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The Crux

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ISO 9001

Editorial

In keeping with not forgetting our social responsibilities as per the times and ongoing medical problems afflicting the world at large, we, in this issue bring to you all clinico-diagnostic aspects as related to H1N1, influenza A also known as Swine Flu. As of 2009, the known SIV strains include influenza C and the subtypes of influenza A known as H1N1, H1N2, H3N1, H3N2, and H2N3. Swine influenza virus is common throughout pig populations worldwide. Transmission of the virus from pigs to humans is not common and does not always lead to human influenza, often resulting only in the production of antibodies in the blood. If transmission does cause human influenza, it is called zoonotic swine flu. People with regular exposure to pigs are at increased risk of swine flu infection. The meat of an infected animal poses no risk of infection when properly cooked.

According to WHO, by the time the current H1N1 loses its force, it would have infected a third of the world's population. Though mortality rate is just about 0.1% (i.e., one in a thousand), the fear factor drains a person mentally. Those otherwise having some other unrelated/ respiratory disorders, pregnant women are at greater risk. If started early, the treatment is usually curative. Historically important outbreaks have occurred in 1918, 1976, 1988, 1998, 2007 and the latest current one of 2009. It usually comes in two or three waves. For further amplification/ elucidation, just dip into the next few pages outlined under DISEASE DIAGNOSIS!

INTERPRETATION segment deals in length with a newly propped up (though known for quite sone time) marker for renal function evaluation. Something that is better than urea/ creatinine, inulin/ creatinine clearance tests etc. To gather an insight into the world of Cystatin C and to understand the clinical utility please flip through the concerned pages within this communique.

Of late, a few issues have been raised about the Malaria RDTs. If manufacturer's recommendations/ test protocol are not followed to the hilt, erroneous results are bound to occur. Most important factors being – (1). Perform the test immediately after taking blood sample, (2). Judge the efficacy of a test unit against wild strains and not the cultured ones and lastly do not deviate from the manufacturer's advise. TROUBLE SHOOTING portion more than amply describes the working of Malaria RDTs and suggests how best to obviate problems/ anomalies.

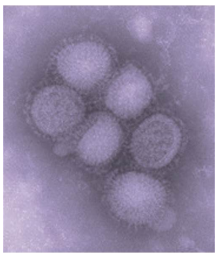
BOUQUET is there in its usual flavour and fervour. Brain teasers are pictorial in this issue too!

Tulip Group

DISEASE DIAGNOSIS

SWINE INFLUENZA

Swine influenza is endemic in pigs



Electron microscope image of the reassorted H1N1 influenza virus. The viruses are 80–120 nanometres in diameter.

INTRODUCTION: Swine influenza (also called swine flu, hog flu, and pig flu) is an infection by any one of several types of swine influenza virus. Swine influenza virus (SIV) is any strain of the influenza family of viruses that is endemic in pigs. During the mid-20th century, identification of influenza subtypes became possible, allowing accurate diagnosis of transmission to humans. Since then, only 50 such transmissions have been confirmed. These strains of swine flu rarely pass from human to human. Symptoms of zoonotic swine flu in humans are similar to those of influenza and of influenza-like illness in general, namely chills, fever, sore throat, muscle pains, severe headache, coughing, weakness and general discomfort.

Classification: Of the three genera of influenza viruses that cause human flu, two also cause influenza in pigs, with influenza A being common in pigs and influenza C being rare. Influenza B has not been reported in pigs. Within influenza A and influenza C, the strains found in pigs and humans are largely distinct, although due to reassortment there have been transfers of genes among strains crossing swine, avian, and human species boundaries. **Influenza C:** Influenza C viruses infect both humans and pigs, but do not infect birds. Transmission between pigs and humans have occurred in the past. For example, influenza C caused small outbreaks of a mild form of influenza amongst children in Japan and California. Due to its limited host range and the lack of genetic diversity in influenza C, this form of influenza does not cause pandemics in humans. **Influenza A:** Swine influenza is known to be caused by influenza A subtypes H1N1, H1N2, H2N3, H3N1, and H3N2. In pigs, three influenza A virus subtypes (H1N1, H1N2, and H3N2) are the most common strains worldwide. In the United States, the H1N1 subtype was exclusively prevalent among swine populations before 1998; however, since late August 1998, H3N2 subtypes have been isolated from pigs. As of 2004, H3N2 virus isolates in US swine and turkey stocks were triple reassortants, containing genes from human (HA, NA, and PB1), swine (NS, NP, and M), and avian (PB2 and PA) lineages.

History: Swine influenza was first proposed to be a disease related to human influenza during the 1918 flu pandemic, when pigs became sick at the same time as humans. The first identification of an influenza virus as a cause of disease in pigs occurred about ten years later, in 1930. For the following 60 years, swine influenza strains were almost exclusively H1N1. Then, between 1997 and 2002, new strains of three different subtypes and five different genotypes emerged as causes of influenza among pigs in North America. In 1997-1998, H3N2 strains emerged. These strains, which include genes derived by reassortment from human, swine and avian viruses, have become a major cause of swine influenza in North America. Reassortment between H1N1 and H3N2 produced H1N2. In 1999 in Canada, a strain of H4N6 crossed the species barrier from birds to pigs, but was contained on a single farm. The H1N1 form of swine flu is one of the descendants of the strain that caused the 1918 flu pandemic. As well as persisting in pigs, the descendants of the 1918 virus have also circulated in humans through the 20th century, contributing to the normal seasonal epidemics of influenza. However, direct transmission from pigs to humans is rare, with only 12 cases in the U.S. since 2005. Nevertheless, the retention of influenza strains in pigs after these strains have disappeared from the human population might make pigs a reservoir where influenza viruses could persist, later emerging to reinfect humans once human immunity to these strains has waned. Swine flu has been reported numerous times as a zoonosis in humans, usually with limited distribution, rarely with a widespread distribution. Outbreaks in swine are common and cause significant economic losses in industry, primarily by causing stunting and extended time to market. For example, this disease costs the British meat industry about £65 million every year.

