V. False negativity

On the other hand, false negative tests have been observed even in severe malaria with parasitemias >40000 parasites/µl. This has been attributed to possible genetic heterogeneity of PfHRP2 expression, deletion of HRP-2 gene, presence of blocking antibodies for PfHRP2 antigen or immune-complex formation, prozone phenomenon at high antigenemia or to unknown causes.

VI. Cross reactions between Plasmodia species and problems in identifying non-falciparum species

Cross reaction of PfHRP2 with non-falciparum malaria could give false positive results for P. falciparum and mixed infections containing asexual stages of P. falciparum could be interpreted as negative in about one third of the patients.

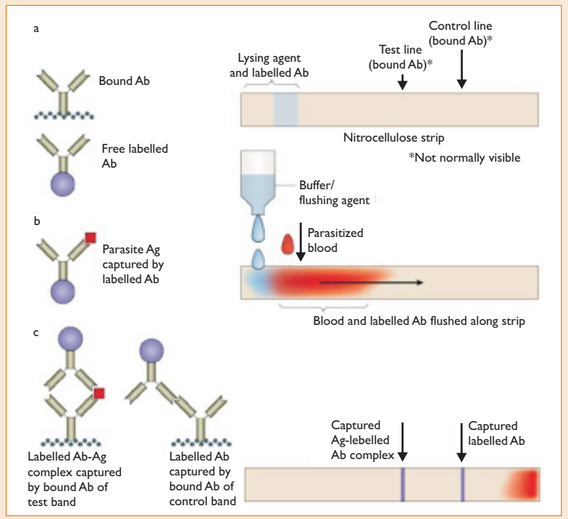
Comparison of Rapid Diagnostic Tests for Malaria Antigens

	PfHRP2 tests	PfHRP2 and PMA test	pLDH test
Target antigen	Histidine rich protein 2 of P. *falciparum*, water soluble protein expressed on RBC membrane	Pan-specific *Plasmodium* aldolase. parasite glycolytic enzyme produced by all species and PfHRP2	Parasite lactate dehydrogenase. parasite glycolytic enzyme produced by all species
Capability	Detects *P. falciparum* only	Can detect all 4 species	Can detect all 4 species
Non-falciparum species	Not detected	Detected; differentiation between the 3 not possible	Detected; differentiation between the 3 not possible
Mixed infections of P. *falciparum* with non-falciparum species	Appear as *P. falciparum;* differentiation not possible	Appear as *P. falciparum;* differentiation not possible	Appear as *P. falciparum;* differentiation not possible
Detection limit	>40-100 parasites/µL	Higher for P. *vivax* and other non-falciparum species	> 100-200 parasites/µL for *P. falciparum* and *P. vivax;* may be higher for *P. malariae* and *P. ovale*
Post-treatment persistence of antigens	Reported up to 31 days	Reported; longer for pan specific antigenemia than for PfHRP2	Reported up to 1 -3 weeks
Cross-reactivity between malarial species	Reported	Reported	Reported
Cross-reactivity with auto antibodies	Reported, high (up to 83% with rheumatoid factor)	Not known	Reported. low (3.3% with rheumatoid factor)
Indication of viability of parasites	No	No	Positive test indicates presence of viable parasitemia

Comparison of Peripheral Blood Smear Examination and RDTs for Malaria

	Peripheral Smear	Rapid Diagnostic Tests
Capability	Detects and differentiates all plasmodia at different stages	Detects malaria antigens (PfHRP2/ PMA/pLDH) from asexual and/or sexual forms of the parasite
Detection threshold	5-10 parasites/µL of blood	100-500/µL for P. *falciparum*, higher for non-falciparum
Species differentiation	Possible	Cannot differentiate among non-falciparum species; mixed infections of *P. falciparum* and non-falciparum appear as *P. falciparum*
Quantification	Possible	Not possible
Differentiation between sexual and asexual stages	Possible	Not possible
Disadvantages	Availability of equipment and skilled microscopists, particularly at remote areas and odd hours	Unpredictable efficiency at low and very high parasitemia; cross reactions among plasmodial species and with auto-antibodies; persistence of antigens
Status	Gold standard	Not yet approved by the FDA

Principle of RDT



Dr. Vaishali Jaurade,
Consultant Pathologist and Chief of Lab, LPL Faridabad, Dr. Lal PathLabs

FROM THE EDITOR'S DESK

"The report is wrong" - is a common response to sterile pyuria - not an uncommon laboratory finding. The article on this topic explores the major causes of sterile pyuria, including infectious, systemic, structural and physiologic, and drug-related causes. It also discusses the differential diagnosis and the basic workup of sterile pyuria. So the next time we are faced with such a report we can go beyond the thought that the lab goofed up.

