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Wrong Sample Dispensing May Cause False Positive Malaria Test

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ABSTRACT

Both false positive (FP) and false negative are known limitations of any diagnostic test. Malaria parasite (MP) rapid diagnostic tests (RDTs) may give FP results due to interference by substance in blood sample. We detected a FP in a MP RDT for first time in 36-year-old female whole blood donor due to incorrect sample dispensing technique. As per manufacturer's instructions, while allowing all kit components and blood specimen to come to room temperature before testing, blood samples usually separate into lower layer of red blood cells (RBC) and upper layer of plasma. Technician performing the test took the sample from the bottom of the vacutainer thus taking RBC instead of whole blood (WB-recommended by manufacturer). This test showed reactive result and as per our standard protocol was re-tested to confirm the result. This second test was performed after re-mixing the same sample, which now tested as non-reactive sample, buffer and other kit component mix-up were ruled out. Repeated test on another sample of same donor produced same results. Thick and thin peripheral blood smear examination for malaria was found negative. This case highlights wrong MP RDT result due to wrong sample dispensing.

Keywords: Malaria parasite, Rapid diagnostic tests, Rheumatoid factor

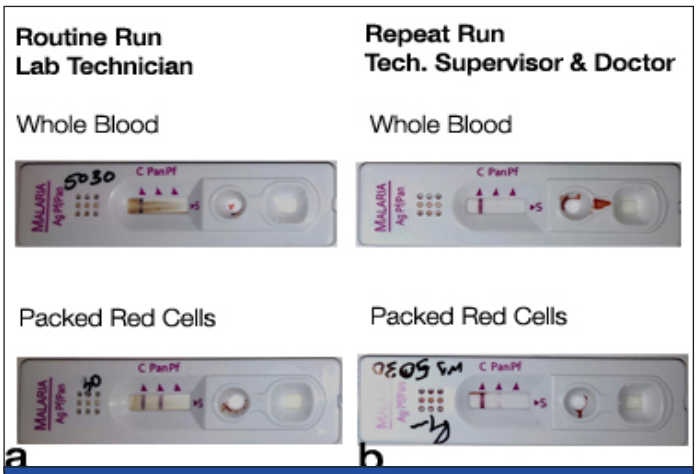
Currently available malaria parasite (MP) rapid diagnostic tests (RDTs) detect *Plasmodium* antigens in blood by antigen-antibody interactions on a nitrocellulose test strip. The targeted antigens include those specific to *Plasmodium falciparum* (histidine-rich protein-2 and *P. falciparum*-specific parasite lactate dehydrogenase) and/ or antigens common to all the *Plasmodium* species (pan-species pLDH and aldolase). RDTs use a control line with one, two or three antigen detecting test lines, and are referred to as two-, three and four-band RDTs respectively.

Both false positives (FP) and false negatives are known limitations of any diagnostic test and MP RDTs are no exception. MP RDTs may give FP results due to interference by substances in the blood sample, e.g. rheumatoid factor, infection with Hepatitis C, lipemic, hemolytic sample, etc [1]. Similarly, there may be false negative reactions with these RDTs. Most false-negative results occur at lower parasite densities. However, false-negative results have been reported at high parasite densities also [2]. We present here a hitherto unreported cause of FP result in an MP rapid diagnostic test.

We detected a FP in a MP RDT for first time in 36-year-old female whole blood donor due to an incorrect sample dispensing technique. All blood donor samples are routinely screened for MP using 3 band RDT (SD Bioline, SD Bio Standard Diagnostics Pvt. Ltd, Gurgaon – 05FK60I) at our blood center. Owing to a very low positive MP yield in healthy blood donors [3], further investigation of all positive cases is done. First test on this donor sample was done using EDTA sample. Blood samples usually separate into a lower layer of red blood cells (RBC) and an upper layer of plasma while waiting for all the RDT kit components and blood specimen to come to the room temperature before testing. In this particular case, the technician performing the test did not mix the sample by inverting and took the sample for testing from the bottom of the vacutainer. Thus instead of taking the whole blood sample as per the manufacturer's recommendation, RBC sample was used for MP testing. This test showed reactive result and as per our standard protocol was re-tested to confirm the result. This second test was performed after re-mixing the same sample, which now tested as non-reactive [Table/Fig-1a]. Sample mix-up was ruled out by blood sample key re-evaluation and re-testing of adjacent samples. Test was repeated on another sample of same donor by another technician using both RBC as well as WB. Buffer and other kit components were checked to rule out mix-up with another kit reagent – a cause of FP results [4]. However, the test result was same, i.e. reactive with RBC (although with a weaker band now) and non-reactive by whole blood saple [Table/Fig-1b]. Thick and thin peripheral blood smear examination of the donor blood sample was done in Microbiology Department and was found to be negative. This ruled out probable FP due to a high parasite density [5].

Other routine tests performed on the donor sample were negative, viz. markers for HIV I/II, hepatitis B & C, VDRL and irregular antibody against RBC (by Indirect Coombs Test). Although, RDT manufacturer claims non-interference by known substances in specimen (e.g. rheumatoid factor (RF), lipaemic, hemolyzed, icteric sample, etc.) with test result, RF on donor sample was done using qualitative test (Agappe Diagnostics, India). Same was found to be positive.

Donor's available medical history form was re-evaluated and no significant medical or surgical history was found. So, the donor was called (nearly 15 days after blood donation) and interviewed



[Table/Fig-1a,b]: Wrong sample type leading to false positive results in malarial antigen testing

for detailed medical history again, especially for malaria illness and treatment – past and present. Medical history was un-revealing and donor claimed to be in good health. Donor was requested to inform the blood centre in case of developing any illness within few weeks of interview. Since donor did not contact back, donor was again contacted after 6 weeks of donation. Donor was fit and did not suffer from any ailment.

This case highlights wrong MP RDT result due to wrong sample dispensing technique. This type of error may be happening routinely in all laboratories and thus the need to carefully evaluate technique of testing at the bench side. FP result in our case although could have been due to interfering RF present in the donor sample, negative result with whole blood and positive with RBC was intriguing. Nonetheless, such false positive result in malaria antigen testing has never been reported and this is the first such case. All positive results in malaria antigen testing thus need to be carefully re-checked before reporting the sample as positive for malaria.

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