Turn Around Time – Setup Schedule	Same Day
Stability	8 hours @ 15-25°C
Units - Reference Ranges	0.0-0.50 µg/mL
Source	Roche Cardiac D-Dimer Assay Kit Insert – 2012-11, V4 English

Mon	Tue	Wed	Thu	Fri
✓	✓	✓	✓	✓