The issue of drug abuse in the workplace, in schools or at home is an unpleasant one to face. Most deny that the problem exists; others feel sure that they would be able to recognize the problem when and if it occurred. However, taking the appropriate steps to understand the impact of alcohol and drug abuse will help eliminate a slew of negative consequences. Physicians have an opportunity to identify substance abusers years before they have medical complications or present for drug treatment. Occult substance abuse can be effectively identified by screening questions and careful attention to clinical indicators during the history and physical examination.

Drug testing though not undertaken as a stand-alone response to a drug problem is a component of broader prevention, intervention and treatment programs, with the common goal of reducing drug abuse. The results of a positive drug test should be used to intervene through counseling and follow-up testing.

All of us feel an immense pride in being a part of LPL, the proud winner of the Emerging India Awards- 2008 in the Health care category (pharmaceuticals and chemicals). This creates another landmark for LPL, poised to take its service to the next level of growth across the country.

It is indeed encouraging to read your appreciative feedback to the previous issue of INSIGHT and we continue to look forward to your valuable views and suggestions.

Dr. REENA NAKRA

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Dr Lal PathLabs
INSIGHT
Volume 11 Sept. 2008 News Letter

Dr. LAL PATHLABS – Winner of "Emerging India Award 2008" India's Biggest Business Award

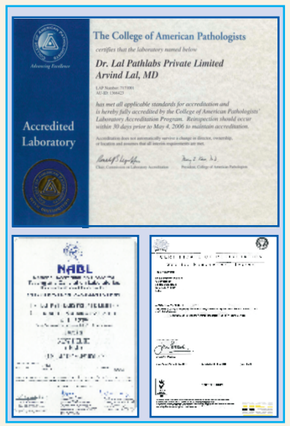
(Health Care Category, Pharmaceuticals and Chemicals)

We are proud to announce that Dr. Lal PathLabs (LPL) is the winner of the prestigious "Emerging India Award 2008". This award is sponsored by ICICI Bank and CNBC TV 18. The award was presented to LPL at a glittering ceremony held at London on 8th July, 2008 by the British Minister for Trade and Industry, Mr. John Hutton. The award was received by Dr. Arvind Lal, Chairman and Managing Director and Dr. Om Manchanda, CEO on behalf of Dr. Lal PathLabs. The function was attended by a large number of business leaders from around the world that included Mr. L. N. Mittal, Chairman of the biggest steel company in the world, Arcellor Mittal, Shri Kamal Nath, Union Minister of Commerce and many others.

The award is given in 15 different business categories and this year there were a record number of three lakh contestants to choose from! The Emerging India Award is India's biggest business award that is given to Small and Medium Enterprises (SME's), the emerging India's dynamic new growth drivers.

The awards are given to honour India's most globally competitive SME's. These companies are not only selected for being the best in their respective fields but should epitomize the best practices in their Business Operations also. CRISIL, the nation's leading credit rating agency, were the auditors for the award and the winners had to go through three rounds of a grueling selection process. This award is in its fourth year of being instituted and creates a global mark with its theme 'Think Global Go Global.' By winning this award, it creates a landmark for LPL that shall propel its service to the next level of growth across the country. This is a dual honour for the lab, one for being the best in the field of Laboratory Medicine and the other for being a top company that has the best business processes.

The lab has an envious record for being the highest accredited lab in India. Out of approximately 40,000 laboratories in the country, only about 100 are recipients of the coveted NABL accreditation. LPL is proud to be the only laboratory services provider in India whose eight labs are accredited. The lab is also the pioneering lab to be awarded the prestigious CAP (College of American Pathologists) accreditation. Currently LPL runs 31 laboratories all over India that are supported by nearly 26 Patient Service Centres (PSC's), 650 other Collection Centres and a record number of 1,500 pick-up points (PUP's) from other labs, doctors clinics, nursing homes and prestigious hospitals. It has the largest test menu in India of more than 1,650 tests and panels and has more than 55 pathologists and 1,200 other staff on its rolls.



