REAGENT PREPARATION

1. **Packages Powdered Phosphate Buffer:** (PBS) pH 7.4 ± 0.2
2. **Vial (2 mL) Buffered Glycerol
3. **Packages (5 each) Bottlers

*These components may be interchanged between different master lots. Additional information is available from Hemagen Diagnostics, Inc.

MATERIALS REQUIRED BUT NOT SUPPLIED

Test tubes and racks for making dilutions Pipettes for preparing dilutions Coverslips, 22 x 55 mm, No. 1 thickness Humidified culture chamber Magnetic stir plate (optional)

Staining dish and slide-holder rack

Fluorescence microscope. Refer to manufacturer’s instruction manual for the filter system that gives optimum results for FITC. (Maximum excitation wavelength = 490 nm. Mean emission wavelength = 520 nm.)

PRECAUTIONS

1. **Health Lab ASSAY SPECIMENS, SLIDES, POSITIVE AND NEGATIVE CONTROLS AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS.**

All human blood components of the kit have been tested by approved FDA methods and found to be negative for both hepatitis B surface antigen (HBsAg) and for antibodies to human immunodeficiency virus type 1. Because no test method can offer complete assurance that HIV, hepatitis B viruses, or other blood-borne viruses are absent, specimen kits and reagents should be handled at the Biosafety Level 2, as recommended for any potentially infectious human serum or blood specimen.6

2. **The antisera are fixed in acetone.**

3. **Do not pipette by mouth.**

4. **Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.**

5. **All materials used in this assay, including reagents, samples and washing materials should be disposed of in a manner that will inactivate infectious agents.**

HANDBOOKING PRECAUTIONS

1. **For In Vitro Diagnostic Use.**

2. **Do not use the kit or individual reagents beyond their labeled expiration dates.**

3. **The components of this kit have been tested as a unit. Do not interchange components from other sources or from different master lots, except as follows.**

4. **Protect the conjugate from prolonged exposure to light.**

REAGENT STORAGE AND STABILITY

1. **Store kit at 2-8°C. Powdered PBS and Buffered Glycerol can be stored at -20°C if desired; the last kit can be used through the expiration date on the outer box label.**

2. **After rehydration, Positive Control, Negative Control and FITC Conjugate solutions should be stored at 2-8°C for 2 weeks.**

The second step involves adding a fluorescing labeled anti-human antibody to the test wells. If the specific antigen-antisera complex is not formed, the non-fluorescing labeled antibody will attach to the antibody moiety of the complex in step two.

**It is necessary to test 1 Positive Control, 1 Negative Control, and 1 PBS Buffer Control for each batch of slides tested.**

3. **Cover each well with diluted samples or controls (+10-20 µL per well).**

4. **Incubate in a humidified chamber at 37°C for 30 to 60 minutes.**

5. **Rinse the slides briefly in a light stream of PBS. Do not dry the slides or allow the test serum to remain on the slides for any length of time.**

6. **Blot the painted mask of the slide with the blotters provided. Do not allow the wells to dry before conjugate addition.**

7. **Incubate for 30 minutes at 2-37°C for 30 to 60 minutes.**

8. **Rinse the slides thoroughly for 7 minutes in a staining dish of PBS.**

9. **Change the buffer and wash the slide 2-3 times with PBS.**

10. **Gently agitate the buffer is necessary for efficient and complete washing.**

11. **Protect from intense light.**

12. **Repeat steps 8 and 9.**

13. **Blot the painted mask of the slides with the blotters provided. Do not allow the wells to dry before the addition of glycerol.**

14. **Place a small drop of Buffered Glycerol in each well and cover with a coverslip.**

15. **For best results, the slides should be read immediately at a magnification of 10X. The results obtained are out of range, the test is invalid and should be repeated.**

CRITERIA FOR GRADING FLUORESCENCE INTENSITY

1. **Brilliant apple-green fluorescence**
2. **Bright apple-green fluorescence**
3. **Clear distinguishable apple-green fluorescence**
4. **Dull apple-green fluorescence, lacking in sharpness but readable**
5. **No fluorescence or barely visible fluorescence**

GUIDELINES FOR CHARACTERIZING FLUORESCENCE

A positive mumps antibody reaction is identified by the presence of a brightly fluorescent cytoplasm in the infected cells with typical inclusions. As an additional control, uninfected cells are mixed with the infected cells. Each high power field should contain cells that exhibit specific fluorescence. These uninfected 'negative' cells should exhibit red or orange staining of the cytoplasm with a greenish-black to black nucleus.

Non-specific Fluorescence

All the cells exhibit positive apple-green fluorescence, either nuclear, cytoplasmic, or both. A reaction produced by a disease state unrelated to or in addition to a mumps infection, e.g., antinuclear antibody or rheumatic fever. Such a reaction should be considered invalid.8

No Fluorescence

Absence of less than 1 fluorescence in the cytoplasm of infected cells.