Historically Important outbreaks: 1918 pandemic in humans, 1976 U.S. outbreak, 1988 zoonosis, 1998 US outbreak in swine, 2007 Philippine outbreak in swine, 2009 outbreak in humans.

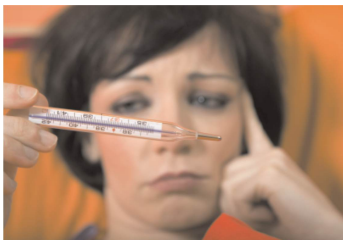
2009 outbreak in humans: The H1N1 viral strain implicated in the 2009 flu pandemic among humans often is called "swine flu" because initial testing showed many of the genes in the virus were similar to influenza viruses normally occurring in North American swine. But further research has shown that the outbreak is due to a new strain of H1N1 not previously reported in pigs. In late April, Margaret Chan, the World Health Organization's director-general, declared a "public health emergency of international concern" under the rules of the WHO's new International Health Regulations when the first cases of the H1N1 virus were reported in the United States. Following the outbreak, on May 2, 2009, it was reported in pigs at a farm in Alberta, Canada, with a link to the outbreak in Mexico. The pigs are suspected to have caught this new strain of virus from a farm worker who recently returned from Mexico, then showed symptoms of an influenza-like illness. The new strain was initially described as an apparent reassortment of at least four strains of influenza A virus subtype H1N1, including one strain endemic in humans, one endemic in birds, and two endemic in swine. Subsequent analysis suggested it was a reassortment of just two strains, both found in swine. Although initial reports identified the new strain as swine influenza (i.e., a zoonosis originating in swine), its origin is unknown. Several countries took precautionary measures to reduce the chances for a global pandemic of the disease. The 2009 swine flu has been compared to other similar types of influenza virus in terms of mortality: "in the US it appears that for every 1000 people who get infected, about 40 people need admission to hospital and about one person dies"..There are fears that swine flu will become a major global pandemic in the winter months, with many countries planning major vaccination campaigns.

Transmission: Transmission between pigs: Influenza is quite common in pigs, with about half of breeding pigs having been exposed to the virus in the US. Antibodies to the virus are also common in pigs in other countries. The main route of transmission is through direct contact between infected and uninfected animals. These close contacts are particularly common during animal transport. Intensive farming may also increase the risk of transmission, as the pigs are raised in very close proximity to each other. The direct transfer of the virus probably occurs either by pigs touching noses, or through dried mucus. Airborne transmission through the aerosols produced by pigs coughing or sneezing are also an important means of infection. The virus usually spreads quickly through a herd, infecting all the pigs within just a few days. Transmission may also occur through wild animals, such as wild boar, which can spread the disease between farms. **Transmission to humans:** People who work with poultry and swine, especially people with intense exposures, are at increased risk of zoonotic infection with influenza virus endemic in these animals, and constitute a population of human hosts in which zoonosis and reassortment can co-occur. Vaccination of these workers against influenza and surveillance for new influenza strains among this population may therefore be an important public health measure. Transmission of influenza from swine to humans who work with swine was documented in a small surveillance study performed in 2004 at the University of Iowa. This study among others forms the basis of a recommendation that people whose jobs involve handling poultry and swine be the focus of increased public health surveillance. Other professions at particular risk of infection are veterinarians and meat processing workers, although the risk of infection for both of these groups is lower than that of

farm workers. **Interaction with avian H5N1 in pigs:** Pigs are unusual as they can be infected with influenza strains that usually infect three different species: pigs, birds and humans. This makes pigs a host where influenza viruses might exchange genes, producing new and dangerous strains. Avian influenza virus H3N2 is endemic in pigs in China and has been detected in pigs in Vietnam, increasing fears of the emergence of new variant strains. H3N2 evolved from H2N2 by antigenic shift. In August 2004, researchers in China found H5N1 in pigs.

Signs and symptoms: In swine: In pigs influenza infection produces fever, lethargy, sneezing, coughing, difficulty breathing and decreased appetite. In some cases the infection can cause abortion. Although mortality is usually low (around 1-4%),the virus can produce weight loss and poor growth, causing economic loss to farmers. Infected pigs can lose up to 12 pounds of body weight over a 3 to 4 week period. **In humans the symptoms of H1N1 (swine) flu** are similar to the symptoms of regular human flu and include fever, cough, sore throat, body aches, headache, chills and fatigue. Some people have reported diarrhea and vomiting associated with H1N1 (swine) flu. In the past, severe illness (pneumonia and respiratory failure) and deaths have been reported with H1N1 (swine) flu infection in people. Like seasonal flu, H1N1 (swine) flu may cause a worsening of underlying chronic medical conditions. In children emergency warning signs that need urgent medical attention include: Fast breathing or trouble breathing, Bluish or gray skin color, Not drinking enough fluids, Severe or persistent vomiting, Not waking up or not interacting, Being so irritable that the child does not want to be held, Flu-like symptoms improve but then return with fever and worse cough. In adults, emergency warning signs that need urgent medical attention include: Difficulty breathing or shortness of breath, Pain or pressure in the chest or abdomen, Sudden dizziness, Confusion, Severe or persistent vomiting, Flu-like symptoms improve but then return with fever and worse cough.

