

The features identification of *Plasmodium* at different developmental stages

(1) Ring stage (RS): dense nucleus, the cytoplasm becomes slender to form the ring-like shape, the change of the volume of erythrocytes was not so dramatic. The volume of erythrocytes infected with *Plasmodium vivax* will distend, the volume of erythrocytes infected with *Plasmodium malariae* will shrink.

(2) Late trophozoite (LT): the nucleus of *Plasmodium* is loose and coarse, the cytoplasm becomes thicker and harbors vacuoles of various sizes, the formation of pseudopods is obvious in erythrocytes infected with *Plasmodium vivax*. The cytoplasm extension is full of scattered granular brown malaria pigments; the erythrocytes parasitized by late trophozoites of *Plasmodium vivax* become swollen and show highly irregular shape; the erythrocytes parasitized by *Plasmodium malariae* and *Plasmodium falciparum* shrink or remain the same volume.

(3) Stage II-III gametocytes of *Plasmodium vivax*: the nucleus of *Plasmodium vivax* become loose and coarse, the cytoplasm was thicker, the vacuoles inside the cytoplasm gradually disappear, the formation of pseudopod protrusions gradually decreased, and the brown pigments in the extended cytoplasm were finely granular in shape. The swelling of erythrocytes with large trophozoites was relieved, and the irregular shape was gradually changed in a round shape.

(4) Stage-IV gametocytes of *Plasmodium vivax*: the nucleus of *Plasmodium vivax* was loose and coarse; the vacuoles in the cytoplasm disappeared to become a whole body, more coarse granules, or short rods of dark brown pigment in the extended cytoplasm area were observed, the parasitized erythrocytes remained swollen and the shape became round, and *Plasmodium vivax* had not yet swollen to occupy the whole erythrocytes.

(5) Stage-V gametocytes of *Plasmodium vivax*: the morphology of the nuclei, cytoplasm and pigments of Stage-V *Plasmodium vivax* is similar to that of Stage-IV, except for the erythrocytes infected by *Plasmodium vivax* were distended to the full and the round shape was more complete [40].

(6) Stage II-IV gametocytes of *Plasmodium malariae*: the morphological change of *Plasmodium* was similar to that of *Plasmodium vivax*, except for the volume of the parasitized erythrocytes was reduced.

(7) Stage III-V gametocytes of *Plasmodium falciparum*: the nuclei of *Plasmodium falciparum* increased and gradually clustered in the center; the cytoplasm thickened and the shape was gradually changed from semi-circular (stage III) and column (stage IV) to crescentic or salami (stage V); the volume of the parasitized erythrocytes remained normal [41]. Meanwhile, the density of *Plasmodium vivax* in the bone marrow aspirate was calculated by using the erythrocyte counting method.