STERILE PYURIA

Sterile pyuria is the presence of elevated numbers of white cells (>10/cubic mm) in a urine which appears sterile using standard culture techniques. Often found in female patients with symptoms of urinary tract infection and presence of pyuria but no bacterial growth. However, these results may be misleading for various reasons:

- Standard laboratory culture conditions may not be optimal for growth of atypical organisms.
- Laboratory may not report significant growth either because it was not a single organism or recognized urinary pathogen.
- Less than 100,000 colony-forming units per ml reported, e.g. may be urine was diluted by high fluid intake or organism may be slow growing.

Studies have shown that approximately half of women presenting with symptoms and counts of 100 -10,000 CFU/ml have genuine bladder infections.

Presence of pyuria increases significance of a low bacterial count. Cell count per high power field is inaccurate and use of counting chamber or similar gives more accurate results with 10 white cells/mm3 being diagnostic of infection.

Causes of Sterile Pyuria

- A recently (within last 2 weeks) treated UTI or inadequately treated UTI.
- UTI with 'fastidious' organism.
- Renal tract tuberculosis; Chlamydial urethritis.
- False negative culture due to contamination with antiseptic.
- Contamination of sample with vaginal leucocytes.
- Interstitial nephritis: analgesic nephropathy, sarcoidosis (lymphocytes not neutrophils).
- Urinary tract stones.
- Renal papillary necrosis: diabetes, sickle-cell disease, analgesic nephropathy.

- Urinary tract neoplasm.
- Interstitial cystitis .
- Corticosteroid
- Viral infections
- Exercise (some studies).
- Recent sexual intercourse in young women
- Urinary catheterization or other instrumentation
- Diabetic women- more likely to have pyuria in some studies.
- Polycystic kidneys.
- Prostatitis.
- Balanitis
- Foreign bodies
- Cyclophosphamide (Cytoxan)
- Use of spermicide in young women
- Male patients with AIDS.

Other associations include appendicitis, systemic lupus erythematosus and Kawasaki disease.

They can be conveniently categorized as:

Infective causes	Non-Infective causes
◆ Virsues ◆ Fungi ◆ Atypical or fastidious organism including: ⊕ Chlamydia trachomatis ⊕ Ureaplasma urealyctium ⊕ Pseudomonas aeruginosa ⊕ Mycobacterium tuberculosis	◆ Systemic and localized diseases: ⊕ Malignant hypertension ⊕ Systemic inflammatory diseases ◆ Structural and physiological abnormalities of the genitourinary tract: ⊕ Stones ⊕ Polycystic kidneys ◆ Certain drug treatments: ⊕ Indinavir ⊕ Olsalazine ⊕ Cyclophosphamide ⊕ Glucocorticoids ◆ Recent antibiotic treatment

Epidemiology: Common. Often found in female patients with symptoms of UTI. Also frequent in elderly.

Presentation: Common presentations are:

- UTI: severe dysuria, frequency, urgency, urge incontinence, hematuria, constant suprapubic discomfort, non-specific malaise.
- Interstitial cystitis: similar symptoms to UTI with sterile pyuria. Cystoscopy shows inflammation, sometimes with ulceration. May progress to cause contracture of the bladder. Cause is unknown.

Investigations

- Ask laboratory to culture under conditions allowing identification of fastidious or slow growing organisms.
- Always consider TB: culture for AFB's (3 early morning urines).
- With urine obtained direct from the bladder, any organism grown is significant and should be treated with a prolonged course of appropriate antibiotics.
- Otherwise cystoscopy to exclude non-infective causes.

Before further investigation, it is important to ensure that repeat specimens are collected appropriately. A mid-stream clean catch sample can help avoid contamination with WBCs from vaginal or prostatic secretions. Culture-negative results can be seen in a single sample if the patient has taken antibiotics immediately before giving the sample. But if persistent sterile pyuria is seen and the patient has clinical symptoms consistent with UTI, a further specimen should be taken and the culture repeated to check for fastidious, atypical or resistant organisms. Standard laboratory media kill many of the possible pathogens so the laboratory must be informed of the clinical suspicion of atypical or fastidious organisms.

Sterile Pyuria and SLE

The results of various studies suggest that isolated hematuria and isolated pyuria are associated with active renal and non-renal disease activity. Thus they should be considered manifestations of active SLE.