The signs of H1N1 infection, which are similar to other forms of influenza, include: fever, coughing, headaches, muscle or joint pain, sore throat, chills, fatigue, runny nose, diarrhea and vomiting (in some cases). People at higher risk of serious complications include: people age 65 years or older, children younger than 5 years old, pregnant women, people of any age with conditions like asthma, diabetes, obesity, heart disease, HIV/AIDS or other conditions that weaken the immune system. According to the U.S. Centers for Disease Control (CDC), more than 70% of hospitalizations in the U.S. have been people with such underlying conditions. However, there is some evidence that H1N1, like previous strains of pandemic influenza, can also cause serious illness in some otherwise healthy children and young adults.



Symptoms that may require medical attention: Certain symptoms may require emergency medical attention. **In children:** signs of respiratory distress, blue lips and skin, dehydration, rapid breathing, excessive sleeping, extreme irritability and unwillingness to be held, persistent vomiting, the return of flu-like symptoms that include a fever and cough. **In adults:** shortness of breath, pain in the chest or abdomen, sudden dizziness or confusion, persistent vomiting, the return of flu-like symptoms that include a fever and cough.

DIAGNOSIS: Specimens: Upper respiratory tract specimens as recommended for seasonal influenza investigation are the most appropriate. Samples should be taken from the deep nostrils (nasal swab), nasopharynx (nasopharyngeal swab), Nasopharyngeal aspirate, throat or bronchial aspirate. It is not yet known which clinical specimen gives the best diagnostic yield. Appropriate precautions should be taken in collecting specimens since this may expose the collector to respiratory secretions from patients. There is, as yet, no information on the diagnostic value of non-respiratory specimens, e.g., stool samples. Acute and convalescent serum specimens should be used for the detection of rising antibody titres. **Laboratory tests - Molecular diagnostics:** Molecular diagnostics are currently the method of choice for influenza A (H1N1) swine lineage (swl) virus (A/California/4/2009-like viruses). The use of different target gene assays is more appropriate for correct identification of this virus. The following gene targets are important: type A influenza matrix gene; haemagglutinin gene specific for influenza A (H1N1)swl virus and haemagglutinin gene specific for seasonal influenza A H1/H3 and other subtypes. The following protocols are currently available: 1) influenza A type-specific conventional and realtime PCR (see Annexes 1 and 2); and CDC realtime RT-PCR (rRT-PCR) protocol for the detection and characterization of influenza A (H1N1) (version 2009). Sequence analyses of the type A influenza matrix gene PCR product using the primers in the WHO protocols will differentiate between M genes of swine-lineage and seasonal H1N1 viruses, however, additional analysis should be performed to confirm the origin of the virus. Conventional RT-PCR assays are currently being evaluated. Virus isolation and typing by haemagglutination inhibition or immunofluorescence: Current protocols for virus isolation of seasonal influenza viruses using MDCK cells and egg inoculation can be used, although their sensitivity remains to be determined. Turkey, chicken, guinea pig and human red blood cells will agglutinate with the influenza A (H1N1)swl virus. Polyclonal antibodies specific for subtype H1 seasonal influenza viruses from the WHO influenza reagent kit will not react in the haemagglutination inhibition (HAI) test with the current influenza A (H1N1)virus. Results obtained using the H1 monoclonal antibodies in the WHO kit should not be taken as conclusive and further verification is recommended. **Rapid tests or immunofluorescence:** The sensitivity and specificity of rapid-point-of-care or immunofluorescence tests designed for direct detection of influenza A viruses are currently unknown. It should be emphasized that these tests will not differentiate seasonal influenza from influenza A (H1N1)swl virus. **Serology:** HAI and microneutralization tests using influenza A (H1N1)swl virus are expected to be able to detect antibody responses following infection. **Interpretation of laboratory results:** PCR: A sample is considered positive if results from tests using two different PCR targets (e.g. primers specific for universal M gene and swine H1 haemagglutinin gene) are positive but the PCR for human H1 + H3 is negative. If RT-PCR for multiple haemagglutinin (HA) targets (i.e. H1, H3, and H1-swine-lineage) give positive results in the same specimen, the possibility of PCR contamination should first be excluded by repeating PCR procedure using new RNA extract from the original specimen or RNA extract from another specimen. If repeated positive results for multiple HA targets are obtained, this raises the possibility of co-infection, which should be confirmed by sequencing or virus culture. CDC realtime PCR assays: Results should be interpreted as described in the CDC H1N1real time assay protocol/manual. A negative PCR result does not rule out that a person may be infected with influenza A (H1N1) virus: Results should be interpreted in conjunction with the available clinical and epidemiological information. Specimens from patients whose PCR results are negative but for whom there is a high suspicion of A (H1N1) infection should be further investigated and tested by other methods such as virus culture or serology, to rule out influenza A (H1N1)swl infection. Serology: A four-fold or greater rise in specific influenza A (H1N1)swl virus antibody titres indicates recent infection with the virus. Sequencing: At this stage, sequencing of at least one target product is essential for confirmation of conventional PCR. Virus isolation: Identification and typing of a cultured influenza virus can be carriedout by PCR, indirect fluorescent antibody (IFA) testing using specific NP monoclonal antibodies, or HA and antigenic analysis (subtyping) by HAI using selected reference antisera. **Referral for confirmation and further characterization:** Laboratories with no capacity for diagnosis of influenza A viruses are recommended to send representative specimens from suspect cases of influenza A (H1N1), according to case definition guidance by WHO, 2 to one of the WHO Collaborating Centres for influenza (WHO CCs). Specimens with laboratory results indicative of influenza A that are untypeable (i.e. negative for influenza A(H1) and A(H3)); are not confirmed according to the WHO criteria) should be forwarded to one of the WHOCCs