NOTE: The end-point titer at the highest dilution showing a 1+ fluorescence in the inclusions.

QUALITY CONTROL

1. **The control sera are representative of positive and negative reactions.**

2. **For the screening dilution, the Positive Control represents a strong positive reaction.**

3. **If the fluorescence intensity of the Positive Control is less than the acceptance level, the test is invalid.**

4. **Each lot of Positive Control must be titrated to an endpoint dilution.**

5. **To ensure that the indirect fluorescent antibody (IFA) assay for the determination of mumps in human sera is a distinct advantage of the indirect fluorescence assay is the ability to determine the immunoglobulin class involved in the immune response.**

Addition of the antigen-antibody conjugate to the serum to perform, requires no serum pretreatment and results are rapidly obtained.

The VIRGO Mumps IFA Kit manufactured by Hemagen Diagnostics, Inc. contains all the necessary reagents for the rapid determination of Mumps antibody in human sera. Antigenic substrate, control sera, FITC conjugate, buffer, coverslip mounting media and an instructional insert are included in the kit.

**NOTE:**

6. **All the cells exhibit positive apple-green fluorescence, either nuclear, cytoplasmic, or both. A reaction produced by a disease state unrelated to or in addition to a mumps infection, e.g., antinuclear antibody or rheumatic fever. Such a reaction should be considered invalid.**

No Fluorescence

Absence of less than 1 fluorescence in the cytoplasm of infected cells.
of paired serum samples taken 10-14 days apart.

LIMITATIONS OF THE PROCEDURE
1. The VIRGO Mumps IFA Test Procedure and the Interpretation of Test Results must be followed closely to obtain reliable test results.
2. IgG antibody has been shown to rise rapidly in the course of mumps infection. Demonstration of a four-fold rise in titer may be dependent on obtaining the first serum very early in the course of the infection.
3. Heterologous antibody responses have been reported due to the presence of other paramyxoviruses such as parainfluenza virus.
4. Lack of a significant rise in antibody titer does not exclude the possibility of mumps infection.
5. Results of this test should be interpreted in the light of other clinical findings and diagnostic procedures.

MICROSCOPE SPECIFICATIONS
Comparable filter systems are shown below:

Transmitted Light Fluorescence:

<table>
<thead>
<tr>
<th>Light Source</th>
<th>Exciter Filter</th>
<th>Barrier Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury Vapor</td>
<td>KPF400 + BG38 (4 mm.)</td>
<td>KPS10, K530</td>
</tr>
<tr>
<td>200W</td>
<td>50W</td>
<td>+ BG38 (4 mm.)</td>
</tr>
<tr>
<td>Tungsten</td>
<td>KPF400 + BG38 (4 mm.)</td>
<td>KPS10, K515, K530</td>
</tr>
<tr>
<td>Halogen</td>
<td>KPF400</td>
<td>KPS10, K515, K530</td>
</tr>
<tr>
<td>50W</td>
<td>100W</td>
<td></td>
</tr>
</tbody>
</table>

Incident Light Fluorescence:

<table>
<thead>
<tr>
<th>Light Source</th>
<th>Exciter Filter</th>
<th>Dichroic Beam</th>
<th>Splitting Mirror</th>
<th>Barrier Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury Vapor</td>
<td>KPF500 + BG38 (4 mm.)</td>
<td>TK-510</td>
<td>K510</td>
<td></td>
</tr>
<tr>
<td>200W</td>
<td>100W</td>
<td>(4 mm.) or BG23 (4 mm.) for stronger red suppression.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50W</td>
<td>100W</td>
<td>Edgelfilter 450 nm, 480 nm, for narrow band excitation, suppression of tissue autofluorescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tungsten</td>
<td>KPF500 + BG38 (4 mm.)</td>
<td>TK510</td>
<td>K510</td>
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<tr>
<td>Halogen</td>
<td>KPF500</td>
<td>K515</td>
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<tr>
<td>50W</td>
<td>100W</td>
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</tbody>
</table>

SUMMARY OF VIRGO Mumps IgG IFA

IMPORTANT: It is recommended that one be familiar with the detailed procedure in the package insert before using this summary.

1. Cover wells with the 1:8 screening dilution of sample or control. For quantitative determination, prepare serial two-fold dilutions.
2. Incubate slides in a humidified chamber at 37 ± 2°C for 30 minutes.
3. Rinse slides briefly with PBS. Wash for 15 minutes, changing buffer 1X. Blot slides.
4. Cover each well with FITC Conjugate.
5. Incubate slides in a humidified chamber at 37 ± 2°C for 30 minutes.
6. Repeat wash step described above.
7. Place a small drop of Buffered Glycerol on each well and cover with a coverslip.
8. Read slides immediately at 200-500X magnification on fluorescence microscope.

BIBLIOGRAPHY

FOR IN VITRO DIAGNOSTIC USE

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P/N 890100-10 Rev. I
August 2000