SUMMARY
Infectious Mononucleosis (IM) is an infectious disease produced by Epstein-Barr virus (EBV). It is characterized by irregular fever for up to one or two weeks, clinical lymph nodes’ pain and swelling and pharyngitis. Due to atypical lymphocyte proliferation, the hematological laboratory findings are quite distinctive.

Paul and Bunnell reported that serum from these patients usually contains heterophile antibodies’ high titer, which can agglutinate horse or sheep erythrocytes. These antibodies are not exclusive to Mononucleosis since anti-sheep agglutinins with 1/28 and up to 1/56 titers may appear in serum from healthy individuals; moreover, 1/112 or higher titers are found in several infections and after antigenic stimulation (such as blood transfusion).

Those facts indicate that the evidence of high heterophile antibody titers has only a presumptive value in the diagnosis of IM. Davidsohn introduced the differential adsorption with Guinea pig kidney extract (capable of adsorbing non-IM heterophile antibodies or Forssman antibodies), thus increasing test sensitivity and specificity and enabling the confirmation of the results from the Paul-Bunnell’s test, which, being a quick and practical test, is still used as screening test.

However, approximately 10% patients suffering from Infectious Mononucleosis have low titers of heterophile antibodies, hence results of these tests are considered relevant only for diagnosis when they are compared with the patient’s hematological data and clinical manifestations.

PRINCIPLE
Heterophile antibodies appear in serum from patients with Infectious Mononucleosis (IM). These antibodies are detected by their ability to agglutinate horse erythrocytes, after neutralizing heterophile antibodies not related to IM (Forssman antibodies) with Guinea pig kidney extract. In this way, heterophile antibodies related to Infectious Mononucleosis are specifically bound to erythrocytes, producing a macroscopically visible agglutination.

INSTRUCTIONS FOR USE
Reagent A: empty the dropper and mix vigorously before use, checking that no erythrocytes are left at the bottom of the bottle.
Positive and Negative Controls: ready to use.

WARNINGS
Reagents are for “in vitro” diagnostic use. Controls have been tested and found to be non-reactive for HIV, HCV and HBV. Nevertheless, they should be treated as if capable of transmitting infection.

Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS
Provided Reagents: stable at 2-10°C until the expiration date stated on the box. Do not freeze.

SAMPLE
Serum
a) Collection: obtain serum in the usual way.
b) Additives: not required.
c) Known interfering substances: a thermolabile inhibitor which produces false positive results has been reported, therefore serum should be inactivated to avoid this interference. Hemolysis also interferes with the reaction.
Inactivation: incubate serum for 15 minutes at 56°C before use.
d) Stability and storage instructions: use fresh serum whenever possible. If serum cannot be immediately assayed, store for up to 48 hours at 2-10°C or for up to 3 months at -20°C.

REQUIRED MATERIAL
1- Provided
- Glass slide.
2- Non-provided
- Glass rod or wood sticks.
- Stopwatch.
- Glassware capable of measuring and diluting samples.
- Horizontal lamp or light source.

PROCEDURE
Bring reagents samples to room temperature before use.
I- QUALITATIVE TECHNIQUE
Place 1 drop (50 ul) of inactivated sample in one of the reaction areas of the slide. Add 1 drop (50 ul) of homogenized Reagent A. Mix with the stick to obtain a homogeneous suspension spreading over the entire reaction area. Start stopwatch at the same time, tilting the slide gently and read the result macroscopically within 2 minutes.
For a correct visualization of results, the slide should be held over a black background and slightly above a light source (e.g.: microscope lamp).

II- SEMI-QUANTITATIVE TECHNIQUE
The titer of positive sera detected with Technique I, may be obtained making serial dilutions:
1) Place 200 ul of Saline in several 10 x 75 mm glass tubes.
2) Add 200 ul of inactivated sample to tube #1 and mix. Transfer 200 ul of this dilution to tube #2 and mix, following the same steps with the remaining tubes.
The dilutions obtained in this way are equal to 1:2, 1:4, 1:8, 1:16, etc.
3) Test each dilution according to Technique I.

INTERPRETATION OF RESULTS
Qualitative technique
Negative: homogeneous suspension.
Positive: agglutination within two minutes, which can be scored from 1 to 4 (+):
4+: strong agglutination
3+: moderate agglutination
2+: mild agglutination
1+: slight agglutination
A positive result indicates the presence of heterophile antibodies associated to Infectious Mononucleosis.

Semi-quantitative technique
Titer: is expressed as the inverse of the highest dilution showing a macroscopically visible agglutination.

QUALITY CONTROL METHOD
It is convenient to test simultaneously the provided Positive and Negative Controls as reference.

PROCEDURE LIMITATIONS
See Known interfering substances under SAMPLE.

PERFORMANCE
Sensitivity: fresh (native) erythrocytes are used in Davidsohn’s original technique. These erythrocytes yield unspecific reactions, therefore hemagglutination is considered as a positive reaction for Infectious Mononucleosis only for dilutions higher than 1:4.
Sensitivity of stabilized erythrocytes provided by Monoslide kit has been regulated to provide the same reactivity with inactivated undiluted serum.

WIENER LAB. PROVIDES
- 100 tests:
  1 x 5 ml Reagent A
  1 x 1,5 ml Positive Control
  1 x 1,5 ml Negative Control
(Cat. 1593151)

REFERENCES
Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent kits.

- CE: This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
- Authorized representative in the European Community
- IVD: "In vitro" diagnostic medical device
- Σ: Contains sufficient for <n> tests
- Use by
- Temperature limitation (store at)
- Do not freeze
- Biological risks
- Volume after reconstitution
- Contents
- Batch code
- Calibr.: Calibrator
- CONTROL: Control
- CONTROL +: Positive Control
- CONTROL -: Negative Control
- REF: Catalog number
- Manufactured by:
- Harmful
- Corrosive / Caustic
- Irritant
- Consult instructions for use