for confirmation. Laboratories with no virus isolation capacity (or which do not have the required biosafety containment level) should forward the specimens to any of the WHO CCs. Standard influenza specimen storage, packaging and shipping practice, together with relevant IATA regulations, should be followed. **Biosafety:** Diagnostic laboratory work on clinical specimens from patients who are suspected cases of influenza A (H1N1)swl virus infection should be conducted in BSL-2 containment conditions with the use of appropriate personal protective equipment (PPE). All clinical specimen manipulations should be done inside a certified biosafety cabinet (BSC). Please refer to the WHO Laboratory biosafety manual. Virus isolation currently requires higher biosafety containment measures. Please refer to the document WHO Laboratory biorisk management for laboratories handling human specimens suspected or confirmed to contain influenza A (H1N1) causing the current international epidemics for recommended guidance. **Testing algorithms:** The overall approach to influenza virus detection by RT-PCR should be considered in the context of the national situation, e.g., how many specimens can be handled (throughput), what gene sequence to target for RT-PCR, and whether to use concurrent or sequential testing for RT-PCR of M, NP and HA genes. **Good laboratory practices:** Standard protocols for all procedures should be in place and reviewed regularly. Making sure that the recommended reagents are used and handled properly is critical, as reactions are complex and problems with a single reagent can have large effects on the results obtained. **Validation:** All protocols should always be validated in each laboratory to ensure adequate specificity and sensitivity using the same controls that are employed in each run.

QUALITY ASSURANCE: Standard quality assurance protocols and good laboratory practices should be in place. Participation in the National Influenza Centres (NIC) evaluation exercises (external quality assessment programme) is highly recommended to confirm that laboratories are achieving an adequate level of sensitivity and specificity in their tests. **Training of personnel:** Familiarity with protocols and experience in correct interpretation of results are cornerstones for successful execution of the diagnostic tests. **Facilities and handling areas:** Specimen and reagent handling facilities (including cold chains) with appropriate separation for different steps of RT-PCR must be in place to prevent cross- contamination. Facilities and equipment should meet the appropriate biosafety level. RT-PCR should be performed in a space separate from that used for virus isolation techniques.

EQUIPMENT: Equipment should be used and maintained according to the manufacturer's recommendations.

PREVENTION: Prevention of swine influenza has three components: prevention in swine, prevention of transmission to humans, and prevention of its spread among humans. **Prevention in swine:** Methods of preventing the spread of influenza among swine include facility management, herd management, and vaccination (ATCvet code: QI09AA03). Because much of the illness and death associated with swine flu involves secondary infection by other pathogens, control strategies that rely on vaccination may be insufficient. Control of swine influenza by vaccination has become more difficult in recent decades, as the evolution of the virus has resulted in inconsistent responses to traditional vaccines. Standard commercial swine flu vaccines are effective in controlling the infection when the virus strains match enough to have significant cross-protection, and custom (autogenous) vaccines made from the specific viruses isolated are created and used in the more difficult cases. Present vaccination strategies for SIV control and prevention in swine farms typically include the use of one of several bivalent SIV vaccines commercially available in the United States. Of the 97 recent H3N2 isolates examined, only 41 isolates had strong serologic cross-reactions with antiserum to three commercial SIV vaccines. Since the protective ability of influenza vaccines depends primarily on the closeness of the match between the vaccine virus and the epidemic virus, the presence of nonreactive H3N2 SIV variants suggests that current commercial vaccines might not effectively protect pigs from infection with a majority of H3N2 viruses. The United States Department of Agriculture researchers say that while pig vaccination keeps pigs from getting sick, it does not block infection or shedding of the virus. Facility management includes using disinfectants and ambient temperature to control virus in the environment. The virus is unlikely to survive outside living cells for more than two weeks, except in cold (but above freezing) conditions, and it is readily inactivated by disinfectants. Herd management includes not adding pigs carrying influenza to herds that have not been exposed to the virus. The virus survives in healthy carrier pigs for up to 3 months and can be recovered from them between outbreaks. Carrier pigs are usually responsible for the introduction of SIV into previously uninfected herds and countries, so new animals should be quarantined. After an outbreak, as immunity in exposed pigs wanes, new outbreaks of the same strain can occur. **Prevention in humans:** Prevention of pig to human transmission: Swine can be infected by both avian and human influenza strains of influenza, and therefore are hosts where the antigenic shifts can occur that create new influenza strains. The transmission from swine to human is believed to occur mainly in swine farms where farmers are in close contact with live pigs. Although strains of swine influenza are usually not able to infect humans this may occasionally happen, so farmers and veterinarians are encouraged to use a face mask when dealing with infected animals. The use of vaccines on swine to prevent their infection is a major method of limiting swine to human transmission. Risk factors that may contribute to swine-to-human transmission include smoking and not wearing gloves when working with sick animals.