Sterile Pturia and Tuberculosis

In the past "sterile" pyuria suggested the existence of tuberculosis of the urinary tract. In common with all forms of the disease, the incidence of genitourinary tuberculosis has declined but "Sterile" pyuria is still common. Various studies have been carried out in the past to establish the relationship between the two. The incidence of urinary tract tuberculosis in patients whose urine showed "sterile" pyuria is low, and so the cost effectiveness of surveying all such samples must be equally low. The practice of the laboratory therefore may not be to examine every specimen showing "sterile" pyuria for Mycobacterium tuberculosis but to advise local practitioners to consider the diagnosis of urinary tract tuberculosis when persistent unexplained "sterile" pyuria is observed. When to investigate for tuberculosis? The association of pyuria and microhematuria associated with acid urine, without identification of a putative organism with routine microbiologic studies, should prompt evaluation for tuberculosis.

Management

The finding of pyuria without bacteriuria can be a diagnostic challenge and warrants further investigation. A careful history and physical examination can help determine the cause of sterile pyuria. Always consider TB or non-infective cause and don't prescribe antibiotics if infection is not proven or otherwise suspected.

Dr. Rajni Sabharwal, HOD, Microbiology and Clinical Pathology, Dr. Lal PathLabs

SCREENING TESTS FOR DRUGS OF ABUSE

Drug abuse is deliberate consumption of an illegal drug or overindulgence of a prescribed psychoactive or performance-enhancing drug for non-therapeutic, non-medicinal use/effects. Addicted individuals have uncontrollable, irresistible and compulsive desire and persistence in its use despite negative consequences.

Opiates, Cannabinoids, Cocaine, LSD, Barbiturates, Amphetamines, Alcohol, Diazepam and Ketamine are commonly used drugs of abuse. Prescription drugs that are abused or used for nonmedical reasons can alter brain activity and lead to dependence. Commonly abused classes of prescription drugs include opioids (often prescribed to treat pain), central nervous system depressants (often prescribed to treat anxiety and sleep disorders), and stimulants (prescribed to treat narcolepsy, ADHD, and obesity).

Adverse affects of drugs of abuse

Short term: Even a single use of an intoxicating drug can affect a person's judgment and decisonmaking—resulting in accidents, poor performance in a school or sports activity, unplanned risky behavior, and the risk of overdosing.

Long term: Repeated drug abuse can lead to serious problems, such as poor academic outcomes, mood changes (depending on the drug: depression, anxiety, paranoia, psychosis), and social or family problems caused or worsened by drugs.

Repeated drug use can also lead to the disease of addiction. Studies show that the earlier a teen begins using drugs, the more likely he or she will develop a substance abuse problem or addiction. Conversely, if teens stay away from drugs while in high school, they are less likely to develop a substance abuse problem later in life.

The physical signs could vary from individual to individual and it could be tachycardia and hypertension with cocaine while opiates cause bradycardia and respiratory distress besides other constitutional symptoms and signs. Spreading of infectious diseases like HIV/AIDS, Hepatitis B, pregnancy related complications and deformed newborns, resorting to crime and death due to overdose of drugs are some of the dire consequences of drug abuse.

Drug testing

Some schools, hospitals, or places of employment conduct drug testing. There are a number of ways this can be done, including: pre-employment testing, random testing, reasonable suspicion/cause testing, post-accident testing, return to duty testing, and follow-up testing. This usually involves collecting urine samples to test for drugs such as marijuana, cocaine, amphetamines, PCP, and opiates.

Following the testing models established in the workplace, some schools have initiated random drug testing and/or reasonable suspicion/cause testing. Schools that have adopted random student drug testing are hoping to decrease drug abuse among students via two routes. First, schools that conduct testing hope that random testing will serve as a deterrent, and give students a reason to resist peer pressure to take drugs. Secondly, drug testing can identify adolescents who have started using drugs so that interventions can occur early, or identify adolescents who already have drug problems, so they can be referred for treatment. Drug abuse not only interferes with a student's ability to learn, but it can also disrupt the teaching environment, affecting other students as well. During random testing schools select, using a random process (like flipping a coin), one or more individuals from the student population to undergo drug testing.

Screening tests for drugs of abuse

Screening for dugs of abuse is done on urine and blood specimens by fluorescence Polarization Immunoassay, at Dr. LAL PATHLABS. These screening tests are preliminary analytical tests. The results must be confirmed by Gas chromatography / Mass Spectrometry.

Various testing methods normally test for a "panel" of drugs. Typically, a drug panel tests for marijuana, cocaine, opioids, amphetamines, alcohol and PCP. Alcohol does not remain in the blood long enough for most tests to detect recent use. Breathalyzers and oral fluid tests can detect current use of alcohol.

