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Title of resource
GN_29: Measuring the human IgG and IgM immune response against salivary antigens of *Simulium damnosum* s.l. by ELISA as a proxy of exposure to blackfly bites.
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DOI
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Description
Protocol for performing two separate immunoassays to measure IgG or IgM anti-*S. damnosum* s.l. antibodies in humans. This protocol was developed as part of the Gnatwork Transformative Science project "Understanding host heterogeneity to vector-borne disease exposure: Implications for modelling disease transmission and improving the design of control interventions".
Intended use
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Resource history
N/A

GN_29 Measuring the human IgG and IgM immune response against salivary antigens of Simulium damnosum s.l. by ELISA as a proxy of exposure to blackfly bites.

When using this protocol, the following should be referenced:

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Introduction

Human onchocerciasis is a parasitic disease caused by the filarial nematode *Onchocerca volvulus* and transmitted by bites of female blackflies, with the *S. damnosmum sensu lato* (s.l.) complex representing the most important vectors in Africa. Currently, transmission dynamics models use exposure patterns inferred from hypothetical distributions, as accurate exposure data is lacking. Therefore, we developed two immunoassays aimed at understanding inter-individual exposure, including age- and/or sex-specific patterns of exposure to blackflies of the *S. damnosum* s.l. complex. These immunoassays quantify IgG and IgM antibody responses in human populations to salivary antigens of *S. damnosum* s.l., and were validated in onchocerciasis endemic communities of Ghana. This protocol is complementary to our protocol GN_30 in which we describe the dissection of blackfly salivary glands, an essential component to perform these immunoassays.

Materials

- ✓ Blackfly salivary glands (*see GN_30: Dissecting salivary glands of field collected S. damnosum s.l.)*
- ✓ 96-well microtiter plates
- ✓ Microplate reader
- ✓ Non-fat dry milk
- ✓ Anti-human IgG or IgM antibody-HRP conjugated (Sigma Aldrich; Bethyl Laboratories, Inc.)
- ✓ Orthophenylendiamine (OPD) tablets/ powder
- ✓ Hydrogen peroxide (30%)
- ✓ Sulfuric acid (10 % H_2SO_4)
- ✓ Coating buffer (20 mM carbonate-bicarbonate, pH 9.5)
- ✓ Phosphate buffered saline + Tween 20 (PBS-Tw; 0.05% PBS-Tween 20, pH 7.5)
- ✓ Substrate buffer (phosphate-citrate buffer, pH 5.5)

Preparation of all solutions is explained below

Methods

In each step only 100 µl of solution (coating/ washing/ blocking/ plasma/ conjugate) is added to each well

Make sure to cover the plate with a lid when incubating

A. Coat the wells of a 96-well microtiter plate with the antigen (i.e. blackfly salivary gland proteins) at a concentration of:
    a. 0.2 µg when running the IgG immunoassay
    b. 0.025 µg when running the IgM immunoassay

    antigen is coated in 100 µl of coating buffer
B. Incubate the plate overnight at 4° C (cover the plate with a lid)
C. Wash the plate twice with PBS-Tw (100 µl/well)
D. Block the plate with 6 % low fat dry milk diluted in PBS-Tw (100 µl/well), for 60 min at 37° C (cover the plate with a lid)
E. Wash the plate three times with PBS-Tw (100 µl/well).
F. Dilute plasma samples at 1:100 (for the IgG immunoassay) or 1:50 (for the IgM immunoassay) in 2 % non-fat dry milk in PBS-Tw and add to the corresponding well (test each sample in duplicate in the same plate) (100 µl/well)
G. Incubate for 90 min at 37° C (cover the plate with a lid)
H. Wash the plate five times with PBS-Tw (100 µl/well)
I. Dilute the HRP-conjugated anti-Human IgG antibody (Sigma-Aldrich; A6029)) 1:1000 in PBS-Tw and add to the wells (100 µl/well)
    or the HRP-conjugated anti-Human IgM antibody (Bethyl Laboratories, Inc.; A80-100P)) 1:100,000 in PBS-Tw and add to the wells (100 µl/well)
    and incubate for 45 min at 37° C (cover the plate with a lid)
J. Wash the plate five times with PBS-Tw (100 µl/well)
K. Add OPD and 30 % H_2O_2 to the substrate solution and add to the wells (100 µl/well)
    *** 10 ml of substrate buffer + 0.005 g OPD (or 1 OPD tablet of 5 mg) + 10 µl 30% H_2O_2 ***
L. Incubate in the dark at room temperature for minimally 5-6 minutes
M. Stop the reaction by adding 100 µl of sulfuric acid to each well
N. Read the plate (with a microplate reader) at 492 nm.

Notes

- ✓ Make sure the substrate solution is at room temperature before usage (store in the fridge)
- ✓ OPD is light sensitive, after adding it to the substrate buffer protect the solution from light using aluminum foil, make sure the powder/tablet is dissolved before proceeding
- ✓ Only add the 30 % H_2O_2 to the substrate + OPD solution right before adding it to the wells
- ✓ After adding 100 µl of the substrate + OPD + H_2O_2 solution to each well cover the plate with aluminum foil and leave for 5-6 min
- ✓ Add methodological controls to each plate:

  Control 1 = ADD antigen; NO Serum (only 2% milk); ADD Conjugate

  Control 2 = ADD antigen; NO Serum (only 2% milk); NO Conjugate (only PBS-Tw)

  Control 3 = NO antigen (only carbonate/bicarbonate buffer); NO serum (only 2% milk); ADD conjugate

  BLANK = NO antigen (only carbonate/bicarbonate buffer); NO serum (only 2% milk); NO conjugate (only PBS-Tw)

  Attention

  Make sure that the controls are never dry.

  E.g.: Control 1: you do not add serum, but instead you will add just 100 µl of the 2 % milk in PBS-Tween

Solutions

A. Coating buffer (20mM carbonate/bicarbonate):
   - pH = 9.5
   - 0.106 g Na_2CO_3 in 50 ml distilled water
   - 0.42 g $NaHCO_3$ in 250 ml distilled water
   - to adjust pH to 9, add drops of Na_2CO_3 solution to solution of $NaHCO_3$

B. Substrate buffer (phosphate-citrate buffer):
   - pH = 5.5
   - 0.11 M $Na_2HPO_4.12H_2O$ 3.97 g in 100 ml distilled water
   - 0.50 M citric acid 5.25 g in 50 ml distilled water
   - to adjust pH to 5.5, add drops of citric acid solution to solution of $Na_2HPO_4.12H_2O$

C. PBS (10x):
   - 80 g NaCl
   - 2 g KCl
   - 15.3 g $Na_2HPO_4.12H_2O$
   - 2 g KH_2PO_4
   - add up to 1000 ml by distilled water

D. PBS-Tween:
    - Dilute PBS (10x) 1:9 to get PBS (1x)
    - Adjust pH = 7.5
    - Add 250 µl Tween20 (0.05 %) to 500 ml PBS