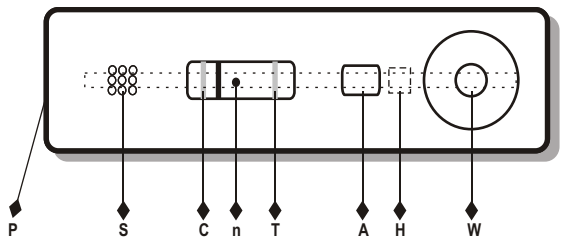
Prevention of human to human transmission: Influenza spreads between humans through coughing or sneezing and people touching something with the virus on it and then touching their own nose or mouth. Swine flu cannot be spread by pork products, since the virus is not transmitted through food. The swine flu in humans is most contagious during the first five days of the illness although some people, most commonly children, can remain contagious for up to ten days. Diagnosis can be made by sending a specimen, collected during the first five days for analysis. Recommendations to prevent spread of the virus among humans include using standard infection control against influenza. This includes frequent washing of hands with soap and water or with alcohol-based hand sanitizers, especially after being out in public. Chance of transmission is also reduced by disinfecting household surfaces, which can be done effectively with a diluted chlorine bleach solution. Experts agree that hand-washing can help prevent viral infections, including ordinary influenza and the swine flu virus. Also avoiding touching eyes, nose and mouth with hands prevents flu. Influenza can spread in coughs or sneezes, but an increasing body of evidence shows small droplets containing the virus can linger on tabletops, telephones and other surfaces and be transferred via the fingers to the mouth, nose or eyes. Alcohol-based gel or foam hand sanitizers work well to destroy viruses and bacteria. Anyone with flu-like symptoms such as a sudden fever, cough or muscle aches should stay away from work or public transportation and should contact a doctor for advice. Social distancing is another tactic. It means staying away from other people who might be infected and can include avoiding large gatherings, spreading out a little at work, or perhaps staying home and lying low if an infection is spreading in a community. Public health and other responsible authorities have action plans which may request or require social distancing actions depending on the severity of the outbreak. **Vaccination:** Vaccines are available for different kinds of swine flu. Although the current trivalent influenza vaccine is unlikely to provide protection against the new 2009 H1N1 strain, vaccines against the new strain are being developed and are being used at field level now.

Thermal scanning of passengers arriving at an airport.

TROUBLESHOOTING

MALARIA - RDTs

Background: Early rapid diagnosis of malaria is crucial to the healthcare programmes in endemic countries. Their increasing importance is in response to increasing drug costs and recognition of the importance of early and correct treatment to attain reduction in malaria morbidity and mortality. Malaria RDT's in the last decade have played a vital role in this regard in reaching objective treatment to affected populations even in resource poor settings, where the traditional microscopic diagnosis is either impractical or impossible. In the last decade there has been an increase in the operational use of RDT's as well as an increase in number of companies manufacturing or dealing in malaria RDT's. Wide range of field and laboratory trials have been conducted in the last decade to assess the accuracy and effectiveness of various products to establish their performance, quality and appropriateness for use in the endemic areas. These trials have given the users and decision makers a huge database to review. In addition, local testing in country of use works to further supplement and validate this performance data for decision making. Published field trials of Malaria RDT's have however shown high variability in performance due to the following reasons: Poor study methods, analysis & reporting. Inexperienced manpower. Incorrect handling. Poor preparation and interpretation. Attempts have been made to undertake larger centralized trials to evaluate the performance of Malaria RDT's. Before discussing the alternative approaches to the performance evaluation of Malaria RDTs, it would be useful to understand the architecture and functioning of Malaria RDTs.



The Essential Components of A Malaria RDT: W = Wicking area for sample / buffer; H = HAMA Blocking Reagents embedded on sample pad; A = Conjugate pad containing the gold conjugate to target antigen specific antibody; T = Test line striped to target antigen specific antibody; n = Nitrocellulose membrane; C = Control line striped with relevant antibody to give a test run validation; S = Soak pad that absorbs the unreacted sample post test conclusion; P = Plastic housing.

Components To Be Used With RDTs:

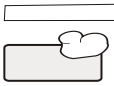

Desiccant with indicator to ensure moisture free pack.


Buffer bottle for test run.


Sample dispensing loop.

Sample dispensing straw.