The Urine Drug Screen is a laboratory-based testing consisting of the following drugs:

- Amphetamines
- Barbiturates
- Benzodiazepines
- Cannabinoids (THC; Marijuana)
- Cocaine
- Methadone
- Opiates
- PCP
- Alcohol

Interpretation of drug tests

Tests are very accurate but not 100 percent accurate. Usually samples should be divided so that if an initial test is positive a confirmation test can be conducted Awareness of possible false-positive results, especially when screening test results for amphetamines or opioids is essential. Over-the-counter cold medications containing pseudoephedrine can cause false-positive screening results for amphetamine, although follow-up testing with gas chromatography and mass spectrometry is highly specific and can reliably confirm the presence of amphetamine. Ingestion of foods that contain poppy seeds makes interpretation of drug testing more difficult, because it can cause screening and gas chromatography and mass spectrometry results to be falsely positive for morphine and/or codeine.

It is fairly easy to defeat drug tests, and most drug-involved youth are all too familiar with ways to do so. Even properly collected specimens must have checks for validity (eg, urine specific gravity and creatinine), because the easiest way to defeat a drug testing is by simple dilution. With the exception of marijuana, the window of detection for most drugs of abuse is 72 hours or less. Therefore, negative test results indicate only that the adolescent did not use a specific drug during the past several days

For how long can drugs be detected in urine?

Each drug is cleared by the body at different rates. When you can find drugs in the urine depends on the drug taken, how often the person takes the drug, and how the drug was taken. The table below shows minimum and maximum times you can detect drugs in urine.

Drug	Drug Street Name	Approximate Detection Time	
		Min	Max
Amphetamine (AMP)	speed, amp, black beauties	2 to 7 hours	2 - 4 days
Cocaine (COC)	coke, snow, blow, candy, crack	1 to 4 hours	2 - 4 days
Ecstasy (MDMA)	E, XTC, X	2 to 7 hours	2 - 4 days
Methamphetamines (MET)	crystal meth, meth, speed, glass	2 to 7 hours	2 - 4 days
Opiates (OPI)	heroin, morphine	2 hours	2 - 3 days
Phencyclidine (PCP)	Angel Dust, rocket fuel	4 to 6 hours	7 - 14 days
Marijuana (THC)	pot, grass, weed, doobie, joint, roach	2 hours	up to 40 days
Benzodiazepines(BZO)	downers	2-7 hours	1-4 days
Barbiturates(BAR)	downers, barbs, red devils	2-4 hours	1-3 weeks
Methadone(MTD)	amdone, chocolate chip cookies, fizzies	3-8 hours	1-3 days
Tri-cyclic Antidepressants (TCA)		8-12 hours	2-7 days
Oxycodone (OXY)	Os, Ox, cotton, blue	1-3 hours	1-2 days

What are drug cut-off levels?

Established "cut - off" levels are conformed to when testing for drugs of abuse. In other words, though some level of drug may be present in a urine sample, the sample would still be considered a NEGATIVE screening result if the drug level is below the cut-off level.

Samples at or near the cut-off level have the greatest margin of error.

Identifier	Drug Name	Cut-off Level
AMP	Amphetamine	1000 ng/ml
COC	Cocaine	300 ng/ml
MDMA	Ecstasy	500 ng/ml
MET	Methamphetamine	1000 ng/ml
OPI	Opiates (morphine,heroin,codeine)	300 ng/ml
PCP	Phencyclidine	25 ng/ml
THC	Marijuana (Tetrahydrocannibinol)	50 ng/ml
BZO	Benzodiazepines	300 ng/ml
BAR	Barbiturates	300 ng/ml
MTD	Methadone	300 ng/ml
TCA	Tri-cyclic Antidepressants	1000 ng/ml
OXY	Oxycodone	100 ng/ml

Admiral Panampilly Sivadas,
Consultant Pathologist and Chief of Lab, LPL Bangalore
Dr. Lal PathLabs

RAPID DIAGNOSTIC TEST FOR MALARIA

Although the peripheral blood smear examination that provides the most comprehensive information on a single test format has been the "gold standard" for the diagnosis of malaria, the immunochromatographic tests for the detection of malaria antigens, developed in the past decade, have opened a new and exciting avenue in malaria diagnosis.

