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Development of flow cytometry based adherence assay for *Nessieria gonorrhoeae* using 5′-carboxy-fluorosceinsuccidyl ester (CFSE) and ME-180 cells

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Statement of the Problem: The microorganism *Nessieria gonorrhoeae* is an obligate human pathogen and its adherence to host cells is essential for its pathogenesis. We devised a flow cytometry-based method to quantify the adherence of piliated *N. gonorrhoeae* strain F62 to human cervical ME-180 cells.

Methodology: Piliated *N. gonorrhoeae* F62 were collected after 10 to 12 hours of growth then stained with cell-permeable fluorescent dye 5'-carboxyfluoroscein succidyl ester (CFSE). The bacteria were incubated with 0.5 µl of 5 mM CFSE in 2.5 ml of PBS and incubated at 37°C for 15 min. ME-180 cells were incubated for 2 hours with fluorescent, piliated *N. gonorrhoeae* (multiplicity of the infection 1:100) then the ME-180 cells were washed with phosphate buffer saline to remove loosely adherent bacteria. Flow cytometry was used to quantify the percentage of ME-180 associated with $CFSE^+$ fluorescent bacteria and a minimum of 30,000 events were recorded.

Finding: Results indicated that 19.2% ± 0.99 (n=4) ME-180 cells were associated with the fluorescent, piliated bacteria. To assess whether antibodies specific for *N. gonorrhoeae* blocked their adherence to ME-180 cells, rabbit hyper-immune anti serum was raised against heat-killed piliated *N. gonorrhoeae* F62. Adherence efficiency, the percentage of cell-associated $CFSE^+$ bacteria divided by the total input $CFSE^+$ bacteria ranged between 37-47% (n=5). Heat-inactivated hyperimmune serum, at 1:10 to 1:80 dilutions, significantly inhibited gonococcal adherence by 6 and 3 fold, respectively. Heat-inactivated negative rabbit serum was significantly (3 to 5 folds) less effective at preventing bacterial adherence suggesting that antibody specificity and not a non-specific serum component were involved. Flow cytometric analysis was amenable to the quick, easy and high-throughput quantification of *N. gonorroheae* association with eukaryotic cells. These approaches may be adapted for use in *in vitro* and *in vivo* adherence studies related to gonococcal pathogenesis.

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