Lancet for obtaining finger prick blood


Alcohol swab

Malaria RDT's: Immunological Considerations

RDT Combinations	Possible Detection Systems	Detects
Pf only	Monoclonal Anti Pf HRP-II specific	*P. falciparum* infection
Pf only	Monoclonal Anti Pf pLDH specific	*P. falciparum* infection
Pf and Pan	Monoclonal Anti Pf HRP-II specific + Monoclonal Anti Pan pLDH specific (co-specific to *P. falciparum, P. vivax, P. ovale* and *P. malariae)*	*P. falciparum* infection & Differentiate *P. falciparum* infection and non *P. falciparum* infection
Pf and Pan	Monoclonal Anti Pf HRP-II specific + Monoclonal Anti Pan Aldolase specific (co-specific to *P. falciparum, P. vivax, P. ovale* and *P. malariae)*	*P. falciparum* infection & Differentiate *P. falciparum* infection and non *P. falciparum* infection
Pf and Pan	Monoclonal Anti Pf pLDH specific + Monoclonal Anti Pan Aldolase specific (co-specific to *P. falciparum, P. vivax, P. ovale* and *P. malariae)*	*P. falciparum* infection & Differentiate *P. falciparum* infection and non *P. falciparum* infection
Pf and Pan	Monoclonal Anti Pf pLDH specific + Monoclonal Anti pan pLDH specific (co-specific to *P. falciparum, P. vivax, P. ovale* and *P. malariae)*	*P. falciparum* infection & Differentiate *P. falciparum* infection and non *P. falciparum* infection
Pf and Pv	Monoclonal Anti Pf HRP-II specific + Monoclonal Anti *P. vivax* pLDH specific	*Speciates P. falciparum* infection & *P. vivax* infection
Pan only	Monoclonal Anti pan pLDH specific (co-specific to *P. falciparum, P. vivax, P. ovale* and *P. malariae)*	All malaria parasite infection
Pf, Pv and Pan	Monoclonal Anti Pf HRP-II specific + Monoclonal Anti *P. vivax* pLDH specific + Monoclonal Anti pan pLDH specific (co-specific to *P. falciparum, P. vivax, P. ovale* and *P. malariae)*	*Speciates P. falciparum* infection, P. vivax infection, Detects non *P. falciparum* and *P. vivax* infections

General Comments: All HRP-II detection tests, have a better analytical sensitivity and a lower limit of detection for P. falciparum malaria as compared to pLDH and Aldolase based assays. Since HRP-II persists in the blood circulation 2-3 weeks even after successful treatment testing within the said "hang over"period will give "false positive results". Hangover period of pLDH assays is much shorter than for HRP-II based assays. HRP-II detection systems by and large do display better thermal stability over other detection systems based on pLDH and aldolase. However, product to product the stability may vary. pLDH based detection assays have lower analytical sensitivity as compared to HRP-II based assays for detection of P. falciparum infections. pLDH and Aldolase based assays also have lower thermal stability as compared to HRP-II assays.

Similarities and Differences in Malaria RDTs: Though the various RDT's appear to be similar, they vary considerably in their functioning due to the intrinsic character of the critical components employed. The performance of each RDT is based on the optimization of all these functional components, which vary from manufacturer to manufacturer in terms of their basic characteristics and processing methods employed during manufacture.

Component	Typical function	Impact on RDT
Sample Pad	Usually contains HAMA blocking agents.	Improves specificity by blocking RF and heterophilic antibodies.
Buffer	Brings about lysis of the red cells, especially of the sequestered parasite and release of target antigens.	The lysing efficiency of the buffer • dictates the revelation of the target antigen from the parasitized red cells. • Formulation effectiveness impacts sensitivity of test.

Component	Typical function	Impact on RDT
Gold Conjugate	Binds to the Target antigen as the lysed blood flows through the conjugate pad.	Primary binding agent to the target antigen, it influences sensitivity, specificity and binding affinity to target antigen.
Nitrocellulose Membrane	Serves as a migration template /platform for the reaction and directs reactants and reaction kinetics of each test.	**Large porosity membranes:** reactants move fast. Better background clearance, however, leads to loss of test sensitivity since accords less time for reagents to react and bind. **Small porosity membranes:** Reactants move slowly, slower background clearance, improves test sensitivity as reagents have more time to react and bind.
Test lines	Capture antibody directed towards the specific malaria antigen in the sample.	Secondary binding agent in tandem with gold conjugate; drives sensitivity, specificity and affinity of binding and signal intensity.
Control line	Reacts with excess / unreacted conjugate to validate test run.	Ideally should be the weakest visible intensity to serve as a practical comparator for test intensity.
Soak pad	Absorbs excess reactants at test culmination.	Absorbance capacity impacts back flow of reactants post test completion.

The final optimization of each product will then have an impact on its functioning and the resultant: Migration and Reaction Kinetics, Sensitivity, Specificity, Thermal stability, Ease of use.

Problems associated with the usage of Malaria RDT's: RDT's retrieved from cold storage (2-8°C must be allowed to come to room temperature (ambient temperature, in case of field conditions) before the pouches are opened and the test used. When specimen is added to a cold device, it attacks a 'moisture rush' thereby altering the migration properties of the membrane. If specimen is added to 'cold' devices the blood flow is usually slowed down affecting the background clearance and visualization of test results; especially of specimen containing low target antigen concentration. At lower temperatures the antigen-antibody binding is less than optimum, leading to loss in sensitivity and resultant signals. Use of specimen transfer devices such as loops and straws are extremely simple to use. However, to ensure accuracy and precision of sample delivery, user training has to be imparted to build usage competence with actual users. The end point of a Malaria RDT reaction is qualitative. Its interpretation has an element of personal subjectivity, that introduces variability. What appears as a weak positive to our reader may well be negative for another. Usually "tie break"method or "best of 3" is the best way to resolve interpretative subjectivity. User training, hands on experience in reading and interpreting RDT end points must be assessed for consistency as a prerequisite. This is especially relevant when samples used during evaluation are low analyte concentrations, nearing the detection limits of RDT's are being used that generate very low signal intensities.