Immunochromatographic Tests for Malaria

Antigens Immunochromatographic tests are based on the capture of the parasite antigens from the peripheral blood using either monoclonal or polyclonal antibodies against the parasite antigen targets. Currently, immunochromatographic tests can target the histidine-rich protein 2 of P. *falciparum*, a pan-malarial *Plasmodium* aldolase, and the parasite specific lactate dehydrogenase. These RDTs do not require a laboratory, electricity, or any special equipment.

Histidine-rich protein 2 of P. *falciparum* (PfHRP2) is a water soluble protein that is produced by the asexual stages and gametocytes of P. *falciparum*, expressed on the red cell membrane surface, and shown to remain in the blood for at least 28 days after the initiation of antimalarial therapy. Several RDTs targeting PfHRP2 have been developed.

***Plasmodium* aldolase** is an enzyme of the parasite glycolytic pathway expressed by the blood stages of P. *falciparum* as well as the non-fa1ciparum malaria parasites. Monoclonal antibodies against *Plasmodium* aldolase are pan-specific in their reaction and have been used in a combined 'P.f/P.v' immunochromatographic test that targets the pan malarial antigen (PMA) along with PfHRP2.

Parasite lactate dehydrogenase (pLDH) is a soluble glycolytic enzyme produced by the asexual and sexual stages of the live parasites and it is present in and released from the parasite infected erythrocytes. It has been found in all 4 human malaria species, and different isomers of pLDH for each of the 4 species exist. With pLDH as the target, a quantitative immunocapture assay, a qualitative immunochromatographic dipstick assay using monoclonal antibodies, an immunodot assay, and a dipstick assay using polyclonal antibodies have been developed.

Principle

The RDTs have been developed in different test formats like the dipstick, strip, card, pad, well, or cassette; and the latter has provided a more satisfactory device for safety and manipulation.

The test procedure varies between the test kits.

In general, the blood specimen (2 to 50µL) is either a finger-prick blood specimen, anticoagulated blood, or plasma, and it is mixed with a buffer solution that contains a hemolyzing compound and a specific antibody that is labeled with a visually detectable marker such as colloidal gold.

In some kits, labeled antibody is pre-deposited during manufacture and only a lysing/washing buffer is added. If the target antigen is present in the blood, a labeled antigen/antibody complex is formed and it migrates up the test strip to be captured by the pre-deposited capture antibodies specific against the antigens and against the labeled antibody (as a procedural control).

A washing buffer is then added to remove the hemoglobin and permit visualization of any colored lines formed by the immobilized antigen-antibody complex.

Problems with RDTs

I. Cross-reactions with autoantibodies:

Studies have reported cross reactivity of the various RDTs with autoantibodies such as rheumatoid factor, resulting in false positive tests for malaria.False positive reactions are higher with the PfHRP2 tests using IgG capture antibody (16.5% to 83%) compared to the PfHRP2 tests using IgM antibodies (6.6%) and the pLDH test (3.3%). Cross reactivity of the PMA antibody with rheumatoid factor does not appear to occur.

II. Sensitivity:

1. Depending upon parasite density: RDTs for the diagnosis of P. falciparum malaria generally achieve a sensitivity of >90% at densities above 100 parasites per µL blood and the sensitivity decreases markedly below that level of parasite density.Many studies have achieved >95% sensitivity at parasitemia of ~500 parasites/µL, but this high parasitemia is seen in only a minority of patients.
2. Depending upon Kit used: For the diagnosis of P. vivax malaria, the PfHRP2/PMA test has a lower sensitivity compared to that for P. falciparum malaria; however, the pLDH test has an equal or better sensitivity for P. vivax malaria compared to P. falciparum malaria. For the diagnosis of P. malariae and P. ovale infections, the sensitivity is lower than that of P. falciparum malaria at all levels of parasitemia on both the PfHRP2/PMA and the pLDH tests.

III. Specificity:

The specificity appears to be better with the pLDH test than the PfHRP2/PMA test for both P. falciparum and non-falciparum malaria.

IV. False Positivity

Potential causes for PfHRP2 positivity, other than gametocytemia, include persistent viable asexual-stage parasitemia below the detection limit of microscopy (possibly due to drug resistance), persistence of antigens due to sequestration and incomplete treatment, delayed clearance of circulating antigen (free or in antigen-antibody complexes) and cross reaction with non-falciparum malaria or rheumatoid factor. Proportion of persistent positivity has been linked to the sensitivity of the test, type of test, degree of parasitemia and possibly the type of capture antibody.

Dr Lal PathLabs