To be continued

BOUQUET

In Lighter Vein

A redhead walks into a sports bar around 9:58 PM. She sits down next to this blonde at the bar and stares up at the TV. The 10:00 news was on. The news crew was covering a story of a man on a ledge of a large building preparing to jump.

The redhead turns to the blonde and says, "You know, I bet he'll jump."

The blonde replied, "Well, I bet he won't."

The redhead placed $20 dollars on the bar and said, "You're on!"

Just as the blonde placed her money on the bar, the guy did a swan dive off of the building, falling to his death. The blonde was very upset and handed her $20 dollars to the redhead and said, "All is fair. Here is your money."

The redhead replies, "Honey, I can't take your money, I saw this earlier on the 5 o'clock news and knew he would jump."

The blonde replies, "I did too, but I didn't think he'd do it again"

There was a blonde who found herself sitting next to a Lawyer on an airplane. The lawyer just kept bugging the blonde wanting her to play a game of intelligence. Finally, the lawyer offered her 10 to 1 odds, and said every time the blonde could not answer one of his questions, she owed him $5.00, but every time he could not answer hers, he'd give her $50.00. The lawyer figured he could not lose, and the blonde reluctantly accepted.

The lawyer first asked, "What is the distance between the Earth and the nearest star?"

Without saying a word the blonde handed him $5.00. then the blonde asked, "What goes up a hill with 3 legs and comes back down the hill with 4 legs?"

Well, the lawyer looked puzzled. He took several hours, looking up everything he could on his laptop and even placing numerous air-to-ground phone calls trying to find the answer. Finally, angry and frustrated, he gave up and paid the blonde $50.00

The blonde put the $50 into her purse without comment, but the lawyer insisted, "What is the answer to you question?"

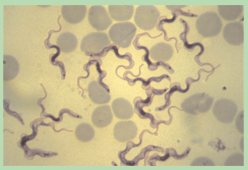
Without saying a word, the blonde handed him $5.

Wisdom Whispers

- Living on Earth is expensive, but it does include a free trip around the sun every year.
- How long a minute is depends on what side of the bathroom door you're on.
- Birthdays are good for you; the more you have, the longer you live.
- Happiness comes through doors you didn't even know you left open.
- Ever notice that the people who are late are often much jollier than the people who have to wait for them?
- You may be only one person in the world, but you may also be the world to one person.
- Some mistakes are too much fun to only make once.
- Don't cry because it's over; smile because it happened.
- We could learn a lot from crayons: some are sharp, some are pretty, some are dull, some have weird names, and all are different colors....but they all exist very nicely in the same box.
- A truly happy person is one who can enjoy the scenery on a detour.

Brain Teasers

Identify the following parasites.

1. 

2. 

3. 

4. 

Answers: 1.Trypanosomes, 2. Ascaris species, 3. Echinococcus granulosus (adult), 4. Adult Tapeworm.

INTERPRETATION

Cystatin C

Cystatin C or **cystatin 3** (formerly **gamma trace**, post-gamma-globulin or neuroendocrine basic polypeptide), a protein encoded by the **CST3** gene, is mainly used as a biomarker of kidney function. Recently, it has been studied for its role in predicting new-onset or deteriorating cardiovascular disease. It also seems to play a role in brain disorders involving amyloid (a specific type of protein deposition), such as Alzheimer's disease. In humans, all cells with a nucleus (cell core containing the DNA) produce cystatin C as a chain of 120 amino acids. It is found in virtually all tissues and bodily fluids. It is a potent inhibitor of lysosomal proteinases (enzymes from a special subunit of the cell that break down proteins) and probably one of the most important extracellular inhibitors of cysteine proteases (it prevents the breakdown of proteins outside the cell by a specific type of protein degrading enzymes). Cystatin C belongs to the type 2 cystatin gene family.

History: Cystatin C was first described as 'gamma-trace' in 1961 as a trace protein together with other ones (such as beta-trace) in the cerebrospinal fluid and in the urine of patients with renal failure. Grubb and Löfberg first reported its amino acid sequence. They noticed it was increased in patients with advanced renal failure. It was first proposed as a measure of glomerular filtration rate by Grubb and coworkers in 1985.

Molecular biology: Cystatin C is a non-glycosylated, basic protein (isoelectric point at pH 9.3). The crystal structure of cystatine C is characterized by a short alpha helix and a long alpha helix running across a large antiparallel, five-stranded beta sheet. Like other type 2 cystatines, it has two disulfide bonds. Around 50% of the molecules carry a hydroxylated proline. Cystatine C forms dimers (molecule pairs) by exchanging subdomains; in the paired state, each half is made up of the long alpha helix and one beta strand of one partner, and four beta strands of the other partner.

Role in medicine: ***Kidney function:*** GFR is best measured by injecting compounds such as inulin, radioisotopes such as ^{51}chromium-EDTA, ^{125}I-iothalamate, ^{99m}Tc-DTPA or radiocontrast agents such as iohexol, but these techniques are complicated, costly, time-consuming and have potential side-effects. Creatinine is the most widely used biomarker of kidney function. It is inaccurate at detecting mild renal impairment, and levels can vary with muscle mass and protein intake. Formulas such as the Cockcroft and Gault formula and the MDRD formula try to adjust for these variables. Cystatin C has a low molecular weight (approximately 13.3 kilodaltons), and it is removed from the bloodstream by glomerular filtration in the kidneys. If kidney function and glomerular filtration rate decline, the blood levels of cystatin C rise. Serum levels of cystatin C are a more precise test of kidney function (as represented by the glomerular filtration rate, GFR) than serum creatinine levels. This finding is based mainly on cross-sectional studies (on a single point in time). Longitudinal studies (that follow cystatin C over time) are scarcer; some studies show promising results. Cystatin C levels are less dependent on age, sex, race and muscle mass compared to creatinine. Cystatin C measurements alone have not been shown to be superior to formula-adjusted estimations of kidney function. As opposed to previous claims, cystatin C has been found to be influenced by body composition. It has been suggested that cystatin C might predict the risk of developing chronic kidney disease, thereby signaling a state of 'preclinical' kidney dysfunction. Studies have also investigated cystatin C as a marker of kidney function in the adjustment of medication dosages. Cystatin C levels have been reported to be altered in patients with cancer, (even subtle) thyroid dysfunction and glucocorticoid therapy in some but not all situations. Other reports have found that levels are influenced by cigarette smoking and levels of C-reactive protein. Levels seem to be increased in HIV infection, which might or might not reflect actual renal dysfunction. The role of cystatin C to monitor GFR during pregnancy remains controversial. Like creatinine, the elimination of cystatin C via routes other than the kidney increase with worsening GFR. ***Death and cardiovascular disease:*** Kidney dysfunction increases the risk of death and cardiovascular disease. Several studies have found that increased levels of cystatin C are associated with the risk of death, several types of cardiovascular disease (including myocardial infarction, stroke, heart failure, peripheral arterial disease and metabolic syndrome) and healthy aging. Some studies have found cystatin C to be better in this regard than serum creatinine or creatinine-based GFR equations. Because the association of cystatin C with long term outcomes has appeared stronger than what could be expected for GFR, it has been hypothesized that cystatin C might also be linked to mortality in a way independent of kidney function. In keeping with its housekeeping gene properties, it has been suggested that cystatin C might be influenced by the basal metabolic rate. ***Neurologic disorders:*** Mutations: Since cystatin 3 also binds amyloid β and reduces its aggregation and deposition, it is a potential target in Alzheimer's disease. Although not all studies have confirmed this, the overall evidence is in favor of are role for CST3 as a susceptibility gene for Alzheimer's disease. Cystatin C levels have been reported to be higher in subjects with Alzheimer's disease. The role of Cystatin C in multiple sclerosis and other demyelinating diseases (characterized by a loss of the myelin nerve sheath) remains controversial. ***Other roles:*** Cystatin C levels are decreased in atherosclerotic (so-called 'hardening' of the arteries) and aneurysmal (saccular bulging) lesions of the aorta. Genetic and prognostic studies also suggest a role for cystatin C. Breakdown of parts of the vessel wall in these conditions is thought to result from an imbalance between proteinases (cysteine proteases and matrix metalloproteinases, increased) and their inhibitors (such as cystatin C, decreased). A few studies have looked at the role of cystatin C or the CST3 gene in age-related macular degeneration. Cystatin C has also been investigated as a prognostic marker in several forms of cancer. Its role in pre-eclampsia remains to be confirmed.

Laboratory measurement: Cystatin C can be measured in a random sample of serum (the fluid in blood from which the red blood cells and clotting factors have been removed) using immunoassays such as nephelometry or particle-enhanced turbidimetry. It is a slightly more expensive test than serum creatinine which can be measured with a Jaffé reaction. Reference values differ in many populations and with sex and age. Across different studies, the mean reference interval (as defined by the 5th and 95th percentile) was between 0.52 and 0.98 mg/L. For women, the average reference interval is 0.52 to 0.90 mg/L with a mean of 0.71 mg/L. For men, the average reference interval is 0.56 to 0.98 mg/L with a mean of 0.77 mg/L. The normal values decrease until the first year of life, remaining relatively stable before they increase again, especially beyond age 50. Creatinine levels increase until puberty and differ according to gender from then on, making their interpretation problematic for pediatric patients. In a large study from the United States National Health and Nutrition Examination Survey, the reference interval (as defined by the 1st and 99th percentile) was between 0.57 and 1.12 mg/L. This interval was 0.55 - 1.18 for women and 0.60 - 1.11 for men. Non-Hispanic Blacks and Mexican Americans had lower normal cystatin C levels. Other studies have found that in patients with an impaired renal function, women have lower and Blacks have higher cystatin C levels for the same GFR. For example, the cut-off values of cystatin C for chronic kidney disease for a 60-year-old White women would be 1.12 mg/L and 1.27 mg/L in a Black man (a 13% increase). For serum creatinine values adjusted with the MDRD equation, these values would be 0.95 mg/dL to 1.46 mg/dL (a 54% increase). Reference values thus clearly are found to be race dependent too. Hence, each laboratory must establish its own normal reference range. Based on a threshold level of 1.09 mg/L (the 99th percentile in a population of 20 to 39-year-olds without hypertension, diabetes, microalbuminuria or macroalbuminuria or higher than stage 3 chronic kidney disease), the prevalence of increased levels of cystatin C in the United States was 9.6% in subjects of normal weight, increasing in overweight and obese individuals. In Americans aged 60 and 80 and older, serum cystatin is increased in 41% and more than 50%. Studies are being conducted in the Indian sub-continent.

TULIP NEWS

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